BREEDING GROUNDNUT (*ARACHIS HYPOGAEA* L.) FOR RUST RESISTANCE IN TANZANIA

By

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Thesis Abstract

Groundnut (*Arachis hypogaea* L., AABB, 2n = 4x = 40) is multi-purpose legume serving millions of farmers and their value chain actors globally. It is the fifth most important oilseed crop in the world in terms of volume of oil production after soybeans (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), rapeseed (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.). Groundnut productivity in Tanzania is less than 1 t/ha compared to the potential yield of up to 2.5 t/ha. The low productivity in the country is attributable to an array of abiotic and biotic constraints. The most notable biotic constraint is the rust disease caused by *Puccinia arachidis* Speg., which can result in a yield loss up to 57 percent. Breeding for host resistance in susceptible groundnut genotypes is cost-effective and environmentally friendly disease control method which is widely regarded as the most sustainable and effective method. Therefore, the objectives of this study were to: (i) document groundnut farmers' major production constraints, farming systems, and varietal trait preferences in selected agroecologies of Tanzania to guide breeding, (ii) determine the extent of genetic variation among diverse groundnut collections using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and complementary genotypes for breeding, (iii) assess genotype and genotype by environment interaction (GEI) on kernel yield and evaluate the adaptability and stability of groundnut genotypes across environments for selection and (iv) determine the combining ability effects and gene action controlling rust resistance and agronomic traits in groundnut genotypes for further breeding.

In the first study, participatory rural appraisal surveys were conducted involving 180 farmers in Mtwara, Dodoma and Shinyanga regions of Tanzania using a semi structured questionnaire, transect walks, and focused group discussion. The results showed that diseases and pests were the main production constraints reported by 87.7% and 84.9% of respondents, respectively. Groundnut rust caused by basidiomycete fungus *Puccinia arachidis* Speg., was the major cause of yield reduction, as reported by 30% of the respondents. Drought stress and non-availability of seed of improved varieties were other important constraints, as reported by 83.9% and 76.1% of the respondents, respectively. Groundnut agronomic attributes preferred by farmers were as follows: high yield (reported by 78.4% of respondents), disease resistance (71.2%), early maturity (66%), drought tolerance (63.0%), and pest resistance (63%). Medium-to large grain size (reported by 62.6%of respondents) and tan and red seed color (59.2%) were the main farmer and market-preferred groundnut seed quality traits. Groundnut variety development programs should therefore integrate the above constraints and farmer-preferred traits for sustainable groundnut production and productivity in Tanzania.

In the second study, 119 genotypes, which included ICRISAT's breeding populations, landrace collections from different agro-ecologies in Tanzania and cultivated varieties were evaluated under field conditions for agronomic traits and susceptibility to rust and leaf spot diseases. The study was conducted in two locations for two seasons. In addition, the 119 accessions were profiled with 13 selected SSR markers. The study revealed that moderate genetic variation was recorded with mean polymorphic information content of 0.34 and gene diversity of 0.63 using the SSR markers. The majority (74%) of genotypes showed high membership coefficients to their respective subpopulations, while 26% were admixtures after structure analysis. Much of the variation (69%) was found within populations due to genotypic differences. Genotypes ICGV-SM 06737, ICGV-SM 16575, ICG 12725 and ICGV-SM 16608 were identified for development of breeding population, which will be useful for groundnut improvement. This study provided a baseline information on characterization and selection of a large sample of groundnut genotypes in Tanzania and selected unique genotypes for effective breeding and systematic conservation. Genotype and genotype by environment interaction effects were significant ($p < 0.05$) for days to flowering (DTF), late leaf spot score at 85 and 100 days after planting, pod yield (PDY), kernel yield (KY), hundred seed weight (HSW) and shelling percentage (SP). Principal components analysis revealed that plant stand, KY, SP, NPP (number of pods per plant), late leaf spot and rust disease scores accounted for the largest proportion of the total variation (71.9%) among the tested genotypes.

In the third study, 120 groundnut genotypes were evaluated at two selected locations (Naliendele and Chambezi) using an 8 x 15 incomplete block design with two replications. The study revealed significant ($p<0.05$) variations among genotypes (G), environments (E) and GEI effects on kernel yield. A relatively higher proportion of the observed variation was due to the environment (34.85%) followed by GEI (24.65%) and genotype (8.25%) effects. The kernel yield of genotypes across environments ranged from 119.6 kgha-1 for ICGV-SM 16574 to 469.0 kgha-1 for ICGV 94124. Genotypes ICGV 94124 and CG 7 had relatively better kernel yield of 469.01 and 450.02 kg ha^{-1}, respectively. The genotype and genotype-by-environment biplot identified ICGV-SM 16556, ICGV-SM 15524, ICGV-SM 15564 and ICGV-SM 15514 as the most stable genotypes across locations, while ICGV-SM 16574 and ICGV-SM 15559 were specifically adapted to Chambezi and Naliendele, respectively. The Naliendele site was the most ideal location for groundnut evaluation and genotype differentiation. Most genotypes exhibited lower mean performance at Chambezi site with average mean yield of 139.76 kg/ha over both seasons compared to Naliendele (431.51 kg/ha). The selected genotypes with high yields and average stability are useful genetic resources as breeding parents for groundnut improvement in Tanzania.

The final study assessed the combining ability effects and gene action controlling rust resistance and agronomic traits in selected groundnut genotypes. Twelve selected and complementary parental lines were crossed in a 12 x 12 diallel design to develop F_2 progeny. Thirty-three successful partial crosses and the 12 parents were field evaluated using a 5 × 9 alpha lattice design with two replications over two seasons in Tanzania. The data were subjected to analysis of variance (ANOVA) using SAS 9.4 and means were separated by Fischers unprotected least significant difference at 5% probability level. There existed significant ($P<0.05$) difference on the general combining ability (GCA) effect of parents and the specific combining ability (SCA) effect of progeny for the assessed traits indicating that both additive and non-additive gene effects conditioned trait inheritance. The Bakers' ratios accounted for non-additive gene effects predominantly controlling rust resistance and yield components. This suggests that transgressive segregants could be selected for improved rust resistance and yield gains in the advanced pure line generations. Genotypes ICGV-SM 05570 and ICGV-SM 15567 were the best general combiners for rust resistance and grain yield. The crosses ICGV-SM 16589 × Narinut and ICGV-SM 15559 × ICGV-SM 15557 were identified as the best specific combiners for rust resistance with moderate yield levels and medium maturity. Genotypes with desirable GCA or SCA effects were selected for further breeding.

Overall, the present study appraised diseases, pests, drought stress and non-availability of seed of improved varieties as the current farmers' major production constraints, and varietal trait preferences of groundnut to guide breeding. Also, the study identified ICGV-SM 15557, ICGV-SM 15559, ICGV-SM 06737, PENDO, ICGV-SM 16601, ICGV-SM 16589, ICGV-SM 05570, Kanyomwa, Narinut 15, ICG 12725, ICGV-SM 15524 and ICGV-SM 15567 as a valuable groundnut genotypes and developed new families with high combining ability for rust resistance and kernel yield. The new families are recommended for genetic advancement and to develop pure lines for cultivar release and deployment in Tanzania

Declaration

I, Happy Makuru Daudi, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other Information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
a. Their words have been re-written but the general information attributed to them has been referenced.
b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference's sections.

Signed

…… ……..

Happy Makuru Daudi

As the candidate's supervisor, I agree to the submission of this thesis:

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Prof. Shimelis Hussein (Supervisor)

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Dedication

This thesis work is dedicated to the almighty God and to my beloved son Paschal Arnold.

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ABBREVIATIONS

ACCI	African Centre for Crop Improvement
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive main effect and multiplicative interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ASV	AMMI stability values
BSA	bulk segregant analysis
CV	coefficient of variation
DF	degrees of freedom
DTF	days to flowering
ENV	environment
FGDs	focused group discussions
FPS	final plant stand
F_1	Filial generation one
F_2	Filial generation two
GCA	general combining ability
GEI	genotype-by-environment interactions
GGE	genotype and genotype × environment
HSW	hundred seed weight
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
IPCA	interaction principal component analysis
IPCA1	first interaction principal component axis
IPCA2	second interaction principal component axis
IPS	initial plant stand
KY	kernel yield
LLSI	late leaf spot infection
LSD	Least significant difference
MR	moderately resistant
NPP	number of pods per plant
PC	principal component
PDY	pod yield
PH	plant height
PRA	participatory rural appraisal
QTL	quantitative trait loci
R	Resistant
RAPD	Random Amplified Polymorphic DNA
REC	Reciprocal
RI	rust infection
R^2	coefficient of determination
S	Selfs
SCA	specific combining ability
SED	Standard error of the mean differences
SP	shelling percent
SSA	sub-Saharan Africa
SSR	Simple sequence repeat
TARI	Tanzania Agricultural Research Institute

Publication pertaining to this thesis

Chapter One

Happy Daudi, Hussein Shimelis, Learnmore Mwadzingeni, Mark Laing and Patrick Okori (2018). Breeding groundnut for rust resistance: A review, Journal of Agricultural Research Communication Centre, 1-9 DOI: 10.18805/LR-416

Chapter Two

Happy Daudi, Hussein Shimelis, Mark Laing, Patrick Okori & Omari Mponda (2018) Groundnut production constraints, farming systems, and farmer-preferred traits in Tanzania, Journal of Crop Improvement, 32:6, 812-828, DOI: 10.1080/15427528.2018.1531801

Chapter Three

Happy Daudi, Hussein Shimelis, Isack Mathew, Richard Oteng-Frimpong, Chris Ojiewo, Rajeev K. Varshney (2020). Genetic diversity and population structure of groundnut (*Arachis hypogaea* L.) accessions using phenotypic traitsand SSR markers: implications for rust resistance breeding, Genetic Resources and Crop Evolution, DOI 10.1007/s10722-020-01007-1)

Chapter Four

Happy Daudi, Hussein Shimelis, Isack Mathew, Chris Ojiewo**.** 2020. Genotype-by-environment interaction analysis of groundnut (*Arachis hypogaea* L.) for kernel yield. Under review in Journal of Agronomy.

Chapter Five

Happy Daudi, Hussein Shimelis, Isack Mathew, Abhishek Rathore, Chris O. Ojiewo. 2020. Combining ability and gene action controlling rust resistance and agronomic traits in groundnut (Arachis hypogaea L.). Under review in Euphytica.

Introduction to the thesis

Background

Cultivated peanut or groundnut (*Arachis hypogaea* L., AABB, 2n = 4x = 40) is an allotetraploid and a predominantly self-pollinated legume crop. It has cleistogamous flowers, but cross pollination can occur due to several reasons. Groundnut is a valuable source of dietary protein, vegetable oil for humans and seedcake and haulm for livestock feed. Groundnut grain is rich in oil (48-50%), protein (26-28%), dietary fiber, minerals, and vitamins (Pasupuleti *et al.* 2013). It is the 5^{h} most important oilseed crop of the world in terms of volume of oil production after soybeans (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), rapeseed (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.). In addition, the crop has the ability to fix atmospheric nitrogen into the soil, which improves soil fertility.

Globally, groundnut is cultivated on about 28.52 million hectares with an annual production of ≈45.95 million tons (FAOSTAT 2018). It is widely grown in more than 100 countries of tropical, subtropical, and warm temperate regions worldwide (Upadhyaya *et al.* 2012).China is leading in the production of groundnut, followed with India and in Africa Nigeria is the leading followed with Sudani and Tanzania is in the third position. According to FAOSTAT (2018), Africa produced about 14,307,084 tonnes of groundnut in 2018, of which Tanzania's output was 940,204 tonnes. Sub-Saharan Africa (SSA) has one of the lowest groundnut productivity levels (<1 t/ha) in the world and local demands are met through imports. FAOSTAT (2020) estimated a monetary value of US$132 for imported groundnut in to Africa in 2020. The mean groundnut yield in Tanzania is <0.7t/ha compared to a potential yield of 2.5 t/ha reported in China and India (FAOSTAT 2018). Although groundnut is of economic, social and cultural importance in Tanzania, its productivity is severely constrained by several biotic and abiotic factors and socio-economic constraints (Daudi *et al.* 2018, Reddy *et al.* 2003).

Production constraints

Groundnut production and productivity is severely constrained by an array of challenges. Drought is the major abiotic constraint affecting groundnut yield and quality worldwide. Two thirds of the global production are under rain-fed systems where rainfall is erratic and insufficient, causing unpredictable drought stress and yield loss (Reddy *et al.* 2003). Diseases are key impediments to groundnut production. The main diseases of the crop are fungal, bacterial, viral pathogens and nematode infestation limiting groundnut yields globally (Daudi *et al.* 2018). Among the fungal diseases, groundnut rust caused by *Puccinia arachidis*. Speg, early leaf spot caused by *Cercospora arachidicola* Hori and late leaf spot (*Phaseoisariopsis*

personata Berk. & Curtis.) are the most prevalent occurring across groundnut growing regions globally including Tanzania (Liu et al., 2013). Groundnut rust and late leaf spot (LLS) often occur simultaneously, causing 50 to 70% yield loss (Khedikar *et al.* 2010). The above diseases have been reported to cause economic losses of up to US$467 m and US$599 m in India and Africa, respectively (FAOSTAT 2004). Groundnut diseases affect yield expression, and quality of pods and haulms.

In Tanzania, groundnut is mainly grown by small-scale farmers, particularly in Shinyanga, Tabora, Dodoma, Mbeya and Mtwara regions (NBS 2012). Groundnut yields in Tanzania are lower compared to other African countries. For example, in 2018, the shelled yield of groundnut was 984 kg/ha in Tanzania compared to 992 kg/ha in Nigeria and 1172 kg/ha in Guinea-Bissau (FAOSTAT 2018). The yield level in Tanzania stagnated over the past decades due to the above constraints (Daudi *et al.* 2020, Daudi *et al.* 2018). The most important biotic factors affecting groundnut production and productivity in the country include groundnut rust, early and late leaf spot and rosette disease caused by a virus (Daudi *et al.* 2018). Groundnut rosette disease is the most devastating under rainfall conditions, while, rust epidemics is favoured under high humid and high temperature conditions. Aflatoxin caused by the fungal pathogen *Aspergillus flavus* affects groundnut yield and quality losses and causes various human health hazards. Socio-economic constraints such as the high cost of seeds, high labor demand, high cost of pesticides, limited land availability and low price of groundnut also contribute to the low production and productivity of the crop in the country (Daudi *et al.* 2018, Katundu *et al.* 2014). In addition, groundnut production is deemed to be a women's business, whereby men do not give a deserved attention in the agronomic management of the crop. This is worsened by gender disparities in land ownership (Ramadhani *et al.* 2002).

Groundnut rust

Groundnut rust causes serious yield losses in Tanzania reaching up to 50% on susceptible varieties. Most farmers in Tanzania use low yielding and disease susceptible groundnut germplasm. Tanzania does not have genetically divergent germplasm; hence, breeding populations have to be developed for future rust resistance breeding programs. There is need to acquire germplasm from leading research institutes such as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in order to develop a population that will be used for rust resistance breeding in the country.

There are various control options against groundnut rust including cultural, chemical and host resistance. Rust can be manged through repeated applications of fungicides (4-8 foliar sprays) based on disease severity. However, majority of smallholder farmers in most African countries

including Tanzania cannot afford fungicides. Also, there is a lack of technical expertise required by farmers to use these chemicals effectively. In the past, there has not been dedicated research program on resistance breeding on groundnut. Though several effective fungicides are available to control rust diseases, host-plant resistance is considered to be the best strategy to surmount additional cost of production and hazardous effect of fungicides on the soil and environment. Genetic approach involving introgression of disease resistance into modern and popular cultivars is the most ideal strategy (Varshney *et al.* 2014). Hence there is a need to develop disease resistant varieties for sustainable groundnut production in Tanzania

Rationale of the study

Groundnut rust is among the major production constraints in most groundnut growing areas in Tanzania. It causes yield losses of up to 50%. Groundnut rust has negatively impacted on the livelihoods of smallholder farmers especially women who depend on this crop for food security and as a source of cash. The presently available groundnut rust control option such as fungicides are not readily available and expensive to smallholder farmers. Recently, the Naliendele Agricultural Research Institute, which has a mandate of oilseeds and legume research in Tanzania developed new varieties with high pod yield, but these varieties still have low level of resistance to rust disease. The major cause of susceptibility of groundnuts varieties to rust disease is the narrow genetic base of cultivated groundnuts. Hence, there is a need to develop groundnut varieties with durable resistance that would withstand the different races of the pathogen. In order to develop rust resistant varieties, it is important to identify sources of resistance and to understand the genetics of rust resistance in groundnuts. In addition, the combining ability of superior parents should be evaluated to determine their capacity to improve local genotypes and to produce superior families. Therefore, in order to achieve this, candidate germplasm from ICRISAT-Malawi, adapted varieties and landraces from Tanzania should be screened and evaluated using phenotypic and diagnostic molecular markers. Simple sequence repeat (SSR) markers showed effectiveness in the selection of cultivated groundnuts. Additionally, inclusion of groundnut farmers in research problem identification and participatory research may facilitate success in adoption of new production technologies such as improved varieties. In the past, the trend of research-extension-farmer linkage in Tanzania was a top-down approach. Therefore, participatory rural appraisal (PRA) study is key to identify groundnut production constraints in general and the impact of rust disease in particular and to assess farmers' preferences and conditions towards the adoption of newly developed resistant groundnut varieties in Tanzania**.**

Research objectives

Overall objective

The overall objective of this study was to develop farmer-preferred, rust resistant, and high yielding groundnut genotypes in Tanzania**.**

Specific objectives

The specific objectives of this study were:

- To document groundnut farmers' major production constraints, farming systems, and varietal trait preferences in selected agro-ecologies of Tanzania to guide breeding.
- To determine the extent of genetic variation among diverse groundnut collections using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and complementary genotypes for breeding.
- To assess the genotype-by-environment interaction (GEI) effect on kernel yield and select best adapted groundnut genotypes in target production environments in Tanzania.
- To determine the combining ability effects and gene action controlling rust resistance and agronomic traits in groundnut genotypes for further breeding.

Research hypotheses

This study was carried out to test the following hypotheses:

1. Farmers will identify leading constraints to groundnuts production and the impact of rust disease that will guide the breeding program.
2. There is extensive genetic diversity among selected groundnut genotypes to provide a broad genetic base for breeding.
3. Changes in environment affects the performance of the groundnut genotypes and that can be exploited to identify genotypes with wide or specific adaptation
4. The selected sources of resistance to rust and their progenies will show good combining ability for groundnut rust resistance, pod yield performance and other agronomic traits.

Outline of this thesis

This thesis consists of five distinct chapters in accordance with a number of activities related to the above objectives (Table 0.1). Chapter 1 is written as a separate review paper, while chapters 2-5 are written in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters.

The referencing system used in the chapters of this thesis is based on the Crop Science referencing system. Chapter 1 was published in the Journal of Agricultural Research Communication Centre (2018, 1–9, DOI: 10.18805/LR-416). Chapter 2 was published in Journal of Crop Improvement (2018, 32 (6): 812-828, DOI:10.1080/15427528.2018.153180). Chapter 3 was published in Genetic Resources and Crop Evolution (2020, DOI 10.1007/s10722-020-01007-1). Chapter 4 has been submitted for publication in Journal of Agronomy (Manuscript ID: agronomy-1022376) and Chapter 5 is under review in Euphytica (Manuscript ID: EUPH-D-20-00746).

Table 0.1 Thesis structure

Chapter	Title
-	Introduction
1	Literature review
2	Groundnut production constraints, farming systems, and farmer-preferred traits in Tanzania
3	Genetic diversity and population structure of groundnut (*Arachis hypogaea* L.) accessions using phenotypic traits and SSR markers: implications for rust resistance breeding
4	Genotype-by-environment interaction analysis of groundnut (*Arachis hypogaea* L.) for kernel yield
5	Combining ability and gene action controlling rust resistance and agronomic traits in groundnut (*Arachis hypogaea* L.)
-	An overview of research findings

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1 CHAPTER ONE

Breeding groundnut (*Arachis Hypogaea* L.) for rust resistance: A review

Abstract

Sustainable groundnut production can be realised through development and adoption of high yielding cultivars possessing durable rust resistance. Integrating conventional breeding with genomic tools in identifying candidate rust resistance genes, and introgressing the genes into adapted elite germplasm, with the aid of molecular makers, could enhance breeding for rust resistance. This review highlights breeding approaches for groundnut rust resistance, with emphasis on integrating conventional breeding with marker-assisted selection. The life cycle, symptoms and epidemiology of the pathogen are also discussed to understand the host-pathogen interaction and guide groundnut rust resistance breeding.

Key words: Epidemiology, Groundnut rust, Host resistance, Marker-assisted selection, *Puccinia arachidis*

This chapter was published in the Journal of Agricultural Research Communication Centre. Happy Daudi, Hussein Shimelis, Learnmore Mwadzingeni, Mark Laing and Patrick Okori. 2018. Breeding groundnut for rust resistance: A review 1-9. doi: 10.18805/LR-41

1.1 Introduction

Groundnut (*Arachis hypogaea* L., AABB, 2n=4x=40), is the fifth world's most economically important oilseed crop after soybeans, cotton, rapeseed and sunflower. It is currently produced on about 26.54 million hectares per year with an annual production of ≈43.92 million tons of shelled grain providing about 16.55 t ha^{-1}across the tropics, subtropics and warm temperate agro-ecologies worldwide (FAOSTAT 2014, Upadhyaya *et al.* 2012). The African continent accounts for about 31.6% of the world's groundnut production and the import trade values for sub-Saharan Africa (SSA) is estimated to be at US$ 54 million by 2020 (Abate *et al.* 2012). Despite the socio-economic and cultural importance of the crop, its productivity and quality are severely constrained by several biotic and abiotic stress factors, particularly fungal diseases including early leaf spot caused by *Cercospora arachidicola* Hori., late leaf spot (*Cercosporidium personatum* Berk. & Curtis.) and groundnut rust (*Puccinia arachidis* Speg) (Reddy *et al.* 2003). Groundnut rust and late leaf spot cause up to 70% yield losses in susceptible cultivars, which most smallholder farmers in developing countries often rely on (Khedikar *et al.* 2010).

Groundnut rust is an economically important disease that was previously prevalent in South and Central America, USSR and Mauritius with sporadic distributions in the People's Republic of China (Stockdale 1914, Subrahmanyam *et al.* 1984, Tai 1937). The disease was later introduced and became established in Asia, Australia, Oceania, and Africa where frequent epidemics occurs (Subrahmanyam *et al.* 1984). Groundnut rust has now become cosmopolitan, reducing seed yield and oil quality of susceptible genotypes globally. Damage symptoms associated with early attacks during the growing season includes early pod maturity, reduced seed size, increased pod senescence, and decreased oil content, while severe infection causes up to 57% economic losses (Mondal and Badigannavar 2015).

There are various control options against groundnut rust including cultural practices, chemical control, use of biological agents and host plant resistance. Cultural practices such as early planting, fertilizer application, removal of volunteer plants, burning of crop residues and intercropping are widely applied to reduce carry-over of rust inoculum from crop to crop (Kokalis *et al.* 1997, Mondal *et al.* 2014). Rust can effectively be controlled through repeated applications of fungicides based on disease occurrence and severity (Mondal and Badigannavar 2015). However, majority of smallholder farmers in sub- Saharan African countries cannot afford fungicides and do not have adequate skills to handle and utilize them without predisposing themselves to health and environmental risks. Breeding and adoption of rust resistant cultivars is the most sustainable control option that can safeguard the crop. Despite several breeding efforts against the disease by private, national and international research institutions, there are still very few improved rust resistant varieties reported globally

(Pasupuleti *et al.* 2013). This could be due to knowledge gaps on the nature of inheritance of rust resistance, pathogenicity of the fungi and breeding approaches for successful selection and introgression of resistance genes (Barro Antoine *et al.* 2017). Therefore, the objective of this review was to summarize the pathogenicity of groundnut rust, inheritance of its resistance, control options and potential breeding methodologies to aid sustainable groundnut production and productivity.

1.2 Life cycle of groundnut rust

The groundnut rust pathogen is a *Pucciniomycetes* classified among higher fungi whose life cycle evolves between haploid and dikaryotic stages that are further characterized by five spore stages such as the spemagonium, dikaryotic aecium, dikaryoticuredium, dikaryotic telium and dikaryotic and/or diploid basidium (Fig. 1.1) (Mondal and Badigannavar 2015). Plasmogamy between two compatible spermatids and receptive hyphae form dikaryotic mycelium. The telial stage, basidium and basidiospores are not common in groundnut rust (Mondal and Badigannavar 2015), which mainly exists as uredinia containing numerous pedicillate uredospores observed on leaf surfaces (Tashildar *et al.* 2012). Due to the rare occurrence of the basidium (sexual stage), limited races or variants of groundnut rust have been reported so far, which could have evolved distinctly due to mutations. Uredospores infect groundnut leaves form uredosori that matures, burst and release numerous uredospores that initiate several cycles of infection under production conditions. Telia containing numerous teliospores are often formed from uredospores under low temperature and nutrient stress, but the existence of teliospores of *P. arachidis*rarely occur in nature, hence their function remains unclear (Mondal and Badigannavar 2015, Tashildar *et al.* 2012). The teliospores and basidia, which are the sexual forms of the rust pathogen, as well as somatic recombination generates the limited genetic sequence variability existing among rust isolates and could cause evolution of new races or pathotypes in future (Tashildar *et al.* 2012). Thus, breeders should constantly pyramid several minor effect genes into elite germplasm to develop durable resistance and safeguard varieties against resistance breakdown.

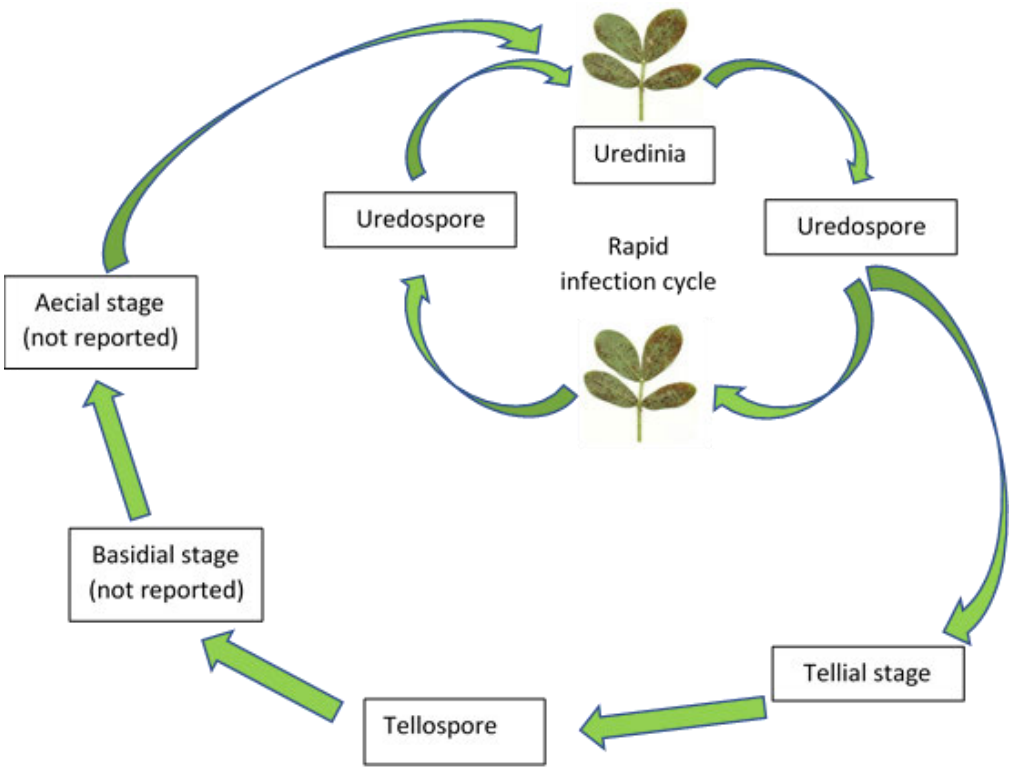

Figure 1.1 Schematic life cycle of groundnut rust *(Puccinia arachidis)*

1.3 Epidemiology of groundnut rust

Uredospores of the groundnut rust pathogen are dispersed by wind, rainfall or together with plant materials (Park and Wellings, 2012). Disease epidemiology is favoured by continuous warm temperatures ranging between 20 and 30 °C and high humidity above 78 % (Mondal and Badigannavar 2015, Peregrine 1971). Uredospores were observed to remain viable for up to 20 days at 25–28 °C (Sunkad and Kulkarni 2007). The disease progresses slowly at 10 °C or less and above 35 °C (Rao *et al.* 1997). Controlled environment experiments can take advantage of these strict temperature and humidity requirements to manipulate the rate of inoculum accumulation. Allowing proper air movement can reduce the build-up and spread of inoculum under a given production condition. A prediction model developed by (Gumpert *et al.* 1987) has been extensively used to describe the epidemic development of airborne foliar fungal diseases in different crops including soybean, groundnut and wheat. Environmental factors such as temperature, wind speed and direction and humidity affect airborne fungi distribution, infection and development (Pivonia and Yang 2006). The following prediction equation has been commonly used in predicting disease severity (Gumpert *et al.* 1987):

$Y = b_0 + b_1x_1 + b_2x_2 + \ldots\ldots\ldots\ldots\ldots b_nx_n$

Where Y = Predicted disease severity

b_0 = intercept

$b_1b_2\ldots\ldots\ldots\ldots b_n$= regression coefficients

$x_1x_2\ldots\ldots\ldots\ldots\ldots x_n$ = independent or predictor variables

The groundnut rust pathogen's host range is confined to the genus *Arachis* making volunteer plants primarily responsible for disease carryover from season to season (Kokalis *et al.* 1997, Mallaiah and Rao 1979). In addition, overlapping crop seasons provide continuous inoculum build up and aerial propagation of uredospores. Rust epidemiology is dependent on the host's genotype and its severity, which is subject to genotype × environment interaction effects (Rao *et al.* 1997). This suggests that rust resistance could be a complex trait that is conditioned by numerous minor genes with additive genetic effect. Light rain showers favor disease dispersal, while heavy showers drastically reduce spore content in the canopy. Therefore, late sowing in the rainy season helps to reduces disease epidemic, whereas early sowing minimizes the severity of rust incidence during summer (Bulbule and Mayee 1997). Spore trapping on the plant canopy is often higher in the morning than during evening hours (Savary and Janeau 1986, Sunkad and Kulkarni 2007).

1.4 Groundnut rust infection process

Groundnut rust disease causes much damage during the flowering, fruiting and vegetative phases of crop growth. Uredospores of the groundnut rust pathogen geminates and protrudes a single unbranched germ tube of ~6 ìmin diameter and 100 to 200 ìm length from one of the equatorial germ pores on its wall (Das *et al.*, 1999). The germ tube grows across the leaf surface until it makes direct contact with the stoma, forming a thin walled ellipsoidal appressorium of about the same size as the spore from which it emerges (Mondal *et al.* 2014). A thin cross wall then forms between the germ tube and the appressorium, confining the dense cytoplasm in the appressorium within 12 hrs of inoculation in susceptible genotypes. This is followed by the growth and penetration of a narrow infection peg from the appressorium through the stomatal apertures (Cook 1980). After traversing the length of the stomatal passage, the infection peg swells and forms a vesicle in the substomatal chamber. Several infection dikaryotic hypha usually grow from the substomatal vesicle within 24 hrs of infection, from which simple knoblike haustoria develop within adjacent mesophyll cells. The pathogen then secretes hydrolytic enzymes like cellulases, glucanases and proteinase that cause dissolution of cell walls and plasma membranes. The infection foci later turn into cloronemic flecks that later develop into orange or reddish brown uredinia or pustules, on the lower surface of groundnut leaves (Fig. 1.2A). An ultrastructure study using a scanning electron microscope detected differences in spore reaction in the lower leaf surfaces of resistant (*A. stenosperma*cv V10309) and susceptible (*A. hypogaea* cv. IAC-Tatu) genotypes (Mondal and Badigannavar 2015). The germ tube elongates sufficiently in susceptible genotypes within 24 hrs of inoculation and makes successful intercellular infection within 72 hrs (Mondal and Badigannavar 2015).

1.5 Symptomatology

Rust symptoms start to appear 8 to10 days after infection with the occurrence of whitish flecks on the abaxial surface followed by yellowish flecks on the upper leaf surface (Figure 1.2B). Orange colored pustules then form on the lower surface of the leaves (Figure 1.2A). Elliptical pustules of 0.3 to 2.0 mm diameter rupture after about 2 days of appearance and expose circular or oval urediniospores, which are dark orange at first but become cinnamon brown with maturity (Leal-Bertioli *et al.* 2009). Pustules occasionally develop on the upper leaf surface but are not as numerous as on the lower. Necrotic areas occur around the pustules and later coalesce causing leaf desiccation. The disease commonly develops in a radiating pattern from a single spot and increase in size in wet and warm weather. Rust spores can clearly be seen with naked eye on above ground parts including the stems and leaves, while it is not easy to diagnose on the seed because the pathogen is internally seed born.

Figure 1.2 Groundnut rust pustule at the lower (A) and upper (B) parts of the leaf

1.6 Control strategies of groundnut rust

1.6.1 Cultural control

Prevention of the outbreak or proliferation of *P. arachidis* in farmers' fields should be prioritized to minimize any damage to the crop and to minimize costs associated with other control strategies. Introduction and spread of the inoculum to areas where it has not been can be avoided through regulating the movement of groundnut plant materials across regions or boarders by enforcing strict phyto-sanitary inspections at quarantine stations. In groundnut producing areas where rust is a constant threat, adoption of crop rotations involving cereals or other nonhost species is effective to avoid disease carryover (Mondal *et al.* 2014). Small-scale farmers particularly in the semiarid tropics often intercrop groundnuts with either pigeon pea (*Cajanus cajan*L.), sorghum (*Sorghum bicolor*), cassava (*Manihot esculenta* Crantz), pearl millet (*Pennisetum glaucum*) or maize (*Zea mays* L). Eradicating volunteer plants, which

are often initial sources of inoculum and implementing fallow periods to break the disease cycle also help to suppress the inoculum since the pathogen is biotrophic. These should be complemented by maintaining field sanitation through weeding and proper spacing of plants (Kokalis *et al.* 1997). Where a new crop has to be planted later during the growing season, adequate isolation distances from old crops should be maintained depending on the direction of the wind and whether the old crop has is infected or not. Cultural control options are however ineffective in the event of severe and unexpected outbreaks or infection, hence the need for constant field inspection and application of fungicides once the economic threshold level is reached.

