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INHERITANCE OF RESISTANCE TO ROSETTE VIRUS DISEASE IN GROUNDNUT (*Arachis hypogaea* L.)

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INHERITANCE OF RESISTANCE TO ROSETTE VIRUS DISEASE IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

A Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology in Partial Fulfilment of the Requirements for the Degree of

MASTER OF PHILOSOPHY

IN

AGRONOMY (PLANT BREEDING)

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(Bsc. Hons. Agriculture)

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DECLARATION

I hereby declare that, I have under supervision undertaken the study and except for specific references which have been duly and appropriately acknowledged, this project is the result of my own research and has not been submitted either in part or whole for other degree elsewhere.

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DEDICATION

This thesis is dedicated to Prof. R. C. Abaidoo and Prof. Richard Akromah for being the inspirational forces behind my studies.

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ABSTRACT

Groundnut rosette disease is one of the most destructive diseases militating against groundnut production in sub-Sahara Africa and Ghana in particular. The disease causes an annual losses of US$156 million across Africa. The development of resistant varieties is necessary to help curb the situation. A study was conducted to understand the inheritance pattern of the disease resistance. Knowledge of inheritance of groundnut rosette resistance is required to accelerate breeding of resistant varieties. Thus F_1, RF_1, F_2, RF_2, BC_1, RBC_1, BC_2, RBC_2 progenies were derived from crosses of Otuhia x Manipintar, Otuhia x Shitaochi, ICGV 01276 x Manipintar and ICGV 01276 x Shitaochi along with their parents were sown in a randomized complete block design at CSIR-CRI, Fumesua, under artificial infection. Disease diagnosis using TAS ELISA revealed the presence of GRAV antigens in the resistant samples analyzed. Resistance genotypes containing GRAV were considered to be resistant to the GRV and its sat RNA, but not the GRAV which causes no obvious symptoms by itself. Generation mean analysis was carried out to detect the nature of gene action responsible for the disease resistance inheritance. The results revealed that inheritance to groundnut rosette disease resistance is governed by both additive and non-additive gene effect. The data suggested that additive gene action effect was predominant on the resistance to the disease in all the crosses. Additive by dominance was the only form of non-allelic interaction observed. Analysis of variance showed significant difference ($P \leq 0.05$) among generation means. Mean reciprocal difference suggested the presence of maternal effect involved in the inheritance of resistance to groundnut rosette disease. Estimate of broad and narrow sense heritability indicates that genetic effect was larger than the environmental effects in this study. Negative heterosis over the mid-parent was observed for the rosette resistance. Pure line breeding with selection from early generation is suggested for the improvement of the

trait, because the additive genetic effect contributed significantly in controlling the inheritance of resistance to GRD.

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

[a]	Additive
[aa]	Additive*additive
[ad]	Additive*dominance
ANOVA	Analysis of variance
AP	Alkaline Phosphate
et al	and others
BC_1	Backcross one
BC_2	Backcross two
CGIAR	Consultative Group for International Agricultural Research
CRI	Crop Research Institute
CSIR	Center for Scientific and Industrial Research
[d]	Dominance,
DAI	Days after Inoculation
[dd]	Dominance*dominance
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EPG	Electric Penetration Graph
F_1	First filial generation
F_2	Second filial generation

FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization Statistics
GRAV	Groundnut Rosette Assistor Virus
GRD	Groundnut Rosette Disease GRV
Groundnut Rosette Virus	
h	hour
ha	Hectare
Hb2	Broad sense heritability
Hn2	Narrow sense heritability
ICRISAT	International Crop Research Institute for Semi – Arid Tropics
IgG	Immuno-γ-globuli
Kb	Kilo base
Kg	Kilogram
LSD	Least significant difference
m	Mean
MAb	Monoclonal antibody
MoFA	Ministry of Food and Agriculture
MT	Metric Tone
NP	Number of plants
ns	not significant
ORFs	Open reading frame
P_1	Parent 1
P_2	Parent 2
PBTools	Plant Breeder Tools
PDI	Percentage disease incidence

RAM	Rapid Antibody medium
RBC_1	Reciprocal Backcross one
RBC_2	Reciprocal Backcross two
RF_1	Reciprocal First filial generation
RF_2	Reciprocal Second filial generation
RT PCR	Reverse transcriptase polymerase chain reaction
SADC	Southern African Development Community
Sat RNA	Satellite RNA
SE	Standard Error
SSA	Sub-Saharan Africa
S_2	Variance
TAS ELISA	Triple-antibody sandwich enzyme-linked immunosorbent assay
VBC_1	Variance of Backcross 1
VBC_2	Variance of Backcross 2
VF_1	Variance of First filial generation
VF_2	Variance of second filial generation
VLP	Virus-like particle
VP_1	Variance of Parent 1
VP_2	Variance of Parent 2
$	United States Dollar

CHAPTER 1

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.) belongs to the family *Leguminoceae* and sub-family *Papilionoideae* (Waele and Swanevelder, 2001). According to Asiedu (1989), groundnut is a herbaceous plant of which there are two major types, bunch and runner. Apart from the bunch and the runner types, many intermediate forms or hybrids exist (Irvine, 1974). Agro-ecologically, groundnut is grown mostly in the northern savanna zone in Ghana, where the highest yield of 1.92 MT/Ha has been recorded (MoFA, 2011). According to Breisinger *et al*. (2008), regional contribution of groundnut to the national total in Ghana stands as follows; Coastal zone 7.7%, Forest zone 9.5%, Southern Savannah zone 7.2%, and Northern Savannah zone 75.6%. Most of the crop is produced in regions with an annual rainfall of 400mm or more under low evaporative demand but there is a minimum requirement for 200mm during the growing season although this is greater in soils that do not store winter rainfall (Gibbon and Pain, 1985). A good rainfall distribution during the vegetative period of growth will encourage adequate flowering and proper development of the nuts (Tweneboah, 2000).

Groundnut seed is rich in oil (38-50%) and contains 22 to 30% protein on dry seed basis, minerals (calcium, potassium, phosphorus, magnesium) and vitamins (Brink and Belay, 2006; Shilling, 2002). It is processed into paste (butter) and widely used by Ghanaians to make soup, stews, and cereal mixtures (Asibuo *et al*., 2008). Groundnut cake from industrial oil processing is mostly used for human and livestock feed especially in the south (Awuah *et al*., 2009). Groundnut is a cash crop providing income and livelihoods to the farmer. It covered 24 million ha area worldwide with a total production of 38 million tons in 2010 (FAOSTAT, 2010).

Despite the recognition of Ghana as one of the leading producers of groundnut in the world, yield on farmers field continue to be below the attainable yield of 2-3 MT/ha due to biotic and abiotic

factors including unstable rainfall patterns, diseases and pest infestation, lack of quality seeds and favourable agronomic practices. These problems have led to low yield and low marketability of groundnut in the international market. Groundnut production is largely constrained by biotic stresses, with groundnut rosette virus disease (GRD) contributing to annual losses of US$156 million across Africa (Nigam *et al*., 2012). Groundnut rosette virus (GRD) is one of the most devastating diseases of groundnut in Africa and Ghana in particular. The disease is one of the most important diseases militating against groundnut production in Ghana (CSIR-SARI, 2014).

Three synergistic agents cause rosette disease. These include groundnut rosette virus (GRV), a satellite RNA of GRV and groundnut rosette assistor virus (GRAV) (Bock *et al*., 1990). The fast spread of GRD is facilitated by the cowpea aphid (*Aphis craccivora* Koch) that is widely distributed in the tropics and Mediterranean regions (Waliyar *et al*., 2007), in a persistent, circulative manner (Okusanya and Watson, 1966).

Although the disease epidemics are sporadic, yield losses approach 100% whenever it occurs in epidemic proportions. For example, an epidemic in northern Nigeria destroyed approximately 0.75 million hectares of groundnut with an estimated loss of US$250 million in regional trade (Yayock *et al.,* 1976). Recurrent epidemics (Olorunju *et al.*, 1992) have limited production since 1975. Similarly, the epidemics that occurred in 1995 in eastern Zambia affected about 43 000 ha, causing an estimated loss of US$4.89 million. In 1996, in the central region of Malawi, groundnut production was reduced 23% by groundnut rosette disease (Anonymous, 1996).While the initial GRD epidemics reported in early 1970's were characteristic of chlorotic rosette symptoms, mosaic and green forms have also been reported in some epidemics (Naidu *et al*., 1999). Plants affected by any of these major forms are often severely stunted and bushy, with leaves being curled and distorted (Nigam *et al*., 2012). Yield loss due to GRD depends on the growth stage at which infection occurs whereby in seedlings infection leads to 100% yield loss while

infection at the pod filling stage causes negligible effects (Naidu *et al*., 1999b; Waliyar *et al*., 2007).

Several methods have been employed by famers to curb GRD. Planting early in the season when the aphid population is low combined with a close plant spacing results in greatly reduced incidence of GRD (Naidu *et al*., 1999b; Taliansky *et al*., 2000; Waliyar *et al*., 2007). However, these methods are not economically feasible for most smallholder farmers in major growing countries. Chemical control measures targeting aphids have been employed for GRD control. The timing, dosage, and type of insecticidal applications are critical for effectively diminishing the aphid vector population especially where spray timing is based on an early forecast of vector migration into the crop (Naidu *et al*., 1999b). Moreover the cost of insecticides and proper application equipment is beyond the economic means of the majority of resource-poor farmers who grow the crop. Again, economic realities and public sensitivity to environmental degradation have currently rendered extensive insecticide use unacceptable. However, the most economic, ecological and environmentally–friendly method of control is the use of rosette resistant lines (Adu-Dapaah *et al.*, 2004). Economic benefits because, crop yields are saved from loss to the disease and money is saved by the farmer by not applying insecticides that would have applied to a susceptible varieties. Ecologically and environmental benefits arise from increases in species diversity in the agro-ecosystem, in part because of reduced use of insecticides.

This has led to concerted efforts to develop varieties that are resistant to the rosette virus to minimize the use of chemicals. Breeding for resistance to diseases remains a principal focus in the groundnut breeding programme in Ghana. Knowledge regarding the amount of genetic variation created through hybridization is prerequisite for groundnut improvement. Although genetics of resistance to the disease has been reported, the mechanism of resistance may be different in the sources of parents. To facilitate the design

of breeding strategies to develop resistance cultivars to groundnut rosette disease (GRD), it would be beneficial to understand more completely the mode of inheritance of this trait.

1.1 OBJECTIVES

The main objective of the study was to investigate the mode of inheritance of resistance to groundnut rosette virus disease.

The specific objectives were:

1. To determine the mechanism of gene action controlling GRVD resistance.
2. To determine the contribution of maternal effects to GRVD resistance.
3. To determine the heritability and heterosis of the trait.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 ORIGIN, DISTRIBUION AND TAXONOMY OF GROUNDNUT

Groundnut (*Arachis hypogaea* L.), which belongs to the family *Leguminosae* and a subfamily *Papilionoideae*, originated in South America and domesticated in the area

covered by Brazil, Argentina, Paraguay, Peru and Bolivia (Tweneboah, 2000; De Waele and Swanevelder, 2001). Today, groundnut is widely distributed and adapted in the tropical, subtropical and warm temperate regions of the world.

