GENETIC ANALYSIS AND IMPROVEMENT OF GROUNDNUT (*Arachis hypogaea* L.) FOR DROUGHT TOLERANCE AND SEED YIELD IN MALAWI

By

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ABSTRACT

Groundnut (*Arachis hypogaea* L,) is one of the major sources of food and income for smallholder farmers in Malawi. It is a valuable food security crop that supplies fats and proteins to the predominantly maize–based Malawian diet. Although, groundnut production is a profitable venture for smallholder farmers in Malawi, its productivity is low averaging 250 – 800 kg/ha as compared to a yield of about 4.0 t/ha obtained at research stations. The decline in productivity of groundnuts is due to several abiotic and biotic constraints that smallholder farmers encounter, among them drought due to inadequate and highly variable rainfall in the country. Information on response of different genotypes to drought stress and the explanation of these variabilities is an important requirement in breeding for drought tolerance improvement in groundnut. The main objectives of the study were: (i) to determine the effect of drought stress on the growth performance of groundnut genotypes with respect to morpho-physiological traits,(ii) to identify the relevant traits related to drought tolerance and their relationship to seed yield under drought stress conditions, (iii) to estimate the relative importance of additive and non-additive gene action in controlling the inheritance of drought tolerance traits under moisture stressed conditions and (iv) to investigate the genetic variation existing among genotypes in relation to morpho-physiological traits related to drought tolerance. Twenty-five genotypes from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Malawi were evaluated during 2016/17 under rainfed field condition at the drought-testing site of Ngabu Agricultural Research Station.

The results indicated high genotypic coefficient of variation (GCV) coupled with high genetic advance (GA), genetic advance as percent of mean (GAM) and heritability estimates for days to maturity (DM), seed yield (SY), relative water content (RWC), biomass (BM), number of filled pods (FP) and pod yield (PY). Seed yield was highly significant and positively correlated with shelling percentage (SHP), hundred seed weight (HSW), SPAD chlorophyll meter reading (SCMR), days to maturity (DM), biomass (BM), relative water content (RWC) and harvest index (HI). Furthermore, path analysis showed that harvest index, biomass, pod yield, shelling percentage, SPAD chlorophyll meter reading, relative water content and days to maturity had the highest direct and indirect effects on seed yield. General combining ability effects were significant for almost all studied traits indicating the importance of additive gene action. Specific combining ability effects were also significant for days to maturity, seed yield, biomass, harvest index, number of field pod and pod yield indicating importance of non-additive gene action controlling the inheritance of these traits. This suggests that both additive and non-additive gene action were important in controlling the majority of the traits. However, additive gene action was more predominant for all traits studied as it was evidenced by its significant (P<0.05) positive GCA effects coupled with high variance components as compared to its interactions. This also, was supported by a high Baker's ratio of close to unity (X>0.5) ranging from 0.78 to 0.96 for all measured traits. Among male parents, ICGV-SM 02724 and ICGV-SM 94139 were identified as good combiners, whereas among females, CG 7 and ICGV-SM 01721 were good combiners. These parents have outstanding breeding value as proven by their high and significant GCA effects. The crosses Pendo x Akwa, ICGV-SM 99555 x ICGV-SM 02724, ICGV – SM 99551 x Baka and ICGV-SM 01721 x ICGV-SM 94139 had significant SCA effects for seed yield, number of filled pod, harvest index and pod yield. The cross, Pendo x ICGV-SM 02724 was identified as potentially useful for developing early maturing varieties. These crosses could be used for further selection in breeding programmes for developing drought tolerant cultivars. Genotypes also showed different degrees of tolerance where seven genotypes with high yield, favourable adaptive traits and useful for breeding were selected. The principal component analysis under moisture stressed condition also showed that specific leaf area, days to maturity, biomass, number of filled pod, hundred seed weight and pod yield had more influence during selection. Based on the current results, breeding for drought tolerance for the material studied will be possible by focussing on relative water content, shelling percentage, number of filled pod, SPAD chlorophyll meter reading, pod yield and hundred seed weight as selection criteria, accompanied with extensive evaluation of the material under multi-located trials.

DECLARATION

I, **Masoud Salehe Sultan**, declare that,

1. The research reported in this thesis, except where otherwise indicated, is my original work.
2. This dissertation has not been submitted for any degree or examination at any university.
3. This dissertation does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.
4. This dissertation 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 rewritten or rephrased, but the general information attributed to them has been referenced.
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5. This dissertation does not contain text, graphic or tables copied from the internet unless specifically acknowledged and the source being detailed in the dissertation and in the reference sections.

Sign 10/08/2018

Masoud Salehe Sultan (Candidate) Date

This thesis has been submitted for examination with our approval as the candidate's supervisors.

Sign……… …………… …………15/08/2018……

Dr. Julia Sibiya (Principal supervisor) Date

Sign ………… …………22/8/2018………….

Dr. Alfred Odindo (Co-supervisor) Date

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ABBREVIATION

ANOVA	Analysis of Variance
ARET	Agricultural Research Extension Trust
BR	Baker's Ratio
BS-CGD	Broad Sense Coefficient of Genetic Determination
DF	Degree of Freedom
DMRT	Duncan Multiple Range Test
FAO	Food and Agriculture Organization
FC	Field Capacity
GA	Genetic Advance
GAM	Genetic Advance as percent per Mean
GCA	General Combining Ability
GCV	Genetic Coefficient of Variation
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
LMM	Linear Mixed Model
NCII	North Carolina Mating Design II
NS-CGD	Narrow Sense Coefficient of Genetic Determination
PCA	Principal Component Analysis
PCV	Phenotypic Coefficient of Variation
RCBD	Random Complete Block Design
RWC	Relative Water Content
SCA	Specific Combining Ability
SCMR	SPAD chlorophyll Meter Reading
SLA	Specific Leaf Area
SPSS	Statistical Package for the Social Sciences
TDR	Time Domain Reflectometry
WUE	Water Use Efficiency

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Chapter 1

INTRODUCTION TO DISSERTATION

1.1 Economic importance of groundnuts in Malawi

Groundnut (*Arachis hypogaea* L.) is both a source of food and income for smallholder farmers in Malawi. It is considered a valuable crop for improving food security by supplying potential nutrient value to the predominantly maize based Malawian diet (Makoka, 2008). Groundnut is considered as one of the country's key export crops and an important earner of foreign exchange. Currently it constitutes well over 25% of agricultural income among smallholder farmers (Derlagen and Phiri, 2012). Apart from its nutritional and cash value, groundnut enriches soil with nitrogen through biological nitrogen fixation, making it an important factor for soil improvement. The haulm and other crop extracts are used as livestock feed since they are rich in digestible crude protein; hence, it increases livestock productivity (Simtowe et al., 2012).

1.2 Production of groundnuts in Malawi

In Malawi, groundnut production is mostly by smallholder farmers who contribute to about 93% of total production (Sangole et al., 2010). It grows well in the mid altitudes and plateau areas with deep, well-drained sandy-loamy soils (Chiyembekeza et al., 1998). From 1990 to date, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in collaboration with the Malawi National Agriculture Research System (NARS) have released several varieties including CG 7, ICGV SM 90704 (Nsinjiro), JL 24 (Kakoma), ICG12991 (Baka), and ICGV SM 99568 (Chitala). Minde et al. (2008) reported that more than half of the country's production is comprised of improved varieties. According to Crop Statistics (2010/2011) in Malawi, legumes covered 27% of the cultivated area of which groundnuts occupied 31% of that total (Monyo and Gowda, 2014). This shows the relative importance of the crop to the farmers and the economy of the country.

1.3 Groundnut producing areas and agro ecological zones

Groundnut remains one of the cash and food crops grown by smallholder farmers in Malawi. It is produced in the entire country. However, over 70% of the crop is grown in the central region districts of Lilongwe, Kasungu, Ntchisi, Dowa, Mchinji, Dedza and Salima. It is grown as an intercrop or monocrop and is rotated with maize, sorghum, millet and other crops (Ngulube et al., 2001). Based on climatic conditions and altitudes of Malawi, groundnut is produced in mainly three agro-ecological zones, namely the plateau zone (mid altitude) which covers 900 to 1200 meter above sea level (masl), the lakeshore and the Shire Valley. The lakeshore and the Shire Valley are often classified as one lowland agro-ecological zone. Generally, groundnuts are grown from near sea level up to more than 1500 masl. However, the mid altitude produces more than 70% of the crop and contributes significantly to the economy of the country compared to the lowland agro-ecological zone.

1.4 Groundnuts production challenges in Malawi

Groundnuts production in Malawi has been increasing in a decreasing trend (Simtowe et al., 2012; Sangole et al., 2010). Although the trend shows that there has been an increase in area planted, yields of groundnuts per hectare are still low, averaging from 250 – 800 kg/ha (Figure 1-1) compared to the yield of about 4 t/ha obtained at research stations (Simtowe et al., 2012; Monyo and Gowda, 2014)

The decline in productivity of groundnuts is due to several constraints that smallholder farmers encounter. These include several abiotic and biotic factors. Major abiotic factors are drought, heat stress, low soil fertility (especially P and Ca) and poor agronomical practices (Akbar et al., 2017). Among the abiotic factors, drought due to inadequate and highly variable rainfall has been reported as the major causes of low groundnut productivity in the country (Minde et al., 2008; Simtowe, 2009). The biotic factors include diseases including groundnut rosette viruses, fungal foliar (rust, early and late leaf spot), aflatoxin contamination and pests (Kumwenda and Madola, 2005). Other factors are low adoption of improved varieties, social economic constraints that include lack of financial, processing, marketing and post-harvesting handling (Monyo and Gowda, 2014).

1.5 Groundnut production trends

In Malawi, groundnut is grown mostly by resource – poor farmers as a sole crop or as an intercrop with maize. It is referred to as a woman's crop since women form the majority of producers (Minde et al., 2008). It is grown in the entire country; however, most of its production is concentrated on the central plains of Kasungu and Lilongwe, which accounts for more than half of total production (Monyo and Gowda, 2014).

Groundnut production area in Malawi has been increasing (Figure 1-1. Groundnut production trends in Malawi (2004 -2014)., while the yield has remained low, about 1/3 of the potential yield per hectare (Longwe – Ngwira et al., 2012). The decline in groundnut yield has been attributed to low, unreliable rainfall often with mid and terminal drought. Terminal drought on groundnut results in yield reduction, high incidence of *Aspergillus flavus* colonization, high aflatoxin contamination and low seed quality (Girdthai et al., 2010; Aninbon et al., 2015). In addition, mid-season drought is of major concern as it occurs at the time of flowering and pod formation, which consequently reduces the yield significantly. Its productivity has also been affected by pests and diseases, especially rosette, which in drought years is more prevalent and yield losses can be as high as 100% depending on the stage of infection (Minde et al., 2008).

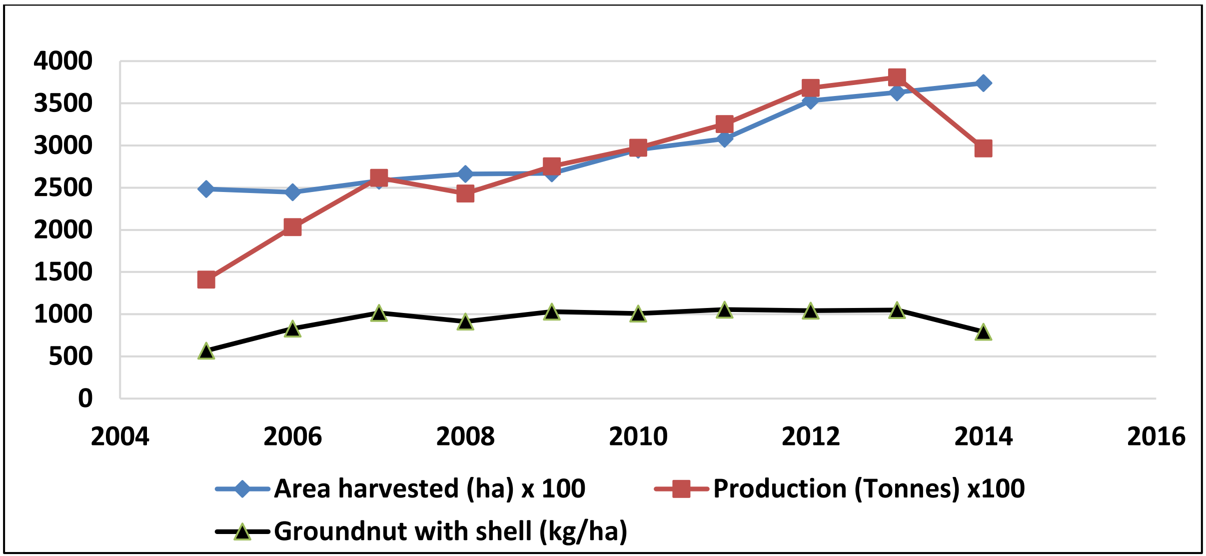

Figure 1-1. Groundnut production trends in Malawi (2004 -2014). Source: FAOSTAT, (2015).

Attempts have been made by the International Crops Research Institute for the Semi – Arid Tropics (ICRISAT) to address these constraints by introducing new breeding technologies. Several high yielding varieties under no stress or with tolerance/resistance to a single factor have been developed and adopted by farmers. These include JL-24, Baka, CG7, Chalimbana and Nsinjiro. Variety JL-24 is a short duration, which has the avoidance mechanism for end-season drought. However, the variety is less preferred by farmers and the groundnut industry, because of its low yield and susceptibility to diseases (Minde et al., 2008). Due to unpredictable rainfall and drought events, there is a need to develop tolerant cultivars that will save the livelihood of smallholder farmers in semi-arid regions. However, genetic information regarding tolerance to drought stress and related traits is limiting.

Selection approaches based on yield under drought conditions have been slow and ineffective because of their time-consuming nature and lack of repeatability across the environments (Girdthai et al., 2010; Nigam, 2014). Therefore, understanding the genetic mechanisms based on morpho – physiological adaptive traits for drought tolerance is important for genetic enhancement of groundnut and will aid in the development of new varieties with drought tolerance.

1.6 Problem statement and justification

Recently, yield trends of groundnuts in Malawi have revealed a yield gap of 53% between the national average and the realizable productivity at research stations (Monyo and Gowda, 2014). The yield gap is mainly attributed to low and unreliable rainfall, which smallholder farmers encounter. Irrigation can considerably increase groundnut productivity and stabilize yields in areas prone to drought. However, the irrigated land in Malawi comprises only 0.6% of the total arable land, which is too small to make significant increase in production (Minde et al., 2008). Therefore, developing groundnuts varieties enhanced with drought tolerance stress would add up to the strategies toward improvement of the livelihood of the farmers. It is suggested that genetic improvement of groundnut under drought stress is an appropriate approach, however, its genetic mechanisms is less known. Therefore, this study seeks to understand the genetic mechanism underlying the tolerance of groundnut to drought in order to enhance breeding progress on crop improvement programmes.

1.7 General objective

The overall goal of the research is to contribute to the improvement of groundnut production in Malawi through identifying genotypes with high tolerance to drought stress and generation of information, which is useful in groundnut drought breeding programmes.

1.7.1 Specific objectives

The specific objectives of this investigation were;

i. To determine the effect of drought stress on the growth performance of groundnut with respect to morpho-physiological traits.
ii. To identify the relevant traits related to drought tolerance and their relationship to seed yield under drought stress conditions.
iii. To estimate the relative importance of additive and non-additive gene action in controlling the inheritance of drought tolerance traits under moisture stressed environment.
iv. To investigate the genetic variation existing among genotypes in relation to morpho-physiological traits related to drought tolerance.

The general and specific objectives are explained in detail for every experiment in the pertinent chapters.

1.7.2 Dissertation outline

The dissertation is structured in the form of separate research chapters, each following the format of a stand-alone research paper. This is the main dissertation format accepted by the University of KwaZulu-Natal. Therefore, there is some inevitable repetition of references and some introductory information between the chapters. The benefit of this format is to simplify publication of research papers since each paper stands as a research article. The outline of the dissertation is presented in Table 1-1.

Table 1-1. The outline of the dissertation

Chapter	Title
1	Dissertation introduction
2	Literature review
3	Early generation evaluation of groundnut (*Arachis hypogaea* L.) crosses for morpho-physiological and seed yield attributes under moisture stressed environment
4	Genetic variability, correlation and path coefficient analysis for drought tolerance improvement in groundnut (*Arachis hypogaea* L.) genotypes
5	Genetic components and combining ability analyses for drought tolerance and associated traits among Malawian groundnut (*Arachis hypogaea* L.) genotypes
6	General overview of the study and implication to plant breeding

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Chapter 2

LITERATURE REVIEW

This chapter provides a review of topics relevant to the study. The following aspects have been reviewed; (1) the origin, distribution and botanical description of groundnut, (2) the effects of drought on groundnut growth and performance as well as (3) combining ability, mating designs, heritability, correlation and path coefficient analysis with particular emphasis on the application of combining ability in groundnut breeding programmes.

2.1 Origin and botanical description

Groundnut (*Arachis hypogaea* L.) originated from Latin America and was introduced to Africa from Brazil by the Portuguese in the 16th century (Adinya et al., 2010). Groundnut is an annual herbaceous leguminous plant growing to a height of 30 to 50 cm. Its leaves are opposite and pinnate with four leaflets (two opposite pairs; no terminal leaflet) and each leaflet is 1 to 7 cm long and 1 to 3 cm across. Like many other legumes, the leaves are nyctinastic (sleep movements), closing at night. The flowers are 1.0 to 1.5 cm across and yellowish orange with reddish veining. Its ovary is not positioned as expected, instead a short stalk at the base of the ovary (termed a pedicel) elongates to form a thread-like structure known as a peg. The peg pushes the ovary down into the soil, where it develops into a mature peanut pod. Pods are 3 to 7 cm long, normally containing one to four seeds (Putnum, 1991).

2.2 Effect of drought stress on growth and yield performance of groundnut

The effects of drought stress are expressed in various morphological, physiological, biochemical and genetic changes on plants. The stress affects different aspects of plant growth and development and finally crop yields. The severity of the drought damage depends on the duration of this stress and varies with growth stages of the crop (Nigam, 2014). The effects of drought on growth parameters and yields of groundnut crops reviewed in this literature, only focus on the traits under study.

2.2.1 Effect of drought on relative water content (RWC)

Groundnut is a relatively drought tolerant crop having improved water-use efficiency mechanisms that allow it to withstand water stress for a certain period (Nautiyal et al., 2002). However, in drought years it suffers leading to significant yield reduction. One of the early responses of drought stress is the decrease of RWC, which is considered as the best physiological measure of plant water status (Sanchez et al., 2010). Sinclair and Ludlow (1985) argued that RWC is a more useful integrator of plant water balance than leaf water potential and should provide universal relationship between physiological traits and level of drought stress. Obviously, stressed plants have lower RWC than non-stressed plants. Relative water content of non-stressed plants ranges from 85 to 98%, while in drought stressed plants it may be as low as 30% (Prabowo and Wright, 1990).