1.6.2 Chemical control

Frequent applications of fungicides at 2-week intervals from the time that signs of rust infection are first observed effectively minimizes crop damage (Kokalis *et al.* 1997). Regular application of chlorothalonil, tridemorph, combinations of mancozeb and zinc, hexaconazole, strobilurinsterol-inhibitors and other sulphur based fungicides effectively reduce groundnut rust incidences (Kokalis *et al.* 1997). Early application of chemicals is more effective in reducing rust epidemics than applications later during the season. However, this should be based on regular monitoring and forecasting according to prevailing weather conditions. Trials conducted at Naliendele Research Institute in Tanzania found chlorothalonil (Daconil) to be the most effective fungicide in controlling groundnut rust (NARI 2001). Fungicides that are effective against both rust and leaf spot diseases such as chlorothalonil and tebuconazole are required in areas where leaf spot and rust occur together (Kokalis *et al.* 1997). The use of costly crop protection chemicals is not economical, cause environmental and health hazards and often leads to resistance build-up among pathogen strains. Since doing away with fungicides is inevitable, proper rotation of fungicides belonging to different chemical groups is required to reduce the chances of resistant mutants. Environmentally friendly interventions such as the use of biological control agents and adoption of resistant cultivars could be more sustainable.

1.6.3 Biological control

Biocontrol agents such as the fungi *Verticillium lecanii* Zimmerm. and *Penicillium islandicum* Sopp. have been reported to inhibit the germination of urediniospore of *P. arachidis* and the severity of rust infection, hence can serve as bio-fungicides (Kokalis *et al.* 1997). *Verticillium lecanii* proliferates within *P. arachidis* disspores, subsequently causing the spores to rupture (Kokalis *et al.* 1997). These antagonistic fungi are a potential biological control agent against groundnut rust, early and late leaf spot, which often occur together (Podile and Kishore 2002).

Treatment of groundnut leaves with the fungus *A.obclavatum* reduces the number of pustules and uredospores, delays maturity and opening of uredosori, and reduces viability of uredospore resulting in significant preservation of seed yield and oil quality (Gowdu and Balasubramanian 1993). The biocontrol agent survives on the crop until the pathogen establishes and is carried along with the rust fungal spores when they are liberated from the pustule (Podile and Kishore 2002). Knowledge gapes still exist on how best to enhance the virulence of different biocontrol agents against the groundnut rust pathogen. Exploring more invasive variance that share similar environmental requirements as the pathogen is a potential study area. Otherwise, integrating host plant resistance into the rust management system will enhance the efficacy of biological control and reduce costs associated with fungicide application.

1.6.4 Host resistance

Adoption of groundnut genotypes that possess inherent resistance against groundnut rust is a sustainable management alternative that can mitigate the shortcomings of other control strategies. To date, some rust resistant groundnut genotypes have been bred by different national and international crop breeding institutions, including the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Mace *et al.* 2006, Singh *et al.* 2003). Some of the genotypes were released for cultivation in Asian and African countries or have been used as parents in national breeding programs (Mace *et al.* 2006, Singh *et al.* 2003). Significant durable resistance could be achieved if different resistance genes harbored in elite cultivated materials could be introgressed into genotypes adapted to various production regions through backcross breeding. In this case, hybridization of elite or superior cultivars or lines will not be hindered by cross incompatibility issues or linkage drag associated with undesirable traits. However, high levels of resistance to late leaf spot and rust are often reported in wild peanut species of groundnut compared to *A. hypogaea* (Mondal and Badigannavar 2015, Singh 2004). Some of these genetic stocks can be utilized through the development of interspecific hybrids and interspecific derivatives such as GPBD 4, developed from the parental genotype ICGV 86855, which is an interspecific derivative of *A. hypogaea* × *A. cardenasii* showing resistance to both late leaf spot and rust (Stalker 1997). However, the use of resistance from wild species is limited because of associated linkage drag, resulting in delayed maturity and undesirable pod and kernel features, requiring several cycles of backcrossing to the recurrent parent with the help of foreground and background selection using genetic makers. Also, ploidy barriers between wild and cultivated species, genetic isolation of several wild species, and genetic incompatibility complicate the use of wild *Arachis* species as sources of resistance (Pasupuleti *et al.* 2013).

1.7 Breeding groundnuts for rust resistance

1.7.1 Genetics of groundnut rust resistance

Resistance to groundnut rust has been reported to be predominantly governed by recessive genes that are expressed in a homozygous state (Bromfiel and Bailey 1972, Paramasivam *et al.* 1990, Tiwari *et al.* 1984). This imply the need to use marker-assisted selection to ensure efficient selection and to reduce hybridization cycles during backcrossing by eliminating the need for test crossing to confirm the presence of the recessive gene. Bromfiel and Bailey (1972) reported of digenic inheritance controlled by recessive resistance genes among F_2 segregants of a natural cross between a rust resistant female parent, PI 298115, and an unknown pollen parent. Similarly, the recessive nature of groundnut rust resistance was confirmed using F_3 derivatives of the same cross at ICRISAT. Other studies at ICRISAT using F_2 genotypes reported digenic inheritance in some crosses and trigenic inheritance in others (Kishore 1981). Continued segregation observed among highly-resistant progenies also suggests that more than two genes influence resistance to groundnut rust (Nigam *et al.* 1980). Based on the F_2 segregation ratios, Joel *et al.* (2006) observed that rust resistance was recessive and controlled in monogenic (3:1), digenic (15:1) and trigenic (63:1) manners. Further studies are required to ascertain the number of genes that govern groundnut rust resistance. Preliminary investigations on the inheritance of rust resistance derived from diploid wild species indicated that F_1 hybrids between *A. hypogaea* and diploid species showed resistant reactions to rust, suggesting that the resistance was governed by a partially dominant gene (Singh *et al.* 1984). The crosses involving wild relatives and wild derivatives often indicate partially dominant or dominant gene actions, which would possibly simplify backcross breeding (Mondal *et al.* 2008). Other studies reported partial resistance, which is described as slow rusting type involving several minor genes that cause decreased infection frequency, pustule size, spore production, and spore viability as well as increased incubation period. (Kokalis *et al.* 1997, Wynne *et al.* 1991). Genetic analysis according to Hayman (1958) revealed preponderance of non-additive, additive × additive, and additive × dominance gene effects on the expression of groundnut rust resistance. Ghewande (2009) reported that resistance to rust was conditioned by additive, additive x additive, and additive x dominance gene effects.

Few studies reported the gene regulation or transcript up-regulation in response to *P. arachidis.* Proite *et al.* (2007) identified 35 putative non-redundant resistance gene analogs (RGAs) and 26 pathogenesis related expressed sequence tags (ESTs) from a rust resistant accession of *A. stenosperma*. Bertioli *et al.* (2003) also reported 78 RGAs based on the nucleotide-binding site (NBS) regions involving *A. hypogaea* and four wild relatives (*A. duranensis, A. cardenasii, A. stenosperma, and Arachis simpsonii*).

1.7.2 Phenotyping for groundnut rust resistance

Accurate phenotyping for rust resistance is important for efficient genotype screening since most critical breeding decisions rely on results obtained from phenotyping (Pasupuleti *et al.* 2013). Selection of plants with a desired combination of traits is a challenging task in breeding programs because a large number of plants and traits are considered and recorded. Further, imposed screening conditions for one trait often have confounding effect(s) on the other. For instance, the rust pathogen being obligate in nature fails to establish and survive on leaf tissues that are already dead following leaf spot pathogen infection making rust screening difficult. Occurrence of chance escapes that get selected also compromises the reliability and reproducibility of phenotyping, particularly when relying on natural infection and limited number of replications (Mondal and Badigannavar 2010). Thus, artificial inoculation under controlled environments is key during initial screening to ensure even distribution of inoculum. Transfer of resistance to the rust disease through hybridization often rely on phenotyping, hence the need to properly define rust symptoms and other traits associated with resistance or susceptibility. Under these circumstances, newly emerging biotechnological tools like marker-assisted selection can play a crucial role in ensuring efficient selection and introgression of genes for disease resistance.

1.7.3 Genotyping of groundnut for rust resistance

Molecular markers are useful in diseases resistance breeding as they can complement phenotypic screening in the early phase of breeding programs. They allow identification of resistant lines at juvenile stage saving time and cost of screening and, allow easy identification, transfer, and tracking of both dominant and recessive genes. Use of both foreground and background selection could help to reduce linkage drag by aiding in the elimination of undesirable traits in a much shorter time than with conventional breeding alone. Several marker systems including Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLPs) and Microsatellites or Simple Sequence Repeat (SSRs) have been used in tagging of genes and selecting genotypes for rust resistance in groundnut. SSR markers are often preferred due to their co-dominance, simplicity, high polymorphism, repeatability, abundance, multi-allelic nature and their transferability within the genus *Arachis* (Moretzsohn *et al.* 2005, Pandey *et al.* 2012, Wang *et al.* 2012).

Pandey *et al.* (2012) studied variation among parental lines and identified microsatellite markers associated with rust resistance in groundnut that can be used in future marker assisted selection and gene introgression. Mace *et al.* (2006) fingerprinted 117 F2 lines segregating for rust resistance derived from the resistant parent VG 9514 and the susceptible parent TAG 24 and tagged the RAPD marker J171300 tightly linked to a rust resistance gene at a genetic distance of 18.5 cM using the modified bulk segregant analysis (BSA).Another

study conducted by Mondal *et al.* (2008) revealed more diagnostic markers associated with rust resistance genes. Analysis of molecular variance (AMOVA) and Kruskal–Wallis one-way ANOVA identified candidate SSR loci that could be valuable for mapping rust and LLS resistance (Kokalis *et al.* 1997, Mace *et al.* 2006). Varma *et al.* (2005) screened 23 SSR markers using 22 groundnut genotypes with. Khedikar *et al.* (2010) screened parental genotypes using 1,089 polymorphic SSR markers and identified a major QTL (QTLrust01) associated with rust resistance, contributing to 6.90–55.20% of the observed variation. Varshney *et al.* (2014) successfully introgressed a major QTL for rust resistance, through marker-assisted backcrossing, in three popular Indian peanut cultivars and generated several promising introgression lines with enhanced rust resistance and higher yield.

1.7.4 Mating design and genetic analysis of groundnut rust resistance

The choice of a mating design for estimating genetic variances is dictated by the objectives of the study, time, space, cost and other biological considerations. Jogloy *et al.* (1999) used the NCD II design involving high yielding and rust resistant lines to generate crosses for genetic analysis of rust resistance and associated agronomic traits. Another genetic study of rust resistance using line x tester mating design was conducted at the Centre for Plant Breeding and Genetics, TNAU, Coimbatore-3 (Tamil Nadu), India and revealed that resistance was recessive and governed in either monogenic, digenic or trigenic manners. Combining ability analysis using half diallel crosses and their parents revealed an additive type of gene action, implying that selection for high yield and for foliar disease resistance should be effective at later selection generations Joel *et al.* (2006). Breeders often use diallel mating schemes to estimate the potential value of genotypes and their combining ability effects for resistance to foliar diseases in groundnut using either a fixed or randomly chosen set of parental lines.

Combining ability studies provide a guideline for selecting of elite parents or crosses. It helps to choose parents and design crosses to accumulate fixable genes and to identify specific cross combinations for use in development of high-yielding rust resistant cultivars. Both specific combining ability (SCA) and general combining ability (GCA) effects have been reported to control resistance to foliar diseases of groundnut (Adamu *et al.* 2008). This suggests that resistance to foliar diseases is controlled by additive and non-additive genetic effect, hence, can be improved through hybridization and selection.

1.8 Conclusions

Developing rust resistant groundnut germplasm requires effective screening techniques and marker-assisted selection in order to identify good source of resistance. ICRISAT scientists identified different molecular markers useful for genomic-assisted breeding of groundnut. Furthermore, several rust resistant varieties were identified through hybridization with

landraces or wild relatives possessing QTL associated with groundnut rust resistance. Genetic control of rust resistance is still not clearly understood, therefore, studying the gene action influencing this trait is important. Further, groundnut rust and Late Leaf spot (LLS) often occur together, hence, their resistance should be selected for simultaneously.

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2 CHAPTER TWO

Groundnut production constraints, farming systems, and farmer-preferred traits in Tanzania

Abstract

Groundnut (*Arachis hypogaea* L.) production in Tanzania is affected by a multitude of biotic and abiotic stresses and socioeconomic constraints. The objective of this study was to document the groundnut farmers' major production constraints, farming systems, and varietal trait preferences in selected agroecologies of Tanzania. A participatory rural appraisal study was conducted in three groundnut-producing zones: Lake, Central, and Southern. Data were collected from 170 groundnut farmers using a semi structured questionnaire, focus group discussions, and field observations. The production constraints were mainly diseases and pests, which were reported by 87.7% and 84.9% of respondents, respectively. Groundnut rust, caused by *Puccinia arachidis* Speg, was the major cause of yield reduction, as reported by 30% of the respondents. Drought stress and non-availability of seed of improved varieties were other important constraints, as reported by 83.9% and 76.1% of the respondents, respectively. Groundnut agronomic attributes preferred by farmers were as follows: high yield (reported by 78.4% of respondents), disease resistance (71.2%), early maturity (66%), drought tolerance (63.0%), and pest resistance (63%). Medium to large grain size (reported by 62.6%of respondents) and tan and red seed color (59.2%) were the main farmer- and market-preferred groundnut seed quality traits. Groundnut variety development programs should therefore address the above constraints and farmer-preferred traits for sustainable groundnut production and productivity in Tanzania.

Key words: Agronomic attributes; *Arachis hypogaea*; farmers' preferences; groundnut rust; participatory rural appraisal; PRA

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2.1 Introduction

Groundnut (*Arachis hypogaea* L., AABB, 2n = 4x = 40) is one of the world's important crops, ranking fifth in oil production after soybeans (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), rapeseed (*Brassica napus* L.), and sunflower (*Helianthus annuus* L.). In addition, Rhizobia, in association with groundnut plant, fix atmospheric nitrogen into the soil, which improves soil fertility. Groundnut seed is a rich food source providing quality vegetable oil (48–50%), protein (26–28%), dietary fiber, minerals, and vitamins (Pasupuleti *et al.* 2013). Globally, groundnut is grown in more than 100 countries situated in tropical, subtropical, and warm temperate regions (Upadhyaya *et al.* 2012). According to FAOSTAT (2015), Africa accounted for about 32% of the global groundnut production in 2015.

Shinyanga, Tabora, Dodoma, Mbeya, and Mtwara regions are the major groundnut production agroecologies in Tanzania (NBS 2012). Tanzania produced 5% of global production of groundnut in 2015, mainly under rain-fed conditions. According to Sibuga *et al.* (1992), the crop is traditionally intercropped with cereals or cassava (*Manihot esculenta* Crantz). Farmers in Tanzania grow groundnut on flat seedbeds or on ridges. Yields of groundnut in Tanzania are reported to be 500 kg ha^{-1} to 1,000 kg ha^{-1} compared with 1,500 kg ha^{-1} to 2, 500 kg ha^{-1} reported in other African countries. For instance, in 2015, the mean groundnut yield (in shell) was 11,300 kg ha^{-1} in Tanzania, compared with 12,376 kg ha^{-1} reported in Nigeria and 11,536 kg ha^{-1} in Guinea-Bissau (FAOSTAT 2015). The lower yields in Tanzania have been attributed to unreliable rainfall, diseases and insects, low-yielding varieties and outdated agronomic practices (NARI 2010).

The most important biotic factors affecting groundnut production and productivity in the country include groundnut rosette disease (groundnut rosette assistor virus, groundnut rosette virus and a satellite RNA), rust (*Puccinia arachidis* Speg), early leaf spot (*Cercospora arachidicola* Hori), and late leaf spot (*Phaseoisariopsis personata* Berk. & Curtis) (Reddy *et al.* 2003). Use of improved groundnut cultivars and production technologies is essential for boosting crop yields. In-depth knowledge of farmers' preferences, production challenges, and priorities are prerequisites for production technology development (Ramadhani *et al.* 2002).

In Tanzania, there is no recent study documenting groundnut production constraints and traits preferred by farmers. The study conducted by Bucheyeki *et al.* (2010) in the Tabora region identified drought and low yielding varieties as the most serious production problems. Participatory rural appraisal (PRA) is a multidisciplinary research approach that aims to incorporate knowledge and opinions of farmers in the planning and management of research development projects and programs. For instance, participatory breeding incorporates farmers' concerns and preferences during variety development, testing, and release (Ceccarelli and Grando 2007). This results in increased adoption of newly developed cultivars

by farmers (Adu *et al.* 2004, Dorward *et al.* 2007). Various PRA techniques include key informant interviews, focus group discussions (FGDs), transect walks, matrix scoring, and ranking. These techniques are effective channels for improving interaction between researchers and farmers (Witcombe *et al.* 2006). In West Africa through farmer participatory selection, the International Crops Research Centre for the Semi-Arid Tropics and regional partners have developed diverse groundnut varieties with desirable attributes including varied maturity groups, resistant to groundnut rosette disease, foliar diseases, and agronomic traits (Ndjeunga *et al.* 2008). Yield increases attributable to the adoption of new cultivars of rice (*Oryza sativa* L.) resulting from participatory plant breeding programs have been reported in South and Southeast Asia (Witcombe *et al.* 2002). Danial *et al.* (2007) reported that improved varieties of potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.), pearl millet (*Pennisetum glaucum* [L.] RBr.), and maize (*Zea mays* L.) were developed in an international project in three Andean countries using participatory varietal selection. Therefore, it is important to consider farmers' needs and preferences in groundnut cultivar development and selection to ensure adoption of improved cultivars by farmers. The objective of this study was to identify the major constraints affecting groundnut production and farmer-preferred groundnut traits in Tanzania to guide future breeding programs.

2.2 Materials and methods

2.2.1 Description of study sites

The study was conducted in three regions: Mtwara (10.3539°S, 40.1682°E; Southern Zone), Dodoma (58.669´S, 35º, 46.093´E; Central Zone), and Shinyanga (3°39´43´´S, 33°25´23´´E; Lake Zone), which are the main groundnut production areas in Tanzania (Figure 2.1). The mean temperature in Mtwara ranges between 24.3°C in July and 27°C in December, with a mean annual rainfall of 820 to 1,245 mm. The site has an altitude of 135 meters above sea level (masl), with a rainfall pattern that is monomodal and erratic. A dry spell of 1–2 weeks often occurs at the end of January or at the beginning of February. Nanyumbu district was selected to represent this region.

Dodoma region was represented by the Bahi district, which has mean monthly temperatures varying between 15°C and 30°C. The area is located at an altitude of 1,080 masl, with an annual rainfall that is marked with large variations in amount and distribution, and it ranges between 300 and 800 mm, with a mean of 600 mm. The rainfall pattern is monomodal (December to April). A long dry season occurs between May and November.

Shinyanga region was represented by Ushetu district, which is located at 1,000 to 1,200 masl. The area is characterized by undulating plains with rocky hills, well-drained soils with low

fertility and a growing season that runs from December to March. The site experiences mean temperatures ranging from 16°C in June to 33°C in October, with prolonged warm conditions.

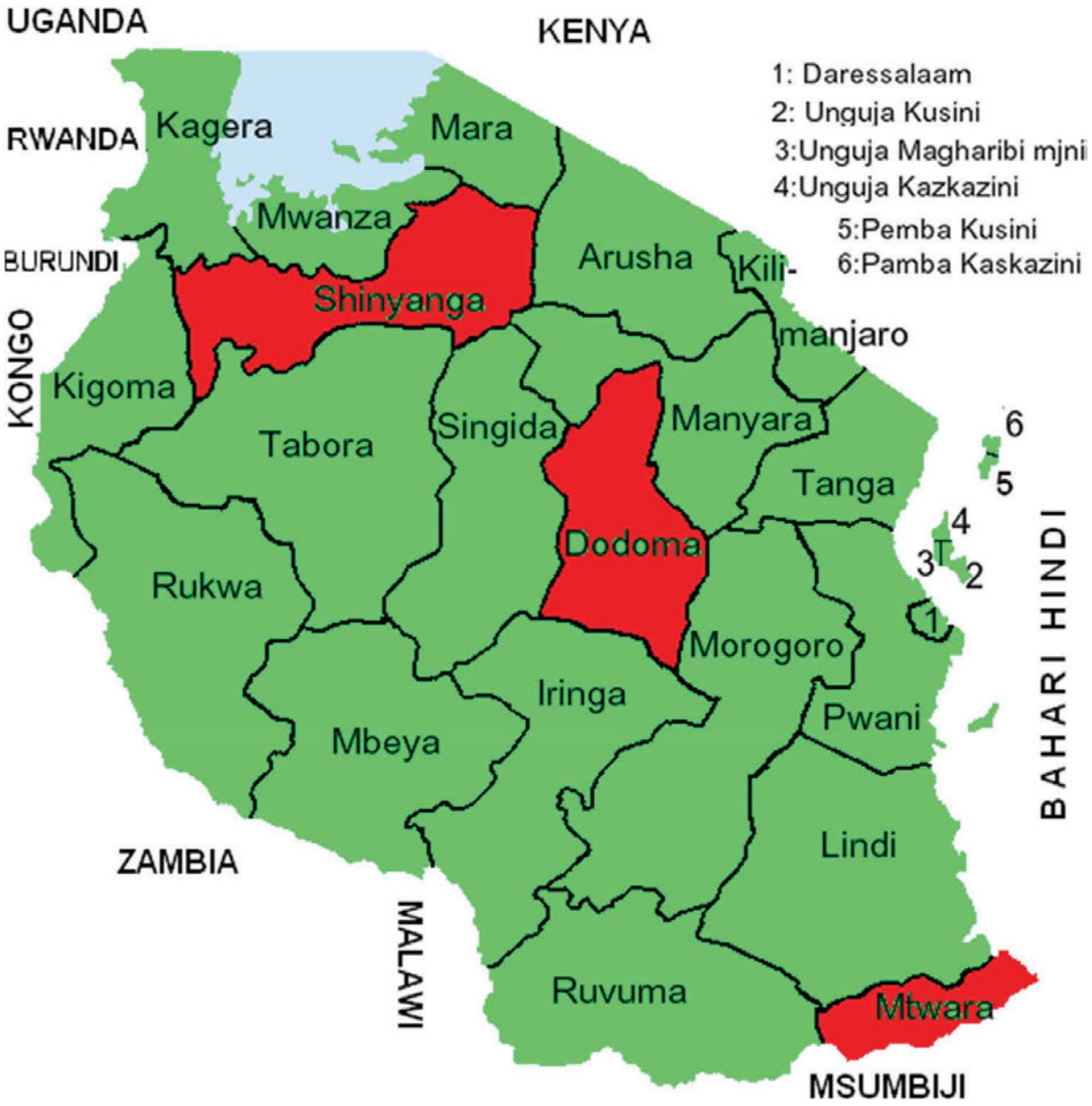

Figure 2.1 Map of Tanzania showing the study sites indicated in red shaded sectors

2.2.2 Questionnaire design, sampling, and data collection

A semi structured questionnaire, transect walks, and FGDs were used to collect information from selected farmers. Data gathered from transect walks and FGDs were used to support and validate the information obtained from the semi structured questionnaire. In each district, two wards were subsampled, which were Mpunze and Sabasabini in the Ushetu district, Kigwe and Ilindi in the Bahi district, and Likokona and Kamundi in the Nanyumbu district. Each ward was represented by two villages that resulted in a total of 12 villages, which were Mpunze,

Bulima, Sabasabini, Iponyanhoro, Kigwe, Mapinduzi, Ilindi, Mindola, Likokona, Msinyasi, Nawaje, and Nahimba. From each village, 10–15 farmers were selected with the assistance of agricultural extension officers and local leaders. In total, 170 farmers were interviewed using the semi structured questionnaire and FGDs. Through the semi structured questionnaire, the following data were gathered: household information, farm size, farming system used, constraints to groundnut production, important crop traits preferred by farmers, and market accessibility. Transect walk was done to make direct observations on a few randomly selected fields in each village. Other PRA tools used to gather information included problem listing and FGDs. In addition, farmers were queried about their understanding of groundnut rust disease and control measures they used. Farmers' preferred groundnut traits were described and ranked using a score of 1 (very important), 2 (intermediate importance), and 3 (least important).

2.2.3 Data analysis

Quantitative and qualitative social survey data collected were coded and analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 16 (SPSS 2007). Cross-tabulation tables were constructed, and descriptive statistics were generated to summarize data from the questionnaires and FGDs. To make statistical inferences, contingency Chi-square tests were conducted to analyze relationships between variables.

2.3 Results

2.3.1 Description of households

Table 2.1 contains a summary of the basic sociodemographic profile of the respondents. Out of the 170 smallholder farmers interviewed, 81 (48%) were females and 89 (52%) males, which suggested that there was gender balance in the study. The gap between number of males and females participating in the study was bigger in Nanyumbu, with >60% males and <40% females. Ushetu had an equal number of male and female participants, and the proportion of females (53%) was greater than that of males (47%) in Bahi. Both male and female farmers produced groundnut as a cash crop, though females used it as their instant cash source by selling it in small quantities to meet the financial needs of their families, whereas males tended to sell the harvest in bulk in a single transaction.

Table 2.1 Sociodemographic profiles of the farmers in the study areas

Variable	Category	District			Total	DF	Chi-Square	P-value
		Bahi	Ushetu	Nanyumbu				
Gender	Male	28	28	33	89	2	2.563	0.278
	Female	32	28	21	81			
Age (years)	15 - 30	7	6	4	17	4	9.237	0.055
	31 - 60	41	45	48	134			
	≥ 61	12	5	2	19			
Education level	Non-formal	9	7	6	22	10	5.628	0.845
	Primary incomplete	10	5	6	21			
	Primary complete	37	36	38	111			
	Secondary incomplete	1	3	2	6			
	Secondary complete	2	3	2	7			
	Tertiary education	1	2	0	3			
Family size (number of individuals per family)	≤ 5	30	13	34	77	4	49.328	0.004
	6-9	30	34	20	84			
	≥10	0	9	0	9			

Ten percent of the participants were under 30 years of age, 79% between 31 and 60 years, and 11% were >60 years of age. Farmers older than 60 years accounted for an average of 11% of the respondents. Most young people did not participate in the agricultural activities, as shown by a small percentage (10%) of respondents. Most respondents (65.3%) had attended primary school and were able to read and write the local language (Kiswahili). On the other hand, 4.1% and 1.8% respondents had obtained secondary and tertiary education; 12.4% and 3.5% of the respondents did not complete their primary and secondary education, respectively. The remainder 12.9% had not attended school at all (Table 2.1). The low level of education in the study areas necessitated the use of vernacular language by extension and research service providers or “change agents” in communicating the nature and value of any new technologies or agricultural inputs to these communities for their rapid adoption. The educated respondents (5.9%) can be useful agents in gathering information regarding farmers’ constraints, needs, and priorities. They can also serve as facilitators when introducing new technologies of value to the smallholder farming communities in the study areas.

About 45.3% of the total households in the three districts comprised ≤5 people and only 5.3% of the households comprised more than 10 people. About half of the families (49%) had 6–10 individuals. The number of individuals per household influenced farming operations requiring human labor. Households with more than five family members were more efficient in groundnut farming than families with fewer members, which predominantly outsourced their labor needs from their communities or cultivated only a small portion of their land. Labor was one of the major constraints affecting groundnut production operations, such as land preparation, planting, weeding, harvesting, and shelling.

2.3.2 Role of male and female farmers in groundnut farming activities

Results from all study sites showed that both men and women participated equally in groundnut farming activities. This contradicted the findings by Katundu *et al.* (2014), who reported that women were the major producers of groundnut in Tanzania. However, there were still some activities in which more women were involved than men, and vice versa. For instance, in threshing activity, females participated the most, whereas males were more involved in the selling activities in all the three districts (Table 2.2). In addition, females frequently engaged their children in farm activities, especially weeding, harvesting, and threshing.

2.3.3 Role of crop production in the study areas

In the study area, farmers depended on both crops and livestock as major sources of food and income. The area of land being cultivated by each interviewed individual farmer ranged from 0.1 to 8.8 ha. Crops grown in the study districts included groundnut, maize, cassava (*Manihot esculenta* Crantz), sesame (*Sesamum indicum* L.), rice (*Oryza sativa* L.), Bambara nut (*Vigna subterranea* Verdc.), cowpea (Vigna unguiculata [L.] Walp.), pigeonpea (*Cajanus cajan* L.), green gram (*Vigna radiata* [L.] Wilczek), cashew nut (*Anacardium occident*ale L.), sorghum (*Sorghum bicolor* L.), cotton (*Gossypium hirsutum* L.), common beans (*Phaseolus vulgaris* L.), sunflower (*Helianthus annuus* L.), watermelon (*Citrullus lanatus* L.), and sweet potato (*Ipomoea batatas* [L.] Lam.) (Figure 2.2). Of the total cultivated land, 9.7% was allocated to groundnut production and 8% to maize in the 2016/2017 cropping season. Some crops were grown in specific locations. For example, cashew nut was grown mostly in Nanyumbu district, occupying 14.7% of the total cultivated land. Furthermore, the amount of land allocated to sorghum in Bahi was almost equal to that of rice grown mostly in Ushetu (Figure 2.2). According to the farmers, most of the crops were grown during the rainy season, i.e. from December to April in Nanyumbu and Bahi and from October to February in Ushetu.

2.3.4 Groundnut production constraints

Production constraints faced by farmers in the three districts are summarized in Table 2.3. The major constraints included diseases, insect pests, drought, and non-availability of improved varieties. In the FGDs, female farmers identified field insect pests as the major constraint, followed by foliar diseases; whereas male farmers identified drought as the main groundnut production constraint, followed by field insect pests and diseases. Farmers' ranking of production constraints across districts showed that 85.7 to 90.7% of the respondents felt that groundnut production was highly constrained by diseases. The main diseases reported

were rosette (58.5%) and rust (30%) (Figure 2.3). Rust disease, reported mainly in Nanyumbu district (48.3%), was promoted by high temperature and humidity in this area. These findings were also observed during the transect walk in farmers' fields in Nanyumbu district (Figure 2.4). Mondal and Badigannavar (2015) reported that the development of rust epidemics was favored by continuous high temperatures (>22°C), along with wet weather or high humidity (>78%). A few farmers mentioned the removal of infected plants from their fields as one of the mitigation strategies against groundnut rosette disease. The ranking of diseases as production constraints did not show significant differences ($\chi^2 = 3.318$; $P = 0.506$) among the districts.

Table 2.2 Percent participation by farmers in various groundnut farming and market activities in Bahi, Ushetu and Nanyumbu district in Tanzania

	Bahi							Ushetu							Nanyumbu						
Activity	M†	F‡	C§	MFC¶	MF#	MC††	FC‡‡	M	F	C	MFC	MF	MC	FC	M	F	C	MFC	MF	MC	FC
Land preparation	8.3	20	0	10	58.3	0	3.3	10.7	8.9	1.8	25	46.4	3.6	3.6	5.6	20.4	0	9.3	59.3	0	5.6
Planting	20	13.3	0	13.3	50	1.7	1.7	3.6	10.7	0	26.8	48.2	3.6	7.1	7.4	11.1	0	14.8	55.6	0	11.1
1st weeding	5	12.5	0	25	51.7	1.7	5	3.6	7.1	0	37.5	42.9	0	8.9	1.9	11.1	0	13	50	0	11.1
2nd weeding	8.3	10	0	21.7	48.3	0	6.7	9	35.4	0	32.1	39.3	0	10.7	1.9	3.7	0	3.7	44.4	0	3.7
Harvesting	3.3	11.7	0	26.7	51.7	1.7	5	3.6	3.6	0	33.9	48.2	0	10.7	3.7	13	0	27.8	46.3	0	9.3
Drying	6.7	28.3	0	13.3	48.3	0	3.3	7.1	12.5	0	30.4	42.9	0	7.1	3.7	11.1	0	25.9	46.3	0	13
Threshing	11.7	30	0	13.3	23.3	1.7	11.7	3.6	17.9	0	23.2	10.7	0	19.6	3.7	11.1	5.6	29.6	22.2	0	9.3
Selling	37	25	1.7	0	35	0	0	42.9	14.3	0	1.8	41.1	0	0	35.2	22.2	0	0	42.6	0	0

†M = Male
‡F = Female
§C = Children
¶MFC = Male, female and children
#MF = Male and female
††MC = Male and children
‡‡FC = Female and children

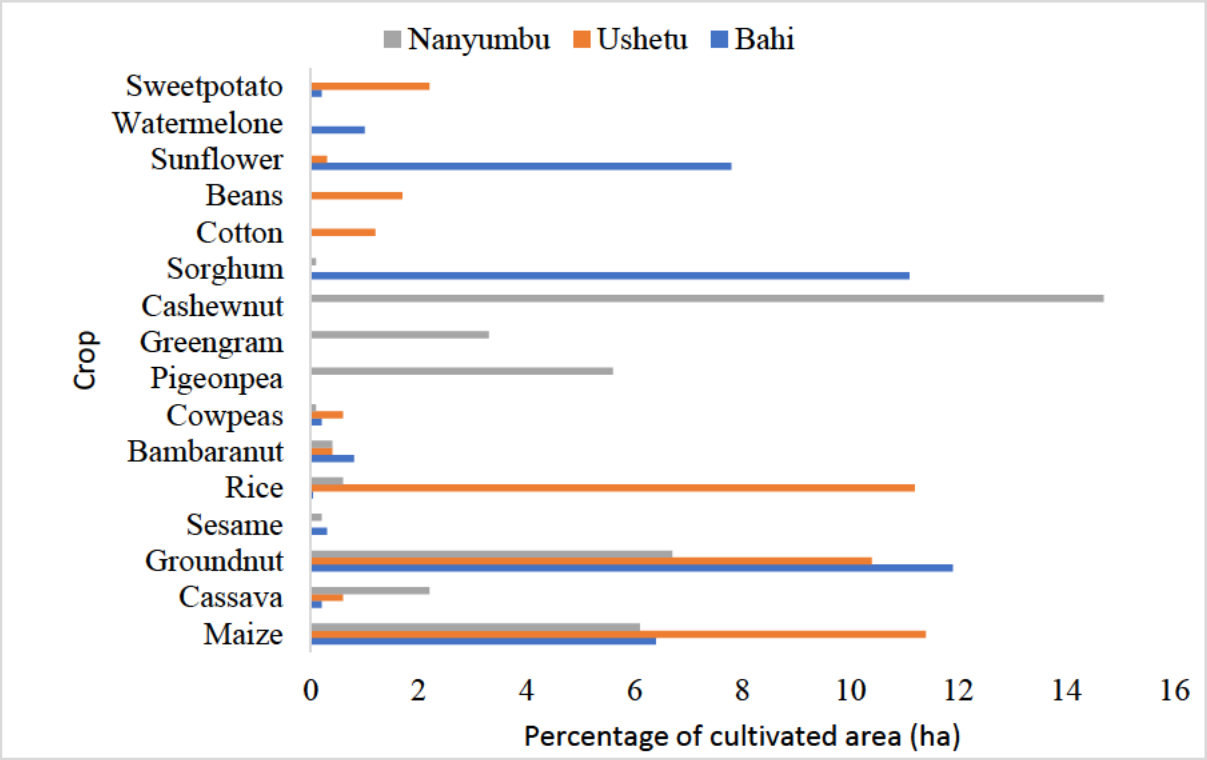

Figure 2.2 Different crop grown in 2016/2017 cropping season in three selected districts in Tanzania

Apart from the diseases, the second most yield-limiting factor in the study areas was field pests (Table 2.3). Groundnut hopper (*Hilda patruelis* Stal.) was one of the insect pests that affected groundnut production, followed by white grub (*Holotrichia consanguinea* Blanch.) and aphids (*Aphis craccivora* Koch.). Drought was ranked the third most yield-limiting factor for groundnut production in the study areas (Table 2.3). Severe droughts were reported by 74.1% to 89.3% of the respondents across all studied districts during the study period. Drought is associated with frequent fluctuations in the general atmospheric circulation in almost all parts of Tanzania. To mitigate drought stress, farmers adopted various strategies, such as mixed crop-livestock farming and early planting. In addition, drought was associated with rosette disease and aphid infestations.

