PHENOTYPIC AND MOLECULER CHARACTERIZATION OF RECOMBINANT INBRED GROUNDNUT LINES FOR RESISTANCE TO GROUNDNUT ROSETTE DISEASE

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ABSTRACT

Groundnut rosette disease is the most constraint to the production of groundnut causing annual economic loss of US $156 millions in sub-Saharan African countries. The present study evaluated 220 (F_5) Recombinant inbred lines (RIL) derived from 12991 X ICGV 86124 resistant and susceptible parents respectively, for resistance to groundnut rosette disease so as to identify resistant Recombinant Inbred Lines (RIL) and markers linked to Groundnut Rosette Disease resistance. The RIL were planted at Sokoine University of Agriculture (SUA) and screening was done by inoculating mechanically all the RILs at seedling stage using rosette virus inoculum prepared from susceptible groundnut plants obtained at SUA. Scoring for rosette disease was done, using a scale of 1-5, 1 being highly resistant and 5 highly susceptible. Forty-two RILs were highly resistant, 109 RILs were moderately resistant while 60 RILs were classified as Susceptible. Chi-square test ($X^2 = 3.30$, $P \leq 0.25$) for this phenotype demonstrated that the resistance was qualitatively controlled. DNA samples from 15 most resistant recombinant inbred lines were pooled to form resistant bulk while DNA samples from 15 most susceptible RILs were pooled to form susceptible bulk. Then the bulks along with the two parents were screened with 30 SSR primer pairs. A total of 10 markers amplified 63 alleles of which 59 alleles were polymorphic while four alleles were monomorphic. The number of alleles per marker ranged from four to eight with the average of six alleles. Five SSR Markers, $tc7a02_{180}$, $pm\ 36_{290}$, $tc7h11_{400}$, $tc9f04_{295}$ and $t11a02_{280}$ amplified DNA fragments in only resistant parent and some of the resistant bulks and produced no amplification in susceptible parent and some of the susceptible bulks. The Polymorphic Information content (PIC) values ranged from 0.32 to 0.52. The identified marker can be used during selection of resistance to rosette disease plants/progenies.

DECLARATION

I, Joseph Athanas do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Joseph Athanas Date

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The above declaration is confirmed;

_____ _____

Prof. Kusolwa, P. M. Date

(Supervisor)

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DEDICATION

The work is dedicated to my grandmother Aurelia Michael who passed away in the course of this study.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFLP	Amplified Fragment Length Polymorphism
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetate
ELS	Early Leaf Spot
GRAV	Groundnut Rosette Assistor Virus
GRD	Groundnut Rosette Disease
GRV	Groundnut Rosette Virus
LLS	Late Leaf Spot
$MgCl_2$	Magnesium Chloride
NaCl	Sodium chloride
ng	Nanogram(s) = 10^{-9}gram
PIC	Polymorphic Information Content
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Lines
RNA	Ribonucleic Acid
SSR	Simple sequence repeat
µg	microgram (s) = 10^{-6} gram
µl	microlitre (s) = 10^{-6} litre

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Groundnut (*Arachis hypogaea* L.) is an important monoecious annual legume in the world mainly grown for oilseed, food and animal feed (Upadhyaya *et al.,* 2006). The genus and species names *Arachis hypogaea* are derived from greek words *arachos,* meaning weed, and *hypogea,* meaning underground chamber (Holbrook and Stalker, 2003). Groundnut is tetraploid with 2n=4x=40 chromosomes (Mamadou, 2011). It is divided into two subspecies, *hypogaea* and *Fastigiata* Waldron (Ferguson and Bramel, 2004). Each of the subspecies is further divided into botanical varieties; subsp. *hypogaea* into var. *hypogaea* and subsp. *fastigiata* Waldron into var. *fastigiata* and var. *vulgaris* (Pandey *et al.,* 2012). It is grown under a wide range of environmental conditions in the semi-arid tropical and sub-tropical regions between 40°N and 40°S (Kayondo *et al.,* 2014).

The largest producers of groundnut are China and India, followed by Sub-Saharan African countries and Central and South America (Chintu, 2013). Groundnut is grown on nearly 23.95 million ha worldwide with the total production of 36.45 million tons and an average yield of 1520 kg/ha in 2009 (Muitia, 2011).

In Tanzania groundnut is mainly grown by small scale farmers and the major producing regions are Mtwara, Dodoma, Tabora, Singida and Shinyanga. The crop is traded both locally and internationally. Locally the crop is consumed raw, roasted with salt, boiled and

as a major ingredient in cereal flours for pregnant women and babies and to a small extent processed and sold as cooking oil (Osei *et al.,* 2013).

Groundnut seeds contain high quality edible oil (44-52%), easily digestible protein (26-28%) and carbohydrates (20%), Vitamin B1 and Vitamin B3, minerals and dietary fiber (Okello *et al.,* 2014). Apart from food groundnuts are used as an important source of income since are sold in the local market as boiled and shelled roasted nuts while some is sold in the confectionery trade (Pandey *et al.,* 2012). The haulms are used as livestock feed and in compost making (Waliya *et al.,* 2007). As a legume, groundnuts improve soil fertility in the farming systems by fixing atmospheric nitrogen (Kanyika, 2013).

However groundnut production is constrained by lack of enough improved groundnut varieties, biotic and abiotic stresses. Biotic stresses include Early leaf spot (ELS) caused by *Cercospora arachidicola*, Late leaf spot (LLS) caused by *Phaeoisariopsis personata*. Rust caused by *Puccinia arachidis,* and Groundnut rosette disease (GRD) is caused by a complex association of three virus, namely Groundnut rosette virus (GRV), Groundnut rosette assistor virus (GRAV) and Satellite RNA virus (SatRNA). It is estimated that early and late leaf spot diseases cause up to 70% yield loss (Monfort *et al.*, 2004) while loses due to rust exceed 50% worldwide (Chintu, 2013). Groundnut is also affected by insect pests such as termites (*Microtermes spp*), white grubs (*Lachnosterna consanguinea* Blanchard), thrips (*Megalurothrips uitatus* Bagnall), aphids (*Aphis crassivora*). Abiotic stress include drought and low soil fertility.

Groundnut rosette disease (GRD) is the major constraint to the production of groundnut in Tanzania, causing chlorosis, reduced leaf size and stunted plants affecting pod formation.

The disease cause losses of up to 100% pod yield if infection occurs before flowering and 5-30% under non epidemic years (Waliyar *et al*., 2007; Okello *et al.,* 2014). GRD is estimated to cause an annual economic loss of US$ 156 million in Africa, Tanzania inclusive (Monyo *et al.,* 2008; Kayondo *et al.,* 2014). This results into reduced yield of groundnut affecting small-scale farmers livelihoods due to reduced households income. The disease (GRD) was reported for the first time in Tanzania in 1905 and it is still a major problem in all-major groundnut growing regions of Tanzania. In 2008/2009 cropping season for example the disease caused total crop failure (100% losses in Mtwara and Dodoma regions (Monyo, 2009).

The use of pesticides has been recommended to control the disease through controlling its vector aphids (Subrahmanyam *et al.,* 2002; Mohammed *et al.,* 2014). However, the use of pesticides leads to increased cost of production to farmers. The use of host resistant varieties is the most effective, economical and sustainable way to control rosette disease (Subrahmanyam *et al.,* 2001). Unfortunately this resistance sources was from late maturing variety as a result available varieties are late maturing hence not suitable to most agro ecological zones of Africa including Tanzania due to erratic and short rains and end of season drought which favour outbreak of rosette disease. In addition to that the resistance was linked to undesirable traits like low pod yield and small seed size (Khedikar, 2008). A new source of resistance from early maturing groundnuts was discovered in wild species of groundnut (van der Merwe and Subrahmanyam, 1997). Breeding efforts to GRD resistance could be more efficient and successful by using

molecular markers linked with GRD resistance in marker-assisted selection and for introgression of disease resistance. The SSR markers which will be identified through this study will be useful to the National Groundnut breeding program for selection and fast truck release of rosette resistant varieties while the potential lines will be advanced further to produce rosette resistant varieties.

1.2 Objectives

1.2.1 Overall objective

To generate potential recombinant inbred groundnut lines and molecular genetic markers for resistance to rosette disease.

1.2.2 Specific objectives

i. Phenotyping and identification of recombinant inbred groundnut lines for resistance to groundnut rosette disease.

ii. To identify molecular markers linked to groundnut rosette disease resistance for genotyping purposes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Groundnut, Botany, Origin and Distribution

Groundnut is an annual plant with an indeterminate growth habit having a distinct main stem and a variable number of lateral branches (Shezi, 2011). The stem is initially solid, upright or prostrate ranging from 120 to 650 mm in length, which then becomes hollow as the plant grows (Chintu, 2013).

The branching pattern and distribution of vegetative and reproductive nodes along the main stem and lateral branches are the main traits which primarily distinguish the two subspecies, subspecie *hypogaea* and subspecie *fastigiata,* from each other (Holbrook and Stalker, 2003). The sub species *hypogaea* has alternate branching to reproductive nodes and either a spreading or a bunching growth habit, while the subspecie *fastigiata* has sequential branching to reproductive nodes and an erect growth habit (Chintu, 2013). The groundnut leaves are mostly tetrafoliate and alternately arranged on the stems, however the subspecie *hypogaea* has dark green leaves while the subspecie *fastigiata* has light green leaves (Shezi, 2011). The groundnut plant produces flowers within four to six weeks after emergence continuing until late in the growing season, depending on the genotype and the environment (Muitia, 2011; Shezi, 2011). Although flowering occurs above ground, seeds are produced below the soil surface. The flowers are variable in colour, ranging from light yellow to deep orange and sometimes white. Flowers are borne in the axils of leaves, usually with three flowers per inflorescence, but only one of these flowers opens at a given time (Shezi, 2011). The groundnut plant produces more flowers than the photosynthetic capacity to fill the pods and less than 20% produce mature pods even under ideal conditions (Muitia, 2011). The flowers are self pollinated. However, at

locations where bee activity is high, some cross-pollination can occur (Chintu, 2013). After fertilization of the ovule, an intercalary meristem becomes active and a pointed carpophores or gynophore, commonly known as a peg, is formed.

The peg exhibits positive geotropism and grows downward into the soil where it becomes diageotropic and ceases to elongate and develops into a pod (Shezi, 2011). The pods are elongated spheres with various amounts of reticulation on the surface and/or constriction between seeds. Although pods usually develop below ground aerial pods can occur (Holbrook and Stalker, 2003). The pods may grow up to 80 mm x 27 mm and normally contain two to five seeds. Although the number of seeds per pod depends on the cultivar, it can also be influenced by season and other factors (Shezi, 2011; Chintu, 2013). Seeds are either round or elliptical with pointed or flattened ends and range in their colours from off white to deep purple. Each seed consists of two large cotyledons, an epicotyl, and a primary root. The cotyledons comprise nearly 96 percent of the seed weight and are the major storage tissue for the developing seedling (Holbrook and Stalker, 2003).

Groundnut is grown in areas between latitudes 40° N and 40° S where temperatures range from 25 to 30 ºC (Kamara, 2010; Okello *et al.,* 2010). It is drought tolerant but high production is only obtained in the presence of well-distributed average annual rainfall ranging from 500-1000 mm and 500-600 mm during the growing season (Kamara, 2010). All soils, other than very heavy ones are suitable for growing groundnut, but the best are deep, well-drained sandy, sandy loam or loamy sand soils (Okello *et al*., 2013).

2.2 Groundnut Rosette Disease

Groundnut rosette disease (GRD) is a viral disease caused by complex association of three agents, groundnut rosette virus (GRV), satellite RNA (sat RNA) and groundnut

rosette assistor virus (GRAV) (Kayondo *et al.,* 2014). GRD is transmitted by an aphid, *Aphis craccivora* Koch (Waliyar *et al*., 2007).

The Groundnut rosette disease occurs as two symptom variants, chlorotic rosette and green rosette (Okello *et al.,* 2014). In chlorotic rosette leaves are bright yellow and leaf lamina is curled while for the case of green rosette, leaves appear dark green to dark green mosaic (Naidu *et al*., 1999). Both forms of the disease cause severe stuntedness, with shortened internodes and reduced leaf size, resulting in a bushy appearance of plants (Waliyar *et al.,* 2007). Chlorotic rosette occurs throughout Sub-Saharan Africa whereas green rosette has been reported from Angola, Kenya, Malawi, Swaziland, Uganda and West Africa (Wangai *et al*., 2001).

Yield losses due to GRD depend on the growth stage at which infection occurs (Olorunju *et al.,* 1991). Infection due to chlorotic or green rosette disease occurring in young plants (prior to flowering) will result in 100% yield loss (Okello *et al*., 2014). For example epidemic in northern Nigeria in 1975 destroyed approximately 0.7 million ha of groundnut, with an estimated loss of US$250 million (Alhassan, 2013). Similarly, an epidemic in 1995 in eastern Zambia affected approximately 43 000 ha causing an estimated loss of US$4.89 million (Waliyar *et al.,* 2007). As per the estimates of ICRISAT, GRD causes an annual yield loss of US$156 million in Sub-Saharan Africa (Kayondo *et al.,* 2014)

Using crosses made with two resistant and six susceptible genotypes in a diallel test it has been found that resistance to groundnut rosette disease (chlorotic and green rosette) is conditioned by two recessive genes (Olorunju *et al*., 1992).

2.3 Groundnut rosette disease (GRD) diagnosis

Groundnut rosette disease can be diagnosed in the field by observing the characteristic symptoms in the host plant (groundnut plant). Mechanical inoculation on to *C. amaranticolor* indicates the presence of GRV; infected plants show rosette symptoms about four days after inoculation (Murant *et al*., 1998). Also reverse transcription-polymerase chain reaction (RT-PCR) is used as a diagnostic tool for detection of GRV, GRAV and SatRNA in plants and aphids (Naidu *et al*., 1998).

2.4 Management of Groundnut Rosette Disease

Various methods are available for protecting groundnut against rosette disease. These include the removal of volunteer groundnut plants that serve as inoculum source, cultural practices that can interfere with vector movement, use of insecticides to control aphids and use of rosette disease resistant cultivars (Naidu *et al.,* 1999)

2.4.1 Chemical control

Insecticides have been used to control *A. craccivora* to minimize or prevent spread of rosette disease in field trials. However, insecticides are an unviable option in Sub-Saharan Africa due to high costs and scarcity, thus seldom preferred by the farmers. Furthermore, insecticide applications pose detrimental effects on health and environment and their usage is being discouraged (Waliyar *et al.,* 2007).

2.4.2 Cultural control strategies

Early sowing in the season to take advantage of low aphid populations, and maintaining good plant density without any gaps since aphids prefer widely spaced plantings for landing have been shown to reduce rosette disease incidence (Subrahmanyam *et al.,* 2002). However, early sowings may not be effective in areas where groundnut is grown

continuously, as this allows perpetuation of virus and vector (Naidu *et al.,* 1998). Intercropping with cereals such as maize, sorghum, finger millet, beans and cowpea were shown to affect aphid colonization, movement and behavior within crops, thereby GRD incidence (Alegbejo, 2002). However, cultural control practices used by smallholder farmers is difficult under subsistence farming conditions due to farmers' reluctance to adopt improved cultural practices (Waliyar *et al.,* 2007).

In addition farmers' pre-occupation with other revenue generating practices, unpredictable climate, small-land holdings and reluctance to adopt improved cultural practices makes early sowing inefficient in controlling rosette disease (Waliyar *et al*., 2007).

2.4.3 Host plant resistance

It is believed that varieties resistant to GRD provide the most economical and practical solution to control GRD in the field (Adu-Dapaah *et al.,* 2007) hence more efforts have been directed in breeding for host resistance and several resistant varieties have been released. Some resistant cultivars available for cultivation in SSA are: ICG 12991, ICGV-SM 99568, ICGV 93437, ICGV-IS 96894, ICGV-SM 99541, ICGV-SM 01513, ICGV-SM 01514, ICGV-SM 01708, ICGV-SM 01731 and ICGV-SM 03701 (Waliyar *et al.,* 2007). However, the varieties had poor yield, small seed size and they were late maturing varieties since source of resistance was from late maturing varieties (Olorunju *et al.,* 2001).

2.5 Mechanisms of Resistance to Groundnut Rosette Disease

Plant virus must move site of infection to the rest of the plant to establish a systemic infection. Taliansky *et al*. (2000) demonstrated that prior infection of *N. benthamiana*

with GRV isolate containing a normal form of the Sat-RNA suppressed expression of symptoms when the plants were subsequently inoculated with an isolate containing brilliant yellow blotch mosaic sat-RNA (YB sat-RNA). The suppression of symptom developments indicate that the host mechanisms for resistance is through inhibition of GRV replication by Olorunju *et al*. (1991) concluded that initial symptoms appearance is greatly delayed in resistant plants suggesting a restriction of virus replication, virus movement within plants or perhaps synthesis of the groundnut rosette virus satellite-RNA.

2.6 Genetics of Resistance to Groundnut Rosette Disease and Markers Linked to Virus Plant Resistance

Breeding for resistance to groundnut rosette disease requires an understanding of the genetic control of resistance and knowledge of the amount of genetic variability available for selection (Alhassan, 2013). Inheritance of resistance to GRVD was done using aphid inoculation in the field with a mixed culture of GRV and its Sat- RNA and GRAV, and with mechanical inoculation in the greenhouse using GRV and its Sat-RNA (Olorunju *et al.,* 1992). Observations from this study using resistant parent (RMP 12) and susceptible parent (M1204.78) lead to conclusion that resistance to GRVD is monogenic and governed with single dominant gene.

Several studies have demonstrated the utility of molecular markers and marker-assisted selection (MAS) to improve the efficiency of conventional breeding especially in the case of low heritable traits, where phenotypic selection is difficult, expensive, lack accuracy or precision (Varshney *et al*., 2009). Currently breeding for resistance to groundnut rosette disease rely on disease pressure based on disease symptoms which is highly influenced by location, year, season and method of infestation since groundnut rosette disease outbreak

is sporadic. Not only that but also identification, multiplication and maintenance of groundnut rosette virus inoculums is not easy, time consuming and costful. In addition selection for lines resistant or susceptible to groundnut rosette disease is difficult based on phenotype alone. At present selection for resistance to groundnut rosette disease can be improved using molecular markers that are tightly linked to groundnut rosette disease (Pandey *et al*., 2012). To date not a single molecular markers for resistance to groundnut rosette disease available, though several markers linked to resistance to rust, root knot nematode resistance, early and late leaf spot have been identified (Pandey *et al*., 2012). This study is aimed at identifying molecular markers associated with resistance to groundnut rosette disease. The identification of molecular markers will speed up selection and consequent release of farmer preferred varieties with high yielding and resistant to groundnut rosette disease, using Marker assisted selection procedures.