The major groundnut producing countries of the world are India, China, Nigeria, Senegal, Sudan, Burma and the USA. In Africa, groundnut is a major cash crop in Senegal, Gambia, Nigeria and Sudan (Brink and Belay, 2006; Martin *et al.*, 2006). According to Tweneboah (2000), out of 6 million tons of groundnuts produced in Africa, about 80% comes from the savanna zone, south of the Sahara. Although groundnut is grown in all the agro-ecological zones of Ghana, about 85 % of the area under groundnut cultivation and the bulk of groundnut production takes place in the Guinea and Sudan savanna agroecological zones in the north (Atuahene-Amankwa *et al*., 1990).

The species of genus *Arachis* are perennial or annual legumes and made up of a large and diverse group of diploid (2n = 2x = 20 or 18) and allotetraploid (2n = 4x = 40) (Stalker, 1997; Burow *et al*., 2008). *Arachis hypogaea* is a recent allotetraploid (David *et al.*, 2012), most probably resulting from hybridization of two wild species followed by natural chromosome duplication (Halward *et al.*, 1991; Young *et al*., 1996; Seijo *et al.*, 2007). It is divided into two subspecies, *hypogaea* and *fastigiata* Waldron. Each of the subspecies is further divided into botanical varieties; subsp. *hypogaea* into var. *hypogaea* and var. *hirsuta*, subsp. *fastigiata* Waldron into var. *fastigiata*, var. *vulgaris*, var. *peruviana* and var. *aequatoriana*. Only three botanical varieties, subsp. *hypogaea* var. *hypogaea*, subsp. *fastigiata* var. *fastigiata* and var. *vulgaris* are widely cultivated in the Americas, Africa, and Asia (Ferguson *et al*., 2004). There are 80 species in the genus *Arachis* divided into nine sections: *Arachis, Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Rhizomatosae, Trierectoides,* and *Triseminatae* based on morphology and cross-compatibility relationships (Valls and Simpson, 2005).

The subspecific and varietal classifications are based on morphological characteristics such as growth habit, branching patterns, pubescence, stem colour, and pod and seed size and shape (Krapovickas and Gregory, 1994). According to Isleib and Wynne (1983), intermediates between the subspecies are rare but do exist, which sometimes makes classification of the cultivated species difficult.

2.2 PRODUCTION, USES AND ECONOMIC IMPORTANCE OF GROUNDNUT

Bunting *et al.* (1985) noted that groundnut is a popular legume crop in the world, valued for its "nuts", oil, meal, and vegetative residue. It is mostly produced in areas where the mean rainfall is 600 - 1200 mm per annum and the mean daily temperatures in the range of 25- 28 $^{\circ}$C (CGIAR, 1994; Maiti, 2002). It is estimated that about 13.5 million ha are grown in Asia, 5.3 million ha in Africa, 1.2 million ha in the Americas. (Carley and Fletcher, 1995). In 2010, the total area under groundnut reached 23.91 million ha worldwide, with an estimated production of 37.95 million tonnes (unshelled) and mean yield of 1.58 tonnes ha^{-1} (FAO, 2012). According to ICRISAT (2014), developing countries in Asia, Africa and South America account for 97% of the area of groundnut and 95% of total production.

Groundnut serves as a major source of protein, essential vitamins and trace minerals in most vegetarian diets. Its oil content and quality varies depending on the cultivar, geographical location, season and growing conditions (Asibuo *et al*., 2008). Most relief agencies supply groundnut pastes to alleviate malnourishment in droughts and famines, mostly in children. Groundnuts play an important dietary role in most developing countries especially Ghana, where they provide high-quality cooking oil and an important source of protein for both humans and animals (Awuah, 2000). According to Carlberg (2012), it serves as a cash crop to provide

income for farmers in developing country. It is eaten raw, roasted, cooked made into candies and the flour is an important ingredient in many foods. The crop produce nodules its roots which host rhizobium that fix atmospheric nitrogen into the soil for its use. This helps increase productivity of semiarid cereal cropping system through the improvement of the soil fertility status (Tweneboah, 2000). Groundnut also provides cash to poor farmers in the developing countries of Asia and sub- Saharan Africa, contributes significantly to food security and poverty alleviation (Naidu *et al*., 1999). Generally, yields of groundnut grown by smallholder farmers in Africa are consistently low (Stalker, 1997; Holbrook and Stalker, 2003). This has led to a wide difference in yields from farms in Africa and those of other parts of the world. For instance, in 2010 the world mean yield for groundnut was 1580.7 kg ha^{-1}, while in Africa the production is pegged at 902.1 kg ha^{-1} compared to 3086.2 kg ha^{-1} realized in Americas (FAO, 2012).

2.3 CONSTRAINTS TO GROUNDNUT PRODUCTION

Groundnut offer an important opportunity to improve livelihoods and nutrition, but its production is subject to important constraints. According to Maiti (2002), groundnut production is constrained by several biotic and abiotic factors such as diseases, pests, aflatoxin contamination, nematodes and drought. Its production is driven by the use of relatively abundant farm labour and extremely limited purchased inputs, with the main purchased input used by those farmers who have larger land areas. In the sub-Saharan region of Africa, diseases are generally regarded as a major constraint to groundnut production (Chiteka *et al.,* 1992). In addition to these, GRD which occurs only in Africa, is also a major production constraint (Nigam, 2008).

Minde *et al*. (2008) noted that diseases such as GRD, early leaf spot and rust are widespread and reduce yields when they occur. It is estimated that early and late leaf spot diseases cause up to 70% yield loss (Monfort *et al.*, 2004), while loses due to rust exceed

50% worldwide (Hagan *et al.,* 2006). GRD also contributes significantly to the low productivity of the crop in Africa with epidemics costing an estimated US$156 million annually (Ntare *et al.,* 2002; Monyo *et al*., 2008). Monyo *et al*. (2008) noted that Africa is the only place where GRD and leaf spot diseases regularly combine to cause devastating yield losses in groundnut crops. Groundnut is also attacked by both pre- and post-harvest insect pests that cause significant economic loses. Over 400 species of pests attack groundnut (Lynch, 1990). Knauft and Wyne (1995) indicated that foliar feeders of groundnut cause maximum yield loss when their feeding reduces photosynthetic area, especially during pod initiation and pod fill period. Insects serve as vectors for viruses and also cause damage to pods and seeds making them undesirable for marketing apart from directly lowering yields (Stalker, 1997).

Lack of access to sufficient quantities of improved seeds is one of the causes of low groundnut productivity because it forces farmers to use low yielding varieties and recycled kernel as seed (Simtowe *et al.,* 2010). There is also a lack of interest by commercial seed companies to breed and sell seeds of self-pollinated crops, because it can be recycled by farmers hence making it uneconomic to breed them (Siambi and Kapewa, 2004). As a result, there is no established groundnut seed enterprise in Ghana which reliably produces and sells good quality groundnut seed.

Aflatoxin contamination of groundnuts is a major constraint to it marketability in Africa (Lubulwa and Davis, 1994). The high level of aflatoxin observed in many products is not unavoidable. Northern Ghana's climatic conditions allow farmers to thoroughly dry their seeds before storage even with local post-harvest handling techniques. In Ghana lack of equipment for harvesting and shelling peanuts makes harvesting laborious, which is one reason why it is widely grown by low-income farmers with few other employment options. Harvesting and plucking are done manually and pods are transported from the

fields either by head, donkey cart, or in rare instances, tractors or trucks (Tsigbey *et al*., 2003).

Erratic or insufficient rainfall is also a major constraint to groundnut production in rainfed environments (Madhava *et al.*, 2003). Groundnut is highly drought tolerant and can grow well in many areas of the world where most other food legumes fail to produce any yield (Holbrook and Stalker, 2003).

2.4 GROUNDNUT ROSETTE DISEASE

Groundnut Rosette Virus disease (GRD) has long been regarded a major limiting biotic constraint to groundnut production in Sub-Saharan Africa (SSA) (Kayondo *et al.*, 2014). GRD usually occurs in small proportions in every growing season, but its severity increases in groundnut crops sown late in the season. When epidemics do occur, groundnut production is significantly reduced and the disease has the potential to cripple rural economies in SSA (Naidu *et al.*, 1999b). An epidemic in northern Nigeria in 1975 destroyed approximately 0.7 million ha of groundnut, with an estimated loss of US$250 million (Yayock *et al*., 1976). Similarly, an epidemic in 1995 in eastern Zambia affected approximately 43,000 ha causing an estimated loss of US$4.89 million. In the following year in the central region of Malawi, groundnut production was reduced by 23% (SADC/ICRISAT Groundnut Project, 1996). Key market class cultivars, including landraces have succumbed to GRD, resulting in yield reduction to as low as 800 kg ha^{-1}, compared with 3,000 kg ha^{-1} reported from on-station plots in Uganda (Okello *et al.*, 2010).

Potential gains due to adequate control of GRD is US$121 million annually, considering mainly improved genetic resistance to the disease (Waliyar *et al*., 2007). GRD is a virus disease, transmitted by an aphid, *Aphis craccivora Koch* (Insecta: Homoptera) (Storey

and Bottomley 1928; Storey and Ryland 1955, 1957; Hull and Adams 1968). Three causal agents involved in GRD etiology are groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV) and a satellite-RNA (SatRNA) (Reddy *et al.,* 1985a, b; Murant *et al*., 1988; Taliansky *et al.,* 2000). The complex association of the three agents in causing GRD makes it a unique and fascinating virus disease whose origin and perpetuation in nature, in spite of significant advance in our knowledge, still remain a mystery.

2.5 SYMPTOMS OF GRD

GRD occurs with two variant symptoms, chlorotic and green rosette with variable symptoms within each type (Murant and Kumar, 1990; Naidu *et al*., 1999; Waliyar *et al*., 2007). Plants affected by the disease are severely stunted, with shortened internodes and reduced leaf size, resulting in a bushy appearance of plants (Naidu *et al*., 1999). Variants of the SatRNA is mainly responsible for symptom variations (Murant and Kumar 1990, Taliansky and Robinson, 1997). 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*., 1998). Leaves of plants affected by chlorotic rosette are usually bright yellow with a few green islands. Whiles in the green rosette leaves appear dark green, with light green to dark green mosaic. Infection due to chlorotic or green rosette disease occurring in young plants (prior to flowering) usually results in 100% yield loss. There is usually 100% yield loss when infection due to chlorotic or green rosette occurred in young plants (prior to flowering). Waliyar *et al*. (2007) reported that 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, but infection after pod setting/ maturation causes negligible effects on pod yield.

The deleterious impact of GRAV or GRV on host plant together with SatRNA in a synergistic manner is not known (Waliyar *et al.*, 2007). Stunting is more severe in diseased groundnut plants containing all the three agents than in diseased groundnut plants containing only GRV and SatRNA (Ansa *et al.*, 1990). According to some reports, 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). But recent studies have demonstrated that, GRAV infection alone affects plant growth and contributes to significant yield losses in susceptible cultivars (Naidu and Kimmins, 2007).

2.6 CAUSAL AGENTS

2.6.1 GROUNDNUT ROSETTE ASSISTOR VIRUS

GRAV belongs to the family *Luteoviridae* and was first identify as a causal agent groundnut rosette disease by Hull and Adams (1968). The virus is anti-genically related to barley yellow dwarf, bean leaf roll, beet western yellows and potato leaf roll viruses (Casper *et al*., 1983; Reddy *et al*., 1985a; Scott *et al*., 1996). Casper *et al*. (1983) and Reddy *et al*. (1985a) characterized the virus and identified it as a luteovirus. Its virions are non-enveloped, isometric shaped with 28 nm diameter particles of polyhedral symmetry and made of single coat protein subunits of size 24.5 kDa. It has a nonsegmented genome, single molecule of linear positive-sense, single-stranded RNA of c.