2.3.5 Groundnut varieties grown in the study areas

The names of groundnut cultivars grown in the three districts were recorded using their local names (see Table 2.4). Improved varieties like Pendo and Mnanje were grown in the study areas by a few farmers. Different landraces were reported to be cultivated in each district, and most of them were maintained by farmers. In each district, the landraces were different because of several specific traits, such as adaptability to environmental stresses, drought tolerance, a high market value, seed availability, and the ability to adapt to different climatic

conditions. (Bucheyeki *et al.* 2008) reported on the adoption of the Pendo variety by farmers in the Tabora region, which was selected for its high yields, and Mamboleo, which was selected for its yield stability. Farmers indicated that Pendo, released in 1998 by Naliendele Agricultural Research Institute (NARI), was susceptible to diseases and insect pests. Mnanje 2009, also released by NARI, was reported to have poor germination and a high level of susceptibility to diseases.

Table 2.3 Percentage of farmers and reported groundnut production constraints in Bahi, Ushetu and Nanyumbu district in Tanzania

Constraints	Importance	District			Mean	DF	Chi-Square	P-value
		Bahi	Ushetu	Nanyumbu				
Limited land availability	Very important	28.6	7.3	9.3	15.1			
	Intermediate	16	10.9	22.2	16.4	4	13.8	0.008
	Less important	60.7	81.8	68.5	70.3			
Poor soil fertility	Very important	45	56.4	31.5	44.3			
	Intermediate	26.7	27.3	33.3	29.1	4	8.055	0.090
	Less important	28.3	16.4	35.2	26.6			
Low yielding varieties	Very important	74.6	60.7	50	61.8			
	Intermediate	8.5	5.6	5.6	6.6	4	10.280	0.036
	Less important	17	34	44.4	31.8			
Unavailability of improved varieties	Very important	83.3	84	61.1	76.1			
	Intermediate	10	7.1	7.4	8.2	4	16.451	0.002
	Less important	6.7	8.9	31.5	15.7			
High cost of seeds	Very important	63.3	61.8	85.2	70.1			
	Intermediate	6.1	9.1	3.7	6.3	4	9.197	0.056
	Less important	30.6	29.1	11.1	23.6			
Poor supply of seeds	Very important	69.5	80.4	54.7	68.2			
	Intermediate	11.9	3.6	9.4	8.3	6	12.849	0.045
	Less important	17	16.1	35.8	23			
Drought	Very important	88.3	89.3	74.1	83.9			
	Intermediate	10	8.9	9.3	9.4	4	13.705	0.008
	Less important	1.7	1.8	16.7	6.7			
Field insects	Very important	88.3	88.9	77.4	84.9			
	Intermediate	10	8.9	5.7	8.2	4	20.311	0.000
	Less important	1.7	32.1	17	16.9			
Storage pests	Very important	57.6	44.6	37.7	46.6			
	Intermediate	11.9	3.6	7.5	7.7	4	9.421	0.051
	Less important	30.5	51.8	54.7	45.7			
High cost of pesticides	Very important	38.6	44.6	44.9	42.7			
	Intermediate	24.6	33.6	10.2	22.8	4	11.103	0.025
	Less important	36.9	21.4	44.9	34.4			
Diseases	Very important	86.7	85.7	90.7	87.7			
	Intermediate	10	5.4	5.6	7	4	3.318	0.506
	Less important	3.3	8.9	3.7	5.3			

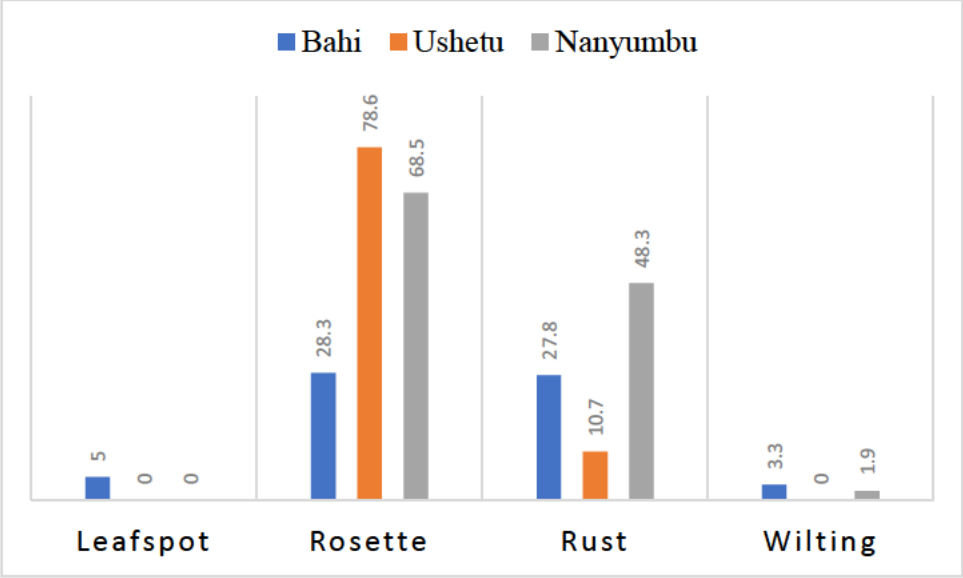

Figure 2.3 Percentage of respondent that reported the main groundnut diseases in the study areas

Figure 2.4 Groundnut rust in one of the farmer fields in Nanyumbu district

2.3.6 Farmer-preferred traits

Farmers in the study areas selected groundnut cultivars for production on the basis of yield, maturity, grain color, grain size, drought tolerance, insect pest resistance, disease resistance, good market price, taste, and oil content (Table 2.5). In addition, women considered taste to be an important trait, especially for making groundnut butter, which is used as an ingredient in preparing foods. Farmers in Bahi considered oil content to be an important trait because they usually received low market prices for varieties with low oil content.

Table 2.4 Groundnut varieties grown in the Bahi, Ushetu and Nanyumbu districts in Tanzania, and their associated characteristics

Districts	Names of varieties	Suggested traits	
		Preferred	Non-preferred
Bahi	Mamboleo,	Early maturity and drought tolerance	Susceptible to diseases and insect, low oil content and in high rainfall restart to germinate
	Pendo	Early maturity, drought tolerance, high yielding and sweet	Susceptible to diseases and insect
Ushetu	Red small	Marketable, early maturity, red in color and high oil content	Susceptible to diseases and insect
	Malumbalala	Early maturity and high yielding	Difficult to harvest and low oil content
	Mnanje	High oil content, red in color and sweet	Poor germination
	Pendo	Soft pod and high oil content	Low market price and susceptible to diseases and insect
Nanyumbu	Pendo	Early maturity, high yielding	Susceptible to diseases and insect and if delay to harvest can restart to germinate
	Johari	High yielding	Susceptible to diseases and insect
	Karanga Njugu	Hard pod cannot re-germinate	Susceptible to diseases
	Mnanje	High yielding	Poor germination and late maturity

Table 2.5 Farmer- preferred traits (% farmers) in groundnut varieties in Bahi, Ushetu and Nanyumbu district in Tanzania

Trait	District			Mean
	Bahi	Ushetu	Nanyumbu	
Yield potential	71.2	75	88.9	78.4
Maturity	63.3	67.9	66.7	66
Grain color	45	71.4	61.1	59.2
Grain size	55	67.9	64.8	62.6
Drought tolerance	70	76.8	64.8	63.0
Insect pest resistance	60	66.1	63	63.0
Disease resistance	68.3	69.6	70.4	71.2
Good market price	65	66.1	64.8	62.3
Taste	58.3	66.1	55.6	60
Oil content	16.7	3.6	7.4	9.2

Farmers preferred early-maturing varieties, which could escape drought and diseases. Some varieties had distinct, market-preferred traits, such as grain color, which varied across markets. For instance, in Bahi and Nanyumbu, tan color was preferred, whereas farmers in Ushetu preferred a red color. Large size of groundnut seed and resistance to diseases were some of the other traits preferred by farmers in the study areas.

2.3.7 Farmers' knowledge of groundnut diseases and management options

About 86.7% of farmers had knowledge about groundnut rust, whereas 11.3% had no knowledge about rust (Table 2.6). Common symptoms for groundnut rust disease mentioned by farmers included yellow and brown leaf color. However, it was noted that most interviewed farmers confused the rust disease with other foliar diseases, such as leaf spot. About 82% of the respondents did not know how the rust disease spread. Only a limited number of the respondents knew that rust was spread by wind and that the primary inoculum could arise from volunteer plants. All respondents described that they did not know how to control the rust disease, and all varieties cultivated were susceptible to the disease. This suggests that farmers' training is important, especially regarding rust control. Furthermore, it indicated the need for developing groundnut varieties with resistance to rust, in addition to other farmer-preferred traits, such as improved yield, early maturity, tolerance to drought stress, and medium grain size.

Table 2.6 Perception of farmers about groundnut rust in the study areas

		District						
Variable	Response	Bahi	Ushetu	Nanyumbu	Mean	DF	Chi-Square	P-value
Knowledge of groundnut rust	Yes	98.3	71.4	96.3	86.7	2	25.572	0.000
	No	1.7	28.6	3.7	11.3			
Rust spread	Do not know	76.7	89.3	83.3	82.1	6	73.957	0.000
	Volunteer	0	3.6	3.7	2.4			
	Wind	23.3	7.1	11.1	13.8			
	Soil	0	0	1.9	0.6			

2.4 Discussion

PRA is an important tool to learn from rural farming communities (Chambers 1994). In the present study, both male and female farmers were well-represented (Table 2.1), which reflected gender equality in groundnut production and planning for their community development (Table 2.1). In smallholder farming communities, the household is the major source of labor (Mendola 2007). Therefore, the larger the household size, the greater the labor force available, and, in turn, the larger the area of land cultivated. Households with only two members (wife and husband) or three members had limited labor, and therefore, they usually cultivated areas of less than one hectare. Households of four or more members cultivated areas of more than 2 ha. The study also showed that most active farmers were between 30 and 60 years of age in all districts (Table 2.1). This was because people of less than 30 years

of age had other jobs in nearby towns or they were selling goods, such as cold drinks and clothes in the villages.

Groundnut was grown for food and cash. Other crops, such as cassava, maize, sorghum, and cowpea, were grown specifically for food security and watermelon, sunflower, and cashew nut were grown for cash. Farmers used groundnut as a source of cooking oil or snacks (roasted or boiled groundnuts).

Most of the farmers in the study areas preferred groundnut cultivars that were characterized by high yield, early maturity, red and tan grain color, medium-to-large grain size, drought tolerance, insect pest resistance, disease resistance, good market prices, taste, and oil content. Kitch *et al.* (1998) reported that farmer-preferred cultivars had large red seed.

The results from this study indicated that most of the farmers were aware of the constraints affecting their crops. Constraints, such as diseases, insect pests, drought, and non-availability of improved cultivars, were reported to be the primary limiting factors in groundnut production in the study areas (Table 2.3). Groundnut rust was among the main diseases reported by farmers in the study areas. Respondents related rust symptoms to crop maturity since the disease appeared late in the season when the crop was about to mature.

This study-initiated dialog between groundnut farmers and groundnut researchers helped understand the main constraints to groundnut production encountered by farmers in the Lake, Central, and Southern zones of Tanzania. This dialog, through the participatory approach, confirmed that farmers were aware of the various issues affecting their daily lives, including crop production. According to Biggs (1978) farmers possess valuable knowledge and they can contribute to agricultural research and development and education.

During this study, farmers' participation in research activities occurring in their districts was somewhat low, which had led to a low rate of adoption of new technologies. The farmers continued to grow their local varieties, resulting in low yields. Farmer participation in agricultural research and development is important because it empowers them (Sperling *et al.* 1993) and increases the efficiency of the research by orienting it to their needs (Witcombe *et al.* 2006). Biggs (1989) proposed that farmers should be consulted to diagnose problems and influence research objectives, thus making them active partners in the research.

2.5 Conclusions

Groundnut is a food security crop and a source of income for rural households in sub-Saharan Africa. However, its productivity in the region is relatively low. Diseases, pests, drought, and no availability of improved seeds were identified as the main production constraints. Farmers in the study areas depended on agricultural activities, such as livestock rearing and growing a range of crops, in addition to groundnut, for food and income generation. Groundnut traits preferred by farmers were high yield, resistance to diseases and pests, early maturity, and

drought tolerance. Medium grain size, high oil content, and tan or red seed color were the quality traits preferred by the famers and the market. Researchers could use the identified farmer-preferred traits as selection criteria in their groundnut breeding program to enhance groundnut production in Tanzania.

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3 CHAPTER THREE

Genetic diversity and population structure of groundnut (*Arachis hypogaea* L.) accessions using phenotypic traits and SSR markers: implications for rust resistance breeding

Abstract

Groundnut (*Arachis hypogaea* L.) is a multi-purpose legume serving millions of farmers and their value chain actors globally. Use of old poor performing cultivars contributes to low yields (<1 t/ha of groundnut in sub-Saharan Africa including Tanzania). The objectives of this study were to determine the extent of genetic variation among diverse groundnut collections using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and complementary genotypes for breeding. One hundred and nineteen genotypes were evaluated under field conditions for agronomic traits and susceptibility to rust and leaf spot diseases. The study was conducted at two locations for two seasons. In addition, the 119 accessions were profiled with 13 selected SSR markers. Genotype and genotype by environment interaction effects were significant ($p<0.05$) for days to flowering (DTF), late leaf spot score at 85 and 100 days after planting, pod yield (PDY), kernel yield (KY), hundred seed weight (HSW) and shelling percentage (SP). Principal components analysis revealed that plant stand, KY, SP, NPP (number of pods per plant), late leaf spot and rust disease scores accounted for the largest proportion of the total variation (71.9%) among the tested genotypes. Genotypes ICGV-SM 08587 and ICGV-SM 16579 had the most stable yields across the test environments. Moderate genetic variation was recorded with mean polymorphic information content of 0.34 and gene diversity of 0.63 using the SSR markers. The majority (74% of genotypes showed high membership coefficients to their respective subpopulations, while 26% were admixtures after structure analysis. Much of the variation (69%) was found within populations due to genotypic differences. The present study identified genotypes ICGV-SM 06737, ICGV-SM 16575, ICG 12725 and ICGV-SM 16608 to be used for development of mapping population, which will be useful for groundnut improvement. This study provided a baseline information on characterization and selection of a large sample of groundnut genotypes in Tanzania for effective breeding and systematic conservation.

Keywords: Agronomic traits; Gene diversity; Molecular variance; Polymorphism; Principal component analysis; Rust disease; SSR markers; Structure analysis; Tanzania

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3.1 Introduction

Cultivated groundnut (*Arachis hypogaea* L., AABB, 2n = 4x = 40) is an allotetraploid and a predominantly self-pollinating legume crop cultivated in most parts of the world. About 26.54 million hectares of groundnut is cultivated globally with an annual production of approximately 43.92 million tons of shelled grain (FAOSTAT 2014, Upadhyaya *et al.* 2012). Africa accounts for about 31.6% of the global production. However, most African countries do not meet their domestic demand for groundnuts. The sub-Saharan Africa (SSA) region has one of the lowest groundnut productivity levels (<1 t/ha) in the world. FAOSTAT (2020) estimated monetary value of US$132 for importation of groundnut in Africa by 2020 to cover the shortfall due to low productivity in the region.

Groundnut productivity in Tanzania is <1 t/ha compared to a mean yield of 2.5 t/ha elsewhere in Africa (FAOSTAT 2018). The low productivity is attributable to an array of abiotic and biotic constraints. The most notable biotic constraints include rust and late leaf spot diseases. Rust disease, caused by *Puccinia arachidis* Speg, is an important disease of cultivated groundnut that causes up to 57% yield loss (Mondal and Badigannavar 2015), while late leaf spot, *Cercosporidium personatum*, causes up to 50% yield loss (Branch and Culbreath 2013). Yield losses of up to 70% can be incurred when the two diseases occur simultaneously (Khedikar *et al.* 2010, Subrahmanyam *et al.* 1985). The damage symptoms associated with the occurrence of early rust attack include early pod maturity, reduced seed size, increased pod senescence, and decreased oil content (Mondal and Badigannavar 2015). Late leaf spot causes the plants to lose most or all the leaves, which significantly reduces photosynthetic efficiency (Branch and Culbreath 2013). Both rust and late leaf spot diseases can be controlled using a combination of methods such as cultural practices, biocontrol agents and host plant resistance (Mondal *et al.* 2014). Chemical control using fungicides requires repeated applications leading to concerns over high costs of production, environmental pollution, low quality of produce due to chemical residue, health of the farmer and the possibility of development of fungicide resistance in the pathogen. The use of chemicals to control rust and leaf spot is widespread but most of the smallholder farmers who depend on groundnut production in Tanzania cannot afford crop protection chemicals or may use sub-optimal rates leading to high yield losses due to the disease (Bucheyeki *et al.* 2010)

The incorporation of host resistance in susceptible groundnut genotypes is cost-effective and environmentally friendly disease control method and is widely regarded as the most sustainable and effective method (Joel *et al.* 2006). Improving rust and leaf spot resistance in groundnut will effectively improve productivity and reduce cost of production. Developing disease resistant cultivars depends on the availability and identification of sources of resistance. Resistance genes for rust and late leaf spot diseases have been identified in a wild

relative of cultivated groundnut (*A. hypogaea*), elite inbred lines and commercial cultivars (Fávero *et al.* 2015, Han *et al.* 2018, Pande and Rao 2001). Improving resistance to rust in cultivated groundnut by introgressing resistance genes from wild Arachis species has been limited due to linkage drag associated with poor shelling, prominent reticulation and deep constriction in the pods (Dwivedi *et al.* 2003). There is a need to circumvent the unfavourable gene linkage by crossing divergent cultivated groundnut genotypes that harbour resistance genotypes. Hence, genetic variation among cultivated lines and landraces of groundnuts is more valuable for improving disease resistance because cultivated and elite inbred lines provide a readily available source of genes with potentially other farmer preferred traits.

Most groundnut genotypes grown in Tanzania are genetically diverse and unimproved landraces. These have not been tested for rust and leaf spot resistance, which could limit their use in breeding programs for developing rust or late leaf spot resistant cultivars with farmer-preferred traits. Therefore, screening the diverse germplasm maintained in Tanzania will contribute vital baseline information to facilitate selection of parental lines for cultivar development. The genetic pool initially acquired from ICRISAT-Malawi and maintained at Tanzania Agricultural Research Institute (TARI-Naliendele station), forms part of important groundnut genetic resources in Tanzania.

Several studies that documented genetic variation in groundnut focused on using morphological traits (Bertioli *et al.* 2011, Ferguson *et al.* 2004, Nautiyal *et al.* 2011). Significant differences in growth habit, leaf number, number of pods, kernel weight and yield have been reported widely. This suggests that adequate morphological variation exists in groundnut for selection of genetically complementary and unique parents for breeding (Huang *et al.* 2015, Upadhyaya *et al.* 2009, Zhang *et al.* 2017). Despite significant morphological variation in groundnut, the limited genetic variability for enhanced yield and yield related traits has been often cited as one of the reasons for little progress in genetic improvement of the crop (He *et al.* 2003). Morphological variations are largely influenced by environmental factors, which may affect the degree of trait heritability. Therefore, genotype screening should involve both phenotypic and molecular markers to elucidate the genetic potential of groundnut collections. In addition, there is a need to assess genetic variation and population structure of groundnut genetic resources using high throughput molecular markers.

Different molecular markers including amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), single nucleotide polymorphisms (SNP) and microsatellites or simple sequence repeat (SSR) markers have been used in genetic variation studies on groundnut (Dwivedi *et al.* 2001, Mondal *et al.* 2008, Pandey *et al.* 2014, Vishwakarma *et al.* 2017). The choice of using each of the techniques is influenced by factors such as ease of application, genome coverage, costs, and automation compatibility. SSRs are highly preferred for their ability to

detect high degrees of polymorphism, high reproducibility and abundant coverage of the genome (Pandey *et al.* 2012). In addition, SSR markers can be used for loci with multiple alleles and with co-dominant system (Gupta and Varshney 2000). Ren *et al.* (2014) and Wang *et al.* (2011) assessed genetic diversity and population structure in groundnut and found significant variation among Chinese cultivars and United States mini-core collections, respectively. Other studies have also reported the use of SSR markers in genetic analysis in groundnut (Mace *et al.* 2006, Mondal and Badigannavar 2010). However, the differences in the level of diversity across different germplasm collections and populations suggest that each population must be assessed in a target production environment for selection and systematic breeding program. Therefore, the objectives of this study were to determine the extent of genetic variation among germplasm from ICRISAT Malawi and landraces and varieties from Tanzania using phenotypic traits and SSR markers to select distinct and complementary genotypes for breeding. Data presented in the test populations provide useful information to deduce the population structure to devising a breeding strategy for enhanced yield and yield components and improved rust resistance by incorporating farmer-preferred traits in Tanzania.

3.2 Materials and methods

3.2.1 Plant materials

A total of 119 groundnut accessions (Table 3.1) were used in this study. The test accessions included ICRISAT's breeding populations, landrace collections from different agro-ecologies in Tanzania and cultivated varieties (Table 3.1). Genotypes from ICRISAT of constituting the following: 68-lines are selections from preliminary rust screening nurseries, 20 lines (selected from advance rust nurseries) and 20 (selected from regional rust trails).

Table 3.1 Origin and description of groundnut genotypes used in the study

SN	Line	Pedigree	Origin*
1	ICGV-SM 16554	(CG 7 X ICGV 02194) F2-P9-P1-B1-B1-B1-B1	ICRISAT-Malawi
2	ICGV-SM 16555	(JL 24 X ICGV 02194)- F2-P2-P1-B1-B1-B1-B1	ICRISAT-Malawi
3	ICGV-SM 16556	(PENDO X ICGV 99557) F2-P4-P1-B1-B1-B1-B1	ICRISAT-Malawi
4	ICGV-SM 16557	(ICGV-SM 01711 X ICGV 02194) F2-P9-P1-B1-B1-B1-B1	ICRISAT-Malawi
5	ICGV-SM 16558	ICGV-SM 05701 X ICGV 02194) F2-P1-P1-B1-B1-B1-B1	ICRISAT-Malawi
6	ICGV-SM 16559	(ICGV-SM 01514 X ICGV 02194) F2-P7-P1-B1-B1-B1-B1	ICRISAT-Malawi
7	ICGV-SM 16560	(ICG 11426 X ICGV-SM 90704) F2-P14-P1-B1-B1-B1-B1	ICRISAT-Malawi
8	ICGV-SM 16561	(ICG 11426 X PENDO) F2-P11-P1-B1-B1-B1-B1	ICRISAT-Malawi
9	ICGV-SM 16562	(ICG 11426 X ICGV-SM 01721) F2-P21-P1-B1-B1-B1-B1	ICRISAT-Malawi
10	ICGV-SM 16563	(ICGV-SM 90704 X ICG 11426) F2-P3-P1-B1-B1-B1-B1	ICRISAT-Malawi
11	ICGV-SM 16564	PENDO X ICG 11426	ICRISAT-Malawi
12	ICGV-SM 16565	(ICGV-SM 01711 X ICG 11426) F2-P11-P1-B1-B1-B1-B1	ICRISAT-Malawi
13	ICGV-SM 16566	(ICGV-SM 99555 X ICG 11426) F2-P8-P1-B1-B1-B1-B1	ICRISAT-Malawi
14	ICGV-SM 16567	(ICGV-SM 99557 X ICG 11426) F2-P14-P1-B1-B1-B1-B1	ICRISAT-Malawi
15	ICGV-SM 16568	(ICGV-SM 05701X ICG 11426) F2-P11-P2-B2-B1-B1-B1	ICRISAT-Malawi
16	ICGV-SM 16569	(ICGV 01276 X CHALIMBANA) F2-P14-P1-B1-B1-B1-B1	ICRISAT-Malawi
17	ICGV-SM 16570	(ICGV 01276 X ICGV-SM 90704) F2-P15-P1-B1-B1-B1-B1	ICRISAT-Malawi
18	ICGV-SM 16571	(ICGV 01276 X ICGV-SM 90704) F2-P22-P1-B1-B1-B1-B1	ICRISAT-Malawi
19	ICGV-SM 16572	(ICGV 01276 X JL 24) F2-P3-P1-B1-B1-B1-B1	ICRISAT-Malawi
20	ICGV-SM 16573	CHALIMBANA X ICGV 01276	ICRISAT-Malawi
21	ICGV-SM 16574	ICGV-SM 90704 X ICGV 01276	ICRISAT-Malawi
22	ICGV-SM 16575	(CG 7 X ICGV 01276) F2-P8-P13-B1-B1-B1-B1	ICRISAT-Malawi
23	ICGV-SM 16576	(JL 24 X ICGV 01276) F2-P16-P1-B1-B1-B1-B1	ICRISAT-Malawi
24	ICGV-SM 16577	(PENDO X ICGV 01276) F2-P18-P1-B1-B1-B1-B1	ICRISAT-Malawi
25	ICGV-SM 16578	(ICGV-SM 01721 X ICGV 01276) F2-P6-P1-B1-B1-B1-B1	ICRISAT-Malawi
26	ICGV-SM 16579	(ICGV-SM 99555 X ICGV 01276) F2-P4-P1-B1-B1-B1-B1	ICRISAT-Malawi
27	ICGV-SM 16580	(ICGV-SM 05701 X ICGV 01276) F2-P8-P1-B1-B1-B1-B1	ICRISAT-Malawi
28	ICGV-SM 16581	(ICGV-SM 01514 X ICGV 01276) F2-P1-P2-B1-B1-B1-B1	ICRISAT-Malawi
29	ICGV-SM 16582	ICGV 02286 X CHALIMBANA	ICRISAT-Malawi
30	ICGV-SM 16583	ICGV 02286 X ICGV-SM 90704	ICRISAT-Malawi
31	ICGV-SM 16584	(ICGV 02286 X CG 7) F2-P21-P1-B1-B1-B1-B1	ICRISAT-Malawi
32	ICGV-SM 16585	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
33	ICGV-SM 16586	(ICGV 02286 X ICGV-SM 05701) F2-P1-P3-B1-B1-B1-B1	ICRISAT-Malawi
34	ICGV-SM 16587	(ICGV 02286 X ICGV-SM 05701) F2-P1-P4-B1-B1-B1-B1	ICRISAT-Malawi
35	ICGV-SM 16588	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
36	ICGV-SM 16589	(ICGV 02286 X ICGV-SM 05701) F2-P1-P14-B1-B1-B1-B1	ICRISAT-Malawi
37	ICGV-SM 16590	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
38	ICGV-SM 16591	(ICGV 02286 X ICGV-SM 05701) F2-P1-P20-B1-B1-B1-B1	ICRISAT-Malawi
39	ICGV-SM 16592	(ICGV 02286 X ICGV-SM 05701) F2-P1-P24-B1-B1-B1-B1	ICRISAT-Malawi
40	ICGV-SM 16593	(ICGV 02286 X ICGV-SM 05701) F2-P1-P27-B1-B1-B1-B1	ICRISAT-Malawi
41	ICGV-SM 16594	(ICGV 02286 X ICGV-SM 05701) F2-P1-P28-B1-B1-B1-B1	ICRISAT-Malawi
42	ICGV-SM 16595	(ICGV 02286 X ICGV-SM 05701) F2-P1-P29-B1-B1-B1-B1	ICRISAT-Malawi
43	ICGV-SM 16597	(ICGV 02286 X ICGV-SM 05701) F2-P1-P31-B1-B1-B1-B1	ICRISAT-Malawi
44	ICGV-SM 16598	(ICGV 02286 X ICGV-SM 05701) F2-P1-P39-B1-B1-B1-B1	ICRISAT-Malawi
45	ICGV-SM 16599	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
46	ICGV-SM 16600	(ICGV 02286 X ICGV-SM 05701) F2-P1-P41-B1-B1-B1-B1	ICRISAT-Malawi
47	ICGV-SM 16601	(ICGV 02286 X ICGV-SM 05701) F2-P1-P44-B1-B1-B1-B1	ICRISAT-Malawi
48	ICGV-SM 16602	(ICGV 02286 X ICGV-SM 05701) F2-P1-P49-B1-B1-B1-B1	ICRISAT-Malawi
49	ICGV-SM 16603	(ICGV 02286 X ICGV-SM 05701) F2-P1-P50-B1-B1-B1-B1	ICRISAT-Malawi
50	ICGV-SM 16604	(ICGV 02286 X ICGV-SM 05701) F2-P1-P53-B1-B1-B1-B1	ICRISAT-Malawi
51	ICGV-SM 16605	(ICGV 02286 X ICGV-SM 05701) F2-P1-P54-B1-B1-B1-B1	ICRISAT-Malawi
52	ICGV-SM 16606	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
53	ICGV-SM 16607	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
54	ICGV-SM 16608	(ICGV 02286 X ICGV-SM 05701) F2-P1-P257-B1-B1-B1-B1	ICRISAT-Malawi
55	ICGV-SM 16609	(ICGV 02286 X ICGV-SM 05701) F2-P1-P58-B1-B1-B1-B1	ICRISAT-Malawi
56	ICGV-SM 16610	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
57	ICGV-SM 16611	(ICGV 02286 X ICGV-SM 05701) F2-P1-P60-B1-B1-B1-B1	ICRISAT-Malawi
58	ICGV-SM 16612	(ICGV 02286 X ICGV-SM 05701) F2-P1-P62-B1-B1-B1-B1	ICRISAT-Malawi
59	ICGV-SM 16613	(ICGV 02286 X ICGV-SM 05701) F2-P1-P64-B1-B1-B1-B1	ICRISAT-Malawi
60	ICGV-SM 16614	(ICGV 02286 X ICGV-SM 05701) F2-P1-P65-B1-B1-B1-B1	ICRISAT-Malawi
61	ICGV-SM 16615	(ICGV 02286 X ICGV-SM 05701) F2-P1-P67-B1-B1-B1-B1	ICRISAT-Malawi
62	ICGV-SM 16616	(ICGV 02286 X ICGV-SM 05701) F2-P1-P68-B1-B1-B1-B1	ICRISAT-Malawi
63	ICGV-SM 16617	(ICGV 02286 X ICGV-SM 01514) F2-P1-P2-B1-B1-B1-B1	ICRISAT-Malawi
64	ICGV-SM 16618	(ICGV 02286 X ICGV-SM 01514) F2-P1-P5-B1-B1-B1-B1	ICRISAT-Malawi
65	ICGV-SM 16619	(ICGV 02286 X ICGV-SM 01514) F2-P1-P6-B1-B1-B1-B1	ICRISAT-Malawi
66	ICGV 93542	ICGV 93542	ICRISAT-Malawi
67	ICGV-SM 15510	ICGV 93437 x ICGV 95342	ICRISAT-Malawi
68	ICGV-SM 15514	(ICGV 93437 x ICGV 95342) F2-P35-P6-B1-B1-B1-B1	ICRISAT-Malawi
69	ICGV-SM 15524	(ICGV 93437 x ICGV 95342) F2-P55-P53-B1-B1-B1-B1	ICRISAT-Malawi
70	ICGV-SM 15529	(ICGV 93437 x ICGV 95342) F2-P63-P41-B1-B1-B1-B1	ICRISAT-Malawi
71	ICGV-SM 15531	ICGV 95342 x ICGV 93437	ICRISAT-Malawi
72	ICGV-SM 15534	(ICGV 95342 x ICGV 93437) F2-P3-P23-B1-B1-B1-B1	ICRISAT-Malawi

SN	Line	Pedigree	Origin*
73	ICGV-SM 15536	(ICGV 94114 x JL 24) F2-P51-P10-B1-B1-B1-B1	ICRISAT-Malawi
74	ICGV-SM 15537	(ICGV 94114 x JL 24) F2-P50-P19-B1-B1-B1-B1	ICRISAT-Malawi
75	ICGV-SM 15538	(ICGV 94114 x JL 24) F2-P50-P14-B1-B1-B1-B1	ICRISAT-Malawi
76	ICGV-SM 15542	(ICGV 94114 x JL 24) F2-P35-P13-B1-B1-B1-B1	ICRISAT-Malawi
77	ICGV-SM 15546	ICGV 94114 x JL 24	ICRISAT-Malawi
78	ICGV-SM 15548	(ICGV 94114 x JL 24) F2-P9-P21-B1-B1-B1-B1	ICRISAT-Malawi
79	ICGV-SM 15554	(JL 24 x ICGV 94114) F2-P134-P7-B1-B1-B1-B1	ICRISAT-Malawi
80	ICGV-SM 15556	(JL 24 x ICGV 94114) F2-P113-P1-B1-B1-B1-B1	ICRISAT-Malawi
81	ICGV-SM 15557	(JL 24 x ICGV 94114) F2-P102-P13-B1-B1-B1-B1	ICRISAT-Malawi
82	ICGV-SM 15558	(JL 24 x ICGV 94114) F2-P93-P11-B1-B1-B1-B1	ICRISAT-Malawi
83	ICGV-SM 15559	(JL 24 x ICGV 94114) F2-P93-P4-B1-B1-B1-B1	ICRISAT-Malawi
84	ICGV-SM 15562	(JL 24 x ICGV 94114) F2-P65-P33-B1-B1-B1-B1	ICRISAT-Malawi
85	ICGV-SM 15564	(JL 24 x ICGV 94114) F2-P65-P22-B1-B1-B1-B1	ICRISAT-Malawi
86	ICGV-SM 15567	(JL 24 x ICGV 94114) F2-P27-P27-B1-B1-B1-B1	ICRISAT-Malawi
87	ICGV-SM 90704	(RG 1 x Manipintar) F2-P23-P59-P59-B1-B1-B13-B1	ICRISAT-Malawi
88	ICGV 94114	(J11 x CS 31) F2-B1-B1-B1-B1-B2-B1-B1-B2-B1	ICRISAT-Malawi
89	ICGV-SM 08578	ICGV 90082 X ICGV-SM 94581	ICRISAT-Malawi
90	ICGV-SM 08587	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
91	ICGV-SM 08586	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
92	CG 7	(USA 20 x TMV 10) F2-P3-B1-B1-B1-B1-B1B1-B1-B1	ICRISAT-Malawi/released variety
93	ICGV-SM 08581	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
94	ICG 12725	ICG 12725	ICRISAT-Malawi
95	ICGV-SM 05570	ICGV 90103 X PC 223 K9	ICRISAT-Malawi
96	ICGV 94124	(ICGV 87314 x NCAC 343) F2-B2-B1-B1-B1	ICRISAT-Malawi
97	ICGV-SM 06718	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
98	ICGV-SM 05611	ICGV 92092 X ICG 9991	ICRISAT-Malawi
99	ICGV-SM 05569	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
100	ICGV-SM 08584	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
101	ICGV-SM 06735	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
102	ICGV 95342	[(ICG(FDRS)33 x ECZ1135) x (ICG (FDRS) x J11)] F2-F1-B1-B2-B2-B1-B1-B1-B1-B2-B1-B1-B1	ICRISAT-Malawi
103	ICGV-SM 05616	ICGV 90100 X JL 24	ICRISAT-Malawi
104	ICGV-SM 87157	ICGV-SM 87157	ICRISAT-Malawi
105	ICGV-SM 06711	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
106	ICGV-SM 06737	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
107	ICG 10879	ICG 10879	ICRISAT-Malawi
108	ICGV-SM 01514	(ICGV 93437 X ICGV-SM 93561)-ICGX-SM 95041/6/P15/P3	ICRISAT-Malawi
109	Masasi 09	ICGV-SM 87727 x ICGV-SM 83708	TARI-Naliendele/released variety
110	Pendo 98	ICGMS -33	TARI-Naliendele/released variety
111	Narinut 15	ICGV-SM 87727 x ICGV-SM 83708	TARI-Naliendele/released variety
112	Mangaka 09	ICGV 93437 x ICGV-SM 94586	TARI-Naliendele/released variety
113	Naliendele 09	ICGV-SM 93437 x ICGV-SM 94586	TARI-Naliendele/released variety
114	Nachingwea 09	ICGV-SM 90704 x ICGV-SM83708	TARI-Naliendele/released variety
115	Kanyomwa	Na	Landrace (Nanyumbu)
116	Local Dodoma	Na	Landrace (Dodoma)
117	Mamboleo	Na	Landrace (Dodoma)
118	Local Tandahimba	Na	Landrace (Tandahimba)
119	Ndulima	Na	Landrace (Nanyumbu)

SN serial number, Na = not available, ICRISAT International Crops Research Institute for the Semi-Arid Tropics, TARI Tanzania Agricultural Research Institute

*names in parenthesis show collections areas in Tanzania

3.2.2 Phenotyping

3.2.2.1 Site description

The 119 accessions were evaluated at two research sites of the Tanzania Agriculture Research Institute (TARI) namely Naliendele Agricultural Research Centre and Chambezi Experimental Station. The genotypes were screened for resistance to rust disease and late leaf spot during the 2018 and 2019 seasons. TARI-Naliendele (10.3539^{0}S, 40.1682^{0}E) is situated at an altitude of 135 m above sea level (masl). The mean monthly temperatures for TARI-Naliendele ranges between 24.3^{0}C in July and 27^{0}C in December while the mean annual rainfall is between 820 and 1245 mm with a unimodal rain distribution. A dry spell of one to two weeks often occurs at the end of January or at the beginning of February. The soils at TARI-Naliendele described as sandy loam with pH of 4.5. Chambezi Experimental Station (06.5167^{0}S, 38.9167^{0}E is located at an altitude of 12 masl. The monthly temperatures at Chambezi vary between 24^{0}C in September and 30^{0}C in February. The site is characterized by a bi-modal rainfall pattern, commencing from October to December and April to June with expected dry spells from January to March. The annual rainfall ranges between 600 and 1000 mm, which is marked by high variation in amount and distribution. The soils at Chambezi were also sandy loam with a pH of 5.0.