The use of molecular markers for selection of disease resistance is believed to be most accurate and efficient method in comparison to visual assessment selection alone. Various molecular markers have been used in different crops including groundnut for breeding disease resistance plants. Markers used include Random Amplified Polymorphism DNAs (RAPDs), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repearts (SSRs). For example, Selvi *et al.* (2006) identified three RAPD markers in resistant and susceptible bulks linked with Mungbean Yellow Mosaic Virus (MYMV) resistance in mungbean *(Vigna radiata,* L. Wilczek) in the cross ML 267 X CO4. Unfortuanately this marker is highly sensitive to small changes in laboratory conditions, and minor modifications of protocols, and therefore, there is a low reproducibility within and between laboratories in addition RAPD is a dominant marker.

Amplified Fragment Length Polymorphism (AFLP) is a molecular marker technique based on PCR analysis and restriction enzymes. Moon (2006) identified 32 AFLP primers linked to Tomato Spotted Wilt Virus resistance in 23 Doubled Haploid Tobacco lines. However, the use of these markers for virus plant characterization is laborious and expensive, also being dominant marker homozygotes can not be distinguished from heterozygotes.

Simple Sequence Repeat (SSR) is a motif of one to six bases arranged in simple internal repeat structures that are frequently and randomly distributed throughout the eukaryotic genomes (Tang *et al.,* 2007). Polymorphism is based on variation in the number of repeats in different genotypes owing to polymerase slippage and point mutations (Kruglyak *et al*., 1998). Unlike RAPDs and AFLP markers, SSR markers are highly informative, locus-specific and frequently show co-dominant inheritance, adaptable to high-throughput genotyping and simple to maintain and distribute. SSR markers have been widely applied in various crops including groundnut. In groundnut it has been used to determine markers for resistance to Peanut bud necrosis (Bera *et al*., 2014), root-knot nematode (Choi *et al.,*1999), late leaf spot (Mace *et al.,* 2006), the aphid vector causing groundnut rosette disease (Herselman *et al.,* 2004), seed infection by *Aspergillus flavus* (Yong *et al*., 2005) and early leaf spot (Bera *et al.,* 2014). Also in maize markers linked to maize streak virus resistance (Danson *et al*., 2006), in cowpea markers linked to cowpea yellow mosaic virus resistance (Gioi *et al*., 2012) have also been identified.

Bera (2014) using 21 interspecific pre-breeding lines and three cultivars of groundnut differing in degree of resistance to peanut bud necrosis disease (PBND) identified three out of 45 SSR markers ($PM15_{190}$, $PM188_{165}$ and $PM201_{130}$) linked to resistance in PBND

Similarly, Gioi *et al.* (2012) identified four out of 60 SSR markers, linked to cowpea yellow mosaic virus disease in 40 cowpea genotypes. Out of 20 resistant genotypes 16 genotypes were amplified by producing 200 bp DNA fragments whereas 180bp DNA fragments were produced in 18 susceptible genotypes out of 20 susceptible genotypes analyzed.

Wang *et al*. (2008) identified two SSR markers linked to a root knot nematode resistance using F_2 population derived from Huayu-22 and D099. Jiang *et al*. (2007) identified SSR markers linked to bacterial wilt resistance in groundnut using (F_7), recombinant inbred lines (RILs) derived from Yuanza 9102 and Chico resistant and susceptible parents respectively. Shoba *et al*. (2012) identified three markers (PM 375, PM 384 and PM 3) linked to resistance in Late Leaf Spot disease. The identified markers will be useful in breeding and selection of resistance to groundnut rosette disease.

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CHAPTER THREE

3.0 PHENOTYPING AND IDENTIFICATION OF RECOMBINANT INBRED GROUNDNUT LINES FOR RESISTANCE TO GROUNDNUT ROSETTE DISEASE

3.1 Abstract

The groundnut (*Arachis hypogaea* L.) is a valuable legume, which supports livelihood of millions of farmers for food, edible oil, animal feeds and as an important source of income. However groundnut rosette disease caused by three viruses; Groundnut rosette virus (GRV), Groundnut rosette assistor virus (GRAV) and Sat-RNA is the most constraint to the production of groundnut causing annually economic loss of US $156 millions in sub-Saharan African countries. The present study evaluated 220 (F_5) Recombinant inbred lines (RILs) derived from ICG 12991 X ICGV 86124 resistant and susceptible parents respectively, for resistance to groundnut rosette disease. The RILs were planted at Naliendele Agricultural Research Institute, Mtwara, Tanzania, using Infector row technique using the variety ICGMS 33. No GRD symptoms were observed, all RILs were resistant to GRD. But when evaluated using mechanical inoculation at Sokoine University of Agriculture (SUA), 42 RILs were highly resistant, 109 RILs were moderately resistant and 60 RILs were susceptible. Chi-square test ($X^2 = 3.30$, $P \leq 0.25$) for segregation ratio of the phenotypes for resistant versus susceptible demonstrated that the resistance of these RILs is controlled by single dominant gene.

3.2 Introduction

Groundnuts (*Arachis hypogaea L.*) is the thirteenth most important food crop of the world, fourth most important source of edible oil and third most important source of

vegetable protein (Taru *et al.,* 2010). Groundnut seeds contain 40-50% edible oil, 20-50% protein and 10-20% carbohydrate (Okello *et al*., 2014). It is also a nutritional source of vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium (Pandey *et al.,* 2012).

Groundnut seeds are used as food and can be consumed raw, roasted or boiled, also used in food industry to make cooking oil, margarine, peanut butter and confectionary products. The groundnut shells are used for making particle boards, used as fuel, filler in fertilizer and feed industry. Groundnut haulms constitute nutritious fodder for livestock (Janila *et al.,* 2013). They contain protein (8–15%), lipids (1–3%), minerals (9–17%), and carbohydrate (38–45%) at levels higher than cereal fodder.

The Tanzanian groundnut industry is affected by many diseases that limit crop production in Tanzania. Among the diseases is the groundnut rosette disease (GRD) caused by Groundnut rosette virus (GRV) genus *Umbravirus* and its satellite RNA, and Groundnut rosette assistor virus (GRAV) genus *Luteovirus* (*Murant et al*., 1995). The disease cause severe stuntedness of plants, with shortened internodes and reduced leaf size resulting in yield loss of 5% to 30% under non-epidermic situation while yield loss of 100% under epidermic situations has been reported (Waliyar *et al.,* 2007). The outcome of the disease is the reduced productivity leading to poor income for farmers relying on this crop. GRD causes an annual yield loss of US$156 million in Sub-Saharan Africa (Waliyar *et al*., 2007).

For example in 2009 due to groundnut rosette disease outbreak following prolonged dry spell estimated yield loss of 30% was observed in Central zone of Tanzania (Bahi and Chamwino Districts in Dodoma region) (Monyo, 2009). The groundnut rosette disease

can be controlled by use of insecticides and cultural practices purposely to reduce vector pressure (Kapewa *et al*., 2001). However despites the effectiveness of insecticides in controlling the disease it is unaffordable to small-scale farmers in Tanzania due to high cost of insecticides. Also, it has been reported that early sowing in the season and at optimum plant population densities could reduce the groundnut rosette disease (Naidu *et al*., 1999). Although cultural practices such as early sowing and planting at optimum plant densities has been demonstrated to reduce groundnut rosette disease, many farmers plant groundnut late due to differential crop priorities (Muitia, 2011). At present breeding and use of groundnut varieties resistant to groundnut rosette disease is the most economical and sustainable means to control groundnut rosette disease. Sources of resistance were first discovered in 1952, from Burkina Faso and Côte d'Ivoire and resistant varieties such as RMP 12, RMP 91, KH 241 D and RG 1 were developed. Resistance among these cultivars was found effective against both chlorotic and green rosette forms of the disease and was governed by two independent recessive genes (Nigam and Bock 1990; Olorunju *et al*., 1992). However such varieties were late maturing making them susceptible to drought during the end of season, small pod size, were also low yielding, hence were not adopted by many farmers. A new source of resistance to GRD was discovered in early maturity source (90-100days) and high yielding parent and at present 300 recombinant inbred lines at F_5 has been developed at ICRISAT (Waliyar *et al.,* 2007). These RILs are available and freely shared among groundnut breeders in the region. Hence there is a need to evaluate these materials so as to identify potential resistant lines or develop and upgrade into varieties for use in National groundnut breeding programme.

3.3 Materials and Methods

3.3.1 Phenotyping experiment at Naliendele (March 2013)

A population of 220 F_5-derived from ICGV 12991(Resistant Parent) and ICG 86124 (Susceptible parent) used in this study were obtained from ICRISAT- Malawi. The list of all 220 recombinant inbred lines (RILs) used for this study (Appendix 1). ICG 12991 is resistant to groundnut rosette disease, early maturing (90-100 days) while ICGV 86124 is susceptible to groundnut rosette disease, drought tolerant but also an early maturing variety (90-100 days). The 220 RILs were developed through crossing of the two parents followed by selfing F_1 producing the F_2, single superior plants were selected and planted in progeny rows where single seeds were retained (Single seed descent selection method). About 25 groundnut plants severely infected with chlorotic rosette and highly infested with aphids (*Aphis crasivora)* were collected in January 2013 from groundnut fields in Masasi and Nachingwea districts. In the glasshouse about 700 seedlings of groundnut plants were raised using a susceptible groundnut variety ICGMS 33. The seedlings were inoculated with Groundnut rosette virus (GRV) at 2-3 leaf stage when still very young using aphids feeding from groundnut plant with chlorotic rosette symptoms.

A total of 220 RILs were planted including infector rows at Naliendele Agricultural Research Institute, Mtwara using Alpha Lattice design (Incomplete Block Design) with three replications, spacing of 10 cm and 50 cm, intra and inter row spacing respectively. To increase disease pressure infector rows were planted using susceptible groundnut variety ICGMS 33 where four plots each consisted of one row per RIL were surrounded on two sides with each side having double rows of infector row. Aphids feeding from groundnut plants infected with rosette disease (chlorotic rosette) were identified around farmers fields about 200km from Naliendele. Aphids were collected and fed on 4-7days old groundnut seedlings from susceptible groundnut variety ICGMS 33 in the glasshouse.

This was done so as to increase the number of virulent aphids and as a way to maintain groundnut rosette virus disease. The RILs were inoculated with groundnut rosette virus by infestation with aphids fed from rosette affected groundnut plants. Also the rosette groundnut plants from glasshouse raised on polythene bags were transplanted close to each of infector rows. This was done to increase disease pressure and to prevent possibilities of escapes as described by Chintu (2013). The field was maintained weed free for the entire period of the trial, no fertilizers or insecticides were applied. The disease severity and incidence was assessed according to *Olorunju et al.* (2001) and Waliyar *et al.* (2007) using a rating scale of 1-5, where 1= Highly resistant and 5= Highly susceptible.

3.3.2 Phenotying experiment at Sokoine University of Agriculture August 2014

The 211 F_5 Recombinant Inbred lines from Naliendele were planted at Sokoine University of Agriculture, Morogoro. The RIL were planted at a spacing of 30 cm by 10 cm inter and intra-rows respectively. Each RIL, six seeds per plot were planted in unreplicated trial. The sources of the rosette virus inoculums were from groundnut volunteer plants with chlorotic rosette symptoms (Plate 3.1) identified at SUA crop Museum. When the plants were still young with two to three leaves, they were inoculated with groundnut rosette virus inoculums as described by Hull and Adams (1968). Evaluation of the disease development was done according to Olorunju *et al. (*2001) using a scale of 1-5, where 1= Highly resistant and 5= Highly susceptible. The segregation of 211 Recombinant Inbred Lines for resistance versus susceptibility were tested using Chi-square test of the segregating classes as described by Gomez and Gomez (1984).

Plate 3.1: Symptomatic plants with GRD used as source of rosette virus inoculums

3.4 Results

3.4.1 Phenotyping experiment at Naliendele

The pod yield per plant was significantly ($p \leq 0.05$) different among inbred lines (Table 3.1 and Appendix 2) which ranged from 2.46g to 14.23g as recorded from RILs (ICGX-SM 08036/5/P19-1 and ICGX-SM 08035/5/P7-7). Similarly the 8.51g and 5.59g pod yield per plant were recorded on resistant and susceptible parents. The pod yield among the RILs were significantly ($p \leq 0.05$) different, among inbred lines Table 3.1 and Appendix 2) which ranged from 493kg/ha to 2845kg/ha recorded from RILs (ICGX-SM 08036/5/P19-1 and ICGX-SM 08035/5/P7-7).

The height of the Recombinant Inbred Lines were highly significant ($p \leq 0.05$) which ranged from 26.33cm (Table 3.1 and Appendix 2) for ICG 12991 a resistant parent to 17.80cm observed from susceptible parent ICGV 86124. Among the RILs the height ranged from 33.22cm to 13.4cm (ICGX-SM 08036/5/P5-2 and ICGX-SM 08035/5/P9-2)

Pod number per plant was significantly ($p \leq 0.05$) different among inbred lines (Table 3.1 and Appendix 2) which ranged from 23 to 13.70 as observed from parents (ICG 12991 and ICGV 86124). Among the RILs the highest pod number per plant (30.91) was recorded in ICGX-SM 08035/5/P9-7 while the lowest pod number per plant (5.52) was recorded in ICGX-SM 08036/5/P1-3. There were no significantly ($p \leq 0.05$) difference among the 220 RILs for either rosette severity or rosette disease incidence as there were no rosette disease symptoms were observed in the field (Plate 3.2 and Appendix 2 and Table 3).

Tables 3.1: Yield and disease reactions of 220 RILs evaluated at Naliendele

Recombinant Inbred Lines (RILs)	Height (cm)	Pod number $Plant^{-1}$	Pod number $plot^{-1}$	Pod yield kg/ha	Pod yield $plant^{-1}$	Disease status
ICGX-SM 08035/5/P7-7	27.42	21.09	101.79	2845	14.23	Resistant
ICGX-SM 08036/5/P20-5	17.12	18.6	84.03	2512	12.56	Resistant
ICGX-SM 08035/5/P9-7	23.39	30.91	174.78	2089	10.45	Resistant
ICGX-SM 08035/5/P10-4	22.02	20.21	101.71	1941	9.71	Resistant
ICGX-SM 08035/5/P10-8	18.91	17.16	83.59	1804	9.02	Resistant
ICGX-SM 08035/5/P8-3	21.65	20.6	88.04	1801	9.01	Resistant
ICGX-SM 08035/5/P21-2	25.26	23.43	97.67	1708	8.54	Resistant
ICGX-SM 08035/5/P7-4	14.81	20.84	79.98	1688	8.44	Resistant
ICGX-SM 08035/5/P9-4	22.1	20.34	64.96	1671	8.36	Resistant
ICGX-SM 08035/5/P20-5	24.66	13.36	63.19	1661	8.31	Resistant
ICGX-SM 08036/5/P17-5	19.51	14.68	42.88	768	3.84	Resistant
ICGX-SM 08036/5/P1-3	16.58	5.52	17.19	644	3.22	Resistant
ICGX-SM 08036/5/P9-12	13.46	12.12	35.31	631	3.16	Resistant
ICGX-SM 08036/5/P8-2	18.07	22.29	90.66	628	3.14	Resistant
ICGX-SM 08035/5/P15-3	13.81	13.07	47.4	625	3.13	Resistant
ICGX-SM 08035/5/P20-6	16.02	10.83	38.2	608	3.04	Resistant
ICGX-SM 08036/5/P4-5	22.61	10.77	29.58	565	2.82	Resistant
ICGX-SM 08035/5/P4-1	16.59	13.48	43.81	517	2.58	Resistant
ICGX-SM 08036/5/P4-1	15.75	8.39	22.78	494	2.47	Resistant
ICGX-SM 08036/5/P19-1	15.64	7.52	20.47	493	2.46	Resistant
Check						
ICG 12991 (R)	**26.33**	**23**	**112.11**	**1702**	**8.51**	
ICGV 86124 (S)	**17.8**	**13.7**	**49.73**	**1118**	**5.59**	
Mean	**21.66**	**16.52**	**66.5**	**1170**	**5.85**	
LSD 0.05%	**8.43**	**10.05**	**51.29**	**761.9**	**3.81**	
CV%	**23.02**	**36.03**	**45.67**	**38.54**	**38.54**	
Significance Level	**	NS	NS	**	*	

Plate 3.2: Highly resistant Recombinant Inbred groundnut lines observed under field experiment at Naliendele Agricultural Research Institute.

3.4.2 Phenotypic experiment at Sokoine University of Agriculture

Results show that 42, 109 and 60 Recombinant inbred lines were higly resistant, moderately resistant and susceptible respectively (Appendix 3 and Plates 3.3 & 3.4) and Table 3.2). There were no significant difference between observed ratio 42: 109: 60 when compared with Mendelian ratio of 1: 2: 1 using Chi-square test (x^2 =3.30, p≤0.25).

Table 3.2: Segregation of 211 Recombinant Inbred lines tested with Chi-square test

Pedigree	Generation	Observed	Expected	X^2	p-value
ICG 12991 X ICG 86124	F_5	42 Resistant	52. 75		
		109 Moderate Resistant	105.5		
		60 Susceptible	52.75	3.30	0.25

The highest pod yield and seed yield was 2817kg/ha and 2224kg/ha, with 17 number of pods per plant was recorded on resistant parent ICGV 12991 (Table 3.3 and Appendix 4). The RIL (ICGX-SM 08035/5/P3-2) had pod yield, seed yield and number of pods per plant of 248.7kg/ha, 1985.59 and 15 respectively. The low pod yield and seed yield of

470.5kg/ha and 329.02kg/ha respectively was recorded on susceptible parent ICGV 86124.