6900 nucleotides that encodes for structural and non-structural proteins (Murant *et al*., 1989). GRAV is thought to encode for six Open Reading Frames (ORFs) unlike other members of the luteovirus. The virus replicates autonomously in the cytoplasm of phloem tissue and transmitted by *A. craccivora* in a persistent manner. The virus on its own causes symptomless infection or transient mottle, and can cause significant yield loss in

susceptible groundnut cultivars (Naidu and Kimmins, 2007). The only known natural host of the virus is groundnut.

2.6.2 GROUNDNUT ROSETTE VIRUS

GRV is an umbravirus but has no recognizable virus-like particle (VLP). It was first isolated and characterized by Reddy *et al.* (1985b). According to Taliansky and Robinson (2003), the virus has no structural (coat) protein and thus no conventional virus particles are formed. Moreover, enveloped bullet-shaped structures discovered in the ultra-thin sections of infected cells were shown to be cytopathological structures due to GRV infection, as opposed to real virions (Taliansky and Robinson, 2003). The virus genome is a non-segmented positive sense with RNA of size c. 4019 that codes for four ORFs and is single liner molecule (Taliansky *et al*., 1996). The genome of an isolate was completely sequenced (Gene Bank Accession# z66910) and several partial sequences are available in the Gene Bank. Taliansky and Robinson, 2003 again reported that it replicates autonomously in the cytoplasm of the infected tissues. GRV on its own causes transient symptoms, but a SatRNA associated with GRV is responsible for rosette disease symptoms. GRAV is responsible for encapsidation of its RNA transmission in a persistent mode by *A. craccivora* (Robinson *et al.*, 1999). Waliyar *et al*. (2007) reported that the virus can be transmitted by grafting and mechanical inoculation, but not through seed, pollen or contact between the plants.

2.6.3 SatRNA

It is an ssRNA with a molecular weight of 0.9 kb. It's required for both rosette symptom production and replication (Murant *et al*., 1988; Diennier *et al*., 1996) and aphid transmission (Murant, 1990). The SatRNA (subviral RNAs) of GRV belongs to the

Subgroup-2 (small linear) satellite RNAs. It is linear, single-stranded and non-segmented RNA of 895 to 903 nucleotides (Murant *et al.,* 1988; Taliansky *et al.,* 2000). It entirely depends on GRV for its replication, movement and encapsidation, both within and between the host plants. It is responsible for rosette symptoms and plays a critical role in GRAV.

2.7 DISTRIBUTION OF GRD

Both green and chlorotic rosette are very common in Africa with green rosette predominant in West Africa (Cote d'Ivoire, Ghana and Nigeria) and Uganda in East Africa. However, the chlorotic rosette is widespread (Senegal, Gambia, Burkina Faso, Cote d'Ivoire, Ghana, Nigeria, Uganda, Somalia, Tanzania, Zambia, Zimbabwe, Mozambique, South Africa and Malawi) (Alegbejo and Abo, 2002) .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 and *A. craccivora* is found in almost all these groundnut growing regions (Waliyar *et al.,* 2007).

2.8 SYNERGISTIC INTERACTION AMONG THE GRD AGENTS FOR VECTOR TRANSMISSION

All the three agents intricately dependent on each other in GRD etiology, which is crucial in the biology and perpetuation of the disease (Taliansky and Robinson, 1997; Naidu *et al.,* 1999b). In the absence of GRAV, aphid's fails to transmit GRD and plants lacking GRV and SatRNA do not show any symptoms (Waliyar *et al.,* 2007). GRAV and GRV replicates autonomously in the host plants. But Sat RNA depends entirely on GRV for its replication. GRV must associate itself with its SatRNA for its packaging in the GRAV coat protein and subsequent transmission by the vector. GRAV alone causes no obvious symptoms, but variants of SatRNA have been shown to be responsible for different rosette

symptoms, such as green and chlorotic rosette (Murant and Kumar, 1990: Taliansky and Robinson, 1997). SatRNA is also mechanically transmissible along with the GRV (Blok *et al.*, 1994).

Aphid craccivora commonly is the only known principal vector involved in the transmission of all the GRD agents in a persistent and circulative way (Storey and Bottomley, 1928; Storey and Ryland, 1955; Watson and Okusanya, 1967; Hull and Adams, 1968). Studies have shown that all the GRAV particles whether they contain GRAV RNA or GRV RNA and SatRNA are acquired by the aphid vector from phloem sap in 4 h and 8 h to cause the disease (Misari *et al.*, 1988). Moreover there is a latent period of 26 h 40 min and 38 h 40 min for chlorotic and green rosette and the inoculation access feeding period of 10 min for both forms (Waliyer *et al.*, 2007). Once acquired, aphid can transmit virus particles for up to two weeks and beyond. 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*., 1999b). Under natural conditions, some GRD-affected plants (GRV and SatRNA positive) can be free from GRAV, while GRAV can be detected in some non-symptomatic plants (no GRV and SatRNA) (Naidu *et al.*, 1999b).

This situation is due to difference in inoculation feeding behavior of the vector leading to transmission of (i) all the three agents together, (ii) only GRAV or (iii) GRV and SatRNA, as demonstrated by the electrical penetration graph (EPG) studies of aphid stylet activities (Naidu *et al.*, 1999b). The studies indicated that, the vector explored the leaves without reaching the phloem, leading to only GRV and SatRNA transmitted during short inoculation feeding. 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). Disease plants lacking GRAV become dead-end sources because aphids

cannot acquire or transmit GRV and SatRNA from it. If such plants receive GRAV later due to feeding the vector, such plants serve as source of inoculum (Waliyar *et al*., 2007).

2.9 DIAGNOSIS OF GROUNDNUT ROSETTE DISEASE

Groundnut rosette disease can be diagnosed by using several methods based on biological, serological (protein-based) and genomic properties (Nucleic acid) of the GRD agents. Serological and nucleic acid –based methods can only be used for the detection of GRAV, but only the nucleic acid based method can be used to detect the GRV and SatRNA. Triple antibody sandwich- enzyme –linked immunosorbent essay method (TAS-ELISA) has been developed for detection of GRAV (Rajeswari *et al*., 1987) and dot-blot hybridization and reverse transcription-polymerase chain reaction (RT-PCR) to detect all the three GRD agents in plants and aphids (Blok *et al.*, 1995; Naidu *et al*., 1998). GRD can be diagnosed in the field based on the characteristic symptoms on groundnut. The use of the symptom based and the TAS- ELISA method is widely used, because the utilisation of the RT-PCR is not economical and need advanced biotechnological equipment and skills.

TABLE 1. Properties of groundnut rosette disease agents and methods for their detection

			Transmission on Groundnut			Detection	
Agent	**Genus**	**Replication**	**Mechanical**	**Aphid**	**Symptoms**	**TAS-ELISA**	**RT-PCR**
GRAV	*Luteovirus*	Autonomous	No	Yes	Symptomless infection (trasient mottle)	Yes	Yes
GRV	*Umbravirus*	Autonomous	Yes	Yes, requires GRAV & satRNA	Symptomless infection	No	Yes
satRNA	……	Requires GRV	Yes	Yes, requires GRAV	Chlorotic, Green , Mosaic etc	No	Yes

[a]Adapted from Naidu *et al.*, 1999 & GRV

[b]GRAV = groundnut rosette assistor virus, GRV = groundnut rosette virus, and sat RNA = satellite RNA.

[c]TAS- ELISA = triple-antibody sandwich enzyme-linked immunosorbent assay, and RT-PCR = reverse-transcription polymerase chain reaction.

2.10 EPIDEMIOLOGY OF GROUNDNUT ROSETTE DISEASE

According to Waliyar *et al*. (2007) the epidemiology of GRD is complex involving interactions between GRV, GRAV, SatRNA, the vector, and the host plant and environment. Since none of the causal agents is seed-borne, initial infection of crops depend on the survival of infected plants (virus sources) and vectors (aphids) (Naidu *et al.,* 1998). Infested groundnut plants surviving between cropping seasons are possible source through which the disease could spread. In regions where there are no sources of infection, initial infection may depend on the influx of viruliferous aphids from other parts of Africa on prevailing wind currents (Bunting, 1950; Adams, 1967). The vector *A. craccivora* is polyphagous and can survive on as many as 142 plant species in addition to groundnut and some of these plant species could be a source of the rosette complex (Adam, 1967; Eastop, 1981; Naidu *et al*., 1998). Winged aphids are responsible for primary spread of the disease. Secondary spread from the initial foci of disease within the fields also occurs by way of the movement of aphid vector, but largely apterae and nymphs (Naidu *et al*., 1998).

The disease is polycyclic because each infected plant serves as a source for initiating subsequent disease spread in the field. In general, primary infection at early stages of the crop growth provides a good opportunity for repeating cycles of infection to occur before crops mature and vector populations decline. The nature and pattern of disease spread is influenced by cultivar, plant age, time of infection, crop density, climatic conditions transmission efficiency of aphids and closeness to the source of infection (Waliyar *et al.,*

2007).

2.11 VIRUS HOST RANGE

Groundnut and some of its wild relatives are the only natural hosts of GRAV, GRV and SatRNA (Okusanya and Watson, 1966). Since GRV is mechanically transmissible and GRAV is not, many host range studies relate to GRV and not to GRAV. GRV has been transmitted to a limited range of species in the *Leguminosae, Chenopodiaceae or Solanaceae* (Okusanya and Watson, 1966; Adams, 1967; Hull and Adams, 1968; Dubern, 1980; Reddy *et al.*, 1985; Naidu *et al.*, 1998). Under experimental conditions using viruliferous *A. craccivora*, GRAV has been transmitted to *Pisum sativum* L., *Stylosanthes gracilis Taub., S. hamata* (L) *Taub., S. mucronata* Wild., *S. sundaica Taub., Trifolium incarnatum* L., *T. pratense* L., *Caspella bursa-pastoris* (L) *Medicus, Gomphrena globosa* L., *Montia perfoliaeta* L. *and Spinacia oleracea* L. (Adams, 1967; Hull and Adams, 1968; Okusanya and Watson, 1966; Murant, 1989). According to Naidu *et al.* (1998), all these plants showed symptomless infection with the exception of *C. bursa-pastoris*, which was reported to show chlorotic symptoms and virus replication in these plants was confirmed by diagnostic assay. By artificial mechanical sap inoculations, experimental hosts of GRV and SatRNA were identified in several species in Leguminosae, Chenopodiaceae and Solanaceae (Okusanya and Watson, 1966; Adams 1967; Hull and Adams, 1968; Dubern, 1980; Reddy *et al.*, 1985a; Murant *et al.*, 1998). Nonetheless, groundnut is the only naturally infected host yet known for the entire rosette disease complex (Naidu *et al.*, 1998). Reddy (1991) reported that, the crop was introduced into Africa from South America sometime during the 16th century by the Portuguese, however the pathogens causing rosette appear to be indigenous to Africa as they have not been recorded elsewhere .

2.12 MANAGEMENT OF GROUNDNUT ROSETTE DISEASE

GRD can be control by several methods, which have investigated and known to protect the plant against the disease. Some of these methods are use of insecticides to control the aphids, use of resistance cultivars, cultural practices that interfere with the vector movement and removal of volunteer groundnut plants that serves as inoculum sources (Naidu *et al.*, 1998; 1999). Earlier studies have shown that use of pesticides such as organophosphates can effectively control aphid populations hence reduce disease incidences (Naidu *et al.*, 1999; Ntare *et al.*, 2002). Chemical treatment Seed treatment with imidaclorprid and followed by regular systemic insecticide spray in the early stages of the crop growth (from emergence to 40th day) will control vector aphids, and consequent protection against GRD (Waliyar *et al.*, 2007). The timing of spray, dosage and type of pesticide used are crucial for efficient control of aphid populations (Waliyar *et al.*, 2007), but the approach is not economically feasible for most smallholder farmers in major growing countries.