Babu and Rao (1983) examined drought stress effects on groundnut over 35 days from 20 to 55 days after sowing. The relative water content ranged between 100 and 87% on the first day of stress imposition. At the end of 35-days dry period, the plants wilted and the lowest relative water content recorded was 29.70%. Shinde et al. (2010) recorded maximum RWC percentage under well-watered conditions in all varieties while at higher water stress level, the variety TG-26 and TG-24 showed maximum reduction in RWC of 19.11% and 9.72%, respectively, over the control. Related findings by Aninbon et al. (2015) and Koolachart et al. (2013), reported significant differences between non-stressed and stressed plants, with the non-stressed treatment having higher RWC compared to stress treatment. Therefore, RWC is a useful selection tool in breeding programmes, which can be used to identify cultivars with high water content under drought conditions.

2.2.2 Effect of drought on SPAD Chlorophyll Meter Reading (SCMR)

A SPAD chlorophyll meter reading provides a useful tool to screen for genotypic variation in potential photosynthetic capacity under drought conditions (Nageswara et al., 2001; Songsri et al., 2008). It is among the surrogate traits that can be used to achieve more effective and rapid progress in selection for drought tolerance (Nigam et al., 2005). Several studies have drawn different conclusions on SPAD Chlorophyll meter readings. For example, Reddy and Rao (1968) reported that severe drought stress decreased the levels of chlorophyll *a, b* and total chlorophyll. However, Jongrungklang et al. (2008) reported an increase in chlorophyll content under drought stress. A similar study by Painawadee et al. (2009) found that drought increased SPAD chlorophyll meter readings but in addition to that, there was no significant difference in SCMR between water regimes.

Nageswara et al. (2001) found that there were significant interrelationships among specific leaf area (SLA), specific leaf nitrogen (SLN) and SCMR and they suggested that SCMR can be used as a reliable and rapid measure to identify genotypes with low SLA or high SLN under drought conditions in groundnut breeding programmes. In addition, SCMR has been suggested as a simple and useful selection criterion for drought tolerance in groundnut as it has high heritability (Songsri et al., 2008). Therefore, the use of SCMR can provide an opportunity for selection of genotypes with drought stress tolerance. However, it is suggested that SCMR can be recorded at any time after 60 days of the crop growth, preferably under moisture deficit conditions (Nigam, 2014). Serraj et al. (2004) added that measurements for

SCMR should be recorded after imposition of moisture stress and particularly at mid-way through stress.

2.2.3 Effect of drought on specific leaf area (SLA)

Specific leaf area is a reflection of leaf thickness and it is defined as the ratio of leaf area to the leaf dry weight. Specific leaf area (SLA) is one of the mostly used and widely accepted key leaf characteristics considered in the study of leaf traits (Hoffman et al., 2005). Drought stress has varying effects on specific leaf area but low SLA is preferable since it indicates high drought tolerance. It is suggested that peanut genotypes with low SLA have more photosynthetic machinery per unit area and hence potential for greater assimilation under drought. Painawadee et al. (2009) found that drought significantly reduced SLA, and groundnut genotypes were also significantly different in SLA at all water regimes. The decrease in leaf surface area indicates that the plant reduces ways for water to be lost through transpiration. Girdthai et al. (2012) demonstrated that low SLA indicates thicker leaves. The thicker leaves represent high dry matter content stored in the leaves since they have greater photosynthetic capacity compared with thinner leaves. The genotypes with low specific leaf area values are considered to be important for conservation of acquired resources. However, Nageswara et al. (2001) suggested that if SLA has to be used as a screening tool for drought, then sampling should be undertaken on clear and full sunlight days. Under high radiation conditions, a variation in SLA is largely governed by photosynthetic capacity. Therefore, the use of SLA as an economically surrogate trait for identification of genotypes with drought tolerance is important in breeding programmes.

2.2.4 Effect of drought on root growth traits

Root traits associated with drought tolerance are important in identifying drought resistant mechanisms of plants (Koolachart et al., 2013). It has been reported that root response to drought is another mechanism enhancing drought resistance (Ludlow and Muchow, 1990; Taiz and Zeiger, 2006). Root traits such as deep rooting, root length and distribution have been identified as drought adaptive traits that can be used as selection criteria for drought resistance (Matsui and Singh, 2003; Taiz and Zeiger, 2006). Peanut genotypes with higher root length density in the deeper soil layers potentially have an enhanced drought tolerance and this could aid peanut genotypes to obtain higher pod yield under long-term drought conditions (Songsri et al., 2008).

Previous, studies have been reported on the response of roots at both mid-season drought (Jongrungklang et al., 2011) and end-season drought (Songsri et al., 2008). They found that varieties with low relative water content (RWC) tend to have higher root density weight (RDW) indicating that drought stress induces root production. Girdthai et al. (2010) observed significant differences in drought tolerance of groundnut genotypes at end-season drought, which was due to the differences in root responses. Painawadee et al. (2009) studied root traits but there were no significant differences in all traits except root density (RD) and they concluded that the lack of variation in root traits might be due to limitation of root growth due to confinement of roots in the pots. Jongrungklang et al. (2011) reported that root length (RL) and root density (RD) might be the only two of several factors contributing to high pod yield under drought conditions. Therefore, the groundnut genotypes that have higher root length density in the deeper soil layers potentially have an enhanced drought tolerance and this can help peanut genotypes to obtain higher pod yield under drought conditions.

2.2.5 Effect of drought on flowering of groundnut

The start of flowering is not affected by drought stress (Boote and Ketring, 1990). The rate of flower production is reduced by drought stress during flowering but the total number of flowers per plant is not affected due to an increase in the duration of flowering (Janamatti et al., 1986; Meisner and Karnok, 1992). A significant burst in flowering on alleviation of stress is a unique feature in the pattern of flowering under moisture stress, particularly when drought is imposed just prior to reproductive development (Janamatti et al., 1986). When stress is imposed during 30 – 45 days after sowing, the first flush of flowers produced up to 45 days do not form pegs during that time. However, flowers produced after re-watering compensate for this loss (Gowda and Hegde, 1986).

2.2.6 Effect of drought on pod formation

Groundnut experience water stress during pegging and pod development stage resulting in a drastic reduction of yield. However, the magnitude of the reduction depends on groundnut genotypes. The effect is not only on the yield, but also on the quality of groundnut products decreases under drought stress (Rucker et al., 1995). It has been reported that under water stress, pegging and seed set response of various groundnut genotypes varies substantially, leading to a reduction in pod yield (Nageswara et al., 2001).

Number of pods per plant is the most susceptible parameter affected by drought stress. The effect of drought stress on the pod yield of three bean cultivars showed that stress at flowering stage reduced the number of pods per plant and seeds per pod in all the three varieties (Fienebaum et al., 1991). Karimian et al. (2015) reported that with increasing drought stress to 50% and 70% of the field capacity, the number of pods per plant reduced by 14.09% and 23.72%, respectively. In addition, immature or undeveloped pods per plant increased under water stress during pod development stage (Patel and Golakiya, 1988). The response of groundnut to drought stress is an important trait that should be incorporated in breeding schemes. Therefore, selection for more number of mature pods per plant can help in breeding for drought tolerance in groundnut.

2.2.7 Effect of drought on pod yield

Selection of groundnut for drought tolerance is usually based on biomass production and pod yield under water stress conditions (Duarte et al., 2013; Santos et al., 2010). Groundnut stressed plants lose moisture from pods, leading to a reduction in physiological activities of the seeds, and finally affecting both seed yield and nutritional quality (Songsri et al., 2008). Recent studies have reported a decrease in pod yield when groundnut is subjected to moisture stress (Boontang et al., 2010; Koolachart et al., 2013; Pereira et al., 2015). Ravindra et al. (1990) also reported that pod yield was significantly reduced during drought stress at pod development stage. Patel and Golakiya (1988) agreed that yield reduction was higher when stress was imposed between pegging and pod development stages and lowest when drought stress was imposed from pod development to maturation. This indicates that the period from pegging to pod development phase is the most sensitive stage to moisture stress. Therefore, the presence of genotypic differences under drought conditions is essential for improvement of drought tolerance in this crop.

2.2.8 Effect of drought on hundred seed weight

Crop improvement strategies under drought stress have recognized 100 seed weight as a selection tool under drought conditions. The results have shown that the weight of 100 seeds in groundnut is reduced due to drought stress (Janamatti et al., 1986). Gowda and Hegde (1986) reported that 100 seed weight was not affected by moisture stress at early growth stages, but was greatly reduced under moisture stress at pod development stage (Vanangamudi et al., 1987) and at seed development stage (Yao et al., 1982). Hundred-seed weight was greater in the irrigated crop than in the rainfed under rainy season conditions (Padma and Subba Rao, 1992). Karimian et al. (2015) showed that with increasing drought stress to 50% and 70% of field capacity, 100 seed weight decreased by 11.24% and 22.22%, respectively. Water deficit in the root zone during pegging was reported to decrease pod and seed growth during drought stress by approximately 30% and to decrease weight per seed from 563 to 428 mg (Sexton et al., 1997). In addition, Boote and Ketring, (1990) reported that pod and seed development are progressively affected by drought stress due to insufficiency of plant turgor and lack of assimilates. Therefore, these situations have an impact on the final weight of the seeds and the result is reduction of the 100 seed weight.

2.2.9 Effect of drought on shelling percentage

Shelling percentage is among the traits that are affected when groundnut encounters drought stress conditions. Reddy (1978) and Pallas et al. (1977) reported a decrease in shelling percentage and increase of the proportion number of unfilled pods when groundnut crop was subjected to moisture stress. An experiment performed by Rasve et al. (1983) reported that, shelling percentage increased to 71.90% with the application of 540 mm of water at 10 day-interval. These findings are similar to those of Saini and Sunder (1973) who reported an increase in shelling percentage after the application of two irrigations, one at flowering and the second at fruiting compared to no irrigation. Golakiya and Patel (1992) reported that the decrease in shelling percentage was maximum under stress during pod development stage. In addition, Janamatti et al. (1986) reported that shelling percentage was reduced by moisture stress during seed development. Since shelling percentage is usually lesser under moisture-stress conditions than under normal conditions, genotypes with relatively high shelling percentage under drought conditions can be considered as drought tolerant. Therefore, selection based on this trait adds some improvement in groundnut breeding programmes for drought tolerance.

2.2.10 Effect of drought on harvest index (HI), drought tolerance index (DTI) and dry matter production

Harvest index (HI) has been identified as a drought resistant trait in groundnut (Nigam et al., 2005). The high harvest index of peanut genotypes under drought conditions is important for sustaining pod yield under drought conditions. Researchers have reported a decrease in biomass and pod yield when groundnut is subjected to terminal drought (Boontang et al., 2010; Girdthai et al., 2010). Painawadee et al. (2009) found a significant difference among groundnut genotypes for biomass, pod yield and HI under drought stress and non-drought stress conditions. Drought significantly reduced biomass by 29%, pod yield by 42% and HI by 18%. Koolachart et al. (2013) reported a significant reduction in harvest index under drought conditions and the results were similar to those reported previously by Nautiyal et al. (2002).

High pod yield under drought stress is another trait to consider during selection of drought tolerant materials. Previous studies have reported that Tifton 8 variety had low harvest index because of low pod yield under drought conditions (Songsri et al., 2008; Jongrungklang et al., 2011). Painawadee et al. (2009) reported that ICGV 98348 had the highest drought tolerance index (DTI) for pod yield, biomass and HI because of low reduction in pod yield, biomass and HI under terminal drought. Songsri et al. (2008) suggested that high yield under non-stress conditions may be important for high yield under drought stress conditions in some genotypes. Therefore, selection of groundnut genotypes with high pod yield both under drought stressed conditions and non-stressed conditions may increase breeding gains in drought tolerance improvement programmes.

2.3 Moisture sensitive stages and screening for drought tolerance in groundnuts

Understanding of critical moisture sensitive development stages in groundnut is very important in screening for drought tolerance. The pre-flowering phase is less sensitive to moisture stress than the flowering phase. Naveen et al. (1992) found that water stress imposed during the flowering and pegging stages of variety JL-24 produced the greatest reductions in pod yield under water stresses at the early and late pod stages.

Screening germplasm for drought tolerance can be done in dry environment or in a glasshouse by withholding water. Nigam (2014) suggested the following; for early-season drought, withholding water for 40 days after planting (DAP) followed by normal watering; for mid-season drought, withholding water after 40 DAP up to 80 DAP then followed by normal watering; for end-of-season drought, withholding water from 80 DAP onward till maturity.

2.4 Correlation, path coefficient analysis and heritability

2.4.1 Concept of correlation and path coefficient analysis

Correlation analysis is a biometrical technique, which explains the nature and the extent of relationship between various morpho-physiological traits; while path analysis partitions the correlation coefficient into direct and indirect effect in order to measure the relative importance of each explanatory (independent) trait to a dependent trait such as seed yield (Babariya and Dobariya, 2012). Direct or indirect effects of yield-related traits in peanut selection has gained importance as demonstrated by several studies (Kotzamanidis et al., 2006; Sharma and Dashora, 2009; Seyyed and Seyyed Ali, 2012; Shoba et al., 2012).

2.4.2 Correlation analysis of seed yield and its component traits

Genetic association plays a significant role in the study of interrelationships and relative contribution of different traits towards crop improvement. Simple correlation coefficient between yield and yield components of groundnut showed that seed yield was significantly and positively correlated with number of pods per plant, pod yield per plant, shelling percentage and hundred seed weight (Korat et al., 2010; Shoba et al., 2012). Painawadee et al. (2009) indicated that pod and seed yields showed significant positive association with number of mature pods per plant, plant height and hundred-seed weight. In addition, Manoharan et al. (1990) reported that pod yield was positively correlated with harvest index. Shoba et al. (2012) concluded that for seed yield per plant improvement in peanut, selection has to consider the number of pods, pod yield per plant, 100-seed weight and shelling percentage.

Correlations among physiological traits have been used to identify drought tolerant genotypes in breeding programmes (Wright et al., 1994; Nigam and Aruna, 2008). Significant correlations between SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) with other physiological traits for drought tolerance, such as harvest index, and transpiration efficiency, have been observed over a wide range of environments (Sheshshayee et al., 2006; Arunyanark et al., 2008; Nigam and Aruna, 2008). The SLA was associated with variation in photosynthetic capacity and chlorophyll density expressed as SCMR (Wright et al., 1994; Nageswara Rao et al., 2001). In addition, relative water content (RWC) was reported to be positive and significantly correlated with pod yield (Ravindra et al., 1990).

2.4.3 Path coefficient analysis on direct and indirect effects

Direct and indirect effects from path analysis have demonstrated different results. Korat et al. (2010) revealed that seed yield was positively associated with 100-seed weight and shelling percentage, while days to maturity had negative association with seed yield. Path coefficient analysis reported by Mane et al. (2008) indicated that seed yield had the highest direct effect on 100-seed weight followed by number of pods per plant and number of seeds per pod. While number of pods per plant, number of seeds per pods and shelling percentage had indirect effects on seed yield through 100-seed weight. Parameshwarappa et al. (2008) found that the number of pods and weight had higher positive direct effect on seed yield. Sharma and Dashora (2009) showed that 100-seed weight had positive direct effect on seed yield.

Seyyed and Seyyed Ali (2012) reported that total number of seeds per plant, hundred seed weight and number of pods per plant had direct effects on seed yield under non-stressed conditions. Bera and Das (2000), Sumathi and Muralidharan (2007), and Mane et al. (2008), also reported similar results. In drought conditions, Seyyed and Seyyed Ali (2012) demonstrated that 100-seed weight, number of seed per plant and biomass had highest positive and direct effect on seed yield. John et al. (2007), Painawadee et al. (2009) and Raut et al. (2010) showed similar results, although number of pods, biomass and hundred seed weight showed a direct effect, but indirect effect of these traits was high and positive in both non-drought and drought conditions. In addition, Shoba et al. (2012) reported that plant height, hundred seed weight and shelling percentage had positive indirect effect on seed yield.

2.4.4 Heritability, genotypic and phenotypic coefficient of variations in groundnut

Researchers have identified a number of traits that will help breeders to develop and identify moisture stress tolerant genotypes with high yield potential. However, the degree of success depends on the magnitude of heritability as it determines the relative heritable portion of variation. John et al. (2012) argued that to achieve the possible improvement in selection, heritability should be used along with genetic coefficient of variations and genetic advance.

Under both water stress and non-stress conditions, broad sense heritability for pod yield were reported as high by Reddy et al. (1987) and Chavan et al. (1992), moderate by Bansal et al. (1992) and Ali et al. (1996) and low by Manoharan et al. (1993). Reddy and Gupta (1992) reported high heritability and genetic advance (GA) for harvest index (HI) in three treatments, namely, entire rainfed, rainfed supplemented with irrigation and irrigated at ten day-interval. Songsri et al. (2008) reported broad sense heritability estimates ranging from 0.73 to 0.96 for biomass, 0.93 to 0.97 for pod yield and 0.54 to 0.93 for DTI of biomass under drought and non-drought conditions. John et al. (2008) reported high heritability, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) estimates for pod yield per plant, mature pod per plant, haulms yield per plant, seed yield per plant and harvest index among F2 population of single crosses both under water stress and non-stress conditions. Similar results were reported by Kumar and Rajamani (2004) who observed high GCV and PCV for seed yield, hundred seed weight, moderate PCV and GCV for shelling percentage and low values for days to maturity.

Studies have recognized the contribution of physiological traits in groundnut under both non-stressed and stressed conditions for peanut improvement (Songsri et al., 2008; Girdthai et al., 2010; Arunyanark et al., 2012). Songsri et al. (2008) reported heritability estimates ranging from 0.89 to 0.97 for harvest index (HI), 0.81 to 0.95 for specific leaf area (SLA), and 0.89 to 0.97 for SPAD chlorophyll meter reading (SCMR) under non-drought and drought conditions. Girdthai et al. (2012) observed high heritability estimates for HI, SLA and SCMR ranging from 0.55 to 0.85, 0.72 to 0.91, and 0.61 to 0.90, respectively. In addition, SLA and SCMR have been shown as traits with high stability across the environments (Nageswara et al., 2001; Arunyanark et al., 2008; Girdthai et al., 2010). Girdthai et al. (2012) suggested that because of stability and high heritability and good correlation with pod yield, SLA and SCMR are the best selection criteria for drought tolerance in groundnut. Therefore, the estimates of genetic variation, heritability and genetic advance are critical values, which can help in making decisions about the selection methods to be used in order to identify drought tolerant and superior genotypes.

