Evaluation of Diverse Cowpea (*Vigna unguiculata* [L.] Walp.) Germplasm for Field Performance and Drought Tolerance

By

Nkoana K.D
15013231

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Department of Plant Production

School of Agriculture
University of Venda
Thohoyandou, South Africa

Supervisor: Prof. E.T. Gwata
Co-Supervisor: Dr. A.S. Gerrano

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ABSTRACT

Cowpea (*Vigna unguiculata*), a nutritious food legume with most edible parts, has the potential to improve the food and nutritional security particularly in sub-Saharan Africa. Despite the drought tolerant nature of cowpea, smallholder famers suffer low yield return in most cowpea production regions of South Africa due to unimproved varieties and drought stress that is often aggravated by high temperatures. High yielding cowpea genotypes that tolerate moisture stress will serve as future potential parents in cowpea breeding programmes for improvement of high yield and drought tolerance within the species. Therefore, this study was conducted to evaluate the agronomic performance and to determine the variability in response to moisture stress among cowpea genotypes in South Africa. The study consisted of both field experiment (Experiment # 1) and greenhouse experiment (Experiment # 2). In experiment # 1, 28 cowpea genotypes were evaluated for field (agronomic) performance based on 50% flowering, hundred seed weight and grain yield among other indicators. The experiment was arranged in a randomized complete block design (RCBD) replicated three times at the Agricultural Research Council (ARC), Roodeplaat experimental research farm in 2015/2016 summer growing season. Similarly, in experiment # 2, 28 selected cowpea genotypes including two controls viz. IT96D-602 (drought tolerant) and TVU7778 (susceptible to drought) were evaluated for drought tolerance in the drought screening house using plastic box evaluation method in January, 2017. The genotypes were subjected to water stress treatment to determine leaf wilting index, relative water content and proline content followed by re-watering to determine genotype(s) with ability to recover from drought stress. Analyses of variance showed that there were highly significant differences among the genotypes for most phenotypic and physiological traits studied indicating the present of a wide genetic variability among the cowpea genotypes. Results from experiment # 1, revealed the potential of improving cowpea for traits of interest. The highest grain yield was obtained from Cwp4 (2624.89 kg/ha), while Cwp1 (264.18 kg/ha) produced the lowest grain yield. However, Cwp1 outperformed other genotypes with the dry weight per plant (2380.33 g).

The principal component analysis also showed that Acc1168, Glenda, L-cwp3 and Acc5352 were the most phenotypically distinct genotypes which are desirable for crosses with the potential for improving traits of interest from transgressive segregations. Results of the greenhouse experiment (experiment # 2) showed highly significant variation in response to moisture stress among the cowpea genotypes for the selected physiological traits except for leaf wilting index at week two of drought stress. Stem greenness and recovery appeared to be a reliable indicator of drought tolerant genotypes which was observed in Acc1257, Acc2355, Acc1168, IT96D-602 and Acc5352 which also exhibited highest and lowest values for relative water content, proline content and leaf wilting index, respectively. Correlation between relative water content, leaf wilting index and proline content with stem greenness indicated that such traits were significant in discriminating among drought tolerant and susceptible cowpea genotypes at vegetative stage. The local drought tolerant genotype (Acc1168) which produced relatively high grain yield and earliest genotype (Acc5352) could be recommended to growers for their suitability in rainfed cowpea production for most production regions of South Africa. The Lack of information on the grain yield potential under drought stress in field conditions indicate a need for field validation of drought tolerant genotypes. The genotypes used in the study presented an ample genetic variability among cowpea genotypes for drought tolerant, yield and yield component traits, which indicate the potential for further exploitation of genetic potential for improved yield and drought tolerance in South Africa.

Key words: Yield, genetic variability, path analysis, water, heritability, physiological response, growers.

DEDICATION

This work is dedicated to my parents; the mother, Nare Reginah Nkoana, and the father, Ngoako Edward Motadi.

DECLARATION

I, ____ hereby declare that the research submitted for the degree Master of Science in Agriculture at University of Venda is my own original work and has not previously been submitted to this or any other university. I further declare, that the source cited or quoted herein has been dully acknowledged by means of complete list of references.