Furthermore, insecticide applications pose detrimental effects on health and environment and mankind. Cultural practices that are known to reduce the disease infestation are; early sowing in the raining season to take advantage of low aphid population, removal and destroying of early infested plants, sowing fast- growing cereals such as maize, pearl millet and sorghum as border crops which interferes with the vector movement and lastly dense population in the field which covers ground thereby discourage the landing of vector on the crop. Breeding work has led to the development of several GRD resistant cultivars that have been released in the sub-Saharan Africa (Van der Merwe *et al.*, 2001; Deom *et al.*, 2006). The earlier developed resistant varieties were seriously flawed in that they had a long growth period, making them unsuitable for areas where droughts are

frequent, and therefore short duration cultivars would have been more appropriate (Naidu *et al*., 1998).

However, early maturing sources of GRD resistance have been identified in the Spanish type of groundnut (*Arachis hypogaea* subsp. *fastigiata*, var. 19 vulgaris) (Naidu *et al.*, 1999). Ntare *et al.* (2002) noted that most of the very few early maturing cultivars available also have some poor agronomic characteristics. As a result, despite the fact that GRD resistant varieties have been available for the last 20 years, adoption of these varieties has been very low and as a result farmers continue to grow susceptible varieties whose yields are far below the world average (Edriss, 2003; Minde *et al* ., 2008). Generally, host-plant resistance is considered to be the most cost-effective management measure against GRD because smallholder farmers seldom use the cultural or chemical control methods.

2.13 BREEDING FOR RESISTANCE TO GROUNDNUT ROSETTE DISEASE

Breeding for resistance to diseases remains a principal focus in the groundnut breeding programme in Ghana. Information about the mode of gene action conferring resistance to diseases is prerequisite to the development of a focused breeding program. Breeding for resistance to any disease demands a good knowledge of the breeding methodologies as well as a good understanding of the disease and its causal organisms.

Olorunju and Ntare (2002) noted that breeding for GRD resistance involves making crosses between both resistant and susceptible varieties followed by selections in the segregating populations which are done through bulk and pedigree systems or their modifications. Determining the proper parents, sources of resistance and knowledge of amount of variability is very important for any successful groundnut breeding program. 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).

Breeding work started when GRD resistant varieties were discovered among late maturing landraces of Virginia type (*Arachis hypogaea subsp. hypogaea var. hypogaea*), during an epidemic of GRD that occurred in the 1950s in Senegal (Naidu *et al.*, 1999; Olorunju and Ntare, 2002). However, the earlier developed varieties were unsuitable for most areas in the sub-Saharan regions having short rain seasons. This meant that there remained a need to breed short duration, GRD resistant varieties (Naidu *et al*., 1998).

Berchoux, (1960) indicated that inheritance of resistance to groundnut rosette virus was controlled by two recessive genes in West Africa with Virginia x Virginia crosses. Adamu *et al*. (2008) reported that additive effects were predominant over non-additive effects in governing GRD resistance. Berchoux (1960) attributed this resistance to production in the plants of antiviral substances. He noted that when subjected to massive inoculum pressure from viruliferous aphids, the resistant plants could be infected with GRV. He attributed this to the plants' inability under these conditions to produce a sufficient quantity of antiviral sub- stances: this hypothesis was later confirmed (Daniel and Berchoux, 1965). Olorunju *et al.* (2001) stated that, breeding for host plant resistance programs by ICRISAT has contributed to the development of several groundnut genotypes and identification of germplasm lines with acceptable levels of field resistance to GRD. Generally, resistance to rosette disease in a genotype was assessed by lack of symptom expression and therefore such resistance was largely against GRV and SatRNA (the two components responsible for rosette symptoms) (Bock *et al*., 1990; Subrahmanyam *et al*., 1998; Olorunju *et al.*, 2001). Waliyar *et al*. (2007) noted that in spite of the availability of several sources of resistance, all the ICRISAT varieties seem to have the same resistance genes.

The mechanism of resistance is reported to be to initial infection, restriction of virus movement, and restricted production of satRNA which induces symptoms (Ntare *et al.*,

2002). It has been observed that all GRD resistant cultivars and germplasm lines contain resistance to GRV and satRNA only and not to GRAV (Naidu *et al.,* 1999; Taliansky *et al.*, 2000; Waliyar *et al.,* 2007). Plants infected with GRAV show significant reduction in seed weight, meaning that GRAV infection without GRV and sat RNA affects plant growth and contribute to yield loss (Naidu and Kimmins, 2007). The complexity in the interaction of GRD viruses poses a challenge to breeders trying to develop groundnut lines with durable resistance.

2.14 SCREENING TECHNIQUES FOR GROUNDNUT ROSETTE DISEASE RESISTANCE

Groundnut rosette disease research in the 1980s led to a better understanding of the epidemiology of the disease, including its transmission by aphids (Ntare *et al.,* 2002). Most Screening for resistance to rosette used to rely on natural infestations, with many of the plants that looked resistant were in fact escapes that had not simply caught the disease. A comprehensive and effective field screening technique was developed (Bock, 1987; Bock and Nigam, 1988). This involves planting a test row of uninfected plants flanked on either side by a row of plants infested with aphids that have been mass reared in the glass house. This infector row technique has permitted rapid field evaluation of large numbers of segregating populations and breeding lines to identify those with different growth characteristics and resistance to groundnut rosette diseases. Groundnut genotypes grown in pots under greenhouse conditions or genotypes sown in fields can be evaluated for resistance to all the three GRD agents by using viruliferous aphids and grafting (Olorunju *et al*., 1992 and Naidu *et al.,* 1999b). Mechanical sap inoculation can be adopted to

transmit and genotypes can be evaluated for resistance to only GRV and SatRNA. Genotypes can be evaluated for resistance to only GRAV by grafting using scions from GRAV-infected groundnut plants (Olorunju *et al.,* 1992; Naidu and Kimmins, 2007). Diagnostic assays such as TAS-ELISA or RT-PCR can be used to confirm the presence or lack of GRD agents (GRV, SatRNA and GRAV) during genotype evaluation (Waliyar *et al.,* 2007). Two methods are being used for routine evaluation of GRD resistance in groundnut genotypes. The rating scale used in both methods primarily accounts for resistance to GRV-SatRNA. One method uses 1-5 disease rating score to evaluate GRD resistance (Pande *et al*., 1997 and Olorunju *et al*., 2001). Whiles the other which is widely used is based on percent disease incidence (PDI)

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

The research was conducted at CSIR-Crop Research Institute (CSIR-CRI), Fumesua, Ghana, (6° 45´ N, 1° 25´ W) from May 2014 to May, 2015. The research field area falls within the semi-deciduous rain forest zone and is characterized by a bimodal rainfall pattern, from April to July and then from September to December, with an average annual rainfall of 1500 mm. The soil is Ferric Acrisol (FAO/UNESCO legend, 1986).

3.2 EXPERIMENTAL MATERIALS

Four groundnut lines used for this studies were selected from germplasm previously screened by CRI for GRD resistance (Table 2). Seeds of these genotypes were obtained from CRI, Fumesua, Ghana. The resistant line were Otuhia, ICGV 01276 while the susceptible lines were Shitaochi and Manipintar.

Table 2 : Characteristics, source and type of reaction to GRD of parental genotypes used for population development

Parent	Source	GRD Reaction
Otuhia	CRI	Resistance (R)
ICGV 01276	CRI	Resistance (R)
Shitaochi	CRI	Susceptible (S)
Manipintar	CRI	Susceptible (S)

3.3 METHODOLOGY

The experiment was conducted in three stages. The first and second stages were carried out in plastic pots under full insecticide protection from May to December, 2014 and the third stage in the field from February to May, 2015.

3.4 SEEDLING ESTABLISHMENT

Seeds were sown in plastic bowls/ pots measuring 45 cm (top diameter) x 39 cm (base diameter) x 12 cm (height) with drainage holes. The pots were filled with 16.5 kg sterilized soil in the ratio of two parts top soil or black soil to one part river sand. Two seeds were planted into each pot and thinned to one plant per pot one week after germination. Sowing of parents was staggered over a period of five days to synchronize flowering. Pots were placed on a table to facilitate appropriate agronomic practices and ease hybridization activities.

3.4.1 STAGE 1

In the first stage, the four parental genotypes were grown and direct and reciprocal crosses made to produce F_1 plants and their reciprocals as follows;

S/N **F_1**

1 Shitaochi x Otuhia

2 Manipintar x Otuhia

3 Shitaochi x ICGV 01276

4 Manipintar x ICGV 01276

S/N	Reciprocal F_1 (RF_1)
1	Otuhia x Shitaochi
2	Otuhia x Manipintar
3	ICGV 01276 X Shitaochi
4	ICGV 01276 x Manipintar

3.4.2 STAGE 2

In the second stage, some of the F_1 progenies (F_1 and R F_1) were allowed to self to produce the F_2 progenies and at the same time backcrossing carried out to produce the backcrossed progenies. The following genotypes were obtain during the cross;

S/N	F_2 Genotypes
1	Shitaochi x Otuhia
2	Manipintar x Otuhia
3	Shitaochi x ICGV 01276
4	Manipintar x ICGV 01276

S/N	Reciprocal F_2 (RF_2) Genotypes
1	Otuhia x Shitaochi
2	Otuhia x Manipintar
3	ICGV 01276 x Shitaochi
4	ICGV 01276 x Manipintar

S/N	Backcross one (BC_1) Genotypes
1	(Shitaochi x Otuhia) x Otuhia
2	(Manipintar x Otuhia) x Otuhia
3	(Shitaochi x ICGV 01276) x ICGV 01276
4	(Manipintar x ICGV 01276) x ICGV 01276

S/N	Reciprocal backcross one(RBC_1) Genotypes
1	(Otuhia x Shitaochi) x Otuhia
2	(Otuhia x Manipintar) x Otuhia
3	(ICGV 01276 x Shitaochi) x ICGV 01276
4	(ICGV 01276 x Manipintar) x ICGV 01276

S/N	Backcross two (BC_2) Genotypes
1	(Shitaochi x Otuhia) x Shitaochi
2	(Manipintar x Otuhia) x Manipintar
3	(Shitaochi x ICGV 01276) x Shitaochi
4	(Manipintar x ICGV 01276) x Manipintar

S/N	Reciprocal Backcross two (RBC_2) Genotypes
1	(Otuhia x Shitaochi) x Shitaochi
2	(Otuhia x Manipintar) x Manipintar
3	(ICGV 01276 x Shitaochi) x Shitaochi
4	(ICGV 01276 x Manipintar) x Manipintar

3.4.3 STAGE 3

Disease evaluation of all the test materials obtained in Stage one and two was carried out under a high disease pressure environment created through aphid infestation on the field. The trials were laid out in randomized complete block design with 3 replications. Each replicate consisted of one plot of each of the Parents, F_1, RF_1, backcross and two plots of each F_2 and RF_2 generations. Each plot was made up of a row, 2m long with 0.4m between rows and 0.2m within plants giving 10 plants per row. Plants were sown at a rate of 1 seed per planting station. Aphid colonies were reared on a highly infested genotype Manipintar in netted cages prior to planting of the experiments. Five wingless aphids were transferred onto 7 to 14 days old seedlings on the test materials using wet camel's hair brush following the method by Naidu and Kimmins (2007). It is rare to find plants without aphids in choice tests because the aphids are free to roam to find suitable plant hosts.