3.2.2.2 Experimental design and trial establishment

The experiment was conducted under field conditions over two seasons and laid out using an 8 × 15 alpha lattice design with two replications. Each genotype was planted on a plot consisting of two rows that were four metres long. The inter-row spacing was 50 cm with an intra-row spacing of 10 cm. The total plot size for each genotype was 4.0m^2. The recommended practices for fertilizer application and weeding in Tanzania were followed (NARI 2001). The trials at Chambezi were established under natural rainfall and TARI-Naliendele under natural rainfall and supplemental sprinkler irrigation when required. These sites are hotspots for rust and late leaf spot diseases. Hence, the genotypes were evaluated under natural disease infection. A susceptible genotype, Pendo 98, was planted next to each plot serving as a disease spreader through maintaining effective inoculum source for test genotypes.

3.2.2.3 Data collection

Data on yield and yield components were recorded during plant growth and at harvest maturity. The initial plant stand (IPS) was determined by counting the number of plants in each plot after germination. Days to 75% flowering (DTF) were recorded by counting the number of

days from sowing to the time when 75% of the plot stand had reached flowering. Plant height (PH, expressed in cm) was measured from ten randomly sampled plants in each plot from the soil surface to the tip of main stem. The number of pods per plant (NPP) was recorded as the average number of pods from ten randomly sampled plants. Final plant stand (FPS) was recorded as the number of plants in each plot before harvesting. Pod yield (PDY) was measured by weighing the dried pods from each plot and was recorded in grams per plot. Shelling percentage (SP) for each genotype was calculated from a random sample of pods weighing 200 g, as the proportion of shelled seed weight to the total weight of the unshelled pods. Additionally, 100 seed weight (HSW, expressed in grams) for each genotype was recorded as an average weight of two samples of 100 randomly selected kernels per plot. Kernel yield (KY, expressed in t ha^{-1}) was estimated as the product of pod yield per plot and shelling percentage and was converted to t ha^{-1} accordingly, using the plot size after adjusting for moisture content.

Rust severity was scored twice at 85 and 100 days after planting. The severity score at 85 days is represented as %RI85 while at 100 days it is designated as %RI100. Severity was scored using a scale of 1 (least affected) to 9 (most affected) (Das *et al.* 1999). Plants with no symptoms of infection were assigned a disease score of 1 (for 0% infection) while leaves with 1–5% infection were assigned a score of 2, 6–10% infection (score 3), 11–20% infection (score 4), 21–30% (score 5), 31–40% infection (score 6), 41–60% infection (score 7), 61–80% infection (score 8) and 81–100% infection (score 9) (Subbarao *et al.* 1990). Plants with a disease score of 1–3, 4–6 and 7–9 were considered to be resistant, moderately resistant and susceptible, respectively (Pande *et al.* 2002). In addition, late leaf spot reaction was assessed as a secondary trait. Late leaf spot disease often occurs simultaneously with rust disease. The screening procedure and scoring for late leaf spot was like the one used for rust disease.

3.2.3 Genotyping

Seeds of the 119 groundnut accessions were sown under greenhouse conditions at TARI-Naliendele, Tanzania. Ten seeds per genotype were planted and allowed to establish for 20 days. Five healthy and randomly selected leaves were sampled per genotype for DNA extraction. The leaves were sun dried after collection and then packed in paper bags with silica gel before shipment to the Centre of Excellence in Genomics and Systems Biology, ICRISAT in India. The Cetyl-tetramethyl ammonium bromide (CTAB) procedure was followed during DNA extraction (Cuc *et al.* 2008). The DNA quality and quantity were checked on nanodrop and DNA concentration was normalized to ~ 10 ng/µl for further genotyping with the linked markers. A total of 13 SSR markers were used in the study (Table 3.2).

Table 3.2 Names and sequence information of the 13 SSR markers used for genetic analysis

SN	Marker	Forward sequence	Reverse sequence	Reference
1	IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA	Cuc et al. (2008)
2	GM2301	GTAACCACAGCTGGCATGAAC	TCTTCAAGAACCCACCAACAC	Varshney et al. (2014)
3	TE 360	GGGATATGATGCCCATAGCTGA	TGCTGACTACTTGCAATGCC	Mondal et al. (2014)
4	TE 498	ATGACTTACATGTAGCAATTG	TGAAAGGAGTCAAAGGTCATG	Mondal et al. (2014)
5	PM 050	CAATTCATGATAGTATTTTATTGGACA	CTTTCTCCTCCCCAATTTGA	He et al. (2003)
6	PM179	CTGATGCATGTTTAGCACACTT	TGAGTTGTGACGGCTTGTGT	He et al. (2003)
7	pPGPseq-17F6	CGTCGGATTTATCTGCCAGT	AGTAGGGGCAAGGGTTGATG	Mace et al. (2006)
8	pPGPseq-16C6	TTGCTACTAAGCCGAAAATGAAG	CTTGAAATTAACACATATGCACACA	Mace et al. (2006)
9	pPGPseq-8E12	TCTGTTGAGAACCACCAGCA	GTGCTAGTTGCTTGACGCAC	Moretzsohn et al. (2005)
10	pPGPseq-10D4	ATCCCTGATTAGTGCAACGC	CGTAGGTGGTTTTAGGAGGG	Moretzsohn et al. (2005)
11	pPGPseq-12F7	TGTCGTTGTAAGACCTCGGA	TTGGTTTCCTTAAGGCTTCG	Moretzsohn et al. (2005)
12	pPGPseq-13A10	AACTCGCTTGTACCGGCTAA	AGGAATAATAACAATACCAACAGCA	Moretzsohn et al. (2005)
13	SSR_HO115759	TATCAACGCAACCTTTTGCAG	GACTTGTGTGGCTGAAACTTGA	Mondal et al. (2012)

The markers used in this study were purposefully selected because of their suitability indiscriminating groundnut genotypes for rust resistance. The markers showed high polymorphic information content and recommended for genetic analysis in groundnut. These were amplified using the polymerase chain reaction (PCR) following the procedures outlined by (Khedikar *et al.* 2010, Sujay *et al.* 2012). The PCR amplicons of the linked markers were separated as described in Varshney *et al.* (2009a).

A 10 µl PCR mix containing 15 mM of magnesium chloride, 2 µl dNTPs, 5u/ul Taq, 10 pm/ul primer, 10 × PCR buffer and 5.95 MilliQ H_2O was used for PCR amplification. The initial denaturation temperature was set at 94^0C with subsequent 10 rounds of denaturing at -1^0C. Annealing was conducted at 55^0C for 10 secs while the PCR substrates were set for at 72^0C for 20 s to allow for extension. Thereafter, the samples were visualized by fluorescence using the Genetic Analyser 3130xl and electrophoresis was conducted on an ABI 3013 automatic

sequencer. Allele sizing of the electropherograms was carried out using GeneMapper V4 software and the fragment sizes were provided as Excel output.

3.2.4 Phenotypic data analyses

The phenotypic data was subjected to analysis of variance (ANOVA) to test the effects of genotypes and locations and their interaction using the restricted maximum likelihood model (REML) procedure for alpha lattice designs in GenStat 18th edition (Payne 2015). The means were separated by the Fischer's unprotected least significant difference at 0.05. The correlations among the traits were based on the Pearson correlation coefficients conducted in R (RCoreTeam 2019). Multivariate analysis using the principal components was conducted using the Statistical Package for Social Science (SPSS) software version 24 (Kirkpatrick and Feeney 2012). The genotype and genotype × environment interaction (Singh *et al.* 2012) analysis was performed to test the effects of genotypes and environments, and their interaction. The effects of genotype, genotype × environment interaction were visualized graphically using the GGE biplot constructed in Genstat 18^{h} edition (Goedhart and Thissen 2010). The GGE biplots were based on the first two principal components (PC1 and PC2) after compressing multi-environment data into a single value (Yan *et al.* 2001). Two GGE biplots were constructed for visual assessments, one focused on the genotype differences while the other depicting the environmental variation.

3.2.5 Genotypic data analyses

The major allele frequency, the number of effective alleles, heterozygosity and gene diversity were calculated using the simple allele frequency estimator while polymorphic information content values were estimated using the equation below (Botstein *et al.* 1980).

PIC = $1–\Sigma(pi^2)$, where pi is the frequency of ith allele.

Hierarchical cluster analysis was conducted based on Ward minimum variance test using R statistical software (RCoreTeam 2019). The cluster patterns were visualized using factoextra package (Kasambara and Mundt 2017) in the R statistical software. The population structure was inferred using Structure 2.0 software (Falush *et al.* 2003). The optimal number of subpopulations (K) was identified based on maximum likelihood and delta K (ΔK) values (Evanno *et al.* 2005). The STRUCTURE program was run 10 times for each K value using the admixture model and correlated allele frequency, with 20,000 burn-in period and 10 000 Markov Chain Monte Carlo (MCMC) iterations during analysis. A repeat run with 50,000 burn in and 100,000 MCMC iterations was carried out to confirm the best K value.

Analysis of molecular variance (AMOVA) was conducted using PowerMarker software version 3.25 (Liu and Muse 2005) to partition genetic variation between and among populations. Significance of estimated variance components was based on 10,000 random permutations.

3.3 Results

3.3.1 Genetic variation among groundnut accessions

The ANOVA revealed that the 3-way interaction involving genotype, location and season had significant ($p<0.05$) impact on IPS, FPS, DTF, PH, NPP, PYD, KY, HSW and SP (Table 3.3). The days to 75% flowering, %LLSI at 85 and 100 days after planting, PDY, KY, HSW, and SP were also significantly ($p<0.05$) different due to the interaction effect between genotype and location. All the traits were significantly ($p<0.05$) affected by the genotype x season interaction except number of pods per plant and rust score at 100 days after planting. Rust score at 85 days after planting did not show significant ($p<0.05$) difference across seasons and locations. There was wide genotypic variation for most assessed traits ($p<0.001$) due to genotype main effect for all traits except NPP and SP.

The top 10 accessions with high pod yield and the five bottom performing genotypes are summarized in Table 3.4. These included ICGV-SM 16579 (967.5 $kgha^{-1}$), ICGV-SM 16613 (926.8 $kgha^{-1}$) and ICGV-SM 08587 (893.7 $kgha^{-1}$) with moderate rust disease scores except for ICGV-SM 08587, which showed resistant to rust disease at hundred days after planting (Table 3.4). The mean pod yield across locations was 567.45 $kgha^{-1}$ and kernel yield were 291 $kgha^{-1}$. The highest average rust (35.17%) and late leaf spot (31.96%) scores were observed 100 days after planting compared to 85 days after planting. Pendo 98, which was used as a susceptible check showed moderate infection to both diseases (Supplementary Table 1) and it attained an average pod yield of 692.5$kgha^{-1}$ The five bottom performing accessions in terms of pod yield were Narinut 15 (252.5 $kgha^{-1}$), ICGV-SM 16574 (310.6 $kgha^{-1}$), ICGV 95342 (318.1 $kgha^{-1}$), ICGV-SM 08584 (338.4 $kgha^{-1}$) and ICGV-SM 06711 (338.7 $kgha^{-1}$). These accessions yielded below average pod yield. Narinut 15 and ICGV-SM 08584 showed resistance reaction to groundnut rust and late leaf spot.

Table 3.3 Analysis of variance showing mean squares and significant tests for eight traits of 119 groundnut accessions evaluated across four environments (2 seasons x 2 locations)

Source of variation	DF	IPS	FPS	PH	DTF	NPP	%LLS 85	%LLS 100	%RS85	%RS100	PDY	KY	HSW	SP
Locations (L)	1	346.34***	97.38***	326.70***	75.76***	183.18***	251.39***	176.43***	0.07	34.57***	2222.70***	1589.26***	483.14***	24.65***
Rep	1	1.70*	6.30**	10.10**	0.56	0.61	39.54***	16.22***	6.27*	18.63***	1.01	0.40*	0.52	0.18
Block	7	2.33*	1.92	0.67	1.21	0.98	3.01**	2.52*	1.78	2.73**	0.48	0.63	2.51	1.33
Genotypes (G)	119	2.08***	2.68***	2.68***	1.71***	1.09	3.43***	4.66***	2.34***	4.00***	4.25***	3.17***	2.01***	1.04
Seasons (S)	1	101.81***	553.12***	312.41***	55.60***	1089.90***	584.17***	476.99***	1.42	5.67*	37.68***	31.98***	500.32***	14.91***
GxL	119	0.91	1.2	1.06	1.27*	0.96	1.33*	1.57***	1.01	0.90	4.58***	3.78***	2.27***	1.30*
GxS	119	1.29*	1.97***	1.31*	1.30*	0.80	1.32*	1.35*	1.34*	1.07	4.43***	3.68***	0.80	1.27*
SxL	1	899.66***	479.86***	2659.56***	43.37***	508.89***	164.81***	669.82	25.19***	116.62***	460.79***	436.14***	251.12***	54.05***
GxLxS	119	0.69	0.77	1.04	1.12	0.78	1.02***	1.71***	1.02	0.93	4.45***	3.26***	0.77	1.04
Residual		184.1	106.3	12.52	12.38	18.18	70.19	113	88.67	187.3	39529	14165	67.91	345.8.

DF= degrees of freedom, IPS= initial plant stand, FPS =final plant stand, PH= plant height, DTF= days to flowering, NPP number of pods per plant, %LLSI 85= Percentage late leaf spot infection at 85 days after planting, %LLSI =100 percentage late leaf spot infection at 100= days after planting, %RI 85= percentage rust infection at 85 days after planting, %RI 100= percentage rust score infection at 100= days after planting, PDY= pod yield, KY= kernel yield, HSW= hundred seed weight, SP shelling percent *,** and *** represent significant differences at 0.05, 0.01 and 0.001 probability levels, respectively

Table 3.4 Mean values for agronomic traits of 119 groundnut genotypes showing the top 10 and bottom 5 ranked genotypes based on mean pod yield (kg/ha) across four environments

Genotypes	IPS	FPS	PH	DTF	NPP	%LLS85	%LLS100	%RS85	%RS100	PDY	KY	HSW	SP
Top 10 genotypes													
ICGV-SM 16579	46.46	38.49	15.49	32.42	7.145	14.39	31.96	13.13	35.17	967.5	310.5	33.16	38.68
ICGV-SM 16613	46.53	34.43	12.99	31.28	10.86	7.87	18.88	13.12	27.68	926.8	432.8	34.7	42.57
ICGV-SM 08587	48.39	25.09	12.86	35.4	10.479	0.63	3.83	0.62	6.92	893.7	333.5	30.75	38.5
ICGV-SM 16555	41.95	32.13	14.65	34.14	6.054	12.53	17.02	7.51	19.33	869.4	433.8	26.2	49.05
ICGV-SM 16572	44.2	29.4	18.56	33.09	12.037	10.63	22.4	6.87	24.25	870.3	375.8	30.46	44.06
ICGV-SM 15546	40.91	31.15	13.8	33.04	9.775	5.01	7.04	3.12	8.31	844.7	426.7	28.54	46.59
ICGV 94124	43.54	29.39	13.89	32.84	6.548	6.26	16.97	3.75	7.1	835.0	475.6	27.63	44.42
ICGV-SM 16593	42.32	33.99	16.82	35.67	7.961	13.14	28.83	10.63	23.67	834.4	431.4	23.54	40.08
ICGV-SM 16589	41.73	38.96	17.21	31.92	7.289	15.63	26.46	13.75	29.4	810	431.7	24.33	43.74
ICGV-SM 15510	53.13	39.14	9.56	34	8.365	6.9	11.74	1.88	3.25	803.7	436.3	23.47	49.8
Bottom 5 genotypes													
Narinut 15	42.93	14.46	14.68	35.77	8.706	3.14	9.45	1.87	9.42	252.5	329.5	117.1	38.48
ICGV-SM 16574	48.19	40.24	17.3	33.08	8.729	15.03	30.48	12.52	25.07	310.6	264	118.4	40.44
ICGV 95342	40.28	31.64	16.26	34.06	9.953	13.13	22.7	16.25	23.39	318.1	475.6	163.3	48.46
ICGV-SM 08584	38.41	22.26	13.99	35.72	9.02	3.14	7.89	8.12	7.73	338.4	258.4	159.7	45.26
ICGV-SM 06711	31.27	20.01	15.34	34.34	10.79	7.52	14.14	6.89	22.73	338.7	282.3	165.6	39.71
Mean	40.28	31.2	16.10	33.6	8.69	10.49	18.75	10.14	21.75	567.45	291.16	27.86	39.96
LSD (5%)	19.69	15.61	7.48	3.68	7.07	11.79	16.94	9.68	14.51	341.9	190.2	11.50	16.22
CV %	49.81	51.27	47.23	11.17	82.96	114.09	91.96	97.24	67.92	61.49	66.48	42.02	34.82
R^2	0.40	0.32	0.17	0.02	0.13	0.88	0.01	0.94	0.00	1	0.75	0.86	0.00
SED FIX	10.03	7.96	3.81	1.88	3.60	6.01	8.63	4.93	7.39	174 20	96.89	5.86	8.26

Notes: IPS = initial plant stand; FPS = final plant stand; PH = plant height, DTF = days to flowering; NPP = number of pods per plant; %LLSI 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 = Percentage late leaf spot infection at 100 days after planting; %RI 85 = Percentage rust infection at 85 days after planting,% RI100 = Percentage rust infection at 100 days after planting; PDY = pod yield; KY = kernel yield; HSW = hundred seed weight; SP = shelling percent; LSD = Least significant difference; CV = coefficient of variation; R^2 = coefficient of determination; SED = Standard error of the mean differences.

3.3.2 Genotype × environment interaction effects on pod yield

The two axes in the GGE biplot accounted for 100% of the variation in the tested germplasm collections. Genotype ICGV-SM 16560, which represented with number 7 was found on the vertex of the polygon in the sector belonging to Chambezi site while ICGV-SM 16579, which represented with number 26 was the vertex genotype for TARI-Naliendele (Fig. 3.1). The two sites were distinctly different and did not belong to the same mega environment. Entries such as ICGVSM 08584 (number 100), ICGV-SM 06737 (number 106) and Narinut 15 (number 111) did not show specific adaptation to a particular environment. TARI Naliendele site had higher discriminatory capability and was more representative of the ideal environment compared to Chambezi (Fig. 3.2). In general, most genotypes exhibited lower mean performance at Chambezi site over both seasons compared to TARI Naliendele. The average environment coordinate (AEC) view from the GGE analysis compares the mean performance of each genotype and its stability across the test environments. In this study, the AEC view showed genotype ICGV-SM 08587 (number 90) as the superior genotype and stable in terms of pod yield as located close to ideal genotype (Fig. 3.2).

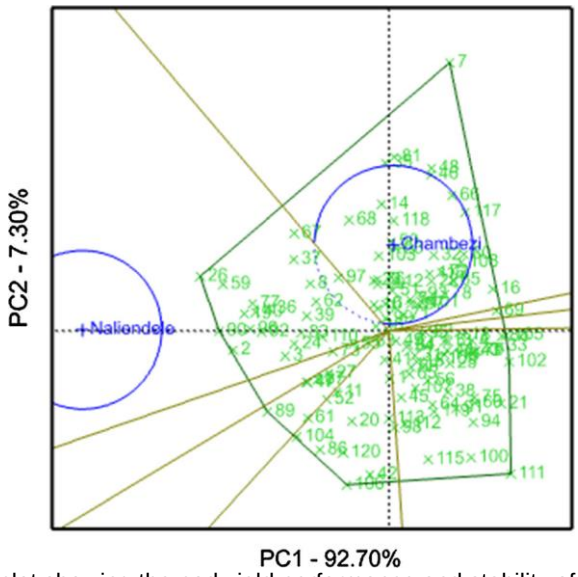

Figure 3.1 GGE-biplot showing the pod yield performance and stability of 119 accessions evaluated across two locations. Note: see codes of accessions in Table 3.1

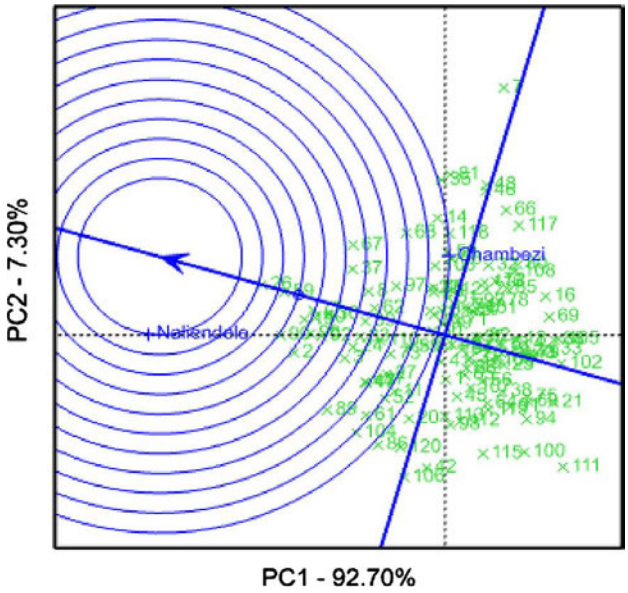

Figure 3.2 GGE-biplot comparing the test environments to the average environment coordinates based on pod yield of 119 accessions. Note: see codes of accessions in Table 3.1

3.3.3 Correlations among traits

The Pearson correlation coefficients (r) among the traits were calculated and presented in Table 3.5. At TARI-Nalindele, the traits that exhibited significant correlation with KY were DTF ($r = 0.133$, $p<0.01$) and NPP ($r = 0.231$, $p<0.01$) (Table 3.5, above diagonal). Traits such as PH ($r = - 0.194$, $p<0.01$), %LLSI85 ($r = -0.275$, $p<0.01$), %LLSI100 ($r = - 0.212$, $p<0.01$) and %RI100 ($r = - 0.204$, $p<0.01$) exhibited negative associations with KY. At Chambezi, KY was

significantly correlated with FPS (r = - 0.392), PH (r = 0.556), NPP (r = 0.637), %LLSI85 (r = -0.153), %LLSI100 (r = 0.192), %RI100 (r = 0.358) and PDY (r = 0.639) at $p<0.01$ (Table 3.5, below diagonal). The percentage LLS and rust infection were positively correlated in both test sites.

Table 3.5 Pearson's correlation coefficients showing the association of phenotypic traits of 119 groundnut genotypes evaluated across two seasons at TARI-Naliendele (above diagonal) and Chambezi (below diagonal)

Traits	IPS	FPS	DTF	PH	NPP	%LLSI85	%LLSI100	%RI85	%RI100	PDY	KY	HSW	SP
IPS		0.54**	0.02	-0.40**	0.22**	-0.26**	-0.32**	0.15**	-0.07	-0.12**	0.08	-0.10*	-0.14**
FPS	0.96**		-0.29**	0.13**	-0.05	0.16**	0.18**	0.07	0.04	0.07	0.01	0.08	0.12**
DTF	-0.23**	-0.26**		-0.54**	0.33**	-0.49**	-0.56**	-0.08	-0.28**	-0.28**	0.13**	-0.11*	-0.23**
PH	-0.43**	-0.41**	-0.07		-0.34**	0.66**	0.75**	-0.06	0.25**	0.32**	-0.20**	0.20**	0.37**
NPP	-0.61**	-0.59**	-0.01	0.69**		-0.45**	-0.44**	-0.06	-0.24**	-0.25**	0.23**	0.01	-0.23**
%LLSI85	0.50**	0.52**	-0.28**	-0.08	-0.24**		0.76**	-0.04	0.37**	0.26**	-0.26**	0.13**	0.25**
%LLSI100	0.24**	0.27**	-0.24**	0.38**	0.17**	0.46**		-0.09	0.37**	0.34**	-0.21**	0.16**	0.33**
5RI85	0.31**	0.34**	-0.22**	0.15**	0-.01	0.46**	0.44**		0.36**	-0.10*	-0.07	-0.14**	-0.11*
%RI100	0.02	0.05	-0.16**	0.47**	0.34**	0.18**	0.66**	0.42**		0.11*	-0.20**	0.10*	0.11*
PDY	-0.20**	-0.16**	-0.12**	0.46**	0.51**	0.02	0.27**	0.13**	0.35**		-0.01	-0.00	0.90**
KY	-0.45**	-0.39**	. -0.08	0.56**	0.64**	-0.15**	0.19**	0.04	0.36**	0.64**		-0.06	-0.01
HSW	-0.09	-0.04	-0.08	0.20**	0.23**	0.03	0.17**	0.08	0.21**	0.20**	0.39**		0.31**
SP	-0.17**	-0.13**	-0.11*	0.45**	0.49**	0.03	0.31**.	0.19**	0.38**	0.93**	0.65**	0.39**	

Notes: IPS = initial plant stand; FPS = final plant stand; PH = plant height, DTF = days to flowering; NPP = number of pods per plant; %LLIS 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 = Percentage late leaf spot infection at 100 days after planting; %RI 85 = Percentage rust infection at 85 days after planting, %RSI 100 = Percentage rust infection at 100 days after planting; PDY = pod yield; KY = kernel yield; HSW = hundred seed weight; SP = shelling percent. * and ** represent significant correlations at 0.05 and 0.01 probability levels, respectively.

3.3.4 Principal component analysis

The multi-variate relationship among traits was elaborated by the principal component analysis to show the contribution of each trait to the overall variation. Traits with high loadings on a given principal component (PC) are important as they account for more variation explained by that PC. The first four principal components accounted for 71.9% of the total variation (Table 3.6). The highest contributor to PC1 was Late leaf spot while the number of pods had the least PC1 contribution. For PC2, plant stand had the highest contribution followed by number of pods. Kernel yield and shelling percent had high contribution on PC3 while rust score had the highest leading on PC4. DAYS 75 had negative contribution on all components.

Table 3.6 Principal component scores and variance of each trait measured among 119 groundnut accessions across two seasons and two sites

Traits	PC1	PC2	PC3	PC4
IPS	-0.071	0.905	0.1	0.171
FPS	0.14	0.887	-0.009	0.186
DTF	-0.505	-0.264	-0.165	-0.18
PH	0.79	-0.288	0.26	0.023
NPP	-0.184	-0.728	0.233	0.28
%LLSi85	0.836	0.253	0.003	0.023
%LLSi100	0.868	0.05	0.119	0.189
%RI85	0.063	0.21	-0.038	0.782
%RI100	0.441	-0.088	0.182	0.653
PDY	0.426	0.183	0.757	-0.231
KY	-0.03	-0.23	0.8	0.158
HSW	0.032	-0.076	0.575	0.332
SP	0.407	0.158	0.818	-0.167
Eigenvalue	3.962	2.582	1.468	1.338
% of Variance	30.47	19.86	11.29	10.29
Cumulative %	30.474	50.333	61.622	71.911

IPS= initial plant stand, FPS= final plant stand, DTF= days to flowering, PH= plant height, NPP= number of pods per plant, %LLSI = Percentage late leaf spot infection at 85 days after planting, %LLSI 100= percentage late leaf spot infection at 100 days after planting, %RI 85 = percentage rust infection at 85 days after planting, %RI 100 = percentage rust score infection at 100 days after planting, PDY= pod yield, KY= kernel yield, HSW hundred seed weight, SP = shelling percent, PC = principal component

3.3.5 Genetic parameters of the SSR markers

In total, the 13 SSR markers used in this study amplified 38 alleles (Table 3.7). The number of alleles per marker ranged from 2 to 5 with a mean of 2.9 alleles per marker. The presence of allelic variants within the population was revealed by allele frequencies ranging from 0.319 to 0.992 with a mean of 0.713. Large variability was also observed among the markers for gene diversity, which ranged from 0.05 for m13_TE360 to a high of 1.56 for m13_PM035. The polymorphic information content values observed in this study ranged from 0.02 to 0.72 with

a mean value of 0.34. Marker m13_TE360 showed the lowest PIC value of 0.02. The results also showed that only three of the markers used had PIC values C 0.5. These were m13_PM035 (with PIC value of 0.72), m13_PGPseq_16C6 (0.66) and m13_PGPseq_10D4 (0.51).

Table 3.7 Genetic diversity estimates in 119 genotypes by using 13 SSR markers

Marker	Allele number	Allele frequency	Gene diversity	PIC
m13_GM2301	2	0.748	0.626	0.32
m13_IPAHM103	2	0.739	0.674	0.34
m13_PGPseq_10D4	3	0.630	1.031	0.51
m13_PGPseq_12F7	3	0.571	0.935	0.46
m13_PGPseq_13A10	3	0.513	0.857	0.43
m13_PGPseq_16C6	5	0.437	1.432	0.66
m13_PGPseq_17F6	3	0.807	0.679	0.31
m13_PGPseq_8E12	2	0.639	0.784	0.40
m13_PM035	5	0.319	1.557	0.72
m13_PM179	3	0.987	0.134	0.03
m13_SSR_HO115759	2	0.941	0.259	0.11
m13_TE360	2	0.992	0.049	0.02
m13_TE498	3	0.941	0.271	0.11
Mean	2.9	0.713	0.626	0.34

3.3.6 Population structure

The Evanno method estimated the best 'K' value to be 2 and, thus, the genotypes could be divided into two subpopulations (Fig. 3.3). The population structure analysis revealed that 74% of the accessions could be stratified into two sub-populations, while 26% could be regarded as admixtures. The two subpopulations were similar in size with sub-population 1 consisting of 36% of the genotypes while subpopulation 2 contained 37% (Fig. 3.4). Results showed that both sub-populations comprised of genotypes collected from different sources although most of the released genotypes were grouped in subpopulation 1 except Mangaka 09, which was grouped in subpopulation 2.

The expected heterozygosity in subpopulation 1 was 0.40 while for subpopulation 2 it was estimated to be 0.22 (Table 3.8). Allele frequency divergence between the two subpopulations was found to be 0.07. The level of genetic differentiation among the subpopulations was measured by estimating the fixation index (F_{ST}). The results showed that sub population 2 with an F_{ST} of 0.47 was more differentiated than subpopulation 1, which had an F_{ST} of 0.01 (Table 3.8).