2.5 Combining ability and mating designs

2.5.1 The concept of combining ability

Identification of the best performing lines to use as parents in future crosses is a basic requirement in any crop breeding programme (Oakey et al., 2006). Combining ability by definition is the ability of the parents to combine among each other during hybridization process, such that the desirable traits are transferred to their progenies (Fasahat et al., 2016). There are two types of combining ability; general combining ability (GCA) and specific combining ability (SCA). According to Sprague and Tatum (1942), GCA describes the average performance of a line in different hybrid combinations, and SCA represents the deviation of an individual cross from the expected performance based on the average performance of the different lines. Parents with high average combining ability are termed to have good GCA or good SCA if their potential to combine well is specifically to a certain cross. A high GCA estimate indicates high heritability, less environmental effects or may also result from less gene interactions and thus high achievement in selection (Chigeza et al., 2014). Statistically, the GCA is a main effect and the SCA is an interaction effect (Kulembeka et al., 2012). Furthermore, GCA is associated with genes, which are additive in effects; while SCA is attributed to non-additive gene effects caused by dominance and epistasis.

2.5.2 Importance of combining ability

Combining ability is generally considered as an efficient method, which evaluates parental lines for their usefulness and has been used to identify the best parental combinations for hybridization (Sibiya et al., 2012; Sing et al., 2014; Ai et al., 2015). Combining ability analysis is an effective tool used in selection of parents based on performance of their progenies, usually the F1 but it has also been used in F2, F3 and later to F-infinite (Fasahat et al., 2016). An advantage of GCA and SCA is that they help to make important decisions in plant breeding. When GCA is significant over SCA, early generation evaluation becomes more efficient and promising genotypes can be recognized and selected based on their prediction from GCA effects (Smith et al., 2008; John et al., 2012). The relative performance of later generations of crosses can be predicted by using GCA of parental genotypes in an early generation evaluation, since the GCA is governed by heritable genetic material, which can be transmitted from parents to the offspring (Lv et al., 2012). However, when SCA is more important than the GCA component, selection has to be undertaken in later generations when homozygous lines are already fixed (Makumbi et al., 2011; Chigeza et al., 2014; Ertiro et al., 2017). Therefore, the use of combining ability analysis makes cultivar improvement more effective and less costly because of less time taken to release varieties and fewer materials being carried forward in breeding programmes.

2.5.3 Mating designs and estimation of GCA and SCA

Several mating designs have been used to estimate the effects of combining ability. They include, top cross developed by Jenkins and Brunaon (1932), polycross by Tysdal et al. (1948), diallel cross by Griffing (1956), line x tester by Kempthorne (1957), partial diallel cross by Kempthorne and Curnow (1961), North Carolina design by Comstock and Robinson (1948), and triallel cross by Rawlings and Cockerham (1962). According to Fasahat et al. (2016), the most used methods are diallel, NCD II and line x tester. However, this review is limited to NCD II, which is important in this study compared to other methods. The NCD II is a factorial design that measures the variance of the males and females' main effects and male x female interaction effects (Comstock and Robinson, 1952). The North Carolina Design II has been frequently used in breeding programmes to measure combining ability (GCA and SCA) and to estimate genetic variance components and heritability (Kaya and Atakisi, 2004; Bosworth and Waldbieser, 2014; Singh et al., 2016). In addition, the design has been applied in plant breeding for selection of testcross performance (Makanda et al., 2010; Qu et al., 2012; Derera et al., 2014).

2.5.4 Advantages of NCD II over Diallel and Line x Tester mating designs

North Carolina design II design has been reported to be similar to L x T design due to its ability to measure the variance of male and female main effects and male x female interaction effects (Comstock and Robinson, 1952). In addition, the male and female main effects, and the male x female interaction effects in NCD II mating design are equivalent to the GCA and the SCA effects in a diallel (Hallauer and Miranda, 1988). However, Fasahat et al. (2016) pointed that the main difference between a diallel and NCD II is that there are two independent estimates for the GCA effects in the NCD II, which is an advantage of the NCD II over the diallel. The two independent estimates of GCA allow determination of maternal effects and calculation of heritability based on male variance, which is free from maternal effects. According to Hallauer (2007), NCD II accommodates more parents and produces fewer crosses compared to the diallel as one can divide the parents into sets. Therefore, this enables a large number of parents to be evaluated. In addition, Hallauer et al. (2010) suggested that NCD II is more adapted to plants with multiple flowers but also is more applicable to self-pollinated crops.

2.5.5 Application of combining ability in peanut breeding programme

Knowledge of the type of gene action involved in the expression of yield and yield component traits helps in selection for desirable genes. Savithramma et al. (2010) reported the importance of additive gene effects in groundnut for days to maturity. However, John et al. (2014) found that the variances due to SCA were greater than that of GCA for all traits under drought conditions, indicating predominance of non-additive gene action for these traits. In addition, Azad et al. (2014) reported predominance of non-additive effects for inheritance of pod yield and related traits in groundnut. John et al. (2011) observed high SCA for SPAD chlorophyll meter readings, indicating the role of inter-allelic interaction in phenotypic expression of the traits. Similar findings by Venkateswarlu et al. (2007) also reported non-additive gene action for SCMR in segregating generations of groundnuts. However, combining ability is not only a method of understanding the genetic nature of quantitative and qualitative traits, but it also provides essential information regarding selection of parents to use in breeding programmes. Therefore, its use in breeding programmes provides useful information for planning suitable breeding approaches.

2.6 Conclusion

The literature review revealed that both midseason and terminal drought stress seriously affect growth performance of groundnut resulting in low yields. The drought effects can have more impact on smallholder farmers who heavily depend on rain-fed agriculture especially in semi-arid and arid areas. Although drought stress affects plant growth and yield, groundnut crop has shown differences in response to midseason and terminal stress among the genotypes. The existence of variations among genotypes in response to drought stress indicates that some genotypes may be tolerant than others. Therefore, it is possible that drought tolerant genotypes can be identified through selection if there are considerable variations existing among population. However, the progress in breeding for polygenically controlled and environmentally influenced traits like drought tolerance is largely determined by the nature and magnitude of their genotypic variability. Therefore, understanding of genetic systems underlying drought tolerance in groundnut will provide insights towards genetic improvement through selection.

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Chapter 3

EARLY GENERATION EVALUATION OF GROUNDNUT (*Arachis hypogaea L.*) CROSSES FOR MORPHO-PHYSIOLOGICAL AND SEED YIELD ATTRIBUTES UNDER MOISTURE STRESS ENVIRONMENT

ABSTRACT

Although groundnut production is considered a profitable venture, farmers in African countries, such as Malawi where it is grown on a small-scale with less application of modern technologies are experiencing a sharp decline in yield. The decline in productivity is caused by several factors including drought due to inadequate and highly variable rainfall. Therefore, developing groundnut cultivars with enhanced drought tolerance would help to improve the livelihoods of the farmers. The current study aimed at determining the level of drought tolerance among segregating populations using agronomical and physiological traits. Twenty-five genotypes from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were evaluated in a randomized complete block design with four replications during the 2016/17 main season under natural water stressed growing condition. Data were collected for variables (traits) included grain yield (GY), hundred seed weight (HSW), shelling percentage (SHP), SPAD chlorophyll meter reading (SCMR), days to maturity (DM), specific leaf area (SLA) and relative water content (RWC). The analysis of variances showed highly significant ($P<0.001$) differences among all the genotypes evaluated. Genotypes showed different degrees of drought tolerance and six genotypes with high yield and favourable adaptive traits for breeding were selected. These are ICGX-SM 14081, ICGX-SM 14101, ICGX-SM 14073, ICGX-SM 14098, ICGX-SM 14075 and ICGX-SM 14078. The principal component analysis under moisture stressed condition also showed that specific leaf area, days to maturity, biomass, number of filled pod, hundred seed weight and pod yield had more influence during selection. Therefore, these traits could be utilized to identify genotypes with relative high level of drought tolerance.

Key words: drought tolerance, groundnut, morpho-physiological traits, yield components.

3.1 Introduction

Groundnut (*Arachis hypogaea* L.) is known by many local names, including ntedza, karanga, peanut, earthnut, monkey-nut and goobers (Mangasini et al., 2014). It is the world's 13th most important food crop, 4th most important source of edible oil and 3rd most important source of vegetable protein (Taru et al., 2010). It is cultivated in more than 100 countries in tropical and warm temperate regions of the world (Nigam, 2014). Although, groundnut production is considered a profitable venture, the total world production has not increased much (Adinya et al., 2010; Taru et al., 2010). Groundnut is grown in many African countries such as Malawi where about 93% of the crop is produced at a small-scale level with less application of modern technologies, and thus farmers are experiencing a sharp decline in yield (Simtowe et al., 2010; Derlagen and Phiri, 2012).

Although the trend shows that there has been an increase in area planted, yields for groundnuts per hectare are still low averaging from 250 – 800 kg/ha compared to the yield of about 4 tons/ha obtained at research stations (Monyo and Gowda, 2014). The low yield of groundnuts is due to several abiotic and biotic constraints that smallholder farmers encounter. Among the abiotic factors, drought due to inadequate and highly variable rainfall has been reported as the major causing factor of low groundnut productivity in the country (Minde et al., 2008; Simtowe et al., 2009). The use of irrigation might have considerable contribution in increasing and stabilize the yield in areas prone to drought. However, in Malawi the irrigated land comprises only 0.6% of the total arable land, which is too small to make significant increase in production (Minde et al., 2008). Therefore, developing groundnut cultivars enhanced with drought stress could be among of the approaches toward improving the livelihoods of the farmers. The information on response of different genotypes to various patterns of drought stress and the explanation of these variabilities is an important requirement in breeding programmes for drought tolerance groundnut. Previous studies have reported the effects of drought on performance of groundnut at different growth stages (Nautiyal et al., 2002; Sanchez et al., 2010; Koolachart et al., 2013; Nigam, 2014). However, there is scanty information on genotypic diversity of groundnut under moisture stressed condition.

Selection of segregating populations under stress conditions has been a standard approach for developing varieties enhanced with drought stress tolerance (Songsri et al., 2008). However, breeding progress for drought tolerance in groundnut based on yield alone as a selection criterion has been slow due to large and uncontrollable genotype x environment interactions (Girdthai et al., 2012; Nigam, 2014). Physiological traits like relative water content (RWC), SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) have been reported to be rapid and reliable measure to identify genotypes enhanced with high water use efficiency in groundnut (Nageswara et al., 2001; Songsri et al., 2008; Painawadee et al., 2009;

Nigam, 2014). Wright et al. (1996), Nageswara et al. (2001) and Nigam et al. (2005) reported low genotype x environment (G x E) interactions for SPAD chlorophyll meter reading traits suggesting high stability across environments. In addition, Songsri et al. (2008) found that the measurement of SPAD chlorophyll meter reading was easier than that of pod yield. This suggest that SPAD chlorophyll meter reading could be used as a rapid, cost effective and simple technique for screening large breeding populations for drought tolerance in groundnut. Therefore, selection approach based on physiological traits would improve the selection efficiency for superior drought tolerant genotypes and supplement the yield-based selection approach. The objectives of the study were to determine the genotypic response to drought stress among 25 genotypes of segregating population based on agronomical and physiological traits, and to identify promising genotypes to be used in breeding programmes for drought tolerance improvement in groundnut.

3.2 Material and Methods

The study used 25 selected F3 groundnut genotypes obtained from the International Crops Research Institute for the Semi – Arid Tropics (ICRISAT) Center at Chitedze in Lilongwe – Malawi. The genotypes were derived from the hybridization of parents selected based on farmers' traits of preference and adaptability to water stress conditions. Materials obtained from the crosses were advanced through Selfing to F3. Having enough seeds and maximum segregation then materials were evaluated under water stressed environment to identify genotypes with high yield and drought tolerance. The trial was conducted during 2016/17 cropping season under rainfed condition.

3.2.1 Field experiment

The field experiment was carried out at Ngabu Agricultural Research Station – Chikwawa region in southern part of Malawi located at 34^0 53'43.04" E, 16^0 27'28.89" S, 425 km south of Chitedze ICRISAT Centre. The site occurs at an altitude of 110 masl in the lower shire of southern Malawi, and it is characterized by warm and dry conditions. The experiment was carried out from December 2016 to June 2017 under natural rain-fed conditions at a drought-testing site of Ngabu Agricultural Station in Malawi. The site is dominated by a clay loam–vertisol soil type with pH ($CaCl_2$) of 7.12, organic carbon (OC) 1.01%, organic matter (OM) 2.05%, Total N 0.30%, phosphorus (P) 8.27 ppm, potassium (K) 1.00 meq/100 g, calcium (Ca) 25.55 meq/100 g, magnesium (Mg) 5.45 meq/100 g and sodium (Na) 0.48 meq/100 g.The potential of using the site is described by the hot weather condition, high evapotranspiration and low seasonal mean rainfall recorded in Table 3-1.

3.2.2 Experimental design and trial establishment

The experiment was laid out in a randomized complete block design with four replications. Seeds were sown in plots of four rows each of 5 m length, spaced 70 cm apart, and the distance from plant to plant in a row was 15 cm. Two seeds were planted per hill and then seedlings were thinned to one plant per hill at 14 days after emergence. JL 24 groundnut variety was grown around the trial as a guard row to avoid damage and boarder effects. Weather data were collected from the meteorological station at Ngabu Agricultural Research Station located about 120 m from the experimental site. Recommended agronomic and plant protection measures were performed as suggested by Santos et al. (2006).

3.3 Data collection

3.3.1 Weather data for field experiment

Weather data for the field trial were obtained from Ngabu meteorological station located 120 m away from the experimental site (Table 3-1). The mean seasonal maximum and minimum air temperature ranged from 36^0C to 20^0C in 2016/17. Daily pan evaporation ranged from 6.4 to 97 mm and the seasonal monthly mean solar radiation ranged from 7.50 to 59.60 Mj m^{-2} d^{-1} during the cropping season. Monthly relative humidity and mean wind speed ranged from 57.90% to 72.97% and 4.33 to 13.3 km/h, respectively. Moisture stress was observed in February and April where minimum and maximum temperature was 24.90 to 34.21 ^{0}C and 21.79 to 31.62 ^{0}C respectively (Table 3-1). At this time, groundnut genotypes were at flowering and pod filling of the reproductive stage, respectively. This was the critical moisture sensitive period for growth development stage in groundnut crop.

Table 3-1. Monthly weather data during the field trial at Ngabu, Chikwawa - Malawi in a season of 2016/2017.

Year	Month	T max (°c)	T min (°c)	Wind (km/h)	RH (%)	SR (Mjm^{-2})	Rain (mm)	ETo (mm)
2016	Nov	36.50	24.60	13.3	57.90	E	25.0	E
2016	Dec	35.80	25.37	9.33	64.50	7.50	149.8	28.17
2017	Jan	34.31	24.39	6.67	75.52	59.07	119.5	62.65
2017	Feb	35.21	24.90	5.73	69.625	44.56	86.3	E
2017	March	32.28	23.11	4.33	72.97	37.56	230.0	55.60
2017	April	31.62	21.79	4.60	72.73	59.60	27.8	E
2017	May	31.74	20.18	5.47	68.74	E	10.5	E

Monthly total rainfall, average wind speed, ETo = average evapo-transpiration, RH = average total relative humidity, SR = average total solar radiation, Tmin = average minimum temperature, Tmax = average maximum temperature, E = not recorded.

3.3.2 Agronomical and physiological data

Relative water content **(**RWC) was recorded from four leaflets of the third fully developed groundnut leaf from the top of the main stem. Leaves were harvested and transported to the laboratory, fresh weight (FW) of the leaf was recorded. The leaf samples were then soaked in distilled water for 8 hours and blotted for surface drying and leaf turgid weight (TW) was determined. The samples were oven dried at 80^{0}C until constant weight was reached and leaf dry weight (DW) was determined. Relative water content was determined based on formula suggested by Bajji et al. (2001) as follows:

$$RWC\ (\%) = \frac{(Fresh\ weight\ -\ Dry\ weight)}{(Turgidity\ weight -\ Dry\ weight)}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots.. \text{ equation 1}$$

The SPAD Chlorophyll Meter Reading (SCMR) and Specific Leaf Area (SLA) were recorded as suggested by Nigam (2014). The third leaf from the terminal bud of the main stem was detached and kept in a plastic cooler box. The leaf samples were transferred to a laboratory for further analysis. The SPAD chlorophyll meter reading was measured by handheld portable SCMR meter (SPAD 502 Plus, Spectrum Technology, USA) at four leaflets per plant. The leaf samples were then oven dried at 80^{0}C until reaching constant weight and leaf dry weight was measured for determination of specific leaf area (SLA) which was further calculated based on the equation suggested by Wilson et al. (1999).

$$Specific\ leaf\ area\ (SLA) = \frac{Leaf\ area\ (cm^2)}{Leaf\ dry\ weight\ (g)} \ldots\ldots\ldots\ldots\ldots\ldots.. \text{ equation 2}$$

After harvest, selected plants were washed to remove the soil particles followed by separating the sample into roots, stem and reproductive structures for measurements. Reproductive parts were separated into mature and immature pods for counting and weight determination after oven drying. The pods were shelled and grain yield, hundred seed weight and shelling percent were measured. Shelling percentage was calculated based on the following formula as suggested by Painawadee et al. (2009).

$$Shelling\ percentage = \frac{Grain\ yield\ (g)}{Total\ pod\ yield\ (g)}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{ equation 3}$$

3.4 Data analysis

The analysis of variance for agronomical and physiological data were analysed using GenStat version 17 software, VSN, International (Payne, 2014). Pairwise multiple comparisons and separation of means was based on Turkey’s procedures (Honestly significant difference test) in GenStat version 17 software. The PCA biplots were plotted using GenStat to show the relationship among studied genotypes based on recorded traits.

3.5 Results

Analysis of variance when groundnut genotypes were evaluated in water stressed environment is summarized in Table 3-2. Groundnut genotypes varied significantly (P<0.001) in all measured traits.

Table 3-2. Analysis of variance when groundnut genotypes were evaluated in the moisture stress condition.

SOV	DF	SY	BM	PY	DM	RL	SCMR	HSW	SHP
REP	3	0.14	1.90	6.43	10.20	29.31	62.67	11.36	41.01
GENOTYPE	24	113.43***	586.07***	164.71***	825.30***	75.52***	104.57***	257.70***	160.84***
ERROR	72	6.71	3.43	5.22	40.38	5.22	24.68	29.14	38.85
TOTAL	99								

Table 1 Continued…

SOV	DF	SLA	RWC	PH	NPB	HI	FP	UFP	RDW
REP	3	45.80	37.85	46.28	2.92	0.001	3.93	3.05	0.08
GENOTYPE	24	1167.20***	42.39***	683.03***	3.35***	0.02***	212.82***	88.96***	5.59***
ERROR	72	135.30	9.90	16.69	1.22	0.001	11.10	4.01	0.24
TOTAL	99								

DM-Days to maturity; RL=root length; SY = seed yield; SCRM=SPAD Chlorophyll meter reading, HSW=hundred seed weight, SHP=shelling percentage, SLA=specific leaf area, RWC=relative water content, PH=plant height, NPB=number of primary branch, BM=biomass, HI=harvest index, FP=number of filled pod, UFP=number of unfilled pod, RDW=root dry weight, PY=pod yield.