3.5 CROSSING PROCEDURE

The conventional technique for hybridization in groundnut which was described by Norden (1973) but, some modification been made by Nigam *et al*. (1980) was adopted in this work. For convenience of operation, hybridization in groundnut was carried out on plants grown in pots or boxes placed on raised benches in the open. Hybridization was

restricted to the rainy season where atmospheric humidity was high and to the early phase of flowering because of higher success rates in the production of mature pods from early formed flowers. Temperature and humidity are very important in groundnut hybridization. Because of that emasculation was carried out in the afternoon or evening depending on the environmental condition. During emasculation a well-developed flower bud on a sufficiently elongated hypanthium was selected, and all other buds at that node were removed with a forceps to ensure one peg set at a node. The bud was treated with care to avoid injury. The bud was then held gently between the thumb and index finger during which the sepal opposite the standard petal was pulled down. The fused sepal was also folded down and held back. The standard was gently and carefully opened with a forceps and held back with the thumb and index finger. The wing petals were pulled down locking them with the standard. The keel was then pulled outwards by its ridge with the forceps to expose the anthers. All the anthers and filament were removed from their bases. This left the stigma and style well exposed for pollination. The standard, wing petals and keel were returned to their normal positions after emasculation to cover the style and stigma to prevent desiccation or damage. The internode just above the emasculated bud was marked with a date-coded nylon thread. Thread of a different colour was used every day to help identify the buds for pollination the next day. Pollination was carried out the day after emasculation as soon as buds start opening in the early hours of the morning (0600-0800 hrs).

For pollination, a healthy flower from a pre-identified male plant was removed by breaking the hypanthium. The calyx, standard, and wing petals were detached for ease of operation. The keel was pressed between the thumb and index finger to squeeze the pollen mass out from the anthers. The pollen was deposited on the tip of the stigma of the emasculated flower. All flowers except those that were artificially pollinated were removed every day soon after pollination from the base of the hypanthium, to help prolong

the duration of flowering of the female plant. The flower removal operation were continue for at least two weeks after the last pollination for the season. This reduced competition for the development of the hybrid pods.

Plate1: Bud emasculation

Plate 2: Pollen sticking to the stigma of a pollinated flower

Plate 3: Hybrid pegs entering the soil

3.6 DATA COLLECTION

Each of the test plant was routinely checked and evaluated for GRD symptoms at weekly interval for the first four weeks and every two weeks thereafter days after aphid infestation. The number of plants showing GRD symptoms per population were computed into percent disease incidence (PDI) and a rating scale used to interpret

genotype response by using the following formula; PDI = (Number of plants showing GRD symptoms ÷ total number of plants per plot) x 100 as described by Waliyar *et al*. (2007). Disease severity was assessed at 45 days after infestation (DAI) by using a 1-5 rating scale (Olorunju *et al*., 2001) (Table 4).

Table 3; Evaluation of groundnut genotypes based on percent disease incidence (PDI)

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

Source: Waliyer *et al.*, 2007

Table 4; Evaluation of groundnut genotypes based on 1 to 5 disease rating score

Score	Genotype reaction
1	No visible symptoms on the foliage
2	Rosette symptoms on 1-20% foliage, but no obvious stunting
3	Rosette symptoms on 21-50% foliage and stunting
4	Severe rosette symptoms on 51-70% foliage and stunting
5	Severe symptoms on 71-100% foliage, stunted or dead plants

Source: Olorunju *et al*., 2001

3.7 DISEASE DIAGNOSIS

3.7.1 TAS-ELISA FOR THE DETECTION OF GRAV

Leaf samples for serological test were taken from field plants rated 1-4 (Susceptible and resistance) and source of inoculum used (Plate 1). An indirect triple antibody sandwich-enzyme –linked immunosorbent essay method which entails the usage of beet western yellow virus (*Luteovirus*) antiserum was used for the detection of GRAV antigen in the various samples. This is because no specific antiserum for GRAV have been developed yet. This is by far one of the most widely used and sensitive serological test for the detection of GRAV. It employs a polyclonal antiserum (IgG) for coating and monoclonal antibodies (MAb) for decorating of the virus coat protein. Since the monoclonal antibodies are not labeled, a secondary, animal species (mouse) antibody is used to react with the bound MAb. This anti mouse (RAM) antibody is labeled with alkaline phosphatase (AP) as reporter group. All TAS-ELISA kits used were supplied by (DSMZ, Germany).

Assessment of results was by visual observation and spectrophotometric measurement after 30-50 minutes after aliquots of the substrate were added to each wells. For the visual observation colour development in wells of the samples were compared to that of controls. The coloured end product correlates to the amount of the analyte (GRAV antigen) in the samples. Samples wells with specific colour development were rated positive, whiles that of the negative control remain virtually clear. The results obtained by the visual assessment were confirm by the Plate reader. Mean Optical densities of the samples in the various well were computed from results obtained from the readings in a plate reader at the correct absorbance of 405nm. Samples that had mean optical densities twice as large as that of the negative were rated as positive which signifies the presence of the GRAV antigen in the samples.

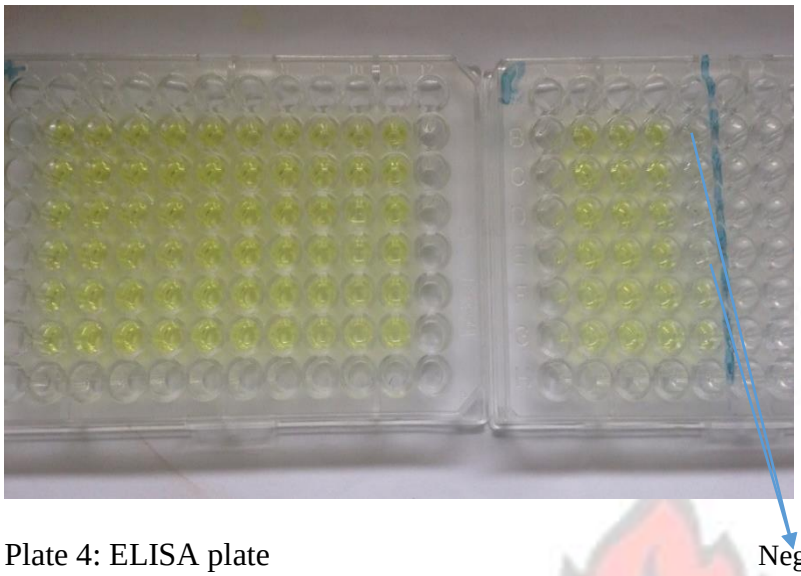

Plate 4: ELISA plate Negative sample wells

3.8 STATISTICAL AND GENETIC ANALYSES

Data collected were subjected to Analysis of Variance (ANOVA) using GENSTAT statistical package (Discovery Edition 4). Least Significant Difference (LSD) at 5% was used to determine the significant differences among the means of the various generations.

Generation mean analysis (GMA) was carried out to determine the types of gene action influencing the expression of groundnut rosette virus disease resistance trait. The additive-dominance model was adopted in the estimation of gene effect for GRVD damage rating. PBTools which uses the weighted regression approach was used for the generation mean analysis. Two full models were fitted to the data. The first one is "mean = 0 + m + a + d + aa + ad" and the other one is "mean = 0 + m + a + d + aa + dd". For each model, backward regression procedure was used to obtain the best model. (Mather and Jinks

,1982) model describes the phenotype in terms of the mid- parental values [m], additive effects [a], dominance effects [d], and additive by additive [aa], additive by dominance [ad], and dominance by dominance [dd] epistatic interaction effects. When these effects had the same sign, the type of epistasis was complementary, while different signs indicated duplicate epistasis.

3.9 HERITABILITY

Broad sense (h^2_b) and narrow-sense (h^2_n) heritability's were estimated using the variance component method (Wright, 1968) and variances of F_2 and back cross generations (Warner, 1952), respectively, as: $(h^2_b) = \{VF_2 – [(VP_1 + VP_2 + 2VF_1) /4]\}/ VF_2$

$$(h^2_n)= [VF_2 – (VBC_1 + VBC_2) /2] / VF_2$$

3.10 MID- PARENT HETEROSIS

Mid–parent heterosis for the various crosses were estimated as the percentage deviation of the mean F_1 value from the mid –parent value using the formula (Wright, 1968).

$$\text{Heterosis} = \frac{(F_1 - MP)}{MP} \times 100$$

Where MP= Mid - parent value (average of the two parents).

CHAPTER 4

4.0 RESULTS

4.1 Crosses success rate of direct and reciprocal crosses in groundnut

The overall success rate recorded for the crosses was 60.21%. The highest success rate recorded was from the reciprocal cross between Manipintar x Otuhia (70.00%) (Table 5). Whiles (ICGV 01276 x Shitaochi) x Shitaochi (RBC_2) recorded the least success rate of 46.15.

All reciprocal crosses for ICGV 01276 in the F_1 population i.e., ICGV 01276 x Shitaochi and ICGV 0176 x Manipintar recorded low success rates in contrast to that of the other reciprocal crosses in that same population. Crosses involving the resistance parent ICGV 01276 consistently recorded low success rate compared to that of the other resistant parent (Otuhia) (Table 5).

Success rates for the first filial generations (F_1s) were higher (70.00-61.54%) compared to that of the reciprocal first filial generations (RF_1s) (63.75-47.89%). (Manipintar x Otuhia) x Manipintar recorded the highest success rate of 67.86% for the backcross population with (ICGV 01276 x Shitaochi) x Shitaochi obtaining the lowest (46.15%) (Table 5).

Table 5: Type of crosses, number of flowers, number of pods and percent success

Type of cross	Number of flowers pollinated	Number of pods obtain	Percent % success
F_1			
Shitaochi x Otuhia	65	40	61.54
Manipintar x Otuhia	70	49	70.00
Shitaochi x ICGV 01276	67	42	62.69
Manipintar x ICGV 01276	66	45	68.18
Reciprocal F_1 (RF_1) Otuhia x Shitaochi	80	51	63.75
Otuhia x Manipintar	66	42	63.64
ICGV 01276 X Shitaochi	71	34	47.89
ICGV 01276 x Manipintar	72	35	48.61
Reciprocal backcross one (RBC_1)			
(Otuhia x Shitaochi) x Otuhia	34	23	67.65
(Otuhia x Manipintar) x Otuhia	37	24	64.86
(ICGV 01276 x Shitaochi) x ICGV 01276	42	23	54.76
(ICGV 01276 x Manipintar) x ICGV 01276	50	25	50.00
Backcross one (BC_1)			
(Shitaochi x Otuhia) x Otuhia	51	29	56.86
(Manipintar x Otuhia) x Otuhia	33	21	63.64
(Shitaochi x ICGV 01276) x ICGV 01276	43	28	65.12
(Manipintar x ICGV 01276) x ICGV 01276	47	27	57.45
Backcross two (BC_2)			
(Shitaochi x Otuhia) x Shitaochi	49	28	57.14
(Manipintar x Otuhia) x Manipintar	56	38	67.86
(Shitaochi x ICGV 01276) x Shitaochi	42	26	61.90
(Manipintar x ICGV 01276) x Manipintar	42	28	66.67
Reciprocal Backcross two (RBC_2)			
(Otuhia x Shitaochi) x Shitaochi	50	32	64.00
(Otuhia x Manipintar) x Manipintar	34	23	67.65
(ICGV 01276 x Shitaochi) x Shitaochi	52	24	46.15
(ICGV 01276 x Manipintar) x Manipintar	45	24	53.33
Total number of flowers pollinated	1264	761	60.21

*F_1 = S x R, RF_1 = R x S, BC_1 = (S x R) x R, RBC_1 = (R x S) x R,

*BC_2= (S x R) x S, RBC_2 = (R x S) x S

4.2 Responses of generations to GRD infection

Groundnut rosette disease on the field was mild to severe. Disease symptoms appeared as early as one (1) week after inoculation with most of them showing leaf symptoms. Average PDIs in the first week ranged between 0-37% with plants showing variant symptoms (chlorotic and green) (Figure 1).

