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GENETIC ANALYSIS ON ROSETTE RESISTANCE IN EXOTIC VALENCIA GROUNDNUTS AND THE RECOVERY OF VALENCIA TRAITS IN SEGREGATING GENERATIONS

BY

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DECLARATION

This study is original and no part of it has ever been presented at any university or tertiary institution for any award

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I dedicate this work to my parents; Dr. Robert Sekimpi and Mrs Rose Sekimpi; my brothers (Isaac & Ronald), sisters (Rinah & Ritah) and my husband Wambi Wilber.

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LIST OF ACRONYMS

ANOVA	Analysis Of Variance
CV%	Percentage Coefficient of Variation
DAP	Days After Planting
F_1	1st Filial generation
F_2	2nd Filial generation
F-val	F-probability value
GRAV	Groundnut Rosette assistor Virus
GRD	Groundnut Rosette Disease
GRV	Groundnut Rosette Virus
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Resources Institute for the Semi Arid Tropics
NaSARRI	National Semi Arid Resources Research Institute
Se	Standard error
SSA	Sub Saharan Africa
USA	United States of America

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ABSTRACT

Groundnut rosette disease (GRD) is the most destructive virus disease of Valencia groundnuts (*Arachis hypogaea* L.) in Africa. To widen the germplasm base of Valencia types grown in Uganda, two exotic Valencia lines namely, NuMex-M_3 and Valencia C, were imported and evaluated for GRD by the Groundnut Improvement Program of National Semi-Arid Resources Research Institute (NaSARRI) but they succumbed to the disease. Sources of GRD resistance were found in Spanish and Virginia types, which could complicate genetic improvement of Valencia type of groundnuts due to lack of information on the genetics of GRD resistance on the available breeding lines. This study was conducted (i) To determine heritability for resistance to GRD in Valencia groundnuts ii) To determine gene action controlling resistance to GRD in Valencia groundnuts and iii) To evaluate F_2 and backcross segregating populations for recovery of Valencia type characteristics . Six crosses were made between; Valencia C (P_1) × ICGV-SM 90704 (P_2), Valencia C (P_1) × ICGV-SM 96801(P_2), Valencia C (P_1) × ICGV-SM 99566 (P_2), NuMex-M_3 (P_1) × ICGV-SM 90704 (P_2), NuMex-M_3 × ICGV-SM 96801 (P_2), and NuMex-M_3 (P_1) × ICGV-SM 99566 (P_2), to generate F_1, F_2, BC_1P_1 and BC_1P_2 populations.The parents ($P_1$and P_2) together with F_1, F_2, BC_1P_1 and BC_1P_2 populations of each cross were evaluated in Randomised Complete Block Design (RCBD). Data on GRD severity were collected on a 1-9 score scale. Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), Genetic Advance as percent of Mean (GAM) and heritability were estimated using variance components. Scaling tests were performed to determine the type of gene effects controlling GRD resistance. Cluster analysis was performed using data on morphological descriptors and similarity

coefficients were generated between Valencia lines and their respective segregating populations.

The PCV and GCV estimates were high (20.04-70.1%) in all the six crosses except for Valencia C × ICGV-SM 96801(18.1%) and NuMex-M_3 × ICGV-SM 96801(17.1%) which exhibited moderate GCV values. Broad and narrow sense heritability estimates for GRD disease score ranged from (64.1 to 73.7%) and (31 to 41.9%) respectively in all the crosses. The GAM was high in all the crosses (21-50.7%) except for Valencia C x ICGV-SM 96801 (14.67), M_3 x ICGV-SM 99566 (18%) and NuMex-M_3 x ICGV-SM 96801 (13.5%) crosses that exhibited moderate GAM.

The study reaveled three types of epistatic gene effects viz., additive × additive [i], additive × dominance [j] and dominance × dominance [l] in control of GRD resistance. The component dominance × dominance [l] was more predominant than additive × additive [i] and additive × dominance [j] in Valencia C× ICGV-SM 96801, NuMex-M_3× ICGV-SM 96801, NuMex- M_3× ICGV-SM 90704, NuMex-M_3 × ICGV-SM 99566 and Valencia C × ICGV-SM 99566 crosses. Opposite and significant signs of dominance [d] and dominance × dominance [l] components indicated the importance of duplicate epitasis in almost all the later crosses for GRD resistance.

The study revealed the presence of variability of GRD resistance, implying that genetic improvement of these exotic materials is possible. Heritability and gene effect estimates depended on the parental backgrounds that were used in the study. The presence of duplicate epitasis in the crosses for GRD resistance revealed a complex nature of inheritance of GRD resistance. Effective improvement of the materials under study will require breeding efforts that exploits both additive and non-additive gene effects.

The current study revealed high recovery (60-98% similarity coefficient) among the populations and Valencia lines (NuMex-M_3 and Valencia C), except for BC_1 ICGV-SM 96801 and F_2 of NuMex-M_3 x ICGV-SM-96801 and Valencia C x ICGM-SM 96801 crosses respectively. Such populations can be considered for further improvements. Therefore, maximum gain in GRD resistance and Valencia trait recovery can be obtained if only those populations that exhibited high resistance and Valencia trait recovery are enhanced in the breeding program.

CHAPTER ONE

1.0 Introduction

1.1 Background

1.1.1 Origin, distribution and uses of groundnut

Groundnut (*Arachis hypogaea L)* is a native of south America and originated somewhere between south Bolivia and north Argentina. The crop was probably brought to Africa from Brazil by the Portuguese in the early sixteenth century. It was later taken from the west coast of south America to Asia (Higgins, 1959). From here, the British imported Indians during the construction of the railway to Uganda, who brought groundnut in Uganda.

Nutritionally, groundnut is rich in protein (25-34%), oil (44-56%), minerals (calcium, phosphorus, magnesium, zinc, iron and potassium) and vitamins (E, B3, B9, riboflavin, thiamine, niacin) (Janila *et al.*, 2013). The haulms can be fed to livestock and may also be used as compost (Singh & Diwakar, 1993). On the socio- economic, unspecified volumes of groundnut products (including roasted nuts, groundnut butter and oil) are processed industrially for sale in supermarkets (Sebunya & Yourtee, 1990). Usually, the Valencia varieties are used for such purposes because of their good and distinctive flavor with a soft skin (Mark *et al.*, 2009). Groundnut is also a principal crop for exportation and an important source of activities for agro industries (Ndiame, 2004) with a variety of industrial end uses which include; the making of paint, varnish and lubricating oil.

1.1.2 Production of groundnut globally and locally

Groundnut is cultivated in the semi-arid tropical and sub-tropical regions. According to FAO, (2011), the world production of groundnut was 38.61 million tonnes where Africa as a continent contributed to about 24.4%, Asia contributed 67.6% and America contributed 7.87%. The total production contributed from East African countries was 86.4 tonnes where Burundi contributed 1.2%, Kenya (1.4%), Rwanda (1.7%), Tanzania (75.3%) and Uganda (20.2%) FAO, 2011.

In Uganda basing on regions, the northern region with 83,000 Mt (34.0%) reported the highest groundnuts production, followed by eastern region with 77,000 Mt (31.6%) while central region with 33,000 Mt (13.4%) had the least percentage of the crop produced. From the district level figures revealed that; Soroti district with 19,000 Mt reported the highest production of groundnuts followed by Nakasongola (18,000 Mt), and then Amuru with 15,000 Mt. Production of groundnuts within regions indicated that the districts of Nakasongola (18,000 Mt), Soroti (19,000 Mt), Amuru (15,000 Mt) and Kibaale (12,000 Mt) reported the biggest production of groundnuts in the Central, Eastern, Northern and Western Regions respectively (UBOS & MAAIF, 2010).

1.1.3 Groundnut production constraints in Uganda

Uganda's productivity is at 0.8 t/ha of dried pods, which is below the average yield of 3 t/ha obtained in countries with developed agriculture (FAO STAT 2011). The low yields at farm level have been attributed to a combination of factors that include: unreliable rains, limited supply of breeders seed, use of poor agronomic practices, use of minimal amounts of inputs such as fertilizers (Okello & Deom, 2010), pests (aphids, whiteflies,

leaf miners) and diseases (groundnut rosette disease, rust, and early and late leaf spots) (Okello *et al.*, 2010; Page *et al.*, 2002).

1.2 Statement of the problem

Among diseases, groundnut rosette disease (GRD) is the most devastating viral disease in Sub-Saharan Africa (SSA) of Valencia botanical group of groundnuts (Subrahmanyam *et al.*, 2002; Okello *et al.*, 2010). It is responsible for annual yield loss worth over US dollars 150 million (Naidu *et al.*, 1999; ICRISAT, 2005; Waliyar *et al.*, 2007). There have been efforts to control groundnut rosette disease using a combination of cultural, biological and chemical measures, however with little success because small scale farmers seldom use them. The use of host plant resistance provides the most effective and economically viable management option for the resource poor farmers. In addition to the existing Valencia landraces, two exotic Valencia breeding lines (NuMex-M_3 and Valencia C) with superior characteristics were introduced by NaSARRI from USA so as to broaden the Valencia germplasm base in Uganda and evaluated for biotic and abiotic stresses but they also succumbed to GRD. For these superior lines to find utility in Uganda, they need further improvement by introducing resistance to GRD. However, there is inadequate information on the genetics of groundnut rosette resistance in these exotic Valencia breeding materials which would make genetic improvement difficult. Knowledge of the inheritance of resitance to GRD in the available breeding materials will facilitate selection of effective breeding method in the breeding program for GRD resistance. The current study was conducted to generate genetic information on GRD resistance and to determine recovery of Valencia type characteristics in segregating populations.

1.3 Justification of the study

Breeding work has led to the development of several GRD resistant cultivars in SSA (Deom *et al.*, 2006). In Uganda cultivars with durable resistance to GRD such as serenut 6T and Mali belonging to Spanish and serenut 2 belonging to Virginia botanical were released but are associated with some inferior attributes in comparison with the Valencia group (Okello *et al.*, 2010). Valencia botanical group are largely preferred by the local market for their superior sweet taste, high oil content, early-maturing and high yield based on number of seeds per pod (4-5 seeds/pod) (Sheidow *et al.*, 1993; Patte *et al.*, 2001; Kaaya & Warren, 2005).

Knowledge of the inheritance of GRD in these exotic varieties will enhance the development and utilisation of genotypes with GRD resistance which will reduce productions costs, boost groundnut production, improve household income as well as sustainable environments.

1.4 Objectives of the study

To enhance productivity of groundnut in Uganda through development of Valencia varieties resistant to groundnut rosette disease (GRD).

Specifically, the study was to:

1. To determine heritability for resistance to GRD in Valencia groundnuts
2. To determine gene action controlling resistance to GRD in Valencia groundnuts.
3. To evaluate F_2 and backcross segregating populations for recovery of Valencia type characteristics

1.5 Hypotheses of the study

1. Resistance to groundnut rosette is highly heritable.

2. Additive gene effects predominantly control resistance to GRD.

3. Among the segregating populations, F_2's will exhibit characteristics of the Valencia botanical group than the F_1 backcrosses.

CHAPTER TWO

2.0 Literature review

2.1 Classification and morphological diversity of groundnut

The most recent classification by Krapovickas & Gregory, (1994) divides groundnut into two subspecies (see Singh & Nigam, 1997, for an English translation), subsp. *hypogaea* and subsp. *fastigiata* (Waldron.), with two and four botanical varieties respectively. Classification into subspecies is based on the presence (subsp. *fastigiata*) or absence (subsp. *hypogaea*) of flowers on the main axis and on lateral branches such as sequential (subsp. *Fastigiata*) as opposed to alternate (subsp. *hypogaea*) branching of vegetative and reproductive lateral branches. The differences are likely to be due to variation in a few major genes (Kochert *et al.*, 1996). It can either exhibit a prostrate (runner) or erect (bunch) growth habit. In contrast, subsp. *fastigiata* is generally short duration with no dormancy and is entirely erect in growth habit. Intermediate forms between the subspecies are rare, but do exist, particularly in South America. *Arachis hypogaea* subsp. *hypogaea* is further divided into varieties *hypogaea* and *hirsute*. Therefore, morphological diversity includes qualitative (growth habit, branching pattern, leaf shape, stem pigmentation, pubescence, pod beak, pod reticulation, pod constriction, flower color and seed color and quantitative traits (days to 50% flowering, primary and secondary branches, plant height, pods/plant, pod length and pod yield) that have been described (IBPGR & ICRISAT,1992).

2.3 Groundnut rosette disease

Groundnut rosette disease is the most destructive virus disease that affects groundnuts in Africa. The disease is sporadic and unpredictable but can result in yield losses of up to 80%-100% (Naidu *et al.*, 1999; Subrahmanyam *et al.*, 2001). Rosette disease has been and continues to be responsible for substantial losses to groundnut production in Africa. For example, the rosette epidemics in 1994/95 in central Malawi and eastern Zambia destroyed the crop to such an extent that the total area of groundnut grown in Malawi fell from 92,000 ha in 1994/95 to 65,000 ha in 1995/96 (Subrahmanyam *et al.*, 2001). The disease is responsible for annual groundnut yield loss worth over US dollars 150 million (Waliyar *et al.*, 2007). Waliyar *et al.*, (2007) further reported that in seasons of rosette epidemics, the entire groundnut can be lost to the disease though in the majority of years approximately 30% average yield loss from all diseases combined. In Uganda, the great impact of rosette virus disease was put forward by Kimmins, (1999), who identified rosette disease as the most devastating in the teso farming system where groundnuts are among their known staple foods. More still, the disease has been identified further as the most important problem in groundnut growing areas mainly in the eastern part of the country (Chancellor, 2002).

2.3.1 Distribution, epidemiology and symptoms of the disease

Groundnut rosette disease is a polycyclic disease because each infected plant serves as a source for initiating subsequent disease spread in the field. Within Africa, 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 Democratic Republic of Congo (Naidu *et al.*, 1999).

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 plant age, cultivar, crop density, time of infection, transmission efficiency of aphids, proximity to the source of infection and climatic conditions.

The epidemiology of GRD is complex, involving interactions between and among two viruses and a Sat RNA. 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). Possible sources from which rosette could be spread are the infected groundnut plants surviving between cropping seasons. 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. The disease vector, the aphid (*Aphis craccivora*), was described as an economically important pest on groundnut in SSA (Naidu *et al.*, 1998). This aphid is polyphagous and can survive on as many as 142 plant species mainly in the Leguminosae family and several non-crop plant hosts serve as reservoirs of *A. craccivora*. One or more of these 142 plant species could be a source of the rosette complex (Naidu *et al.*, 1998).

Groundnut rosette disease occurs as two symptom variants, chlorotic rosette and green rosette, with considerable variation within each type (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. 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. Chlorotic rosette occurs

throughout the SSA, whereas green rosette has been reported from Angola, Kenya, Malawi, Swaziland, Uganda and West Africa (Naidu *et al.*, 1999). 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).

2.3.2 The causal agents of disease

The causal agents of rosette are groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV), and satellite RNA. GRAV is asymptomatic but it acts as a helper virus in vector transmission of GRV and its satellite RNA as they must be packaged in the coat protein of GRAV to form particles that can be transmitted by the aphid, *Aphis craccivora.* The satellite RNA is largely responsible for symptom expression and depends on GRV for replication while GRV depends on satellite RNA for aphid transmission. However, GRV can replicate independently. All three agents must occur together for transmission by the aphid vector and subsequent disease development. Prolonged probes by the aphid vector are needed for the inoculation of GRAV-containing particles into the phloem, where the virus can replicate, while particles containing GRV and satellite RNA can be inoculated into mesophyll cells during short probes (Naidu *et al.*, 1999).

2.3.3 Disease management

Long acquisition access feeding period required by the vector provides an opportunity to control aphids with chemical sprays before they can spread the disease. Various insecticides have been used to control *Aphis craccivora* to minimize or prevent spread of rosette disease in field trials. Timing of spray, dosage and type of insecticide utilized is

critical for controlling aphids. However, insecticides are an unviable option in SSA due to high costs and scarcity; chemical control is, thus, seldom preferred by the farmers. Furthermore, insecticide applications pose detrimental effects on health and environment and their usage is being discouraged (Subrahmanyam *et al.*, 2002; Dennis & Katttingu, 2012).

Removal of disease and aphid vector sources such as infected plants after harvest, volunteer plants or weeds that can harbor vector aphids are viable cultural control options. Planting fast-growing cereals such as maize, pearl millet and sorghum, as inter and border cropping, has been reported effective in interfering with the vector aphid movement. Dense planting maintains optimum plant population in the field which covers the ground thereby discouraging landing of vector aphids on the crop. Early planting in the rainy season would also allow the plant to take advantage of low-aphid populations; maintaining good plant density without any gaps (aphids prefer widely spaced plantings for landing) would subsequently reduce rosette disease incidence. Early planting may not be effective in areas where groundnut is grown continuously, as this allows perpetuation of virus and vector. Rouging of voluntary sources and early-infected plants prevent primary and secondary spread of the disease. Intercropping with cereals such as maize, sorghum, finger millet, beans and cowpea have also been shown to affect aphid colonization, movement and behavior within crops, thereby GRD incidence (Dennis & Katttingu, 2012). However, control by cultural practices by smallholder farmers is difficult under subsistence farming conditions due to farmers' pre-occupation with other revenue generating practices, unpredictable climate, small-land holdings and farmers' reluctance to adopt improved cultural practices.