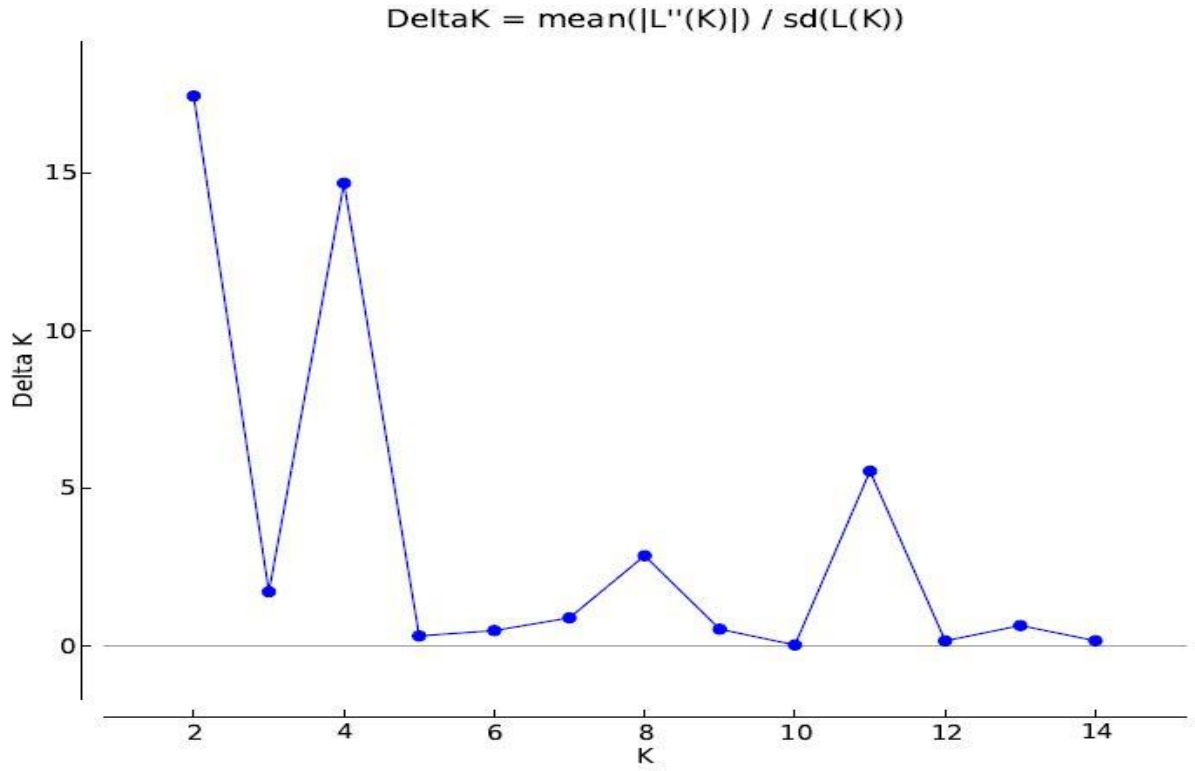

Figure 3.3 The best Delta K value for population structure among 119 groundnut genotypes

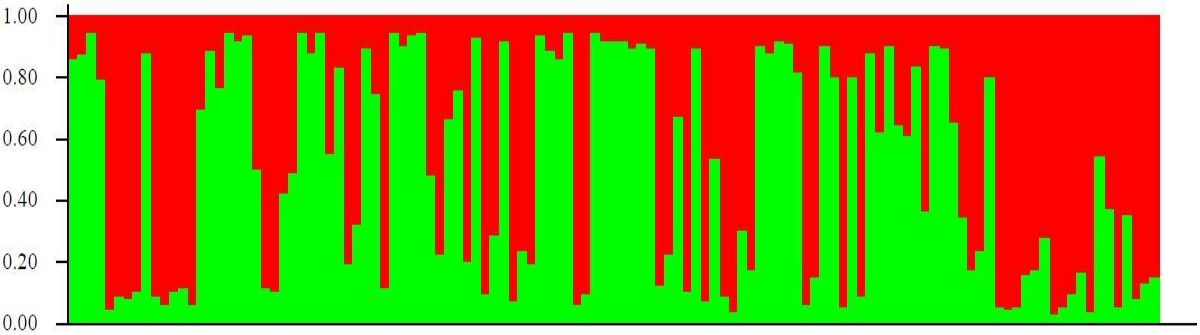

Figure 3.4 Estimated population structure of 119 groundnut genotypes with 13 SSR markers for K = 2 (Red = cluster 1, Green = cluster 2)

Table 3.8 Genetic clusters and their member genotypes, proportion of membership, expected heterozygosity and the mean fixation indices for 119 groundnut accessions

Cluster	Genotypes	% Membership	Expected heterozygosity	Fixation index (F_{ST})	Allele frequency divergence
1	ICGV-SM 08586, ICGV-SM 06718, ICGV-SM 15554, ICGV-SM 15559, ICGV-SM 16557, ICGV-SM 05570, ICGV-SM 16612, ICGV-SM 16617, CGV-SM 15534, CG 7, ICGV-SM 16565, ICGV-SM 15548 ICGV-SM 16559, Ndulima, ICGV-SM 15536, Nachingwea 09, ICGV-SM 05611, ICGV-SM 15510, ICGV-SM 15556, Narinut 15, ICGV-SM 16571, ICGV-SM 15524, ICG 12725, ICGV-SM 15546, ICGV 94114, ICGV-SM 15562, ICGV-SM 08587, ICGV-SM 15514, ICGV 95342, ICGV-SM 15529, ICGV-SM 06737, ICGV-SM 16558, ICGV-SM 08578, Masasi 09, ICGV-SM 16615, ICGV-SM 15538, ICGV-SM 16587, Kanyomwa, Naliendele 09, ICGV-SM 15567, ICGV-SM 08584,ICGV-SM 16597, ICGV-SM 16567	36	0.40	0.01	-
2	ICGV-SM 16567,ICGV-SM 16572, ICGV-SM 15558, ICGV-SM 16608, ICGV-SM 16601, ICGV-SM 16610, ICGV-SM 16586, ICGV-SM 16609, ICGV-SM 16556, ICGV-SM 16563, ICGV-SM 16595, ICGV-SM 16580, ICGV-SM 05569, ICGV-SM 16593, ICGV-SM 16603, ICGV-SM 16602, Mangaka 09, ICGV-SM 16579, ICGV 10879, ICGV-SM 16611, Local Tandahimba, ICGV-SM 16576, Mamboleo, ICGV-SM 16574,, ICGV-SM 16582, ICGV-SM 16598, ICGV-SM 16606, ICGV-SM 16591, ICGV-SM 16577, ICGV-SM 16568, ICGV-SM 16562, ICGV-SM 16578, ICGV-SM 16566, ICGV-SM 16583, ICGV-SM 16605, ICGV-SM 15542, ICGV-SM 06711, ICGV-SM 16600, ICGV-SM 16560, ICGV-SM 16588, Local Dodoma, ICGV-SM 16604, ICGV-SM 16585, ICGV-SM 16581,ICGV-SM 16599. ICGV-SM 16592	38	0.22	0.47	0.07
Admixture	ICGV-SM 15531, ICGV-SM 16569, ICGV-SM 16570, ICGV-SM 16555, ICGV-SM 05616, ICGV-SM 15537, ICGV-SM 16584, ICGV-SM 16554, ICGV 93542, ICGV-SM 16561, ICGV 94114, ICGV-SM 87157, ICGV-SM 16564, ICGV-SM 16618, ICGV-SM 16594, ICGV-SM 15557, ICGV-SM 90704, ICGV-SM 16607, ICGV-SM 08581, ICGV-SM 06735, ICGV-SM 16575, ICGV-SM 16589, PENDO, ICGV-SM 15564, ICGV-SM 16616, ICGV-SM 16619, ICGV-SM 16590, CGV-SM 01514, ICGV-SM 16573, ICGV-SM 16613, ICGV-SM 16614	26			

3.3.7 Cluster analysis

The accessions were allocated into two main clusters (Fig. 3.5). Each cluster was further divided into two subclusters. Most individuals that were grouped in a cluster and its sub-cluster shared one or both parents showing close relatedness. Landraces were grouped in sub-cluster D within cluster 2 together with some lines from ICRISAT and released varieties. Five accessions (ICG 12725, ICGV-SM 06737, ICGV- SM 05570, ICGV-SM 15524 and ICGV-SM 15559, which were high yielding, but showed susceptibility to rust in the screening trial, and identified as potential parents for breeding were grouped into sub-cluster A. Sub-cluster C contained genotypes identified as high yielding and grouped together with Pendo 98, which is a popular cultivar in Tanzania and susceptible to rust. Landraces Kanyomwa and Narinut 15, which showed low yield but resistance to rust were grouped together in subcluster D. The analysis of molecular variance (AMOVA) among the 119 accessions estimated that 88% of the variation was due to intra-population variation while 2% was due to inter-population variation. There was also significant variation within accessions, which accounted for 10% of the variation (Table 3.9).

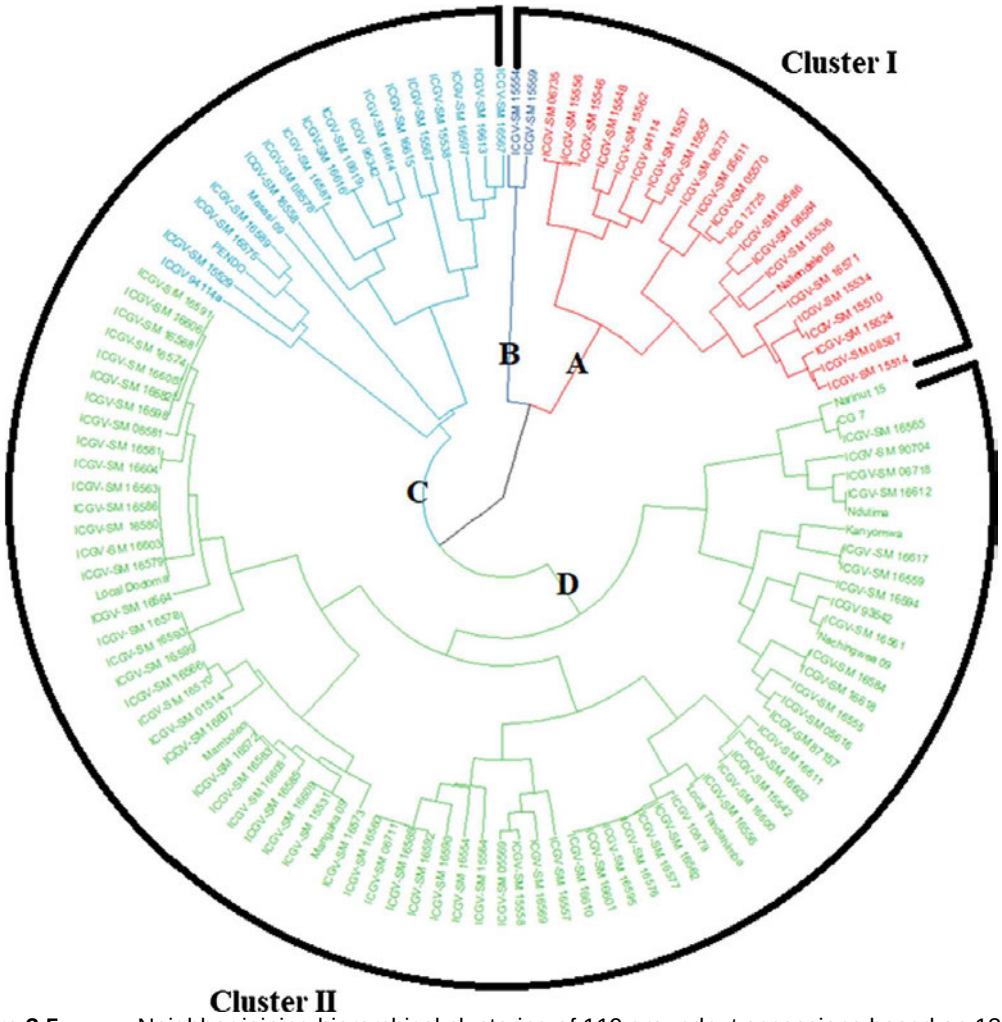

Figure 3.5 Neighbor joining hierarchical clustering of 119 groundnut accessions based on 13 SSR markers

Table 3.9 Analysis molecular variance (AMOVA) showing variation between and within the 119-groundnut accession of different origin

Source	df	SS	MS	Est.Var	(%) Variation	P-value
Between population	1	14.499	14.499	0.065	2	0.160
Among individuals	119	803.833	6.870	3.252	88	0.001
Within individuals	117	43.500	0.366	0.366	10	0.031
Total	237	861.832	-	3.683	100	-

3.4

3.5 Discussion

3.5.1 Genotypic variation and mean performance

This study evaluated genetic variation among 119 accessions of groundnut using phenotypic traits and SSR markers as a preliminary step to identify suitable parental lines for rust resistance breeding. The 119 accessions showed significant ($p<0.05$) variation for yield and yield components showing that the germplasm could potentially provide vital genetic

resources for groundnut improvement in Tanzania. The variation exhibited by phenotypic traits signify differences in genetic composition of the individuals (Liao 2014). The genotypes were sourced from different geographical locations where they could have adapted to local conditions and involved in help to identify the best site for rust disease screening. Accessions such as ICGV-SM 06737, NARINUT 15 and Kanyomwa that scored low values for rust could be possible sources of genes for rust tolerance. Although these lines did not show comparable yield advantage, they can be used in crosses to introgress the resistance genes into genotypes with a high yield potential genetic background. Genotype ICGV-SM 16579 was identified as the best in terms of pod yield and stability while genotype ICGV-SM 08587 was more stable in terms of pod yield across the test environments. These accessions showed high level of rust disease susceptibility across the test environments, and therefore would not be selected as parental lines for rust resistance breeding but can provide the high yield potential genetic background. 16589.

3.5.2 Trait associations

The relationships among yield components and disease response scores are critical in devising a selection strategy since selection of one trait may amplify or negatively affect performance in the other traits. The principal component (PC) analysis highlighted that late leaf spot, kernel yield, plant height, shelling percent and pod yield were mostly associated with PC1, showing that these traits accounted for much of the variation among the genotypes and could be used as the basis for selection. Accessions with higher performance in these traits could be selected for groundnut improvement. Rust scores were associated with PC4 as there was no wide range of variation for rust reaction among the accessions. This showed that most genotypes were more inclined towards susceptibility rather than resistance. Similarly, (Denwar *et al.* 2019) found that trait contribution to different PCs differed depending on the extent of variation for the particular trait among test genotypes. Pod yield, kernel yield and, late leaf spot, rust scored, and shelling percent are important yield components that can be used for indirect selection for yield due to their significantly correlation with yield. The correlations found in this study were in concurrence with Denwar *et al.* (2019), who also found that disease ratings were negatively correlated with yield while selection for number of pods and seeds per pod increased grain yield in soybean. The positive correlation between rust and late leaf spot shown in this study were confirmed in the previous reports (Narasimhulu *et al.* 2012, Narasimhulu *et al.* 2013). These diseases often occur together (Branch and Culbreath 2013, Subrahmanyam *et al.* 1985) and accessions with resistance to these diseases are generally late maturing (Khedikar *et al.* 2010). The results also showed that there existed a highly negative correlation between rust scores and the number of pods per plant, which could be attributed to the decimation of foliage resulting in low photosynthetic capacity of the plant to

accumulate a high number of pods. Leaf diseases are known to reduce yield through interfering with chloroplast integrity and causing abscission of leaves (Singh *et al.* 2011).

3.5.3 Genetic diversity estimates based on the SSR markers

SSR markers are often preferred for genetic diversity study due to their co-dominance, simplicity, high polymorphism, repeatability, abundance, multi-allelic nature and their transferability within the genus Arachis (Moretzsohn *et al.* 2005, Pandey *et al.* 2012, Wang *et al.* 2012). The PIC ranges from 0.02 to 0.72 for the 13 SSR markers used in this study showed that the genotypes were genetically diverse, and the markers were able to discriminate the genotypes. Genetic variability emanates from differences in the genetic constitution of individuals, thus the panel included both closely related and divergent genotypes. It also shows that the markers used were efficient in discriminating the genotypes, which is fundamental in genetic studies to evaluate the extent of genetic variation in the gene pool. The highest PIC obtained in this study was comparably higher than 0.52 and 0.62 obtained by Varma *et al.* (2005) and Mace *et al.* (2006), respectively. Differences in PIC values are concomitant with differences in the markers and genotypes used in the studies. Nonetheless, it shows that the germplasm investigated in each of the studies exhibited adequate genetic variation that can be exploited during groundnut improvement. The variation is important for breeding for Puccinia resistance as it avails genotypes with diverse response to the pathogen and some of the genotypes could harbour resistance genes. The gene diversity obtained in this study (0.93), which is significantly higher than 0.11 and 0.59 obtained by Ren *et al.* (2014) and Wang *et al.* (2011), respectively, showed that there were many variants of the genes in this population because it included diverse genotypes that included released varieties, advanced lines and landraces. The high gene diversity also implies that the SSR markers used were highly polymorphic. Mace *et al.* (2006) asserted that the use of high polymorphic markers increases the potential of identifying high levels of gene diversity among test genotypes. A total of 38 alleles were revealed across the 13 polymorphic SSR loci in the 119 groundnut genotypes with an average of three alleles per locus, which was similar to four alleles per locus reported by Ren *et al.* (2014). There are a few markers that revealed five alleles per locus and were comparable to findings by Mace *et al.* (2006), who reported an average of six alleles per locus. This suggests that there is favourable allelic diversity, which is essential for assessment of genetic diversity. The variability in the number of alleles detected per locus by different reports might be due to the use of diverse genotypes.

3.5.4 Population structure and clustering

The population structure, principal component and hierarchical clustering analyses were able to delineate the 119 accessions into two major clusters (Figs. 3.3 and 3.4). The optimal

number of clusters in the population structure was based on the Evanno method (Earl and VonHoldt 2012), which has been widely used to confirm number of clusters in populations of different crops including cereals and legumes (Denwar *et al.* 2019, Ren *et al.* 2014, Van Inghelandt *et al.* 2010). The two identified clusters grouped the released varieties separately from the landraces while genotypes with similar genetic background were correctly placed in closely linked cluster and sub-clusters. Eighty-eight accessions were grouped into the two clusters while 31 accessions were admixtures. Admixtures could be regarded as separate clusters from the two main ones. The ability to delineate the germplasm is a significant step towards groundnut improvement in Tanzania as these genotypes form part of germplasm collection intended for use in country wide breeding programs. However, the low number of clusters could be a sign of narrow genetic diversity between populations. A narrow genetic base of groundnut had been reported by different authors (Mace *et al.* 2006, Mondal *et al.* 2008, Varshney *et al.* 2010). The narrow genetic variation could be a result of origin since all cultivated groundnuts originated in South America, through a limited number of interspecific hybridization and polyploidization (Pasupuleti *et al.* 2013). Therefore, a wider range of accessions should be introduced to improve the current population for future breeding programs.

The mean fixation index (F_{ST}) of 0.47 within subpopulation 2 indicates a higher genetic diversity within this subpopulation from which parental lines could be selected to produce variable populations for selection. The high FST was similar to 0.47 reported by (Wang *et al.* 2011). In contrast, the low FST found among genotypes in subpopulation 1, which was dominated by the crosses of JL 24, ICGV 94114, ICGV 95342 and ICGV 93437 lines from ICRISAT, could be a bottleneck for groundnut improvement by inter-crossing individuals within this subpopulation. Crosses between individuals in subpopulations 1 and 2 would be recommended to increase genetic variation and enhance genetic gain through active selection.

The first cluster consisted mainly of crosses of JL 24 and ICGV 94114, ICGV 90103 and ICGV 92092, ICGV 93437 and ICGV 95342, showing that the analysis managed to identify and group genetically related individuals (Table 3.8). The second cluster consists of C and D sub-groups of 19 and 76 genotypes, respectively. The D sub-group consisted of more genotypes compared to all subgroups. Ren *et al.* (2014) grouped 196 accessions of groundnut in 5 groups for both cluster and structure analyses. Most of the genotypes used in this study showed resistance to rust and LLS diseases except three genotypes (ICGVSM 16585, ICGV-SM 16587 and ICGV-SM 16575), which showed comparable susceptibility to the susceptible check (Pendo 98).

The results showed that differences among individual accessions accounted for 88% of the variation, which means that the variation was less influenced by sources of collection or

population structure. The remainder of the total variation was found among the populations, which could have been contributed by adaptation to different environments and the number of markers, which showed polymorphisms to groundnut rust. This agreed with Ren *et al.* (2014) who showed that only differences in geographic origin contributed less to the differentiation in groundnut collections from China. The variation within individuals could be attributed to factors such as low frequency mutations that induce localised genetic changes since groundnut is highly self-pollinating. Random mutations occur in nature and have been reported to be contributors to variation observed in most self-pollinating species (Oladosu *et al.* 2016, Sigurbjornsson 1971).

3.6 Conclusions

The accessions exhibited significant phenotypic variation in yield and yield component traits, which were underpinned by the genetic diversity. The trait associations revealed significant correlation between rust and late leaf spot severity and number of pods per plant providing a means for direct selection to improve yield and disease resistance. The SSR markers used in this study were able to deduce genetic variation among groundnut genotypes. The largest proportion of variation was attributed to individual differences, which is essential for improving rust resistance by crossing individuals from divergent clusters. The germplasm was stratified into two sub-populations despite being sourced from diverse collection sources showing that sources of collection were less important. Accessions ICGV-SM 15557, ICGV-SM 15559, ICGV-SM 06737, PENDO, ICGV-SM 16601, ICGV-SM 16589, ICGV-SM 05570, Kanyomwa, Narinut 15, ICG 12725, ICGV-SM 15524 and ICGV-SM 15567 exhibited low scores for rust resistance. Accessions ICGV-SM 16601, ICGV-SM 16589 had high mean performance for pod yield and were clustered in different clusters, which provides opportunity for their selection as divergent parental lines in groundnut breeding for enhanced yield. Furthermore, the current study identified accessions ICGV-SM 06737, ICGVSM 16575, ICG 12725 and ICGV-SM 16608 of high diversity genotypically and in rust diseases could be used for development of rust mapping population, which will be useful resource for groundnut improvement.

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4 CHAPTER FOUR

Genotype-by-environment interaction analysis of groundnut (*Arachis hypogaea* L.) for kernel yield

Abstract

Kernel yield is a wining trait in groundnut breeding and production. However, yield expression is subject to genotype-by-environment interaction effects that reduce selection response. Therefore, it is essential to evaluate genotype-by-environment interactions (GEI) to identify high yielding and stable genotypes for breeding or variety recommendation. The objectives of this study were to assess the GEI effect on kernel yield and select best adapted groundnut genotypes in target production environments in Tanzania. One hundred and twenty groundnut genotypes were evaluated in two selected locations (Naliendele and Chambezi) using an incomplete block design with two replications. Significant ($p<0.05$) variations were detected among genotypes (G), environments (E) and GEI effects on kernel yield. A relatively higher proportion of the observed variation was due to the environment (34.85%) followed by GEI (24.65%) and genotype (8.25%) effects. Genotypes ICGV 94124 and CG 7 had relatively better kernel yield of 469.01 and 450.02 kg ha^{-1}, respectively. The genotype and genotype-by-environment biplot identified ICGV-SM 16556, ICGV-SM 15524, ICGV-SM 15564 and ICGV-SM 15514 as the most stable genotypes across locations, while ICGV-SM 16574 and ICGV-SM 15559 were specifically adapted to Chambezi and Naliendele, respectively. The Naliendele site was the most ideal location for groundnut evaluation and genotype differentiation. The above selected genotypes with high yields and average stability were selected as useful genetic resources for groundnut improvement in Tanzania.

Keywords: Additive main effect and multiplicative interaction, AMMI, *Arachis hypogaea,* genotype and genotype by environment model, kernel yield, stability analysis

4.1 Introduction

Groundnut (Arachis hypogaea L.) is a key food security and commercial crop globally. Groundnut kernel is rich in the contents of oil (48–50%), protein (26–28%), dietary fiber, minerals and vitamins (Pasupuleti *et al.* 2013). Globally, groundnut is grown in more than 100 countries situated in tropical, subtropical, and warm temperate regions (Upadhyaya *et al.* 2012). In sub-Saharan Africa (SSA) Tanzania is third after Nigeria and Sudan in groundnut production (FAOSTAT 2018).

Kernel yield in groundnut remains low in SSA including Tanzania due to a number of production challenges such as poor soil fertility, moisture stress, insect pests and diseases and a lack of improved cultivars with high yield potential. The average yield for groundnut in SSA is below 1000 kgha-1, which is comparably lower than the global average of 2500 kg ha^{-1} (FAOSTAT 2018). There is a need to develop improved cultivars and adapt good agronomic practices for enhanced productivity. The development of improved cultivars depends on identifying adequate genetic variation and identifying stable, superior and complementary genotypes for genetic recombination and selection.

Yield expression is subject to genotype-by-environment interaction effects that reduce selection response. In an attempt to select desirable genotypes for breeding, Daudi *et al.* (2020) evaluated genetically diverse groundnut collections in Tanzania. The authors reported the presence of marked genetic variation for rust resistance, high yield and farmer preferred attributes in the assessed genotypes. Further, the study found a significant influence of the environmental variance on genotype selection. Therefore, there is a need to assess the GEI to facilitate selection of superior genotypes with specific or broad adaptation. This will enhance selection response, identify superior genotypes and favourable test environments. (Lal *et al.* 2019) reported that the identification of superior groundnut genotypes was affected by the GEI effect that influenced selection responses.

Different statistical procedures are available to assess and compare genotype adaptability and stability. These procedures are based on analysis of variance, multivariate analysis, linear regression, non-linear analysis, biplot analyses, among others (De Figueiredo *et al.* 2015, Lal *et al.* 2019). The additive main effects and multiplicative interaction (AMMI) model was found to be one of the most useful procedures. Additionally, Yan *et al.* (2000) proposed a modification of the conventional AMMI analysis and developed the genotype and genotype × environment bi-plot model. The GGE analysis complements the AMMI analysis by apportioning the sum of squares to genotypes (G) and genotypes by environments (GE) interaction, making a graphical illustration more convenient and practical (Yan *et al.* 2007). The AMMI and GGE-biplot analyses are widely used to identify genotypes with broad or specific adaptation (Kaya *et al.* 2006). Both methods consider the genotype and environmental

effects to be additive, while the GEI is multiplicative (Zobel *et al.* 1988). The AMMI combines analysis of variance (ANOVA) to quantify genotype and environment main effects and the principal component analyses (PCA) to quantify genotype-by-environment interactions. The AMMI generates biplots based on the first two principal components to depict the relationship between genotypes and environments (Gabriel 1978). Purchase (1997) proposed the AMMI stability values (ASV) to quantitively identify genotype stability over a number of environments. The ASV is a metric of stability and is related to the distance of a genotype from the origin of an interaction principal component axes (IPCA) bi-plot. Highly stable genotypes have low corresponding ASV values (Purchase 1997).

The GGE biplot provides a visual depiction of GEI after removal of the environmental main effects. The GGE biplot depicts the response of a set of genotypes and their interaction with the environments to guide selection (Yan *et al.* 2000). It investigates genotype ranking based on the mean performance across test environments. The GGE biplot is based on the first two principal components, where the first interaction principal component (IPCA1) relating to mean performance and the second interaction principal component (IPCA2) denoting stability (Yan *et al.* 2000). An ideal genotype would be identified by high IPCA1 and low IPCA2 values. Thus, the GGE biplot complements the AMMI analysis by removing environmental variance, which is known to confound selection. Thus, integrating AMMI and GGE analyses provides opportunities to identify superior and stable genotypes across different environments. This is important to identify high yielding and stable genotypes for breeding or variety recommendation. The objectives of this study were to assess the GEI effect on kernel yield and select best adapted groundnut genotypes in target production environments in Tanzania.

4.2 Materials and Methods

4.2.1 Plant materials

A total of 120 groundnut genotypes were used in this study (Table 4.1). The test accessions included breeding lines obtained from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), landrace collections from different agro-ecologies in Tanzania and cultivated varieties. Genotypes from ICRISAT of constituting the following: 68-lines are selections from preliminary rust screening nurseries, 20 lines (selected from advance rust nurseries) and 20 (selected from regional rust trails).

Table 4.1 Description of groundnut genotypes used in the study

SN	Line name/designation	Pedigree	Source
1	ICGV-SM 16554	(CG 7 X ICGV 02194) F2-P9-P1-B1-B1-B1-B1	ICRISAT-Malawi
2	ICGV-SM 16555	(JL 24 X ICGV 02194)- F2-P2-P1-B1-B1-B1-B1	ICRISAT-Malawi
3	ICGV-SM 16556	(PENDO X ICGV 99557) F2-P4-P1-B1-B1-B1-B1	ICRISAT-Malawi
4	ICGV-SM 16557	(ICGV-SM 01711 X ICGV 02194) F2-P9-P1-B1-B1-B1-B1	ICRISAT-Malawi
5	ICGV-SM 16558	ICGV-SM 05701 X ICGV 02194) F2-P1-P1-B1-B1-B1-B1	ICRISAT-Malawi
6	ICGV-SM 16559	(ICGV-SM 01514 X ICGV 02194) F2-P7-P1-B1-B1-B1-B1	ICRISAT-Malawi
7	ICGV-SM 16560	(ICG 11426 X ICGV-SM 90704) F2-P14-P1-B1-B1-B1-B1	ICRISAT-Malawi
8	ICGV-SM 16561	(ICG 11426 X PENDO) F2-P11-P1-B1-B1-B1-B1	ICRISAT-Malawi
9	ICGV-SM 16562	(ICG 11426 X ICGV-SM 01721) F2-P21-P1-B1-B1-B1-B1	ICRISAT-Malawi
10	ICGV-SM 16563	(ICGV-SM 90704 X ICG 11426) F2-P3-P1-B1-B1-B1-B1	ICRISAT-Malawi
11	ICGV-SM 16564	PENDO X ICG 11426	ICRISAT-Malawi
12	ICGV-SM 16565	(ICGV-SM 01711 X ICG 11426) F2-P11-P1-B1-B1-B1-B1	ICRISAT-Malawi
13	ICGV-SM 16566	(ICGV-SM 99555 X ICG 11426) F2-P8-P1-B1-B1-B1-B1	ICRISAT-Malawi
14	ICGV-SM 16567	(ICGV-SM 99557 X ICG 11426) F2-P14-P1-B1-B1-B1-B1	ICRISAT-Malawi
15	ICGV-SM 16568	(ICGV-SM 05701X ICG 11426) F2-P11-P2-B2-B1-B1-B1	ICRISAT-Malawi
16	ICGV-SM 16569	(ICGV 01276 X CHALIMBANA) F2-P14-P1-B1-B1-B1-B1	ICRISAT-Malawi
17	ICGV-SM 16570	(ICGV 01276 X ICGV-SM 90704) F2-P15-P1-B1-B1-B1-B1	ICRISAT-Malawi
18	ICGV-SM 16571	(ICGV 01276 X ICGV-SM 90704) F2-P22-P1-B1-B1-B1-B1	ICRISAT-Malawi
19	ICGV-SM 16572	(ICGV 01276 X JL 24) F2-P3-P1-B1-B1-B1-B1	ICRISAT-Malawi
20	ICGV-SM 16573	CHALIMBANA X ICGV 01276	ICRISAT-Malawi
21	ICGV-SM 16574	ICGV-SM 90704 X ICGV 01276	ICRISAT-Malawi
22	ICGV-SM 16575	(CG 7 X ICGV 01276) F2-P8-P13-B1-B1-B1-B1	ICRISAT-Malawi
23	ICGV-SM 16576	(JL 24 X ICGV 01276) F2-P16-P1-B1-B1-B1-B1	ICRISAT-Malawi
24	ICGV-SM 16577	(PENDO X ICGV 01276) F2-P18-P1-B1-B1-B1-B1	ICRISAT-Malawi
25	ICGV-SM 16578	(ICGV-SM 01721 X ICGV 01276) F2-P6-P1-B1-B1-B1-B1	ICRISAT-Malawi
26	ICGV-SM 16579	(ICGV-SM 99555 X ICGV 01276) F2-P4-P1-B1-B1-B1-B1	ICRISAT-Malawi
27	ICGV-SM 16580	(ICGV-SM 05701 X ICGV 01276) F2-P8-P1-B1-B1-B1-B1	ICRISAT-Malawi
28	ICGV-SM 16581	(ICGV-SM 01514 X ICGV 01276) F2-P1-P2-B1-B1-B1-B1	ICRISAT-Malawi
29	ICGV-SM 16582	ICGV 02286 X CHALIMBANA	ICRISAT-Malawi
30	ICGV-SM 16583	ICGV 02286 X ICGV-SM 90704	ICRISAT-Malawi
31	ICGV-SM 16584	(ICGV 02286 X CG 7) F2-P21-P1-B1-B1-B1-B1	ICRISAT-Malawi
32	ICGV-SM 16585	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
33	ICGV-SM 16586	(ICGV 02286 X ICGV-SM 05701) F2-P1-P3-B1-B1-B1-B1	ICRISAT-Malawi
34	ICGV-SM 16587	(ICGV 02286 X ICGV-SM 05701) F2-P1-P4-B1-B1-B1-B1	ICRISAT-Malawi
35	ICGV-SM 16588	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
36	ICGV-SM 16589	(ICGV 02286 X ICGV-SM 05701) F2-P1-P14-B1-B1-B1-B1	ICRISAT-Malawi
37	ICGV-SM 16590	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
38	ICGV-SM 16591	(ICGV 02286 X ICGV-SM 05701) F2-P1-P20-B1-B1-B1-B1	ICRISAT-Malawi
39	ICGV-SM 16592	(ICGV 02286 X ICGV-SM 05701) F2-P1-P24-B1-B1-B1-B1	ICRISAT-Malawi
40	ICGV-SM 16593	(ICGV 02286 X ICGV-SM 05701) F2-P1-P27-B1-B1-B1-B1	ICRISAT-Malawi
41	ICGV-SM 16594	(ICGV 02286 X ICGV-SM 05701) F2-P1-P28-B1-B1-B1-B1	ICRISAT-Malawi
42	ICGV-SM 16595	(ICGV 02286 X ICGV-SM 05701) F2-P1-P29-B1-B1-B1-B1	ICRISAT-Malawi
43	ICGV-SM 16597	(ICGV 02286 X ICGV-SM 05701) F2-P1-P31-B1-B1-B1-B1	ICRISAT-Malawi
44	ICGV-SM 16598	(ICGV 02286 X ICGV-SM 05701) F2-P1-P39-B1-B1-B1-B1	ICRISAT-Malawi
45	ICGV-SM 16599	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
46	ICGV-SM 16600	(ICGV 02286 X ICGV-SM 05701) F2-P1-P41-B1-B1-B1-B1	ICRISAT-Malawi
47	ICGV-SM 16601	(ICGV 02286 X ICGV-SM 05701) F2-P1-P44-B1-B1-B1-B1	ICRISAT-Malawi
48	ICGV-SM 16602	(ICGV 02286 X ICGV-SM 05701) F2-P1-P49-B1-B1-B1-B1	ICRISAT-Malawi
49	ICGV-SM 16603	(ICGV 02286 X ICGV-SM 05701) F2-P1-P50-B1-B1-B1-B1	ICRISAT-Malawi
50	ICGV-SM 16604	(ICGV 02286 X ICGV-SM 05701) F2-P1-P53-B1-B1-B1-B1	ICRISAT-Malawi
51	ICGV-SM 16605	(ICGV 02286 X ICGV-SM 05701) F2-P1-P54-B1-B1-B1-B1	ICRISAT-Malawi
52	ICGV-SM 16606	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
53	ICGV-SM 16607	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
54	ICGV-SM 16608	(ICGV 02286 X ICGV-SM 05701) F2-P1-P257-B1-B1-B1-B1	ICRISAT-Malawi
55	ICGV-SM 16609	(ICGV 02286 X ICGV-SM 05701) F2-P1-P58-B1-B1-B1-B1	ICRISAT-Malawi
56	ICGV-SM 16610	ICGV 02286 X ICGV-SM 05701	ICRISAT-Malawi
57	ICGV-SM 16611	(ICGV 02286 X ICGV-SM 05701) F2-P1-P60-B1-B1-B1-B1	ICRISAT-Malawi
58	ICGV-SM 16612	(ICGV 02286 X ICGV-SM 05701) F2-P1-P62-B1-B1-B1-B1	ICRISAT-Malawi
59	ICGV-SM 16613	(ICGV 02286 X ICGV-SM 05701) F2-P1-P64-B1-B1-B1-B1	ICRISAT-Malawi
60	ICGV-SM 16614	(ICGV 02286 X ICGV-SM 05701) F2-P1-P65-B1-B1-B1-B1	ICRISAT-Malawi
61	ICGV-SM 16615	(ICGV 02286 X ICGV-SM 05701) F2-P1-P67-B1-B1-B1-B1	ICRISAT-Malawi
62	ICGV-SM 16616	(ICGV 02286 X ICGV-SM 05701) F2-P1-P68-B1-B1-B1-B1	ICRISAT-Malawi
63	ICGV-SM 16617	(ICGV 02286 X ICGV-SM 01514) F2-P1-P2-B1-B1-B1-B1	ICRISAT-Malawi