PDI of all the generations were intermediate between the resistant parents and that of the susceptible parents, but skewed towards the susceptible parents. Disease incidence in plants of the susceptible parent (Manipintar) reached as high as 100%. Moreover, about 10% test plants of Otuhia (resistant parent) became infested to the disease whiles 20% test plants of ICGV 01276 developed symptoms (Figure 1).

Average PDIs for F_1 and RF_1 crosses, progenies ranges from 23-73% respectively, with 23-30% of their plants developing symptoms for the disease in the first week after aphid inoculation. Among the F_2 progenies of S x R, 72% of its plants didn't develop any rosette symptoms at two (2) weeks after inoculation whiles 61% developed symptoms at four weeks after aphid inoculation. Average PDI obtained for the backcross progenies ranges from 23-74%, with RBC_2 obtaining the highest average PDI (Figure 1).

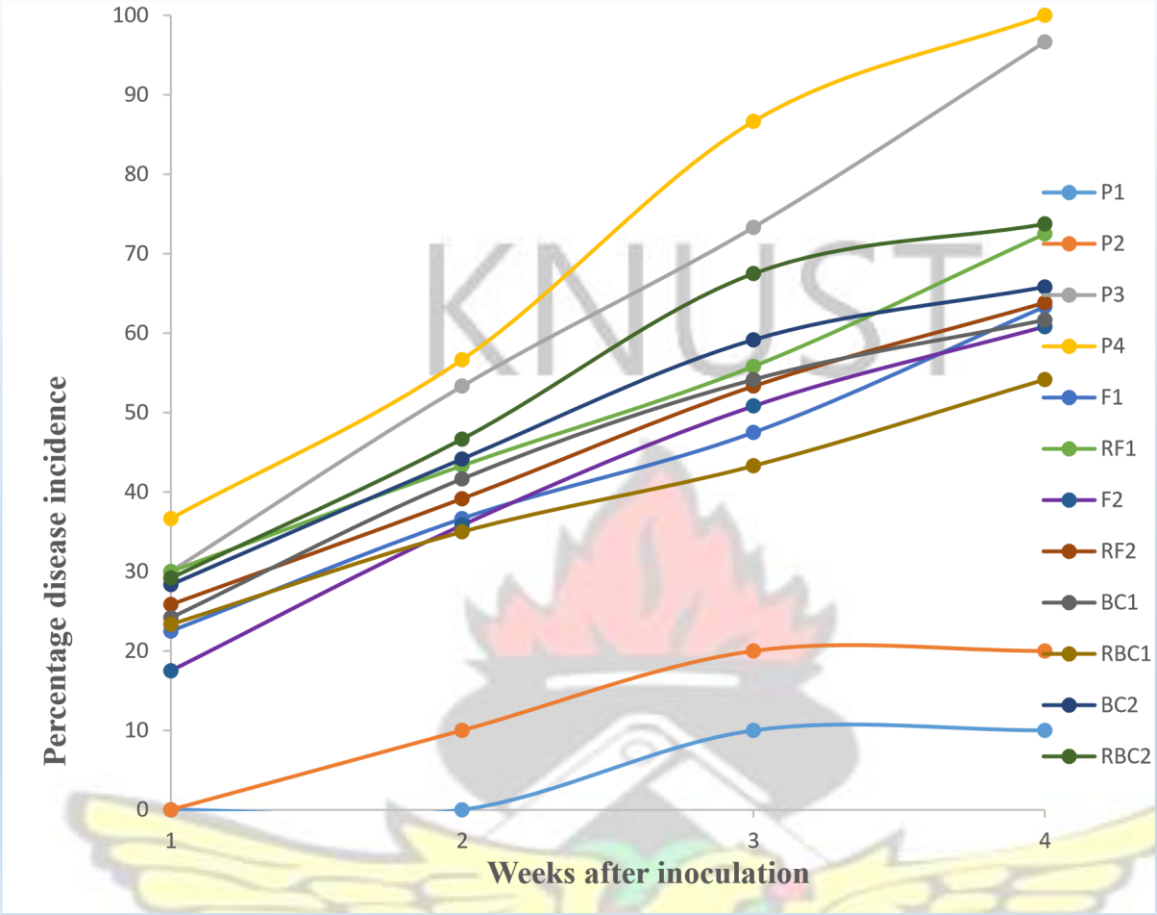

Figure 1; Disease progress curve of GRD over time for twelve generations of direct and reciprocal groundnut crosses

*F_1 = S x R, RF_1 = R x S, *BC_1 = (S x R) x R, *RBC_1 = (R x S) x R, *BC_2 = (S x R) x S, *RBC_2 = (R x S) x S. Where R= resistance and S= Susceptible. Data points represent average PDIs averaged over 3 replications at week 1, week 2, week 3 and week 4 after aphid infestation.

Plate 5: F_1 (Otuhia x Manipintar) plants infested by the rosette virus disease

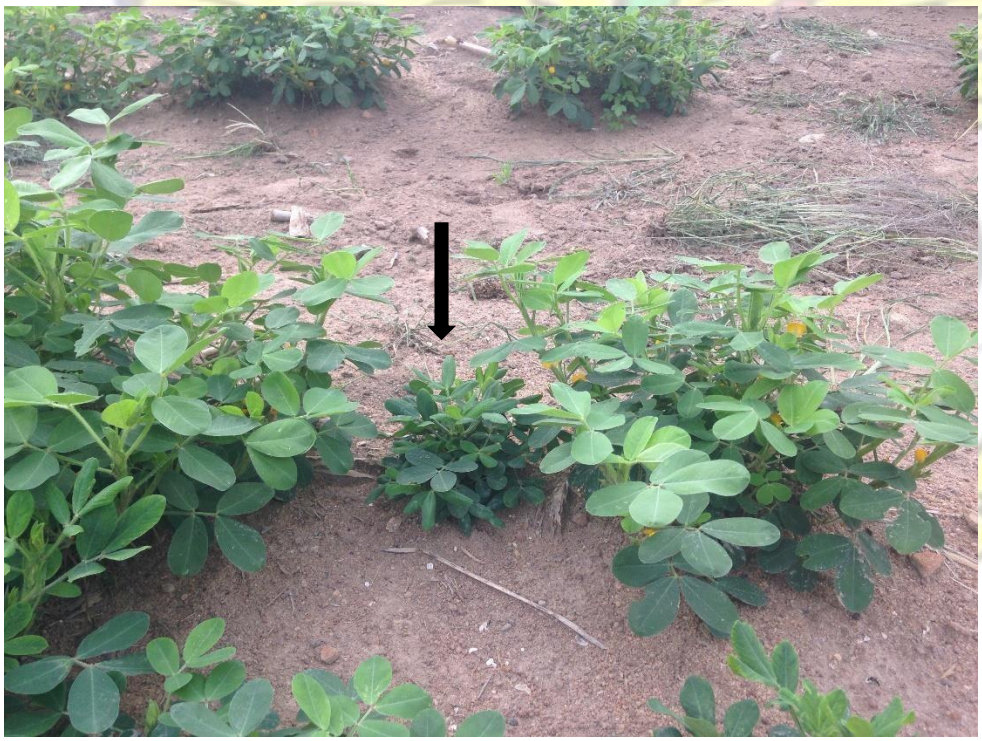

Plate 6: Infested rosette plant sandwich between resistant ones in Shitaochi x Otuhia cross

4.3 Detection of groundnut rosette assistor virus (GRAV) by ELISA in groundnut genotypes resistance and susceptible to groundnut rosette virus (GRV) Readings from the ELISA plate reader showed that the negative samples had a mean optical density range of 0.260-0.283 and 2x range of 0.52-0.566. In contrast all the samples had a mean optical range of 0.567-0.614. In general GRAV antigen occurred frequently in all the samples tested.

Table 6: Detection of groundnut rosette assistor virus (GRAV) by ELISA groundnut genotypes resistance and susceptible to groundnut rosette virus (GRV).

S/N	Genotype	Field status	MOD	GRAV
1	(ICGV 01276 x Manipintar) x Manipintar (S_1)	Susceptible	0.570	+
2	(Shitaochi x ICGV 01276) x ICGV 01276	Susceptible	0.595	+
3	Manipintar x Otuhia	Susceptible	0.587	+
4	Otuhia x Manipintar	Susceptible	0.589	+
5	Shitaochi x Otuhia	Susceptible	0.580	+
6	(Manipintar x Otuhia) x Manipintar (S_1)	Susceptible	0.567	+
7	(ICGV 01276 x Manipintar) x Manipintar (S_2)	Susceptible	0.588	+
8	(Otuhia x Shitaochi) x Shitaochi	Susceptible	0.599	+
9	(ICGV01276 x Shitaochi) x Shitaochi	Susceptible	0.594	+
10	(Otuhia x Manipintar) x Manipintar	Susceptible	0.598	+
11	(Otuhia x Manipintar) x Otuhia	Susceptible	0.599	+
12	(ICGV01276 x Shitaochi) x ICGV 01276 (S_1)	Susceptible	0.584	+
13	(Manipintar x Otuhia) x Manipintar (S_2)	Susceptible	0.587	+
14	ICGV 01276	Susceptible	0.597	+
15	Manipintar	Susceptible	0.595	+
16	Shitaochi	Susceptible	0.594	+
17	Otuhia (S_1)	Susceptible	0.593	+
18	(Shitaochi x Otuhia) x Shitaochi	Susceptible	0.574	+
19	(ICGV 01276 x Shitaochi) x ICGV 01276 (S_2)	Susceptible	0.592	+

20	(Manipintar x ICGV 01276) x Manipintar (S_1)	Susceptible	0.593	+

Table 6. Continued

21	Manipintar x Otuhia	Susceptible	0.598	+
22	Shitaochi x ICGV 01276	Susceptible	0.585	+
23	Manipintar x ICGV 01276	Resistance	0.603	+
24	ICGV 01276 x Manipintar	Resistance	0.594	+
25	(Manipintar x Otuhia) x Otuhia	Resistance	0.582	+
26	Otuhia (S_2)	Resistance	0.582	+
27	(Otuhia x Shitaochi) x Shitaochi	Resistance	0.593	+
28	(ICGV 01276 x Shitaochi) x ICGV 01276 (S_2)	Resistance	0.614	+
29	(Manipintar x ICGV 01276) ICGV 01276	Resistance	0.591	+
30	(Shitaochi x Otuhia) x Otuhia	Resistance	0.578	+
31	(Manipintar x ICGV 01276) x Manipintar	Resistance	0.593	+
32	Shitaochi x ICGV 01276	Susceptible	0.601	+
33	Source of inoculum 1	……	0.580	+
34	Source of inoculum 2	……	0.590	+
35	Source of inoculum 3	……	0.602	+
36	Source of inoculum 4	……	0.592	+
37	Negative Control	……	0.260	
38	Buffer	……	0.283	
39	Positive	……	0.588	

[a] MOD = Mean Optical Density (Average two wells for each sample) [b] + = Positive (GRAV present) [c] - = Negative (GRAV absent) [d] (S_1) = Sample One (1) [e] (S_2) = Sample Two (2) [f] Positive samples had to have OD values twice as large as the negative control

4.4. Mean rosette resistance scores, standard error and variances of ten generations in four groundnut crosses.

Mean values, standard error and variances for the analyzed trait of the four crosses are presented in the Table 7. Parents used in this research showed significant differences in the character studied. *Otuhia (P_1)* was the most resistant followed by *ICGV 01276 (P_1)* and Manipintar (P_4) was highly susceptible.