Varieties resistant to GRD provide the most economical and practical solution to control GRD in the field, and thus, substantial efforts has been made to identify durable GRD resistant sources. Cultivation of resistant varieties is recommended. Some resistant cultivars available for cultivation in SSA include: ICG 12991, ICGV-SM 99568, ICGV 93437, ICGV-IS 96894, ICGV-SM 99541, ICGV-SM 01513, ICGV-SM 01514, ICGV-SM 01708 and ICGV-SM 01731) (Waliyar *et al.*, 2007). Genetic engineering provides unique possibilities to introduce foreign DNA into plant cells to alleviate the problem of incompatibility (Sharma & Anjaiah, 2000). The potential of this development to genetically transform groundnut to obtain resistance to viruses and other biotic and abiotic constraints can positively impact groundnut productivity, especially in the resource poor agricultural systems of the semi arid tropics (Sharma & Anjaiah, 2000). To date, tissue culture and genetic engineering research in groundnut have been carried out in India and USA (Maina *et al.*, 2010).

2.4 Sources of host resistance

Sources of resistance to rosette were first discovered in 1952, when an epidemic of the disease destroyed a large collection of groundnut germplasm in Senegal, with the exception of few germplasm lines originating from the frontier region between Burkina Faso and Cote d' Ivoire (Sauger & Catharinet, 1954). These lines have since been extensively used in rosette resistance breeding programs throughout Africa. Resistance was effective against both chlorotic rosette and green rosette and is governed by two independent recessive genes (Nigam & Bock, 1990). Resistance has also been found in other *Arachis* species by way of introducing genes from resistant varieties to non resistant ones. *Arachis chacoense* has been reported to be highly resistant in green house tests

(Murant *et al.*, 1991). An interspecific hybrid derivative, 83/372-2-22 Bl, originating from a cross between *Arachis hypogaea* and *Arachis chacoense,* has been reported to be resistant to groundnut rosette in Malawi (Moss *et al.*, 1995).

2.5 Screening for disease resistance

Screening methods have been developed for large-scale evaluation of groundnut genotypes against rosette (Bock, 1987). Breeders can use an effective screening technique that permits rapid evaluation of large segregating populations and inbred lines to identify GRD resistance (Naidu et al., 1999). The infector row technique is used in rosette resistance whereby a rosette susceptible cultivar is sown after two test rows. The technique involves planting a test row of uninfected plants flanked on either side by a row of susceptible cultivar infested with aphids. To minimize chances of escape, each infector row is planted approximately 2 weeks before the test crop. This technique leads to a 99% success rate in spreading the disease to susceptible plants hence resistant cultivars are easily detected (Ntare *et al.*, 2002). Another screening method would be mechanical sap inoculation to transmit and evaluate resistance but this only works for GRV and sat RNA (Waliyar *et al.*, 2007). Grafting using scions from GRAV infected groundnut plants can be used to evaluate resistance to GRAV (Olorunju *et al.*, 1992). Virus inoculation of groundnut seedlings before transplanting is an effective method to create high levels of infection for determining the effect of single infection of GRAV on the growth and yield of groundnuts and/or to compare different varieties or germplasm lines of groundnut for their reaction to GRAV under natural conditions (Naidu and Kimmins, 2007). Another screening method is using hot spots for rosette because of the available disease inoculums that build over the season. In Uganda, National Semi-Arid

Resources Research Institute in Serere district is known as the hot spot for groundnut rosette disease and the susceptible varieties succumb to the disease (Okello et al., 2010). However, for convention breeding, the infector row technique ranks the most appropriate. There are two rating methods that are being used to quantify GRD resistance in groundnut cultivars. Both methods use a rating of symptoms of infected plants, hence they evaluate resistance to GRV and sat RNA which are responsible for producing symptoms (Waliyar *et al.*, 2007). The first method employs a visual rating score one method uses the 1-5 disease rating score to evaluate GRD resistance (Olorunju *et al.*, 2001). Score 1=no visible symptoms on the foliage, therefore highly resistant, score 2=rosette symptoms on 1-20% foliage but no obvious stunting, thus resistant, score 3= rosette symptoms on 21-50% foliage and stunting, moderately resistant, score 4 = severe rosette symptoms on 51-70% foliage and stunting, susceptible, and score 5 =severe symptoms on 71-100% foliage, stunted or dead plants, highly susceptible. However, in Uganda, a scale of 1-9 is used by the Groundnut Improvement Program at NaSARRI in Serere, to determine the disease incidence and severity where 1-3 no symptoms (resistant), 4-5 slight leaf symptoms with no stunting (Slightly resistant) , 6-7 moderate leaf symptoms with < 50% stunting (moderately susceptibly), 8-9 severe leaf symptoms with >50% stunting (highly susceptible). The other widely used is based on percent disease incidence (PDI), measured when the crop is at early pod filling stage. The total number of plants in each row and the plants showing rosette symptoms (chlorosis with severe stunting) are counted once at 80 days and again at 100 days after germination. Percent disease incidence in each row and the mean percentage incidence for each plot over the two counts are then computed to assess the genotype resistance to GRD. The

scheme of screening groundnut genotypes for GRD resistance and interpretation of genotype response is depicted as follows. PDI of less than 10% is highly resistant, PDI of 11-30% is resistant, 31-50% is moderately resistant and 50% is susceptible (Waliyar *et al.*, 2007).

2.6 Mechanism of resistance to groundnut rosette

The natural genetic resistance mechanisms include; inhibition of virus replication, hypersensitive resistant (local necrotic lesion and systemic necrosis), extreme resistance, resistance to movement of the virus, resistance to symptom development (tolerance), resistance to seed transmission and resistance to transmission by vectors (Tolin, 2012). The mechanisms of resistance in groundnuts were speculated first by Nutman, (1964), Bock & Nigam, (1988), and Olorunju, (1990). The latter reported resistance to initial infection, restriction of virus movement, and restricted production of satellite RNA which induces rosette symptoms. Resistance in groundnut to *Aphis craccivora* was reported by Padgham *et al.*, (1990) and later by Herselman *et al.*, (2004) through the mechanisms of non-preference and antibiosis. Detailed feeding studies revealed that virus transmission by the aphid vector might be inhibited through the collapse and death of plant cells at the feeding site (Chancellor, 2002).

Varieties that are available in Uganda, are vector resistant and these include, Serenut 4T (ICG 12991), while Serenut 3R (ICGV-SM 93530) and ICGV-SM 90704 are resistant to groundnut rosette virus. It is difficult from the present screening trials to distinguish between virus and vector resistance because the criterion used is based on presence or absence of rosette symptoms and does not involve aphid population counts. Given the

large numbers found in aphid colonies (range 2-1,000 individuals) and the relatively small size of the individual insects, such counts will not be practical, although rough estimations of colony sizes can be made. However, genotypes with resistance to both virus and the vector are expected to be more stable than those to either one of the components. It is therefore suggested that field screening tests using disease incidence measures of resistance should be complemented by laboratory screening to the vector. Application of molecular markers and transgenic resistance mediated mechanism should facilitate the rapid identification of resistance to rosette or the mechanism governing the resistance (Taliansky & Robinson, 1998). Under natural conditions, it is possible to identify vector resistance on cultivars by the comparative level of aphid colony establishment (Chancellor, 2002). In that instance, resistance would be determined by the effect of the plant on the aphid physiological aspects: the development of instars, reduced survival, and reduced fecundity of adult aphids. The adverse effect of the plant on aphid survival, longevity and fecundity would be termed as antibiosis, whereas the effect whereby the aphid is directed away from a plant is called antixenosis or non-preference (Thomas and Waage, 1996).

2.7 Heritability and genetics of rosette resistance and some yield traits

Harkness, (1977) and Nigam & Bock, (1990) reported double independent recessive genes in control of groundnut rosette resistance However, other findings that proceeded revealed that the inheritance of resistance to rosette disease was governed by dominant genes (Olurunju *et al.*, 1992; Akpan & Olurunju, 2009). Many other scientists highlighted that in cases where the coat protein is involved in the replication of the virus as in the case of GRD where GRAV coat protein control the disease transmiision, dominant resistant

genes are expected to be present in the inheritance of the resistance genes (Bendahmane *et al.*, 2000, Cooley *et al.*, 2000, Takahashi *et al.*, 2001). Olorunju *et al.*, 1992, reported that in F_2 generation, the plant segregated into 1 susceptible: 3 resistant, suggesting dominant gene action governing rosette resistance. (Adu Dapaah *et al.*, (2007), reported broad sense heritability for rosette resistance as high (75%) showing that the trait is heritable when other genetic imbalances like the dominance and epistatic interactions are taken to be null. Nigam *et al.*, (2012) indicated that there is limited information concerning the nature and type of gene interactions that exist in groundnuts particularly for viruses. According to Singh & Oswalt, (1991) variation due to dominance effects and their interactions cannot be exploited effectively in groundnut while additive × additive epistatic variation is potentially useful, as it can be fixed in homozygous cultivars.

2.8. Breeding for resistance to GRD

Early studies used conventional breeding in the inheritance of resistance in groundnut rosette were results were got and still stand as the basis for molecular work. In Uganda, efforts in breeding for host plant resistance have contributed to the identification and development of several groundnut germplasm lines with acceptable levels of field resistance to GRD. The Uganda National Groundnut Improvement Programme has released 13 rosette resistant commercial varieties (Okello *et al.*, 2010).

Conventional breeding approaches have been successful in development and release of several improved cultivars not just for increased yield but also for resistance/tolerance to biotic and abiotic stresses thus offering protection against losses and for consumer/trader preferred traits (Janila *et al.*, 2013*)*. However, the progress has been limited in improvement of some traits. It is possible to specifically target such traits for

improvement, and also enhance the efficiency of overall breeding program through use of molecular breeding approaches.

Conventional breeding methods have been used intensively to enhance crop productivity, the results have not been encouraging. Therefore, conventional breeding procedures along with the tools for phenotyping have to be largely used in groundnut improvement programs especially in cases where there is presence of dominance variance and epistasis variance control the trait of interest especially due to allotetraploid nature of groundnut cannot be exploited in a self pollinated crops like groundnut.

2.9 Section summary

The commercial varieties grown by farmers in Uganda mostly are Spanish and Virginia (serenut series) while Valencia's landraces (Red Beauty, Acholi White and Roxo), are on small scale due to their susceptibility to rosette. Therefore, for broadening the Valencia base in Uganda, Valencia C and NuMex $-M_3$ lines were imported from USA and they needed further improvement for rosette resistance so as to find their utility in Uganda. The knowledge about heritability and nature of gene effects prevailing in the breeding material is necessary to decide on a breeding procedure to be chosen.

CHAPTER THREE

3.0 Determination of heritability for resistance to GRD in exotic Valencia groundnuts

3.1 Introduction

Valencia groundnuts are known for their quality attributes like good and distinctive flavor, with a soft skin, sweet taste, early maturity, relatively bigger seeds that makes it key for commercial purposes (Mark *et al.*, 2009). Despite of their importance, production is still constrained by GRD. The disease is sporadic and unpredictable but can result in yield losses of up to 80% or even 100% (Waliyar, 1999; Subrahmanyam *et al.*, 2001; Adu Dapaah *et al.*, 2004).

There have been efforts to control groundnut rosette disease using a combination of cultural, biological and chemical measures, however little success has been achieved because small scale farmers seldom use them. In addition, chemical control of aphids is not economically viable because of its persistent nature in the disease transmission .The use of host plant resistance is the most effective and economically viable management options for the resource poor farmers especially in Uganda. However, it's limited by lack of resistant varieties and information on heritability of GRD resistance on the available Valencia breeding materials

Estimation of genetic variability with the help of suitable parameters such as genetic coefficient of variation, heritability estimates and genetic advance are absolutely necessary to start an efficient breeding program (Atta *et al.*, 2008; Janila *et al.*, 2013). Kayondo *et al.*, 2014 reported high (93%) heritability estimate for GRD. However,

heritability estimates depends on the genetic background of the materials used and the environment in which the populations are evaluated from (Kearsey and Pooni, 1996; Wambi, 2014; Wambi *et al.*, 2014).Therefore, this study was conducted to estimate genotypic and phenotypic coefficient of variations, and heritability for GRD resistance in Valencia groundnut genotypes. The information generated will be used in suggesting a future breeding program for GRD resistance which will enhance development and utilization GRD resistant genotypes.

3.2 Material and methods

3.2.1 Study Area

The research was conducted in Uganda at the National Semi-Arid Resources Research Institute (NaSARRI) of the National Agricultural Research Organisation (NARO) located 01^0 30 00N and 33^0 33 00E in serere district. It receives an annual rainfall of 1,000-1,200 mm.

3.2.2 Materials

In this study, a total of five parents that were characterised for GRD resistance were used (Table 1). Rosette resistant lines; Serenut 6T (ICGV SM 99566), Serenut 2 (ICGV-SM 90704) and Mali (ICGV-SM 96801) were kindly provided by the Groundnut Improvement Program at the National Semi-Arid Resources Research Institute-(NaSARRI) in Serere. The exotic susceptible Valencia lines; Valencia C and NuMex-M_3 were provided by the Plant Breeding Department New Mexico State University.

Table 1. Characteristics of the groundnut parental germplasm used in the study

Genotype	Other name	Origin	Pedigree	Botanical type	Seeds per pod	GRD reaction
ICGV-SM 99566	Serenut 6T	Malawi	Introduction from ICRISAT Malawi	Spanish	1-2	Resistant
ICGV-SM 90704	Serenut 2	Malawi	RG-1 x Manipintar	Virginia	1-2	Resistant
ICGV-SM 96801	Mali	Mali	Selection from Mali	Spanish	1-2	Resistant
Valencia C	Valencia C	USA	Selection from Colorado Manfredi	Valencia	3-5	Susceptible
NuMex-M_3	M_3	USA	Valencia C x ICGV 87157	Valencia	3-5	Susceptible
Redbeauty[a]	Redbeauty	Uganda	Landrace	Valencia	3-5	Susceptible

[a]=It is not among the parents used in the study but rather used as a spreader to increase disease pressure during the experiment

3.2.3 Generation of first filial generation

Valencia C and NuMex-M_3 were used as female exotic susceptible lines while ICGV-SM 90704, ICGV-SM 96801 and ICGV-SM 99566 were used as male local resistant lines. In August 2011, nine seeds from each of the parents were planted in plastic pots of diameter 45 cm and height 15 cm containing loam and sandy soil. The parents were grown in a glass house and later thinned to two. Plants were watered after every two days up to harvest. Staggered planting of parents was done where the male parents were planted 10 days before the female parents so as to properly synchronize the flowering such that continuous availability of flowers .

Crossing of parents was done at the onset of anthesis at about 25-30 days after planting (DAP) by hand pollination using the emasculation and hooking method (Okonkwo & Clayberg,1984). During the flowering period, the parents were emasculated using a pair of forceps in the morning between 6:30 and 8:30 and evening between 6:30 and 7:20pm. This was followed by careful rubbing the pollen from resistant parents on to the stigma of the susceptible parents using hands. The nodes of the flowers that were crossed were tagged with a label that showed the two parents that crossed. Bi-parental mating design was employed where a total of six crosses (Valencia C × ICGV-SM 90704, Valencia C × ICGV-SM 96801 , Valencia C × 99566, NuMex-M_3 × ICGV-SM 90704, NuMex-M_3 × ICGV-SM 96801 and NuMex-M_3 × ICGV-SM 99566) were got. In each cross, 20 flowers were pollinated and at physiological maturity, the pods from each parent were harvested, dried, packed, and stored in labeled envelops.

3.2.4 Generation of F_1, F_2, and backcross to parents

In 2011, 20 F_1 seeds generated above from each cross together with their respective parents were planted in plastic pots containing loam soil and sandy soil under glass house of NaSARRI. The F_1 seeds were raised in close proximity with their parents so as to eliminate the selfed off types. During flowering, ten plants were selfed to generate F_2 seeds while five plants were backcrossed to susceptible parents and other five plants backcrossed to resistant parents. The process of pollination was done as in F_1 above and the backcrosses to either parent done. The crossing scheme used is summarized in Figure 1.