64	ICGV-SM 16618	(ICGV 02286 X ICGV-SM 01514) F2-P1-P5-B1-B1-B1-B1	ICRISAT-Malawi
65	ICGV-SM 16619	(ICGV 02286 X ICGV-SM 01514) F2-P1-P6-B1-B1-B1-B1	ICRISAT-Malawi
66	ICGV 93542	ICGV 93542	ICRISAT-Malawi
67	ICGV-SM 15510	ICGV 93437 x ICGV 95342	ICRISAT-Malawi
68	ICGV-SM 15514	(ICGV 93437 x ICGV 95342) F2-P35-P6-B1-B1-B1-B1	ICRISAT-Malawi
69	ICGV-SM 15524	(ICGV 93437 x ICGV 95342) F2-P55-P53-B1-B1-B1-B1	ICRISAT-Malawi
70	ICGV-SM 15529	(ICGV 93437 x ICGV 95342) F2-P63-P41-B1-B1-B1-B1	ICRISAT-Malawi
71	ICGV-SM 15531	ICGV 95342 x ICGV 93437	ICRISAT-Malawi
72	ICGV-SM 15534	(ICGV 95342 x ICGV 93437) F2-P3-P23-B1-B1-B1-B1	ICRISAT-Malawi
73	ICGV-SM 15536	(ICGV 94114 x JL 24) F2-P51-P10-B1-B1-B1-B1	ICRISAT-Malawi
74	ICGV-SM 15537	(ICGV 94114 x JL 24) F2-P50-P19-B1-B1-B1-B1	ICRISAT-Malawi
75	ICGV-SM 15538	(ICGV 94114 x JL 24) F2-P50-P14-B1-B1-B1-B1	ICRISAT-Malawi
76	ICGV-SM 15542	(ICGV 94114 x JL 24) F2-P35-P13-B1-B1-B1-B1	ICRISAT-Malawi
77	ICGV-SM 15546	ICGV 94114 x JL 24	ICRISAT-Malawi
78	ICGV-SM 15548	(ICGV 94114 x JL 24) F2-P9-P21-B1-B1-B1-B1	ICRISAT-Malawi
79	ICGV-SM 15554	(JL 24 x ICGV 94114) F2-P134-P7-B1-B1-B1-B1	ICRISAT-Malawi
80	ICGV-SM 15556	(JL 24 x ICGV 94114) F2-P113-P1-B1-B1-B1-B1	ICRISAT-Malawi
81	ICGV-SM 15557	(JL 24 x ICGV 94114) F2-P102-P13-B1-B1-B1-B1	ICRISAT-Malawi
82	ICGV-SM 15558	(JL 24 x ICGV 94114) F2-P93-P11-B1-B1-B1-B1	ICRISAT-Malawi
83	ICGV-SM 15559	(JL 24 x ICGV 94114) F2-P93-P4-B1-B1-B1-B1	ICRISAT-Malawi
84	ICGV-SM 15562	(JL 24 x ICGV 94114) F2-P65-P33-B1-B1-B1-B1	ICRISAT-Malawi
85	ICGV-SM 15564	(JL 24 x ICGV 94114) F2-P65-P22-B1-B1-B1-B1	ICRISAT-Malawi
86	ICGV-SM 15567	(JL 24 x ICGV 94114) F2-P27-P27-B1-B1-B1-B1	ICRISAT-Malawi
87	ICGV-SM 90704	(RG 1 x Manipintar) F2-P23-P59-P59-B1-B1-B13-B1	ICRISAT-Malawi
88	ICGV 94114	(J11 x CS 31) F2-B1-B1-B1-B1-B2-B1-B1-B2-B1	ICRISAT-Malawi
89	ICGV-SM 08578	ICGV 90082 X ICGV-SM 94581	ICRISAT-Malawi
90	ICGV-SM 08587	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
91	ICGV-SM 08586	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
92	CG 7	(USA 20 x TMV 10) F2-P3-B1-B1-B1-B1-B1B1-B1-B1	ICRISAT-Malawi/commercial variety
93	ICGV-SM 08581	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
94	ICG 12725	ICG 12725	ICRISAT-Malawi
95	ICGV-SM 05570	ICGV 90103 X PC 223 K9	ICRISAT-Malawi
96	ICGV 94124	(ICGV 87314 x NCAC 343) F2-B2-B1-B1-B1	ICRISAT-Malawi
97	ICGV-SM 06718	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
98	ICGV-SM 05611	ICGV 92092 X ICG 9991	ICRISAT-Malawi
99	ICGV-SM 05569	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
100	ICGV-SM 08584	ICGV 90082 X ICGV 90092	ICRISAT-Malawi
101	ICGV-SM 06735	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
102	ICGV 95342	[(ICG(FDRS)33 x ECZ1135) x (ICG (FDRS) x J11)] F2-F1-B1-B2-B2-B1-B1-B1-B1-B2-B1-B1-B1	ICRISAT-Malawi
103	ICGV-SM 05616	ICGV 90100 X JL 24	ICRISAT-Malawi
104	ICGV-SM 87157	ICGV-SM 87157	ICRISAT-Malawi
105	ICGV-SM 06711	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
106	ICGV-SM 06737	ICGV 90103 X ICGV 92092	ICRISAT-Malawi
107	ICG 10879	ICG 10879	ICRISAT-Malawi
108	ICGV-SM 01514	(ICGV 93437 X ICGV-SM 93561-ICGX-SM 95041/6/P15/P3	ICRISAT-Malawi
109	Masasi 09	ICGV-SM 87727 x ICGV-SM 83708	TARI-Naliendele/commercial varety
110	Pendo 98	ICGMS -33	TARI-Naliendele/ commercial varieties
111	Narinut 15	ICGV-SM 87727 x ICGV-SM 83708	TARI-Naliendele/commercial variety
112	Mangaka 09	ICGV 93437 x ICGV-SM 94586	TARI-Naliendele/commercial variety

113	Naliendele 09	ICGV-SM 93437 x ICGV-SM 94586	TARI-Naliendele/commercial variety
114	Nachingwea 09	ICGV-SM 90704 x ICGV-SM83708	TARI-Naliendele/commercial variety
115	Kanyomwa	Na	Nanyumbu landraces
116	Local Dodoma	Na	Dodoma landraces
117	Mamboleo	Na	Dodoma landraces
118	Local Tandahimba	Na	Tandahimba landraces
119	Ndulima	Na	Nanyumbu landraces
120	ICGV-SM 16596	ICGV 02286 x ICGV-SM 057 F2-P1-P30-B1-B1-B1-B1	ICRISAT-Malawi

Note: SN = serial numbers representing genotypes in the AMMI and GGE analyses sections; Na = not available.

4.2.2 Study sites

The genotypes were evaluated under field conditions in two selected sites, namely, Naliendele and Chambezi during the 2018 and 2019 cropping season in Tanzania. Table 4.2 presents the geographic location, altitude, weather, and soil characteristics of the study sites. The different seasons presented variable climatic conditions resulting in different site × season combinations. Chambezi is located in the Coastal region in Bagamoyo district of Tanzania. The site experiences high temperatures ranging between 24^{o}C in September and 30^{o}C in February. Long term annual rainfall at the site is 1000 mm while the rainy season is bimodal. The total amount of rainfall received during the experiments were 901.2 and 897.5 mm and the average temperatures were 31.55^{o}C and 30.7^{o}C in seasons I and II, in that order. The soils at the Chambezi site are sandy loam with pH of 5.0. Naliendele is located in Mtwara region of the Mtwara municipal. It has unimodal rain ranging between 820 to 1245mm. The average temperatures during the growing period at this site in 2018 and 2019 were 29.73 and 21.37 ^{o}C, respectively. The total rainfall in 2018 was 1086.75mm, while in 2019 at 996.98 mm. The soils at Naliendele are defined as sandy loam with a pH of 4.5. This study consisted of four environments. Briefly, the trial conducted at Chambezi in 2018 represented Environment 1, while the 2019 trail is Environment 2. Environments 3 and 4 represented trails conducted at the Naliendele site in 2018 and 2019, respectively.

Table 4.2 Geographic coordinates, climatic and soil properties of the study locations

Location	Latitude (^{0}S)	Longitude (^{0}E)	Altitude (masl)	Soil		Max Temp (^{0}C)		Min Temp (^{0}C)		Total rainfall (mm)	
				Type	Ph	2018	2019	2018	2019	2018	2019
Chambezi	6.5167	38.9167	12	Sandy loam	5.00	30.20	32.90	29.90	31.50	901.20	897.50
Naliendele	10.3539	40.1682	135	Sandy loam	4.50	28.20	31.26	20.33	22.40	1086.75	996.98

Min = minimum; Max = maximum; mm= millimetre, masl= metre above sea level

4.2.3 Experimental design and field planting

The study was conducted using an 8 × 15 alpha lattice design with two replications at each site. Each plot consisted of two rows that were 4 m long with a spacing of 50 cm between the rows and an intra-row spacing of 10 cm with one seed planted per hole. The trials at Chambezi were established in March in both seasons, while at Naliendele the trials were established in January 2018 and September 2019. Standard crop management practices were followed as recommended for the areas (NARI 2001). The trials at Chambezi were established under natural rainfall, while the Naliendele trials were under natural rainfall with supplemental sprinkler irrigation (Figure 4.1).

Figure 4.1 Partial view of the evaluation trial at Naliendele site in 2018

4.2.4 Data collection

Pod yield (PDY) was measured by weighing the dried pods from each plot and was recorded in grams per plot. Shelling percentage (SP) for each genotype was calculated from a random sample of pods weighing 200g, as the proportion of shelled seed weight to the total weight of

the unshelled pods. Kernel yield (KY, expressed in t ha^{-1}) was estimated as the product of pod yield per plot and shelling percentage and was converted to kg ha^{-1} using the plot size ($4m^2$) after adjusting for moisture content.

4.2.5 Data analysis

A combined analysis of variance was conducted in R (RCoreTeam 2019) after testing for homogeneity of variance. Subsequently, the data was subjected to the additive main effect and multiplicative interaction (AMMI) analysis to deduce the effects of genotype, genotype × environment interaction. The following AMMI model was adopted (Crossa 1990):

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda n \, Y_{gn} \, \eta_{en} + \theta_{ge}$$

Where; Yge is the yield of genotype, g, in environment, e; μ is the grand mean; αg is the genotype mean deviation; βe is the environment mean deviation; λn is the Eigen value of the principal component (PCA)axis, n; Ygn and ηen are the genotype and environment PCA scores for the PCA axis, n; N is the number of PCA axes retained in the model; and θge is the residual.

The AMMI stability values (ASV) were calculated using the formula proposed by Purchase (1997). The ASV were calculated as follows:

$$AMMI\ Stability\ Value\ (ASV) = \sqrt{\left[\left(\frac{\text{SSIPCA1}}{SSIPCA2}(IPCA1)\right)^2 + [IPCA2]^2\right]}$$

Where, SSIPCA1 and SSIPCA2 are the sum of squares for the first and second interaction principal component axes, respectively.

The genotype and genotype × environment interaction effects were visualized using the GGE biplot constructed in Genstat 18th edition (Payne 2015). The GGE biplots were based on the first two principal components (PC1 and PC2) after compressing multi-environment data into a single value (Yan *et al.* 2001).

4.3 Results

4.3.1 Combined analysis of variance

The analysis of variance revealed that the GEI was highly significant. Also, highly significant ($P < 0.001$) effects were noted among the tested groundnut genotypes (Table 4.3). Kernel yield exhibited highly significant ($P < 0.001$) environmental variability. The kernel yield of genotypes across environments ranged from 119.6 $kgha^{-1}$ for ICGV-SM 16574 to 469.0 $kgha^{-1}$ for ICGV 94124 (Table 4.4). Genotypes ICGV 94124, CG 7 and ICGV-SM 15510 had

the highest kernel yield, while ICGV-SM 16574, Narinut 15 and ICG 12725 were among the poor performers.

Table 4.3 Analysis of variance for kernel yield among 120 groundnut genotypes evaluated in four environments in Tanzania

Source of variation	df	Sum of squares	Mean squares
Environment (E)	3	24871134	8290378***
Replication (Site)	1	1054229	263557
Block (Rep)	56	703790	12568
Genotype (G)	119	5885095	49455***
G × E	357	17588442	50110***
Residuals	373	4541488	12176
Trial statistics			
Mean (kg ha^{-1})	307.87		
R^2	80.00%		
CV	35.84%		

Notes: DF = degrees of freedom, *** represent significant differences at 0.001 probability level, CV = coefficient of variation and R^2 = coefficient of determination.

Table 4.4 Mean kernel yield (kg ha^{-1}) for the top 10 and bottom five performing groundnut genotypes evaluated across four environments in Tanzania

	Location and year				
	Chambezi		Naliendele		
Genotypes	2018	2019	2018	2019	Overall
Top 10 genotypes					
ICGV 94124	19.97	216.21	1194.34	445.32	469.01
CG 7	45.97	296.90	1094.60	403.51	450.02
ICGV-SM 15510	55.03	353.62	775.01	571.13	438.71
ICGV-SM 15546	54.93	170.60	1144.53	379.60	437.42
ICGV-SM 15514	259.98	183.71	793.50	476.61	428.51
ICGV-SM 16555	25.00	215.03	1027.20	427.90	423.80
ICGV-SM 16556	20.01	235.91	856.21	599.10	422.81
ICGV-SM 16613	139.97	137.52	1237.31	172.31	421.82
ICGV-SM 16601	70.07	228.70	963.02	379.02	410.21
ICGV-SM 15559	50.03	137.50	737.20	607.71	401.90
Bottom 5 genotypes					
ICGV-SM 16574	75.01	88.70	96.01	218.53	119.61
Narinut 15	-20.04	87.51	150.03	293.71	132.80
ICG 12725	54.97	71.23	255.70	206.91	147.21
ICGV 95342	94.97	121.32	170.02	214.92	150.32
ICGV-SM 08584	34.96	57.50	238.14	310.14	160.23
Mean	79.47	200.04	536.44	326.57	291.16
LSD (5%)	160.1	233.5	268.4	193.3	190.2
CV%	101.11	60.29	24.82	28.47	66.48

LSD = least significant difference, CV = coefficient of variation

4.3.2 AMMI analysis

The AMMI model showed that genotype and environment main effects and their interaction had highly significant (P<0.001) impact on kernel yield productivity in groundnut (Table 4.5). The environment and genotype accounted for 34.85 and 8.25% of the total variance, respectively. The remaining 24.65% was due to the GEI effects. The GEI effects were further partitioned into two interaction principal component axes that were highly significant. The first interaction principal component axis (IPCA1) explained 76.9% of the total GEI variance, while the second principal component axis (IPCA2) explained 17.2% of the total GEI variance.

AMMI stability values (ASV) revealed variations in yield stability among the 120 genotypes (Table 4.6). According to (Purchase 1997), a stable variety is defined as one with low ASV. Consequently, genotype ICGV-SM 16614 with ASV value of 0.97 was the most stable while genotypes such as ICGV-SM 16613, ICGV-SM 15546, ICGV 94124, ICGV-SM 16606 and ICGV-SM 16560 were the least stable. The AMMI analysis identified the first four best performing genotypes in each environment (Table 4.7). In environment 1 (Chambezi in 2018) genotype number 69 (ICGV-SM 15524) had the best performance followed by genotype 96 (ICGV 94124), which was also second in environment 3 (Naliendele in 2018). In 2019 at Chambezi site, the best performing genotype was number 7 (ICGV-SM 16560) followed by number 16 (ICGV-SM 16569). At the Naliendele genotype number 59 (ICGV-SM 16613) was the best in 2018 and while 61 (ICGV-SM 15531) performed best in 2019

Table 4.5 AMMI analysis of variance among 120 groundnut accessions evaluated in four environments in Tanzania

Source of variation	Df	Sum of square	Mean square	Total variation explained (%)	Total G×E explained (%)	Cumulative (%)
Environments (E)	3	24871134	8290378**	34.85		
Replication	1	1054229	263557***	1.48		
Blocks	7	703790	12568ns	0.99		
Genotype (G)	119	5885095	49455***	8.25		
G × E	357	17588442	50110***	24.65		
IPCA1	121	12848070.7	106182.40***		76.9	76.9
IPCA2	119	2878685	24190.63***		17.2	94.1
G × E residuals	117	987531.7	8440.44		5.9	100
Pooled error	373	4541488	12176	6.36		

Notes: AMMI = Additive main effect and multiplicative interaction; DF = degrees of freedom; NS = non-significant ($P < 0.05$) and *** represent significant differences at 0.001 probability level, IPCA= interaction principal component analysis

Table 4.6 AMMI adjusted mean kernel yield (kg ha^{-1}), IPCA scores and AMMI stability value (ASV) of 120 groundnut genotypes evaluated across four environments in Tanzania

Genotype	Mean	IPCA1	IPCA2	ASV
10 most stable genotypes				
ICGV-SM 16614	236.17	0.02	0.96	0.97
ICGV-SM 16559	217.14	0.07	0.99	1.03
Kanyomwa	190.35	0.53	0.9	2.51
ICGV-SM 16591	207.83	-0.52	1.4	2.73
ICGV-SM 08581	274.88	-0.6	0.59	2.74
ICGV-SM 16577	209.57	-0.3	2.41	2.75
ICGV-SM 15531	244.75	-0.63	0.12	2.83
ICGV-SM 15510	503.06	0.58	-1.42	2.95
ICGV-SM 15562	277.03	-0.61	1.17	2.97
ICGV-SM 06718	354.33	0.82	-2.33	4.33
5 least stable genotypes				
ICGV-SM 16613	421.77	13.22	2.35	59.07
ICGV-SM 15546	437.41	10.17	-1.52	45.41
ICGV 94124	555.11	9.41	-1.45	42.04
ICGV-SM 16606	352.75	8.81	-1.01	39.33
ICGV-SM 16560	305.71	-8.48	8.11	38.69

Notes: AMMI = Additive main effect and multiplicative interaction; IPCA= interaction principal component analysis; IPCA1 = first interaction principal component axis; IPCA2 = second interaction principal component axis.

Table 4.7 The first four AMMI selection of groundnut genotypes per environment

Environment	Mean (kg ha^{-1})	Standard deviation	Score	1st	2nd	3rd	4th
Chambezi 2018	107.19	70.79	-190.54	ICGV-SM 15524	ICGV 94124	CG 7	ICGV-SM 16556
Chambezi 2019	195.36	89.19	-102.38	ICGV-SM 16560	ICGV-SM 16569	Mamboleo	ICGV-SM 16604
Naliendele 2018	545.75	264.54	248.01	ICGV-SM 16613	ICGV 94124	ICGV-SM 15546	CG 7
Naliendele 2019	342.65	118.58	44.91	ICGV-SM 16615	ICGV-SM 15536	Local Tandahimba	ICGV-SM 16556

4.3.3 Stability and performance of genotypes

Figure 4.2 presents genotype-focused biplot showing genotype comparisons based on mean performance and stability across environments within a mega-environment. The first two principal components explained 87.61% of the total variation. Genotypes 3 (ICGV-SM 16556), 69 (ICGV-SM 15524), 85 (ICGV-SM 15564) and 68 (ICGV-SM 15514) were the most stable genotypes, as they were located almost on the average-environment coordination (AEC) abscissa and had a near zero projection onto the AEC ordinate. This indicates that their rankings were highly consistent across environments. In contrast, genotype 21 (ICGV-SM

16574) and 111 (Narinut 15) were the least stable genotypes with below average mean performance.

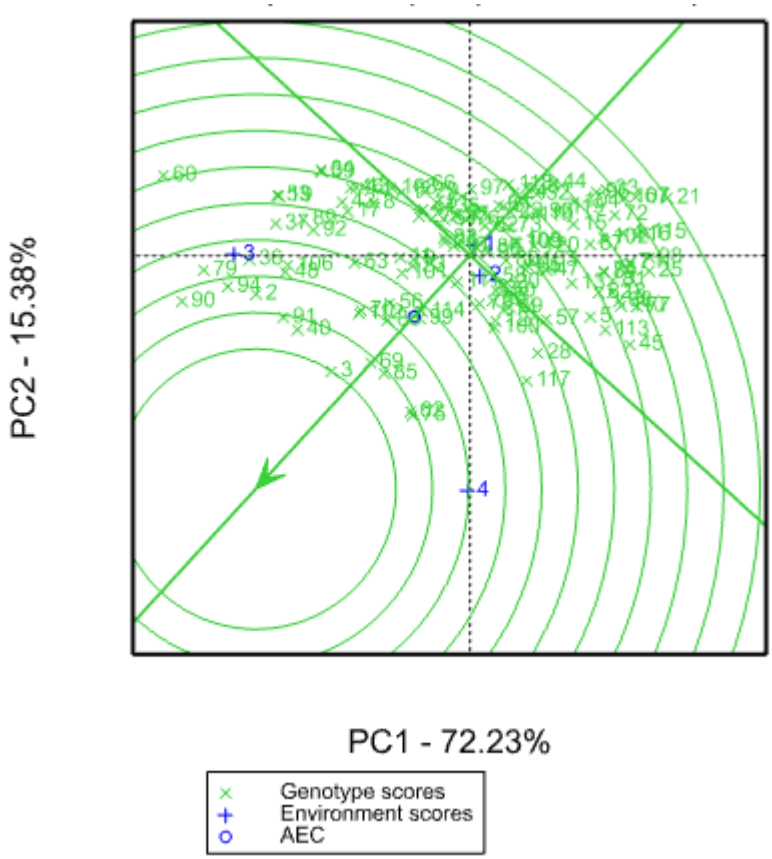

Figure 4.2 The average-environment coordination (AEC) view comparison biplot comparing genotypes relative to an idea genotype (the centre of the concentric circles). Numbers in blue denote environments 1 = Chambezi in 2018, 2 = Chambezi in 2019, 3 = Naliendele in 2018 and 4 = Naliendele in 2019. Dotted vertical and horizontal lines indicate points where the PC1 and PC2 axes had respective values of zero. Blue circle on the arrowed line represents the average environment and the green arrow represents ideal genotypes. See codes for genotypes (1-120) in Table 4.1

4.3.4 Test environment evaluation

Figure 4.3 shows GGE-biplot for comparison of the test environments with the ideal environment. The biplot accounted for 87.61% of the total variation relative to genotype and genotype-by-environment interaction. Environment 3 (Naliendele in 2018) was closest to the ideal environment, showing that it had the highest discriminatory capability and provided more information among the four test environments. Chambezi in 2018 and 2019 were classified as the least favorable testing environments for genotype evaluation.

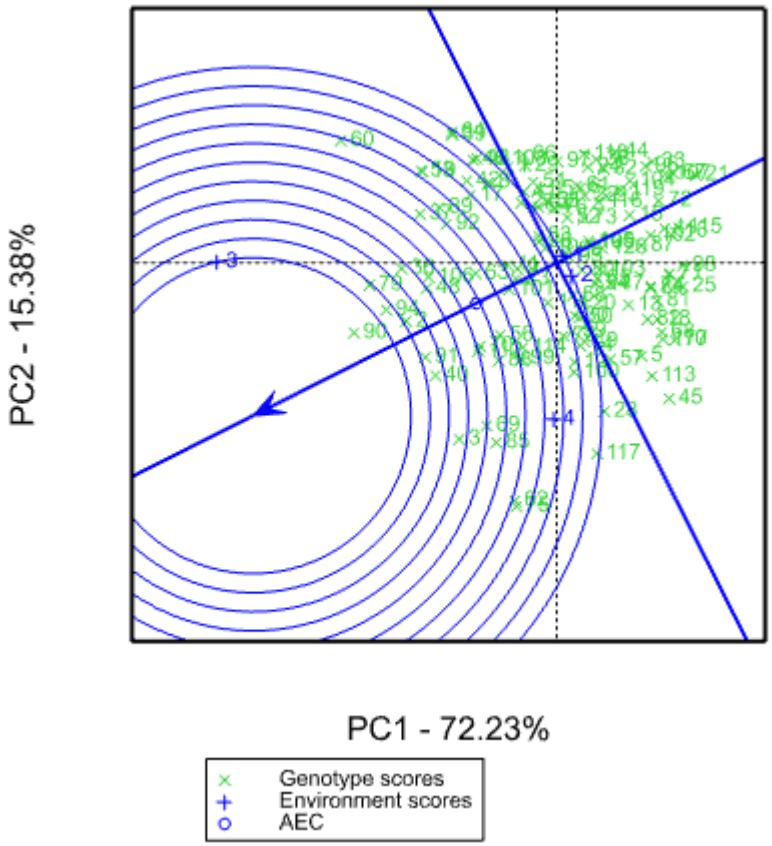

Figure 4.3 The average-environment coordination (AEC) view comparison biplot comparing environments relative to an ideal environment (the centre of the concentric circles). Numbers in blue denote environment: 1= Chambezi in 2018. 2 = Chambezi in 2019, 3= Naliendele in 2018 and 4 = Naliendele in 2019. Dotted vertical and horizontal lines indicate point where the PC1 and PC2 axes had respective values of zero. Blue circle on the arrowed line represents the average environment and the blue arrow represents ideal environment. See codes genotypes (1-120) in Table 4.1

4.3.5 Mega-environment and which-won-where

Mega-environment is defined as a group of locations that consistently share the best set of genotype or cultivars across years (Yan and Rajcan 2002). The GGE-biplot was divided into nine sectors and grouped testing sites into three mega environments. Chambezi in 2018 and 2019 makes one mega-environment (Figure 4.4). Naliendele in 2018 and 2019 were grouped in different mega-environments identified as environments 3 and 4, in that order. Environment 3 was distinctly different from the rest and was considered as a mega-environment. The genotypes plotted on the vertices were identified as the best suited for the particular mega-environment. Genotype 21 (ICGV-SM 16574) was the best performing genotype in the mega-environment comprising environments 1 and 2. Similarly, genotypes 90 (ICGV-SM 08587) and 83 (ICGV-SM 15559) were best suited for environments 3 and 4, respectively (Figure 4.4).

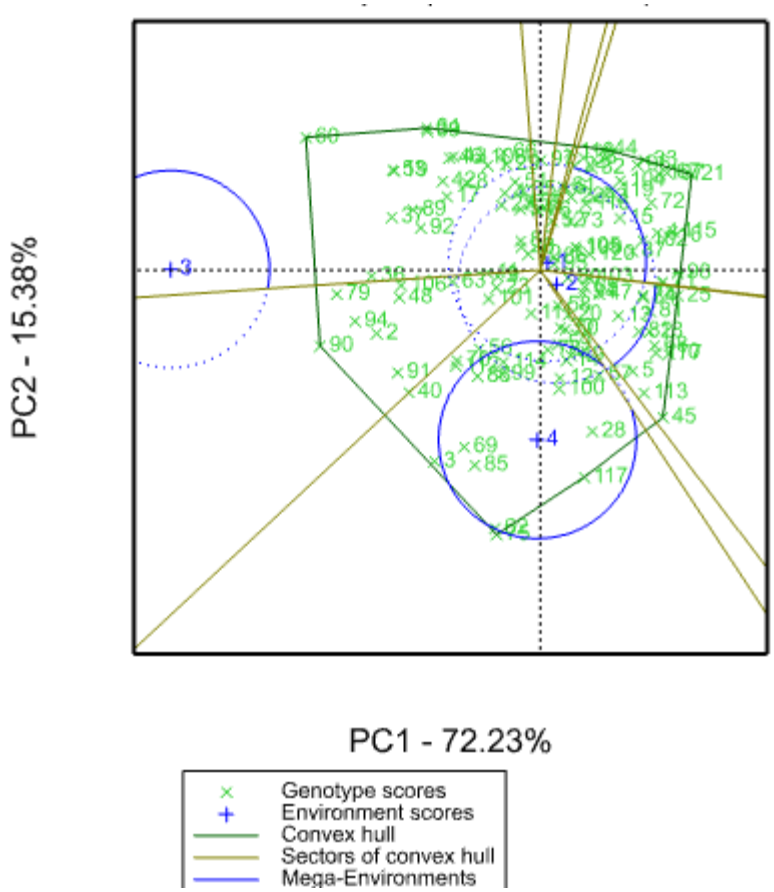

Figure 4.4 The 'which-won-where' polygon view of the GGE biplot showing which genotypes performed best in which environment. Numbers in blue denote environments: 1 = Chambezi in 2018, 2 = Chambezi in 2019, 3 = Naliendele in 2018 and 4 = Naliendele in 2019. Dotted vertical and horizontal lines indicate points where the PC1 and PC2 axes had respective values of zero. Vertices of the polygon indicate superior genotypes in each sector. See codes for genotypes (1-120) in Table 4.1

4.4 Discussion

4.4.1 Genotypic variation

Significant genotype × environment interactions were detected in the present study when comparing 120 groundnut genotypes in four environments in Tanzania (Table 4.3). This presents differential response of genotypes to environmental conditions confounding genotype selection across the test environments. Selecting superior genotypes is complicated when genotype performance is not consistent over a number of test environments, which prolongs the breeding process (Funnah and Mak 1980). The differential performance of genotypes across environments was indicative of variation in climatic and soil conditions in the different growing environments. Variability in climatic factors such as temperature, rainfall and humidity are important factors affecting plant growth and development that ultimately lead to differences in yield productivity. Previously, variation in yield due to differences in soil properties, rainfall and planting dates was reported (Ikeogu and Nwofia 2013). The genotypic variation observed in kernel yield within a site is a result of differences in the genetic composition of test genotypes. Yield is a quantitative trait conditioned by polygenes and its

expression varies across test genotypes and environments. Genetic variation is fundamental in plant breeding programs which allows identification of superior genotypes for enhanced genetic gain. Previous studies reported significant genetic variation after evaluating a large panel of groundnut germplasm (Daudi *et al.* 2020, Lal *et al.* 2019, Narasimhulu *et al.* 2012).

4.4.2 AMMI analysis

The AMMI analysis discerns the proportion of variance attributable to the genotype, environment and their interaction (Najafian and Kaffashi 2010). The high proportion (34.85%) of variation attributable to the environment main effects (Table 4.5) indicated that the environments were diverse and significantly impacted kernel yield. Grain yield is a complex polygenic trait that is affected by environmental variance, which has been identified as a major impediment to selection efficiency (Faye *et al.* 2015). Environmental variability in temperature, rainfall, humidity and soil properties among other factors influence yield potential in different crops including groundnut. Several studies have reported large environmental variance based on GEI analysis. For instance, Kebede and Getahun (2017) found that the environment accounted for 69.8%of the variation in yield among groundnut genotypes evaluated in Ethiopia The GEI accounted for 24.65% of the variation in kernel yield, which presents opportunities to identify suitable and adapted genotypes for a particular environment. The presence of GEI reduces correlations between genotype and phenotype expression (Bustos-Korts *et al.* 2018) leading to longer breeding cycles or failure to identify superior genotypes. The low proportion of genotype variance shows that genotypic expression was limited by the environmental component. It has been reported that the environment accounted for up to 80% of the total variation, while the genotype and GEI contributed only 20% in heterogenous environments (Yan et al. 2000).

The ASV is the distance from the coordinate point to the origin in a two dimensional of IPCA1 against IPCA2 scores in the AMMI model (Purchase *et al.* 2000). Genotypes with small ASV are more stable, accordingly genotypes 60 (ICGV-SM 16614, 6 (ICGV-SM 16559) and 115 (Kanyomwa) were the most stable, while genotypes 59 (ICGV-SM 16613), 77(ICGV-SM 15546) and 96 (ICGV 94124) were the least stable (Table 4.6). The genotypes with high stability are ideal candidate for breeding most adapted genotypes with high yield potential.

4.4.3 Genotype evaluation

Newly developed crop varieties must have high yield potential and also exhibit high stability across diverse environment. In the present study, genotypes with consistently high kernel yield across environments or genotypes that are high yielders but well adapted to the specific environment were selected. Yan *et al.* (2001) defined an ideal genotype based on both mean performance and stability, and the genotypes can be ranked based on their biplot distance

from the ideal genotype. Consequently, genotypes 3 (ICGV-SM 16556), 69 (ICGV-SM 15524), 85 (ICGV-SM 15564) and 68 (ICGV-SM 15514) were the most stable genotypes, being located close to the AEC abscissa. The rankings for these genotypes were due to their relatively consistent performance across the environments within the three mega-environments. Genotype, ICGV-SM 16574 was adapted to environments 1 and 2 showing its suitability to the Chambezi site, which was characterised by higher temperatures and rainfall. Genotypes ICGV-SM 08587 and ICGV-SM 15559 were more suitable for environments 3 and 4. Environment 3 experienced lower temperature and rainfall compared to environment 4. Hence it could be posited that genotype ICGV-SM 08587 was more suited to colder and dry conditions. Identification of genotypes with stable yield performance using GGE biplot analysis was successfully used by Chaudhari *et al.* (2019) and Pradhan *et al.* (2010). The stable genotypes across the environments can be released after multilocation evaluation and comparison with popular national checks.