Table 3-3 summarizes the mean response of groundnut genotypes evaluated under water stressed condition. The genotypes were found to vary significantly (P<0.001) in all measured traits. Genotypes ICGX-SM 14054, ICGX-SM 14046, ICGX-SM 14052, ICGX-SM 14055 and ICGX-SM 14080 matured 39 days earlier than the others did. Late maturing genotypes matured in 118 to 125 days from planting. Other genotypes had average days to maturity that ranged from 95 to 117. Their number of branches ranged from 2.25 to 6.0. Among them, ICGX-SM 14057, ICGX-SM 14060, ICGX-SM 14095, ICGX-SM 14098 and ICGX-SM 14055 had high number of primary branches.

The SPAD chlorophyll meter reading of 36 to 60 were determined among all evaluated genotypes**.** Genotypes ICGX-SM 14091, ICGX-SM 14085, ICGX-SM 14081, ICGX-SM

Table 3-3. Means of the water use efficiency traits for 25 groundnut genotypes evaluated under field stressed moisture condition.

Genotype	DM	RL(cm)	SY(g)	SCMR	HSW(g)	SHP(%)	SLA(cm^2/g)	RWC(%)	PH(cm)	NPB	BM(g)	HI	FP	UFP	RDW(g)	PY(g)
ICGX-SM 14046	92.00	17.75	4.91	39.75	38.30	62.89	140.10	75.64	53.80	3.25	64.91	0.11	6.25	7.50	1.49	7.53
ICGX-SM 14047	105.50	26.52	8.34	36.48	38.33	73.19	142.50	82.86	72.90	4.25	52.01	0.22	12.50	7.25	1.67	11.42
ICGX-SM 14050	122.80	28.27	8.86	38.55	36.05	72.13	153.70	85.83	47.13	4.25	68.45	0.18	18.75	13.75	2.00	12.26
ICGX-SM 14052	90.00	21.05	10.29	42.78	27.67	70.79	139.90	80.38	35.75	3.50	63.63	0.23	17.75	7.00	1.36	14.53
ICGX-SM 14053	118.80	23.82	8.65	43.58	37.60	69.71	130.60	78.76	24.05	4.00	61.06	0.20	15.00	13.25	3.56	12.38
ICGX-SM 14054	89.80	19.55	8.02	39.70	45.95	71.19	139.50	79.54	41.78	3.25	56.71	0.20	15.25	5.25	1.54	11.28
ICGX-SM 14055	91.00	19.80	10.39	38.58	56.67	63.59	139.30	78.98	64.63	6.00	52.57	0.31	16.00	7.25	2.42	16.24
ICGX-SM 14057	115.80	22.12	8.90	45.30	49.34	57.47	171.10	81.39	31.73	5.25	59.65	0.26	25.25	12.75	4.06	15.49
ICGX-SM 14059	117.00	27.37	9.36	38.38	52.00	65.35	158.90	81.14	37.10	5.50	78.55	0.18	22.00	11.25	2.12	14.33
ICGX-SM 14060	108.20	25.65	13.00	44.90	52.38	71.57	162.80	85.44	31.88	5.25	92.40	0.20	27.50	9.50	3.73	18.15
ICGX-SM 14073	122.20	17.12	21.20	44.80	53.67	78.98	136.20	81.12	62.55	4.25	73.80	0.36	24.75	11.50	1.68	26.71
ICGX-SM 14075	106.50	22.75	18.59	44.55	60.46	74.25	133.90	85.29	68.43	3.50	75.32	0.33	30.75	12.25	3.25	24.80
ICGX-SM 14078	125.80	24.80	18.57	42.70	51.48	75.08	151.70	86.03	32.63	4.00	77.70	0.32	35.00	9.00	3.36	24.75
ICGX-SM 14080	87.00	28.87	9.27	43.88	53.95	59.46	119.70	77.13	46.40	4.50	62.25	0.25	10.75	13.50	2.02	15.60
ICGX-SM 14081	121.20	20.60	23.71	47.60	57.85	78.57	108.10	87.37	34.28	3.50	85.83	0.35	24.25	9.25	2.15	30.28
ICGX-SM 14083	93.80	19.75	8.72	42.85	47.40	61.81	143.40	80.25	52.50	4.25	60.35	0.23	13.75	17.00	2.01	13.94
ICGX-SM 14085	95.80	32.88	18.76	46.10	47.05	64.52	137.30	78.82	59.40	5.00	66.73	0.44	16.50	21.75	3.49	29.09
ICGX-SM 14088	121.80	28.35	9.97	46.13	54.60	59.58	147.90	79.33	37.50	4.75	67.10	0.25	22.00	15.50	5.62	16.77
ICGX-SM 14090	93.50	30.65	9.39	46.10	44.94	64.33	165.80	82.42	36.08	4.00	61.90	0.24	24.00	25.50	5.30	14.63
ICGX-SM 14091	123.80	30.30	8.43	50.75	50.00	67.62	156.20	78.76	32.75	2.25	72.27	0.17	21.00	8.75	3.05	12.43
ICGX-SM 14093	96.50	20.90	8.31	47.25	47.45	72.25	158.40	78.71	38.30	3.00	68.37	0.17	14.75	8.00	2.46	11.49
ICGX-SM 14095	94.20	23.72	12.32	51.63	52.45	66.47	173.60	80.87	51.70	5.25	85.27	0.22	29.50	11.00	2.79	18.55
ICGX-SM 14098	121.20	29.80	18.69	50.63	53.15	76.86	163.50	87.10	49.05	5.50	92.97	0.26	24.50	8.00	4.33	24.32
ICGX-SM 14100	105.80	22.82	11.42	48.95	48.80	68.29	179.50	80.95	35.05	5.00	56.45	0.30	20.75	11.50	3.43	16.75
ICGX-SM 14101	124.00	24.25	22.32	59.65	60.40	78.97	163.80	85.48	50.05	4.00	88.41	0.32	34.75	15.25	4.19	28.09
MEAN	**107.35**	**24.40**	**12.41**	**44.86**	**48.72**	**70.15**	**148.69**	**81.58**	**45.09**	**4.00**	**69.78**	**0.25**	**20.93**	**12.00**	**2.92**	**17.67**
CV (%)	**5.10**	**9.40**	**20.90**	**11.10**	**11.10**	**13.20**	**7.80**	**3.90**	**9.10**	**25.70**	**2.70**	**12.50**	**15.90**	**17.10**	**16.60**	**12.90**
SE	**3.84**	**1.62**	**1.83**	**3.51**	**3.82**	**6.53**	**8.23**	**2.23**	**2.89**	**0.80**	**1.31**	**0.02**	**2.36**	**1.40**	**0.34**	**1.62**
LSD (5%)	**7.65**	**3.22**	**3.65**	**7.00**	**7.61**	**13.02**	**16.40**	**4.44**	**5.76**	**1.60**	**2.61**	**0.04**	**4.70**	**2.80**	**0.68**	**3.22**

DM-Days to maturity; RL=root length, SY=seed yield, SCRM=SPAD Chlorophyll meter readings, HSW=hundred seed weight, SHP=shelling percentage, SLA=specific leaf area, RWC=relative water content, PH=plant height, NPB=number of primary branch, BM=biomass, HI=harvest index, FP=number of filled pod, UFP=number of unfilled pod, RDW=root dry weight, PY=pod yield.

14095, ICGX-SM 14050 and ICGX-SM 14083 had higher SCMR that ranged from 47 to 59. Measurement of specific leaf area showed that, six genotypes had small specific leaf area that ranged from 108.1 to 137.3. Measurement of relative water content showed significant difference among genotypes. The genotypes ICGX-SM 14075, ICGX-SM 14060, ICGX-SM 14101, ICGX-SM 14050, ICGX-SM 14078, ICGX-SM 14098 and ICGX-SM 14081 had high relative water content ranged from 81% to 87% compared with others. Measurement of hundred seed weight, shelling percentage and harvest index identified ICGX-SM 14081, ICGX-SM 14073, ICGX-SM 14101, ICGX-SM 14098, ICGX-SM 14075 and ICGX-SM 14078 as potential genotypes with heavy seed weight, good shelling ability, and high productive efficiency. Their relatively low number of unfilled pods and high seed yield proved their good yielding ability as compared to the others (Table 2). Genotypes ICGX-SM 14046, ICGX-SM 14047, ICGX-SM 14050, ICGX-SM 14091, ICGX-SM 14088, ICGX-SM 14054, ICGX-SM 14053, ICGX-SM 14059 and ICGX-SM 14059 were associated with low seed yield, pod yield, SPAD chlorophyll meter reading, biomass, harvest index, number of filled pods, root dry weight and number of primary branch.

3.6 Principal Component Analysis

The rotated component matrix shows the proportion of total variance explained by different principal components and their correlations with variable traits. The results show that three principal components were important, contributing 76.15% of the total variation observed. The first two principal components were the most influential with a cumulative contribution of 63.75% to the total variation. Traits including number of filled pods and specific leaf area had high positive loading into the first principal component while seed yield had positive loading into the second principal component and shoot dry weight into the third principal component. The traits biomass and days to maturity had positive loading into both first and second principal component while pod yield and hundred seed weight loading positively into the second and third principal component.

3.7 Principal Component Biplot Analysis

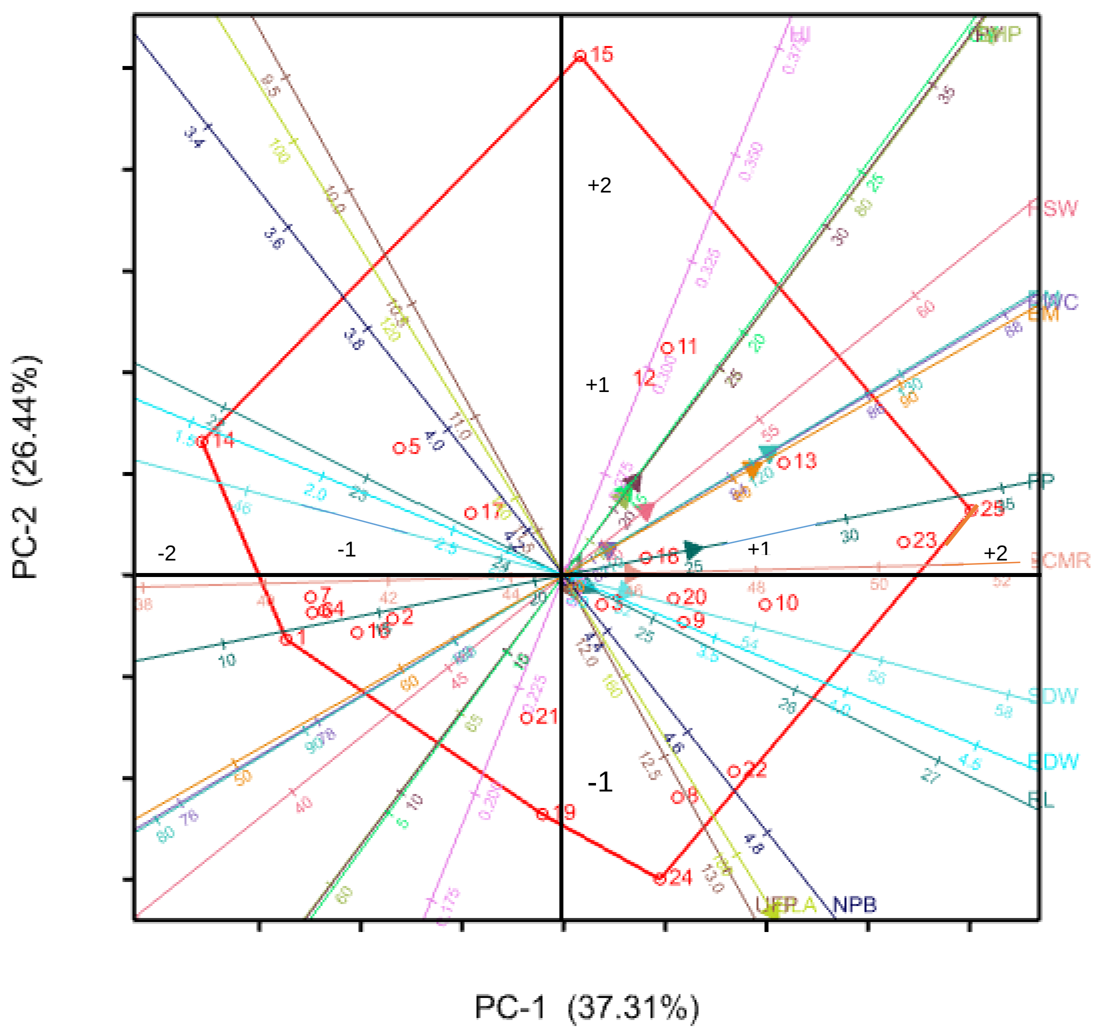
Principal components analysis of groundnut water use efficiency, yield and yield component traits in water stressed environment is presented in Figure 3-1. The smaller angles between dimension vectors in the same direction were observed. The genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular traits, often on the vertices of the convex hull. Most of the genotypes were scatted in the positive side of the

Table 3-4. Rotated component matrix of sixteen phenotypic and physiological traits evaluated under moisture stressed conditions.

TRAITS	PC-1	PC-2	PC-3
BM	0.46	0.26	-0.22
DM	0.50	0.30	-0.24
FP	0.31	0.06	0.04
GY	0.15	0.21	0.15
HI	0.00	0.00	0.00
HSW	0.20	0.16	0.20
NPB	0.01	-0.01	0.01
PY	0.17	0.23	0.21
RDW	0.03	-0.01	0.02
RL	0.06	-0.03	0.00
RWC	0.11	0.06	0.00
SCMR	0.17	0.00	0.05
SDW	0.15	-0.04	0.88
SHP	0.14	0.19	0.00
SLA	0.51	-0.82	-0.07
UFP	0.02	-0.03	0.11
Explained variance (eigenvalue)	**3.73**	**2.64**	**1.24**
Proportion of total variance (%)	**37.31**	**26.44**	**12.40**
Cumulative variance (%)	**37.31**	**63.75**	**76.15**

DM-Days to maturity; RL=root length, GY=grain yield, SCRM=SPAD Chlorophyll meter readings, HSW=hundred seed weight, SHP=shelling percentage, SLA=specific leaf area, RWC=relative water content, PH=plant height, NPB=number of primary branch, BM=biomass, HI=harvest index, FP=number of filled pod, UFP=number of unfilled pod, RDW=root dry weight, PY=pod yield.

first principal component. The genotypes ICGX-SM 14073, ICGX-SM 14075, ICGX-SM 14078, ICGX-SM 14081, ICGX-SM 14098 and ICGX-SM 14101 outclassing in seed yield which was contributed by shelling percentage, harvest index, hundred seed weight, relative water content, and biomass as well as optimum values for other yield components.



Keys:				
01=ICGX-SM 14046	06=ICGX-SM 14054	11=ICGX-SM 14073	16=ICGX-SM 14083	21=ICGX-SM 14093
02=ICGX-SM 14047	07=ICGX-SM 14055	12=ICGX-SM 14075	17=ICGX-SM 14085	22=ICGX-SM 14095
03=ICGX-SM 14050	08=ICGX-SM 14057	13=ICGX-SM 14078	18=ICGX-SM 14088	23=ICGX-SM 14098
04=ICGX-SM 14052	09=ICGX-SM 14059	14=ICGX-SM 14080	19=ICGX-SM 14090	24=ICGX-SM 14100
05=ICGX-SM 14053	10=ICGX-SM 14060	15=ICGX-SM 14081	20=ICGX-SM 14091	25=ICGX-SM 14101

Figure 3-1. Principal component biplot showing genotypic grouping under moisture stressed condition. DM-Days to maturity, RL=root length, SY=seed yield, SCRM=SPAD Chlorophyll meter readings, HSW=hundred seed weight, SHP=shelling percentage, SLA=specific leaf area, RWC=relative water content, PH=plant height, NPB=number of primary branch, BM=biomass, HI=harvest index, FP=number of filled pod, UFP=number of unfilled pod, RDW=root dry weight, PY=pod yield.

3.8 Discussion

Drought is the most significant constraint that affects groundnut productivity in rain-fed agriculture. Currently, various agronomical and physiological traits such as biomass, pod yield, number of filled pod, hundred seed weight, specific leaf area, SPAD chlorophyll meter reading and relative water content have been reported to be associated with water use efficiency under drought condition (Shinde et al., 2010; Kachout et al., 2011; Pereira et al., 2015). Therefore, if selection for drought tolerance in groundnut were trait based, rapid improvement in developing drought tolerant cultivars would be granted in breeding programmes. The analysis of variance showed high significant variation among the genotypes evaluated for all traits (Table 3-1). The observed significant differences among genotypes indicated the variability in genetic composition and thus adaptation to adverse environmental condition. This variation permits selection for appropriate diverse material to utilize in breeding programmes. Existence of significant genotypic variation for specific leaf area, SPAD chlorophyll meter reading (SCMR), relative water content, hundred seed weight, shelling percentage, and pod yield in moisture stress environment has been reported in early studies conducted by Girdthai et al. (2012), Aninbon et al. (2015) and Pereira et al. (2015).

Selection for improved yield and yield attributing traits under stressed conditions offers an opportunity for genotypes to maintain their performances even in stress free conditions. High seed yield observed for ICGX-SM 14098, ICGX-SM 14081, ICGX-SM 14101, ICGX-SM 14075, ICGX-SM 14073, ICGX-SM 14085 and ICGX-SM 14078 concur with Duarte et al. (2013) who reported high yield for some groundnut genotypes under moisture stress environment. Their high relative water content and SPAD chlorophyll meter reading recorded indicated their high ability in photosynthetic capacity and improved water-use efficiency mechanisms that allow them to withstand moisture stress condition. Genotypes with higher relative water content and SPAD chlorophyll meter reading under moisture stressed condition were genetically reported to be enhanced with drought tolerance (Koolachart et al., 2013; Nigam, 2014; Aninbon et al., 2015). Good adaptability to water stress environment for these genotypes has been confirmed by their high shelling percentage, hundred seed weight, number of filled pods, pod yield, biomass and harvest index. Poor performance of the other genotypes for these traits contributed to their inability to withstand moisture stress environmental conditions. Groundnut in water stress environment loses moisture from pods and this leads to a reduction in physiological activities of seeds, and consequently affecting pod yield and seed weight. This confirms findings by Boontang et al. (2010) and Koolachart et al. (2013) that genotypes with relatively high number of filled pods, shelling percentage and hundred seed weight under moisture stress conditions are drought tolerant. Painawadee et al. (2009) reported groundnut genotype ICGV 98348 to have highest drought tolerance because of low reduction in pod yield, biomass and harvest index under drought condition. Therefore, selection of these genotypes would have a positive impact toward breeding for drought tolerance in groundnut.