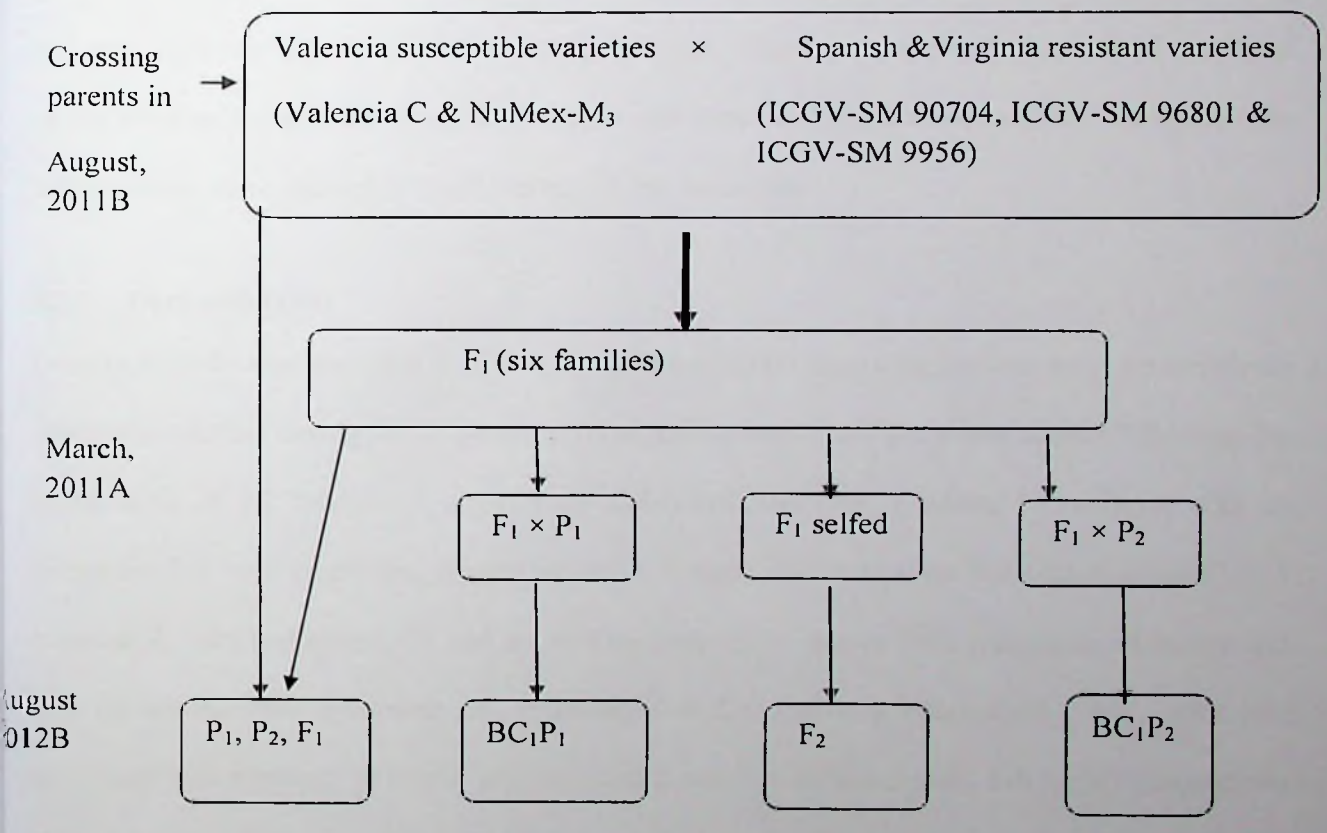


Figure 1: Crossing Scheme used to generate F_1, F_2, BC_1P_1 & BC_1P_2

3.2.5 Evaluation of the six generations of six cross crosses

3.2.5.1 Field layout

The six generations (P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P2) of each cross were evaluated in the experimental field of NaSARRI a known hot spot for groundnut rosette disease in a randomized complete block experimental design (RCBD) with three replications, in June 2012. The plot sizes of 3m × 3m, comprising 6 rows of 3m length at spacing of 45 x 15 cm.

3.2.5.2 Inoculation

The infector row technique was used to build up disease pressure on the experimental materials that were naturally inoculated. Acholi white, (a highly rosette susceptible local variety) was used as the infector line and was planted in a single row between every two rows of test materials. The infector rows were planted 14 days before the test materials.

3.3 Data collection

Disease severity was recorded at 115 days after planting (at physiological maturity) on ten plants randomly selected among the 12 plants in three replications. Each plant was scored following the rating scale of 1-9 where 1-3 represented highly resistant ,HR, (where 1= resistant with no symptom, 2 = very slight leaf symptoms and 3 = slight leaf symptoms but still negligible), 4-5 resistant,R, with leaf symptoms and no stunting (where 4 = shows 50% symptoms on leaves and 5 = all leaves show symptom of chlorosis) , 6-7 moderately susceptible , MR, with leaf symptoms and stunting (where 6 is 25% stunted and 7 = 50% stunted), 8-9 highly susceptible, HS, with severe leaf symptoms with >50% stunt (where 8 = has few pods while 9 = no pod at all is expected). This rating scale was adopted from the Groundnut Improvement Program at NaSARRI in Serere, Uganda.

3.4 Data analysis

3.4.1 Analysis Of Variance (ANOVA)

Data on disease severity at harvesting maturity on the generations of each cross were subjected to one way ANOVA using a computer program Genstat version 13 to test for the significance of the differences between the generations of each cross for GRD score. The ANOVA was based on the linear mathematical model: $Yij = \mu + ri + gj + eij$; where Yij= observed effect for ith replication and jth genotype, μ = grand mean of the experiment, ri = effect of the ith replication, gi = effect of the jth genotype, eij = residual effect. Where the ANOVA showed significant differences, the treatment means were separated using by Fisher's protected least significant difference at 5% probability level (Payne *et al.*, 2010).

3.4.2 Estimation of variance components

The phenotypic and genotypic coefficients of variability, heritability and genetic advance for GRD resistance, variance components that include, environmental, genotypic, additive and dominance, were obtained following (Kearsy and Pooni, 1996).

Environmental variance or error $(\sigma^2 e) = (\sigma^2 P_1 + \sigma^2 P_2 + 2\sigma^2 F_1)/4$

Where; $\sigma^2 P_1$ =variance of susceptible parents, $\sigma^2 P_2$ =variance of resistant parents and

$\sigma^2 F_1$ =variance of first filial generations

Phenotypic Variance = $[\sigma^2 F_2]$ = variance of F_2 generation

Genotypic variance in F_2 $[\sigma^2 G (F_2)] = \sigma^2 F_2 - \sigma^2 e$

Where; $\sigma^2 F_2$ = variance of F_2 generation and $\sigma^2 e$ = Environmental variance

Additive variance in F_2 $[\sigma^2 A (F_2)] = (2\sigma^2 F_2) - [\sigma^2 BC_1 + \sigma^2 BC_2]$

Where; σ^2F_2 = variance of F_2 generation, and σ^2BC_1 and σ^2BC_2 = variance of backcross to female and male parents, respectively.

Dominance variance in F_2 $[\sigma^2D\ (F_2)] = \sigma^2G\ (F_2) - \sigma 2A\ (F_2)$

Where; $[\sigma^2G\ (F_2)]$ = Genotypic variance in F_2 and $\sigma^2A\ (F_2)$ = Additive variance in F_2

3.4.3 Coefficient of variability

Both the genotypic coefficient of variation (GCV) and phenoptypic coefficient of variation (PCV) were estimated following the method suggested by Singh and Chaudhury, 1985 where the GCV and PCV values were classified as low (0-10), medium (11-20) and high (20 and above) Sivasubramanian and Menon, 1973.

$PCV=(\sqrt{Vp})/\bar{X} * 100$

$GCV=(\sqrt{VG})/\bar{X} * 100$

V_P = Phenotypic variance

V_G =Genotypic variance

$\bar{X}$ =Grand mean of the character

3.4.4 Estimation of heritability

The variance components descibed above were used to determine broad sense heritability (h^2_b)and narrow sense heritability (h^2_n) following (Keasey & Pooni, 1996) in all the six crosses as detailed below;

Broad-sense $(h^2_b) = 100[\sigma^2G\ (F_2)/V_{F2}]$

Where; $\sigma^2G\ (F_2)$ = Genotypic variance in F_2 and V_{F2} = variance of F_2 generation

Narrow-sense heritability $(h^2n) = 100[\sigma^2A\ (F_2)/V_{F2}]$

Where; $\sigma^2 A$ (F_2) = Additive variance in F_2 and V_{F2} = variance of F_2 generation

3.4.5 Estimation of Genetic advance (GA)

Genetic advance was estimated following Singh & Chaudhury (1985) method.

Genetic advance (GA) = $h^2_n \times k \times \sigma^2_p$

Where,

h^2_n = Narrow sense heritability estimate

σ^2_p = Phenotypic standard deviation

K = Selection intensity at 5% is equal to 2.06

3.4.6 Genetic advance as percent of mean (GAM)

GAM% = $(GA/\overline{X})*100$

Where X= Grand mean of the trait

GA = Genetic advance

The Genetic Advance as percent of Mean (GAM%) was categorized as described by Johnson *et al.*, 1955) as low (0-10), medium (10-20) and high (21 and above)

3.5 Results

3.5.1 ANOVA results

There was significant variation observed in the generations for disease severity at $P<0.05$ (Table 2). The donor parents: ICGV-SM 99566 and ICGV-SM 90704 were highly resistant with mean scores (1.33-2.83) while ICGV-SM 96801 was slightly resistant with the score that ranged 4-6 whereas all the susceptible genotypes Valencia C and NuMex-M_3 were highly susceptible with the score that ranged (7.5-8).

All the six F_1's showed high resistance to GRD (mean score range 1.67 to 2.0) and the mean disease score in F_2 were moderately resistant. The segregants of F_2 from ICGV-SM 90704 donor line were highly resistant with mean score in the range of 2.3-2.73 whereas those from ICGV-SM 96801 were susceptible with mean score in the range of 5- 6.

Table 2: Results of GRD mean score and standard error for the six generations of the 6 crosses

Generation	Valencia C × ICGV-SM 99566	Valencia C × ICGV-SM 90704	Valencia C × ICGV-SM 96801	NuMex-M_3 × ICGV-SM 99566	NuMex-M_3 × ICGV-SM 90704	NuMex-M_3 × ICGV-SM 96801
P_1 (S)	7.5c	7.83d	8.167c	8.67b	7.67c	7.83c
P_2(R)	2.17a	1.35a	5.05a	2.83a	1.33a	4.67a
F_1	1.67a	1.83a	1.83a	2.0a	1.67a	1.67a
F_2	4.67b	2.3a	6.0c	4.3b	2.73a	5.0b
BC_1P_1	6.33c	6.33c	7.5c	8.67b	7.0c	6.0a
BC_1P_2	2.83a	3.0a	5.0c	1.83a	2.33b	1.67ab
F cal	**12.16****	**8.27****	**5.47****	**48.1****	**34.0****	**59.4****
MS	**20.2**	**23.2**	**21.1**	**16.1**	**13.3**	**11.8**
CV %	**29.2**	**26.8**	**27.1**	**24.9**	**30.2**	**20.4**

P_1 (S) =Parent 1elite parents, (Valencia C and NuMex-M_3) P_2= P_2(R) - the donor parents .F_1=1st Filial generation, F_2= 2nd Filial generation, BC_1P_1= Backcross to sucesptible parent(P_1) and BC_1P_2 = Backcross to resistant parent (P_2) ,**=significant at 95%, CV = Coefficient of variation, Fcal=F-value, MS=Mean sum of square

The Resistance rating scale: 1-3 (highly resistant), 4-5 slight (Slightly resistant), 6-7 (moderately susceptibly), 8-9 (highly susceptible)

3.5.2 Estimation of coefficients of variability and heritability

The results in table 3, shows phenotypic and genotypic coefficients of variation, heritability and GAM estimates for GRD resistance in six crosses. In all crosses, dominance variance component (V_D) exhibited relatively higher magnitudes as compared to additive component (V_A) except for Valencia C x ICGM-SM 99566, Valencia C x ICGM-SM 90704 and The PCV and GCV estimates were high (20.04-70.1%) in all the six crosses except for Valencia C × ICGV-SM 96801(18.1%) and NuMex-M_3 × ICGV-SM 96801(17.1%) crosses which exhibited moderate GCV values. Broad and narrow sense heritability estimates for GRD disease score ranged from (64.1 to 73.7%) and (31 to 41.9%) respectively in all the crosses. The GAM was high in all the crosses (21-50.7%) except for Valencia C x ICGV-SM 96801 (14.67), M_3 x ICGV-SM 99566 (18%) and NuMex-M_3 x ICGV-SM 96801 (13.5%) crosses that exhibited moderate GAM.

Table 3: Genetic variance components and parameters for resistance to groundnut rosette in groundnut

Generation	Valencia C × ICGV-SM 99566	Valencia C × ICGV-SM 90704	Valencia C × ICGV-SM 96801	NuMex-M_3 × ICGV-SM 99566	NuMex-M_3 × ICGV-SM 90704	NuMex-M_3 × ICGV-SM 96801
V_E	0.83	0.9	0.9	0.7	0.6	0.5
V_G	1.5	1.93	1.63	1.93	1.07	1.2
V_A	0.83	0.99	0.79	0.97	0.7	0.55
V_D	0.67	0.94	0.82	0.99	0.37	0.65
V_P	2.33	2.83	2.51	2.66	1.67	1.7
$\bar{X}$	5.3	2.4	7	6.8	2.2	6.4
PCV	28.8	70.1	22.6	23.6	58	20.4
GCV	23.1	57.8	18.1	20.5	47.1	17.1
h^2_b	64.4	68.2	64	73.7	64.1	70.6
h^2_n	35.6	34.9	31	36.4	41.9	32.4
GA	1.12	1.2	1.02	1.23	1.1	0.86
GAM %	21	50.5	14.67	18	50.7	13.5

V_E=Environmental variance, V_G=Genotypic variance, V_A=Additive variance, V_D=Dominance variance, PCV and GCV=Phenotypic and Genotypic Coefficient of Variation respectively, $h2_b$ and $h2_n$=Broad and narrow sense heritability respectively , $\bar{X}$=Grand mean, GAM%=Genetic Advance as percent of mean

3.6 Discussion and conclusion

The ANOVA for the 6 crosses showed highly significant differences ($P<0.01$) among generations for GRD resistance, suggesting presence of variability for GRD disease score in the generations. The significant variation implies that genetic improvement of groundnut for GRD resistance is possible hence such variability can be exploited by breeders for groundnut improvement. The presence of such variability would be as a result of wide genetic distance between the parental backgrounds that were used in the study. The results of the current study are comparable to those Naidu *et al.*, 1999, who reported that GRD variations are said to be due to diversity among the causal agents (sat RNA variants), diferences in genotype response, variable climatic conditions and mixed infections with other viruseses. Monyo *et al.*, (2007) also reported variability for GRD resistance among 143 accessions that were evaluated. High resistance was exhibited by donor parents ICGV-SM 99566 and ICGV-SM 90704 which results are comparable with Waliyar *et al.*, (2007) and Okello *et al.*, (2010) who reported the two lines as universal donor for GRD resistance.

The mean of F_1 generations tended towards the mean of resistant parents indicating that resistance to GRD was controlled by dominant genes or epistatic gene action. These results support the earlier reports that resistance to GRD is controlled by dominant genes (Olurunju *et al.*, 1992: Akpan & Olurunju, 2009). In contrast, Harkness, (1977) and Nigam & Bock, (1990) reported recessive genes controlling resistance to GRD. Tolin, (2012), approximated that over 80% of known virus resistance is 50% completely dominant with the remaining being polygenic which comply with the current study where dominant genes control the resistance to GRD. The presence of dominant genes implies that the F_1's would be utilized due to relative heterosis where hybrid vigour could be exploted. However in groundnuts; commercial production of F_1

seed can't be achieved since it is a self fertilizing crop and its a tetraploid nature makes the F_1's unstable. Wambi, (2014), suggested that in situations, selection should take place at later stages such that that the dominant genes are decreased. To exploit such heterosis, breeding methods such as recurrent seletion and single seed descent may be used due to the presence of dominance effects.

Success of genetic improvement is attributed to the magnitude and nature of variability present for a specific character. Moderate to high level of GCV (17.1-57.8%) and high PCV (20.4-70.1%) was noticed for all crosses indicating higher magnitude of variation in these crosses which could be attributed to genotype with very little effect of the environment. In the current study, the magnitude of PCV (20.4 -70.1%) were superior over that of GCV (17.1-57.8%) indicating greater influence of environment on the trait which could reduce possible response to selection for GRD resistance on phenotypic basis. According to Oyiga & Iguru, (2011) when the magnitude of genetic variance is higher than the environmental variance, it indicates that the genetic component is the major contributor to the total variance of the trait under study. Vishnuvardhan *et al.*, (2012), high GCV may indicate a predominant role of additive gene actions and amenability for phenotypic selection in early generations. The high values of PCV and GCV in almost all the crosses indicated a wide scope of selection for GRD resistance due to wide spectrum of genotypic variation of the trait.

The information on genotypic coefficient of variation revealed the existence genetic variability present in the genotypes for GRD resistance; however, it does not provide full scope to assess the variation that is heritable. In the current study, all the crosses had high broad sense heritability values (64.1-73.7%). The findings are comparable with those of (Van der Merwe, 1998; Adu Dapaah, *et al.*, 2007; Kayondo *et al.*, 2014) who reported high broad sense

heritability estimates for GRD resistance (67- 93%). The high heritability could indicate a high response to selection due to reduced environment influence and predominance role of additive gene effects (Chattopadhyay & Dutta, 2010; Tafere *et al.*, 2013), in the control of GRD resistance which could result to efficient response to selection in early generations.

Moderate values (31-41.9%) for narrow sense heritability were observed in all crosses which indicated greater dominance effects on GRD resistance than the additive. In all crosses, the dominance variance component (V_D) exhibited relatively higher magnitudes as compared to additive component (V_A) except for Valencia C x ICGM-SM 99566, Valencia C x ICGM-SM 90704 and NuMex-M_3 x ICGV-SM 90704. It is generally verified that an increase in magnitude of V_D implies a decrease in h^2n in the reference to F_2 generation. Hence selection of genotypes from initial generations for GRD disease score in these crosses may be difficult due to the higher influence of dominance effects. According to Kormsa-art *et al.*, (2002), selection for such traits controlled by dominance becomes ineffective when carried out in early generations; therefore, selection based on this trait is more effective when undertaken in subsequent generations of all crosses. In this way, the occurrence of heterozygotes would be reduced and the available additive variance for selection is increased, thereby providing higher possibilities of selection gains for the trait.