Table 3.3: Yield and disease reaction of 211 RILs evaluated at Sokoine University of Agriculture

Recombinant Inbred Lines	Number of pods plant	Severity	Pod yield (kg/ha)	Seed yield kg/ha	Disease status
ICG 12991	17	1	2817	2223.93	Highly resistant
ICGX-SM 08035/5/P3-2	15	1	2487	1985.59	Highly resistant
ICGX-SM 08035/5/P7-1	30	1	2068	1278.47	Highly resistant
ICGX-SM 08035/5/P5-1	17	1	1922	1381.86	Highly resistant
ICGX-SM 08036/5/P4-6	13	1	1882	1448.69	Highly resistant
ICGX-SM 08035/5/P6-6	14	1	1786	1325.38	Highly resistant
ICGX-SM 08035/5/P6-10	15	1	1783	1303.05	Highly resistant
ICGX-SM 08035/5/P1-10	17	1	1691	1185.12	Highly resistant
ICGX-SM 08035/5/P1-2	14	1	1671	1230.18	Highly resistant
ICGX-SM 08035/5/P10-9	11	1	1654	1275.83	Highly resistant
ICGX-SM 08035/5/P1-1	11	1	1644	1275.07	Highly resistant
ICGX-SM 08035/5/P6-8	9	1	1637	1322.68	Highly resistant
ICGX-SM 08035/5/P1-15	16	1	1633	1153.28	Highly resistant
ICGX-SM 08035/5/P1-1	14	1	1603	1159.18	Highly resistant
ICGX-SM 08035/5/P6-11	19	1	1600	1053.39	Highly resistant
ICGX-SM 08036/5/P1-11	13	1	1594	1173.98	Highly resistant
ICGX-SM 08035/5/P10-4	18	4	544.5	320.29	Susceptible
ICGX-SM 08035/5/P7-16	15	4	538	334.16	Susceptible
ICGX-SM 08035/5/P12-6	19	4	535.5	306	Susceptible
ICGX-SM 08035/5/P7-14	14	4	527	337.82	Susceptible
ICGX-SM 08035/5/P18-33	16	4	522.5	312.87	Susceptible
ICGX-SM 08036/5/P11-1	8	4	512	307.83	Susceptible
ICGX-SM 08035/5/P12-2	15	4	510.5	305.34	Susceptible
ICGX-SM 08035/5/P7-17	12	4	506.5	333.22	Susceptible
ICGX-SM 08035/5/P7-15	12	4	502	336.91	Susceptible
ICGX-SM 08035/5/P12-4	14	4	499	311.34	Susceptible
ICGX-SM 08035/5/P10-2	12	4	490.5	327.21	Susceptible
ICGX-SM 08035/5/P7-19	16	4	488	364.21	Susceptible
ICGX-SM 08035/5/P18-34	12	4	481.8	317.65	Susceptible
ICGX-SM 08035/5/P11-3	12	4	478.5	318.52	Susceptible
ICGX-SM 08035/5/P14-6	13	4	477	315.76	Susceptible
ICGV 86124	10	5	470.5	329.02	Susceptible

Plate 3.3: GRD Resistant groundnut plant

Plate 3.4: GRD susceptible groundnut plant

The correlation between pod yield and GRD severity was highly significant and negatively correlated (r = - 0.84, P≤0.01) (Table 3.4). The seed yield was highly significant and negatively correlated with GRD severity (r = - 0.84, P≤0.01). Pod number per plant was significantly and negatively correlated with GRD severity (r = - 0.40, P≤0.01). Seed yield and pod yield was significantly and positively correlated (r = 0.89, P≤0.001). The pod number was significantly and positively correlated (r = 0.46, P≤0.001) with pod yield. The pod number was also significantly and positively correlated (r = 0.34, P≤0.001) with seed yield.

Table 3.4: Correlation between GRD severity and yield performance of 211 RILs planted at Sokoine University of Agriculture, Morogoro

	Severity	Pod yield	Seed yield	Pod number
Severity	1			
Pod yield	-0.84**	1		
Seed yield	-0.84**	0.89***	1	
Pod number	-0.40**	0.46***	0.34***	1

** Significant at P≤ 0.01, *** Significant at P≤ 0.001

3.5 Discussion

The pod yield per plant of the 220 RILs at Naliendele (Table 3.1 and Appendix 2) were significant at ($p \leq 0.05$) which implies high genetic variability among the RILs, hence selection can be done. The high pod yield per plant on RIL (ICGM-SM 08035/5/P7) implies that this RIL can be selected and used as a source of high yielding and resistance to rosette disease for use by farmers and for improvement of existing low yielding and rosette susceptible groundnut varieties currently in use by farmers so as to increase groundnut production.

There were no significant ($p \leq 0.05$) difference on either rosette incidence or severity among the 220 RILs observed. This implies that all the 220 RILs are resistant to rosette disease and can be used as a good source of resistance to rosette disease for either direct use by farmers or for use by National breeding programme to incorporate this resistance to existing farmer preferred but rosette susceptible varieties. The resistance of 220 RILs to rosette disease observed in this study can be attributed to ICG 12991, parent which is resistant to groundnut rosette virus and its vector the *Aphis crassivora* (Olorunju *et al.,* 1992). On the other hand, probably the resistance observed in these RILs might be due to the influence of seasonal variations including relative humidity, rainfall and temperature which have been reported to affect rosette development (Muitia, 2011).

The results for resistance to GRD (Table 3.2 and Appendix 3) at Sokoine University Agriculture shows that 42 RILs were highly resistant, 109 RILs were moderately resistant, while 60 RILs were suceptible to GRD. These results were in line with Mendelian ratio of single gene inheritance pattern since no significant difference were observed between observed ratio and expected ratio of (X^2 3.30, $P \leq 0.25$). The results suggest that groundnut rosette disease (GRD) in this population is governed qualitatively with single dominant

gene. Similar findings were reported by Olorunju *et al.* (1992), using mechanical inoculation using parents RMP 12 X M1204.781 who found that groundnut rosette disease is controlled by one dominant gene.

Similarly, Basamma (2011) used Urdbean (*Vigna mungo* L.) to study the inheritance of resistance to mungbean yellow mosaic virus (MYMV) by crossing BDU-4 X TAU-1 resistant and susceptible parents respectively with the following results in F_3: 92 resistant, 139 moderate resistant and 67 susceptible with a goodness of fit (1:2:1) X^2=5.54, p≤0.34, suggesting single dominant inheritance to MYMV resistance. Ikram (2004) demonstrated similar findings after crossing six resistant parents AC-58, No. 411, No 2127, MSP, S.A.D and White Star with a susceptible parent AC-62, F3 which segregated into 511 resistant, 1036 tolerant and 506 susceptible (X^2=0.985, p≤0.70-0.80) and observed ratio were in good fit to 1:2:1 expected ratio suggesting that the resistance to yellow mosaic virus in cowpea is controlled by a dominant gene at a single locus. Aslam *et al.* (2000) crossed *G. barbadense* L. (Giza-45) to *G. hirsutum* L. (Reba P-288) susceptible and resistant parents to cotton leaf curl virus (CLCuV). None of the F1 showed symptoms of the disease, and among 285 F_2 plants, 223 were resistant; 62 were susceptible, using chi-square, the data were not significantly different from a 3: 1 ratio (χ^2=1.606, p≤0.2) and concluded that resistance to cotton curl virus is controlled by a single dominant gene. Sugimoto *et al.* (2007) crossed Tanbakuro to PI103091 susceptible and resistant parents respectively and F_2 segregated as 33 homozygous resistant, 61 segregating and 29 homozygous susceptible. The chi-squared test gave a goodness-of-fit for the expected ratio of 1: 2: 1 (X_2=6.40, P≤0.87), suggesting that the inheritance of resistance to phytopthora in soyabean to gene Rps1-d is controlled by a monogenic dominant gene. In constrast to these findings, Bock and Nigam (1990) using six F_2 crosses with resistant

parents RG 1 and RM 40 and three susceptible cultivars JL 24, Mani Pintar and ICG 48 were able to fit 15:1 F_2 ratio for susceptibility to resistance suggesting that GRD resistance was controlled by two recessive genes. Probably the differences in inheritance to GRD reported is due to differences of the populations used, this study used recombinant inbred lines F_5, while other studies used F_2 populations or the strain of GRD might also be different interms of virulence.

Among the 211 RILs (Table 3.3 and Appendix 3) the highest pod yield, seed yield and number of pods per plant were 2487kg/ha, 1986kg/ha, and 15 respectively were recorded on highly resistant recombinant inbred line ICGX-SM 08035/5/P3-2. The RIL (ICGX-SM 08035/5/P3-2) had also high pod yield and seed yield close the resistant parent which was 248.7kg/ha and 1985.47kg/ha respectively. The high seed yield and pod yield observed in this study is due its high level of resistance to rosette disease. Similar results to this study were reported by Olorunju *et al.* (1991) and Muitia (2011) who reported high yield on groundnut lines resistant to rosette diasease. The low pod yield and seed yield of 470.5kg/ha and 329.02kg/ha respectively was observed on susceptible parent ICGV 86124. This yield in comparison to that recorded on resistant parent ICG 12991, implies low yield. This can be due to genotype itself but also probably can be due to effect of groundnut rosette disease which reduce yield. The GRD severity was negatively ($r = - 0.84$, $P \leq 0.01$) and significantly correlated with pod yield, seed yield ($r = - 0.84$, $P \leq 0.01$) and number of pods per plant ($r = -0.40$, $P \leq 0.01$) This implies that as GRD severity increases from score 1 to 5, the pod yield, seed yield and number of pods per plant decreases. Probably the yield reduction observed in susceptible RILs in this study might be due to the effect of GRD. Similar findings to this study was reported by Ntare and Olorunju (2001); Chintu (2011); Adelana (1980) and Muitia (2011).

On the other hand, highly significant positive correlations were recorded between pod yield and seed yield ($r = 0.89$, $P \leq 0.001$), number of pods per plant and pod yield ($r = 0.46$, $P \leq 0.001$) and number of pods per plant with seed yield ($r = 0.34$, $P \leq 0.001$). This implies that as pod yield increase also seed yield increases and as number of pods per plant increase also pod yield and seed yield increases.

As from this study it seems that, there is very strong correlation between pod yield and seed yield. This implies that at harvest if pod yield is high also the seed yield is expected to be high. The number of pods per plant correlated moderately to seed yield and pod yield, probably because you can have more number of pods with no seeds/ with few single seeds. Similarly to this study Chintu (2011), evaluated 100 groundnut genotypes for resistance to GRD, yield and yield components and found that percentage disease incidence was significant and negatively correlated with pod yield while highly significant positive correlations were recorded between pod yield and seed yield. This was also reported by Muitia (2011) who was working on relationship between GRD and yield performance of groundnut lines.

The identified highly resistant but high yielding RIL (ICGX-SM 08035/5/P3-2) will be very useful source of resistance to rosette disease for use by farmers to increase groundnut production but also will be used by National breeding programmes to improve existing farmer preferred but rosette susceptible varieties example ICGM 33 (Pendo variety). The perfomance observed in this study is high 2.8t/ha compared to 1.5t/ha the standard and current pod yield on National breeding programmes from ICGM 33 (Pendo variety).

3.6 Conclusion

The Groundnut rosette disease is among the biotic constraint for the increased productivity and production of groundnut in Tanzania and efforts to control the disease can be achieved through the use of groundnut varieties with resistance to the disease. Results from evaluation of 220 RILs for resistance to rosette disease at Naliendele shows that there is wide genetic variability for pod yield among the 220 RILs (Table 3.1 and Appendix 2) which implies that RILs with high pod yield can be selected and used as varieties for resistance to rosette disease by farmers.

The ICG 12991 and ICGV 86124 resistant and susceptible parents respectively along with 211 Recombinant Inbred Lines (RIL) were inoculated mechanically with rosette virus inoculums in the field and found that no rosette symptoms developed in the resistant parent while symptoms developed on susceptible parent. The development of rosette symptoms on susceptible parent lacking in resistant parent implies that ICG 12991 has the genes for resistance to rosette disease which lacks in ICGV 86124. It is also shows that the rosette virus inoculums used were well isolated and prepared, and also the mechanical inoculation was successful at screening for rosette disease. Hence the breeding programs can use the two parents and mechanical inoculation procedure for breeding groundnut lines with resistance to rosette disease.

Among the 211 RILs evaluated for resistance to rosette disease found that 109 RILs were moderately resistant, 42 RILs highly resistant and 60 RILs were susceptible. This implies that the 42 RILs posses the genes for resistance to rosette disease which lacks in the 60 RILs. Hence the study has been successful at identifying new sources of resistance to groundnut rosette disease to the groundnut breeding programs. To the breeder the knowledge of genetic heritability is very crucial, hence among the 211 RILs evaluated for

resistance to rosette disease, it was found that 151 RILs were resistant while 60 RILs were susceptible to rosette disease which conformed with ratio of 3:1 resistant versus susceptible, concluding that heritability for resistance to rosette disease is through dominant genes. This information is so useful for the groundnut breeder, as it implies that incorporation of resistance genes using the sources identified can be done since the disease is qualitatively inherited. The study, also demonstrated that groundnut rosette disease is negatively correlated with pod yield and seed yield which implies that GRD is responsible for yield reductions observed in some of RILs. Hence for increased groundnut yield the highly resistant but high yielding RILs should be used.

3.7 Recommendations

Since the present study identified 42 Recombinant Inbred Lines which are highly resistant to rosette disease and having high pod yield greater than 1500kg/ha, there is a need for the groundnut breeders in the country to utilize these sources to improve the available farmer preferred groundnut varieties which are well adapted but only susceptible to rosette disease.

Further screening and search for additional source of resistance to rosette virus is highly encouraged so as to incorporate various sources of resistant genes into single susceptible varieties for assurance of durable resistance to groundnut rosette disease.

There may be a need to conduct viroids survey in various regions of the country grown with groundnut to identify if new strains of the virus exist.

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CHAPTER FOUR

4.0 IDENTIFICATION OF DNA MARKERS LINKED TO RESISTANCE TO GROUNDNUT ROSETTE DISEASE FOR GENOTYPING PURPOSES

4.1 Abstract

The use of molecular markers is considered to be most efficient and accurate method for breeding host resistant lines hence identification of molecular markers linked to the trait of interest is the first step towards molecular breeding. In groundnut it has been successful to do molecular breeding using markers linked to traits of interest following the availability of markers linked to various traits such as root knot-nematode, high oleic linoleic acid ratio (L/O), rust resistance, yield and yield parameters, and bacterial wilt resistance. Of recent no report of availability of markers for resistance to groundnut rosette disease. The present study was undertaken to identify molecular markers linked to groundnut rosette disease. A bulk of 15 resistant and 15 susceptible RILs along with resistant and susceptible parents were screened using 30 SSR primer pairs. Based on previous results of rosette scores (chapter 3), Genomic DNA samples from each of the 15 bulks were used. Of the 30 SSR markers, four SSR Markers; pm 36, tc7h11, tc9f04 and t11a02 were polymorphic among the two parents while two SSR markers, tc7h11 and Pm 36 were able to amplify a 400bp and 290bp DNA alleles also observed in both resistant parent and resistant bulk but were not present in susceptible bulk and the susceptible parent. The identified marker can be used by breeding programs during selection to breed for resistance to rosette disease in this population.

4.2 Introduction

Groundnut rosette disease is the major biotic stress capable of causing 100% yield loss limiting groundnut production. The disease is caused by interaction of three components; Groundnut rosette virus (GRV), its satellite RNA (Sat-RNA), and groundnut rosette assistor virus (GRAV). The disease is spread by an aphid vector, *Aphis craccivora* Koch (Waliyar *et al*., 2007). The cost of annual yield losses due to Groundnut Rosette Virus Disease (GRVD) have been estimated as high as US $156 million across Sub-Saharan Africa (Naidu *et al*., 1999).

Genetic studies on GRVD suggest that resistance to this viral disease is complex, polygenic and governed partly by a pair of independent complementary recessive genes (Nigam and Bock, 1990). Okello *et al.* (2010) reported that resistance to GRVD is not simply inherited. However Olorunju (1992) determined inheritance of resistance to Groundnut rosette virus disease, green rosette and chlorotic rosette to be controlled with two recessive genes.

Several studies have demonstrated the utility of molecular markers and marker-assisted selection (MAS) to improve the efficiency of conventional breeding especially in the case of low heritable traits, where phenotypic selection is difficult, expensive, lack accuracy or precision (Varshney *et al*., 2009). Currently breeding for resistance to groundnut rosette disease rely on disease pressure based on disease symptoms which is highly influenced by location, year, season and method of infestation since groundnut rosette disease outbreak is sporadic. Not only that but also identification, multiplication and maintenance of groundnut rosette virus inoculums is not easy, time consuming and costful. In addition selection for lines resistant or susceptible to groundnut rosette disease is difficult based on phenotype alone. At present selection for resistance to groundnut rosette disease can be

improved using molecular markers that are tightly linked to groundnut rosette disease (Pandey *et al*., 2012). To date not a single molecular markers for resistance to groundnut rosette disease available, though several markers linked to resistance to rust, root knot nematode resistance, early and late leaf spot have been identified (Pandey *et al*., 2012). This study is aimed at identifying molecular markers associated with resistance to groundnut rosette disease. The identification of molecular markers will speed up selection and consequent release of farmer preferred varieties with high yielding and resistant to groundnut rosette disease, using Marker assisted selection procedures.

4.3 Materials and Methods

The 220 (F_5) Recombinant Inbred lines were planted for phenotyping at Naliendele Agricultural Research Institute (Mtwara). These planting materials the RILs (Appendix 1) were obtained from ICRISAT Malawi. The 220 RILs were derived from a cross between ICG 12991, a Spanish variety, Groundnut rosette disease resistant female parent and ICGV 86124 a Spanish variety, drought tolerant but rosette disease susceptible. The genotyping were done at Sokoine University of Agriculture (SUA), Crop Science Department, Bean Research Molecular laboratory in 2014. For genotyping purpose, 6 seeds of groundnut per RIL (F_5) were planted at SUA, Crop Museum in 2014. The DNA was extracted from individual RILs including the parents from both susceptible and resistants (Table 4.1 and 4.2).