Principal component analysis under moisture stress condition showed specific leaf area, days to maturity, biomass, number of filled pod, hundred seed weight and pod yield to have more influence during selection. They had high positive loading into the first principal component. This is attributed to their high photosynthetic machinery per unit area that have greater assimilation, and thus accumulates more biomass. This resulted in high number of filled pods, seed weight and high pod yield. Santos et al. (2010), Girdthai et al. (2012), Duarte et al. (2013) and Karimian et al. (2015) have reported the importance of selecting groundnut genotypes under moisture stress condition based on these traits. Therefore, simultaneous selection based on these traits could improve yield significantly.

3.9 Conclusion

The effects of drought stress are expressed in various morphological, physiological, biochemical and genetic changes in plants. Groundnut genotypes displayed different responses for traits associated with drought tolerance. Genotypes ICGX-SM 14075, ICGX-SM 14078, ICGX-SM 14060, ICGX-SM 14098, ICGX-SM 14081, ICGX-SM 14101, ICGX-SM 14100 and ICGX-SM 14095 were selected for their high tolerance to moisture stress. They had high biomass, pod yield, number of filled pod, hundred seed weight, specific leaf area, SPAD chlorophyll meter reading, and relative water content, and they can be incorporated into the breeding programme. Differential response of genotypes under moisture stressed conditions reported in this study showed existence of genetic variation that contributed to superiority of the selected genotypes. Therefore, integrating these traits during selection would help in developing groundnut genotypes with high levels of drought tolerance.

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Chapter 4

GENETIC VARIABILITY, CORRELATION AND PATH COEFFICIENT ANALYSIS FOR DROUGHT TOLERANCE IMPROVEMENT IN GROUNDNUT (*Arachis hypogaea L.*) GENOTYPES

ABSTRACT

Selection of groundnut genotypes with high seed yield under drought conditions have been useful for yield improvement. However, the approach has been slow and ineffective because seed yield is a complex polygenic trait that is highly influenced by the environment. Therefore, selection based on component traits, which are less complex, simply inherited and less influenced by environmental factors would increase yield. The current study was thus undertaken to estimate variability (phenotypic and genotypic), heritability, genetic advance (GA), correlation and path analysis among traits of 25 groundnut genotypes evaluated under field moisture stressed conditions. The experiment was laid out in a randomized complete block design with four replications. The results indicated high genetic coefficient of variation (GCV), coupled with high genetic advance (GA), genetic advance as percent of mean (GAM) and heritability for number of filled pods per plant (FP), biomass (BM), pod yield per plant (PY), days to maturity (DM) and relative water content (RWC). Seed yield (SY) was highly significant and positively correlated with biomass, days to maturity, relative water content, SPAD chlorophyll meter reading, harvest index and shelling percentage. Furthermore, path analysis showed that biomass, harvest index, SPAD chlorophyll meter reading and shelling percentage had the highest direct and indirect effects on seed yield. Therefore, breeding for high yield and drought tolerance in groundnut should target on these traits.

Key words: correlation and path analysis, genetic variability, groundnut, selection

4.1 Introduction

Groundnut (*Arachis hypogaea* L,) is one of the major sources of food and income for smallholder's farmers in Malawi. It is a valuable food security crop that supplies fats and proteins to the predominantly maize–based Malawian diet. Groundnut is one of the country's key export crops and an important earner of foreign exchange (Derlagen and Phiri, 2012). Apart from that, groundnut enriches the soil through biological nitrogen fixation. The haulm and other crop extracts are used as livestock feeds since they are rich in digestible crude protein; hence, it directly contributes to increased livestock productivity (Simtowe et al., 2012). Although, groundnut production is a profitable venture for smallholder farmers in Malawi, its total production remains low (Minde et al., 2008; Longwe – Ngwira et al., 2012). Groundnut production in Malawi relies on rainfed agriculture, and drought is a major production constraint (Sangole et al., 2010; Simtowe et al., 2010). Monyo and Gowda (2014) reported that current groundnut yields in Malawi have remained low averaging less than 1 tha^{-1} compared to the yield of about 4 tha^{-1} obtained at research stations. The low groundnut yields are attributed to unreliable rainfall, often with midseason and terminal droughts (Simtowe et al., 2012). Therefore, breeding for drought tolerance would be an important strategy for alleviating low yield in groundnut.

Selection based on seed yield under drought conditions has been slow and ineffective because of high genotype x environment (G x E) and the complex nature of seed yield, which is influenced by many interrelated traits directly or indirectly (Girdthai et al., 2010; Shoba et al., 2012; Nigam, 2014). Path coefficient analysis is a helpful tool for making decisions on selection criteria based on the influence of component traits. Additionally, success from selection for an economic trait depends on the magnitude of genotypic variability existing within a particular population (John, 2012). However, the overall genotypic variation needs to be partitioned into heritable and non-heritable portion using genetic parameters such as phenotypic coefficient of variations (PCV), genotypic coefficient of variations (GCV), genetic advance (GA), genetic advance as percent of mean (GAM) and broad sense heritability (BSH). These parameters have mostly been advocated for theoretically, but few attempts have been made to establish genetic control based on them. Therefore, the current study was undertaken to determine the extent of genotypic variability, correlation coefficients and path coefficients among the traits. This information will guide decision regarding the selection procedures to be employed for identifying superior genotypes under drought conditions.

4.2 Material and Methods

4.2.1 Plant material

The experimental material comprised of 25 F3 genotypes developed from crossing of 10 parents selected at ICRISAT, Chitedze, Malawi. Genotypes ICGV-SM 99551, ICGV-SM 99555, ICGV-SM 01721, CG 7 bred in Malawi and Pendo bred in Tanzania; were used as drought susceptible female parents with different attributes. Pendo and ICGV – SM 99551 are *Spanish* bunch types and early maturing varieties released and grown in Tanzania. ICGV – SM 99555 is a *Spanish* bunch type, early maturing and rosette resistant variety released in Tanzania (Monyo, 2010). CG 7 is a *Virginia* bunch type that is high yielding, with high oil content and wide adaptability. It has been released in Malawi, Tanzania, Uganda, Zambia and Mozambique (Subrahmanyam et al., 2000). ICGV – SM 01721 is a high yielding and rosette resistant *Virginia* bunch type bred in Malawi and released in Tanzania (Monyo, 2010). Akwa, Malimba, Baka, ICGV-SM 02724 and ICGV-SM 94139, which are sources of drought tolerance were used as male parents. Baka is an early maturing and aphid resistant *Spanish* bunch type bred in India and has been released in Malawi, Uganda, Zambia and Mozambique (Deom et al., 2006). Akwa is *Valencia* bunch type and early maturing variety released in South Africa (Merwe and Joubert, 1995). ICGV – SM 02724 is a drought tolerant, high yielding and rosette resistant line, which has not been released. Malimba and ICGV – SM 99139 are *Spanish* bunch types bred in Malawi and recommended for low lands (altitude of 200 to 300 masl) and rosette resistance, respectively.

Crosses evaluated in this study were developed in 2015/2016 and 2016/2017 seasons at International Crops Research Institute for Semi-Arad Tropics (ICRISAT), Chitedze, Malawi, station using a 5 x 5 NCD II mating design. The F1 seeds of the 25 progenies obtained from the crosses were selfed to obtain F2 and F3 generations. Selfing was done to multiply seeds for evaluation and to allow maximum segregation among genotypes.

4.2.2 Experimental condition and design

The field experiment was carried out at National Agricultural Research Station (NARS), Ngabu in Chikwawa region, southern Malawi (34^0 53’43.04” E, 16^0 27’28.89” S, altitude of 110 masl), located 425 km south of Chitedze ICRISAT Centre. It is characterized by warm and dry conditions and it is used as a drought-testing site. The site has clay loam–vertisol soils with pH 7.12, organic carbon (OC) 1.01%, organic matter (OM) 2.05%, total N 0.30%, phosphorus (P) 8.27 ppm, potassium (K) 1.00 meq/100g, calcium (Ca) 25.55 meq/100g, magnesium (Mg) 5.45 meq/100g and sodium (Na) 0.48 meq/100g. The experiment was carried out from December 2016 to June

2017 in a drought-testing site under natural rain-fed conditions. It was laid out in a randomized complete block design with four replications. Seeds were sown in plots of four rows of 5 m length, with inter-row spacing of 70 cm and intra-row spacing of 15 cm. Two seeds were planted per hill and then seedlings were thinned to one plant per hill 14 days after emergence. The variety JL 24 was grown around the trial as a guard row to avoid damage from animals and boarder effects. Recommended agronomic and plant protection measures were performed as suggested by Santos et al. (2006).

4.3 Data collection

Relative water content (RWC) was recorded from four leaflets of the third fully developed leaves from the top of the main stem. Leaves were harvested and transported to the laboratory where fresh weight (FW) was recorded. The leaf samples were then soaked in distilled water for eight hours and blotted for surface drying and leaf turgidity weight (TW) was determined. The samples were oven-dried at 80^0C until they reached constant weight and leaf dry weight (DW) was determined. Relative water content was determined based on the following formula suggested by Bajji et al. (2001).

$$RWC\ (\%) = \frac{(Fresh\ weight\ -\ Dry\ weight)}{(Turgidity\ weight -\ Dry\ weight)}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{ equation 1}$$

The SPAD, SCMR and SLA were recorded as suggested by Nigam, (2014). The third leaf from the terminal bud of the main stem was detached and kept in a plastic cooler box. The leaf samples were then transferred to a laboratory. The SPAD chlorophyll meter reading was measured using a handheld portable SCMR meter (SPAD – 502 Plus, Spectrum Technology, USA) on four leaflets per plant. The leaf samples were then oven-dried at 80^0C until they reached constant weight and leaf dry weight was recorded for determination of specific leaf area (SLA), which was calculated based on the equation suggested by Wilson et al. (1999) as follows:

$$Specific\ leaf\ area\ (SLA) = \frac{Leaf\ area\ (cm^2)}{Leaf\ dry\ weight\ (g)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ equation 2}$$

After harvest, selected plants were washed to remove the soil particles followed by separating the sample into roots, stem and reproductive structures. Reproductive parts were separated into mature and immature pods for counting and weighting after oven drying. Pod yield, hundred seed weight and shelling percent were recorded. Shelling percentage was calculated based on the following formula as suggested by Painawadee et al. (2009):

$$Shelling\ percentage = \frac{Grain\ yield\ (g)}{Total\ pod\ yield\ (g)} x\ 100 \ldots\ldots\ldots\ldots\ldots.. \text{ equation 3}$$

Root and above ground samples were oven- dried at 80^{0}C for 48 hours and dry weights were recorded. The harvest index (HI) was calculated based on the relationship suggested by Nautiyal et al. (2002) as:

$$Harvest\ Index\ (HI) = \frac{Total\ dry\ pod\ mass\ at\ final\ harvest}{Total\ dry\ biomass\ at\ final\ harvest} \ldots\ldots.. \text{ equation 4}$$

4.4 Data analysis

The analysis of variance was performed using GENSTAT 17th Edition (Payne, 2014). The significant difference between the means was tested using the Least Significance Difference (LSD) at 5% level of significance. The means were extracted and used in correlation analysis. Pearson’s correlation coefficients (*r*) were calculated using IBM SPSS version 25 software (SPSS, 2012) to determine the relationship between yield and the yield attributing traits. Correlation coefficients were further partitioned into direct and indirect effects on seed yield through path coefficient analysis using the procedures suggested by Dewey and Lu, (1959). The variance components were analysed using SPSS version 25 software (SPSS, 2012) and were used to calculate the genetic parameters. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated as suggested by Singh and Chaudhury (1985). The heritability and genetic advance (GA) were calculated according to Johnson et al. (1955). The genetic advance as percent of mean (GAM) was analysed as suggested by Shukla et al. (2006). The formulas used were as follows; -

$$PCV = (\sigma_p^2 / x)\ x\ 100 \ldots\ldots\ldots\ldots \text{ equation 5}$$

$$GCV = (\sigma_g^2 / x)\ x\ 100 \ldots\ldots\ldots\ldots \text{ equation 6}$$

$$GA = (\sigma_g^2 / \sigma_p^2)\ x\ K \ldots\ldots\ldots\ldots \text{ equation 7}$$

$$GAM = (GA / x)\ x\ 100 \ldots\ldots\ldots\ldots \text{ equation 8}$$

Where: σ_p^2 = phenotypic variance, σ_g^2 = genotypic variance, x = the mean from general analysis of variance, K = selection differential, a constant (z/p) at 5% which is a value of 2.06.

The coefficients of variation (GCV, PCV) were categorized as proposed by Sivsubramanian and Menon (1973) as 0-10% = low, 11-20% = medium and >20% = high. Heritability values were classified as proposed by Robison et al. (1949) as 0-30% = low, 31-60% = medium and >60% = high. The genetic advance was categorized as proposed by Murugan et al. (2010) as <10% = low, 10-20% = medium and >20% = high.

4.5 Results

4.5.1 Genetic variability, heritability and genetic advance of drought tolerance traits

The results for genetic variability among the genotypes for all traits studied are presented in Table 4-1. The highest GCV (%) was recorded for days to maturity, root length, seed yield, SPAD chlorophyll meter reading, hundred seed weight, shelling percentage, relative water content, biomass per plant, number of filled pod per plant and pod yield per plant. The number of primary branch exhibited moderate GCV (%), whereas specific leaf area and harvest index displayed very low GCV (%) values (Table 4-1). High variability in PCV (%) was observed for days to maturity, root lenght, seed yield, hundred seed weight, relative water content, biomass, number of filled pod and pod yield. A moderate PCV (%) was recorded for SPAD chlorophyll meter reading, shelling percentage and root dry weight whereas harvest index and specific leaf area exhibited low PCV (%) value. High genetic advance (GA) was recorded for biomass followed by moderate GA for days to maturity, root length, seed yield, hundred seed weight, relative water content, harvest index, number of filled pod, root dry weight and pod yield. Low GA was recorded for SPAD chlorophyll meter reading, shelling percentage, number of primary branches and specific leaf area. The genetic advance as percent per mean (GAM %) was high for all measured traits except for days to maturity, shelling percentage, relative water content and number of primary branches (Table 4-1).

High broad sense heritability (BSH %) estimates were observed for days to maturity, root length, seed yield, hundred seed weight, relative water content, biomass, harvest index, number filled pod, root dry weight and pod yield, ranging from 65.60 to 97.70%. On the other hand, heritability values were moderate for SPAD chlorophyll meter reading, shelling percentage and specific leaf area ranging from 43.98 to 45.07%, and the lowest BSH (30.39%) was exhibited by number of primary branches (Table 4-1). High GCV %, GAM % and BSH % coupled with moderate GA was observed for seed yield, hundred seed weight, biomass, number of filled weight and pod yield.

Table 4-1. Estimates of broad sense heritability (BSH), PCV, GCV, GA and GAM for drought tolerant traits under moisture stress conditions

TRAIT	MEAN	RANGE	PCV	GCV	GA	GAM	BSH
DM	107.36	87.00-126	91.83	76.16	17.08	15.91	82.93
RL	24.38	17.12-32.88	38.96	30.04	15.88	65.16	77.11
SY	12.42	4.91-23.71	112.06	89.54	16.46	132.57	79.90
SCMR	44.86	36.48-59.65	41.47	18.55	9.21	20.54	44.73
HSW	48.72	27.67-60.46	73.79	48.87	13.64	28.00	66.23
SHP	69.00	59.47-79.98	41.88	18.42	9.06	13.13	43.98
SLA	148.70	119.7-179.5	5.05	2.28	9.28	6.24	45.07
RWC	81.58	75.64-87.37	100.43	65.88	13.51	16.56	65.60
NPB	4.29	2.25-6.00	17.02	5.17	6.26	145.91	30.39
BM	69.78	52.01-92.97	89.02	86.97	20.13	28.84	97.70
HI	0.25	0.11-0.44	0.95	0.79	17.06	67.87	82.81
FP	20.93	6.25-34.75	122.49	100.39	16.88	80.67	81.96
RDW	2.92	1.36-5.62	22.44	19.09	17.52	59.96	85.06
PY	17.67	7.53-30.28	106.32	94.02	18.22	103.09	88.43

Where; RWC = relative water content (%), SLA = specific leaf area (gcm^{-2}), SCMR = SPAD chlorophyll meter reading, HSW = hundred seed weight (g), SHP = shelling percentage (%), RDW = root dry weight (g), HI = harvest index, DM = days to maturity, SY = seed yield per plant (g), FP = number of filled pod per plant, BM = biomass per plant (g), PY = pod yield per plant (g).

4.5.1 Correlation of seed yield and drought tolerant traits of the F3 population.

Correlations between seed yield, yield attributing traits and water-use efficiency traits are presented in Table 4-2. Except for specific leaf area, other traits were positively correlated with seed yield. Highly significant and positive ($P<0.01$) correlation coefficient were recorded for seed yield with harvest index (r=0.800), shelling percentage (r=0.664), relative water content (r=0.645), biomass (r=0.634) and hundred seed weight (r=0.577). Except for root length (RL), number of primary branches (NPB) and root dry weight (RDW), seed yield exhibited significant ($P<0.05$) positive correlation with all other traits.

The inter-trait correlation showed that, except for root length, specific leaf area, number of primary branches, harvest index and root dry weight other traits were significant ($P<0.01$) and positively correlated with biomass. Days to maturity, shelling percentage and biomass had significant ($P<0.01$) positive correlation with relative water content of r=0.559, r=0.669 and r=0.617, respectively. Biomass, relative water content and days to maturity had significant positive correlation of r=0.520, r=0.669, and r=0.449 with shelling percentage, respectively (Table 4-2).