The genetic advance as percent of mean (GAM) was high in all the crosses (21-50.7%) except for Valencia C x ICGV-SM 96801 (14.67%), M_3 x ICGV-SM 99566 (18%) and NuMex-M_3 x ICGV-SM 96801 (13.5%) exhibited moderate GAM. According Wambi, (2014) high GAM indicates predominant role additive gene actions in control of GRD resistance which is very important for the improvement of a crop through breeding while moderate GAM reflects additive and non-additive effects in control of the trait under study. Breeding methods such

recurrent selection and biparental mating would useful for such traits controlled by additive and non-additive effects (Nidagundi *et al*, 2012).

Conclusion and recommendation

The study revealed presence of considerable variability for GRD resistance which could be attributed to genotype with very little effect of the environment indicating a greater success of these exotic materials to genetic improvement. Moderate narrow sense heritability was observed in all crosses as a result greater dominance effects on GRD resistance than the additive. Hence selection of genotypes from initial generations for GRD disease score in these crosses may be difficult due to the higher influence of dominance effects. However, the heritability estimates depended on the genetic background of the parents that were used in the study. Breeding methods such as recurrent selection and single seed descent methods may be used for improvement of the materials.

CHAPTER FOUR

4.0 Determination of gene action controlling resistance to GRD in exotic Valencia groundnuts.

4.1 Introduction

Development of improved cultivars requires an understanding of the nature of gene action governing key traits such as GRD resistance in the germplasm used for breeding. However, there is limited information on the nature and type of gene interactions that exist in groundnuts particularly for GRD resistance. This information is necessary for planning appropriate breeding and selection strategies (Zhang *et al.*, 2005; Wambi *et al.*, 2014). According to Singh & Oswalt, (1991) variation due to dominance effects and their interactions cannot be exploited effectively in groundnut while additive × additive epistatic variation is potentially useful, as it can be fixed in homozygous cultivars. Adamu *et al.*, (2008) reported that additive effects were predominant over non-additive effects in governing GRD resistance and other yield related traits. Many methods including analysis of mating designs has been employed in study of gene effects but have no capacity to estimate individual interacting effects precisely. The presence or absence of epistasis can be detected by generation mean analysis which measures epistasis accurately whether complimentary or duplicate even with low populations at the digenic level (Keasey & Pooni 1996). Furthermore Wambi *et al* (2014) concluded that the type of gene effect depends on genetic background of the parents and variation in environmental conditions in which the populations under study is evaluated. In this study, a generation mean analysis was employed to obtain information on the nature of gene action and information obtained will be used by breeders to design sound breeding program for GRD resistance and hence developing groundnut genotype with GRD resistance.

4.2 Materials and methods

The six generations (P_1, P_2, F_1, F_2, $BC_1P_1$and BC_1P_2) of crosses between Valencia C × ICGV-SM 90704, Valencia C × ICGV-SM 96801, Valencia C × ICGV-SM 99566, NuMex-M_3 × ICGV-SM 90704, NuMex-M_3 × ICGV-SM 96801 and NuMex-M_3 × ICGV-SM 99566 were used for this study. The generations were developed as in chapter 3.

4.3 Data collection and analysis

GRD severity data scored at 115 days after planting using a modified scale of 1-9 was used in this study. The disease reaction on individual plants from the six generations (P_1, P_2, F_1, F_2, $BC_1P_1$and BC_1P_2) from six crosses (Valencia C × ICGV-SM 90704, Valencia C × ICGV-SM 96801, Valencia C × ICGV-SM 99566, NuMex-M_3 × ICGV-SM 90704, NuMex-M_3 × ICGV-SM 96801 and NuMex-M_3 × ICGV-SM 99566) were used to calculate the generation means and variances. The generation means of the six generations from the GRD severity scores were used to estimate the individual scaling tests; A, B and C components (Marther and Jinks, 1982) so as to test the adequancy of additive-dominance model. The A and B scaling tests provide the evidence for the presence of additive × additive [*i*], additive × dominance [*j*] and dominance × dominance [*l*] type gene interactions while the C scaling test provide a test for type dominance × dominance [*l*] epistasis.

The scales were tested for their significance by *t*- test at 5% level of significance as, t_A =A-0/SE_A, t_B =B-0/SE_B and t_C =C-0/SE_C where SE_A,SE_B and SE_C are standard errors of A,B and C scaling tests, respectively. The null hypothesis for test of significance (H_o) was that A = 0 or B and C in place of A of the scaling test. The additive-dominance model was considered adequate when the *t*- test of any one of the three scales was found not significant. The following

assumptions were made while performing the scaling test; (1) all generations have been raised in the same environment,(2) only autosomal inheritance is considered (3) non-allelic interaction is absent and (4) no differential fertility and viability.

The individual scaling tests; A, B and C components described by (Marther and Jinks, 1982;

$A = 2BC_1P_1 - P_1 - F_1$,

$B = 2BC_1P_2 - P_2 - F_1$,

$C = 4F_2 - 2F_1 - P_1 - P_2$.

The variances of the generation means were used to compute variances of the scaling tests as follows:

$V_A = 4V_{BC1P1} + V_{P1} + V_{F1}$

$V_B = 4V_{BC1P2} + V_{P2} + V_{F1}$

$V_C = 16V_{F2} + 4V_{F1} + V_{P1} + V_{P2}$

The standard errors of A, B and C scales were obtained as the square root of the variances of the scaling tests. .The scales were tested for their significance by t-test at 5% level of significance as;

$t_A = A—0/SE_A$,

$t_B = B-0/SE_B$ and

$t_C = C-0/SE_C$

Where SE_A, SE_B and SE_C are standard errors of A, B and C scale, respectively.

To estimate the gene effects, the following calculations were done basing on the method described by Kearsey &Pooni, (1996). Estimates gene effects were computed using generation means and the standard errors of these estimates. The parameters (m), average value between

parents, additive effect [*a*], dominace effects [*d*], additive × additive [*i*], additive × dominance [*j*] dominance × dominance effects [*l*] were obtained as shown below:

$\mathbf{m}=0.5P_1+0.5P_2+4F_2-2BC_1-2BC_2$

$SE^2_m= 0.25SE_{P1}+0.25SE_{P2}+16SE_{F2}+4SE_{BC1}+ 4SE_{BC2}$

$[a]=0.5P_1-0.5P_2$

$SE^2_{.d}=0.25SE_{P1}+0.25SE_{P2}$

$[d]=6BC_1+6BC_2-8F_2-F_1-1.5P_1-1.5P_2$

$SE^2_{(h)} =36SE_{BC1}+36SE_{BC2}+64SE_{F2}+SE_{F1}+2.25SE_{P1}+2.25SE_{P2}$

$[i]=2BC_1+2BC_2-4F_2$

$SE^2_{(i)}=4SEBC1+4SE2BC2+16SE2F2$

$[j]=2BC_1-P_1-2BC_2+P_2$

$SE^2_{(j)} =4SE^2_{BC1}+SE^2_{P1}+4SE^2_{BC2}+SE^2_{P1}$

$[l]=P_1+P_2+2F_1+4F_2-4BC_1-4BC_2$

$SE^2_{(l)} =SE^2_{P1}+SE^2_{P2}+4SE^2_{F1}+16SE^2_{F2}+16SE^2_{BC1}+16SE^2_{BC2}$

4.5 Results

The results of the scaling tests along with their standard error and t-test were presented in table 4. Atleast 1 of the 3 (A, B and C) scaling tests was found significantly different from zero by t-test (Table 4) and therefore, interacting terms were computed. Results on gene effects were presented in (Table 5).The results on gene effects showed significant contribution of additive gene effects [a] in controlling resistance to GRD in all the crosses except for NuMex-M_3 × ICGV-SM 96801 (Table 5). Dominance [d] gene effects were significant in controlling resistance to GRD in all

the six crosses except in Valencia C × ICGV-SM 99566 and Valencia C × ICGV-SM 90704 crosses (Table 5). The magnitude of dominance gene effects was generally larger than that of additive gene effect in all crosses except Valencia C × ICGV-SM 99566.

Epistatic type effects were all non significant for Valencia C × ICGV-SM 90704 cross, only dominance × dominance (l) gene effects were found siginficant in Valencia C × ICGV-SM 96801, NuMex-M_3 × ICGV-SM 96801, NuMex-M_3 × ICGV-SM 90704 and NuMex-M_3 × ICGV-SM 99566 crosses, and cross Valencia C × ICGV-SM 99566 had all the three types of epistatic effects such as additive × additive [i], additive × dominance [j] and dominance × dominance [l] significant. (Table 5). The magnitude of dominance × dominance interaction [l] effects was generally larger than other genetic interactions except for Valencia C × ICGV-SM 90704 cross.

Table 4. Scaling test estimates with their standard errors and *t*-test for six crosses

	Valencia C x ICGV-SM 99566		Valencia C x ICGV-SM 90704		Valencia C x ICGV-SM 96801		NuMex-M_3 x ICGV-SM 99566		NuMex-M_3 x ICGV-SM 90704		NuMex-M_3 x ICGV-SM 96801	
Scale	Value observed	t val	Value observed	t val	Value observed	t val	Value observed	t val	Value observed	t val	Value observed	t val
A	3.9 ± 1.6ns	1.57	**-3.7*±1.45**	-1.72	**7*±0.75**	15.59	**6.67*±0.59**	24.98	**-0.92*±0.95**	-5.12	**-6.16*±0.6**	-14.4
B	1.3 ± 0.7ns	0.39	-5.3±1.89ns	0.67	9.6±1.7ns	0.31	11.5±2.34ns	0.475	0.57*±0.6ns	0.1	3.7±0.57ns	0.707
C	**7.7* ± 1.8**	2.27	8±4.77ns	0.35	11.67±3.9ns	0.73	**-5.17*±1.18**	-3.73	3.93*±3.9ns	1.01	7.16±7.2ns	0.138

A=scaling test A, B=scaling test B and C=scaling test C, and t=calculated t-values and *=significant 5% respectively,

Table 5. Genetic parameters for GRD disease score for six crosses

G E	Valencia C × ICGV-SM 99566	Valencia C × ICGV-SM 96801	Valencia C × ICGV-SM 90704	NuMex-M_3 × ICGV-SM 96801	NuMex-M_3 × ICGV-SM 90704	NuMex-M_3 × ICGV-SM 99566
[m]	6.7±2.8	1.3±4	11.2±5.4	1.2±8.1	19.2±4.1	18.1±0.2
[*a*]	**3.2*±0.2**	**3.17*±0.36**	**3.0*±0.26**	1.8±0.85	**3.2*±0.2**	**3.4*±0.2**
[*d*]	2.5±0.6	**22.2*±0.6**	13.9±0.4	**22.2*±0.8**	**31.7*±0.6**	**61*±1.2**
[*i*]	**-2.3*±2.8**	5±3.96	-6.3±5.4	5.9±8.7	-14.7±4	23.3±2.5
[*j*]	**2.7*±2.7**	-2.7±1.8	-9±2.69	-25±3.7	-10.3±1.3	-4.8±2.4
[*l*]	**-2.9*±4.8**	**-21.6*±4.8**	-4.6±7.2	**-19.2*±10**	**-13.6*±4.7**	**-41*±4.8**

[m] = represents mean; [*a*] = additive; [*d*] = dominance; [*i*] = additive × additive; [*j*] = additive × dominance, and [*l*] = dominance × dominance effects.

4.7 Discussion and conclusion

Atleast 1 of the 3 (A, B and C) scaling tests was found significant (Table 4), implying that additive –dominance model was not adequate to explain the inheritance of GRD resistance in all the six crosses. Therefore, epistatic interaction and maternal effects could be involved in the inheritance of GRD resistance. The presence of significant additive gene effects [a] implied that genetic improvement of resistance to GRD could be possible through selection methods. The dominance gene effects [d] were significant and greater in magnitude than the additive effect [a] in all crosses except Valencia C × ICGV-SM 99566, indicating a predominant role of dominance gene action in controlling GRD resistance. The results are comparable with those of (Olurunju *et al.*, 1992; Akpan & Olurunju, 2009) which revealed that the inheritance of resistance to rosette disease was governed by dominant genes.

For Valencia C × ICGV-SM 90704 cross, additive gene action was the most important for GRD disease score; while other effects were less important which indicates that selection in early generations of segregating populations of this cross could be effective for GRD resistance. The results are comparable with those of Chintu, (2013) and Adamu *et al.*, (2008), which revealed additive gene action predominantly controlling GRD resistance. However, Wambi *et al.*, (2014) reported that effective selection in early generations of segregating materials can be accomplished only when additive genetic effects are substantial and heritability is high. Therefore, in the Valencia C × ICGV-SM 90704 cross, selection in early generations of segregating materials may be effective if heritability is high.

The study reaveled three types of epistatic gene effects viz., additive × additive [i], additive × dominance [j] and dominance × dominance [l] in control of GRD resistance which is contrary

to findings by many previous workers who reported predominace of additive (Chintu, 2013; Adamu *et al.*, 2008) and dominace (Olurunju *et al.*, 1992; Akpan & Olurunju, 2009) gene effects. Such variations in the results could be probably due to the genetic background of the parents and variation in environmental conditions in which the populations were evaluated. Therefore, knowledge of gene effects on a given breeding material in a particular environment is paramount for successful genetic improvement of such a trait.

For interaction components, the component dominance × dominance [l] was more than additive × additive [i] and additive × dominance [j] in Valencia C× ICGV-SM 96801, NuMex-M_3× ICGV-SM 96801, NuMex- M_3× ICGV-SM 90704, NuMex-M_3 × ICGV-SM 99566 and Valencia C × ICGV-SM 99566 crosses, adding more evidence on the importance of dominance in controlling rosette resistance. Opposite and significant signs of dominance effects [d] and dominance × dominance [l] components indicated the importance of duplicate epitasis in almost all the later crosses for GRD resistance Revealing a complex nature of inheritance which would hinder improvement of this trait through selection. In this situation breeding methods such as reciprocal recurrent selection is recommended for effective utilization of both additive and non additive gene effects simultaneously. According to Shoba *et al.*, (2010), duplicate epistasis observed hinders the rapid improvement of a trait by selection. This could be overcome by delaying the selection to later generation when dominance effects disappear and intermating of segregants in F_2 followed by recurrent selection. The selection in later generations and maintenance of large populations prior to selection may provide the maximum opportunity for advantageous combinations of genes to occur.

In Valencia C × ICGV-SM 99566 cross additive [a] (3.2*±0.2), additive × additive [i] (-2.3*±2.8) additive × dominance [j] (2.7*±2.7) and dominance × dominance [l] (-2.9*±4.8) were

significant. Presence of significant additive and additive × additive [i] revealed potentially useful variation which can be fixed easly. According to Singh & Oswalt, (1991) variation due to additive × additive epistatic variation is potentially useful, as it can be fixed in homozygous cultivars while dominance effects and their interactions cannot be exploited effectively in groundnut. The breeding method that exploits both additive and non additive gene effects such as recurrent selection (Singh & Oswalt, 1991; Nidagundi *et al.*, 2012; Janila *et al.*, 2013; Wambi *et al.*, 2014), reciprocal recurrent selection Janila *et al.*, (2013) and biparental mating Dabholkar, (1992) could be suitable for improving traits controlled by both additive and non-additive effects.

For traits controlled by additive and non additive gene effect, Wambi *et al*, 2014 also recommended that selection should be done in later generations.

Conclusion and recommendation

Considering overall results, it is apparent that the inheritance of GRD in all the crosses was found to be under the control of additive and non-additive gene effects coupled with duplicate type of epitasis. The duplicate epitasis revealed a complex nature of inheritance GRD resistance. Therefore, breeding strategies should be designed accordingly to get desired results. Selection should be done in later generation when dominance effects are decreased. Heterosis breeding and recurrent selection would be more useful for the improvement of groundnut for rosette resistance.

CHAPTER FIVE

5.0 Recovery of traits associated with exotic Valencia botanical in segregating groundnut generations

5.1 Introduction

The cultivated groundnut (*A. hypogaea*) is an annual herb with two subspecies; *Hypogaea* and *fastigiata* . The subspecies *hypogaea* has two botanical varieties; *hypogaea* (*Virginia* bunch *and* Virginia *runner* types) and *hirsuta.* The *fastigiata* subspecies has four botanical varieties, *fastigiata* (Valencia type), *vulgaris* (Spanish type), *peruviana,* and *aequatoriana* (Krapovickas and Gregory, 1994).