Table 4.1: Resistant Recombinant Inbred lines used for Genotyping and forming Resistant bulk

S/No	Recombinant Inbred Groundnut Lines	Plants with 1-5 score 1	2	3	4	5	Severity	Remarks
1	ICGX-SM 08035/5/P3-2	6	0	0	0	0	1	Highly resistant
2	ICGX-SM 08036/5/P4-6	6	0	0	0	0	1	Highly resistant
3	ICGX-SM 08035/5/P5-1	6	0	0	0	0	1	Highly resistant
4	ICGX-SM 08035/5/P6-6	6	0	0	0	0	1	Highly resistant
5	ICGX-SM 08035/5/P6-8	6	0	0	0	0	1	Highly resistant
6	ICGX-SM 08035/5/P6-10	6	0	0	0	0	1	Highly resistant
7	ICGX-SM 08035/5/P7-1	6	0	0	0	0	1	Highly resistant
8	ICGX-SM 08035/5/P10-9	6	0	0	0	0	1	Highly resistant
9	ICGX-SM 08035/5/P1-1	6	0	0	0	0	1	Highly resistant
10	ICGX-SM 08035/5/P1-2	6	0	0	0	0	1	Highly resistant
11	ICGX-SM 08035/5/P1-1	6	0	0	0	0	1	Highly resistant
12	ICGX-SM 08035/5/P1-6	6	0	0	0	0	1	Highly resistant
13	ICGX-SM 08035/5/P1-9	6	0	0	0	0	1	Highly resistant
14	ICGX-SM 08035/5/P1-10	6	0	0	0	0	1	Highly resistant
15	ICGX-SM 08036/5/P1-11	6	0	0	0	0	1	Highly resistant
	ICG 12991 (Resistant Parent)	6	0	0	0	0	1	Highly resistant

Table 4.2: Susceptible Recombinant Inbred lines used for Genotyping and forming Susceptible bulk

S/NO	Recombinant Inbred line	Plants with 1-5 score 1	2	3	4	5	Severity	Remarks
1	ICGX-SM 08035/5/P7-14	0	0	2	2	2	5	Susceptible
2	ICGX-SM 08035/5/P7-15	0	1	2	1	2	5	Susceptible
3	ICGX-SM 08035/5/P7-16	0	2	2	1	1	5	Susceptible
4	ICGX-SM 08035/5/P7-17	0	0	2	1	3	5	Susceptible
5	ICGX-SM 08035/5/P7-18	1	1	2	1	1	5	Susceptible
6	ICGX-SM 08035/5/P7-19	0	1	2	1	2	5	Susceptible
7	ICGX-SM 08035/5/P7-10	2	1	2	1	0	4	Susceptible
8	ICGX-SM 08035/5/P10-2	2	1	2	1	0	4	Susceptible
9	ICGX-SM 08035/5/P10-3	2	2	1	1	0	4	Susceptible
10	ICGX-SM 08035/5/P10-4	2	3	1	0	0	4	Susceptible
11	ICGX-SM 08035/5/P18-34	2	3	1	0	0	4	Susceptible
12	ICGX-SM 08035/5/P11-3	2	3	1	0	0	4	Susceptible
13	ICGX-SM 08035/5/P18-33	2	2	2	0	0	4	Susceptible
14	ICGX-SM 08035/5/P11-6	2	2	2	0	0	4	Susceptible
15	ICGX-SM 08035/5/P12-2	2	3	1	0	0	4	Susceptible
	ICGV 86124	0	0	0	2	3	5	**Susceptible**

Following phenotyping, a bulk of 15 susceptible individuals was generated from plants that scored 4/5 on GRD severity while another bulk of 15 resistant individuals was generated from plants that scored 1 on GRD severity. DNA samples from each of these plants were pooled to make susceptible and resistant bulks respectively. The DNA samples were PCR profiled using SSR primers (Table 4.3) by the method described by Doyle and Doyle (1990).

Table 4.3: List of SSR primers used for genotyping 220 RILs

S/No	Marker name	Primer sequence	Linkage group	Tm	Reference
1	pm 3-F	Gaaagaaattatacactccaattatgc	3	52^{0}C	He *et al.,* 2003
	pm 3-R	Cggcatgacagctctatgtt	3	52^{0}C	He *et al.,* 2003
2	pm32-F	Agtgttgggtgtgaaagtgg	2	52^{0}C	He *et al.,* 2003
	pm32-R	Gggactcggaacagtgtttatc	2	52^{0}C	He *et al.,* 2003
3	pm35-F	Tgtgaaaccaaatcactttcattc	5	52^{0}C	He *et al.,* 2003
	pm35-R	Tggtgaaaagaaaggggaaa	5	52^{0}C	He *et al.,* 2003
4	pm36-F	Actcgccatagccaacaaac	5	52^{0}C	He *et al.,* 2003
	pm36-R	Cattcccacaactcccacat	5	52^{0}C	He *et al.,* 2003
5	pm42-F	Acgggccaagtgaagtgat	3	52^{0}C	He *et al.,* 2003
	pm42-R	Tcttgcttctttggtgattagc	3	52^{0}C	He *et al.,* 2003
6	pm45-F	Tgagttgtgacggcttgtgt	5	52^{0}C	He *et al.,* 2003
	pm45-R	Gatgcatgtttagcacacttga	5	52^{0}C	He *et al.,* 2003
7	pm188-F	Gggcttcactgcttttgatt	8	52^{0}C	He *et al.,* 2003
	pm188-R	Tgcgacttctgagaggacaa	8	52^{0}C	He *et al.,* 2003
8	pm204-F	Tgggcctaaacccaacctat	7	52^{0}C	He *et al.,* 2003
	pm204R	Ccacaaacagtgcagcaatc	7	52^{0}C	He *et al.,* 2003
9	pm238-F	Ctctcctctgctctgcactg	3	52^{0}C	He *et al.,* 2003
	pm238-R	Acaagaacatggggatgaaga	3	52^{0}C	He *et al.,* 2003
10	tc6e01-F	ctccctcgcttcctctttct	A5	52^{0}C	Moretzohn *et al.,* 2005
	tc6e01-R	acgcattaaccacacaccaa	A5	52^{0}C	Moretzohn *et al.,* 2005
11	tc6g09-F	Ggaggttgcatgcatcatagt	A7	520C	Moretzohn *et al.,* 2005
	tc6g09-R	Tcattgaacgtatttgaaagctc	A7	52^{0}C	Moretzohn *et al.,* 2005
12	tc6h03-F	Tcacaatcagagctccaacaa	A8	52^{0}C	Moretzohn *et al.,* 2005
	tc6h03-R	Caggttcaccaggaacgagt	A8	52^{0}C	Moretzohn *et al.,* 2005
13	tc7a02-F	Cgaaaacgacactatgaaactgc	A8	52^{0}C	Moretzohn *et al.,* 2005
	tc7a02-R	Ccttggcttacacgacttcct	A8	52^{0}C	Moretzohn *et al.,* 2005
14	tc7c06-F	Ggcaggggaataaaactactaact	A6	52^{0}C	Moretzohn *et al.,* 2005
	tc7c06-R	Ttttccttccttctcctttgtc	A6	52^{0}C	Moretzohn *et al.,* 2005
15	tc7e04-F	Gaaggaccccatctattcaaa	A3	52^{0}C	Moretzohn *et al.,* 2005
	tc7e04-R	Tccgatttctctctctctctc	A3	52^{0}C	Moretzohn *et al.,* 2005
16	tc7g10-F	Aatggggttcacaagagagaga	A4	52^{0}C	Moretzohn *et al.,* 2005
	tc7g10-R	Ccagccatgcactcatagaata	A4	52^{0}C	Moretzohn *et al.,* 2005
17	tc7h02-F	Tcaggataatgacagagtgagt	A9	52^{0}C	Moretzohn *et al.,* 2005
	tc7h02-R	Ggaagaagacctttgatgag	A9	52^{0}C	Moretzohn *et al.,* 2005
18	tc7h09-F	Aactttatgccagtcccctctt	A1	52^{0}C	Moretzohn *et al.,* 2005
	tc7h09-R	Ggatgatgacaagggtgatttc	A1	52^{0}C	Moretzohn *et al.,* 2005
19	tc7h11-F	Aggttggaactatggctgattg	A2	520C	Moretzohn *et al.,* 2005
	tc7h11-R	Ccagtttagcatgtgtggttca	A2	52^{0}C	Moretzohn *et al.,* 2005
20	tc9b08-F	Ggttgggttgagaacaagg	A1	52^{0}C	Moretzohn *et al.,* 2005
	tc9b08-R	Accctcaccactaactccatta	A1	52^{0}C	Moretzohn *et al.,* 2005
21	tc9b12-F	Ggctgggctatgttgatgt	A1	52^{0}C	Moretzohn *et al.,* 2005
	tc9b12-R	Tgcagtacctaaaccaccactac	A1	52^{0}C	Moretzohn *et al.,* 2005
22	tc9e08-F	Gaaacagccgcgagagaa	A4	52^{0}C	Moretzohn *et al.,* 2005
	tc9e08-R	Ccctaacctctcttcattgtgc	A4	52^{0}C	Moretzohn *et al.,* 2005
23	tc9f04-F	Cctaaacaacgacaaacactca	A8	52^{0}C	Moretzohn *et al.,* 2005
	tc9f04-R	Aagcacaacacagaaccctaaa	A8	52^{0}C	Moretzohn *et al.,* 2005
24	tc9f10-F	Atcacaatcacagctccaacaa	A8	52^{0}C	Moretzohn *et al.,* 2005
	tc9f10-R	Ggcaagtctaatctcctttcca	A8	52^{0}C	Moretzohn *et al.,* 2005
25	tc9h08-F	Gccaaaggggaccataaac	A7	52^{0}C	Moretzohn *et al.,* 2005
	tc9h08-R	Tccatcttccatctcatccac	A7	52^{0}C	Moretzohn *et al.,* 2005
26	tc11a02-F	Aatcggaatggcaagagaca	A2	52^{0}C	Moretzohn *et al.,* 2005
	tc11a02-R	Agagcaaagggcgaatctatg	A2	52^{0}C	Moretzohn *et al.,* 2005
27	tc11a04-F	Actctgcatggatggctacag	A6	52^{0}C	Moretzohn *et al.,* 2005
	tc11a04-R	Catgttcggtttcaagtctcaa	A6	52^{0}C	Moretzohn *et al.,* 2005
28	rm15c11-F	Ggactgaacatccggcac	A8	52^{0}C	Proite *et al.,* 2007
	rm15c11-R	Ggaccaaatgactgctctctct	A8	52^{0}C	Proite *et al.,* 2007
29	rm11h06-F	Tcaagggtccactaataagacca	A8	520C	Proite *et al.,* 2007
	rm11h06-R	Tgcaactgataaggaagctgaa	A8	52^{0}C	Proite *et al.,* 2007
30	rm5g08-F	Atagtccatgatagccccatgt	A8	52^{0}C	Proite *et al.,* 2007
	rm5g08-R	Ttacaaccgaatctgcaaagac	A8	52^{0}C	Proite *et al.,* 2007

Approximately 100 mg of young leaves from 2 weeks old young groundnut leaves were collected from the field in 1.5 ml Eppendorf tube. Then 500 µl of CTAB buffer (1 M Tris HCl pH 8.0, 5 M NaCl, of 0.5 M EDTA, 20 g of CTAB salt with addition of 0.08g of polyvinylpyrrolidone were added into the eppendorf tube, followed by grinding the sample using the Teflon pastle. The leaf materials were homogenized by placing into the water bath at 65^0C for 1hour with occasional shaking after every 15 minutes. The sample was removed from the water bath, on fume hood chamber man equal volume of chloroform: Isoamyl alcohol (24:1) was added and mixed by gently shaking tubes. Centrifugation was done for 7 minutes at maximum speed. The aqueous phase was transferred into the new labeled tube. The 0.08 volumes cold 7.5 M ammonium acetate was added followed by addition of 0.54 volumes of cold isopropanol. Then it was mixed by inverting tubes 20-30 times. Incubatation on ice for 30-40 minutes. Centrifugation for 3 minutes at maximum speed. The supernatant was discarded followed by addition of 700 ul 70% Ethanol and invertion of tubes 5-10 times. Centrifugation for 1 minute at maximum speed. The supernatant discarded again followed by addition of 700 ul 95% Ethanol, tubes inverted 5-10 times. Centrifugation for 1 minute at maximum speed. Finally the Supernatant was discarded and the tubes were Inverted on a clean kimwipe and allowed to dry for 10-15 minutes upside down, or until pellet looks dry. The DNA pellets were hydrated with 50uL TE buffer (1 M Tris HCl pH 8.0, 0.5 M EDTA) and allowed to resuspend overnight at room temperature. The quality and concentration of the extracted DNA was determined using 1% Agarose in 1X TBE buffer (Tris base, Boric acid, EDTA and Distilled water). 6ul DNase free water was mixed with 2 ul loading dye and genomic DNA was 2ul, the mixture was prepared on paraffin paper and loaded into gel wells 6ul along with lamda DNA 25ng and 50ng. The voltage was 100v for 30minutes, followed by staining in eithidium bromide (0.5ug/ml) for 15minutes followed by photographing the gel under UV-Transilluminator with canon camera mounted on

Camera hood. The extracted DNA was of high quality following the sharp, bright and clear bands observed which allowed the extracted DNA to be used for PCR.

The concentration of the extracted DNA was estimated to be 10-15ng. Based on the score results, 15 RILs most resistant to groundnut rosette disease (GRD) and 15 most susceptible RILs to GRD, including the resistant parent and susceptible parent were used for genotyping. The individual DNA from 15 RILs was bulked together by mixing 10ul from each 15 RILs forming the susceptible bulk and resistant bulk. 30 SSR primers (Table 4.3 above) were used to amplify the bulks and parents resistant and susceptible respectively so as to determine SSR markers linked to resistance in groundnut rosette disease. Each 20ul of reaction mixture contained 30ng of groundnut genomic DNA, 10uM of each primer pair, 10x PCR buffer, 25mM $Mgcl_2$, 10mM dNTPs, 0.2 units of Taq DNA polymerase.

PCR amplification profiles consisted of 4 minutes of denaturation at 94^0C, 32 cycles of 1minute denaturation at 94^0C, 1minute of annealing temperature at 52^0C and 2 minutes extension at 72^0C followed by final 8minutes extension at 72^0C. The amplification was done in the thermocycler (GeneAmp PCR system 9700, Applied Biosystem, Cary California, USA). All PCR products were analyzed in 2% Agarose gels in 1X TBE buffer, with a 100 bp (Qiagen). Electrophoresis was perfomed at 100v for 2: 30 hours, followed by staining the gel in the eithidium bromide with 0.5g/1ml concentration for 15 minutes. The gel was then placed in Uv-Transilluminator and canon Camera was placed on Camera hood and finally the gel was photographed. The gel were scored for presence of bands by recordings as “1” and absence of bands by recordings as “0” among the parents and bulks.

4.4 Results

The amplification was observed on ten markers (Table 4.4) of 30 SSR markers used in this study.

Table 4.4: Amplification of four resistant and susceptible bulks and parents

SSR markers	Size in bp	Rp	Sp	Rb1	Rb2	RB3	RB4	Sb1	Sb2	Sb3	Sb4
pm 36	290	1	0	1	1	1	1	1	1	1	0
tc6g09	120	1	1	1	1	0	1	1	0	1	0
tca027	180	0	1	0	1	1	1	0	0	1	0
tc7h11	400	1	0	0	0	1	0	1	0	1	0
tc9b12	190	0	0	1	0	0	1	1	1	1	0
tc9e08	300	1	1	0	1	0	1	1	0	0	1
tc9f04	295	1	0	1	0	1	0	1	1	1	1
tc9f10	300	0	0	1	0	1	1	1	1	1	1
tc11a02	280	1	0	0	1	1	1	1	1	1	1
tc11a04	250	0	0	1	1	1	0	1	1	1	0

Rp=Resistant parent, Sp= Susceptible parent, Rb=Resistant bulk, Sb= Susceptible bulk
1= presence of DNA allele, 0=absence of DNA allele for the marker following PCR amplification.

Four markers (pm 36, tc7h11, tc9f04 and t11a02) gave band on resistant parent but not susceptible parents. Marker pm 36 had DNA fragments on resistant parent and resistant bulks 1, 2, 3 and 4, absent in susceptible parent and susceptible bulk 4. The marker tc7h11 had DNA fragments on resistant parent and resistant bulk 3, absent in susceptible parent and susceptible bulks 2 and 4. Marker tc9f04 had DNA fragments on resistant parent and resistant bulks, 1 and 3, which were also present on susceptible bulks 1, 2, 3 and 4 but not on susceptible parent. Marker t11a02 had DNA fragments on resistant parent and resistant bulks 2, 3 and 4, which were also present in susceptible bulks 1, 2, 3 and 4, but not on susceptible parent.

Of the four markers polymorphic among the parents markers, pm 36 and tc7h11 were polymorphic among the two parents and at least one bulks (Plates 4.1 and 4.2). However from this study, marker tc7a02 had DNA fragments on susceptible parent and susceptible bulks 3 absent in resistant parent and resistant bulks 3 (Plates 4.3).

Plate 4.1 Presence of 400bp from marker tc7 h11 in Resistant parent and Resistant bulk 5 but absent in Susceptible parent and Susceptible bulk 8 and 10

1=Resistant Parent, 2=Susceptible parent, 3-6 are Resistant bulks
7-8 are Susceptible bulks, M= 100 DNA base pairs

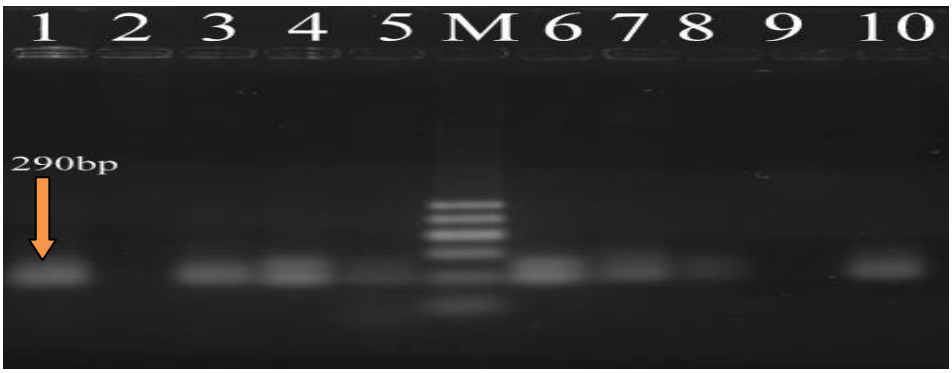

Plate 4.2: Presence of 290bp from marker pm 36 in Resistant parent and Resistant bulks but Absent in Susceptible parent and Susceptible bulk 9.

1= Resistant parent, 2=Susceptible parent, 3-6=Resistant bulks
7-10 =Susceptible bulks, M=100 DNA bp

Plate 4.3: Presence of 180bp from marker t11a02 in Susceptible parent and Susceptible bulks 9 but Absent in Resistant parent and Resistant bulks 3.