Table 4-2. Correlation coefficient (*r*) estimates of morphological and physiological traits for water use efficiency (WUE) in F_3 population

TRAIT	DM	RL	SY	SCMR	HSW	SHP	SLA	RWC	NPB	BM	HI	RDW
DM	1.000											
RL	0.203	1.000										
SY	0.448*	0.010	1.000									
SCMR	0.287	0.179	0.504*	1.000								
HSW	0.275	0.027	0.577**	0.471*	1.000							
SHP	0.449*	-0.176	0.664**	0.253	0.128	1.000						
SLA	0.132	0.215	-0.181	0.362	0.021	-0.112	1.000					
RWC	0.559**	0.148	0.645**	0.236	0.301	0.669**	0.136	1.000				
NPB	-0.007	0.191	0.091	-0.073	0.267	-0.284	0.314	0.131	1.000			
BM	0.483*	0.171	0.634**	0.557**	0.476*	0.520**	0.168	0.617**	0.086	1.000		
HI	0.167	0.066	0.800**	0.286	0.489*	0.261	-0.248	0.319	0.273	0.126	1.000	
RDW	0.350	0.523**	0.195	0.528**	0.360	-0.153	0.452*	0.246	0.273	0.247	0.219	1.000

** Correlation is significant at the 0.05 level (2-tailed).*

*** Correlation is significant at the 0.01 level (2-tailed).*

Where; RWC = relative water content (%), SLA = specific leaf area (gcm^{-2}), SCMR = SPAD chlorophyll meter reading, HSW = hundred seed weight (g), SHP = shelling percentage (%), RDW = root dry weight (g), HI = harvest index, DM = days to maturity, SY = seed yield per plant (g), FP = number of filled pod per plant, BM = biomass per plant (g), PY = pod yield per plant (g).

Table 4-3. Direct (diagonal) and indirect (non-diagonal) effects of 11 traits on seed yield in 25 groundnut genotypes

TRAIT	DM	RL	SCMR	HSW	SHP	SLA	RWC	NPB	BM	HI	RDW	TC
DM	**0.0376**	-0.0152	0.0169	0.0002	0.0846	-0.0097	0.0310	0.0002	0.1905	0.1094	0.0025	0.4480*
RL	0.0076	**-0.0750**	0.0105	0.0000	-0.0332	-0.0158	0.0082	-0.0068	0.0675	0.0432	0.0037	0.0100
SCMR	0.0108	-0.0134	**0.0588**	0.0003	0.0477	-0.0266	0.0131	0.0026	0.2197	0.1873	0.0037	0.5040*
HSW	0.0103	-0.0020	0.0277	**0.0007**	0.0241	-0.0015	0.0167	-0.0095	0.1878	0.3202	0.0025	0.5770**
SHP	0.0169	0.0132	0.0149	0.0001	**0.1885**	0.0082	0.0371	0.0101	0.2051	0.1709	-0.0011	0.6640**
SLA	0.0050	-0.0161	0.0213	0.0000	-0.0211	**-0.0734**	0.0076	-0.0112	0.0663	-0.1624	0.0032	-0.1810
RWC	0.0210	-0.0111	0.0139	0.0002	0.1261	-0.0100	**0.0555**	-0.0047	0.2434	0.2089	0.0017	0.6450**
NPB	-0.0003	-0.0143	-0.0043	0.0002	-0.0535	-0.0230	0.0073	**-0.0356**	0.0339	0.1788	0.0019	0.0910
BM	0.0181	-0.0128	0.0327	0.0003	0.0980	-0.0123	0.0343	-0.0031	**0.3945**	0.0825	0.0017	0.6340**
HI	0.0063	-0.0049	0.0168	0.0003	0.0492	0.0182	0.0177	-0.0097	0.0497	**0.6549**	0.0015	0.8000**
RDW	0.0131	-0.0392	0.0310	0.0002	-0.0288	-0.0332	0.0137	-0.0097	0.0974	0.1434	**0.0070**	0.1950

Diagonal values (Bolded letters) indicate direct effects of respective characters and indirect effects for above and below bolded values. Yellow colour shows high direct effect while light blue colour shows indirect effects of the respective direct effects. Residual effects=0.05.

Where: BM = Biomass, PY = Pod yield per plant, RWC = Relative water content, RDW = Root dry weight, SCMR = SPAD chlorophyll meter reading, SLA = Specific leaf area, SHP = Shelling percentage, DM = Days to maturity, NPB = Number of primary branch, PH = Plant height, HI = Harvest index, TE = total effects of correlation with seed yield.

Hundred seed weight exhibited significant ($P<0.5$) and positive correlation with SPAD chlorophyll meter reading, biomass and harvest index. Root dry weight showed significant positive correlation with root length, SPAD chlorophyll meter reading and specific leaf area. Number of primary branch was negatively correlated with days to maturity, SPAD chlorophyll meter reading and shelling percentage (Table 4-1).

4.5.2 Estimates of path coefficients for direct and indirect effects on seed yield

The path coefficient analysis for 11 characters with direct and indirect effect on seed yield are summarized in Table 4-3. The results showed that, among the 11 characters, only harvest index exhibited the highest positive direct effect (0.6549) on seed yield per plant. Positive direct effects of 0.3945 and 0.1885 on seed yield was also exhibited by biomass and shelling percentage respectively. Other traits with positive direct effects on seed yield were biomass SPAD chlorophyll meter reading (0.0588), relative water content (0.0555), days to maturity (0.0376), hundred seed weight (0.0007) and root dry weight (0.0070). The negative direct effects were observed for root length (-0.0750), specific leaf area (-0.0734) and number of primary branch (-0.0356).

Relative water content, hundred seed weight, SPAD chlorophyll meter reading and relative water content had indirect effects through both harvest index and biomass (Table 4-3). Relative water content (0.1261), biomass (0.0980) and days to maturity (0.0846) had high positive indirect effects via shelling percentage. Biomass, relative water content and shelling percentage had high positive indirect effects through days to maturity, SPAD chlorophyll meter reading and relative water content.

4.6 Discussion

4.6.1 Genetic variability, heritability and genetic advance of drought tolerance traits

Genetic advance (GA) is a measure of genetic gain under selection that depends on genetic variability, heritability and selection intensity. High GA from selection is due to high genetic variability or trait heritability. Analysis of variance revealed the presence of extensive genetic variation among the studied genotypes. The highest GCV was recorded for number of filled pod, pod yield per plant, seed yield per plant, biomass per plant, days to maturity and relative water content, which indicated existence of extensive genetic variations among genotypes. Shoba et al. (2009) and Padmaja et al. (2013) reported similar results on these traits.

Moderate GCV were exhibited by SPAD chlorophyll meter reading, shelling percentage and root dry weight, whereas harvest index and specific leaf area displayed very low GCV value. The low GCV exhibited by these traits indicated high influence of the environment in the expression of these traits. This may also be attributed by their polygenic nature, resulting in limited scope for selection. Dolma et al. (2010), and Padmaja et al. (2013) reported similar results for these traits. High PCV was observed for days to maturity, root length, seed yield, SPAD chlorophyll meter reading, hundred seed weight, relative water content, number of filled pod, biomass and pod yield. The high PCV revealed by these traits suggested a greater contribution of the environmental factor on the expression of these traits. Padmaja et al. (2013) also reported high PCV for these traits. The PCV for biomass, number of filled pod, days to maturity and pod yield were nearer to their corresponding GCV values indicating that, the environment had little influence on both phenotype and genotype expression of these traits. Similar results were reported by Kalpande et al. (2014) who found closer values of PCV and GCV for days to maturity, number of filled pod and biomass.

High broad sense heritability estimates were observed for all measured traits except SPAD chlorophyll meter reading, shelling percentage, specific leaf area and number of primary branches. High heritability for these traits indicates an opportunity for improvement under drought conditions. These results agree with the findings reported by Songsri et al. (2008), Shoba et al. (2009), and Dolma et al. (2010). In addition, previous studies reported that inheritance of drought tolerance traits are predominantly controlled by additive gene action (Surihan et al., 2005; Songsri et al., 2008; Nigam, 2014).

High GA coupled with high GAM were noted for biomass; and high GAM coupled with moderate GA were recorded for pod yield, number of filled pods, harvest index, root dry weight, root length and hundred seed weight. This indicated that genetic control had more influence in the expression of these traits than environmental effects; hence, selection for drought tolerance among groundnut genotypes under these traits may be effective. Vasanthi et al. (2004) and Padmaja et al. (2013) reported similar results for these traits. Furthermore, low GA coupled with low GAM were observed for specific leaf area, shelling percentage and number of primary branches. This indicated that, the traits were highly influenced by environmental factors; hence, phenotypic selection for improvement and breeding progress under drought conditions would be ineffective and slow. Similar findings were reported in earlier studies conducted by Manoharan et al. (1993), Misra et al. (2000) and Naik et al. (2000). They reported low heritability coupled with low genetic advance for shelling percentage and number of primary branches.

4.6.2 Correlation of yield and drought tolerant traits of the F3 population

Highly significant and positive correlation of seed yield with the yield attributing traits was observed for days to maturity, hundred seed weight, shelling percentage and biomass.

Simultaneous selection based on these traits would be effective for improving seed yield under drought conditions. Similar results were reported in previous studies conducted by Sharma and Dashora, (2009), Sadeghi and Seyyed, (2012) and Padmaja et al. (2013). Importance of yield components on selection for seed yield improvement in groundnut has been reported in previous studies (Parameshwarappa et al., 2008; Vaithiyalingan et al., 2010).

Among the water-use efficient traits, SPAD chlorophyll meter reading, harvest index and relative water content had positive significant correlation with seed yield, while specific leaf area exhibited negative correlation. It has reported that, genotypes with low specific leaf area have high dry matter content stored in their leaves, representing greater photosynthetic capacity compared to those with low specific leaf area. Selection for low specific leaf area under moisture stress could help to identify genotypes with high drought tolerance. Songsri et al. (2008), Girdthai et al. (2012) and Nigam (2014) reported similar results for specific leaf area. With the incorporation of relative water content, harvest index and SPAD chlorophyll meter reading in selection, it would be possible to improve groundnut yield in drought tolerance breeding programmes. Consistently, SPAD chlorophyll meter reading is an indicator of the photo-synthetically active radiation transmittance traits of the leaf and it is positively correlated with chlorophyll content, chlorophyll density and water use efficiency (Akkasaeng et al., 2003; Sheshshayee et al. 2006; Arunyanark et al., 2008). Therefore, integration of these traits in drought tolerance breeding scheme would be advantageous in selecting for groundnut genotypes that are more efficient in water use.

4.6.3 Estimates of path coefficients for direct and indirect effects on seed yield

The path coefficient analysis for 11 characters with direct and indirect effect on seed yield showed that harvest index, biomass and shelling percentage exhibited the highest positive direct effect. The high direct effects revealed in this study suggested that selection based on these traits would result in genetic gain toward groundnut yield improvement under drought stress. Sumathi and Muralidharan (2007), Raut et al. (2010) and Shoba et al. (2012) reported similar results on these traits.

Other positive direct effects on seed yield per plant were observed for relative water content, SPAD chlorophyll meter reading, days to maturity. The low magnitude of positive direct effects exhibited by root dry weight and hundred seed weight indicated that, the direct effects may be confounded with indirect effects. Therefore, improvement of seed yield under drought conditions based on these traits would be more effective if the indirect effects would be considered. John et al. (2007), Painawadee et al. (2009), and Sadeghi and Seyyed (2012) obtained similar results for shelling percentage, relative water content, biomass and SPAD chlorophyll meter reading.

Negative direct effects on seed yield were observed for specific leaf area, number of primary branch and root length. Similar results were reported in previous studies for number of primary branches and specific leaf area (Arjunan et al., 1999; Lakshmidevamma, 2004).

The indirect effects of SPAD chlorophyll meter reading, hundred seed weight, shelling percentage and relative water content through harvest index and biomass were positive. These results support the findings of Alam et al. (1985), Manoharan et al. (1990), Moinuddin (1997). Lakshmidevamma (2004) concluded that, simultaneous selection based on direct and indirect effects of these traits would be of paramount importance for improving yield in groundnut. Further, indirect effects were noted for biomass through relative water content, shelling percentage, SPAD chlorophyll meter reading and days to maturity. Padmaja et al. (2013) reported similar results for these traits. The indirect effects of days to maturity through biomass, shelling percentage and relative water content could be explained that, genotypes with increased days to maturity have enough time to accumulate more biomass resulting in high shelling turn-out and seed yield. Selecting for these traits would benefit the groundnut breeding programmes for drought tolerance. From the above findings, it may be concluded that selecting for pod yield, shelling percentage, SPAD chlorophyll meter reading, biomass and relative water content in groundnut improvement could result in high overall seed yield under moisture stressed conditions.

4.7 Conclusion

The study results revealed sufficient variations among the evaluated variables, which also had high heritability, indicating the possibility of improving groundnut yield through selection. High genotypic variations in number of filled pod, pod yield, days to maturity, biomass, relative water content, hundred seed weight and SPAD chlorophyll meter reading; coupled with high BSH, GA and GAM (%) confirms considerable existence of genetic variation in the population, and that selection for superior genotypes in early generations is possible. Significant positive correlations of seed yield with harvest index, days to maturity, biomass, hundred seed weight, shelling percentage, relative water content and SPAD chlorophyll meter indicated the ability to improve seed yield through selection based on these attributes. The path analysis showed that, harvest index, biomass, shelling percentage, relative water content and pod yield had direct and indirect effects on seed yield, and thus breeding for high yielding and drought tolerant groundnut genotypes should be based on these traits. Traits like SPAD chlorophyll meter reading present rapid and cost effective screening methods for identifying genotypes with enhanced drought tolerance. In addition to that, the yield attributing traits are less complex and simply inherited.

Therefore, incorporating these traits during selection would lead to great progress in drought tolerance breeding.

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Chapter 5

GENETIC COMPONENTS AND COMBINING ABILITY ANALYSES FOR DROUGHT TOLERANCE AND ASSOCIATED TRAITS AMONG MALAWIAN GROUNDNUT (*Arachis hypogaea L.*) GENOTYPES

ABSTRACT

Several studies have been conducted on use of morpho-physiological traits as selection criteria for drought tolerance improvement in groundnut. However, there is limited information on the genetic control and inheritance of the drought tolerance traits. This study was undertaken to determine the combining ability of parents and mode of gene action controlling the inheritance of drought tolerance traits including relative water content, specific leaf area (SLA), SPAD chlorophyll meter content and yield components. Ten parental genotypes were crossed in a 5 x 5 North Carolina II mating design (NCD II). The resultant twenty-five crosses were advanced to F3 generation and were evaluated under random drought stress field conditions. The experiment was laid out in a randomized complete block design with four replications. Both general combining ability (GCA) effects and specific combining ability (SCA) effects were significant for all the traits, indicating importance of both additive and non-additive gene action in controlling most of the traits. However, additive gene action was more predominant for all the traits studied. Among the male parents, ICGV-SM 02724 and ICGV-SM 94139 were identified as good combiners, whereas among females, CG 7 and ICGV-SM 01721 were good combiners. These parents had good breeding values as evidenced by their high and significant GCA effects. Pendo x ICGV-SM 02724 was identified as a useful cross for developing early maturing varieties. The crosses Pendo x Akwa, ICGV-SM 99555 x ICGV-SM 02724, ICGV – SM 99551 x Baka and ICGV-SM 01721 x ICGV-SM 94139 showed high SCA effects for seed yield, number of filled pods, harvest index and pod yield. These crosses could be selected and be incorporated in breeding programmes for developing drought tolerant genotypes. The breeding procedures also have to be properly amended by delaying selection to later generations especially for these crosses in order to exploit this type of gene action. Alternatively, inter-mating of F2 segregants followed by recurrent selection and pedigree breeding could harness the different types of gene action.

Key word: Combining ability, drought tolerant groundnut, gene action, NCD II.

5.1 Introduction

Groundnut (*Arachis hypogaea* L.) is an important oleaginous food, widely cultivated in tropical and semi-arid regions of Africa including Malawi. In the major part of cultivation regions, groundnut is mostly constrained by severe droughts that cause yield losses approximately up to 70% (Bakht et al., 2010; Khan et al., 2010). The magnitude of yield losses varies depending on timing, intensity and duration of drought coupled with other stress factors such as high irradiance and heat (Nigam, 2014). Irrigation systems do not provide critical solutions for groundnut yield improvement in Malawi as irrigation water is always insufficient to meet normal plant requirements (Pereira et al., 2012). Therefore, breeding for drought tolerance could be the best option to mitigate drought effects and ensure sustained food security for the benefit of resource poor farmers in the semi-arid regions.

Several breeding strategies for drought tolerance in groundnuts have been employed to increase crop productivity in drought prone areas (Santos et al., 2013). However, the behaviour and inheritance of traits associated with drought tolerance in groundnut is likely to be genetically complex, due to the number and arrangement of genes governing quantitative traits (Leal-Bertioli et al., 2012). Some of the serious bottlenecks are the narrow genetic base and the tetraploid and complex nature of the genome of cultivated groundnut (Wang et al., 2011). Selection for drought tolerant groundnut varieties based on morpho-physiological traits has been reported in earlier studies conducted by Vadez, (2014), Karimian et al. (2015) and Aninbon et al. (2015). However, there is scanty information on the type of gene action involved in expression of these traits. John et al. (2011), Agoyi et al. (2016) and Fasahat et al. (2016) reported the importance of understanding the genetic composition and the nature of gene action controlling traits in selection of groundnut parents to be used in a breeding programme.

Combining ability analysis is an effective and quick method that provides information on nature and magnitude of gene action for different qualitative and quantitative traits (Crestani et al., 2012). The method can further provide information on selection of parents based on the performance of their progenies (Ali et al., 2001). In this method, general combining ability (GCA) is associated with additive gene effects and specific combining ability (SCA) is associated with dominance and epistatic gene effects (Rojas and Sprague, 1952). The method has been widely used in improving the quality, yield and disease resistance in different crops (Sibiya et al., 2011; Parkes et al., 2013; Chigeza et al., 2014; Ai et al., 2015; Agoyi et al., 2016).

Several mating designs have been used for evaluation of combining ability in groundnuts (Hariprasanna et al., 2008; Jivani et al., 2009; John et al., 2011; John et al., 2014). However, North Carolina II mating design (NCD II) was reported as the best method for evaluating the combining ability in self-pollinated crops including groundnut (Fasahat et al., 2016). It can effectively estimate the genetic variance components and heritabilities (Singh et al., 2011; Bosworth and Waldbieser, 2014). The objective of this study was to estimate the combining ability and the genetic components of inheritance and deduce the impact of drought stress on yield of 10 groundnut parental lines in a 5 x 5 NCD II mating design. This was done to guide selection strategies for developing high yielding and drought tolerant groundnut lines and to determine the appropriate procedures that could be utilized efficiently in breeding programmes.

5.2 Materials and Methods

5.2.1 Planting material

Twenty-five F3 progenies developed by crossing five male lines of *A. hypogaea* [(ICGV-SM 99551, ICGV-SM 99555, ICGV-SM 01721, CG 7 and Pendo)] and five female lines [(Akwa, Malimba, Baka, ICGV-SM 02724 and ICGV-SM 94139)] in a 5 x 5 NCD II mating scheme suggested by Comstock and Robinson, (1952) were evaluated in this study. The crosses were made in 2015/2016 and 2016/2017 seasons at ICRISAT – Malawi station. The 25 F1 progenies were then selfed to F2 and F3 seeds, to allow maximum gene segregation and at the same time, multiplying seeds for evaluation. To confirm hybrids (successful crosses), plants resembling their parents were rogued out. The progenies and their parents were then evaluated under moisture stressed environment for combining ability and gene action studies. A detailed description of the main characteristics of the 10 parents used in this study is presented in Table 5-1.