In Uganda, most of the released and grown groundnut varieties belong to Virginia and Spanish botanicals. However, these varieties are associated with relatively relatively poor agronomic traits such as late maturity, smaller seeds and in addition they have lower oil content (Okello *et al.*, 2010). The Valencia type varieties are largely preferred by the local market for their superior sweet taste (Patte *et al.*, 2001), high oil content (Kaaya & Warren, 2005), early-maturing and high yield based on number of seeds per pod (4-5 seeds/pod) (Okello *et al.*, 2010). The groundnut improvement programme (NaSARRI) introduced Valencia varieties (Valencia C and NuMex- M_3) from USA to broaden the Valencia genetic base in Uganda. The varieties were evaluated for abiotic and biotic stresses however, they succumbed to rosette disease (Kalule *et al.*, 2010). The introgression of rosette resistant genes into exotic Valencia varieties would provide a more viable option of controlling rossette disease. The available sources of rosette resistance were found in other botanicals (Virginia and Spanish) however, introgression of resistance genes is alwalys associated with the transfer of undesirable traits from the donor parents. Use of morphological descriptors that distinguish between parents and their populations

in relation to their phenotypic variability allow users (plant breeders) to interpret the differences and similarities in the population data. In this study, cluster analysis was conducted to determine the recovery of Valencia traits in early segregating and promising rosette resistant populations.

5.2 Materials and methods

The six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) of crosses between Valencia C × ICGV-SM 99566, Valencia C × ICGV-SM 99566, Valencia C × ICGV-SM 96801, NuMex-M_3 × ICGV-SM 99566, NuMex-M_3 × ICGV-SM 90704, and NuMex-M_3 × ICGV-SM 96801 were used for this study. The generations were developed as illustrated earlier in chapter 3.

5.3 Data collection

Data on ten health plants was collected on growth habit (GH), branching pattern (BP), number of primary branches (NPB), stem (SP) and peg pigmentation (PP), pod beak (PB), pod reticulation (PR) and pod constriction (PC) following, IBPGR & ICRISAT, (1992) groundnut descriptor guideline. Data on vegetative descriptor was recorded 60 days after planting as follows; number of primary branches, growth habit was recorded as either erect (1) or decumbent (2), stem pigmentation was recorded as either present (1) or abscent (2), stem hair occurence was recorded as either present (1) or abscent(2) and peg pigmentation was recorded as either present (1) or abscent (2).

Pod data was collected after drying as follows; pod reticulation was recorded as smooth (0), slight (3), moderate (5) or prominent (7) (Figure 2A), pod constriction was recorded as none (0), slight (3), moderate (5), deep (7) or very deep (9) (Figure 2B) and pod beak was recorded as abscent (0), slight (3), prominent (5), or very prominent (9) (Figure 2C).

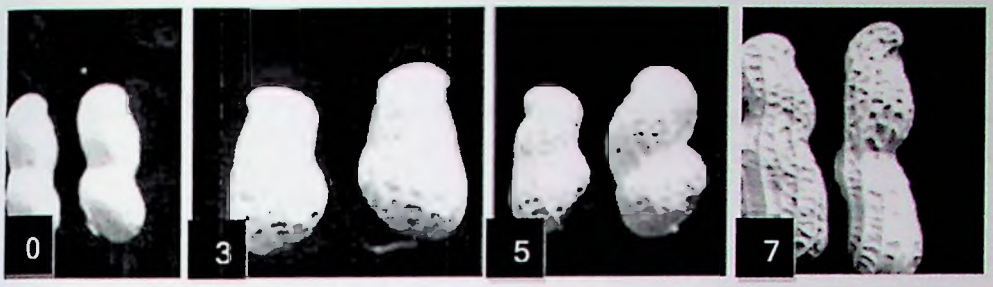


Figure 2A=pod reticulation

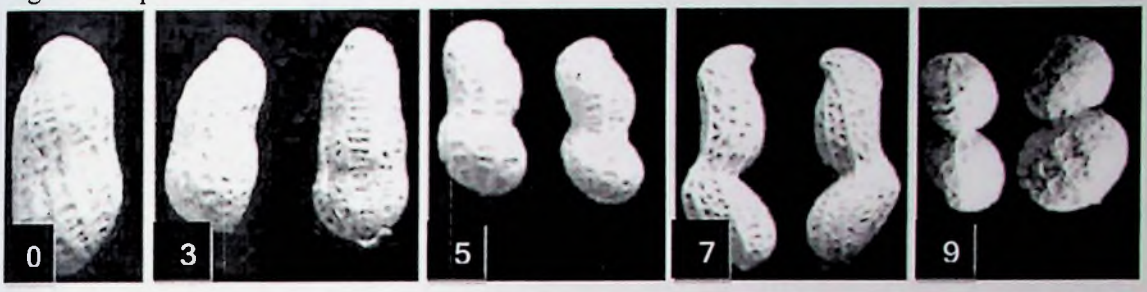


Figure 2B=pod constriction

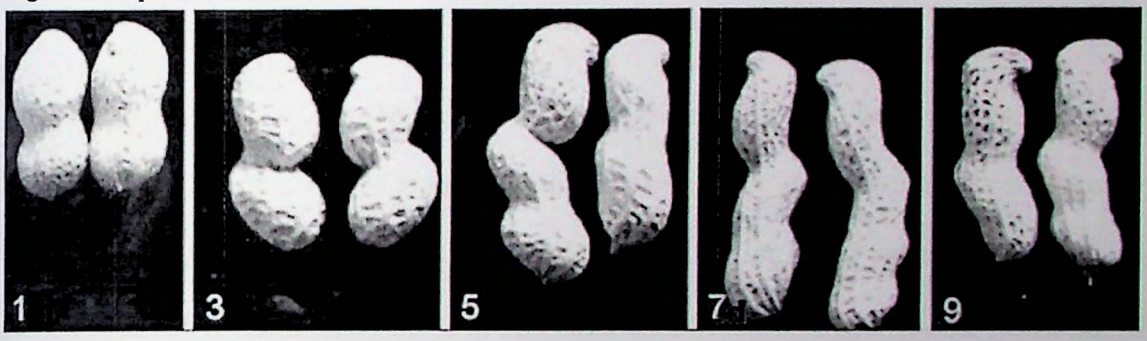


Figure 2C=pod beak

Figure 2 (A, B, &C): IBPGR & ICRISAT, 1992 groundnut descriptor scale for scoring pod reticulation constriction and beak characteristics respectively

5.4 Data analysis on morphological descriptors

Plants were counted and percentage frequencies were computed for morphological shoot and pod traits. Hierarchical cluster analysis was performed where estimates of similarity were based on euclidean coefficients. The similarity matrices were then analysed using the clustering method UPGMA (Unweighted Pair Group Method of the arithmetic average) Sneath & Sokal, (1973) to construct dendrogram using the GenStat computer package (13th edition Version 13.3).

5.5 Results

5.5.1 Percentage frequency distribution of morphological shoot and pod traits in the parental, F_2 and backcross populations for the crosses between Valencia lines and donor lines

The result on percentage frequency distribution in parental and population of six crosses for morphological shoot and pod characters are presented in Table 6 and 7. Considering growth habit; only two classes of growth habit were observed in parental lines and their populations which included; erect and decumbent. All the segregating populations from crosses which involved the Valencia (Valencia C and NuMex-M_3) with two donor parents (ICGV-SM 96801 and ICGV-SM 99566), the populations exhibited erect growth habit (100% frequency) except for the crosses where ICGV-SM 90704 donor line was used (50-65% frequency) (Table 6).

Considering stem pigmentation; many of the segregating populations segregated towards the Valencia traits for presence of stem pigmentation trait with 45-84% frequency range compared with those that lacked pigmentation 16-65% frequency (Table 6 and appendix 1 A, B, F,G).
Considering stem hairiness; all the segregating populations from crosses between Valencia lines (Valencia C and NuMex-M_3) and Spanish lines (ICGV-SM 96801 and ICGV-SM 99566) exhibited 100% frequency for stem hairness while for crosses where Valencia lines (Valencia C and NuMex-M_3) were crossed with Virginia (ICGV-SM 90704) had 20-67% frequency (Table 6). For number of primary branches; all populations between Valencia lines (Valencia C and NuMex-3) and Virginia (ICGV-SM 90704) had a range of 4-5 branches while for cross between Valencia lines (Valencia C and NuMex-3) and Spanish (ICGV-SM 96801 and ICGV-SM 99566) produced branches on a range of 6-11% frequency (Table 6 and Appendix 4 A, B, C, D).
For morphological pod characteristics, peg color in all populations where Valencia lines (Valencia C and NuMex-M_3) and Virginia (ICGV-SM 90704) were crossed exhibited pigmented

pegs (100% frequency) while for crosses between the Valencia lines (Valencia C and NuMex-M_3) varieties and Spanish donor lines (ICGV-SM 96801 and ICGV-SM 99566) segregated more towards Valencia (Valencia C and NuMex-M_3) (42-75% frequency) (Table 7 and Appendix 2, A, B,C,&D). In pod beak characteristics, among the segregating populations there was a wide variation as regards pod beak from absent-moderate; however, prominent and very prominent pod beak was not exhibited at all in all the crosses (Table 7and Appendix 3, C, D, &E). In pod constriction, majority of the segregating populations revealed slight and moderate pod constriction in the range of 25-92% in all the crosses, however, for crosses where Virginia (ICGV-SM 90704) were involved, very deep pod constriction was seen (40-75% frequency) (Table 7). In pod reticulation, most of the segregating populations showed slight-moderate reticulation in the range of 30-75% was seen in all the crosses between Valencia lines (Valencia C and NuMex-M_3) and Spanish lines (ICGV-SM 96801 and ICGV-SM 99566) while for crosses between Valencia C (Valencia C and NuMex-M_3) and Virginia (ICGV-SM 90704), exhibited a prominent pod reticulation a range of 35-82% frequency (Table 7).

Table 6: Percentage frequency distribution in parental and population of six crosses for morphological shoot characters

Genotype generation	Growth Habit		Stem color		peg color		No of branches	Stem Hair	
	erect	Dec	absent	present	absent	present		absent	present
Valencia C^{S}	100	0	0	100	0	100	4	0	100
NuMex-M$_3$ ^{S}	100	0	0	100	0	100	5	0	100
ICGV-SM 99566^{R}	100	0	100	0	100	0	7-10	0	100
ICGV-SM 90704^{R}	0	100	100	0	0	100	16	100	0
ICGV-SM 99801^{R}	100	0	100	0	100	0	7-10	0	100
F_2 (Valencia C × ICGV-SM 99566)	100	0	65	45	77	23	3	0	100
BC$_1$Valencia C (Valencia C × ICGV-SM 99566)	100	0	40	60	75	25	4	0	100
F_2(Valencia C × ICGV-SM 90704)	40	60	16	84	0	100	4	54	45
BC$_1$Valencia C(Valencia C × ICGV-SM 90704)	45	55	29.8	70.2	0	100	9	25	75
F_2(Valencia C × ICGV-SM 96801)	100	0	37.2	62.8	79	21	4	0	100
BC$_1$Valencia C(Valencia C × X ICGV-SM 96801)	100	0	42	58	80	20	11	31	69
F_2(NuMex-M$_3$ × ICGV-SM 99566)	100	0	47	53	81	19	5	0	100
BC$_1$NuMex-M$_3$ (NuMex-M$_3$ × ICGV-SM 99566)	100	0	22	78	82	18	5	0	100
F_2(NuMex-M$_3$ × ICGV-SM 90704)	35	65	54	46	0	100	4	67	23
BC$_1$NuMex-M$_3$ (NuMex-M$_3$ × ICGV-SM 90704)	50	50	38	62	0	100	5	20	80
F_2(NuMex-M$_3$ × ICGV-SM 96801)	100	0	45	55	75	25	4	0	100
BC$_1$NuMex-M$_3$ (NuMex-M$_3$ × ICGV-SM 96801)	100	0	41	49	77	23	10	0	100

Table 7: Percentage frequency distribution in parental and population of six crosses for morphological pod characters

Genotype generation	Pod reticulation				Pod constriction					Pod beak				
	Smooth (0)	Slight (3)	Mode (5)	Prom (7)	None (0)	Slight (3)	Mode (5)	Deep (7)	V deep((9)	Absc(0)	Slight (3)	Mode (5)	Prom (7)	V Prom (9)
Valencia C[S]	100	0	0	0	100	0	0	0	0	100	0	0	0	0
NuMex-M_3 [S]	100	0	0	0	100	0	0	0	0	100	0	0	0	0
ICGV-SM 99566[R]	100	0	0	0	0	100	0	0	0	0	100	0	0	0
ICGV-SM 90704[R]	0	0	0	100	0	0	0	0	100	0	0	0	0	100
ICGV-SM 99801[R]	100	0	0	0	0	100	0	0	0	0	0	0	0	100
F_2 (Valencia C × ICGV-SM 99566)	0	75	25	0	0	25	75	0	0	0	86	14	0	0
BC_1Valencia C (Valencia C × ICGV-SM 99566)	0	60	40	0	15	85	0	0	0	0	88	12	0	0
F_2(Valencia C × ICGV-SM 90704)			18	82	0	0	0	25	75	0	25	75	0	
BC_1Valencia C(Valencia C × ICGV-SM 90704)			65	35	0	0	0	60	40	90	10	0	0	
F_2(Valencia C × ICGV-SM 96801)	0	16	82	0	0	28	72	0	0	8	92	0	0	0
BC_1Valencia C(Valencia C × X ICGV-SM 96801)	0	80	20	0	80	20	0	0	0	25	75	0	0	0
F_2(NuMex-M_3 × ICGV-SM 99566)		90	10	0	0	92	8	0	0	80	20	0	0	0
BC_1NuMex-M_3 (NuMex-M_3 × ICGV-SM 99566)	0	95	5	0	0	22	78	0	0	90	10	0	0	0
F_2(NuMex-M_3 × ICGV-SM 90704)	0	0	60	40	0	0	0	30	70	0	50	50	0	0
BC_1 NuMex-M_3 (NuMex-M_3 × ICGV-SM 90704)	0	0	52	48	0	80	20	0	0	0	75	25	0	0
F_2(NuMex-M_3 × ICGV-SM 96801)	0	82	18	0	0	0	90	10	0	0	20	80	0	0
BC_1NuMex-M_3 (NuMex-M_3 × ICGV-SM 96801)	0	30	70	0	24	76	0	0	0	0	90	10	0	0

5.5.2 Results on cluster analysis

There were three clusters (A, B, and C) that were revealed by the similarity coefficients from crosses where NuMex-M_3 was involved as the elite Valencia group (Figure 3). Cluster A and B were 0.6 (60%) related morphologically to each other. In cluster B, where the elite parent NuMex-M_3 was found, BC_1 ICGV-SM 90704 (NuMex-M_3 × ICGV-SM 90704) was 0.91 (91%) related to NuMex-M_3 therefore showing the highest recovery while F_2's from cross NuMex-M_3 × ICGV-SM 99566, NuMex-M_3 × ICGV-SM 96801 and backcrosses to NuMex-M_3 from the cross NuMex-M_3 × ICGV-SM 90704; NuMex-M_3 × ICGV-SM 96801 were 0.88 (88%) related to NuMex-M_3 (Figure 3).

There were also four clusters (A, B, C and D) revealed by similarity coefficients from the crosses where Valencia C was involved as the elite parent (Figure 4). Cluster A, B and C were 0.62 (62%) related to cluster D, while Cluster B and C were 0.7 (70%) related to each other each other. Considering cluster C where elite Valencia C parent was found, Valencia C was 0.85 (85%) related to BC_1 Valencia from cross Valencia C × ICGV-SM 90704 while in cluster B where all backcrosses to the donor parents and one F_2 from cross Valencia C × ICGV-SM 99566 were 0.7 (70%) related to Valencia C parent (Figure 4).