1= Resistant parent, 2=Susceptible parent, 3-6=Resistant bulks

7-10 =Susceptible bulks, M=100 DNA bp

Marker Pm 36 from linkage group 5 of cultivated groundnut (AABB genome) with the sequence F: 5'-actcgccatagccaacaaac-3'and R: 3'-cattcccacaactcccacat-5' produced 290bp DNA fragments which were present in resistant parent and all four resistant bulks but was not present in susceptible parent and susceptible bulks 4. Also marker tc7h11 from linkage group A_2 with the sequence 5'-3' aggttggaactatggctgattg and 3'- 5' ccagtttagcatgtgtggttca produced 400bp DNA fragment which were present in resistant parent and resistant bulk 3 absent in susceptible parent and absent in susceptible bulks 2. The total numbers of alleles detected with the 10 SSR primers were 63, with a range of four to eight alleles per primer and a mean of 6.3 alleles. Also the Polymorphic Information content (PIC) of markers ranged from 0.32 to 0.52 (Table 4.5). Markers (pm 36 and tc11a02) detected eight alleles with 0.32 PIC, followed by markers (tc6g09, tc9f04, tc9f10) which detected seven alleles with 0.42 PIC, markers tc9e08 and tc11a04 detected six alleles with 0.52 PIC, and five alleles were detected with markers tc7a02 and tc9b12 with 0.5 PIC, while the marker tc7h11 detected four alleles with 0.5 PIC. Three markers (tc9f10, tc9b12, tc11a04) amplified DNA fragments from the bulks not parents whereas two markers (tc6g09, tc9e08) gave amplificons of monomorphic bands between the parents.

Table 4.5: Polymorphic Information content observed on four resistant bulks, four susceptible bulks and parents

SSR marker	Allele size (bp)	No. alleles	Pol. Alleles	Mono. Alleles	Percent Pol.	PIC
pm 36	290	8	8	0	100	0.32
tc6g09	120	7	5	2	71	0.42
tc7a02	180	5	5	0	100	0.5
tc7h11	400	4	4	0	100	0.5
tc9b12	190	5	5	0	100	0.5
tc9e08	300	6	4	2	100	0.52
tc9f04	295	7	7	0	67	0.42
tc9f10	300	7	7	0	100	0.42
tc11a02	280	8	8	0	100	0.32
tc11a04	250	6	6	0	100	0.52
Total	2605	63	59	4	938	
Mean	260.5	6.3	5.9	0.4	93.8	0.44

No. allele = Total number of alleles amplified, Pol. Allele = Number of polymorphic alleles, Mono. Alleles = Total number of monomerphic alleles, Perent. Pol= Percent Polymorphism, PIC = Polymorphic Information content

4.5 Discussion

The marker tc7h11 amplified 400bp DNA fragment on resistant parent and resistant bulk 5 (Plate 4.1) absent in susceptible parent and susceptible bulks 8 and 10. This implies that marker tc7h11 is linked to GRD resistance. The marker pm 36, amplified 290bp DNA fragment on resistant parent and resistant bulks 3, 4, 5 and 6 (plate 4.2) absent in susceptible parent and susceptible bulks 9. This marker is also linked to GRD resistance. Hence, the two markers can be used to distinguish resistant progenie/plants from susceptible ones during breeding for GRD resistance. However, inconsistent amplification of markers was observed. For example, marker tc7h11 amplified 400 DNA fragments on susceptible bulks 7 and 8, while no amplification were observed on resistant bulks 3, 4 and 6. This implies that, probably some individual recombinant inbred lines in susceptible bulks 7 and 8 though phenotypically scored as susceptible to groundnut rosette disease they were heterogenic alleles for resistant to groundnut rosette disease. Altinkut (2003) reported that scoring the disease visually basing on the disease symptoms is not always 100% accurate due to environmental and observational variation.

On the other hand marker pm 36 amplified 290 DNA fragment on susceptible bulks 7, 8 and 10. This implies that probably susceptible bulks did not certainly express high level of susceptible scores of 5 as compared to parent. It can be recalled that some of classified resistant by virtual absent of symptoms might be a failure of inoculation of the virus. Failure of inoculations leading to lack of expression of symptoms has been observed in other similar assays in plant (Olorunju, 1990). Marker tc7a02 amplified a 180bp DNA fragment on susceptible parent and susceptible bulks 3 which were absent in resistant parent and resistant bulks 1. This implies that this marker is linked with susceptibility gene for GRD resistance. The marker is also useful during during selection of plants resistant to GRD. Marker tc9e08 amplified 300bp DNA fragment on resistant bulks 2 and 4, absent in susceptible bulks 2 and 3 (Table 4.4). This marker is also useful for breeding GRD resistance, since distinguished two resistant bulks from two susceptible bulks.

The Polymorphic Information Content (PIC) is the measure of the usefulness of each marker in distinguishing one individual from another and it is influenced by the number and frequency of alleles (Liu *et al*., 2000). PIC values ranged from 0.32 to 0.52, which implies that the markers used in this study are moderately informative. Five markers tc7h11, tc7a02, tc9b12, tc9e08 and tc11a04 had the highest PIC values of 0.5. The high PIC value indicates that the markers can distinguish high variation among progenies in relation to resistance from susceptible during selection for resistance to resistance in groundnut rosette disease. On the other hand markers pm 36, tc11a02, tc9f04 and tc6g09 had the PIC value below 0.5. But still useful markers for groundnut rosette resistance breeding. The low PIC values are due to small proportion of alleles that distinguish variations among resistant and susceptible to rosette disease and probably can have resulted from misclassification of some lines as resistant phenotypically but genetically susceptible.

Similarly Holeyachi and Savithramma (2013) reported on using 20 RAPD markers to screen $88F_5$ (recombinant inbred lines) obtained a single 700 bp band in the genotype BL 849 (resistant parent) and Mungbean Yellow Mosaic Virus (MYMV) resistant bulk which was absent in Chinamung (susceptible parent) and MYMV susceptible bulk. Indicating that primer UBC 499 was linked to (MYMV) resistance. Hou *et al*. (2007) identified two AFLP markers linked to rust resistance in groundnut using an F_2 population derived from Yuanza 9102 (a rust susceptible and ICGV86699 (a rust resistant). Furthermore, Xia *et al*. (2007) identified two AFLP markers linked to rust resistance in groundnut using an F_2 population derived from the cross of ICGV 86699 (resistant to late leaf spot) and Zhonghua-5 (susceptible to late leaf spot). Hong *et al*. (2009) reported the identification of five SSR markers that was associated with resistance to *Aspergillus flavus* infection in groundnut. One of the markers, pPGSseq19D9, could distinguish all resistant cultivars from susceptible ones. These findings conforms to the present study, that five markers has been identified as useful for genotyping GRD resistance. Similar to this study among the five markers marker tc7h11 successfully distinguished resistant from susceptible parents and also distinguished two susceptible bulks out of four susceptible bulks but also distinguished 1 resistant bulk out of four resistant bulks. The marker pm 36, successfully, identified all the 2 parents and all 4 resistant bulks and 1 susceptible bulk. These markers will be very useful for selection of plant/progenies for GRD resistance in breeding programs.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based from this study it is concluded that it is possible to isolate the groundnut rosette virus from infected groundnut plants for mechanical inoculation when evaluating groundnut for resistance to groundnut rosette disease. The present study using mechanical inoculation successfully screened 211 recombinant inbred lines (RIL) which resulted into 42 RILs classified as highly resistant, 109 RILs Moderately resistant and 60 RILs susceptible. Breeding for disease resistance followed by selecting lines visually for absence or presence of symptoms alone is not always accurate due to induction by host plants symptoms similar to those induced by other pathogens and due to influence of environment or failure of inoculums to produce symptoms on the host. Hence the selection for resistance to groundnut rosette disease should be done aided with genetic markers as this will increase accuracy during selection for rosette resistant lines. The SSR marker tc7h11 400bp and Pm 36 290bp are linked to resistance in groundnut rosette disease. For example in this study marker PM 36 were able to amplify 290bp regions in the resistant parent ICGV 12991 which were also present in all four resistant bulks. These markers will be useful in groundnut breeding programs for breeding and selection of resistance to groundnut rosette disease.

5.2 Recommendations

More confirmatory results may confirm by screening individual line from the 42 RILs classified as resistant and also challenging the susceptible lines. Confirmation of innoculum in susceptibles is also required to ascertain the fidelity of the linked marker.

For the case of markers tc11a04, tc9b12, tc9f10 although no amplification observed on the parents but observed on the bulks, are still useful to breeders hence more work should be done to verify their usefulness of these markers to other groundnut populations.

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APPENDICES

Appendix 1: List of 220 Recombinant Groundnut Inbred Lines used in the phenotyping resistance to groundnut rosette disease (GRD)

Entry No.	Recombinant Inbred Lines	Pedigrees
1	ICG 12991	ICG 12991 X ICG 86124
2	ICGX-SM 08035/5/P1-1	ICG 12991 X ICG 86124
3	ICGX-SM 08035/5/P1-2	ICG 12991 X ICG 86124
4	ICGX-SM 08035/5/P1-4	ICG 12991 X ICG 86124
5	ICGX-SM 08035/5/P1-6	ICG 12991 X ICG 86124
7	ICGX-SM 08035/5/P1-8	ICG 12991 X ICG 86124
8	ICGX-SM 08035/5/P1-9	ICG 12991 X ICG 86124
9	ICGX-SM 08035/5/P1-10	ICG 12991 X ICG 86124
10	ICGX-SM 08036/5/P1-11	ICG 12991 X ICG 86124
11	ICGX-SM 08035/5/P1-12	ICG 12991 X ICG 86124
12	ICGX-SM 08035/5/P1-14	ICG 12991 X ICG 86124
13	ICGX-SM 08035/5/P1-15	ICG 12991 X ICG 86124
14	ICGX-SM 08035/5/P1-17	ICG 12991 X ICG 86124
15	ICGX-SM 08035/5/P2-1	ICG 12991 X ICG 86124
16	ICGX-SM 08035/5/P3-1	ICG 12991 X ICG 86124
17	ICGX-SM 08035/5/P3-2	ICG 12991 X ICG 86124
18	ICGX-SM 08035/5/P4-1	ICG 12991 X ICG 86124
19	ICGX-SM 08035/5/P4-5	ICG 12991 X ICG 86124
20	ICGX-SM 08035/5/P4-5	ICG 12991 X ICG 86124
21	ICGX-SM 08035/5/P4-4	ICG 12991 X ICG 86124
22	ICGX-SM 08035/5/P4-5	ICG 12991 X ICG 86124
23	ICGX-SM 08036/5/P4-6	ICG 12991 X ICG 86124
24	ICGX-SM 08035/5/P5-1	ICG 12991 X ICG 86124
25	ICGX-SM 08035/5/P6-1	ICG 12991 X ICG 86124
27	ICGX-SM 08035/5/P6-3	ICG 12991 X ICG 86124
28	ICGX-SM 08035/5/P6-4	ICG 12991 X ICG 86124
29	ICGX-SM 08035/5/P6-5	ICG 12991 X ICG 86124
30	ICGX-SM 08035/5/P6-6	ICG 12991 X ICG 86124
31	ICGX-SM 08035/5/P6-7	ICG 12991 X ICG 86124
32	ICGX-SM 08035/5/P6-8	ICG 12991 X ICG 86124
33	ICGX-SM 08035/5/P6-9	ICG 12991 X ICG 86124
34	ICGX-SM 08035/5/P6-10	ICG 12991 X ICG 86124
35	ICGX-SM 08035/5/P6-11	ICG 12991 X ICG 86124
36	ICGX-SM 08035/5/P6-12	ICG 12991 X ICG 86124
37	ICGX-SM 08035/5/P6-13	ICG 12991 X ICG 86124
38	ICGX-SM 08035/5/P6-14	ICG 12991 X ICG 86124
39	ICGX-SM 08035/5/P7-1	ICG 12991 X ICG 86124
40	ICGX-SM 08035/5/P7-2	ICG 12991 X ICG 86124
41	ICGX-SM 08035/5/P7-3	ICG 12991 X ICG 86124
42	ICGX-SM 08035/5/P7-4	ICG 12991 X ICG 86124
43	ICGX-SM 08035/5/P7-5	ICG 12991 X ICG 86124
44	ICGX-SM 08035/5/P7-6	ICG 12991 X ICG 86124

Entry No.	Recombinant Inbred Lines	Pedigrees
45	ICGX-SM 08035/5/P7-7	ICG 12991 X ICG 86124
46	ICGX-SM 08035/5/P18-35	ICG 12991 X ICG 86124
47	ICGX-SM 08035/5/P7-9	ICG 12991 X ICG 86124
48	ICGX-SM 08035/5/P7-10	ICG 12991 X ICG 86124
49	ICGX-SM 08035/5/P7-11	ICG 12991 X ICG 86124
50	ICGX-SM 08035/5/P7-12	ICG 12991 X ICG 86124
51	ICGX-SM 08035/5/P7-13	ICG 12991 X ICG 86124
52	ICGX-SM 08035/5/P7-14	ICG 12991 X ICG 86124
53	ICGX-SM 08035/5/P7-15	ICG 12991 X ICG 86124
54	ICGX-SM 08035/5/P18-37	ICG 12991 X ICG 86124
55	ICGX-SM 08035/5/P8-2	ICG 12991 X ICG 86124
56	ICGX-SM 08035/5/P8-3	ICG 12991 X ICG 86124
57	ICGX-SM 08035/5/P8-5	ICG 12991 X ICG 86124
58	ICGX-SM 08035/5/P9-1	ICG 12991 X ICG 86124
59	ICGX-SM 08035/5/P9-2	ICG 12991 X ICG 86124
60	ICGX-SM 08035/5/P9-3	ICG 12991 X ICG 86124
61	ICGX-SM 08035/5/P9-4	ICG 12991 X ICG 86124
63	ICGX-SM 08035/5/P9-6	ICG 12991 X ICG 86124
64	ICGX-SM 08035/5/P9-7	ICG 12991 X ICG 86124
65	ICGX-SM 08035/5/P9-8	ICG 12991 X ICG 86124
66	ICGX-SM 08035/5/P9-9	ICG 12991 X ICG 86124
67	ICGX-SM 08035/5/P9-10	ICG 12991 X ICG 86124
68	ICGX-SM 08035/5/P10-2	ICG 12991 X ICG 86124
69	ICGX-SM 08035/5/P10-3	ICG 12991 X ICG 86124
70	ICGX-SM 08035/5/P10-4	ICG 12991 X ICG 86124
72	ICGX-SM 08035/5/P10-6	ICG 12991 X ICG 86124
73	ICGX-SM 08035/5/P10-8	ICG 12991 X ICG 86124
74	ICGX-SM 08035/5/P10-9	ICG 12991 X ICG 86124
75	ICGX-SM 08035/5/P18-34	ICG 12991 X ICG 86124
76	ICGX-SM 08036/5/P10-13	ICG 12991 X ICG 86124
77	ICGX-SM 08035/5/P11-1	ICG 12991 X ICG 86124
78	ICGX-SM 08035/5/P11-2	ICG 12991 X ICG 86124
79	ICGX-SM 08035/5/P11-3	ICG 12991 X ICG 86124
80	ICGX-SM 08035/5/P11-4	ICG 12991 X ICG 86124
81	ICGX-SM 08035/5/P18-33	ICG 12991 X ICG 86124
82	ICGX-SM 08035/5/P11-6	ICG 12991 X ICG 86124
83	ICGX-SM 08036/5/P11-7	ICG 12991 X ICG 86124
84	ICGX-SM 08035/5/P11-8	ICG 12991 X ICG 86124
85	ICGX-SM 08035/5/P11-9	ICG 12991 X ICG 86124
86	ICGX-SM 08035/5/P12-8	ICG 12991 X ICG 86124
87	ICGX-SM 08035/5/P12-2	ICG 12991 X ICG 86124
88	ICGX-SM 08035/5/P12-3	ICG 12991 X ICG 86124
89	ICGX-SM 08035/5/P12-4	ICG 12991 X ICG 86124
90	ICGX-SM 08035/5/P18-27	ICG 12991 X ICG 86124
91	ICGX-SM 08035/5/P12-6	ICG 12991 X ICG 86124
92	ICGX-SM 08035/5/P12-7	ICG 12991 X ICG 86124
93	ICGX-SM 08035/5/P12-8	ICG 12991 X ICG 86124
94	ICGX-SM 08035/5/P12-9	ICG 12991 X ICG 86124

Entry No.	Recombinant Inbred Lines	Pedigrees
95	ICGX-SM 08035/5/P14-1	ICG 12991 X ICG 86124
96	ICGX-SM 08035/5/P14-2	ICG 12991 X ICG 86124
97	ICGX-SM 08035/5/P14-3	ICG 12991 X ICG 86124
98	ICGX-SM 08035/5/P14-4	ICG 12991 X ICG 86124
99	ICGX-SM 08035/5/P14-5	ICG 12991 X ICG 86124
100	ICGX-SM 08035/5/P14-6	ICG 12991 X ICG 86124
102	ICGX-SM 08035/5/P15-2	ICG 12991 X ICG 86124
103	ICGX-SM 08035/5/P15-3	ICG 12991 X ICG 86124
105	ICGX-SM 08035/5/P15-5	ICG 12991 X ICG 86124
106	ICGX-SM 08035/5/P15-6	ICG 12991 X ICG 86124
107	ICGX-SM 08035/5/P18-40	ICG 12991 X ICG 86124
108	ICGX-SM 08035/5/P18-42	ICG 12991 X ICG 86124
109	ICGX-SM 08035/5/P15-10	ICG 12991 X ICG 86124
110	ICGX-SM 08035/5/P15-11	ICG 12991 X ICG 86124
111	ICGX-SM 08035/5/P15-12	ICG 12991 X ICG 86124
112	ICGX-SM 08035/5/P18-39	ICG 12991 X ICG 86124
113	ICGX-SM 08035/5/P18-1	ICG 12991 X ICG 86124
114	ICGX-SM 08035/5/P18-2	ICG 12991 X ICG 86124
115	ICGX-SM 08035/5/P18-38	ICG 12991 X ICG 86124
116	ICGX-SM 08035/5/P18-4	ICG 12991 X ICG 86124
117	ICGX-SM 08035/5/P18-5	ICG 12991 X ICG 86124
118	ICGX-SM 08035/5/P18-6	ICG 12991 X ICG 86124
119	ICGX-SM 08035/5/P18-7	ICG 12991 X ICG 86124
120	ICGX-SM 08036/5/P18-8	ICG 12991 X ICG 86124
121	ICGX-SM 08035/5/P18-9	ICG 12991 X ICG 86124
122	ICGX-SM 08035/5/P18-10	ICG 12991 X ICG 86124
123	ICGX-SM 08035/5/P18-11	ICG 12991 X ICG 86124
125	ICGX-SM 08035/5/P19-1	ICG 12991 X ICG 86124
126	ICGX-SM 08035/5/P19-2	ICG 12991 X ICG 86124
127	ICGX-SM 08035/5/P19-3	ICG 12991 X ICG 86124
128	ICGX-SM 08035/5/P19-4	ICG 12991 X ICG 86124
129	ICGX-SM 08035/5/P19-5	ICG 12991 X ICG 86124
130	ICGX-SM 08035/5/P21-3	ICG 12991 X ICG 86124
132	ICGX-SM 08036/5/P19-8	ICG 12991 X ICG 86124
133	ICGX-SM 08035/5/P18-32	ICG 12991 X ICG 86124
134	ICGX-SM 08035/5/P18-43	ICG 12991 X ICG 86124
135	ICGX-SM 08035/5/P20-3	ICG 12991 X ICG 86124
136	ICGX-SM 08035/5/P20-4	ICG 12991 X ICG 86124
137	ICGX-SM 08035/5/P20-5	ICG 12991 X ICG 86124
138	ICGX-SM 08035/5/P20-6	ICG 12991 X ICG 86124
139	ICGX-SM 08035/5/P20-7	ICG 12991 X ICG 86124
140	ICGX-SM 08035/5/P20-8	ICG 12991 X ICG 86124
141	ICGX-SM 08035/5/P21-1	ICG 12991 X ICG 86124
142	ICGX-SM 08035/5/P21-2	ICG 12991 X ICG 86124
143	ICGX-SM 08035/5/P21-3	ICG 12991 X ICG 86124
144	ICGX-SM 08035/5/P21-4	ICG 12991 X ICG 86124
146	ICGX-SM 08035/5/P21-6	ICG 12991 X ICG 86124
147	ICGX-SM 08035/5/P21-7	ICG 12991 X ICG 86124