Means of the direct and reciprocal filial generation F_1 was significantly different in 3 of the crosses except *Otuhia x Manipintar* cross. The F_1 and F_2 were more resistant than that of their respective reciprocals. The mean of the F_{1s} was less than the mid-parent value but higher than the mean of the parent with lowest disease score (P_1). Significant mean differences were detected in all the Backcrosses except that of the *Otuhia* and *Manipintar* backcross, with reciprocal cross of Otuhia x Manipintar recording the highest mean score (Table 7).

Mean scores for BC_1 and BC_2 were significantly different from each other in two of the crosses i.e *Otuhia x Shitaochi* and *Otuhia x Manipintar*. The differences among analyzed generation were sufficient to perform generation mean analysis. This made it possible to assess whether the variation observed in the generation means could be explained on an additive by dominance basis or whether the interaction between genes at different loci (epistasis) was important (Table 7).

Table 7: Mean rosette resistance scores, standard error and variance in ten generations of direct and reciprocal crosses in groundnut.

Geneation	NP	O/S		O/M		I/S		I/M	
		Mean ± SE	S^2	**Mean ± SE**	S^2	**Mean ± SE**	S^2	**Mean ± SE**	S^2
P1	30	1.13 ± 0.06 a	0.12	1.13 ± 0.06 a	0.12	1.67 ± 0.08 a	0.23	1.67 ± 008. a	0.23
P2	30	4.00 ± 0.05 g	0.07	4.13 ± 0.06 e	0.12	4.00 ± 0.05 g	0.07	4.13 ± 0.06 e	0.12
MP		2.67		2.73		2.84		2.9	
F1	30	2.20 ± 0.07 d	0.17	3.03 ± 0.03 d	0.03	2.60 ± 0.09 cd	0.25	2.67 ± 0.11 c	0.27
RF1	30	2.63 + 0.09 e	0.24	2.87 ± 0.06 d	0.12	3.20 ± 0.07 f	0.17	3.10 ± 0.06 d	0.09
F2	60	1.57 ± 0.10 b	0.55	2.40 ± 0.15 c	1.29	2.23 ± 0.14 b	1.21	2.03 ± 0.13 b	0.95
RF2	60	2.13 ± 0.04 cd	0.12	3.13 ± 0.16 d	1.47	3.00 ± 0.13 ef	1.02	2.43 ± 0.13 c	1.00
BCI	30	2.17 ± 0.17 d	0.83	2.13 ± 0.13 c	0.53	2.70 ± 0.11 de	0.36	2.63 ± 0.12 c	0.45
RBCI	30	1.80 ± 0.07 c	0.17	1.67 ± 0.14 b	0.58	2.30 ± 0.09 bc	0.22	3.06 ± 0.20 d	1.17
BC2	30	2.67 ± 0.08 e	0.23	2.97 ± 0.09 d	0.24	2.73 ± 0.13 de	0.5	2.60 ± 0.16 c	0.73
RBC2	30	3.27 ± 0.08 f	0.20	2.93 ± 0.10 d	0.34	3.00 ± 0.15 ef	0.76	3.13 ± 0.15 d	0.67

*NP= Number of plants evaluated, O/S= Otuhia x Shitaochi, O/M= Otuhia x Manipintar

*I/S= ICGV 01276 x Shitaochi, I/M= ICGV 01276 x Manipintar

*SE= Standard error

*S^2 = Variance

4.5 Estimate of gene effects for groundnut rosette resistance in *Otuhia/ Shitaochi* cross.

Gene effect was estimated using PBTools software which uses the weighted regression approach and are listed in the Table 8. The overall mid parent value was 0.14 and not significant (P≤0.05). Additive and dominance by dominance gene effect were negative towards the resistant parent Otuhia, but only additive gene effect was significant at p≤0.05. Dominance, additive by additive and additive by dominant were all positive towards the susceptible parent but not significant at p≤0.05. Dominance had the largest estimated value (3.6) compared to the estimated parameters.

Table 8: Estimates of gene effects for groundnut rosette resistance in *Otuhia/ Shitaochi* cross.

Parameter	Estimate	S.E -Means	t- value	Probability
m	0.14	1.30	0.11	0.91
a	-1.43*	0.25	-5.77	0.01
d	3.60	3.37	1.07	0.34
aa	2.40	1.19	2.00	0.11
ad	1.50	1.15	1.30	0.26
dd	-1.17	2.20	-0.53	0.62

[m]-mean, [a]-additive, [d]-dominance, [aa]-additive*additive, [ad]-additive*dominance, [dd]-dominance*dominance effect

4.6 Estimate of gene effects for groundnut rosette resistance in Otuhia/ Manipintar cross.

Results from the estimated parameters indicates that, the mid parent value was 3.6 and not significant at P≤0.05. Additive, dominance and additive by additive were all negative towards the resistant parent, but only additive was significant (Table 9). Additive by dominance and dominance by dominance were all positive towards the susceptible parent.

Table 9: Estimates of gene effects for groundnut rosette resistance in *Otuhia/ Manipintar* cross.

Parameter	Estimate	S.E -	Means	t- value	Probability
m	3.60*	1.06	3.40	0.03	
a	-1.50*	0.19	-7.74	0.02	
d	-2.88	2.63	-1.09	0.36	
aa	-0.96	1.04	-0.93	0.41	
ad	1.01	0.77	1.32	0.26	
dd	2.18	1.65	1.32	0.26	

[m]-mean, [a]-additive, [d]-dominance, [aa]-additive*additive, [ad]additive*dominance, [dd]-dominance*dominance effect

4.7 Estimate of gene effects for groundnut rosette resistance in *ICGV 01276/ Manipintar* cross.

Mid parent value estimated was 0.93 and not significant. Both Additive and dominance by dominance were the only significant parameter estimated (Table 10). Their estimated mean value was -1.23 and -1.62 respectively. Dominance, additive by additive and additive by dominance were all positive towards the susceptible parent with a mean values ranged from 1.98 - 3.62.

Table 10: Estimates of gene effects for groundnut rosette resistance in (*ICGV 01276/ Manipintar)* cross.

Parameter	Estimate	S.E -Means	t- value	Probability
m	0.93 1.07 0.86	0.40 a	-1.23* 0.21 -5.86	0.01 d 3.62
2.60 1.39	0.24 aa	1.98 1.05 1.88	0.13 ad	2.32* 0.75 3.11
0.03 dd	-1.62 1.62 -0.20	0.37		

[m]-mean, [a]-additive, [d]-dominance, [aa]-additive*additive, [ad]additive*dominance, [dd]-dominance*dominance effect

4.8 Estimate of gene effects for groundnut rosette resistance in ICGV 01276/ Shitaochi cross.

Mid parent value recorded after the analysis was 4.01 and significant (Table 11). Additive, dominance and additive by additive gene effect was negative towards the resistant parent

(Otuhia). Both additive by dominance and dominance by dominance were all positive towards the susceptible parent, but none was significant at P≤0.05. Additive gene effect was the only significant gene effect in all the parameters estimated (Table 11).

Table 11: Estimate of gene effects for groundnut rosette resistance in (*ICGV 01276/ Shitaochi)* cross.

Parameter	Estimate	S.E	Means	t- value	Probability
m	4.01*	1.04	3.94	0.02	
a	-1.17*	0.29	-4.06	0.01	
d	-3.74	2.78	-1.35	0.25	
aa	-1.24	1.00	-1.25	0.28	
ad	1.57	1.00	1.64	0.18	
dd	2.75	1.83	1.50	0.20	

[m]-mean, [a]-additive, [d]-dominance, [aa]-additive*additive, [ad]additive*dominance, [dd]-dominance*dominance effect

4.9 Genetic effect and mode of inheritance for groundnut rosette disease in four crosses.

Generation mean analysis used to test the six-parameter model to explain the genetic control of resistance to groundnut rosette virus disease in the four crosses are listed in (Table 12). Mid parent value was significant in the cross of Otuhia x Manipintar and Shitaochi x ICGV 01276. Additive gene action was significant in three of the crosses i.e.; Otuhia x Shitaochi, Otuhia x Manipintar and ICGV 01276 and Shitaochi, whiles both Additive and Additive by dominance was significant in the cross of ICGV 01276 and Manipintar (Table 12).

Table 12: Summary of main genetic effect and mode of inheritance for groundnut rosette disease in four crosses.

Crosses	Parameter					
	m	a	d	aa	ad	dd

Otuhia x Shitaochi		*		
Otuhia x Manipintar	*	*		
ICGV 01276 x Manipintar		*		
ICGV 01276 x Shitaochi	*	*		
				*

* = Significant at the 0.05 level of probability
[m]-mean, [a]-additive, [d]-dominance, [aa]-additive*additive, [ad]-additive*dominance, [dd]-dominance*dominance effect

5.0 Heritability and heterosis estimates

Broad sense, narrow sense heritability and heterosis (based on mid parent value) for the groundnut rosette resistance in four different crosses are presented in Table 13. Heritability estimates varied between crosses. The broad sense heritability ranged between 94-76.40 in the various crosses with Otuhia and Manipintar cross recording the highest broad sense. Mean broad sense heritability in all the four crosses was 82.92% whilst mean narrow sense heritability was 43.77%. In sharp contrast Otuhia x Shitaochi cross obtained the lowest narrow sense heritability of 3.63% which varied considerably from results of other crosses. Negative heterosis was recorded for three of the crosses, only Otuhia x Manipintar cross obtained positive heterosis (Table 13).

Table 13: Percentage heritability and heterosis of rosette virus disease resistance in groundnut crosses

Cross	Heritability (%)		Heterosis (%)
	Broad sense	Narrow Sense	
Otuhia x Shitaochi	76.40	3.63	-17.60
Otuhia x Manipintar	94.80	69.77	10.00
ICGV 01276 x Manipintar	83.47	64.46	-8.45
ICGV 01276 x Shitaochi	77.00	37.00	-7.93

CHAPTER 5

5.0 DISCUSSION

Artificial inoculation of all the test plants in the various crosses with viruliferous aphids was effective in transmitting the groundnut rosette disease. This made it possible for effective assessment of the test plants per the set objectives. Based on percent disease incidence, parental lines used in this studies differed significantly in their reaction to the disease. PDI reached as high as 100% in plants of the susceptible parents. This results confirmed their susceptibility to the disease. Expression of the disease symptoms by few plants of the resistance parents indicates that the resistance genotypes were not totally immuned and that they became infected to the GRV when subjected to a high disease pressure. Similar findings have been reported by Berchoux (1960), Nigam *et al*. (2012), Adu-Dapaah *et al.* (2007), Chancellor *et al*. (2002) and Kayondo *et al*. (2014). Berchoux (1960) attributed this to the plants' inability under these conditions to produce a sufficient quantity of antiviral substances. This hypothesis was later confirmed by Daniel and Berchoux (1965).