5.2.2 Field experiment

A field experiment was carried out at Ngabu Agricultural Research Station – Chikwawa region in southern part of Malawi, located (34^0 53’43.04” E, 16^0 27’28.89” S, at altitude of 110 masl), 425 km south of Chitedze ICRISAT Centre. It is located in the in the lower Shire of southern Malawi, and characterized by warm and dry conditions throughout the year. The site is dominated by a clay loam–vertisol soil type with pH ($CaCl_2$) of 7.12, organic carbon (OC) 1.01%, organic matter (OM) 2.05%, total nitrogen 0.30%, phosphorus (P) 8.27 ppm, potassium (K) 1.00 meq/100g, calcium (Ca) 25.55 meq/100g, magnesium (Mg) 5.45 meq/100g and sodium (Na) 0.48 meq/100g. The experiment was conducted from December 2016 to June 2017 under natural rain-fed conditions. The experiment was laid up in a randomized complete block design with four replications. Seeds were sown in plots consisting of four rows spaced 70 cm apart and 5 m in length, with a 15 cm distance between planting hills in a row.

Table 5-1. The main characteristics of the ten parental lines

Pedigree	Origin	Botanical	Released/year	Recommendation	Reference
Malimba	Malawi	*Spanish*	Malawi (1968).	Recommended for lowland areas and Lakeshore plains altitude of 200 to 300m	-
Baka	India	*Spanish*	Malawi (2001), Mozambique (2002), Uganda (2002) and Zambia (2004).	Early maturity and aphid resistant	Deom et al., (2006)
Akwa	South Africa	*Valencia*	South Africa, (1994).	Early maturity	Merwe and Joubert, (1995).
ICGV-SM-02724	Malawi	*Virginia*	Not released anywhere	Drought tolerance and high yielding	-
ICGV-SM-94139	Malawi	*Spanish*	Not released anywhere	Rosette resistant	-
ICGV-SM-99555	Malawi	*Spanish*	Tanzania, (2009).	Early maturity and rosette resistant	Monyo, (2010).
ICGV-SM-99551	Malawi	*Spanish*	Malawi (2014) and Zimbabwe (2014).	Early maturity and rosette resistant	
CG 7	Malawi	*Virginia*	Malawi (1990), Tanzania (2009), Uganda (1999), Zambia (1990) and Mozambique (2011).	Wide adaptability, high yielding and high oil content	Subrahmanyam et al., (2000)
Pendo	Tanzania	*Spanish*	Tanzania, (2002).	Early maturity	-
ICGV-SM-01721	Malawi	*Virginia*	Tanzania, (2009).	High yielding, rosette resistant	Monyo, (2010).

Two seeds were planted per hill and then seedlings were thinned to one plant per hill at 14 days after emergence. Ten plants from the middle row were selected randomly in each plot and tagged for data collection. Variety JL 24 was grown around the trial as a guard row to avoid damage from animals and border effects. Recommended agronomic and plant protection measures were performed as suggested by Santos et al. (2006).

5.3 Data collection

Relative water content (RWC) was recorded from four leaflets of the third fully developed groundnut leaf from the top of the main stem. Leaves were harvested and transported to the laboratory, where fresh weight (FW) of the leaf was recorded. Afterward, the leaf samples were soaked in distilled water for 8 hours and blotted for surface drying and leaf turgidity weight (TW) was determined. The samples were oven dried at 80^0C until a constant weight was reached and leaf dry weight (DW) was recorded. Relative water content was determined based on formula suggested by Bajji et al., (2001) as follows.

$$RWC\ (\%) = \frac{(Fresh\ weight\ -\ Dry\ weight)}{(Turgidity\ weight\ -\ Dry\ weight)} x\ 100 \ldots\ldots\ldots\ldots\ldots \text{ equation 1}$$

The SPAD Chlorophyll Meter Reading (SCMR) and Specific Leaf Area (SLA) were recorded as suggested by Nigam (2014). The SCMR was measured by handheld SCMR meter (SPAD – 502 Plus, Spectrum Technology, USA) at four leaflets per plant. The leaf samples were then oven dried at 80 ^{0}C until a constant weight was reached and leaf dry weight was measured for determination of specific leaf area that was further calculated based on the equation suggested by Wilson et al. (1999).

$$Specific\ leaf\ area\ (SLA) = \frac{Leaf\ area\ (cm^2)}{Leaf\ dry\ weight\ (g)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ equation 2}$$

After harvest, selected plants were washed to remove the soil particles followed by separating the sample into roots, stem and reproductive structures for measurement. Reproductive parts were separated into mature and immature pods for counting and weight determination after oven drying at 80^OC for 24 hours. The pods were shelled and the hundred seed weight were measured. Roots samples and above ground samples were oven dried at 80^0C for 48 hours, after oven dry root dry weight, shoot dry weight and total dry biomass were determined.

The harvest index (HI) was calculated based on the relationship suggested by Nautiyal et al. (2002).

$$Harvest\ Index(HI) = \frac{Total\ dry\ pod\ mass\ at\ final\ harvest}{Total\ dry\ biomass\ at\ final\ harvest}$$ …….. equation 3

5.4 Data analysis

Data were analyzed using Linear Mixed Model (LMM) with genotypes considered as a fixed effect and replications considered as random effects in GENSTAT 17th Edition (Payne et al., 2014). The genetic variance component was partitioned into general combining ability (GCA) and specific combining ability (SCA) variance according to Dabholker (1992). The linear model used was as follows:

$$Y_{ijkl} = \mu + r_i + g_k + g_l + s_{kl} + \varepsilon_{ijkl}$$ …………………………….. equation 4

Where; Y_{ijkl} = observed value from each experimental unit, μ = general mean, r_i = effect of the i^{th} replication, g_k = GCA effect of the k^{th} female parent, g_l = GCA effect of the i^{th} male parent, s_{kl} = SCA effect of the k^{th} male mated to the l^{th} female and ε_{ijkl} = the residual effect of $ijkl^{th}$ observation.

General combining ability (GCA) effects were estimated as the difference between the grand mean and the average mean of the particular parent in the series of combinations with other parents. The specific combining ability (SCA) effects were estimated as the difference between the predicted means of a particular cross and the observed mean as described by Dabholker (1992). A student's t – test was applied to determine the significance of general and specific combining ability (GCA and SCA) for each of the traits based on the associated standard error of the particular trait.

The estimations of variance components for GCA male, GCA female and SCA were calculated by equating mean squares to their respective expectations, and solving the equations. The narrow sense coefficient of determination (NS–CGD) and broad sense coefficient of determination (BS–CGD) were determined based on the formula given in Equation 5 and 6, respectively as suggested by Ozimati et al. (2014). Baker's ratio components of variance were estimated according to Baker (1978) to determine the relative significance of additive vs. non – additive effects using the formula in Equation 7. The ratio below (X<0.5) indicates predominance of non-additive gene action and predominance of additive gene action when above (X>0.5)

$$\mathrm{NS-CGD}(h^2) = \frac{\sigma_e^{2}\,\mathrm{GCA}i + \sigma_e^{2}\,\mathrm{GCA}j}{\sigma_e^{2}\,\mathrm{GCA}i + \sigma_e^{2}\,GCAj + \sigma_e^{2}\,\mathrm{SCA}ij + \sigma_e^{2}/\mathrm{r}} \quad \text{5 equation}$$

$$\mathrm{BS-CGD}(H^2) = \frac{\sigma_e^{2}\,\mathrm{GCA}i + \sigma_e^{2}\,\mathrm{GCA}j + \sigma_e^{2}\,\mathrm{GCA}ij}{\sigma_e^{2}\,\mathrm{GCA}i + \sigma_e^{2}\,\mathrm{GCA}j + \sigma_e^{2}\,\mathrm{SCA}ij + \sigma_e^{2}/\mathrm{r}} \quad \text{6 equation}$$

$$\text{Baker's ratio} = \frac{\sigma_e^{2}\,\mathrm{GCA}i + \sigma_e^{2}\,\mathrm{GCA}j}{\sigma_e^{2}\,\mathrm{GCA}i + \sigma_e^{2}\,\mathrm{GCA}j + \sigma_e^{2}\,\mathrm{SCA}ij} \quad \text{7 equation}$$

Where; σ^{2} GCA*i* is the GCA effect of parent *i,* σ^{2} GCA*j* is the GCA effect of the parent *j,* σ_e^{2} `SCA*i* is the SCA effect of cross *i x j,* and $\sigma^2{}_e/r$ is the mean square of the effective error.

5.5 Results

5.5.1 Combining ability variances and genetic parameters for drought tolerance traits

The analysis of variance for evaluated morphological and physiological traits among groundnut genotypes are summarized in Table 5-2. The analysis of variance showed significant (P<0.001) difference among genotypes for all measured traits. The general combining ability (GCA) mean squares for male and female parents were significant (P<0.05) in all measured traits except for shelling percentage and hundred seed weight in GCA female. Specific combining ability (SCA) mean squares were also significant (P<0.05) in all studied traits except for SPAD chlorophyll meter reading, hundred seed weight, shelling percentage and relative water content.

The results showed that σ_e^{2}GCA for males were high compared with σ_e^{2}GCA for females in almost all measured traits, except for days to maturity (DM) and relative water content (RWC). The σ_e^{2}GCA for males were also high as compared with σ_e^{2}SCA in all measured traits except for days to maturity. Heritability estimates indicated that both narrow and broad sense heritability were high; 0.84 and 0.96 for pod yield, 0.75 and 0.88 for number of filled pod, 0.82 and 1.00 for harvest index, 0.80 and 0.99 for biomass weight, 0.60 and 0.68 for days to maturity and 0.80 and 0.92 for seed yield, respectively. Heritability estimates were moderate; 0.47 and 0.61 for relative water content, 0.46 and 0.48 for SPAD chlorophyll meter reading and 0.59 and 0.62 for hundred seed weight, respectively.

Table 5-2.Analysis of variance for combining ability, variance components and heritability estimates for drought tolerance related physiological and morphological traits.

SOV	DF	DM	SY	SCMR	HSW	SHP	SLA	RWC	BM	HI	FP	PY
Replication	3	10.20	0.14	62.67	11.36	41.01	45.80	37.85	1.90	0.00	3.93	6.43
Genotypes	24	825.30***	113.43***	104.57***	257.70***	160.84***	1167.20***	42.39***	586.07***	0.02***	212.82***	164.71***
GCA male	4	52.24*	80.97***	102.12***	298.17***	88.73**	1004.30***	9.985	225.10***	0.01***	102.94***	117.01***
GCA Female	4	705.30***	25.85***	32.91*	36.26	38.48	191.20*	19.84**	224.10***	0.00***	86.80***	39.65***
SCA	16	96.14***	15.83***	5.45	13.05	28.50	139.30*	8.51	107.50***	0.00***	32.37***	22.60***
Error		20.19	3.36	12.34	14.57	19.43	67.65	4.95	1.71	0.00	5.55	2.61
Variances												
σ^2 GCA male		2.5874	24.1305	8.2758	20.4647	4.5678	14.8455	2.0172	131.2919	25.0527	18.5477	44.8572
σ^2 GCA female		34.9331	7.7038	2.6665	2.4887	1.9810	2.8263	4.0085	130.7087	10.6671	15.6396	15.2003
σ^2 SCA		4.7618	4.7176	0.4417	0.8957	1.4672	2.0591	1.7190	62.7005	7.9116	5.8324	8.6640
σ^2 Error		20.1900	3.3600	12.3400	14.5700	19.4300	67.6500	4.9500	1.7100	0.0005	5.5500	2.6100
Heritability												
NS - CGD (h^2)		0.6006	0.7977	0.4612	0.5974	0.2386	0.2022	0.4747	0.8027	0.8187	0.7502	0.8420
BS - CGD (H^2)		0.6768	0.9159	0.4799	0.6208	0.2921	0.2258	0.6101	0.9947	1.0000	0.8782	0.9634
Baker's ratio (BR)		0.8874	0.8709	0.9612	0.9624	0.8170	0.8956	0.7780	0.8069	0.8187	0.8543	0.8739

* Significant at 0.05, ** significant at 0.01, *** significant at 0.001.

DM = Days to maturity, SY = Seed yield, SCMR = SPAD chlorophyll meter reading, HSW = Hundred seed weight, SHP = Shelling percentage, SLA = Specific leaf area, RWC = Relative water content, BM = Biomass, HI = Harvest index, FP = Number of field pod, PY = Pod yield, GCA = General combing ability, SCA = Specific combing ability, F = Female, M = Male. NS-CGD (h^2) = Narrow sense coefficient of genetic determination, BS-CGD (H^2) = Broad sense coefficient of genetic determination, DF = Degree of freedom.

The low heritability estimates for narrow and broad sense were recorded for shelling percentage (0.24 and 0.29) and specific leaf area (0.20 and 0.23), respectively. In addition, the results showed a high Baker's ratio close to unity ranging from 0.78 to 0.96 for all measured traits.

5.5.2 General combining ability effects for parental genotypes

The general combining ability (GCA) effects for both male and female parents for the evaluated genotypes are presented in Table 5-3. Male parents, ICGV-SM 02724 and ICGV-SM 94139 had the highest significant (P<0.05) positive GCA effects for all measured traits except for specific leaf area. Malimba had positive GCA effects in all measured traits except for days to maturity and shelling percentage. Male parent Baka had significant (P<0.05) positive GCA effects for all measured traits except in days to maturity, shelling percentage and relative water content. Parent Akwa had negative GCA effects in all studied traits except for shelling percentage. The estimates of GCA effects for female parents showed that parents, ICGV – SM 01721 and CG 7 had the highest, positive and highly significant (P<0.001) CGA effects for all measured traits except for shelling percentage, specific leaf area and SPAD chlorophyll meter reading (Table 5-4). The results also showed that, parents ICGV – SM 99551 had positive and significant (P<0.05) GCA effects in all measured traits except for specific leaf area, shelling percentage, SPAD chlorophyll meter reading and days to maturity. For female parent Pendo, the significant (P<0.05) positive GCA effects were observed for number of filled pods, harvest index and specific leaf area; whereas non-significant positive GCA effects were observed for SPAD chlorophyll meter reading, relative water content and pod yield, while the rest of the traits displayed negative GCA effects. Female parents, ICGV – SM 99555 registered negative GCA effects for all studied traits except for shelling percentage.

5.5.3 Specific combining ability effects for the crosses

The estimates of the SCA effects for yield and yield component traits among groundnuts crosses are presented in Table 5-5. The SCA effects for the evaluated traits varied significantly among genotypes. Highly significant (P<0.001) negative SCA effects for days to maturity were recorded for crosses Pendo x ICGV – SM 02724 (-16.84) whereas significant (P<0.001) positive effects for this trait were observed for Pendo x Malimba (21.34) and ICGV – SM 99555 x ICGV – SM 02724 (18.16). Significant (P<0.001) positive SCA effects for biomass were recorded for crosses Pendo x Malimba (15.80), ICGV – SM 01721 x Malimba (14.20), ICGV – SM 99555 x Akwa (7.90),

Table 5-3.Estimates of general combining ability effects of parents for physiological and morphological traits under moisture stress conditions

SOV	DM	SY	SCMR	HSW	SHP	SLA	RWC	BM	HI	FP	PY
Male parents											
Malimba	-1.768	0.88*	0.21	13.05***	-3.76***	11.49***	0.42	4.41***	0.023***	5.77***	2.26***
ICGV-SM 02724	6.41***	9.22***	3.55***	17.26***	3.68***	-12.91***	2.51***	11.41***	0.12***	9.67***	11.59***
Baka	-0.39	1.99**	5.23***	10.58***	-6.02***	7.29***	-0.96*	2.10***	0.07***	4.02***	4.53***
ICGV-SM 94139	2.21*	5.56***	10.46***	14.23***	2.98**	24.93***	1.75***	14.72***	0.05***	9.42***	7.00***
Akwa	-0.31	-0.84*	-0.93	-2.63***	0.1488	-1.47	-0.18	-1.55***	-0.01**	-1.37**	-1.21***
Female parents											
ICGV-SM 99551	-1.96*	3.01***	0.20	4.01***	-0.93	0.76	1.80***	0.56*	0.08***	4.90***	5.13***
CG 7	20.92***	2.33***	1.39	1.93***	-1.11	13.02***	4.37***	7.36***	0.03***	8.95***	3.83***
Pendo	-1.90*	-0.73	0.75	-1.51	-3.69***	8.20***	0.84	-1.26***	0.02***	2.90***	0.28
ICGV-SM 01721	18.64***	4.55***	6.03***	4.65***	3.95***	-0.26	3.60***	14.18***	0.03***	8.35***	5.38***
ICGV-SM 99555	-1.7	-0.44	-0.40	-0.43	0.09	-1.04	-0.51	-0.99***	-0.01	-1.20	-0.69*

* Significant at 0.05, ****** significant at 0.01, *** significant at 0.001 level.

DM = Days to maturity, SY = Seed yield, SCMR = SPAD chlorophyll meter reading, HSW = Hundred seed weight, SHP = Shelling percentage, SLA = Specific leaf area, RWC = Relative water content, BM = Biomass, FP = Number of filled pod, PY = Pod yield, HI = Harvest index.