Entry No.	Recombinant Inbred Lines	Pedigrees
148	ICGX-SM 08035/5/P21-8	ICG 12991 X ICG 86124
149	ICGX-SM 08035/5/P21-9	ICG 12991 X ICG 86124
150	ICGX-SM 08035/5/P21-10	ICG 12991 X ICG 86124
151	ICGX-SM 08035/5/P21-11	ICG 12991 X ICG 86124
152	ICGX-SM 08035/5/P21-12	ICG 12991 X ICG 86124
153	ICGX-SM 08035/5/P18-41	ICG 12991 X ICG 86124
154	ICGX-SM 08035/5/P22-2	ICG 12991 X ICG 86124
155	ICGX-SM 08035/5/P22-3	ICG 12991 X ICG 86124
156	ICGX-SM 08035/5/P22-4	ICG 12991 X ICG 86124
157	ICGX-SM 08035/5/P22-4	ICG 12991 X ICG 86124
158	ICGX-SM 08035/5/P22-7	ICG 12991 X ICG 86124
160	ICGX-SM 08035/5/P22-9	ICG 12991 X ICG 86124
161	ICGX-SM 08035/5/P22-10	ICG 12991 X ICG 86124
162	ICGX-SM 08036/5/P1-4	ICG 12991 X ICG 86124
163	ICGX-SM 08036/5/P1-2	ICG 12991 X ICG 86124
164	ICGX-SM 08036/5/P2-2	ICG 12991 X ICG 86124
165	ICGX-SM 08036/5/P1-4	ICG 12991 X ICG 86124
166	ICGX-SM 08036/5/P4-7	ICG 12991 X ICG 86124
167	ICGX-SM 08036/5/P6-15	ICG 12991 X ICG 86124
168	ICGX-SM 08036/5/P8-1	ICG 12991 X ICG 86124
169	ICGX-SM 08036/5/P11-2	ICG 12991 X ICG 86124
170	ICGX-SM 08036/5/P11-8	ICG 12991 X ICG 86124
171	ICGX-SM 08036/5/P15-1	ICG 12991 X ICG 86124
172	ICGX-SM 08036/5/P1-11	ICG 12991 X ICG 86124
173	ICGX-SM 08036/5/P16-5	ICG 12991 X ICG 86124
174	ICGX-SM 08036/5/P17-1	ICG 12991 X ICG 86124
175	ICGX-SM 08036/5/P18-1	ICG 12991 X ICG 86124
176	ICGX-SM 08036/5/P19-7	ICG 12991 X ICG 86124
177	ICGX-SM 08036/5/P22-4	ICG 12991 X ICG 86124
178	ICGX-SM 08036/5/P22-8	ICG 12991 X ICG 86124
179	ICGX-SM 08036/5/P22-9	ICG 12991 X ICG 86124
184	ICGX-SM 08036/5/P1-9	ICG 12991 X ICG 86124
185	ICGX-SM 08036/5/P2-1	ICG 12991 X ICG 86124
186	ICGX-SM 08036/5/P5-2	ICG 12991 X ICG 86124
188	ICGX-SM 08036/5/P5-8	ICG 12991 X ICG 86124
190	ICGX-SM 08036/5/P8-12	ICG 12991 X ICG 86124
192	ICGX-SM 08036/5/P10-5	ICG 12991 X ICG 86124
196	ICGX-SM 08035/5/P15-6	ICG 12991 X ICG 86124
197	ICGX-SM 08036/5/P5-7	ICG 12991 X ICG 86124
199	ICGX-SM 08036/5/P19-1	ICG 12991 X ICG 86124
208	ICGX-SM 08036/5/P23-4	ICG 12991 X ICG 86124
209	ICGX-SM 08035/5/P18-44	ICG 12991 X ICG 86124
210	ICGX-SM 08036/5/P24-3	ICG 12991 X ICG 86124
211	ICGX-SM 08036/5/P3-3	ICG 12991 X ICG 86124
212	ICGX-SM 08036/5/P4-5	ICG 12991 X ICG 86124
215	ICGX-SM 08036/5/P5-10	ICG 12991 X ICG 86124
216	ICGX-SM 08036/5/P5-11	ICG 12991 X ICG 86124
217	ICGX-SM 08036/5/P5-12	ICG 12991 X ICG 86124

Entry No.	Recombinant Inbred Lines	Pedigrees
218	ICGX-SM 08036/5/P6-15	ICG 12991 X ICG 86124
220	ICGX-SM 08036/5/P6-13	ICG 12991 X ICG 86124
222	ICGX-SM 08036/5/P21-9	ICG 12991 X ICG 86124
223	ICGX-SM 08036/5/P13-1	ICG 12991 X ICG 86124
226	ICGX-SM 08036/5/P17-2	ICG 12991 X ICG 86124
228	ICGX-SM 08036/5/P20-5	ICG 12991 X ICG 86124
230	ICGX-SM 08036/5/P1-10	ICG 12991 X ICG 86124
231	ICGX-SM 08036/5/P3-1	ICG 12991 X ICG 86124
232	ICGX-SM 08036/5/P4-2	ICG 12991 X ICG 86124
233	ICGX-SM 08036/5/P5-3	ICG 12991 X ICG 86124
234	ICGX-SM 08036/5/P8-2	ICG 12991 X ICG 86124
235	ICGX-SM 08036/5/P9-2	ICG 12991 X ICG 86124
238	ICGX-SM 08036/5/P13-7	ICG 12991 X ICG 86124
242	ICGX-SM 08036/5/P24-6	ICG 12991 X ICG 86124
252	ICGX-SM 08036/5/P10-9	ICG 12991 X ICG 86124
254	ICGX-SM 08036/5/P1-5	ICG 12991 X ICG 86124
255	ICGX-SM 08036/5/P5-1	ICG 12991 X ICG 86124
260	ICGX-SM 08036/5/P9-12	ICG 12991 X ICG 86124
261	ICGX-SM 08036/5/P11-3	ICG 12991 X ICG 86124
265	ICGX-SM 08036/5/P15-7	ICG 12991 X ICG 86124
266	ICGX-SM 08036/5/P3-2	ICG 12991 X ICG 86124
267	ICGX-SM 08036/5/P5-6	ICG 12991 X ICG 86124
273	ICGX-SM 08036/5/P17-5	ICG 12991 X ICG 86124
274	ICGX-SM 08036/5/P15-3	ICG 12991 X ICG 86124
281	ICGX-SM 08036/5/P19-5	ICG 12991 X ICG 86124
283	ICGX-SM 08035/5/P18-36	ICG 12991 X ICG 86124
285	ICGX-SM 08036/5/P9-10	ICG 12991 X ICG 86124
289	ICGX-SM 08036/5/P6-1	ICG 12991 X ICG 86124
290	ICGX-SM 08036/5/P11-1	ICG 12991 X ICG 86124
291	ICGX-SM 08036/5/P17-4	ICG 12991 X ICG 86124
296	ICGX-SM 08036/5/P4-1	ICG 12991 X ICG 86124
297	ICGX-SM 08035/5/P5-14	ICG 12991 X ICG 86124
301	ICGX-SM 08036/5/P1-3	ICG 12991 X ICG 86124
304	ICGV 86124	ICG 12991 X ICG 86124

Appendix 2: **The mean Pod yield (kg/ha), Pod yield/plant, Pod number/plant, severity and Rosette incidence of 220 Groundnut Recombinant Inbred Lines evaluated at Naliendele Agricultural Research Institute, Mtwara, Tanzania February – June 2013**

Recombinant Inbred Lines	Severity	Incidence %	Height (cm)	PN/p	Pod Yield kg/ha	PY/p (g)	Disease status
ICGX-SM 08035/5/P1-1	1.01	11.44	21.10	14.14	1371.00	6.86	Highly resistant
ICGX-SM 08035/5/P1-2	1.01	0.52	20.61	11.23	964.00	4.82	Highly resistant
ICGX-SM 08035/5/P1-4	1.00	0.44	21.37	13.92	1081.00	5.41	Highly resistant
ICGX-SM 08035/5/P1-6	0.99	0.22	24.36	19.01	1151.00	5.75	Highly resistant
ICGX-SM 08035/5/P1-8	1.02	0.28	20.39	22.76	1091.00	5.46	Highly resistant
ICGX-SM 08035/5/P1-9	1.02	0.81	23.27	15.65	1070.00	5.35	Highly resistant
ICGX-SM 08035/5/P1-10	0.99	0.17	25.21	19.51	1321.00	6.61	Highly resistant
ICGX-SM 08036/5/P1-11	1.01	0.34	27.94	17.10	1116.00	5.58	Highly resistant
ICGX-SM 08035/5/P1-12	1.00	0.48	14.41	19.05	850.00	4.25	Highly resistant
ICGX-SM 08035/5/P1-14	0.98	1.00	25.80	19.02	1180.00	5.90	Highly resistant
ICGX-SM 08035/5/P1-15	0.98	1.76	23.61	19.10	1298.00	6.49	Highly resistant
ICGX-SM 08035/5/P1-17	1.01	0.74	24.22	17.19	1310.00	6.55	Highly resistant
ICGX-SM 08035/5/P2-1	1.01	0.57	23.34	19.06	1237.00	6.19	Highly resistant
ICGX-SM 08035/5/P3-1	1.00	0.49	26.97	18.31	1566.00	7.83	Highly resistant
ICGX-SM 08035/5/P3-2	1.00	0.71	17.93	15.17	891.00	4.46	Highly resistant
ICGX-SM 08035/5/P4-1	0.97	1.55	16.59	13.48	517.00	2.58	Highly resistant
ICGX-SM 08035/5/P4-5	1.02	1.56	23.74	20.46	1649.00	8.25	Highly resistant
ICGX-SM 08035/5/P4-5	1.00	0.35	23.10	19.62	1132.00	5.66	Highly resistant
ICGX-SM 08035/5/P4-4	1.00	0.75	24.33	17.51	1109.00	5.54	Highly resistant
ICGX-SM 08035/5/P4-5	1.01	1.39	22.73	14.63	1260.00	6.30	Highly resistant
ICGX-SM 08036/5/P4-6	1.00	0.07	27.02	17.16	1008.00	5.04	Highly resistant
ICGX-SM 08035/5/P5-1	1.01	0.53	24.51	13.44	1008.00	5.04	Highly resistant
ICGX-SM 08035/5/P6-1	0.98	1.41	29.56	17.59	1112.00	5.56	Highly resistant
ICGX-SM 08035/5/P6-3	1.01	0.52	28.46	22.57	1575.00	7.88	Highly resistant
ICGX-SM 08035/5/P6-4	1.00	0.50	22.66	15.76	852.00	4.26	Highly resistant
ICGX-SM 08035/5/P6-5	1.01	0.18	26.59	13.21	1447.00	7.24	Highly resistant
ICGX-SM 08035/5/P6-6	1.00	0.72	25.13	19.29	1496.00	7.48	Highly resistant
ICGX-SM 08035/5/P6-7	1.01	0.03	19.07	17.60	1112.00	5.56	Highly resistant
ICGX-SM 08035/5/P6-8	0.97	1.74	22.08	18.00	911.00	4.56	Highly resistant
ICGX-SM 08035/5/P6-9	1.01	0.25	21.49	14.76	1342.00	6.71	Highly resistant
ICGX-SM 08035/5/P6-10	1.00	0.54	21.56	12.97	827.00	4.13	Highly resistant
ICGX-SM 08035/5/P6-11	1.00	0.50	23.69	15.80	1433.00	7.17	Highly resistant
ICGX-SM 08035/5/P6-12	1.01	0.11	22.43	15.44	830.00	4.15	Highly resistant
ICGX-SM 08035/5/P6-13	1.01	0.37	26.71	17.17	1326.00	6.63	Highly resistant
ICGX-SM 08035/5/P6-14	0.99	0.01	21.15	15.97	1443.00	7.22	Highly resistant
ICGX-SM 08035/5/P7-1	0.98	0.63	14.72	16.56	1353.00	6.77	Highly resistant
ICGX-SM 08035/5/P7-2	0.99	1.04	20.96	8.47	1024.00	5.12	Highly resistant
ICGX-SM 08035/5/P7-3	1.01	0.45	24.13	11.70	715.00	3.58	Highly resistant
ICGX-SM 08035/5/P7-4	1.01	0.33	14.81	20.84	1688.00	8.44	Highly resistant
ICGX-SM 08035/5/P7-5	1.02	0.77	20.33	18.20	1234.00	6.17	Highly resistant
ICGX-SM 08035/5/P7-6	1.01	0.02	19.73	14.73	934.00	4.67	Highly resistant
ICGX-SM 08035/5/P7-7	0.98	1.34	27.42	21.09	2845.00	14.23	Highly resistant
ICGX-SM 08035/5/P18-35	1.02	1.02	17.94	16.42	1488.00	7.44	Highly resistant
ICGX-SM 08035/5/P7-9	0.99	0.07	20.02	22.88	1616.00	8.08	Highly resistant
ICGX-SM 08035/5/P7-10	1.00	0.37	18.38	18.83	1595.00	7.97	Highly resistant
ICGX-SM 08035/5/P7-11	1.00	0.29	24.93	19.65	1339.00	6.69	Highly resistant
ICGX-SM 08035/5/P7-12	1.01	0.31	17.41	12.00	698.00	3.49	Highly resistant
ICGX-SM 08035/5/P7-13	1.01	0.82	18.82	15.01	1338.00	6.69	Highly resistant
ICGX-SM 08035/5/P7-14	0.96	1.55	17.73	18.56	1341.00	6.70	Highly resistant
ICGX-SM 08035/5/P7-15	0.99	0.28	24.15	13.90	1269.00	6.35	Highly resistant
ICGX-SM 08035/5/P18-37	0.99	0.21	21.82	12.49	1029.00	5.15	Highly resistant
ICGX-SM 08035/5/P8-2	0.98	0.06	26.71	17.14	1250.00	6.25	Highly resistant
ICGX-SM 08035/5/P8-3	1.01	0.79	21.65	20.60	1801.00	9.01	Highly resistant
ICGX-SM 08035/5/P8-5	1.01	0.92	20.90	20.68	1588.00	7.94	Highly resistant