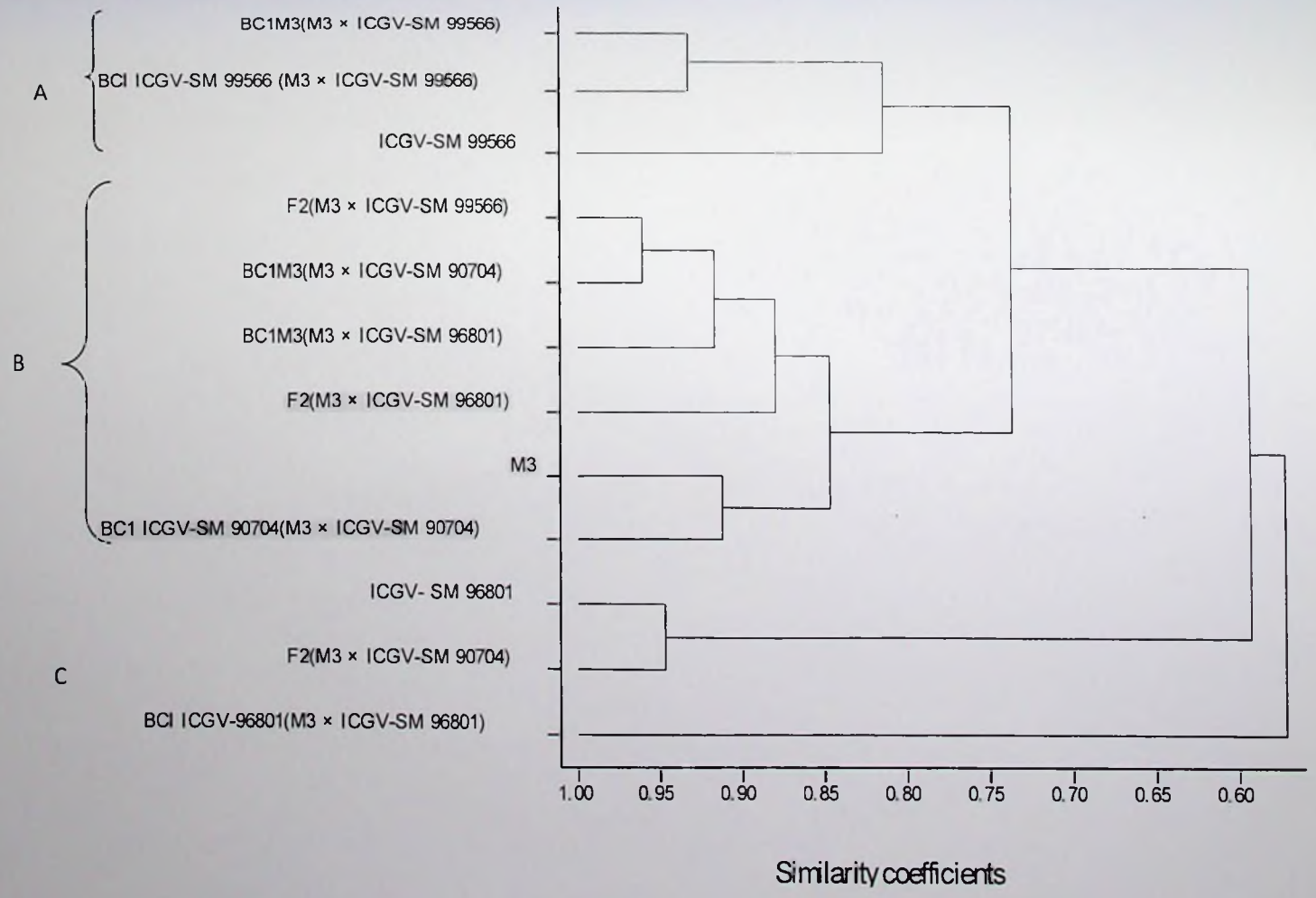


Figure 3: Dendrogram obtained by hierarchical cluster analysis for qualitative characters in cross where NuMex-M_3 (M_3) parent was used

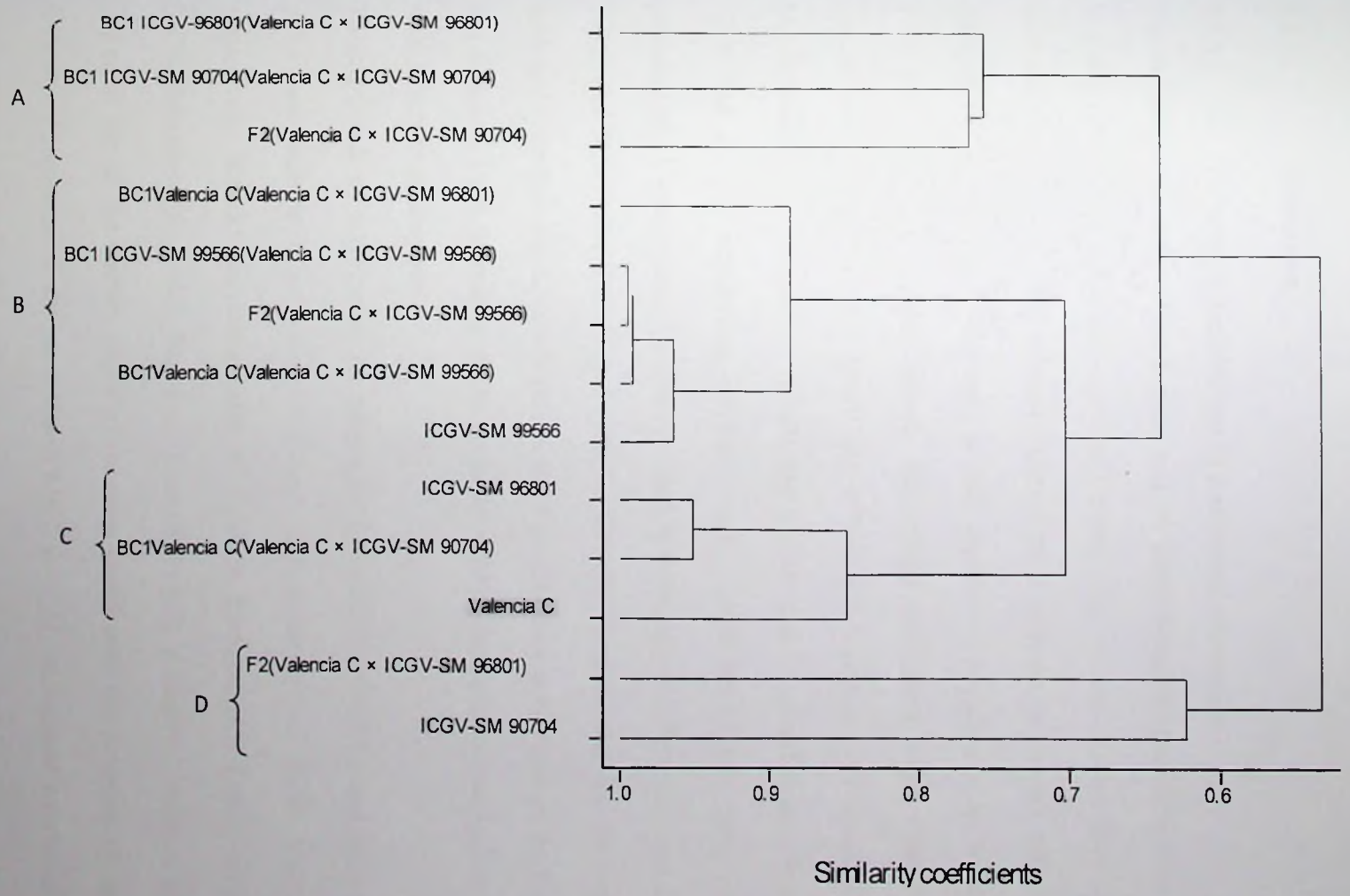


Figure 4. Dendrogram obtained by hierarchical cluster analysis for qualitative characters in cross where Valencia C parent was used

5.5.3 Discussion

5.5.3.1 Pattern of distribution of qualitative characters

The distribution patterns of the populations for morphological characters were presented in Table 6 and 7. Crosses that involved parents with common traits, their segregating populations recovered 100% of their parental traits whereas in parents with contrasting traits reflected a diversity of the traits in their segregating populations for traits analyzed. Two types of growth habit namely decumbent and erect were observed in the populations. F_2 (Valencia C × ICGV-SM 90704), BC_1Valencia C (Valencia C × ICGV-SM 90704) F_2 (NuMex-M_3 × ICGV-SM 90704) and BC_1NuMex-M_3 (NuMex-M_3 × ICGV-SM 90704) populations exhibited both growth habits but erect pattern was predominant. Valencia lines (Valencia C and NuMex-M_3) and Spanish lines (ICGV-SM 96801 and ICGV-SM 99566) and their populations were similar for the erect growth habit and stem hair characters. Valencia and Spanish lines were reported by Krapovickas and Gregory, 1994 to belong in the same subspecies *fastigata* probably they share the some common descriptors.

Varying levels of segregation for presence of peg color (18-25%) and stem color (45-84%) was exhibited indicating low and high recovery of these Valencia traits respectively. Results are comparable to those of Pattanashetti, (2005) who reported 9.52% frequency peg color. However, Kumari, (2008) in contrast reported presence of peg color for all the entries. Color of the stem varies with the presence or absence and intensity of pigments in the stem tissue which is highly influenced by the environment particularly exposure to the sunlight.

Considering number of branches, the F_2 segregated towards the Valencia lines with 3-4 branches whereas wide variation was seen in the backcrosses indicating high recovery of this trait in the segregating population.

Pod and kernel traits are important as they largely determine consumer preference and marketability of the produce. In groundnuts, the tip of the indehiscent fruit may end in an appearance called beak. In the present investigation beaklessness –moderate beak were observed with no prominent and very prominent pod beak exhibited in all populations of the crosses. Results were comparable to those of Pattanashetti, (2005).

Pod constriction is an interesting characteristic as it affects the developing seed. The study revealed slight and moderate pod constriction in the range of 25-92% in all the crosses, except for crosses where Virginia line (ICGV-SM 90704) was involved which showed very deep pod constriction with 40-75% frequency range. Kumari, (2008) also reported deep and moderate pod constrictions in mutant genotypes, followed by none, slight and very deep pod constrictions which is comparable to the results of current study.

Reticulation on the pods is a prominent characteristic; it also contributes to the cleanness of pods at the time of harvest and had an impact on quality and marketability of the produce. The study revealed slight-moderate reticulation in the range of 30-75% frequency in all crosses between Valencia lines (Valencia C and NuMex-M_3) and Spanish line (ICGV-SM 96801 and ICGV-SM 99566) and prominent pod reticulation in crosses between Valencia lines (Valencia C and NuMex-M_3) and Virginia (ICGV-SM 90704) with a range of 35-82% frequency. The findings are comparable to those of Kumari,

(2008) which revealed, predominant moderate reticulation, followed by prominent, slight and very prominent in TMV 2, VL 1, DER, and their mutants.

The Cluster analysis of the six crosses showed differences among the generations for the traits analyzed with major and sub-clusters (Figure 2 and Figure 3). The Valencia lines and donor lines were found in different clusters indicating that the lines were distinct for most of the morphological traits. This revealed variability among the generations for the traits analyzed. Results are comparable with those of (Upadhyaya *et al.*, 2003; Ajay, 2006; Kumari, 2008; Kroonenberg *et al.*, 2012) which indicated significant phenotypic diversity in groundnuts using morphological descriptors.

According to Khedikar, (2008) more similarity means less genetic diversity between the parents. The current study, cluster analysis revealed less diversity between Valencia C, and the donor ICGV-SM 96801 parental lines. These lines have been reported to belong in the same subspecies *fastigiata* (Krapovickas & Gregory, 1994) and probably they shared many common descriptors. In addition, the higher similarity could be attributed to greater influence of the environment on the morphological traits that were analysed or the small population size and less number of descriptors that were used in the current study.

Morphologically, intermediate populations in each cross with different similarity coefficient were observed indicating all kinds of segregation and recombination events that had taken place to generate such variability, which may provide opportunity for selection of desirable segregants from these populations. Pattee *et al.*, (2001) reported

that segregation within crosses provides additional opportunity for progress from selection.

The current study indicated a range of 0.6 – 0.98 (60-98%) similarity among the population and Valencia lines, indicating a high recovery of Valencia traits in the populations. Such populations can be considered for further improvements. Therefore, maximum gain in GRD resistance and Valencia trait recovery can be obtained if only those populations that exhibit high resistance and Valencia trait recovery are enhanced in the breeding program. These findings are comparable to Dwivedi *et al.*, (2001) who reported similarity of 59-98.8% among 26 accessions.

The donor parental traits including resistance to GRD was recovered mostly in the populations of cross where ICGV-SM 99566 compared to recurrent parent (Valencia) traits especially in all the backcrosses to either parents and F_2 (Valencia C x ICGV-SM 99566). This implies that if the populations of this cross are to be used in the breeding program little gain in Valencia traits can be obtained unless more backcrosses are made to recover the Valencia traits in the advanced generations.

Based on hierarchical clustering of all the six crosses, it is concluded that all the donor lines and Valencia lines were quite distinct and placed in different clusters showing high morphological diversity; with exception of Valencia C and ICGV-SM 96801 which was found in the same cluster.

CHAPTER SIX

6.0 General discussions, conclusions and recommendation

6.1 General discussions

The ANOVA for the 6 crosses showed highly significant differences ($P<0.01$) among generations for GRD resistance, suggesting presence of variability for GRD disease score in the generations. The significant variation implies that genetic improvement of groundnut for GRD resistance is possible hence such variability can be exploited by breeders for groundnut improvement. The presence of such variability would be as a result of wide genetic distance between the parental backgrounds that were used in the study. The results of the current study are comparable to those of Naidu *et al.*, 1999, who reported that GRD variations are said to be due to diversity among the causal agents (sat RNA variants), diferences in genotype response, variable climatic conditions and mixed infections with other viruses.

The mean of F_1 generations tended towards the mean of resistant parents indicating that resistance to GRD was controlled by dominant genes or epistatic gene action. These results support the earlier reports that resistance to GRD is controlled by dominant genes (Olurunju *et al.*, 1992: Akpan & Olurunju, 2009). In contrast, Harkness, (1977) and Nigam & Bock, (1990) reported recessive genes controlling resistance to GRD. The presence of dominant genes implies that the F_1's would be utilized due to relative heterosis where hybrid vigour could be exploted. However in groundnuts; commercial production of F_1 seed can't be achieved since it is a self fertilizing crop and its tetraploid nature makes the F_1's unstable. Wambi, (2014), suggested that in situations, selection

should take place at later stages such that the dominant genes are decreased. To exploit such heterosis, breeding methods such as recurrent seletion and single seed descent may be used due to the presence of dominance effects.

Success of genetic improvement is attributed to the magnitude and nature of variability present for a specific character. Moderate to high level of GCV (17.1-57.8%) and high PCV (20.4-70.1%) was noticed for all crosses indicating higher magnitude of variation in these crosses which could be attributed to genotype with very little effect of the environment. According to Oyiga & Iguru, (2011) when the magnitude of genetic variance is higher than the environmental variance, it indicates that the genetic component is the major contributor to the total variance of the trait under study. Vishnuvardhan *et al.*, (2012), high GCV may indicate a predominant role of additive gene actions and amenability for phenotypic selection in early generations. The high values of PCV and GCV in almost all the crosses indicated a wide scope of selection for GRD resistance due to wide spectrum of genotypic variation of the trait.

The information on genotypic coefficient of variation revealed the existence genetic variability present in the genotypes for GRD resistance; however, it does not provide full scope to assess the variation that is heritable. In the current study, all the crosses had high broad sense heritability values (64.1-73.7%). The findings are comparable with those of (Van der Merwe, 1998; Adu Dapaah, *et al.*, 2007; Kayondo *et al.*, 2014) who reported high broad sense heritability estimates for GRD resistance (67- 93%). The high heritability could indicate a high response to selection due to reduced environment influence and predominance role of additive gene effects (Chattopadhyay & Dutta, 2010;

Tafere *et al.*, 2013), in the control of GRD resistance which could result to efficient response to selection in early generations.

Moderate values (31-41.9%) for narrow sense heritability were observed in all crosses which indicated greater dominance effects on GRD resistance than the additive. In all crosses, the dominance variance component (V_D) exhibited relatively higher magnitudes as compared to additive component (V_A) except for Valencia C x ICGM-SM 99566, Valencia C x ICGM-SM 90704 and NuMex-M_3 x ICGV-SM 90704. It is generally verified that an increase in magnitude of V_D implies a decrease in h^2n in the reference to F_2 generation. Hence selection of genotypes from initial generations for GRD disease score in these crosses may be difficult due to the higher influence of dominance effects. According to Kormsa-art *et al.*, (2002), selection for such traits controlled by dominance becomes ineffective when carried out in early generations; therefore, selection based on this trait is more effective when undertaken in subsequent generations of all crosses. In this way, the occurrence of heterozygotes would be reduced and the available additive variance for selection is increased, thereby providing higher possibilities of selection gains for the trait.

The additive –dominance model was not adequate to explain the inheritance of GRD resistance in all the six crosses. Therefore, epistatic interaction and maternal effects could be involved in the inheritance of GRD resistance. The presence of significant additive gene effects [a] implied that genetic improvement of resistance to GRD could be possible through selection methods. The dominance gene effects [d] were significant and greater in magnitude than the additive effect [a] in all crosses except Valencia C × ICGV-SM 99566, indicating a predominant role of dominance gene action in controlling GRD

resistance. The results are comparable with those of (Olurunju *et al.*, 1992; Akpan & Olurunju, 2009) which revealed that the inheritance of resistance to rosette disease was governed by dominant genes.

For Valencia C × ICGV-SM 90704 cross, additive gene action was the most important for GRD disease score; while other effects were less important which indicates that selection in early generations of segregating populations of this cross could be effective for GRD resistance. The results are comparable with those of Chintu, (2013) and Adamu *et al.*, (2008), which revealed additive gene action predominantly controlling GRD resistance. However, Wambi *et al.*, (2014) reported that effective selection in early generations of segregating materials can be accomplished only when additive genetic effects are substantial and heritability is high. Therefore, in the Valencia C × ICGV-SM 90704 cross, selection in early generations of segregating materials may be effective if heritability is high.

The study reaveled three types of epistatic gene effects viz., additive × additive [i], additive × dominance [j] and dominance × dominance [l] in control of GRD resistance which is contrary to findings by many previous workers who reported predominace of additive (Chintu, 2013; Adamu *et al.*, 2008) and dominace (Olurunju *et al.*, 1992; Akpan & Olurunju, 2009) gene effects. Such variations in the results could be probably due to the genetic background of the parents and variation in environmental conditions in which the populations were evaluated. Therefore, knowledge of gene effects on a given breeding material in a particular environment is paramount for successful genetic improvement of such a trait.