4.4.4 Environment evaluation

The test environments were delineated into three mega-environments. A mega-environment is defined as a group of locations that consistently share the best set of genotypes or cultivars across years (Yan and Rajcan 2002). It is imperative to identify homogenous environments to reduce breeding costs since evaluations could be conducted in one of the sites identified in a mega-environment. Fewer and representative test environments enable effective genotype comparison with minimum costs and high selection efficiency. The “which-won-where” view of the GGE biplot is an effective visual tool in mega-environment analysis (Yan *et al.* 2000). It consists of an irregular polygon and a set of lines drawn from the biplot origin and intersecting each of the sides at right angles. Genotype 21(ICGV-SM 16574) was the highest yielding in environments 1 and 2, genotypes 60 (ICGV-SM 16614) and 90 (ICGV-SM 08587) were the highest in environment 3 and genotype 83 (ICGV-SM 15559) was the highest yielding in environment 4. Hence these genotypes could be recommended for the respective environments. High performance of a particular genotype in an environment could be due to high and favorable interactive effects between the genotypes and environment. (Yan *et al.* 2007) reported that different sectors separate environments that support different genotypes. In relation to representativeness and discriminatory capability of the sites, the GGE identified Naliendele as the best environment in the present study for groundnut genotype evaluation. It could be attributed to the conducive temperatures and good rainfall that was relatively similar between the two sites. An “ideal” test environment should be both discriminating of the genotypes and representative of the mega-environment (Yan *et al.* 2007).

4.5 Conclusions

Kernel yield is a wining trait in groundnut breeding and production. Therefore, it is essential to evaluate GEI adequately to identify high yielding and stable genotypes for breeding or variety recommendation. Genotypes 3 (ICGV-SM 16556), 69 (ICGV-SM 15524), 85 (ICGV-SM 15564) and 68 (ICGV-SM 15514) are recommended for wide cultivation across the two test sites or for developing breeding populations for groundnut improvement. These genotypes expressed high and stable kernel yield across the test environments. The Naliendele site was found to be the most suitable site for the identification of best performing groundnut genotypes. The selected genotypes with high kernel yield and average stability are useful genetic resources for groundnut improvement in Tanzania.

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5 CHAPTER FIVE

Combining ability and gene action controlling rust resistance and agronomic traits in groundnut (*Arachis hypogaea* L.)

Abstract

Groundnut rust caused by *Puccinia arachidis* Speg. is a major cause of yield and quality losses in groundnut (*Arachis hypogaea* L.) in the warm-humid tropics including Tanzania. Breeding and deployment of rust resistant cultivars with farmer-preferred attributes will bolster groundnut production and productivity. The objective of this study was to determine the combining ability effects and gene action controlling rust resistance and agronomic traits in groundnut genotypes for breeding. Twelve selected and complementary parental lines were crossed in a diallel design to develop F_2 progeny. Thirty-three successful partial crosses and the 12 parents were field evaluated using a 5 × 9 alpha lattice design with two replications over two seasons in Tanzania. The tested genotypes exhibited significant ($P<0.05$) variation for rust resistance, yield and yield-related traits. There existed significant ($P<0.05$) difference on the general combining ability (GCA) effect of parents and the specific combining ability (SCA) effect of progeny for the assessed traits indicating that both additive and non-additive gene effects conditioned trait inheritance. The Bakers' ratios accounted for non-additive gene effects predominantly controlling rust resistance and yield components. This suggests that transgressive segregants could be selected for improved rust resistance and yield gains in the advanced pure line generations. Genotypes ICGV-SM 05570 and ICGV-SM 15567 were the best general combiners for rust resistance and grain yield. The crosses ICGV-SM 16589 × Narinut and ICGV-SM 15559 × ICGV-SM 15557 were identified as the best specific combiners for rust resistance with moderate yield levels and medium maturity. Genotypes with desirable GCA or SCA effects were selected for further breeding.

Keywords

Agronomic traits; cultivar development; diallel analysis; inheritance; gene action; rust resistance

5.1 Introduction

Groundnut (*Arachis hypogaea* Speg., AABB, 2n = 4x = 40) is cultivated in more than 100 countries in tropical, subtropical and warm temperate regions globally (Upadhyaya *et al.* 2012). World groundnut production is estimated at approximately 45.95 million tons of shelled grain per year which is mainly used for oil (FAOSTAT 2018). Groundnut production in Tanzania is estimated at 0.9 million tonnes per year with an average productivity of less than one tonne per hectare (FAOSTAT 2018) . Despite its importance, groundnut production and productivity are challenged by a number of biotic and abiotic stress factors. Among biotic stresses, groundnut rust disease caused by *Puccinia arachidis* Speg. is a major constraint to groundnut production in the hot humid tropics causing yield losses reaching up to 57% (Mondal and Badigannavar 2015). Reportedly, about 48.3% of groundnut farmers in the hot and humid production environments in Tanzania indicated groundnut rust as the major constraint to low yields and quality (Daudi *et al.* 2018).

Groundnut rust causes early pod senescence, reduced seed size, and low seed oil content (Mondal and Badigannavar 2015) reducing the economic value of the crop. Yield losses of up to 70% can be incurred when rust and late leaf spot diseases occur simultaneously (Khedikar et al. 2010, Subrahmanyam et al. 1985). Late leaf spot causes leaf senescence significantly reducing the photosynthetic efficiency and leading to yield and quality losses (Branch and Culbreath 2013).

Both groundnut rust and late leaf spot diseases can be controlled through a combination of methods such as cultural practices, chemical fungicides, biological control agents and host-plant resistance. Each method has its own merits and demerits when applied in isolation. Host plant resistance is potentially the most economically viable, technically feasible, environmentally friendly, and socially acceptable disease management strategy for groundnut rust integrated disease control (Mondal *et al.* 2014). In sub-Sharan Africa host-plant resistance is not widely used as the main rust control strategy due to a lack of varieties with durable disease resistance and enhanced yields. Hence breeding for groundnut rust resistance is the principal consideration to develop better performing varieties with rust resistance and improved productivity. Successful development of improved varieties depends on the genetic variability present in a breeding population and selection of farmer- and market-preferred parents with good combining ability for rust resistance and agronomic traits.

Knowledge on the gene action conditioning economic traits is a prerequisite for groundnut resistance breeding (Ashish *et al.* 2014, Joel *et al.* 2006, Mehan *et al.* 1994, Usman *et al.* 2015). Evaluating the combining ability of candidate lines is important to identify superior and good combiner parents and progeny, to deduce the type of gene action conditioning trait

inheritance and to discern suitable selection methods (Böhm *et al.* 2014). Sprague and Tatum (1942) devided combining ability effects into general combining ability (GCA) of parents and the specific combining ability (SCA) of progeny. The GCA and SCA effects are associated with additive and non-additive gene action, respectively (Falconer and Mackay 1996). Both GCA and SCA effects have been reported in foliar disease resistance breeding programs including groundnut using various mating designs (Adamu *et al.* 2008, Joel *et al.* 2006, Vishnuvardhan *et al.* 2014).

The diallel mating design is the most commonly used method to estimate GCA and SCA effects (Griffing 1956). It is a more appropriate design for self-pollinated species where the success rate for generating crosses is often low such as in groundnut and soybean (Tai 1976). It has been used in genetic analysis of traits of various legume crop species such as cowpea (Barro Antoine *et al.* 2017, Jean-Baptiste *et al.* 2011, Kwaye *et al.* 2008), soyabean (Kurasch *et al.* 2017, Mebrahtu and Devine 2009) and chickpea (Karami 2011, Kumar *et al.* 2001, Saxena *et al.* 2010). To initiate groundnut pre-breeding for rust resistance and farmer-preferred agronomic traits, genetically diverse collections were characterised using agronomic traits and polymorphic simple sequence repeat (SSR) markers. This enabled selection of potential and complementary parents for strategic breeding (Daudi *et al.* 2020). The combining ability effects of the selected parents and their progeny should be assessed to develop new breeding populations adapted to Tanzania. Therefore, the objectives of this study were to determine the combining ability effects and gene action controlling rust resistance and agronomic traits in selected groundnut genotypes to develop breeding populations.

5.2 Materials and methods

5.2.1 Description of the study environment

The study was conducted at Tanzania Agriculture Research Institute (TARI), Naliendele Agricultural Research Centre. TARI-Naliendele (10.3539^{o}S, 40.1682^{o}E) is situated at an altitude of 135 metres above sea level. The mean monthly temperatures range between 24.3^{o}C in July and 27^{o}C in December while the mean annual total rainfall is between 820 and 1245 mm with a unimodal rainfall distribution. A dry spell of one to two weeks often occurs at the end of January or at the beginning of February. The soils at TARI-Naliendele are described as sandy loam with pH of 4.5. The prevailing temperatures and rainfall conditions of the test sites during the experiments are summarised in Table 5.1.

Table 5.1 Total monthly rainfall and mean maximum and minimum temperature of Naliendele during 2019 and 2020

Year	**2019**	**2020**

Month	Total rainfall (mm)	Mean maximum temperature (^{0}C)	Mean minimum temperature (^{0}C)	Month	Total rainfall (mm)	Mean maximum temperature (^{0}C)	Mean minimum temperature (^{0}C)
September	1.0	32.0	21.0	January	289.8	30.8	24.7
October	20.2	32.2	22.8	February	198.4	31.5	24.4
November	55.7	32.2	24.1	March	300.7	32.0	24.1
December	229.9	31.6	24.2	April	102.7	32.3	23.9

5.2.2 Plant materials

The study used 12 parents selected from preliminary evaluation trials based on rust resistance, agronomic performance and SSR markers (Daudi et al. 2020). The lines consisted of accessions, a landrace and two released varieties (Table 5.2). Accessions were selected based on low severity for rust disease or better yield responses (Table 5.2). A released variety Pendo was included because it is susceptible to rust disease and popular among local farmers in Tanzania. The tested lines included Virginia and Spanish botanical groups (Table 5.2). The Spanish type have erect growth type and set flowers on their main axis with small capsule (Kumazawa and Nishimura 1953, Naito *et al.* 2008). The Virginia type have creeping growth type, highly branched main stem with large capsule (Kumazawa and Nishimura 1953, Naito *et al.* 2008). The selected parents showed varied seed colour and size to cater farmer- and market-preferences (Table 5.2).

Table 5.2 Description of groundnut parents used in the crosses

Genotype	**Botanical group**	**Origin**	**Seed coat colour**	**Yield (kg/ha)**	**Rust reaction**
ICGV-SM 06737	Spanish	ICRISAT-Malawi	Red	562.50	R
ICGV-SM 05570	Virginia	ICRISAT-Malawi	Red	503.13	R
ICGV-SM 15524	Spanish	ICRISAT-Malawi	Tan	370.63	R
ICGV-SM 15567	Spanish	ICRISAT-Malawi	Tan	543.13	R
ICG 12725	Spanish	ICRISAT-Malawi	Red	308.16	R
ICGV-SM 15559	Spanish	ICRISAT-Malawi	Tan	734.38	R
ICGV-SM 15557	Spanish	ICRISAT-Malawi	Tan	653.13	MR
ICGV-SM 16589	Spanish	ICRISAT-Malawi	Tan	810	MR
ICGV-SM 16601	Spanish	ICRISAT-Malawi	Tan	756.07	MR
Narinut	Virginia	Naliendele/released variety	Tan	202.74	R
Pendo	Spanish	Naliendele/released variety	Tan	753.57	MR
Kanyomwa	Virginia	Landrace	Tan	420.00	R

ICRISAT = International Crop Research Institute for the Semi-Arid Tropics, R = resistant, MR = moderately resistant

5.2.3 Crosses and mating design

Crosses were performed using a full diallel mating design involving the 12 lines according to the scheme shown in Table 5.3. Crossing blocks were established in a greenhouse during the off-season in September 2018. The 12 parents were stagger-planted with a 2-weeks interval to synchronize flowering and pollen supply. Hand emasculation and pollination of the flowers were carried following the procedure described by Nigam *et al.* (1991) and Pasupuleti *et al.* (2018). Crosses were made during August–October 2018. Emasculation was done between 14:00 and 16:00 hrs when the hypanthium was sufficiently elongated, the bud was large enough for easy handling during emasculation, and the anthers were not dehisced. Pollination was carried out between 06:30 and 08:00 hrs the following day. Each cross was labelled appropriately using white tags. A total of 132 cross combinations were expected from the full diallel, however, only 33 crosses had enough seed set (100-200 seeds per cross) for genetic analysis. The F_1 seed of all successful crosses was planted after three weeks for seed bulking and genetic analysis in the F_2.

Table 5.3 A 12 X 12 diallel mating scheme in groundnut showing the overall and successful crosses

Parents	ICG 12725	ICGV-SM 05570	ICGV-SM 06737	ICGV-SM 15524	ICGV-SM 15557	ICGV-SM 15559	ICGV-SM 15567	ICGV-SM 16589	ICGV-SM 16601	Kanyomwa	Narinut	Pendo
ICG 12725	S_1	ICG 12725 × ICGV-SM 05570	(1) ICG 12725 × ICGV-SM 06737	ICG 12725 × ICGV-SM 15524	ICG 12725 × ICGV-SM 15557	ICG 12725 × ICGV-SM 15559	ICG 12725 × ICGV-SM 15567	ICG 12725 × ICGV-SM 16589	ICG 12725 × ICGV-SM 16601	ICG 12725 × Kanyomwa	ICG 12725 × Narinut	(2) ICG 12725 × Pendo
ICGV-SM 05570	ICGV-SM 05570 × ICG 12725	S_2	(3) ICGV-SM 05570 × ICGV-SM 06737	ICGV-SM 05570 × ICGV-SM 15524	ICGV-SM 05570 × ICGV-SM 15557	ICGV-SM 05570 × ICGV-SM 15559	ICGV-SM 05570 × ICGV-SM 15567	ICGV-SM 05570 × ICGV-SM 16589	ICGV-SM 05570 × ICGV-SM 16601	ICGV-SM 05570 × Kanyomwa	ICGV-SM 05570 × Narinut	(4) ICGV-SM 05570 × Pendo
ICGV-SM 06737	ICGV-SM 06737 × ICG 12725	(5) ICGV-SM 06737 × ICGV -SM 05570	S_3	(6) ICGV-SM 06707 × ICGV-SM 15524	ICGV-SM 06737 × ICGV-SM 15557	ICGV-SM 06737× ICGV-SM 15559	ICGV-SM 06737 × ICGV-SM 15567	(7) ICGV-SM 06737 × ICGV-SM 16589	ICGV-SM 06737 × ICGV-SM 16601	ICGV-SM 06737 × Kanyomwa	(8) ICGV-SM 06737 × Narinut	(9) ICGV-SM 06737× Pendo
ICGV-SM 15524	ICGV-SM 15524 × ICG 12725	(10) ICGV-SM 15524 × ICGV-SM 05570	ICGV-SM 15524 × ICGV-SM 06737	S_4	ICGV-SM 15524 × ICGV-SM 15557	ICGV-SM 15524 × ICGV-SM 15559	ICGV-SM 15524 × ICGV-SM 15567	ICGV-SM 15524 × ICGV-SM 16589	ICGV-SM 15524 × ICGV-SM 16601	ICGV-SM 15524 × Kanyomwa	(11) ICGV-SM 15524 × Narinut	ICGV-SM 15524 × Pendo
ICGV-SM 15557	ICGV-SM 15557 × ICG 12725	ICGV-SM 15557 × ICGV-SM 05570	ICGV-SM 15557 × ICGV-SM 06737	ICGV-SM 15557 × ICGV-SM 15524	S_5	(12) ICGV-SM 15557 × ICGV-SM 15559	ICGV-SM 15557 × ICGV-SM 15567	ICGV-SM 15557 × ICGV-SM 16589	ICGV-SM 15557 × ICGV-SM 16601	ICGV-SM 15557 × Kanyomwa	ICGV-SM 15557 × Narinut	ICGV-SM 15557 × Pendo
ICGV-SM 15559	ICGV-SM 15559 × ICG 12725	(13) ICGV-SM 15559 × ICGV-SM 05570	(14) ICGV-SM 15559 × ICGV-SM 06737	ICGV-SM 15559 × ICGV-SM 15524	ICGV-SM 15559 × ICGV-SM 15557	S_6	(15) ICGV-SM 15559 × ICGV-SM 15567	ICGV-SM 15559 × ICGV-SM 16589	ICGV-SM 15559 × ICGV-SM 16601	ICGV-SM 15559 × Kanyomwa	ICGV-SM 15559 × Narinut	ICGV-SM 15559 × Pendo
ICGV-SM 15567	ICGV-SM 15567× ICG 12725	ICGV-SM 15567 × ICGV-SM 05570	ICGV-SM 15567 × ICGV-SM 06737	(15) ICGV-SM 15567 × ICGV-SM 15524	ICGV-SM 15567 × ICGV-SM 15557	ICGV-SM 15567 × ICGV-SM 15559	S_7	ICGV-SM 15567 × ICGV-SM 16589	ICGV-SM 15567× ICGV-SM 16601	ICGV-SM 15567 × Kanyomwa	ICGV-SM 15567 × Narinut	ICGV-SM 15567 × Pendo
ICGV-SM 16589	ICGV-SM 16589 × ICG 12725	(16) ICGV-SM 16589 × ICGV-SM 05570	(17) ICGV-SM 16589 × ICGV-SM 06737	ICGV-SM 16589 × ICGV-SM 15524	ICGV-SM 16589 × ICGV-SM 15557	ICGV-SM 16589 × ICGV-SM 15559	(18) ICGV-SM 16589 × ICGV-SM 15557	S_8	(19) ICGV-SM 16589 × ICGV-SM 16601	ICGV-SM 16589 × Kanyomwa	(20) ICGV-SM 05570 × Narinut	ICGV-SM 16589 × Pendo
ICGV-SM 16601	ICGV-SM 16601 × ICG 12725	ICGV-SM 16601 × ICGV-SM 05570	(21) ICGV-SM 16601 × ICGV-SM 06737	ICGV-SM 16601 × ICGV-SM 15524	ICGV-SM 16601× ICGV-SM 15557	ICGV-SM 16601 × ICGV-SM 15559	ICGV-SM 16601× ICGV-SM 15567	ICGV-SM 16601 × ICGV-SM 16589	S_9	ICGV-SM 16601 × Kanyomwa	(22) ICGV-SM 05570 X Narinut	ICGV-SM 16601 × Pendo
Kanyomwa	Kanyomwa × ICG 12725	(23) Kanyomwa × ICGV-SM 05570	Kanyomwa × ICGV-SM 06737	Kanyomwa × ICGV-SM 15524	Kanyomwa × ICGV-SM 15557	(24) Kanyomwa × ICGV-SM 15559	Kanyomwa × ICGV-SM 15567	Kanyomwa × ICGV-SM 16589	Kanyomwa × ICGV-SM 16601	S_{10}	(25) Kanyomwa × Narinut	Kanyomwa × Pendo
Narinut	Narinut × ICG 12725	(26) Narinut × ICGV-SM 05570	(28) Narinut × ICGV-SM 06737	Narinut × ICGV-SM 15524	Narinut × ICGV-SM 15557	Narinut × ICGV-SM 15559	Narinut × ICGV-SM 15567	Narinut × ICGV-SM 16589	Narinut × ICGV-SM 16601	Narinut × Kanyomwa	S_{11}	Narinut × Pendo
Pendo	Pendo × ICG 12725	(29) Pendo × ICGV-SM 05570	Pendo × ICGV-SM 06737	(30) Pendo × ICGV-SM 15524	Pendo × ICGV-SM 15557	Pendo × ICGV-SM 15559	Pendo × ICGV-SM 15567	(31) Pendo × ICGV-SM 16589	(32) Pendo × ICGV-SM 16601	Pendo × Kanyomwa	(33) Pendo × Narinut	S_{12}

Numbers (1) to (33) denote successful crosses, which were used for genetic analyses

S_1 to S_{12} denote selfs

5.2.4 Experimental design and trial management

Thirty-three successful progeny and their parents were evaluated in the field using a 5 × 9 alpha lattice design with two replications at TARI-Naliendele in two seasons (2019 and 2020). The genotypes were evaluated for rust resistance, agronomic performance and yield potential during the off-season in 2019 and the rainy season in 2020. The off-season trial was conducted under irrigation, while the main season trial was done under rainfed condition. Each genotype was planted on two rows of 4m length, with a spacing of 50cm between the rows and 10cm between plants in a row. The plot size for each genotype was 2.0m^2. The recommended practices for fertilizer application and weeding for groundnut were followed (NARI 2001). This site is a hotspot for rust and late leaf spot diseases. Hence genotypes were evaluated under natural rust and late leaf spot infection and disease development.

5.2.5 Data collection

Data on yield and yield components were recorded during plant growth and at harvest maturity. Days to flowering (DTF) were recorded by counting the number of days from sowing to the time when 75% of the plants in a plot had emerging flowers. Plant height (PH, expressed in cm) was measured from ten randomly sampled plants in each plot from the soil surface to the tip of main stem. The number of pods per plant (NPP) was recorded as the average number of pods from ten randomly sampled and tagged plants per plot. Final plant stand (FPS) was recorded as the number of plants in each plot before harvesting. Pod yield (PDY) was measured by weighing the dried pods from each plot and was recorded in grams per plot. Shelling percentage (SP) for each genotype was calculated from a random sample of pods weighing 200g, as the proportion of shelled seed weight to the total weight of the unshelled pods. Additionally, 100 seed weight (HSW, expressed in grams) for each genotype was recorded as an average weight of two samples of 100 well-developed whole air-dried kernel per plot. Kernel yield (KY, expressed in t ha^{-1}) was estimated as the weight of kernels harvested from a plot.

Rust severity was scored at 85 days after planting (%RI85) and 100 days after planting (%RI100). The severity was scored using a scale of 1 (least affected) to 9 (most affected) following Das *et al.* (1999). Plants with no symptoms of infection were assigned a disease score of 1 (for 0% infection) while leaves with 1–5% infection were assigned a score of 2, 6–10% infection (score 3), 11–20% infection (score 4), 21–30% (score 5), 31–40% infection (score 6), 41–60% infection (score 7), 61–80% infection (score 8) and 81–100% infection (score 9) (Subbarao et al. 1990). Plants with a disease score of between 1 and 3, 4 and 6, and 7 and 9 were considered as resistant, moderately resistant and susceptible, respectively (Pande *et al.* 2002). In addition, late leaf spot reaction was assessed as a secondary trait due

to the simultaneous occurrence with rust disease. Late leaf spot severity was assessed at 85 days after planting (%LLSI85) and 100 days after planting (%LLSI100) as in rust severity.

5.2.6 Data analyses

5.2.6.1 Estimation of combining abilities

The data were subjected to analysis of variance (ANOVA) using SAS 9.4 (SASInstituteInc 2018) and means were separated by Fischers unprotected least significant difference at 5% probability level. General combining ability (GCA) effects of parents and specific combining. The general linear model used was as follows:

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

where Y_{ijk} is the observed measurement for the ij^{th} cross grown in the k^{th} replication or environment; μ is the population mean; g_i, and g_j are the GCA effects; s_{ij} the SCA effect; r_{ij} is the reciprocal cross effect between i^{th} and j^{h} parents; b_k is the effect of the kth block and e_{ijk} the error term associated with the ij^{th} cross evaluated in the k^{th} replication or environment.

The relative importance of GCA and SCA effects was estimated using the GCA-SCA prediction ratio proposed by Baker (1978) as follows:

$$\frac{2\sigma^2{}_{GCA}}{2\sigma^2{}_{GCA} + \sigma^2{}_{SCA}}$$

Where: $\sigma^2{}_{GCA}$ and $\sigma^2{}_{SCA}$ are estimated variance components for GCA and SCA effects, respectively

A trait whose Bakers' ratio is close to 1.00 indicate that the GCA effects were more important in conditioning the heritability of that trait, whereas a ratio close to zero would indicate that SCA effects would be more important in controlling trait heritability.

5.3 Results

5.3.1 Genetic variation and mean yield and yield component response of parents and progeny

The ANOVA revealed that the genotype × season interaction effects had significant (P<0.001) impact on DTF and NPP (Table 5.4). There was wide genotypic variation for all the assessed traits except SP. The traits DTF, NPP, DTM and SP exhibited significant (P<0.05) seasonal variability (Table 5.4).

Days to flowering varied from 31 days (for cross ICGV-SM06737 × Pendo) to 45 days (cross ICGV-SM16589 × ICGV-SM05570) (Table 5.5). The earliest maturing genotypes included

crosses ICG12725 × Pendo, ICGV-SM06737 × ICGV-SM16589, ICGV-SM15524 × Narinu, and Pendo × ICGV-SM16601, which matured in 99 days. Cross Narinut × ICGV-SM 05570 had the highest number of pods per plant (32 pods plant^{-1}). The highest HSW was recorded for the cross ICG 12725 × ICGV-SM 06737 (46.35 g/100 seed). The highest pod yield of 4712.82 kg ha^{-1} was attained by the cross ICGV-SM 15524 × ICGV-SM 05570. The crosses that had better yield response than their mid parents included ICGV-SM 15524 × ICGV-SM 05570 (1548.72 kg ha^{-1}), Narinut × ICGV-SM 05570 (957.23 kg ha^{-1}), Pendo × ICGV-SM 15524 (908.36 kg ha^{-1}), and Pendo × ICGV-SM 16601 (864.37 kg ha^{-1}).

Table 5.4 Analysis of variance showing F-statistic values for four disease parameters and eight agronomic traits of 45 groundnut genotypes evaluated in two seasons in Tanzania

		Disease parameters				Agronomic traits							
Source of variation	DF	%RI85	%RI100	%LLS85	%LLS100	DTF	PH	NPP	DTM	PDY	HSW	SP	KY
Replication	1	0.29	2.40	0.29	0.09	5.61*	0.48	1.89	0.01	0.22	0.75	0.38	0.13
Block (Replication)$	8	2.70	3.40	0.10	3.30	0.10	0.50	0.80	0.50	8.20**	3.50	0.10	7.20**
Genotype (G)	44	5.46***	5.39***	4.09***	4.26***	6.16***	62.26***	2.68***	3.66***	6.86***	5.61***	1.27	5.72***
Season (S)	1	0.48	0.41	0.01	1.12	5.14*	1.36	150.05***	11.39**	0.01	0.01	9.48**	0.70
G × S	44	0.22	0.33	0.35	0.81	2.15***	0.62	1.98***	0.24	0.26	0.99	0.54	0.32
Residual		0.06	0.07	0.03	0.05	7.15	6.12	0.95	32.95	333741	65.64	0.02	164336

Notes: DF = degrees of freedom; %RI85 = percentage rust infection at 85 days after planting, %RI100 = percentage rust score infection at 100 days after planting; %LLSI 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 =percentage late leaf spot infection at 100 days after planting; DTF = days to flowering; PH = plant height; NPP = number of pods per plant; DTM = days to maturity; PDY = pod yield; HSW = hundred seed weight; SP = shelling percent; KY = kernel yield; $ indicates chi-square statistic; *,** and *** represent significant differences at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 5.5 Mean values of four disease parameters and eight agronomic traits of 12 parental genotypes of groundnut and their 33 F2 families evaluated in two seasons in Tanzania

	Disease parameters					Agronomic traits						
Entry	%RI85	%RI100	%LLSI85	%LLSI100	DTF	PH	NPP	DTM	PDY	HSW	SP	KY
Parents												
ICG12725	21.05	35.54	8.75	11.01	37	20.30	14	113	890.10	30.51	64.74	587.23
ICGV-SM05570	6.58	11.52	2.50	6.05	38	23.25	13	110	1146.10	28.01	63.56	725.30
ICGV-SM06737	3.84	8.14	3.92	12.38	40	25.16	13	112	1044.43	26.73	58.41	640.33
ICGV-SM15524	12.70	18.63	7.43	17.55	38	22.64	13	107	975.67	24.99	64.77	660.45
ICGV-SM15557	4.92	14.01	2.26	4.75	38	24.18	9	99	489.41	19.06	56.15	268.56
ICGV-SM15559	5.97	10.69	1.82	6.66	38	22.47	11	110	1053.29	22.39	56.02	595.72
ICGV-SM15567	0.71	3.95	22.67	34.96	38	24.78	15	110	1724.95	21.77	58.19	970.97
ICGV-SM16589	15.51	26.71	6.40	14.98	38	29.87	11	110	779.79	23.79	54.20	436.63
ICGV-SM16601	14.79	29.22	6.24	16.34	39	23.60	10	108	887.70	28.60	64.13	587.70
Kanyomwa	11.30	18.54	0.61	4.00	39	27.31	16	109	832.47	25.51	56.12	491.22
Narinut	16.96	28.12	4.36	14.31	38	20.44	12	110	768.35	22.92	57.25	441.51
Pendo	28.85	39.61	15.65	28.38	37	23.74	8	107	841.04	27.31	62.72	530.49
Crosses												
ICG12725× ICGV-SM06737	1.43	2.60	2.31	4.10	35	37.15	12	113	1639.31	46.35	58.31	943.05
ICG12725 × Pendo	61.26	63.81	30.06	57.55	35	43.30	19	99	1294.64	26.49	63.49	839.17
ICGV-SM05570 x ICGV-SM06737	0.00	0.00	1.31	1.93	36	39.40	22	110	1666.27	20.95	60.58	1004.14
ICGV-SM05570 × Pendo	4.70	10.38	0.00	0.00	34	28.70	10	113	1581.73	34.60	57.94	813.71
ICGV-SM06737 × ICGV-SM15524	0.36	0.36	3.76	7.54	36	28.55	24	107	1689.11	10.42	59.89	984.49
ICGV-SM06737 × ICGV-SM16589	36.82	55.17	21.58	40.88	35	39.33	14	99	1560.88	21.54	58.86	892.50
ICGV-SM06737 × Narinut	32.47	39.98	1.99	15.30	36	32.23	15	114	1280.38	40.16	62.13	776.83
ICGV-SM06737 × Pendo	15.72	32.47	7.31	11.00	31	26.50	12	106	1502.81	5.35	58.10	825.30
ICGV-SM15524 × Narinut	43.75	57.51	7.55	20.03	33	45.95	10	99	656.53	27.20	52.85	336.97
ICGV-SM15557 × ICGV-SM15559	4.67	6.83	2.00	6.55	42	25.35	19	114	1510.60	27.78	61.85	930.13
ICGV-SM 15559 × ICFV-SM 15567	23.2	17.8	21.30	20.2	38	42.13	9	114	850	22.45	54.05	459.43

ICGV-SM16589 × ICGV-SM16601	55.01	75.09	19.46	33.46	35	41.06	14	110	1318.93	26.60	58.24	774.16
ICGV-SM16589 × Narinut	1.93	11.17	1.36	9.25	36	26.30	13	113	1461.05	19.67	50.02	740.25
ICGV-SM16601 × Narinut	7.30	17.43	3.74	8.32	35	22.03	18	100	1610.67	35.26	54.37	958.20
Kanyomwa × Narinut	4.67	4.67	0.00	2.65	39	36.29	14	114	1321.45	30.04	53.98	744.00
ICGV-SM06737 × ICGV-SM05570	1.32	8.32	0.35	1.32	35	40.33	15	114	1212.34	39.75	64.38	789.30
ICGV-SM15524 × ICGV-SM05570	0.00	0.00	1.27	2.88	37	41.28	30	114	4712.82	37.17	63.99	3018.25
ICGV-SM15559 × ICGV-SM05570	4.67	9.87	3.99	8.99	35	39.78	21	113	1818.15	31.98	54.49	1033.01
ICGV-SM15559 × ICGV-SM06737	1.32	1.32	0.00	0.00	45	19.15	6	114	1740.56	19.11	52.88	914.63
ICGV-SM15567 × ICGV-SM15524	0.71	0.00	0.01	0.00	35	29.45	15	113	1337.45	30.77	66.82	884.32
ICGV-SM16589 × ICGV-SM05570	0.36	1.32	0.01	0.00	45	32.28	23	113	1565.74	21.91	71.22	1085.73
ICGV-SM16589 × ICGV-SM06737	0.36	1.32	6.01	8.32	39	49.98	18	114	1099.27	3.55	43.06	496.12
ICGV-SM16589 × ICGV-SM15557	42.49	57.51	0.95	13.33	36	33.19	14	113	1106.97	42.69	59.52	641.75
ICGV-SM16601 × ICGV-SM06737	0.00	0.00	0.00	3.29	38	27.65	13	113	1837.97	36.68	53.58	985.58
Kanyomwa × ICGV-SM05570	3.29	5.39	4.60	11.17	33	31.63	18	106	1663.36	22.20	53.40	1003.44
Kanyomwa × ICGV-SM15559	1.32	5.00	0.36	1.32	36	21.20	12	114	2240.61	27.82	64.95	1464.50
Narinut × ICGV-SM05570	0.00	0.00	0.35	1.32	37	27.08	32	114	2452.21	41.29	63.41	1576.17
Narinut × ICGV-SM06737	6.83	9.06	4.75	5.39	36	36.43	20	110	1131.60	18.65	55.62	635.25
Pendo × ICGV-SM05570	0.00	0.00	0.01	0.36	38	28.20	18	113	1260.74	28.12	47.88	600.81
Pendo × ICGV-SM15524	1.32	13.50	17.38	22.45	36	43.13	17	114	2082.72	31.39	68.95	1440.49
Pendo × ICGV-SM16589	46.18	55.01	27.61	37.42	33	39.38	19	100	1470.73	25.37	59.53	853.52
Pendo × ICGV-SM16601	8.60	22.45	1.34	17.13	44	21.20	14	99	1686.72	16.34	63.89	1106.66
Pendo × Narinut	24.29	32.05	14.08	27.98	35	27.75	16	113	1173.45	44.91	60.77	696.97
Mean	12.64	19.19	6.09	12.56	37	30.66	15	110	1434.57	27.13	58.98	857.40
CV (%)	81.68	69.79	89.64	69.85	7.24	8.07	25.07	5.24	40.27	29.86	14.25	47.28
LSD (5%)	0.30	0.33	0.22	0.27	3.30	2.92	1.20	7.19	730.49	10.24	-	512.20

Notes: %RI85 = percentage rust infection at 85 days after planting, %RI100 = percentage rust score infection at 100 days after planting; %LLSI 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 =percentage late leaf spot infection at 100 days after planting; DTF = days to flowering; PH = plant height; NPP = number of pods per plant; DTM = days to maturity; PDY = pod yield; HSW = hundred seed weight; SP = shelling percent; KY = kernel yield; LSD = least significant difference; CV = coefficient of variation

5.3.2 Combining ability of groundnut genotypes for rust and leaf spot resistance and agronomic traits

The mean square of GCA, SCA and reciprocal variance for disease parameters and eight agronomic traits are presented in Table 5.6. The GCA, SCA and reciprocal effects were highly significant for all the traits except for NPP whose GCS variance was not significant (Table 5.6). Seasonal effects significantly affected the GCA, SCA and reciprocal variances for NPP and HSW only (Table 5.6).