Recombinant Inbred Lines	Severity	Incidence %	Height (cm)	PN/p	Pod Yield kg/ha	PY/p (g)	Disease status
ICGX-SM 08035/5/P9-2	1.00	0.47	13.41	18.29	1162.00	5.81	Highly resistant
ICGX-SM 08035/5/P9-3	1.10	5.03	15.99	22.30	1293.00	6.47	Highly resistant
ICGX-SM 08035/5/P9-4	1.01	0.44	22.10	20.34	1671.00	8.36	Highly resistant
ICGX-SM 08035/5/P9-6	1.01	0.52	23.95	20.96	1600.00	8.00	Highly resistant
ICGX-SM 08035/5/P9-7	0.99	0.18	23.39	30.91	2089.00	10.45	Highly resistant
ICGX-SM 08035/5/P9-8	1.02	1.03	23.57	17.69	1423.00	8.12	Highly resistant
ICGX-SM 08035/5/P9-9	0.98	1.57	22.27	14.01	1565.00	7.83	Highly resistant
ICGX-SM 08035/5/P9-10	1.00	0.96	22.56	17.59	1279.00	6.39	Highly resistant
ICGX-SM 08035/5/P10-2	1.03	0.97	15.38	21.35	1297.00	6.49	Highly resistant
ICGX-SM 08035/5/P10-3	0.99	0.32	16.45	14.07	1074.00	5.37	Highly resistant
ICGX-SM 08035/5/P10-4	1.08	0.85	22.02	20.21	1941.00	9.71	Highly resistant
ICGX-SM 08035/5/P10-6	1.01	1.16	16.47	12.96	991.00	4.95	Highly resistant
ICGX-SM 08035/5/P10-8	1.01	0.53	18.91	17.16	1804.00	9.02	Highly resistant
ICGX-SM 08035/5/P10-9	1.00	0.59	24.34	19.87	1245.00	6.23	Highly resistant
ICGX-SM 08035/5/P18-34	1.01	0.32	24.43	21.24	1171.00	5.85	Highly resistant
ICGX-SM 08036/5/P10-13	1.00	0.06	21.83	15.34	1080.00	5.40	Highly resistant
ICGX-SM 08035/5/P11-1	1.02	1.05	21.35	20.93	1407.00	7.04	Highly resistant
ICGX-SM 08035/5/P11-2	1.00	0.31	26.21	17.49	1341.00	6.70	Highly resistant
ICGX-SM 08035/5/P11-3	1.01	0.70	19.21	11.64	713.00	3.56	Highly resistant
ICGX-SM 08035/5/P11-4	0.99	0.40	26.64	23.94	1597.00	7.99	Highly resistant
ICGX-SM 08035/5/P18-33	1.00	0.61	23.75	15.76	1023.00	5.12	Highly resistant
ICGX-SM 08035/5/P11-6	1.01	0.10	24.82	19.80	1181.00	5.90	Highly resistant
ICGX-SM 08036/5/P11-7	1.00	0.02	18.56	16.35	1055.00	5.28	Highly resistant
ICGX-SM 08035/5/P11-8	1.01	0.24	25.17	19.98	1399.00	6.99	Highly resistant
ICGX-SM 08035/5/P11-9	1.00	0.67	20.73	11.13	814.00	4.07	Highly resistant
ICGX-SM 08035/5/P12-4	1.00	0.05	19.83	19.28	923.00	4.62	Highly resistant
ICGX-SM 08035/5/P12-2	1.00	0.71	23.45	18.68	1100.00	5.50	Highly resistant
ICGX-SM 08035/5/P12-3	0.99	0.03	17.86	19.74	918.00	4.59	Highly resistant
ICGX-SM 08035/5/P12-4	1.03	1.59	24.03	15.01	1063.00	5.32	Highly resistant
ICGX-SM 08035/5/P18-27	0.99	0.58	29.70	20.20	1619.00	8.10	Highly resistant
ICGX-SM 08035/5/P12-6	1.00	0.36	26.87	18.17	1039.00	5.19	Highly resistant
ICGX-SM 08035/5/P12-7	1.07	0.75	29.19	15.22	1266.00	6.33	Highly resistant
ICGX-SM 08035/5/P12-8	1.02	1.80	24.84	17.40	1187.00	5.94	Highly resistant
ICGX-SM 08035/5/P12-9	1.09	0.62	29.60	21.24	1383.00	6.92	Highly resistant
ICGX-SM 08035/5/P14-1	0.98	1.40	30.01	20.94	1470.00	7.35	Highly resistant
ICGX-SM 08035/5/P14-2	1.01	0.63	20.12	15.08	702.00	3.51	Highly resistant
ICGX-SM 08035/5/P14-3	0.99	0.43	22.33	15.27	1014.00	5.07	Highly resistant
ICGX-SM 08035/5/P14-4	0.98	1.35	21.04	13.78	1371.00	6.86	Highly resistant
ICGX-SM 08035/5/P14-5	1.01	0.76	18.52	15.56	964.00	4.82	Highly resistant
ICGX-SM 08035/5/P14-6	0.97	1.75	24.57	14.92	883.00	4.42	Highly resistant
ICGX-SM 08035/5/P15-2	1.03	0.81	21.03	19.33	1065.00	5.33	Highly resistant
ICGX-SM 08035/5/P15-3	1.01	0.84	13.81	13.07	625.00	3.13	Highly resistant
ICGX-SM 08035/5/P15-5	0.99	0.43	22.46	21.58	1407.00	7.03	Highly resistant
ICGX-SM 08035/5/P15-6	1.09	7.65	20.41	18.67	1464.00	7.32	Highly resistant
ICGX-SM 08035/5/P18-40	1.02	1.00	21.26	19.09	1342.00	6.71	Highly resistant
ICGX-SM 08035/5/P18-42	1.01	1.12	27.32	17.92	1244.00	6.22	Highly resistant
ICGX-SM 08035/5/P15-10	1.01	0.70	18.54	18.64	1056.00	5.28	Highly resistant
ICGX-SM 08035/5/P15-11	1.01	0.16	26.50	24.57	1247.00	6.24	Highly resistant
ICGX-SM 08035/5/P15-12	0.99	1.54	23.84	21.13	1250.00	6.25	Highly resistant
ICGX-SM 08035/5/P18-39	1.02	1.03	24.55	17.64	1423.00	7.12	Highly resistant
ICGX-SM 08035/5/P18-1	1.00	0.31	21.83	18.88	1466.00	7.33	Highly resistant
ICGX-SM 08035/5/P18-2	1.09	10.30	18.32	16.06	1149.00	5.74	Highly resistant
ICGX-SM 08035/5/P18-38	1.05	0.35	27.88	16.31	1114.00	5.57	Highly resistant
ICGX-SM 08035/5/P18-4	1.00	0.07	20.29	14.52	1115.00	5.58	Highly resistant
ICGX-SM 08035/5/P18-5	0.99	0.83	18.62	9.42	1216.00	6.08	Highly resistant
ICGX-SM 08035/5/P18-6	1.02	1.02	19.73	16.57	1195.00	5.97	Highly resistant
ICGX-SM 08035/5/P18-7	0.98	0.89	16.41	14.58	1610.00	8.05	Highly resistant
ICGX-SM 08036/5/P18-8	1.01	0.24	23.16	11.61	1249.00	6.24	Highly resistant
ICGX-SM 08035/5/P18-9	1.01	0.87	24.02	16.28	1901.00	9.51	Highly resistant
ICGX-SM 08035/5/P18-10	1.01	0.54	18.77	15.05	929.00	4.65	Highly resistant

Recombinant Inbred Lines	Severity	Incidence %	Height (cm)	PN/p	Pod Yield kg/ha	PY/p (g)	Disease status
ICGX-SM 08035/5/P19-1	1.00	0.46	19.82	11.71	1009.00	5.05	Highly resistant
ICGX-SM 08035/5/P19-2	1.00	0.46	23.50	15.97	1069.00	5.35	Highly resistant
ICGX-SM 08035/5/P19-3	1.01	0.20	22.45	12.94	1126.00	5.63	Highly resistant
ICGX-SM 08035/5/P19-4	1.01	0.52	24.63	14.91	1194.00	5.97	Highly resistant
ICGX-SM 08035/5/P19-5	1.08	9.72	22.94	18.67	1425.00	7.13	Highly resistant
ICGX-SM 08035/5/P19-6	1.00	0.22	22.06	16.11	1476.00	7.38	Highly resistant
ICGX-SM 08036/5/P19-8	1.03	1.59	25.03	20.67	1521.00	7.60	Highly resistant
ICGX-SM 08035/5/P18-32	1.00	0.28	22.17	12.14	1168.00	5.84	Highly resistant
ICGX-SM 08035/5/P18-43	1.01	0.58	23.72	12.60	1203.00	6.01	Highly resistant
ICGX-SM 08035/5/P20-3	0.95	2.79	28.28	15.59	1654.00	8.27	Highly resistant
ICGX-SM 08035/5/P20-4	0.99	0.11	21.52	18.50	921.00	4.60	Highly resistant
ICGX-SM 08035/5/P20-5	0.99	0.49	24.66	13.36	1661.00	8.31	Highly resistant
ICGX-SM 08035/5/P20-6	1.00	0.07	16.02	10.83	608.00	3.04	Highly resistant
ICGX-SM 08035/5/P20-7	1.02	1.40	25.64	13.73	1217.00	6.09	Highly resistant
ICGX-SM 08035/5/P20-8	1.10	5.05	24.90	24.68	1118.00	5.59	Highly resistant
ICGX-SM 08035/5/P21-1	1.01	0.52	21.42	18.20	1051.00	5.25	Highly resistant
ICGX-SM 08035/5/P21-2	1.01	0.31	25.26	23.43	1708.00	8.54	Highly resistant
ICGX-SM 08035/5/P21-3	1.01	0.50	17.61	11.32	707.00	3.53	Highly resistant
ICGX-SM 08035/5/P21-4	0.96	2.52	24.88	17.28	1488.00	7.44	Highly resistant
ICGX-SM 08035/5/P21-6	1.01	0.67	27.62	17.04	1088.00	5.44	Highly resistant
ICGX-SM 08035/5/P21-7	1.01	0.87	16.12	6.31	675.00	3.37	Highly resistant
ICGX-SM 08035/5/P21-8	1.01	0.34	18.24	14.82	973.00	4.87	Highly resistant
ICGX-SM 08035/5/P21-9	0.97	1.84	26.06	12.62	1319.00	6.60	Highly resistant
ICGX-SM 08035/5/P21-10	0.99	0.06	15.25	7.47	741.00	3.71	Highly resistant
ICGX-SM 08035/5/P21-11	0.98	0.09	21.88	13.07	1051.00	5.25	Highly resistant
ICGX-SM 08035/5/P21-12	1.02	0.35	19.01	19.26	940.00	4.70	Highly resistant
ICGX-SM 08035/5/P18-41	1.00	0.30	25.96	16.99	991.00	4.95	Highly resistant
ICGX-SM 08035/5/P22-2	1.00	0.39	21.43	12.25	963.00	4.82	Highly resistant
ICGX-SM 08035/5/P22-3	0.99	0.12	22.87	18.39	1257.00	6.29	Highly resistant
ICGX-SM 08035/5/P22-4	1.02	0.20	22.44	14.24	728.00	3.64	Highly resistant
ICGX-SM 08035/5/P22-4	0.98	0.06	26.88	15.81	1441.00	7.20	Highly resistant
ICGX-SM 08035/5/P22-7	1.17	16.49	24.80	16.31	898.00	4.49	Highly resistant
ICGX-SM 08035/5/P22-9	1.00	0.49	17.31	14.15	1022.00	5.11	Highly resistant
ICGX-SM 08035/5/P22-10	1.00	0.76	18.00	15.74	982.00	4.91	Highly resistant
ICGX-SM 08036/5/P1-4	1.00	0.08	26.60	23.80	1082.00	5.41	Highly resistant
ICGX-SM 08036/5/P1-2	1.01	0.71	22.47	17.13	1403.00	7.02	Highly resistant
ICGX-SM 08036/5/P2-2	1.05	4.17	22.51	15.89	1085.00	5.43	Highly resistant
ICGX-SM 08036/5/P1-4	1.01	0.45	21.71	12.60	1189.00	5.94	Highly resistant
ICGX-SM 08036/5/P4-7	1.01	0.41	18.67	13.32	891.00	4.46	Highly resistant
ICGX-SM 08036/5/P6-15	1.00	0.26	26.76	23.20	1409.00	7.04	Highly resistant
ICGX-SM 08036/5/P8-1	1.01	0.47	21.11	19.55	1386.00	6.93	Highly resistant
ICGX-SM 08036/5/P11-2	1.02	1.30	24.05	14.81	1345.00	6.72	Highly resistant
ICGX-SM 08036/5/P11-8	0.98	2.32	20.41	14.28	1444.00	7.22	Highly resistant
ICGX-SM 08036/5/P15-1	1.00	0.45	19.18	10.59	706.00	3.53	Highly resistant
ICGX-SM 08036/5/P1-11	1.00	0.28	24.70	25.41	1031.00	5.16	Highly resistant
ICGX-SM 08036/5/P16-5	1.01	0.92	19.50	10.72	878.00	4.39	Highly resistant
ICGX-SM 08036/5/P17-1	0.98	0.90	23.65	19.20	1413.00	7.06	Highly resistant
ICGX-SM 08036/5/P18-1	1.01	0.56	22.70	20.73	1105.00	5.52	Highly resistant
ICGX-SM 08036/5/P19-7	1.01	0.49	16.95	20.15	1318.00	6.59	Highly resistant
ICGX-SM 08036/5/P22-4	1.00	0.11	16.37	16.53	841.00	4.21	Highly resistant
ICGX-SM 08036/5/P22-8	1.01	0.78	23.45	17.97	1063.00	5.31	Highly resistant
ICGX-SM 08036/5/P22-9	1.01	1.12	18.90	17.46	1310.00	6.55	Highly resistant
ICGX-SM 08036/5/P1-9	1.00	0.59	28.52	20.60	1042.00	5.21	Highly resistant
ICGX-SM 08036/5/P2-1	1.01	0.27	27.41	22.04	1157.00	5.79	Highly resistant
ICGX-SM 08036/5/P5-2	1.01	0.54	33.22	19.83	1367.00	6.83	Highly resistant
ICGX-SM 08036/5/P5-8	0.99	0.06	24.42	10.30	674.00	3.37	Highly resistant
ICGX-SM 08036/5/P8-12	1.00	0.58	23.73	13.82	866.00	4.33	Highly resistant
ICGX-SM 08036/5/P10-5	1.00	0.21	24.07	10.48	830.00	4.15	Highly resistant
ICGX-SM 08035/5/P15-6	0.99	0.46	22.09	10.66	1205.00	6.03	Highly resistant
ICGX-SM 08036/5/P5-7	0.98	1.80	29.01	19.03	1284.00	6.42	Highly resistant
ICGX-SM 08036/5/P19-1	1.02	0.62	15.64	7.52	493.00	2.46	Highly resistant
ICGX-SM 08036/5/P23-4	1.02	0.78	17.22	13.61	886.00	4.43	Highly resistant

Recombinant Inbred Lines	Severity	Incidence %	Height (cm)	PN/p	Pod Yield kg/ha	PY/p (g)	Disease status
ICGX-SM 08036/5/P24-3	1.11	0.13	21.11	17.38	1414.00	7.07	Highly resistant
ICGX-SM 08036/5/P3-3	1.01	0.41	20.75	20.91	1069.00	5.34	Highly resistant
ICGX-SM 08036/5/P4-5	1.00	0.37	22.61	10.77	565.00	2.82	Highly resistant
ICGX-SM 08036/5/P5-10	1.01	0.34	18.86	13.79	628.00	3.14	Highly resistant
ICGX-SM 08036/5/P5-11	1.01	0.20	27.55	15.81	895.00	4.48	Highly resistant
ICGX-SM 08036/5/P5-12	1.00	0.31	17.33	11.88	1075.00	5.37	Highly resistant
ICGX-SM 08036/5/P6-15	1.03	1.06	18.29	13.33	1230.00	6.15	Highly resistant
ICGX-SM 08036/5/P6-13	1.44	10.14	14.45	14.24	654.00	3.27	Highly resistant
ICGX-SM 08036/5/P21-9	1.11	2.68	20.29	12.94	1136.00	5.68	Highly resistant
ICGX-SM 08036/5/P13-1	1.02	1.21	17.82	14.83	1050.00	5.25	Highly resistant
ICGX-SM 08036/5/P17-2	0.99	0.07	14.70	13.44	934.00	4.67	Highly resistant
ICGX-SM 08036/5/P20-5	0.98	1.24	17.12	18.60	2512.00	12.56	Highly resistant
ICGX-SM 08036/5/P1-10	0.99	1.37	22.46	19.03	950.00	4.75	Highly resistant
ICGX-SM 08036/5/P3-1	1.00	0.02	19.71	13.35	1166.00	5.83	Highly resistant
ICGX-SM 08036/5/P4-2	0.99	1.50	19.80	18.93	1221.00	6.11	Highly resistant
ICGX-SM 08036/5/P5-3	1.01	0.93	18.26	17.21	967.00	4.84	Highly resistant
ICGX-SM 08036/5/P8-2	1.01	0.25	18.07	22.29	628.00	3.14	Highly resistant
ICGX-SM 08036/5/P9-2	1.01	0.61	18.55	14.74	1419.00	7.09	Highly resistant
ICGX-SM 08036/5/P13-7	1.01	0.24	17.57	16.66	1036.00	5.18	Highly resistant
ICGX-SM 08036/5/P24-6	1.00	0.12	20.40	9.29	1091.00	5.45	Highly resistant
ICGX-SM 08036/5/P10-9	1.01	0.38	19.69	21.35	1413.00	7.07	Highly resistant
ICGX-SM 08036/5/P1-5	0.99	0.66	21.18	15.31	1083.00	5.42	Highly resistant
ICGX-SM 08036/5/P5-1	1.01	0.86	23.17	21.99	1145.00	5.73	Highly resistant
ICGX-SM 08036/5/P9-12	0.99	0.50	13.46	12.12	631.00	3.16	Highly resistant
ICGX-SM 08036/5/P11-3	0.98	1.80	11.84	10.70	707.00	3.54	Highly resistant
ICGX-SM 08036/5/P15-7	1.00	0.16	17.75	15.42	857.00	4.29	Highly resistant
ICGX-SM 08036/5/P3-2	0.99	0.05	21.06	17.07	880.00	4.40	Highly resistant
ICGX-SM 08036/5/P5-6	0.96	1.72	18.75	16.42	1087.00	5.44	Highly resistant
ICGX-SM 08036/5/P17-5	1.17	0.14	19.51	14.68	768.00	3.84	Highly resistant
ICGX-SM 08036/5/P15-3	1.00	0.63	19.84	16.73	1174.00	5.87	Highly resistant
ICGX-SM 08036/5/P19-5	1.29	30.21	21.35	7.30	1606.00	8.03	Highly resistant
ICGX-SM 08035/5/P18-36	1.01	0.25	18.78	20.80	1406.00	7.03	Highly resistant
ICGX-SM 08036/5/P9-10	1.01	0.37	16.70	13.97	845.00	4.23	Highly resistant
ICGX-SM 08036/5/P6-1	0.97	1.39	21.11	15.34	911.00	4.55	Highly resistant
ICGX-SM 08036/5/P11-1	0.97	2.12	22.73	16.44	1279.00	6.39	Highly resistant
ICGX-SM 08036/5/P17-4	1.00	0.03	18.42	15.86	1115.00	5.58	Highly resistant
ICGX-SM 08036/5/P4-1	1.00	0.17	15.75	8.39	494.00	2.47	Highly resistant
ICGX-SM 08035/5/P5-14	1.11	11.39	20.38	13.56	1006.00	5.03	Highly resistant
ICGX-SM 08036/5/P1-3	1.16	7.84	16.58	5.52	644.00	3.22	Highly resistant
Control check							
ICG 12991 (R)	**0.99**	**1.43**	**26.33**	**23.00**	**1702.00**	**8.51**	
ICGV 86124 (S)	**1.00**	**0.05**	**17.80**	**13.70**	**1118.00**	**5.59**	
Mean	**1.01**	**0.56**	**21.66**	**16.52**	**1170.00**	**5.85**	
LSD 0.05%	**0.12**	**6.24**	**8.43**	**10.05**	**761.90**	**3.81**	
CV%	**7.23**	**655.35**	**23.02**	**36.03**	**38.54**	**38.54**	
Significance Level	NS	NS	**	NS	**	*	

Appendix 3: Disease status, yield and yield components of 211 groundnut Recombinant Inbred Lines evaluated for rosette resistance at Sokoine University of Agriculture, Morogoro, 2013