Opposite and significant signs of dominance effects [d] and dominance × dominance [l] components indicated the importance of duplicate epitasis in almost all the later crosses for GRD resistance revealing a complex nature of inheritance which would hinder improvement of this trait through selection. In this situation breeding methods such as reciprocal recurrent selection is recommended for effective utilization of both additive and non additive gene effects simultaneously. According to Shoba *et al.*, (2010), duplicate epistasis observed hinders the rapid improvement of a trait by selection. This could be overcome by delaying the selection to later generation when dominance effects disappear and intermating of segregants in F_2 followed by recurrent selection. The selection in later generations and maintenance of large populations prior to selection may provide the maximum opportunity for advantageous combinations of genes to occur.

The distribution patterns of the populations for morphological characters were presented in Table 6 and 7. Crosses that involved parents with common traits, their segregating populations recovered 100% of their parental traits whereas in parents with contrasting traits reflected a diversity of the traits in their segregating populations for traits analyzed. The Cluster analysis of the six crosses showed differences among the generations for the traits analyzed with major and sub-clusters (Figure 2 and Figure 3). The Valencia lines and donor lines were found in different clusters indicating that the lines were distinct for most of the morphological traits. This revealed variability among the generations for the traits analyzed. Results are comparable with those of (Upadhyaya *et al.*, 2003; Ajay, 2006; Kumari, 2008; Kroonenberg *et al.*, 2012) which indicated significant phenotypic diversity in groundnuts using morphological descriptors.

Morphologically, intermediate populations in each cross with different similarity coefficient were observed indicating all kinds of segregation and recombination events that had taken place to generate such variability, which may provide opportunity for selection of desirable segregants from these populations. Pattee *et al.*, (2001) reported that segregation within crosses provides additional opportunity for progress from selection.

The current study indicated a range of 0.6 – 0.98 (60-98%) similarity among the population and Valencia lines, indicating a high recovery of Valencia traits in the populations. Such populations can be considered for further improvements. Therefore, maximum gain in GRD resistance and Valencia trait recovery can be obtained if only those populations that exhibit high resistance and Valencia trait recovery are enhanced in the breeding program. These findings are comparable to Dwivedi *et al.*, (2001) who reported similarity of 59-98.8% among 26 accessions.

The donor parental traits including resistance to GRD was recovered mostly in the populations of cross where ICGV-SM 99566 compared to recurrent parent (Valencia) traits especially in all the backcrosses to either parents and F_2 (Valencia C x ICGV-SM 99566). This implies that if the populations of this cross are to be used in the breeding program little gain in Valencia traits can be obtained unless more backcrosses are made to recover the Valencia traits in the advanced generations.

6.2 Conclusions

The study revealed presence of considerable variability for GRD resistance which could be attributed to genotype with very little effect of the environment indicating a greater success of these exotic materials to genetic improvement. High broad sense heritability (64.1-73.7%) and moderate narrow sense heritability (31-41.9%) was observed in all crosses and the inheritance of GRD resistance was found to be under the control of additive and non-additive gene effects coupled with duplicate type of epitasis. The current study indicated a range of 0.6 – 0.98 (60-98%) similarity among the population and Valencia lines, indicating a high recovery of Valencia traits in the populations with backcrosses showing a higher recovery of these traits more than the F_2,s. Such populations can be considered for further improvements. Therefore, maximum gain in GRD resistance and Valencia trait recovery can be obtained if only those populations that exhibit high resistance and Valencia trait recovery are enhanced in the breeding program. Therefore, backcrossing can be used to improve other adapted germplasm that are not resistant to GRD and breeders have choices to work in terms of variation observed in the gene action.

6.3 Recommendations

Genetic improvement of Valencia groundnuts for resistance to GRD is possible in all the crosses; therefore, selection based on individual plants for GRD resistance is more effective when undertaken in later generations in all crosses as selection of genotypes from initial generations for GRD resistance may be difficult due to the higher influence of dominance effects. Recurrent selection breeding methods, followed by selection on family mean can ensure high genetic gain among the populations. Breeding methods such

as recurrent selection and single seed descent methods may be used for improvement of the materials. For future breeding, use of marker assisted backcrossing and marker assisted selection would ensure early selection. Further studies should address the number of major genes or QTLs, their locations and determine how much of the variation do each account for.

REFERENCES

Adu Dapaah, H.K., Asibuo, J. Y., Danquah, O. A., Asumadu, H., Halcegoah, J, & Asafo, A. B. (2004). Farmer participation in groundnut rosette resistant varietal selection in Ghana. In: Fisher *et al.*, (eds). New direction for a diverse planet: Proceedings for the 4th International crop Science. Congress, Brisbane, Australia.

Ajay, P.M. (2006). *Evaluation of groundnut varieties for confectionery traits and selection of donors for their improvement*. Master of Science Thesis. University of Agricultural Sciences, Dharwad 580 pages.

Akpan, G.I., & Olorunju, P.E. (2009). Yield stability and resistance to leaf spot diseases and rosette in groundnut *Czech journal of genetics and plant breeding*, 45 (1): 18–25.

Bendahmane, A., Kanyuka, K., & Baulcombe, D, C. (1999). The Rx gene from potato controls separate virus resistance and cell death responses. *The plant cell*, 11: 781-791. In the 7th International IPM symposium. Genetics, genomics and R genes virus disease management. Are ecologically based IPM strategies relevant for sustainable management of virus disease in the 21st Century? Mephis, Tennessee, March 27-29, 2012.

Bock, K.R. (1987). Rosette and early leaf spot diseases. A review of research progress 1984/1985 pages 5-14 in proceedings of regional workshop for southern Africa.

Chancellor, T.C.B. (2002). Groundnut rosette management: livelihood, capacity building and promotional issues. CPP Semi-arid cluster meeting, Natural Resources Institute, 7.

Cooley, M. B., Pathirana, S., Wu, H., Kachrop, J., & Klessig, D. F. (2000). In the 7th International IPM symposium. Genetics, genomics and R genes virus disease management. Are ecologically based IPM strategies relevant for sustainable management of virus disease in the 21st Century? Mephis, Tennessee, March 27-29, 2012.

Dennis, T., & Kattingu, C. (2012). Groundnut rosette disease, Fact sheets for farmers. Sierra Leone CAB.

Deom, C.M., Naidu, R.A, Chiyembekez ,A.J., Ntare, B.R & Subrahmanyam, P. (2000). Sequence diversity within the three agents of groundnut rosette disease. *Phytopathology* 90(3), 214-219.

FAO STAT. (2011). Food and agriculture Organisation of the united Nations, FAO Stastical Databasa and Data set, http://faostat.org/site/291 accesses by June, 2013.

Gorbet, D. W., Kucharek, T. A., Shokes, F. M., & Brenneman, T. B. (2004). Field Evaluations of Peanut Germplasm for Resistance to Stem Rot Caused by *Sclerotium*

rolfsii. Peanut Science, 31: (2) 91-95.

Harkness, C. (1977). The breeding and selection of groundnut varieties for resistance to rosette virus disease in Nigeria. Pages 1-45 *in* Submission to the African Groundnut Council, June 1977. Institute for Agricultural Research, Ahmadu Bello University, P.M.B. 1044, Zaria, Nigeria. IAR.

Herselman, L. (2003). Genetic variation among Southern African cultivated peanut (Arachis Hypogaea L), genotypes as revealed by AFLP analysis. *Euphytica* 133: 319-327.

Higgins, B.B., & Bailey, W. K. (1959). Peanut seed and seed treatment. Mimeo. Ca. Agricultural Experiment Station No. 70.

IBPGR & ICRISAT. (1992). Descriptors for groundnut. International Board for Plant Genetics resources. Rome, Italy; International crop research Institute for the semi arid tropics, Patancheru, India.

Kaaya, N. A., & Warren, H. L. (2005). A review of past and present research on aflatoxin in Uganda. *African journal of food agriculture and nutritional development* (AJFAND), 5:(1). Available on line. www.ajfand.net

Keasey, J. M., & Pooni,S.H. (1996). The genetic analysis of quantitive Traits.1st ED. Chapman and hall.

Khedikar, P.Y. (2008). *Molecular Tagging and Mapping of Resistance to Late Leaf Spot and Rust in Groundnut* (*Arachis hypogaea* L.). phD Thesis University of Agricultural Sciences, Dharwad 502 pages.

Kimmins, F. (1999). Groundnut rosette disease epidemiology. DFID Crop Protection Programme, Final Technical Report, Project R6811. Natural resources institute, University of Greenwich, Chatham, Kent, UK. 12-14.

Kochert, G., Stalker, H.T., Gimenes, M., Galgaro, L., Romero, L. C. & Moore, K. (1996). RFLP and cytogenetic evidence on the origin nut landraces and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (*Leguminosae*). *Annals Journal of Botany,* 83(12): 82–1291.

Krapovickas, A., & Gregory, W. C. (1994), "Taxonomfa del G6nero Arachis (Leguminosae)," Bonplandia, 8, 1-186.

Lane, P.W., & Payne, R.W. (1996) GenStat for Windows TM Introductory Course 2nd Lawes, Agricultural Trust Rothamsted Experimental Station.

Mark, A., Marsalis, Naveen,P., Natalie, P., Goldberg, J.A., Sanoga, S.& Trostle, C. (2009). New Mexico Peanut Production circular-645.

Mather, K., & Jinks, J. L. (1982), Biometrical genetics, cornell Iniversity Press. Thaca. N.Y.

Monyo, E.S., Osiru, M.O., Waliyar, F., & Charlie, H. J. (2007). Screening groundnut varieties for resistance to the groundnut rosette disease. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), P.O. Box 1096, Lilongwe, Malawi. ICRISAT, Patancheru 502 - 324, Andhra Pradesh, India.

Moss, J. P., and Ramanatha, R. V. (1995). The peanut—reproductive development to plant maturity. p. 1–13. In: H. E. Pattee and H. T. Stalker (eds.), Advances in peanut science. Am. Peanut Res. Educ. Soc., Stillwater, OK.

Mukankusi, C., Adipala, E., Kyamanywa, S., Epieru, G., Wareen, H., Willson, H. R. & Odeke, V. (1999). Efficacy and economic benefit of different chemical spray regime on the management of the major pests and diseases of groundnut in Eastern Uganda. *African journal of plant protection*, 9: 65-81.

Murant, A. F., Kumar, I. K., & Robinson, D.J. (1991). Current Research on groundnut Rosette at SCRI. Page 7-8 in Groundnut Virus Diseases in Africa: Summary proceedings of the Consultative Group Meeting, 18:20, September 1990, Montpillier France, Patencheru 502:302, Andhra Pradesh India: ICRISAT.

Naidu, R. A., & Kimmins, F. M. (2007). The effect of groundnut rosette assistor virus on the agronomic performance of four groundnut (*Arachis Hypogaea* L.) genotypes. *Journal of phytopathology,* 155 (6): 350-356.

Naidu, R.A., Kimmins, F.M., Deom, C.M., Subrahmanyam, P., Chiyembekeza, A.J. &Van der Merwe. P.J.A. (1999). Groundnut Rosette. A Virus Disease Affecting the Sustainability of Groundnut Production in Sub-Saharan Africa. *Plant Disease* 83. 700-709.

Naidu, R.A., Bottenberg, H., Subrahmanyam, P., Kimmins, F. M, Robinson, D. J., & Thresh, J.M. (1998). Epidemiology of groundnut rosette virus disease: Current status and future research needs. *Annals of applied biology* 132:525-548.

Ndiame, D., John, B., & Mirvat, S. (2004). Groundnut policies, global trade dynamics and the impact of trade liberalization of World bank policy research. Working Paper, 3226.

Nidagundi, J.M., Patil, S.S., Salimath, P.M., Kajjidoni, S. J., Patil, B. C., & Hegde, M.G. (2012). Genetic analysis of seed cotton yield and its component traits in *Gosspium hirsutum* L. *Kanataka journal of agricultural science;* 25: 260-261.

Nigam, S. N & Bock, K.R. (1990). Inheritance of resistance to groundnut rosette virus in groundnut (*Arachis hypogaea* L). *Annals of applied Biology.* 117:533-560.

Nigam, S. N., Prasada, R., Bhatnagar, M. P., & Sharma, K. K. (2012). Genetic Management of Virus Diseases in Peanut. In: *Plant breeding reviews.* Wiley & Sons, Inc, 293-356.

Ntare, B.R., & Waliyar, F. (1999). Genotype and Environmental effects of resistance to

late leafspot in groundnut. *African crop science journal*; 7(**2**) 109 -115.

Ntare, S.N., & Olurunju, P.E. (2001). Variation in yield and resistance to groundnut rosette disease in early and medium maturing groundnut genotype in Nigeria. *African crop science journal,* 9:451-461.

Nutman, F.J., Roberts, F.M & Williamson, J.G. (1964). Studies on varietal resistance in the groundnut (Arachis Hypogaea L) to rosete disease. *Rhodesia journal of agricultural research*, 2:63-77.

Okello, D. K., Biruma, M., & Deom, C. M. (2010). Overview of groundnuts research in Uganda. Past, present and future. *African journal of biotechnology*, 9(**39**): 6448-6459.

Okonkwo, C. A., & Clayberg, C. D. (1984). The wrapping method, a new technique for effective crossing of common bean. Bean improvement cooporation *Annals of repectives*, 27:156.

Olorunju, P.E., Kuhn, C.W., Demski, J.W., Misari, S.M., & Ansa, O.A. (1990). Mechanical inoculation to study resistance to groundnut rosette virus in groundnut (Peanut). (Abstract) 1990 proc. *Annals of peanut respectives education Society*, 22:48.

Olorunju, P. E., Kuhn, C.W., Demski, J. W., Misari, S. M., & Ansa, O.A. (1991). Disease reaction and yield performance of peanut genotypes grown under groundnut rosette and rosette-free field environments. *Plant Disease,* **75**: 1269–1273.

Olorunju, P.E., Kuhn, C.W., Demski, J.W., Misari, S.M., & Ansa, O.A. (1992). Inheritance of resistance in peanut to mixed infection of groundnut rosette virus (GRV) and groundnut rosette assistor virus and single infection of GRV. *Plant disease* **76**. 95–100 on groundnut rosette virus disease. Summary proceedings of the Consultative Group.

Olorunju, P.E., Ntare, B.R., Boye-Goni, S.R., Alabi, O., & Tanimu B., (2001). Recommendation for registration and release of two extra early rosette resistant and two early maturing rosette resistant groundnut varieties ICGV-IS-96894, ICGV-1S 96891, ICGV-IS-96808 and ICGV-IS- 96855. Paper presented at the Professional and Academic Board of the Institute for Agricultural Research, Zaria, Nigeria.

Padgham, D.E., Kimmins, F.M., & Ranga, R.G.V. (1990). Resistance in groundnut (*Arachis hypogaea* L.) to *Aphis craccivora* Koch. Annals of applied biology, 117: 285-294.

Pandey, M. K., Monyo, E., Ozias, A., P. J., Liag, X., Gumarales, P., Nigam, S.N. (2012). Advances in *Arachis* genomics for peanut Improvement. *Biotecnol*ogy advancement 30: 639-651.

Janila, J., Nigam, S.N., Manish, K., Pandey, P.N., & Rajeev, K. V. (2013). Groundnut improvement: Use of genetic and genomic tools. *Peanut sci*ence; 10.3389.

Patte, H.E., Isleib, T. G., Gorbert, D.W., Giesbrecht, F. G & Cui, Z. (2001). Parental selection in breeding for roasted peanut flavor quality. *Peanut sci*ence; 28:51-58.

Sebunya, T. K. & Yourtee, D. M. (1990). Aflatoxigenic Aspergilli in foods and feeds in Uganda. *Food Quality*, **13**: 97 – 107.

Sharma, K.K., & Anjaiah, V. (2000). An efficient method for the production of transgenic plants of peanut (*Arachis hypogaea* L.) through *Agrobacterium tumefaciens* mediated genetic transformation. *Plant science.* 159:7-19.

Shoba, D., Manivarannan, N., & Vindhiyavarman, P. (2010). Gene effects of pod yield and its components in three crosses of groundnut (*Arachis hypogaea* L). Dept of Oil seeds, Tamil Nadu Agricultural University, Coimbatore 641003.

Singh. F., & Diwakar. B. (1993). Nutritive value and uses of pigeonpea and groundnut. Human resource development program. ICRISAT. Patancheru, Andhra Pradesh 502 324, India.

Singh.F. & Oswalt.D.L. (1991). Genetics and Breeding of Groundnut. ICRISAT. Human Resource Development Program. Patancheru, Andhra Pradesh 502 324, India 11.

Sneath, P. H. A., & Sokal, R. R. (1973). Numerical Taxonomy, WH Freeman, San Francisco.

Subrahmanyam, P., Naidu, R. A., Reddy, L. J, Kumar, P. L., & Ferguson, M. (2001). Resistance to groundnut rosette disease in wild *Arachis* species. *Annals of applied biology,* 139: 45-50.

Subrahmanyam, P., Vander merwe, P.J.A., Chiyembekeza, A.J., & Chandra, S. (2002). Integrated management of groundnut rosette disease. *African crop science journal,* 10(**1**): 99-110.

Tolin, S. (2012). Genetics, genomics and R genes for virus disease management. 7th International IPM Symposium. Virginia Tech. Are ecologically based IPM strategies relevant for sustainable management of virus disease in the 21st Century? March 27-29, 2012, Memphis, Tenessee, USA.

Maina, S. M., Quinata, E., Kiran, K.S., Simon, T. G., Moses, G., & Santie, V. (2010). Surface sterilant effect on the regeneration efficiency from cotyledon explants of groundnut (*Arachis hypogeal* L.) varieties adapted to eastern and southern Africa. *African journal of biotechnology* 9(**20**): 2866-2871.