Table 5-4. **Estimates of specific combining ability effects of progenies for physiological and morphological traits under moisture stress conditions**

CROSSES	DM	GY	SCMR	HSW	SHP	SLA	RWC	BM	HI	FP	PY
ICGV – SM 99555 x Akwa	-5.32	-1.12	1.51	4.87	-7.28	3.90	-2.52	7.90***	-0.04	-1.82	-0.61
ICGV – SM 99551 x Akwa	8.44	-1.13	-2.35	0.47	4.04	4.50	2.39	-6.60***	-0.02	-1.67	-2.55
CG 7 x Akwa	2.86	0.07	-1.48	0.25	3.16	3.50	2.78	3.00*	-0.01	0.53	-0.41
Pendo x Akwa	-7.12	4.55*	3.40	-4.67	4.40	-5.50	0.87	6.80***	0.05*	5.58*	5.41**
ICGV – SM 01721 x Akwa	1.14	-2.37	-1.08	-0.92	-4.32	-6.40	-3.51	-11.10***	0.02	-2.62	-1.84
ICGV – SM 99555 x Malimba	-6.06	0.27	0.32	-3.15	4.93	-9.60	0.77	-6.30***	0.01	0.03	-0.34
ICGV – SM 99551 x Malimba	-4.60	-0.81	-1.4	3.13	-1.65	-11.60	-2.10	-12.00***	0.03	-5.32*	-1.20
CG 7 x Malimba	-2.68	-1.62	4.13	-2.13	-7.59	7.90	-2.26	-11.80***	0.03	-0.12	-0.65
Pendo x Malimba	21.34***	1.90	-2.15	3.98	2.87	0.50	1.02	15.80***	-0.03	2.68	1.74
ICGV – SM 01721 x Malimba	-8.00	0.26	-0.91	-1.82	1.44	12.90	2.56	14.20***	-0.03	2.73	0.45
ICGV-SM 99555 x ICGV-SM 02724	18.16***	5.12**	2.09	0.35	5.28	11.50	0.26	3.80**	0.07***	5.63*	5.77***
ICGV-SM 99551 x ICGV-SM 02724	2.72	-0.94	1.24	2.7	1.57	7.40	2.12	3.70**	-0.04*	5.53*	-1.97
CG 7 x ICGV – SM 02724	-0.86	-0.28	-1.81	-4.21	2.58	12.90	0.29	-0.70	-0.01	5.73*	-0.72
Pendo x ICGV – SM 02724	-16.84***	-6.53***	0.02	1.71	-10.46*	-14.30	-5.08*	-7.50**	-0.06**	-12.47***	-6.32***
ICGV-SM 01721 x ICGV-SM 02724	-3.18	2.64	-1.54	-0.56	1.01	-17.40*	2.40	0.60	0.03	-4.42	3.26*
ICGV – SM 99555 x Baka	-3.44	-0.15	-1.54	0.77	-2.19	-1.50	2.87	-0.30	0.00	0.28	0.05
ICGV – SM 99551 x Baka	-1.18	6.44***	1.11	-4.02	1.54	-9.40	-0.88	4.40**	0.12***	-3.07	9.38***
CG 7 x Baka	1.94	-1.67	-0.06	5.6	-3.22	-11.10	-2.94	-2.00	-0.02	-1.62	-1.64
Pendo x Baka	-3.54	0.81	0.56	-0.61	4.11	11.60	3.68	1.50	-0.02	6.43**	-0.24
ICGV – SM 01721 x Baka	6.22	-5.43**	-0.07	-1.73	-0.24	10.50	-2.73	-3.60**	-0.09***	-2.02	-7.53***
ICGV-SM 99555 x ICGV-SM 94139	-3.34	-4.12*	-2.38	-2.84	-0.75	-4.20	-1.38	-4.90***	-0.05*	-4.12	-4.87**
ICGV-SM 99551 x ICGV-SM 94139	-5.38	-3.56	1.4	-2.27	-5.51	9.20	-1.53	10.40***	-0.09***	4.53	-3.64*
CG 7 x ICGV – SM 94139	-1.26	3.5	-0.79	0.49	5.06	-13.10	2.13	11.30***	0.01	-4.52	3.44*
Pendo x ICGV – SM 94139	6.16	-0.72	-1.83	-0.40	-0.93	7.70	-0.49	-16.70***	0.06*	-2.22	-0.59
ICGV-SM 01721 x ICGV-SM 94139	3.82	4.90**	3.59	5.02	2.11	0.40	1.28	-0.10	0.07**	6.33**	5.66***

*, **, *** significant at 0.05, 0.01, and 0.001 respectively.

DM = Days to maturity, SY = Seed yield, SCMR = SPAD chlorophyll meter reading, HSW = Hundred seed weight, SHP = Shelling percentage, SLA = Specific leaf area, RWC = Relative water content, BM = Biomass, FP = Number of filled pod, PY = Pod yield, HI = Harvest index.

ICGV – SM 99551 x ICGV – SM 94139 (10.40) and CG 7 x ICGV – SM 94139 (11.30). The highest significant (P<0.05) positive SCA effects for seed yield were observed for crosses Pendo x Akwa (4.55), ICGV-SM 99555 x ICGV-SM 02724 (5.12), ICGV – SM 99551 x Baka (6.44) and ICGV-SM 01721 x ICGV-SM 94139 (4.90). In addition, crosses Pendo x Akwa, ICGV – SM 99551 x Baka, ICGV-SM 99555 x ICGV-SM 02724 and ICGV-SM 01721 x ICGV-SM 94139 had high significant (P<0.05) positive SCA effects for harvest index, number of filled pods, and pod yield. Furthermore, significant (P<0.05) negative SCA effects for specific leaf area were observed for cross ICGV-SM 01721 x ICGV-SM 02724 with a value of -17.40.

5.6 Discussion

The genotypes varied (P<0.001) significantly for all studied traits suggesting the existence of genetic variability among genotypes. The significance of general combining ability (GCA) mean squares (P<0.001) for both male and female parents indicated high additive gene effects, implying that the performance of the progenies can be easily predicted based on the parental performance and the GCA effects (Baker, 1978; Bernado, 2010). The significant differences of specific combining ability (SCA) mean squares (P<0.001) for all studied traits also suggested that non-additive gene action played a role in the inheritance of these traits. However, the magnitude of GCA effects was higher than the SCA effects indicating additive gene action was more important than non-additive. Swe and Branch (1986), Holbrook (1990), John et al. (2011) and Alam et al. (2013) reported similar results for GCA and SCA effects.

The variance components, σ_e^2 GCA female, σ_e^2 GCA male and σ_e^2 SCA were significant for almost all studied traits. However, the σ_e^2 GCA male and σ_e^2 GCA female were higher than the σ_e^2 SCA suggesting that there was an effective transmission of heredity material between donor and recipient parents (Hallauer et al., 2010; Alam et al., 2013). Consistent with this, the high variances for σ_e^2 GCA for males and females indicated that most of the variances were due to additive gene action as suggested by Upadhyaya et al. (1992). This explains the significant contribution of additive genes effects controlling these traits and that drought resistance in groundnut varieties may be improved through selection in early generations by focusing on SPAD chlorophyll meter reading, hundred seed weight, shelling percentage and relative water content as proposed by Bernado (2010). In line with this, the SCA was significant for days to maturity, seed yield, specific leaf area, biomass, harvest index, number of filled pod and pod yield indicating the importance of non – additive gene action in expression of the traits. These traits can be improved through selection of the crosses with high SCA effects and advancing them to later filial generations through selection. The significant GCA and SCA effects reported in this study concurred with earlier findings reported by John et al. (2012), Alam et al. (2013) and John et al. (2014).

High heritability estimates (broad and narrow sense) for days to maturity, seed yield, biomass, harvest index, number of filled pod and pod yield indicated that a large proportion of the phenotypic variations was a result of genetic effects, therefore performing selection based on performances in different environments would guarantee a substantial genetic gain (Acquaah, 2012; Bi et al., 2015). In addition, the Baker's ratios were close to unity for these traits suggesting the high influence of additive gene effects, and thus high predictability of progenies from parental performance. The low heritability estimates for shelling percentage, specific leaf area and SPAD chlorophyll meter reading showed that the traits were influenced by both genetic and environmental effects, with t relatively low genetic effects (Acquaah, 2012). Therefore, genetic gain based on these traits might be difficult to achieve. However, selection based on multi environments trials may result in some improvement if G x E effects are accounted for (Holland et al., 2003).

Significant positive and negative GCA effects for days to maturity were observed for male and female parents. The male parents ICGV-SM 02724, ICGV-SM 94139 and female parents CG 7, ICGV-SM 01721 had the highest positive significant GCA effects suggesting that they are good combiners for developing late maturity varieties. On the other hand, the high negative GCA effects for male parent Malimba and female parents ICGV – SM 99551 and Pendo for days to maturity, indicated that these parents are good combiners for developing early maturity varieties. Therefore, there is high possibility of improving early maturity groundnut cultivars based on these parents as an avoidance mechanism for terminal drought stress. Rekha et al. (2009), Savithramma et al. (2010) and John et al. (2011) reported similar results. The results showed that, ICGV – SM 02724, ICGV – SM 94139, Malimba, and Baka were good combiners for seed yield, hundred seed weight, biomass, harvest index, number of filled pods and pod yield for male parents. The good combiners for female parents for these traits were ICGV – SM 01721 and CG7. These parents can thus be incorporated into breeding programmes for developing high yielding cultivars under drought conditions. Jivan et al. (2009) reported similar results for hundred seed weight, Swe and Branch (1986) for biomass, Hariprasad (1990) for number of filled pods and Ganesan et al. (2010) for pod yield.

The analysis of GCA effects for physiological traits revealed that the genotypes differed significantly for all studied traits. The males, ICGV – SM 02724 and ICGV – SM 94139 were identified as good combiners for SPAD chlorophyll meter reading and relative water content, whereas the good combiner female parents for these traits were CG 7 and ICGV – SM 01721. John et al. (2014) reported similar results for SPAD chlorophyll meter reading in groundnut for drought tolerance traits. Mohyaji et al. (2014) have reported related studies for relative water content in sunflower, Goyal et al. (2013) in sorghum and Golparvar (2012) in bread wheat under drought conditions. The GCA estimates revealed that, ICGV – SM 02724 for males and ICGV – SM 99555 for female parents were the best combiners for specific leaf area. The genotypes had low specific leaf area value indicating high photosynthetic capacity per unit leaf area and thus greater assimilation under drought stress (Nageswara et al., 2001; Songsri et al., 2008). These results support the findings of Vasanthi et al. (2004), Venkateswarlu et al. (2007) and John et al. (2014) for specific leaf area in groundnuts.

Estimates of the SCA effects for DM among groundnuts crosses showed that Pendo x ICGV – SM 02724 had the most desirable SCA effects for developing early maturity cultivars as evidenced from its high significant negative SCA effects. In agreement with these findings, John et al. (2011) and John et al. (2014) also reported the importance of both additive and non-additive gene action for this trait. This cross is recommended for further selection in breeding for early maturity varieties. Significant positive SCA effects for biomass were recorded in crosses ICGV – SM 99555 x Akwa, Pendo x Malimba, ICGV – SM 01721 x Malimba and CG 7 x ICGV – SM 94139. Swe and Branch (1986) also reported the significant SCA effects for biomass. In addition, the study by Dwivedi et al. (1998) on combining ability for biomass and harvest index under short and long day conditions in groundnut indicated that biomass was controlled by both additive and non-additive gene action.

Yield is a complex trait that is determined by various yield – attributing traits and is conditioned by both additive and non-additive gene actions (Bhattarai et al., 2016). From the results, both additive and non-additive gene action were important in the inheritance of seed yield, harvest index, number of filled pods, and pod yield. The results showed that the best crosses with desirable direction for SCA effects for these traits were Pendo x Akwa, ICGV-SM 99555 x ICGV-SM 02724, ICGV – SM 99551 x Baka and ICGV-SM 01721 x ICGV-SM 94139. The crosses were derived from poor by poor cross combinations of parents indicating the role of inter-allelic interactions in expressing the higher SCA effects on these traits (John et al., 2014). Similar observation was reported in earlier studies conducted by Savithramma et al. (2010), Mothilal and Ezhil (2010), John et al. (2011) and Alam et al. (2013). In addition, cross ICGV-SM 01721 x ICGV-SM 02724 was the best cross for specific leaf area (SLA) due to its highest significant negative SCA effects. The results are supported by findings of John et al. (2014) who reported the importance of both non-additive and additive gene action in the inheritance of specific leaf area.

Generally, significant SCA effects portrayed by some crosses for the mentioned traits indicated the importance of non-additive gene action in controlling the inheritance of the traits. Therefore, the breeding method that can exploit both additive and non-additive gene action should be implemented. On the other hand, non-significant positive SCA effects in a desirable direction were further noted for SPAD chlorophyll meter reading, relative water content, shelling percentage and hundred seed weight. However, SCA effects were not significant for these traits suggesting additive gene action was more prevalent in the inheritance of these traits. Selection in early generations can be fulfilled in their progenies as proposed by Falconer and Mackay (1996) and Acquaah (2012).

5.7 Conclusion and recommendations

The mean squares for GCA and SCA effects and their interactions portrayed significant variations among parents and crosses, respectively. The significant positive GCA estimates coupled with high heritability observed for most of the measured traits indicated the significance of additive gene action in controlling the inheritance of these traits. Therefore, the traits may be improved through selection in early generations. Male parents identified as good combiners for various attributes were ICGV – SM 02724 and ICGV – SM 94139, whilst good female combiners were CG 7 and ICGV – SM 01721. In addition, male parent Malimba and female parents ICGV – SM 99551 and Pendo, were the best parents for developing short duration cultivars. The parents that showed significant GCA effects in the desired direction for most measured traits are good transmitters of additive gene effects, hence are best parents for use in a breeding programmes for drought tolerance improvement. The significant SCA effects for seed yield, number of filled pods, harvest index and pod yield were observed on Pendo x Akwa, ICGV-SM 99555 x ICGV-SM 02724, ICGV – SM 99551 x Baka and ICGV-SM 01721 x ICGV-SM 94139. Therefore, to exploit both types of gene action in these traits, breeding procedures have to be properly modified by postponing selection to later generations (Baker, 1978). Holbrook (1990) suggested that significance of SCA at F3 is due to additive x additive epistatic gene effects that can be fixed at homozygosity level. Alternatively, intermating of F2 segregants followed by recurrent selection and pedigree breeding can harness the different modes of gene action.

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Chapter 6

GENERAL OVERVIEW OF THE STUDY AND IMPLICATION TO PLANT BREEDING

6.1 Introduction

Drought due to inadequate and highly variable rainfall has been reported as one of the major factors causing low groundnuts productivity in Malawi (Simtowe, 2009). The effects of drought stress on the groundnuts are expressed in various morphological, physiological, biochemical and genetic changes on the plant (Sanchez et al., 2010). The stress affects different aspects of plant growth and development and finally crop yields. The severity of the drought damage depends on the duration of this stress and varies with growth stage of the crop (Nigam, 2014). Groundnut response to drought stress is genotype-specific and varies with growth stage of the crop. Understanding of trait responses under drought stress environments is important in order to design a breeding programme and develop improved cultivars with enhanced drought tolerance.

Studies have shown that groundnut genotypes differ in sensitivity to drought; however, reproductive stages are more sensitive to stresses (Sanchez et al., 2010; Nigam, 2014; Akbar et al., 2017). Limited studies are available on genotypic response of groundnut genotypes to moisture stress conditions. Therefore, understanding of genotypic response based on morpho – physiological adaptive traits for drought tolerance is important for progress of genetic enhancement of groundnut and would help in development of new varieties with drought tolerance.

The current study was undertaken with the following objectives; (i) to determine to determine the effect of moisture stress on the growth performance of groundnut genotypes in respect of morpho-physiological traits, (ii) to identify the relevant traits related to drought tolerance and their relationship to yield, and (iii) to investigate the genetic variation existing among genotypes and to estimate the relative importance of additive and non-additive gene action in controlling the inheritance of drought tolerance traits. Twenty-five genotypes from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Malawi were evaluated under field conditions at the drought-testing site of Ngabu Agricultural Research Station during 2016/17 cropping season.

6.2 Summary of findings

The results indicated high GCV, GA, GAM and heritability for number of filled pods, biomass, seed yield, pod yield and relative water content. This indicated the existence of extensive genetic variations for these traits among the genotypes tested (Padmaja et al. 2013). Seed yield was highly significant and positively correlated with SPAD chlorophyll meter reading, hundred seed weight, shelling percentage, relative water content, biomass, harvest index and days to maturity. Therefore, selection based on these traits would be effective for improving seed yield under drought conditions (Sadeghi and Seyyed. 2012). Furthermore, path analysis showed that harvest index, biomass, shelling percentage, relative water content, SPAD chlorophyll meter reading and days to maturity had the highest direct and indirect effects on seed yield, suggesting that selection based on these traits would result in genetic gain toward groundnut yield improvement under drought conditions (Shoba et al., 2012).

General combining ability mean squares were significant at different levels for all studied traits indicating the importance of additive gene action (Baker, 1978; Bernado, 2010). Specific combining ability mean squares were also significant for some traits like days to maturity, seed yield, biomass, harvest index, number of filled pods and pod yield indicating importance of non-additive gene action for these traits (Alam et al., 2013). This suggests that both additive and non-additive gene action were important in controlling the majority of traits. However, additive gene action was more predominant for all traits studied. Among male parents, ICGV-SM 02724 and ICGV-SM 94139 were identified as good combiners, whereas among females, CG 7 and ICGV-SM 01721 were good combiners. These parents have excellent breeding values as evidenced by their high and significant GCA effects. The cross, Pendo x ICGV-SM 02724 was identified as potentially useful for developing early maturity varieties. Pendo x Akwa, ICGV – SM99555 x ICGV-SM 02724, ICGV – SM 99551 x Baka and ICGV-SM 01721 x ICGV-SM 94139 recorded significant SCA for seed yield, biomass, harvest index, number of filled pod and pod yield. These crosses could be selected and incorporated into the breeding pipeline for developing drought tolerant cultivars.

6.3 General recommendations

High-yielding cultivars that continue to produce well under drought conditions are a priority to enable stability of production. Previous studies have reported the importance of physiological traits that are influenced during drought conditions, such as relative water content, leaf water potential, stomatal resistance, specific leaf area rate of transpiration, SPAD chlorophyll meter reading, leaf temperature and canopy temperature. Several researchers have used these traits as criteria to select groundnut genotypes that are tolerant to drought (Pereira et al., 2012). In the current study, only three physiological traits were used (SLA, RWC and SCMR) accompanied by agronomical traits to identify seven groundnut genotypes tolerant to moisture stress conditions. However, the current study was undertaken in a single year and location;

therefore, it is recommended that further studies to evaluate these genotypes be conducted by targeting more drought testing sites for further validation.

Among the physiological traits studied, SPAD chlorophyll meter reading was found to be a complementary trait to drought tolerance, which is easy to measure and cost effective, and can be used to identify superior genotypes under large-scale breeding programmes. Therefore, it is recommended that more studies be done to validate the use of this trait in screening for drought tolerance in groundnut by focussing on the parents that have good expression for the trait. The study also identified ICGV – SM 02724 and ICGV – SM 94139 as good combiners for male parents, whilst CG 7 and ICGV – SM 01721 were identified as good female combiners. In addition, male parent Malimba, female parents ICGV – SM 99551 and Pendo showed good general combining ability for developing short duration cultivars. Therefore, these genotypes would be beneficial if used in the breeding programmes.

Drought stress occurs along with multiple combinations of stresses like heat especially under field conditions. The maximum temperatures recorded under field conditions ranged from 20^0C to 36^0C, which had an impact on the growth performance of the crop. The response of groundnut to a combination of stresses deserve much more attention. On the other hand, the response of groundnut to multiple stresses cannot be inferred from the response of individual stress. Therefore, it is important to test the newly developed varieties against multiple stresses and conduct extensive field studies under diverse environments to evaluate their tolerance to drought.

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