Recombinant Inbred Lines	Number of pods plant	Severity	Pod yield (kg/ha)	Seed yield kg/ha	Disease status
ICG 12991	17	1	2817	2223.93	Highly resistant
ICGX-SM 08035/5/P3-2	15	1	2487	1985.59	Highly resistant
ICGX-SM 08035/5/P7-1	30	1	2068	1278.47	Highly resistant
ICGX-SM 08035/5/P5-1	17	1	1922	1381.86	Highly resistant
ICGX-SM 08036/5/P4-6	13	1	1882	1448.69	Highly resistant
ICGX-SM 08035/5/P6-6	14	1	1786	1325.38	Highly resistant
ICGX-SM 08035/5/P6-10	15	1	1783	1303.05	Highly resistant
ICGX-SM 08035/5/P1-10	17	1	1691	1185.12	Highly resistant
ICGX-SM 08035/5/P1-1	14	1	1671	1230.18	Highly resistant
ICGX-SM 08035/5/P10-9	11	1	1654	1275.83	Highly resistant
ICGX-SM 08036/5/P1-1	11	1	1644	1275.07	Highly resistant
ICGX-SM 08035/5/P6-8	9	1	1637	1322.68	Highly resistant
ICGX-SM 08035/5/P1-15	16	1	1633	1153.28	Highly resistant
ICGX-SM 08035/5/P1-1	14	1	1603	1159.18	Highly resistant
ICGX-SM 08035/5/P6-11	19	1	1600	1053.39	Highly resistant
ICGX-SM 08036/5/P1-11	13	1	1594	1173.98	Highly resistant
ICGX-SM 08035/5/P1-2	10	1	1590	1254.1	Highly resistant
ICGX-SM 08035/5/P9-6	12	1	1589	1190.73	Highly resistant
ICGX-SM 08035/5/P2-1	16	1	1584	1095.07	Highly resistant
ICGX-SM 08035/5/P4-5	17	1	1581	1082.14	Highly resistant
ICGX-SM 08035/5/P1-9	11	1	1579	1208.88	Highly resistant
ICGX-SM 08035/5/P4-5	17	1	1572	1080.9	Highly resistant
ICGX-SM 08035/5/P6-1	16	1	1559	1076.13	Highly resistant
ICGX-SM 08035/5/P1-17	13	1	1550	1141.28	Highly resistant
ICGX-SM 08035/5/P1-6	10	1	1549	1210.93	Highly resistant
ICGX-SM 08035/5/P7-7	15	1	1506	1048.51	Highly resistant
ICGX-SM 08035/5/P9-2	12	1	1492	1070.71	Highly resistant
ICGX-SM 08035/5/P9-3	16	1	1481	1012.4	Highly resistant
ICGX-SM 08035/5/P6-4	9	1	1475	1067.75	Highly resistant
ICGX-SM 08035/5/P6-5	13	1	1473	1065.85	Highly resistant
ICGX-SM 08035/5/P6-9	13	1	1473	1058.34	Highly resistant
ICGX-SM 08035/5/P7-12	15	1	1461	1022.08	Highly resistant
ICGX-SM 08035/5/P8-3	14	1	1455	1020.21	Highly resistant
ICGX-SM 08035/5/P8-5	14	1	1451	1020.16	Highly resistant
ICGX-SM 08035/5/P7-10	13	1	1449	1036.49	Highly resistant
ICGX-SM 08035/5/P4-5	11	1	1433	1087.41	Highly resistant
ICGX-SM 08035/5/P4-1	10	1	1430	1092.99	Highly resistant
ICGX-SM 08035/5/P6-13	12	1	1429	1051.45	Highly resistant
ICGX-SM 08035/5/P7-4	12	1	1427	1049.61	Highly resistant
ICGX-SM 08035/5/P7-3	12	1	1419	1050.09	Highly resistant
ICGX-SM 08035/5/P9-1	13	1	1411	1019.58	Highly resistant
ICGX-SM 08035/5/P7-5	11	1	1409	1048.8	Highly resistant

Recombinant Inbred Lines	Number of pods plant	Severity	Pod yield (kg/ha)	Seed yield kg/ha	Disease status
ICGX-SM 08035/5/P11-4	14	3	1405	992.02	Moderately resistant
ICGX-SM 08035/5/P11-8	14	3	1400	987.5	Moderately resistant
ICGX-SM 08036/5/P11-7	13	3	1399	990.2	Moderately resistant
ICGX-SM 08035/5/P19-4	16	3	1399	932.32	Moderately resistant
ICGX-SM 08035/5/P10-6	13	3	1395	1001.23	Moderately resistant
ICGX-SM 08035/5/P15-10	15	3	1393	949.97	Moderately resistant
ICGX-SM 08035/5/P18-11	16	3	1393	933.48	Moderately resistant
ICGX-SM 08036/5/P18-8	15	3	1385	939.52	Moderately resistant
ICGX-SM 08035/5/P18-38	15	3	1372	941.39	Moderately resistant
ICGX-SM 08035/5/P21-7	16	3	1369	912.34	Moderately resistant
ICGX-SM 08036/5/P8-1	17	3	1364	895.33	Moderately resistant
ICGX-SM 08035/5/P11-1	11	3	1357	996.87	Moderately resistant
ICGX-SM 08035/5/P15-5	13	3	1353	955.78	Moderately resistant
ICGX-SM 08035/5/P12-7	12	3	1339	968.86	Moderately resistant
ICGX-SM 08035/5/P15-6	12	3	1332	954.08	Moderately resistant
ICGX-SM 08035/5/P11-9	11	3	1329	985.86	Moderately resistant
ICGX-SM 08035/5/P18-39	12	3	1329	947.66	Moderately resistant
ICGX-SM 08035/5/P18-41	14	3	1328	911.42	Moderately resistant
ICGX-SM 08036/5/P15-1	15	3	1328	891.46	Moderately resistant
ICGX-SM 08036/5/P2-2	15	3	1326	899.55	Moderately resistant
ICGX-SM 08035/5/P12-3	11	3	1325	978.21	Moderately resistant
ICGX-SM 08035/5/P21-2	13	3	1313	915.25	Moderately resistant
ICGX-SM 08036/5/P18-1	14	3	1308	886.9	Moderately resistant
ICGX-SM 08035/5/P14-3	11	3	1306	968.58	Moderately resistant
ICGX-SM 08036/5/P6-15	14	3	1305	897.69	Moderately resistant
ICGX-SM 08035/5/P18-2	11	3	1297	944.69	Moderately resistant
ICGX-SM 08035/5/P20-4	12	3	1297	922.26	Moderately resistant
ICGX-SM 08035/5/P6-7	17	3	1287	818.73	Moderately resistant
ICGX-SM 08036/5/P19-7	13	3	1285	886.66	Moderately resistant
ICGX-SM 08036/5/P4-5	14	3	1284	872.24	Moderately resistant
ICGX-SM 08035/5/P7-11	17	3	1280	815.64	Moderately resistant
ICGX-SM 08035/5/P20-8	11	3	1271	920.88	Moderately resistant
ICGX-SM 08035/5/P18-9	10	3	1266	935.6	Moderately resistant
ICGX-SM 08035/5/P21-6	11	3	1256	914.76	Moderately resistant
ICGX-SM 08035/5/P21-12	11	3	1253	912.01	Moderately resistant
ICGX-SM 08035/5/P18-7	10	3	1249	939.92	Moderately resistant
ICGX-SM 08035/5/P22-4	11	3	1247	906.51	Moderately resistant
ICGX-SM 08036/5/P5-2	12	3	1244	885.12	Moderately resistant
ICGX-SM 08035/5/P22-9	11	3	1238	901.16	Moderately resistant
ICGX-SM 08036/5/P3-1	13	3	1238	854.02	Moderately resistant
ICGX-SM 08036/5/P19-8	10	3	1237	927.84	Moderately resistant
ICGX-SM 08035/5/P12-8	15	3	1235	805.85	Moderately resistant
ICGX-SM 08035/5/P21-11	10	3	1233	912.12	Moderately resistant
ICGX-SM 08035/5/P20-6	16	3	1232	791.15	Moderately resistant
ICGX-SM 08036/5/P10-13	15	3	1225	809.57	Moderately resistant

Recombinant Inbred Lines	Number of pods plant	Severity	Pod yield (kg/ha)	Seed yield kg/ha	Disease status
ICGX-SM 08035/5/P3-1	14	3	1222	819.74	Moderately resistant
ICGX-SM 08035/5/P22-7	10	3	1218	906.21	Moderately resistant
ICGX-SM 08035/5/P10-8	14	3	1209	814.78	Moderately resistant
ICGX-SM 08036/5/P23-4	10	3	1205	882.42	Moderately resistant
ICGX-SM 08035/5/P14-5	7	3	1204	966.33	Moderately resistant
ICGX-SM 08035/5/P18-40	7	3	1193	951.26	Moderately resistant
ICGX-SM 08036/5/P6-15	11	3	1191	861.93	Moderately resistant
ICGX-SM 08035/5/P1-8	19	3	1186	702.79	Moderately resistant
ICGX-SM 08035/5/P19-5	13	3	1183	795.06	Moderately resistant
ICGX-SM 08036/5/P13-7	11	3	1182	849.96	Moderately resistant
ICGX-SM 08036/5/P21-9	10	3	1177	860.55	Moderately resistant
ICGX-SM 08035/5/P18-5	13	3	1175	801.19	Moderately resistant
ICGX-SM 08036/5/P17-4	12	3	1169	819.97	Moderately resistant
ICGX-SM 08035/5/P11-2	12	3	1168	808.56	Moderately resistant
ICGX-SM 08036/5/P15-3	11	3	1162	828.06	Moderately resistant
ICGX-SM 08036/5/P17-1	14	3	1159	761.24	Moderately resistant
ICGX-SM 08036/5/P22-9	8	3	1156	885.52	Moderately resistant
ICGX-SM 08036/5/P5-8	8	3	1154	883.65	Moderately resistant
ICGX-SM 08036/5/P17-2	15	3	1150	741.2	Moderately resistant
ICGX-SM 08036/5/P1-10	15	3	1145	736.88	Moderately resistant
ICGX-SM 08035/5/P19-3	11	3	1139	796.48	Moderately resistant
ICGX-SM 08035/5/P21-9	12	3	1138	787.7	Moderately resistant
ICGX-SM 08036/5/P6-13	14	3	1134	741.65	Moderately resistant
ICGX-SM 08035/5/P22-3	12	3	1125	776.05	Moderately resistant
ICGX-SM 08036/5/P1-4	12	3	1121	762.32	Moderately resistant
ICGX-SM 08036/5/P1-2	12	3	1115	763.71	Moderately resistant
ICGX-SM 08035/5/P7-6	16	3	1107	689.02	Moderately resistant
ICGX-SM 08036/5/P6-1	14	3	1105	707.44	Moderately resistant
ICGX-SM 08036/5/P17-5	8	3	1104	831.91	Moderately resistant
ICGX-SM 08035/5/P6-3	15	3	1103	696.96	Moderately resistant
ICGX-SM 08036/5/P4-2	13	3	1101	734.8	Moderately resistant
ICGX-SM 08036/5/P4-1	14	3	1101	706.96	Moderately resistant
ICGX-SM 08036/5/P19-1	12	3	1098	751.93	Moderately resistant
ICGX-SM 08036/5/P1-3	14	3	1094	703.89	Moderately resistant
ICGX-SM 08036/5/P5-1	7	3	1080	847.83	Moderately resistant
ICGX-SM 08036/5/P5-3	12	3	1080	729.26	Moderately resistant
ICGX-SM 08036/5/P10-9	12	3	1077	723.34	Moderately resistant
ICGX-SM 08035/5/P4-4	13	3	1074	698.64	Moderately resistant
ICGX-SM 08035/5/P21-1	9	3	1072	790.26	Moderately resistant
ICGX-SM 08036/5/P8-12	10	3	1070	758.28	Moderately resistant
ICGX-SM 08036/5/P22-4	10	3	1069	761.01	Moderately resistant
ICGX-SM 08035/5/P20-7	9	3	1064	790.78	Moderately resistant
ICGX-SM 08036/5/P20-5	11	3	1063	737.77	Moderately resistant
ICGX-SM 08035/5/P21-10	9	3	1059	786.15	Moderately resistant
ICGX-SM 08036/5/P8-2	11	3	1058	726.42	Moderately resistant

Recombinant Inbred Lines	Number of pods plant	Severity	Pod yield (kg/ha)	Seed yield kg/ha	Disease status
ICGX-SM 08036/5/P1-5	11	3	1058	723.04	Moderately resistant
ICGX-SM 08035/5/P15-6	10	3	1052	756.62	Moderately resistant
ICGX-SM 08036/5/P3-2	12	3	1052	714.36	Moderately resistant
ICGX-SM 08036/5/P15-7	11	3	1044	717.33	Moderately resistant
ICGX-SM 08036/5/P22-8	9	3	1040	759.9	Moderately resistant
ICGX-SM 08036/5/P24-3	9	3	1040	749.41	Moderately resistant
ICGX-SM 08035/5/P7-2	12	3	1040	690.75	Moderately resistant
ICGX-SM 08035/5/P5-14	11	3	1032	705.73	Moderately resistant
ICGX-SM 08035/5/P7-9	12	3	1029	683.39	Moderately resistant
ICGX-SM 08036/5/P1-9	8	3	1026	758.71	Moderately resistant
ICGX-SM 08036/5/P5-11	9	3	1019	744.47	Moderately resistant
ICGX-SM 08035/5/P21-3	7	3	1014	789.07	Moderately resistant
ICGX-SM 08035/5/P18-35	11	3	1012	684.61	Moderately resistant
ICGX-SM 08035/5/P22-4	7	3	999	770.64	Moderately resistant
ICGX-SM 08035/5/P6-14	10	3	998	692.45	Moderately resistant
ICGX-SM 08035/5/P1-12	9	3	981	702.18	Moderately resistant
ICGX-SM 08036/5/P5-6	8	3	972	709.02	Moderately resistant
ICGX-SM 08036/5/P11-3	7	3	954	720.19	Moderately resistant
ICGX-SM 08035/5/P6-12	8	3	953	695.43	Moderately resistant
ICGX-SM 08035/5/P18-1	13	4	931	574.31	Susceptible
ICGX-SM 08035/5/P7-13	8	4	925	676.5	Susceptible
ICGX-SM 08035/5/P14-1	11	4	925	607.17	Susceptible
ICGX-SM 08035/5/P11-6	10	4	920	625.31	Susceptible
ICGX-SM 08035/5/P10-3	9	4	914	640.58	Susceptible
ICGX-SM 08035/5/P19-2	13	4	912	558.29	Susceptible
ICGX-SM 08035/5/P15-12	12	4	909	575.32	Susceptible
ICGX-SM 08035/5/P7-18	8	4	902	655.4	Susceptible
ICGX-SM 08035/5/P18-27	9	4	889	611.19	Susceptible
ICGX-SM 08035/5/P15-11	11	4	886	582.44	Susceptible
ICGX-SM 08035/5/P12-9	9	4	882	608.35	Susceptible
ICGX-SM 08035/5/P15-3	10	4	882	594.92	Susceptible
ICGX-SM 08035/5/P18-42	10	4	877	593.79	Susceptible
ICGX-SM 08035/5/P18-4	11	4	874	565.86	Susceptible
ICGX-SM 08035/5/P14-4	9	4	871	601.94	Susceptible
ICGX-SM 08035/5/P9-10	7	4	869	648.48	Susceptible
ICGX-SM 08035/5/P18-10	11	4	857	560.24	Susceptible
ICGX-SM 08035/5/P20-3	11	4	848	540.44	Susceptible
ICGX-SM 08035/5/P20-5	11	4	843	533.24	Susceptible
ICGX-SM 08035/5/P21-8	12	4	842	525.83	Susceptible
ICGX-SM 08036/5/P1-4	13	4	833	499.33	Susceptible
ICGX-SM 08035/5/P14-2	7	4	822	604.15	Susceptible
ICGX-SM 08035/5/P18-43	10	4	822	544.39	Susceptible
ICGX-SM 08035/5/P18-6	9	4	819	561.41	Susceptible
ICGX-SM 08035/5/P19-1	8	4	806	560.09	Susceptible
ICGX-SM 08035/5/P21-4	8	4	760	529.24	Susceptible

Recombinant Inbred Lines	Number of pods plant	Severity	Pod yield (kg/ha)	Seed yield kg/ha	Disease status
ICGX-SM 08035/5/P18-32	6	4	734	549.72	Susceptible
ICGX-SM 08036/5/P4-7	8	4	721	496.85	Susceptible
ICGX-SM 08036/5/P11-2	7	4	708	495.62	Susceptible
ICGX-SM 08036/5/P10-5	10	4	706	445.98	Susceptible
ICGX-SM 08036/5/P16-5	7	4	700	481.49	Susceptible
ICGX-SM 08036/5/P2-1	8	4	700	478.1	Susceptible
ICGX-SM 08035/5/P22-2	5	4	699	525.36	Susceptible
ICGX-SM 08035/5/P18-44	9	4	695	442.35	Susceptible
ICGX-SM 08036/5/P11-8	7	4	691	491.03	Susceptible
ICGX-SM 08035/5/P22-10	5	4	668	500.64	Susceptible
ICGX-SM 08036/5/P13-1	10	4	667	404.66	Susceptible
ICGX-SM 08036/5/P5-10	7	4	647	434.41	Susceptible
ICGX-SM 08036/5/P5-12	6	4	625	434.12	Susceptible
ICGX-SM 08036/5/P3-3	6	4	622	442.11	Susceptible
ICGX-SM 08036/5/P9-10	4	4	622	298.97	Susceptible
ICGX-SM 08036/5/P9-12	8	4	619	390.83	Susceptible
ICGX-SM 08036/5/P9-2	7	4	602	403.55	Susceptible
ICGX-SM 08036/5/P24-6	5	4	559	395.9	Susceptible
ICGX-SM 08035/5/P10-4	18	4	544.5	320.29	Susceptible
ICGX-SM 08035/5/P7-16	15	4	538	334.16	Susceptible
ICGX-SM 08035/5/P12-6	19	4	535.5	306	Susceptible
ICGX-SM 08035/5/P7-14	14	4	527	337.82	Susceptible
ICGX-SM 08035/5/P18-33	16	4	522.5	312.87	Susceptible
ICGX-SM 08036/5/P11-1	8	4	512	307.83	Susceptible
ICGX-SM 08035/5/P12-2	15	4	510.5	305.34	Susceptible
ICGX-SM 08035/5/P7-17	12	4	506.5	333.22	Susceptible
ICGX-SM 08035/5/P7-15	12	4	502	336.91	Susceptible
ICGX-SM 08035/5/P12-4	14	4	499	311.34	Susceptible
ICGX-SM 08035/5/P10-2	12	4	490.5	327.21	Susceptible
ICGX-SM 08035/5/P7-19	16	4	488	364.21	Susceptible
ICGX-SM 08035/5/P18-34	12	4	481.8	317.65	Susceptible
ICGX-SM 08035/5/P11-3	12	4	478.5	318.52	Susceptible
ICGX-SM 08035/5/P14-6	13	4	477	315.76	Susceptible
ICGV 86124	10	5	470.5	329.02	Susceptible