TAS ELISA results showed that GRAV antigen occurred frequently in all the samples tested irrespective of whether resistance or susceptible. Detection of GRAV in the resistant plants tested, is in agreement with results obtained by Bock and Nigam (1988) who observed GRAV antigen present in all plants of six rosette-resistant groundnut lines that had been exposed to aphid inoculation in Malawi. The six lines were RG 1, RMP 91, RMP 40, RMP 93, RRI/24 and RRI/16. Similar findings were also reported by Olorunju *et al.* (1992), who reported that GRAV was detected in 11 of 15 symptomless plants of R x R and RMP x M1204.781 crosses. Usman (2013), also detected GRAV in 15 of 16 genotypes (Assay RT-PCR) of the genotypes he worked with.

The detection of GRAV in resistance genotype can be attributed to the lower concentration of GRV (SatRNA) in the genotype resulting in no symptoms expression as compared to

the susceptible once (Olorunju *et al.,* 1992). Naidu and Kimmins (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. All resistance samples tested positive, indicates that genes conferring resistance to GRV and its sat RNA was successfully introgressed in those varieties but those genes do not confer resistance to the GRAV. This observations infer that symptomatology alone cannot be a reliable basics for screening of groundnut plants for their resistance to the causal agents of the disease, as demonstrated by this study. According to Olorunju *et al.* (2001), the importance of GRAV in the rosette disease reaction remains unknown because the quantity of GRAV antigen in different genotypes has not been determined and infections with GRAV alone cause no leaf symptoms. Nevertheless, recent studies indicate that GRAV can intensify rosette symptoms in a mixed infection with GRV and that mixed infections can cause a more severe disease than a single infection of GRV, with regard to plant size and seed yield. Therefore, GRAV should not be ignored in groundnut resistance screening and breeding programs.

Results from the mean groundnut rosette resistance scores detected significant difference among some of the direct and reciprocal crosses. This indicate that maternal effect played a major role in the GRD resistance. The character could therefore not be attributed to nuclear gene control. This suggests that the choice of maternal parent is relevant in hybridization programme that focuses on the improvement of groundnut for resistance to the disease. According to Strickberger (1976) maternal effects arise from egg cytoplasm which has been modified by chromosomally transmitted genes. He explained further that, Its distinguishing characteristic is the difference in the results of reciprocal crosses, so that cytoplasm produced by a particular genotype acts differently on a developing zygote than cytoplasm produced by a different genotype; that is, there is a difference in the phenotypes of offspring A♂ x a♀ and a♀ x A♂ .This results contradict earlier results by Misari *et al.*

(1988) where no maternal effect was observed in his studies, but corroborate results obtained by Chintu (2013) who studied genetics of GRD in diallel crosses of groundnut.

Generation mean analysis using the weighted regression approach was adequate to explain the genetic control of resistance to groundnut rosette disease in the four crosses involving two resistance parents and two susceptible parents. One advantage of generation mean analysis, compared with other mating designs such as diallel, is an increased level of sensitivity through a decreased error rate (Hallauer and Miranda, 1988:
Azizi *et al.,* 2006). Additive gene effect was of the greatest importance in crosses of Otuhia x Shitaochi, Otuhia x Manipintar and ICGV 01276 x Shitaochi for resistance to GRD. In converse, both additive and additive x dominance gene effect were important for inheritance of rosette resistance in ICGV 01276 x Manipintar cross. In respect to epistatic effects, additive by dominance gene effect was the only non-allelic interaction observed to play a significant role in the inheritance of resistance to groundnut rosette disease. In general, additive and additive by dominance gene effect were the only form of gene effects involved in the inheritance of resistance to the disease in this study. On the contrary however, Nalugo *et al.* (2013) found the interaction of dominance by dominance with duplicate epistatic effect to be the only type of epistatic effect on the resistance to groundnut rosette disease. Probably this contradiction would have come as a result of the differences in the parent genotypes which were used in both studies. The presence of epistasis has important implications for any plant breeding program. Because selection have to be delayed after several generations of selection until a high level of gene fixation is attained. The negative sign for additive effects depend on which parent is chosen as P_1 (Cukadar- Olmedo and Miller, 1997; Edwards *et al.,* 1975 and Azizi *et al*., 2006). The sign for dominance effect is a function of the F_1 mean value in relation to the mid-parental value and indicates which parent is contributing to the dominance effect (CukadarOlmedo

and Miller, 1997). The large contribution of additive effect to groundnut rosette resistance suggest effective selection for the trait as proposed by Acquaah (2007). It is suggested that pure line selection at early generation would be appropriate because of the large significant contribution of additive gene effect to the inheritance of the resistance trait. Whereas selection at later generation would be appropriate for the additive by dominance type of epistasis because it will allow favorable gene combinations to be in a homozygous state before practicing final selection (Azizi *et al.*, 2006).

High average broad sense heritability of 86 % observed in the study for the four crosses gives a reflection of genetic contribution towards the phenotypic variance (Falconer and Mackay, 2009). This findings is in agreement with a high realized broad sense heritability reported by Kayondo *et al.* (2014). The generally high broad sense heritability estimate in all the four crosses indicates that the environment in which the plants were evaluated had a lower effect on the expression of the trait. A high narrow sense heritability recorded for Otuhia x Manipintar and ICGV 01276 x Manipintar crosses is apparent that resistance to groundnut rosette disease was highly heritable, since it was more than 50% (Singh and Chaudhary, 2004). This further suggested that resistance to GRD is conditioned by both additive and non- additive gene action (Kayondo *et al.*, 2014). Fehr (1987) asserted that selection for resistance to the disease should be effective because of the close corresponding between the phenotype and genotype since environment had a small effect on the phenotype. This implies that dependence on phenotypic predictions/values for GRD as a breeding strategy may be helpful and reliable (Chahal and Gosal, 2002; Dabholkar, 2006; Kayondo *et al*., 2014). Negative heterosis over mid-parent was observed for rosette resistance score indicating heterosis in the direction of the better parent (parent with lower damage score).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study has shown that it is possible to introgress resistance genes in to susceptible but desirable cultivars through hybridization. Artificial infestation of the genotypes made it easier for proper assessment of the genotypes. Detection of GRAV antigens in the resistant samples suggest that introgressed gene conferred resistance to GRV and its sat RNA but not GRAV.

The significant difference between the direct and reciprocals suggested that maternal effect contributed significantly to the inheritance of the resistance to the rosette disease. This indicates that when developing breeding populations for resistance to GRD, the choice of a maternal parent is very important.

Additionally, generation mean analysis revealed that inheritance of resistance to the disease is control by both additive and non-additive gene action. But the additive gene component was predominant over the non-additive. Additive by dominance form of nonallelic interaction was the only form of epistasis revealed in this study. The presence of significant additive gene action would make selection from early generation effective while selection at the advanced generation would be effective because of significant additive by dominance.

High heritability estimates suggest low environment influence on the trait. It is suggested that high selection pressure should be imposed to select as many high-potential recombinants as possible.

6.2 RECOMMENDATION

Resistant GRD materials needs to be properly be evaluated against different variants of GRD agents in different environment. GRAV should not be ignored in groundnut resistance screening and breeding programs.

Pure line breeding with selection from early generation is suggested for the improvement of the trait, because the additive genetic effect contributed significantly in controlling the inheritance of resistance to GRD.

Resistance to Aphid vector have to be given the needed attention in breeding for GRD resistance varieties. Understanding the epidemiological principles of the disease

combined with resistance will lead to the development of sustainable integrated disease management strategies.

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APPENDICES

Appendix 1: TAS ELISA buffers used

1. Coating buffer (pH 9.6)

   1.59g sodium carbonate (Na_2CO_3)

   2.93 sodium bicarbonate ($NaHCO_3$)

   0.20g sodium azide (NaN_3)

   Dissolved in 900 ml H2O

2. PBS (pH 7.4) phosphate buffered saline

   0.80g sodium chloride (NaCl)

   0.2g monobasic potassium phosphate (KH_2PO_4)

   1.15g dibasic sodium phosphate (Na_2HPO_4)

   0.2g potassium chloride (KCl) 0.2g

   sodium azide (NaN_3)

   Dissolved in 900 ml H2O

3. PBS-Tween (PBST)

   PBS + 0.5ml tween 20 per liter

4. Sample extraction buffer (pH7.4)

   PBS + 2% PVP (serva PVP- 15 polyvinyl pyrrolidone)

5. Conjugate buffer

   PBST +2%PVP +0.2% egg albumin (Sigma A-5253)

6. Substrate buffer (pH 9.8)

   97 ml diethanolamine

   600 ml H_2O

   0.2 g sodium azide (NaN_3)

Appendix 2: TAS ELISA protocol used

1. Purified polyclonal antiserum (lgG) was diluted at recommended dilution in coating buffer, 200 µl was added to each well of a microliter plate.
2. It was then incubated at 37˚C for 2-4h
3. Plates were washed with PBS-Tween using wash bottle, soaked for few minutes and repeated washing two times. The plates were then blotted by tapping upside down on tissue paper
4. 200 µl of 2% skim milk in PBS- Tween was added to each well (Blocking), 30 minutes at 37˚C
5. Blocking solution were removed and tapped dried.
6. 200 µl aliquots of the test samples were added to duplicate wells
7. It was then incubated overnight at 4˚C
8. Plates were washed three times after overnight incubation
9. 200 µl of the monoclonal antibodies (MAb) was added in appropriate dilution in conjugate buffer to each well
10. The plates were then incubated at 37˚C for 2-4h
11. The plates were washed three times as in step three

12. 200µl of anti-mouse with alkaline phosphatase RaM-ap was added in appropriate dilution in conjugate buffer to each well of the plates.
13. The plates were then incubated at 37˚C for 2 hours
14. Plates were again washed three time as in step 3
15. 200µl aliquot of freshly prepared substrate (10 mg p-nitrophenyl phosphate (sigma, fluke) dissolved in 10ml of substrate buffer to each well of the plates.
16. The plates were incubated at room temperature for 30-60 min to obtain a clear reaction.
17. Samples were the assessed by visual and spectrophotometric measurement of absorbance at 405 nm.

SUMMARY OF ANOVA

Appendix 3. Genetic analysis of resistance to shattering in ICGV 01276 x Manipintar cross.

Source of variation	d.f.	s.s .	m.s.	v.r	F pr.
Block stratum	2	0.39267	0.19633	9.80	
Block.*Units* stratum					
Treat	9	12.34133	1.37126	68.44	<.001
Residual	18	0.36067	0.02004		
Total	29	13.09467			

Appendix 4. Genetic analysis of resistance to shattering in Otuhia x Manipintar cross.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.25800	0.12900	3.05	
Block.*Units* stratum					
Treat	9	19.21200	2.13467	50.43	<.001
Residual	18	0.76200	0.04233		
Total	29	20.23200			

Appendix 5. Genetic analysis of resistance to shattering in ICGV 01276 x Shitaochi cross.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.69067	0.34533	9.57	
Block.*Units* stratum					
Treat	9	10.67367	1.18596	32.88	<.001
Residual	18	0.64933	0.03607		
Total	29	12.01367			

Appendix 6. Genetic analysis of resistance to shattering in Otuhia x Shitaochi cross.

Source of variation	d.f.	s.s.	m.s	v.r.	F pr.
Block stratum	2	1.23267	0.61633	15.54	
Block.*Units* stratum					
Treat	9	18.72700	2.08078	52.46	<.001
Residual	18	0.71400	0.03967		
Total	29	20.67367			