Table 5.6 Analysis of variance showing F-statistic for combining ability effects for four disease parameters and eight agronomic traits of 45 groundnut genotypes evaluated in two seasons in Tanzania

		Disease parameters				Agronomic parameters							
Source of variation	DF	%RI85	%RI100	%LLS85	%LLS100	DTF	PH	NPP	DTM	PDY	HSW	SP	KY
GCA	11	7.21***	7.79***	6.11***	6.51***	3.45***	91.88***	0.93	2.34**	4.01***	8.25***	0.84	3.45***
SCA	19	4.14***	3.51***	2.81***	2.86***	2.66***	88.75***	2.0**	4.38***	3.74***	7.60***	0.71	2.66***
GCA*ENV	11	0.14	0.25	0.42	0.84	0.29	0.28	2.22*	0.22	0.32	1.49	0.51	0.29
SCA*ENV	19	0.11	0.14	0.32	0.66	0.24	0.66	1.60*	0.21	0.19	2.01**	0.50	0.24
REC	14	2.65**	3.64***	2.78***	3.63***	9.33***	27.25***	2.58**	2.37**	10.84***	6.86***	1.38	9.33***
REC*ENV	14	0.14	0.28	0.27	0.90	0.25	1.02	2.26**	0.16	0.10	1.23	0.34	0.25

Notes: DF = degrees of freedom; %RI85 = percentage rust infection at 85 days after planting, %RI100 = percentage rust score infection at 100 days after planting; %LLSI 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 =percentage late leaf spot infection at 100 days after planting; DTF = days to flowering; PH = plant height; NPP = number of pods per plant; DTM = days to maturity; PDY = pod yield; HSW = hundred seed weight; SP = shelling percent; KY = kernel yield; GCA = general combining ability; SCA = specific combining ability; ENV = environment/season; REC = reciprocal; GCA*ENV= general combining ability by environment/season interaction, SCA*ENV= specific combining ability by environment/season interaction; ; REC*ENV= reciprocal by environment/season interaction; *,** and *** represent significant differences at 0.05, 0.01 and 0.001 probability levels, respectively.

5.3.3 General combining ability effects

The GCA estimates varied among the 12 parental genotypes for the agronomic traits and disease parameters (Table 5.7). The best combiners for %RI85 and %RI100 were ICGV-SM 05570 and ICGV-SM 15567, which had negative and desirable GCA effects of -0.15 and -0.14, respectively. In addition, ICGV-SM 05570 exhibited negative and desirable GCA effects for %LLSI100. Desirable GCA effects for DTF and DTM were exhibited by genotypes ICGV-SM 15524, and ICGV-SM 16601, respectively. There was only one parental line, Kanyomwa, which exhibited desirable and significant GCA effect for pod and kernel yield. Genotype Kanyomwa was the best general combiner for kernel and pod yield with GCA effects of 760.61 and 1007.47, respectively, while Narinut 15 had significant negative effects for both (Table 5.7).

Table 5.7 General combining ability effects with mean squares and significant tests for four disease parameters and eight agronomic traits of 12 parental genotypes evaluated in two seasons in Tanzania

Parents	Disease parameters				Agronomic traits							
	%RI85	%RI100	%LLSI85	%LLSI100	DTF	PH	NPP	DTM	PDY	HSW	SP	KY
ICG 12725	0.07	0.06	0.14**	0.10	1.70*	-1.79**	-0.14	-0.56	-205.07	-3.23	0.04	-68.54
ICGV-SM 05570	-0.15**	-0.16*	-0.06	-0.13*	2.25**	-4.09	-0.10	1.75	-159.42	-6.64**	0.02	-78.59
ICGV-SM 06737	-0.11	-0.10	0.01	-0.02	1.19	-3.77	-0.08	1.27	110.21	-13.06	0.04	107.40
ICGV-SM 15524	-0.08	-0.21	-0.05	-0.08	-6.05*	29.44	1.14	-6.90	-398.30	21.52*	-0.21	-511.29
ICGV-SM 15557	0.26*	0.29*	-0.22*	-0.06	-0.20	-3.67**	-0.14	4.65	-174.42	18.04	0.05	-60.60
ICGV-SM 15559	-0.05	-0.06	-0.02	-0.03	1.49**	-5.57	-0.34*	0.43	-179.85*	-6.52	-0.005	-108.17
ICGV-SM 15567	-0.14*	-0.14*	0.16**	0.14**	1.15	-4.42	-0.01	0.48	155.98	-6.83**	0.01	79.45
ICGV-SM 16589	0.12*	0.16**	0.14**	0.14**	0.58	3.25	-0.06	-0.65	-131.60	-10.79	-0.03	-109.72
ICGV-SM 16601	0.02	0.09	0.04	0.06	3.28	-6.82	-0.17	-3.74*	2.17	-8.19	0.04	74.68
Kanyomwa	-0.17	-0.15	-0.10	-0.18	-0.62	-6.84	-0.16	3.94	1007.47**	-1.09	0.09	760.61**
Narinut	0.04	0.06	0.02	0.03	1.61**	-6.59	-0.23	0.35	-322.32**	-6.26	0.001	-185.28**
Pendo	0.19	0.16	-0.05	0.01	-6.38	10.87	0.31	-1.02	295.15	23.04	-0.04	100.04

Notes: %RI85 = percentage rust infection at 85 days after planting, %RI100 = percentage rust score infection at 100 days after planting; %LLSI 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 =percentage late leaf spot infection at 100 days after planting; DTF = days to flowering; PH = plant height; NPP = number of pods per plant; DTM = days to maturity; PDY = pod yield; HSW = hundred seed weight; SP = shelling percent; KY = kernel yield; *,** and *** represent significant differences at 0.05, 0.01 and 0.001 probability levels, respectively.

5.3.4 Specific combining ability effects

The SCA effects of the 33 crosses for the twelve characters showed a wide variation (Table 5.8). Good specific combiners for rust infection were ICGV-SM 15557 × ICGV-SM 15559, ICGV-SM 16589 × Narinut and ICG 12725 × ICGV-SM 06737, which exhibited negative SCA effects for %RI85 or %RI100 (Table 5.8). Crosses Pendo × ICGV-SM 05570 exhibited desirable negative and significant ($P \leq 0.05$) SCA effects for DTF, while Kanyomwa × ICGV-SM 05570 had positive and significant SCA effect for DTF. Crosses that exhibited negative and significant ($P \leq 0.05$) SCA effects for DTM included ICG 12725 × Pendo and ICGV-SM 16589 × ICGV-SM 06737. None of the families exhibited positive and significant ($P \leq 0.01$) effect for KY, although crosses ICGV-SM 06737 × ICGV-SM 15524 and Kanyomwa × ICGV-SM 05570 showed high and positive SCA effect for KY.

Table 5.8 Specific combining ability effects showing mean squares and significant tests for four disease parameters and eight agronomic traits of 33 F2 families evaluated in two seasons in Tanzania

Crosses	Disease parameters				Agronomic traits							
	%RI85	%RI100	%LLSI85	%LLSI100	DTF	PH	NPP	DTM	PDY	HSW	SP	KY
ICG 12725 × Pendo	0.25	0.21	0.32**	0.41**	4.77**	0.62	0.22	-8.33*	-208.43	-28.75	0.07	-4.39
Narinut × ICGV-SM05570	0.22	0.31*	0.07	0.09	1.95	-4.14**	-2.05**	-2.44	-1520.96	-18.75	-0.04	-1027.97
ICGV-SM 15524 × ICGV-SM 05570	0.06	0.02	-0.04	-0.10	-5.12	17.68	-0.49	-9.69	-3857.55	13.15	-0.26	-2796.06
Pendo × ICGV-SM 05570	0.15	0.22*	-0.01	0.01	-2.25*	0.25	-0.49	-0.05	160.50	3.24	0.05	106.45
ICGV-SM 16589 × ICGV-SM 05570	0.25	0.31*	0.25*	0.33*	-7.28	0.50	-0.99	-2.78	-443.76	-3.91	-0.15*	-461.97
Kanyomwa × ICGV-SM 05570	-0.17	-0.11	-0.20	-0.31	3.50	-8.95	-0.54	8.95*	597.68	5.51	0.15	490.64
ICGV-SM 06737 × ICGV-SM 05570	-0.16	-0.16	0.03	0.01	0.12	-0.46	0.41	-1.69	226.97	-9.40**	-0.02	107.42
Narinut × ICGV-SM 06737	0.17*	0.19*	-0.04	0.09	0.16	-2.1*	-0.25	1.97	74.39	10.75**	0.03	70.79
Kanyomwa × ICGV-SM 15559	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ICGV-SM 15559 × ICGV-SM 05570	-0.08	-0.11	-0.10	-0.15	4.41**	-15.83	-1.14*	-1.98	-744.43*	-9.71*	0.04	-407.71
ICGV-SM 15557 × ICGV-SM 15559	-0.36	-0.43*	0.21	0.03	5.59**	0.98	0.86	-0.65	451.89	-19.17**	0.003	286.84
ICG 12725 × ICGV-SM 06737	0.21	-0.26*	-0.17*	-0.21*	-2.83*	9.10	-0.25	3.20	321.18	27.21	-0.07	92.13
ICGV-SM 06737 × ICGV-SM 15524	-0.06	-0.02	0.06	0.09	5.97*	-30.73	-0.11	3.13	564.21	-33.47**	0.20	576.32
ICGV-SM 16589 × ICGV-SM 06737	0.32**	0.38**	0.12	0.21**	-2.25*	-5.33	-0.22	-7.32**	230.81	8.99**	0.08	198.19
ICGV-SM 16601 × ICGV-SM 06737	0.21	0.40*	0.22*	0.17	2.14	-4.63**	0.13	-6.47	-312.61	-22.50	0.11	8.57
ICGV-SM 15559 × ICGV-SM 06737	0.07	0.19	0.16	0.26*	-7.39	5.12**	1.01	-2.77	-397.21	-3.26	0.07	-103.35
ICGV-SM 15524 × Narinut	0.38	0.51	0.13	0.14	1.77	-10.51	-1.60	-3.61	-35.85	-23.50**	0.17	221.47
ICGV-SM 05570 × ICGV-SM 06737	0.01	0.00	-0.04	-0.04	-3.27**	14.11	0.52	-0.17	75.53	14.62	-0.004	55.84
ICGV-SM 15567 × ICGV-SM 15524	0.13	0.05	0.28	0.40	-4.71	29.18	1.15	10.63	-166.78	19.36	-0.31	-504.09

Kanyomwa × Narinut	0.02	-0.04	-0.10	0.03	2.35	11.74	0.25	0.38	-776.70	1.96	-0.12	-643.4
ICGV-SM 06737 × Narinut	0.016	0.10	-0.02	-0.02	-2.33*	11.07	0.56	0.65	5.11	13.29**	-0.02	-28.14
Pendo × Narinut	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pendo × ICGV-SM 15524	-0.21	-0.90	-0.74	-0.77	-35.86*	141.88	5.56	-23.72	-1660.53	159.07**	-1.26	-2708.43
ICGV-SM 15559 × ICGV-SM 15567	0.15	0.10	0.21	0.17	-1.19	12.05	1.35	-7.15	64.17	2.15	-0.05	78.25
ICGV-SM 05570 × Pendo	0.25	-0.25	-0.06	-0.16	4.99**	-11.94	-0.43	3.39	-127.48	-20.47	-0.02	-126.25
ICGV-SM 16589 × Narinut	-0.36**	-0.33*	-0.22**	-0.19	-1.16	-3.98**	0.03	4.35	501.97	1.28	-0.05	223.18
ICGV-SM 16601 × Narinut	-0.12	-0.15	-0.04	-0.12	-5.01**	1.82	0.71	-6.25*	517.83	14.27**	-0.07	256.73
ICGV-SM 16589 × ICGV-SM 15557	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pendo × ICGV-SM 16589	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ICGV-SM 06737 × ICGV-SM 16589	0.04	0.02	0.04	0.07	-0.19	11.56	0.20	-3.35	-61.53	0.97	-0.07	-115.44
ICGV-SM 16589 × ICGV-SM 16601	0.40**	0.42**	0.10	0.12	-4.01	11.02	0.001	5.08*	35.38	10.14**	-0.002	-2.87
Pendo × ICGV-SM 16601	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ICGV-SM 06737 × Pendo	-0.03	0.09	0.14	0.03	0.57	-14.21	-0.73	-3.20	-315.53	-40.05	0.02	-194.19

Notes: %RI85 = percentage rust infection at 85 days after planting, %RI100 = percentage rust score infection at 100 days after planting; %LLSI 85= Percentage late leaf spot infection at 85 days after planting; %LLSI 100 =percentage late leaf spot infection at 100 days after planting; DTF = days to flowering; PH = plant height; NPP = number of pods per plant; DTM = days to maturity; PDY = pod yield; HSW = hundred seed weight; SP = shelling percent; KY = kernel yield; *,** and *** represent significant differences at 0.05, 0.01 and 0.001 probability levels, respectively.

5.3.5 Gene action

The GCA variances were smaller than the corresponding SCA variances for all assessed traits (Table 5.9). The Bakers ratios which are based on the GCA to SCA variance were all below 0.50 suggesting the preponderance of non-additive genetic effects. In comparison to agronomic traits, disease-related traits such as %RI100, %LLS185, and %LLSI100 had higher Bakers' ratios, each with about a value of 0.20. The heritability of assessed traits ranged between 17.89 and 97.74%. The highest heritability was estimated for plant height at 97.74% (Table 5.9).

Table 5.9 Variance components for four-disease parameters and eight agronomic traits of 45 groundnut genotypes evaluated in two seasons

Traits	GCA	SCA	REC	GCA & SCA ratio	Baker's ratio	Heritability (%)
Disease parameters						
%RI85	0.008	0.092	0.018	0.085	0.145	78.48
%RI100	0.011	0.095	0.039	0.111	0.182	79.89
%LLSI85	0.003	0.029	0.013	0.113	0.184	70.47
%LLSI100	0.005	0.046	0.026	0.117	0.190	68.40
Agronomic traits						
DTF	0.559	22.318	11.635	0.025	0.048	65.38
PH	10.081	256.013	37.641	0.039	0.073	97.74
NPP	-0.005	0.545	0.383	-0.008	-0.017	20.26
DTM	1.136	53.976	14.639	0.021	0.040	70.27
PDY	21812.010	480854.190	830693.490	0.045	0.083	83.13
HSW	9.638	220.665	97.726	0.044	0.080	80.46
SP	-0.000	-0.002	0.001	0.009	0.018	17.89
KY	8655.410	146365.650	348164.330	0.059	0.106	80.53

Notes: %RI85 = percentage rust infection at 85 days after planting, %RI100 = percentage rust score infection at 100 days after planting; %LLSI85= Percentage late leaf spot infection at 85 days after planting; %LLSI100 =percentage late leaf spot infection at 100 days after planting; DTF = days to flowering; PH = plant height; NPP = number of pods per plant; DTM = days to maturity; PDY = pod yield; HSW = hundred seed weight; SP = shelling percent; KY = kernel yield; GCA = general combining ability; SCA = specific combining ability; REC = reciprocal

5.4 Discussion

5.4.1 Genetic variations among parents and progeny

The F_2 progeny and their parents showed significant ($P<0.05$) variation for yield and yield components (Table 5.4) suggesting that the test genotypes were useful genetic resources for groundnut improvement. The groundnut genotypes used in this study included divergent parental lines from Virginia and Spanish botanical groups which invariably contributed to the genetic variation observed in the new breeding population. The parental lines have different genetic constitution, agronomic potential and adaptations providing the F_2 progeny with transgressive segregations. This led to wide genetic variation in the observed performance in the F_2. The presence of high progeny performance exceeding the parental phenotypic values have been reported in segregating populations (Yang *et al.* 1998, Yonas *et al.* 2014, Zhao *et*

al. 2020). Transgressive segregation is useful in crop improvement as one of the mechanisms that contribute to genotype discovery with unique genetic composition and novel adaptations compared to the base population (Rieseberg *et al.* 2000). Crop improvement depends on the availability of genetic variation with stable performance for economic traits (Khan *et al.* 2015). Genotype × season interaction effects were significant for DTF and NPP (Table 5.4) suggesting that seasonal variability and change in climatic conditions affects the phenotypic expression of the tested groundnut genotypes. Seasonal variability presents challenges for selection as it reduces correlation between genotype and phenotypic expression. Genotypes such as ICGV-SM 15524 × ICGV-SM 05570 and Kanyomwa × ICGV-SM 15559 that consistently perform across seasons and locations will be ideal for selection. Traits whose expression was not significantly affected by seasonal or genotype × seasonal variability will be easier to select and improve. Mekontchou *et al.* (2006) and Bucheyeki *et al.* (2008) reported significant genotype, environmental and genotype × environment interaction variations for agronomic traits in groundnuts.

The impact of rust diseases on groundnut production compels breeders to select genotypes that express appreciable levels of rust resistance coupled with high yield potential. Parental lines such as ICGV-SM 15567 and progeny such as ICGV-SM 15524 × ICGV-SM 05570 and Narinut × ICGV-SM 05570 exhibited low rust infection and had high kernel yield response across seasons. Higher mean performance among crosses compared to the parents indicates that there was genetic gain in yield and agronomic performance among the crosses. For groundnut rust resistance breeding, the following families were selected: ICGV-SM 15524 × ICGV-SM 05570, Pendo × ICGV-SM 05570, ICGV-SM 16601 × ICGV-SM 06737 and ICGV-SM 05570 × ICGV-SM 06737. These families exhibited low rust severity than their corresponding parents. This suggests that rust resistance was achieved through gene recombination hence desirable transgressive segregants can be selected in successive generations. For instance, Pendo is known to be susceptible to rust but its progeny, i.e., Pendo × ICGV-SM 05570 was among the crosses with desirable SCA effects for rust resistance.

Incorporating rust resistance, with kernel yield and yield components will enhance groundnut production and productivity. Groundnut breeding should target multiple traits to achieve suitable agronomic performance and high yields. For instance, parental lines Pendo and ICGV-SM 15524 and crosses such as Pendo × ICGV-SM 16589, ICGV-SM 06737 × Pendo and ICGV-SM 15524 × Narinut displayed early flowering and maturity. Hence these genotypes should be selected to improve early maturity for environments with short and erratic rainfall patterns in Tanzania. Chaudhari *et al.* (2019) and Sukruth *et al.* (2015) selected groundnut genotypes that exhibited early flowering for yield improvement under marginal conditions especially drought prone areas. Parental lines and crosses that exhibited desirable mean performance in other desirable traits such as higher shelling percent (ICG 12725, ICGV-SM

15524, ICGV-SM 16601, Pendo × ICGV-SM 15524 and ICGV-SM 15567 × ICGV-SM 15524)5 and hundred seed weight (ICG 12725, ICG 12725 × ICGV-SM 06737, Pendo × Narinut and ICGV-SM 16589 × ICGV-SM 15557) should be selected for direct or indirect selection of grain yield. It is, thus, imperative to consider the correlations existing among the target traits to ensure that appropriate selection methods are devised for simultaneous improvement.

5.4.2 Combining ability

Except NPP and SP, all assessed traits exhibited significant GCA and SCA variance (Table 5.6) suggesting that the assessed traits were conditioned by additive and non-additive genes. For NPP, the SCA effect was only significant indicating non-additive genes controlling this trait. In addition, the GCA and SCA variances were consistent across seasons indicating that allele interactions and additive gene effects were less influenced by the environment and were thus highly heritable. The traits that exhibit significant GCA effects may be improved by selection and crossing of parental lines with favourable performance for that trait. Parents will be expected to additively contribute their favourable alleles to develop better performing offspring. In contrast, SCA effects will be exploited through hybrid breeding instead of pure line selection. Dominance genes occur as a result of interaction between alleles governing the inheritance of a trait. Intra-allelic interaction is not easily predictable. For instance, two different parents with favourable mean performance for a trait may produce an offspring with undesirable performance due to poor gene combinations or intra-allelic interaction (dominance) or inter-allelic interaction (epistasis). On the other hand, genes from poor performing parents may combine favourably well to produce high performing offspring due to favourable SCA effects. Traits such as rust and leaf spot resistance and grain yield have been reported to be controlled by additive and non-additive gene effects (Adamu *et al.* 2008, Ghewande 2009). However, other reports indicated that non-additive gene effects were more important for rust resistance and grain yield (Shoba *et al.* 2010, Vishnuvardhan *et al.* 2011). The significant reciprocal effects in the cross ICGV-SM 16589 × ICGV-SM 06737 (for DTF and DTM) and Narinut × ICGV-SM 06737 for HSW suggested the presence of maternal inheritance effect conditioning trait heritability. Maternal effects are contributed by the female parent and thus it is important to purposefully designate the female and male parents during crosses to exploit any favourable maternally inherited trait. Pasupuleti *et al.* (2013) and Dwivedi *et al.* (1989) reported highly significant reciprocal effects for late leaf spot resistance and kernel yield in groundnut, respectively.

5.4.3 General combining ability of parents

General combining ability analysis is an effective method in selection of parents based on performance of their progeny, usually at the F_1 or F_2 and later generations (Sleper and

Poehlman 2006). Developing groundnut cultivars with rust resistance that are adapted to harsh growing conditions is important for sub-Sahara African region where rust and other foliar diseases are endemic. The development of high-yielding cultivar with resistance to foliar disease is one of the major objectives of the groundnut improvement programme in Tanzania. Developing suitably adapted cultivars is preceded by identifying parental lines with good combining ability for the suitable traits. Parental genotypes that exhibit good GCA effects often have the ability to transfer their favourable characteristics to the offspring (Amegbor *et al.* 2017). Parental genotypes such as ICGV-SM 05570 and ICGV-SM 15567 had negative GCA effects for rust resistance, while genotype Kanyomwa had positive GCA effects for grain yield (Table 5.7). These parents are selected for developing breeding populations. The GCA effects of the parental lines is particularly important for traits controlled by additive traits since their inheritance and expression in the offspring are conditioned by the summation of the allelic effects of the different parents. For improving traits such as earliness to flowering and maturity, parental lines ICGV-SM 15524 and ICGV-SM 16601 that exhibited negative GCA effect will be ideal due to their potential to reduce the average DTF and DTM in the offspring. Early flowering and maturity varieties are ideal for marginal environments such as those mostly found in sub-Sahara Africa region, characterised by inadequate rainfall and high temperatures. However, earliness to maturity can lead to yield penalty in environments where soil moisture is adequate, and the rainy season is long (Caliskan *et al.* 2008). Groundnut rust disease epidemiology is favored by continuous warm temperatures ranging between 20 and 30 °C and high humidity above 78 % (Mondal *et al.* 2008, Peregrine 1971). Under favourable moisture and temperature conditions the following genotypes are recommended for breeding: ICGV-SM 05570, ICGV-SM 15559 and Narinut. These lines had positive GCA effects for DTF and DTM useful for long duration variety development. The parents with positive GCA effects for PH (ICGV-SM 15524 and Pendo), NPP (ICGV-SM 15524 and Pendo), HSW (ICGV-SM 15524 and Pendo) and SP (Kanyomwa) (Table 5.7) will be useful for trait improvement including grain yield. Groundnut genotypes of the Virginia botanical group have high above ground biomass, high number of pods per plant and large seed types which may likely provide higher grain yield. These group of genotypes exhibited medium to late maturity compared to small-biomass types (Wells *et al.* 1991). However, there were no parental lines that exhibited good GCA for all assessed traits in the present study. Therefore, different complementary parents should be selected for breeding purposes based on their GCA effects.

5.4.4 Specific combining ability of crosses

Specific combining ability effect relates to performance of some crosses relatively better or worse than would be expected based on the average performance of the parents involved (Griffing 1956). SCA effects represent the non-additive proportion of variance that is difficult

to exploit in trait improvement in self-pollinating crops due to low heritability and unpredictability of reshuffling of genes. The performance of a specific cross depends on the extent of the favourable genes for a trait from the two parents complementing each other (Sleper and Poehlman 2006). For instance, crosses such as ICGV-SM 15557 × ICGV-SM 15559, ICGV-SM 16589 × Narinut and ICG 12725 × ICGV-SM 06737 exhibited good SCA effects for rust resistance and reduced number of days to flowering (Table 5.8). The families such as ICGV-SM 06737 × ICGV-SM 15524 and Kanyomwa × ICGV-SM 05570 had good SCA effects for kernel yield due to favourable interaction between alleles from the female and male parents. In some cases, `crosses can exhibit good SCA effect even when their parents have poor or unfavourable GCA effects for the trait. This is due to the favourable interaction after recombination showing that there will be potential for selection of transgressive individuals in segregating populations. Crosses such as Narinut × ICGV-SM 05570 had positive SCA effects and high mean values for kernel yield. Conversely, crosses such as ICGV-SM 16589 × Narinut and ICG 12725 × ICGV-SM 06737 had negative SCA effect and lower mean values for rust severity scores compared to their mid parent values. These suggest that the new progeny are transgressive segregants that could be further selected for trait improvement.

5.4.5 Gene action

The ratios of GCA to SCA mean squares for most assessed traits were less than one and had a value close to zero based on Baker's ratio indicating that non-additive gene action had a more prominent role in the control of groundnut rust resistance and agronomic traits. The Baker's ratios (> 0.5) showed the preponderance of non-additive gene effects for most traits and suggested that trait improvement will only be effective after selection in the advanced generations. The non-additive gene action found in this study were in concurrence with Shoba *et al.* (2010), who reported that rust resistance is controlled by non-additive gene action. The significant differences showed by the reciprocal effects indicate that maternal effects have impact on groundnut rust resistance (Table 5.6). Hence, it is important to use appropriate mating design that will allow to exploit cytoplasmic inheritance. According to Joel *et al.* (2006) rust resistance is controlled by few genes with either monogenic, digenic or trigenic inheritance and hence backcross breeding can facilitate accumulation of major genes in progeny.

5.5 Conclusions

The assessed groundnut genotypes exhibited wide genetic variation for rust resistance, agronomic traits and kernel yield useful for breeding. The inheritance of rust resistance is conditioned by dominance gene action, while kernel yield was controlled by additive gene

action. Parental lines ICGV-SM 05570 and ICGV-SM 15567, which were the best combiners for rust resistance and kernel yield, were selected for breeding population development and pure line maintenance. The families Narinut × ICGV-SM 16589 and ICGV-SM 15559 × ICGV-SM 15557 were identified as best specific combiners for rust resistance. The selected families are recommended for genetic advancement and to identify transgressive segregants and develop pure lines for cultivar release and deployment in Tanzania.

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An overview of the research findings

Introduction and objectives of the study

Groundnut (*Arachis hypogaea* L.) is cultivated globally on about 28.52 million hectares with an annual production of ≈45.95 million tons. It is grown in more than 100 countries of tropical, subtropical, and warm temperate regions. The mean groundnut yield in Africa including Tanzania is <1 t/ha far below the attainable yields of 2.5 t/ha. In Tanzania groundnut production is affected by a multitude of biotic and abiotic stresses and socioeconomic constraints. Rust disease, caused by *Puccinia arachidis* Speg, is an important disease of groundnut that causes up to 57% yield loss. The incorporation of host resistance in susceptible groundnut genotypes have been recommended as a cost-effective and environmentally friendly disease control method and to improve groundnut productivity, especially under the smallholder farming system in Tanzania. This chapter summarizes the research objectives and highlights the core findings and implications of the study.

The objectives of this study were:

- To document the groundnut farmers' major production constraints, farming systems, and varietal trait preferences in selected agro-ecologies of Tanzania.
- To determine the extent of genetic variation among diverse groundnut collections using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and complementary genotypes for breeding.
- To assess genotype and genotype by environment interaction (GEI) effect on kernel yield and select best adapted groundnut genotypes in target production environments in Tanzania
- To determine the combining ability effects and gene action controlling rust resistance and agronomic traits in groundnut genotypes for breeding.

Research findings in brief

Groundnut production constraints, farming systems, and farmer-preferred traits in Tanzania

A participatory rural appraisal study was conducted in three selected regions in the southern (Mtwara), Central (Dodoma) and lake zone (Shinyanga), which are the main groundnut

production areas in Tanzania. A semi structured questionnaire, transect walks, and FGDs were used to collect information from 180 participant farmers. The main findings of the study were:

- The major constraints affecting groundnut production in the study areas included diseases and pests, which were reported by 87.7% and 84.9% of respondents, respectively
- Groundnut rust, caused by *Puccinia arachidis* Speg, was the major cause of yield reduction, as reported by 30% of the respondents
- Drought stress and nonavailability of seed of improved varieties were other important constraints, as reported by 83.9% and 76.1% of the respondents, respectively.
- Groundnut agronomic attributes preferred by farmers were high yield (reported by 78.4% of respondents), disease resistance (71.2%), early maturity (66%), drought tolerance (63.0%), and pest resistance (63%).
- The main farmers and market-preferred groundnut seed quality traits were medium-to large grain size (reported by 62.6%of respondents) and tan and red seed color (59.2%).
- The above production constraints, agronomic attributes and farmer-preferred traits are the main drivers of groundnut improvement in Tanzania.

Genetic diversity and population structure of groundnut (Arachis hypogaea L.) accessions using phenotypic traits and SSR markers: implications for rust resistance breeding

The study used a total of 119 groundnut accessions. One hundred and eight lines sourced from the gene bank of International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) which are breeding populations, six cultivated varieties and five were landrace collections from different agro-ecologies in Tanzania. Test genotypes were evaluated at the Naliendele Agricultural Research Centre and Chambezi Experimental Station. The genotypes were screened for resistance to rust and late leaf spot during the 2018 and 2019 seasons. The experiment at each site was laid out using an 8 x 15 alpha lattice design with two replications. Test genotypes were sampled for genotyping using 13 selected SSR markers. The core findings of the study were:

- Genotype and genotype by environment interaction effects were significant ($p<0.05$) for days to flowering (DTF), late leaf spot score at 85 and 100 days after planting, pod yield (PDY), kernel yield (KY), hundred seed weight (HSW) and shelling percentage (SP).

- Principal components analysis revealed that plant stand, KY, SP, NPP (number of pods per plant), late leaf spot and rust disease scores accounted for the largest proportion of the total variation (71.9%) among the tested genotypes.
- Genotypes ICGV-SM 08587 and ICGV-SM 16579 were the most stable yielders across the test environments.
- Moderate genetic variation was recorded with mean polymorphic information content of 0.34 and gene diversity of 0.63 using the SSR markers.
- The majority (74%) of genotypes showed high membership coefficients to their respective subpopulations, while 26% were admixtures after structure analysis.
- Much of the variation (69%) was found within populations due to genotypic differences.
- Genotypes ICGV-SM 06737, ICGV-SM 16575, ICG 12725 and ICGV-SM 16608 were identified to be used for development of breeding population, which will be useful for groundnut improvement.

Genotype-by-environment interaction analysis of groundnut (*Arachis hypogaea* L.) for kernel yield

A total of 120 groundnut genotypes were used in this study. The genotypes were evaluated in two selected sites, namely, Naliendele and Chambezi during the 2018 and 2019 cropping season in Tanzania. The experiments were laid out using an 8 × 15 alpha lattice design with two replications at each site. Each plot consisted of two rows that were 4 m long with a spacing of 50 cm between the rows and an intra-row spacing of 10 cm with one seed planted per hole. The trials at Chambezi were established in March in both seasons, while at Naliendele the trials were established in January 2018 and September 2019. Data were recorded on pod yield, shelling percent and kernel yield. The main outcomes were as follows:

- Significant ($p<0.05$) variations were detected among genotypes (G), environments (E) and GEI effects on kernel yield
- A relatively higher proportion of the observed variation was due to the environment (34.85%) followed by GEI (24.65%) and genotype (8.25%) effects.
- Genotypes ICGV 94124 and CG 7 had relatively better kernel yield of 469.01 and 450.02 kg ha^{-1}, respectively
- The genotype and genotype-by-environment biplot identified ICGV-SM 16556, ICGV-SM 15524, ICGV-SM 15564 and ICGV-SM 15514 as the most stable genotypes across locations, while ICGV-SM 16574 and ICGV-SM 15559 were specifically adapted to Chambezi and Naliendele, respectively

- The Naliendele site was the most ideal location for groundnut evaluation and genotype differentiation

Combining ability and gene action controlling rust resistance and agronomic traits in groundnut (*Arachis hypogaea* L.)

This study determined the combining ability effects and gene action controlling rust resistance and agronomic traits in groundnut. Twelve selected parental lines were crossed in a diallel to generate F2 progenies. Thirty-three successful F_2 progenies and the parents were evaluated in the field using a 9 × 5 alpha lattice design with two replications over two seasons in Tanzania.

The main findings of the study were:

- The tested genotypes exhibited significant ($P<0.05$) variation for rust resistance, yield and yield-related traits
- There existed significant ($P<0.05$) difference on the general combining ability (GCA) effect and the specific combining ability (SCA) effect for the assessed traits indicating that both additive and non-additive gene effects conditioned trait inheritance.
- The Bakers' ratios accounted for non-additive gene effects predominantly controlling rust resistance and yield components.
- Genotypes ICGV-SM 05570 and ICGV-SM 15567 were the best general combiners for rust resistance and grain yield.
- The crosses ICGV-SM 16589 × Narinut and ICGV-SM 15559 × ICGV-SM 15557 were identified as the best specific combiners for rust resistance with moderate yield levels and medium maturity.

Implications of the study to breeding groundnut for rust resistance and higher yield

- The PRA study showed that farmers preferred to grow groundnut varieties with high yield, resistance to diseases and pests, early maturity, and drought tolerance with quality attributes. Also, medium grain size, high oil content, and tan or red seed color were other farmer and market preferred traits. Hence groundnut researchers could use the identified farmer-preferred traits as selection criteria in their breeding program to enhance groundnut production in Tanzania.
- There is considerable genetic variation for groundnut yield and yield component traits. The SSR markers were able to deduce genetic variation among groundnut genotypes.

The largest proportion of variation was attributed to individual differences, which is essential for improving rust resistance by crossing individuals from divergent clusters.

- The study identified genotypes with high kernel yield and average stability which could be useful genetic resources for groundnut improvement in Tanzania.
- The inheritance of rust resistance is conditioned by dominance gene action, while kernel yield was controlled by additive gene action suggesting that breeding gain can be realized through hybridization and targeted selection.
- In general, the study identified valuable groundnut families with high combining ability for rust resistance and kernel yield, which are recommended for genetic advancement and to identify transgressive segregants and develop pure lines for cultivar release and deployment in Tanzania.