Takahashi, H. J., Sato, K., & Takeda, K. (2001). In the 7th International IPM symposium. Genetics, genomics and R genes virus disease management. Are ecologically based IPM strategies relevant for sustainable management of virus disease in the 21st Century? Mephis, Tennessee, March 27-29, 2012.

Taliansky, M. E.,. Ryabov, E. V., & Robinson, D. J. (1998). Two Distinct Mechanisms of Transgenic Resistance Mediated by Groundnut Rosette Virus Satellite RNA Sequences. *Molecular plant microbe interactions* 11(**5**): 367-374.

Uganda Bureau of statistics (UBOS) and Ministry of Agriculture, animal Industries and Fisheris (MAAIF), (2010). Uganda Census of agriculture 2008/2009 Volume 4:

crop area and production Report.

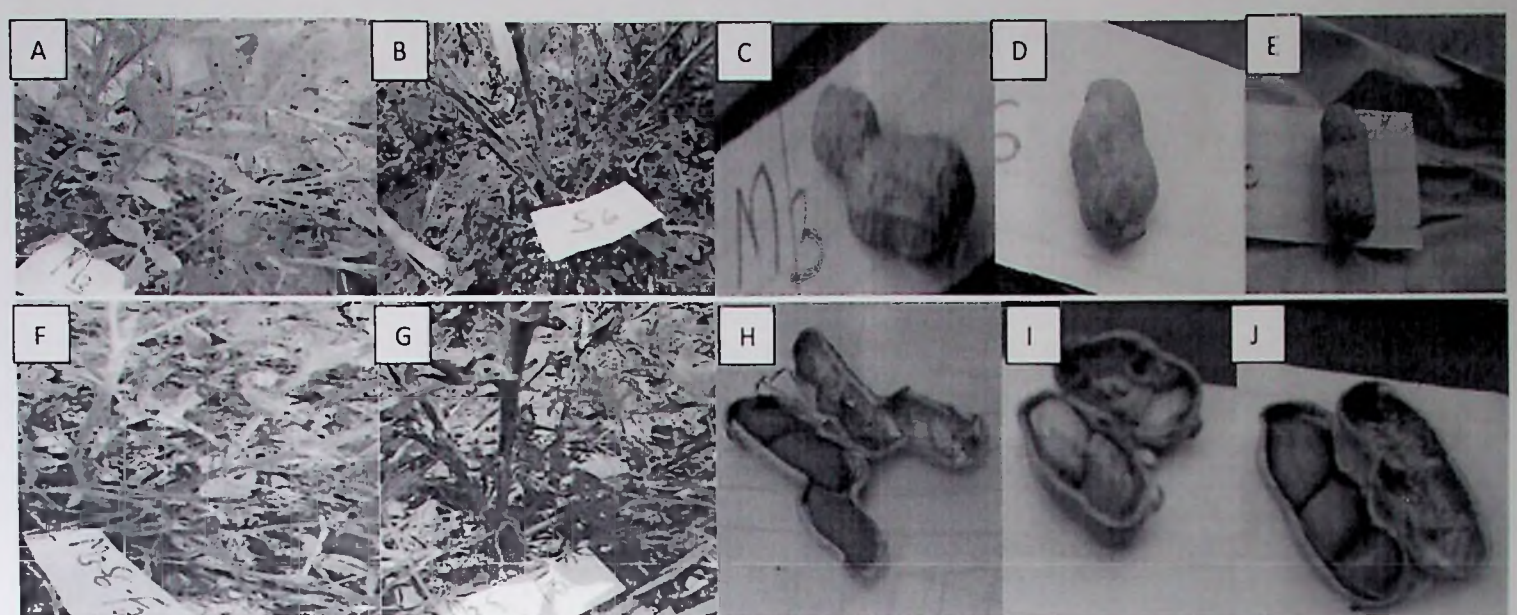
Upadhayaya, H. D., Bramel, P. J., Oritz, R., Sube, S., & Singh, S. (2002). Developing a mini core of peanut for utilization of genetic resources. *Crop Science*, 42(6). 2150-2156.

Vander Merwe, P. J. A. (2001). Project groundnut rosette disease Management Progress Report: June 2010 - 2001. International crops Research Institute for the Semi Arid Tropics (ICRISAT) and Serere agricultural and animal Institute (SAARI).

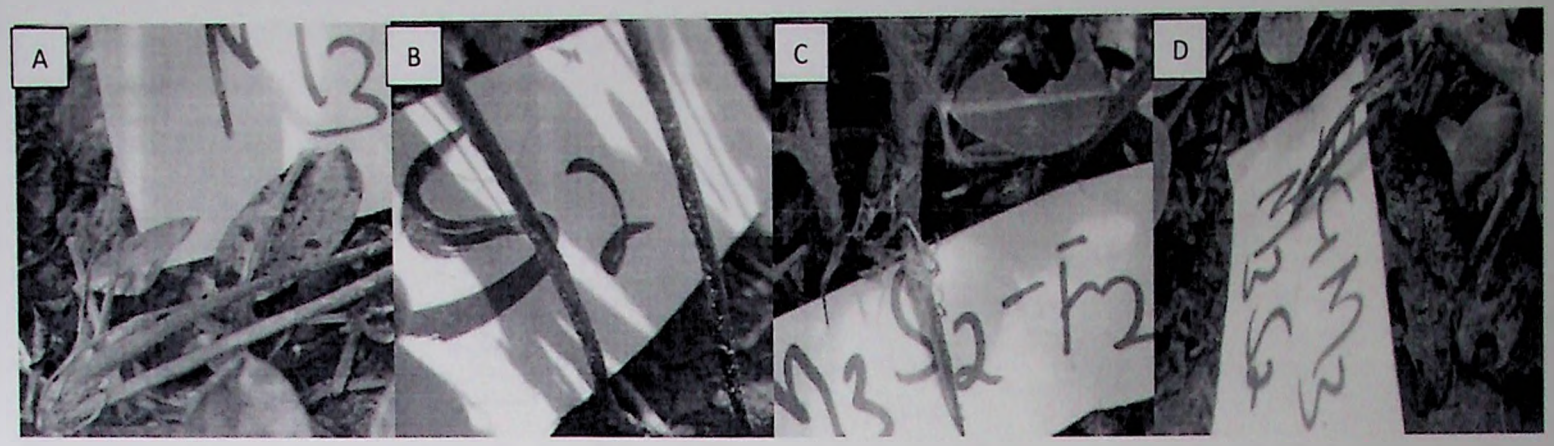
Waliyar, F., Kumar, P. L., Ntare, B. R., Monyo, E., Nigam, S. N., Reddy, A. S, Osiru, M. & Diallo, A.T. (2007). A Century of Research on Groundnut Rosette Disease and its Management. Information Bulletin no. 75. Patancheru 502 324, Andhra Pradesh, India. International Crops Research Institute for the Semi-Arid Tropics. 40 pp.

Waliyar, F., Lava, K., Osiru, P. M., Monyo, E., Ntare, B. R., & Nigam, S. N. (1999). Centennial of research on groundnut rosette disease: what is known and what still needs to be known to achieve effective control of this menace in Sub- Saharan Africa. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; ICRISAT,

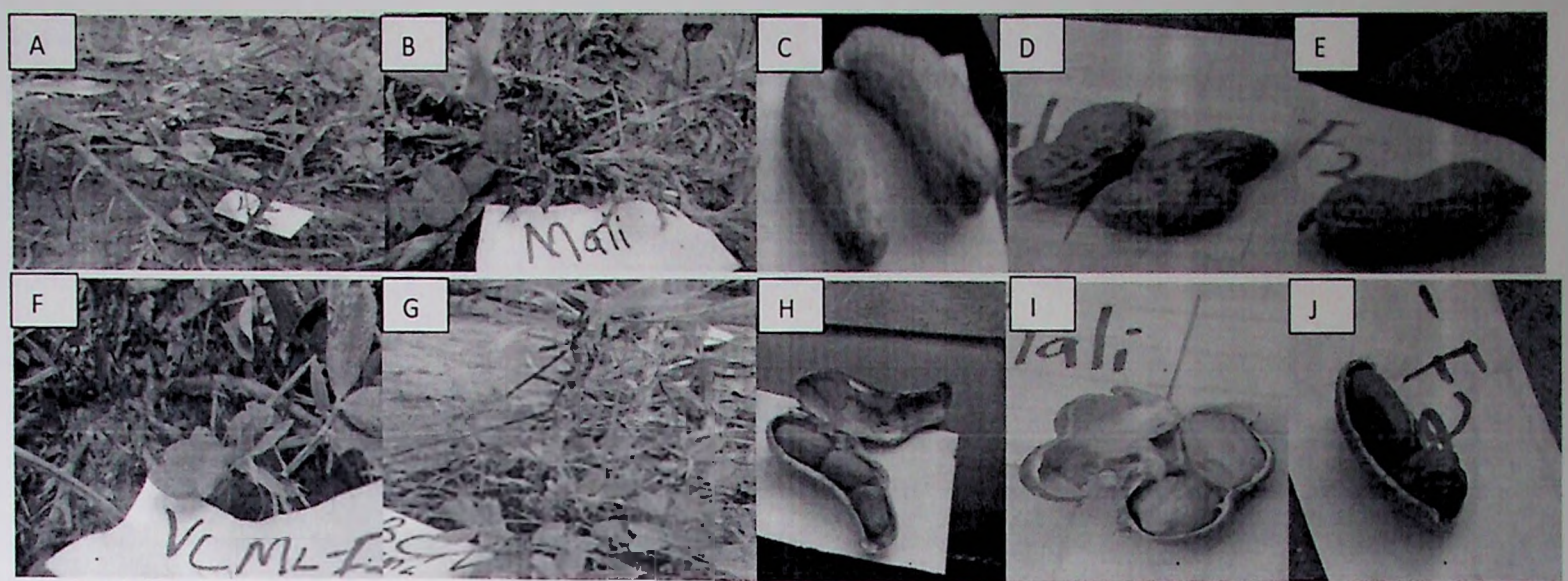
LIST OF APPENDICES



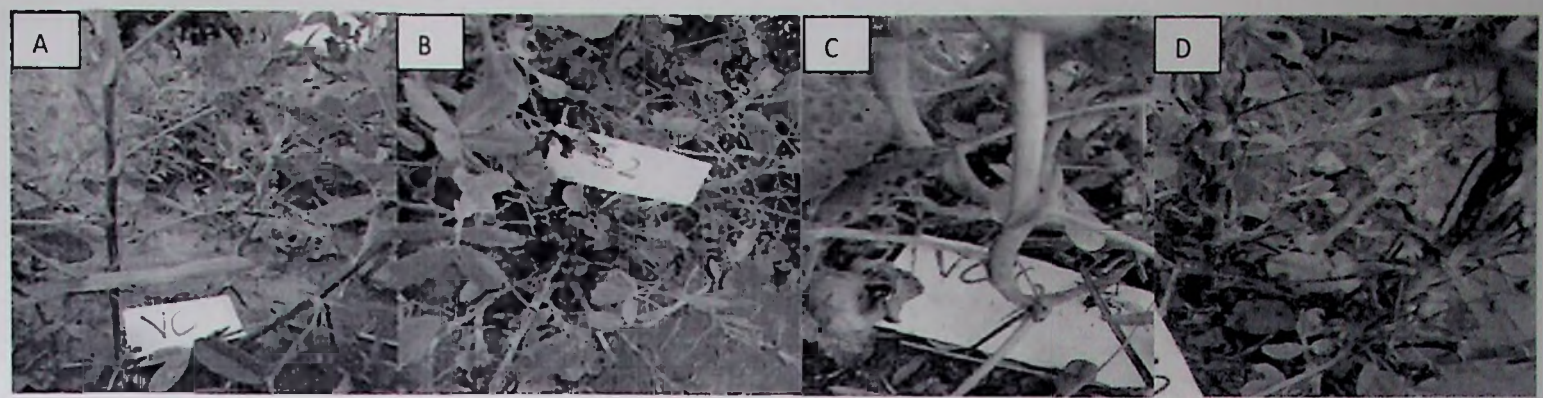
Appendix 1: Morphological shoot and pod characteristics expressed by parental and progeny derived from the cross NuMex-M_3 × ICGV-SM 99566 populations. (A, C, H): NuMex-M_3; (B, D, 1): ICGV-SM 99566; (F, G) : F2, (E, J) : BC_1NuMex-M_3



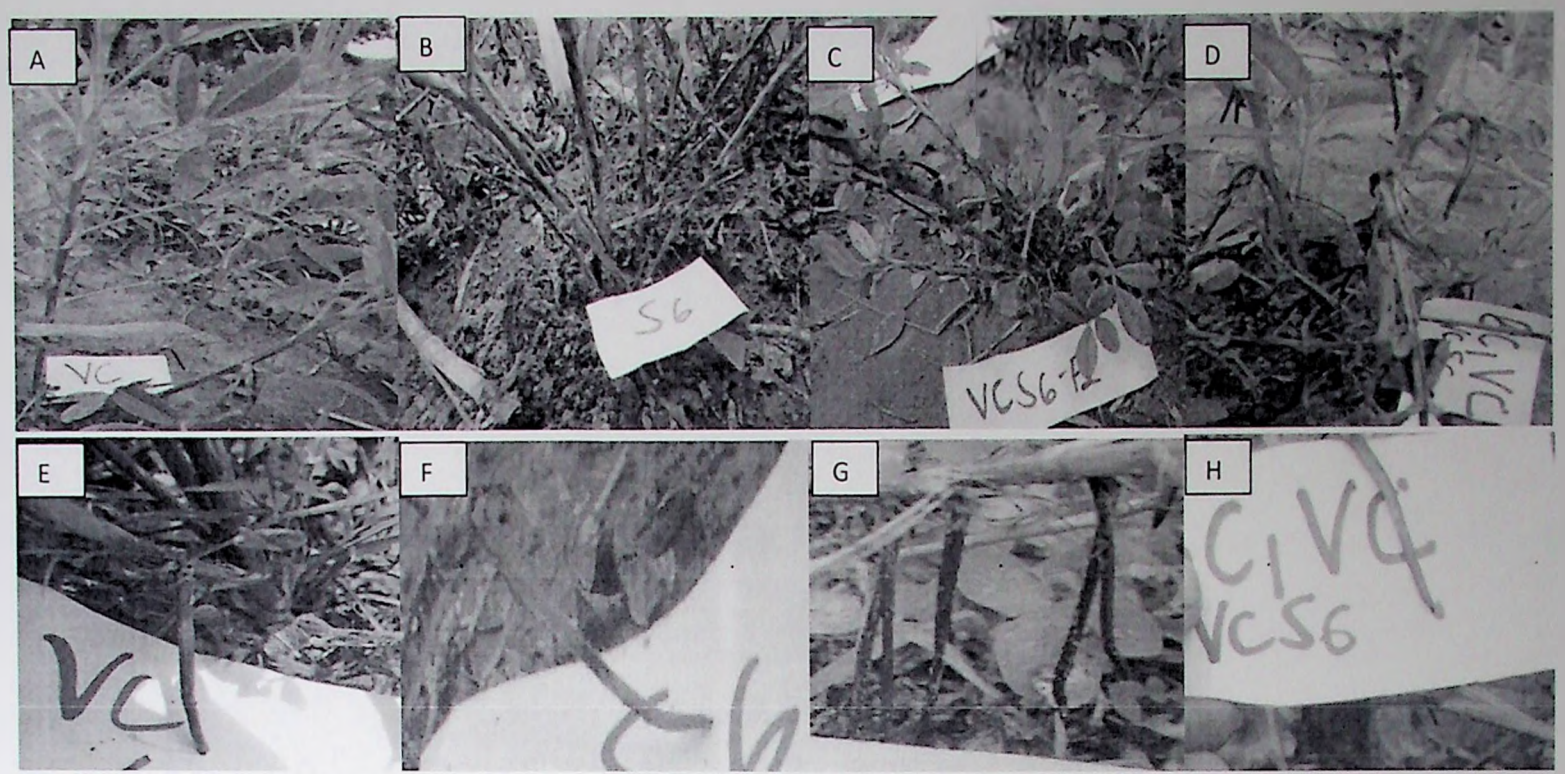
Appendix 2: Morphological shoot characteristics expressed by parental and progeny derived from the cross NuMex-M_3 × ICGV-SM 90704 populations. A: NuMex-M_3, B: ICGV-SM 90704, C: F_2, and D: BC_1NuMex-M_3



Appendix 3: Morphological shoot and pod characteristics expressed by parental and progeny derived from the cross Valencia C × ICGV-SM 96801populations. (A, C, H: Valencia C; (B, D, I) : ICGV-SM 96801; (F, E, J) : F_2; G : BC_1Valencia C



Appendix 4. Morphological shoot and pod characteristics expressed by parental and progeny derived from the cross Valencia C × ICGV-SM 90704 populations. A : Valencia C, B : ICGV-SM 90704, (C, D) : F_2



Appendix 4. Morphological shoot and pod characteristics expressed by parental and progeny derived from the cross Valencia C × ICGV-SM 99566 populations. (A, E) : Valencia C, (B,F) : ICGV-SM 90704, (C, G) : F_2 and (D, H) : BC_1Valencia C