Signature………………………… Date……………………………………

(Candidate: K.D. Nkoana)

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

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Supervisor: Prof. E.T. Gwata

…………………………………………………………………………………………

Co-Supervisor: Dr. A.S. Gerrano (Agricultural Research Council – Vegetable and Ornamental Plants)

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List of Abbreviations

ARC	- Agricultural Research Council
CWR	- Crop wild relatives
D50	- Days to 50% flowering
D95	- Days to 95% maturity
DAFF	- Department of Agriculture, Forestry and Fisheries
DAP	- Days after planting
FAO	- Food and Agriculture Organization
FAOSTAT	- Food and Agriculture Organization Statistical database
GCV	- Genotypic coefficient of variation
GM	- Grand mean
GY	- Total grain yield
GYP	- Grain yield per plant
h^2bs	- Broad sense heritability
HSWT	- Hundred seed weight
IBPGR	- International Board for Plant Genetic Resource
IITA	- International Institute of Tropical Agriculture
LAI	- Leaf area index
LPC	- Leaf proline content
LSD	- Least significant difference
LWI	- Leaf wilting index
MS_e	- Error mean squares
MS_g	- Genotype mean squares
NB	- Number of primary branches
NIP	- Number of internode per plant
NPGRC	- National Plant Genetic Resource Centre
NPP	- Number of pods per plant
NSPP	- Number of seed per pod

PC1	- First principal component
PC2	- Second principal component
PCA	- Principal component analysis
PCV	- Phenotypic coefficient of variation
PGR	- Plant genetic resource
PH	- Plant height
PODL	- Pod length
PWT	- Dry weight per plant
RG2	- Regrowth
ROS	- Reactive oxygen species
RWC	- Relative water content
SACG	- South African Child Gauge
STG2	- Stem greenness
σ^2g	- Genotypic variance
σ^2p	- Phenotypic variance

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1.0 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

Cowpea is an important food legume crop that is cultivated across the world, especially in hot and dry environmental conditions. Cowpea exhibit a diverse crop morphology than most crops which makes cowpea a multipurpose crop for human consumption, livestock feeding and income generation from most edible and nutritious parts harvested at different developmental stages (Singh, 2005). As a drought tolerant crop, cowpea is produced in dry environments to mitigate the risk of crop failure under mixed cropping with cereals. Cowpea is considered an integral part of cropping system with a less developed value chain to suppress its potential for improving the food and nutrition security in South Africa and beyond.

Cowpea is produced throughout the world including Europe, Asia, United State of America and many parts of Africa, which account for about 60 percent of the global production (IITA, 2015). This signifies that cowpea is an important food legume in Africa. In South Africa, substantial cowpea production is realized in North West, Mpumalanga, Limpopo and Kwa-Zulu Natal provinces predominately for subsistence purpose in the smallholder sector (Asiwe, 2009). Most smallholder farmers allocate cowpea production on a small area due to low yield return of less than 500 kg/ha. Production of cowpea is curtailed by several constraints, which include unimproved varieties, drought, lack of market, poor agronomic practices, pest and diseases. However, drought and unimproved varieties are the most important production limiting constrains of cowpea in environment characterized by low and erratic rainfall, where production of cowpea is preferred under little or no irrigation to avert the risk of crop failure (Timko and Singh, 2008). Switching to cultivation of inherently climate resilience crop such as cowpea form an adaptation strategy to hot and dry agro-ecological zones confounded by low and poorly distributed rainfall that results in drought stress that affect the performance of most crop plants (Kurukulasuriya and Mendelsohn, 2008).

1.2 Problem statement

The variability in drought response among local and exotic cowpea has not been determined adequately in South Africa. In addition, the agronomic performance of the germplasm in the country has not been evaluated sufficiently and little information is available. It remains unclear whether the exotic germplasm outperform the local genotypes in the country.

1.3 Rationale of the study

Cowpea is a useful food and nutritional legume crop in many parts of the world. In South Africa, the determination of superior cowpea genotypes that tolerate moisture stress will provide sources of genes that can be exploited in cowpea breeding programs aimed at improving the response to drought in the species. The identification of superior genotypes that produce optimal high yield in local conditions will benefit both growers and end-users in South Africa.

1.4 Objectives

The broad objective of the study was to evaluate the performance of cowpea germplasm. The specific objectives were to:

(i) determine the agronomic performance of cowpea germplasm in local conditions of South Africa and
(ii) determine the variability in response to moisture stress among cowpea germplasm.

1.5. Hypotheses

The study tested the following null hypotheses:

(i) diverse cowpea genotypes perform similarly in the local production condition of South Africa.
(ii) there are no differences in the response to moisture stress among cowpea germplasm.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin, taxonomy and distribution of cowpea

Cowpea (2n=2x=22 chromosomes) is a dicotyledonous plant belonging to the leguminiceae family in the order Fabaceae, subfamily Faboideae (Syn. Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus Vigna, and section Catiang. The genus *Vigna* consist of species between 150 and 170 most of which are indigenous to Africa (Summerfield and Roberts, 1985). Cultivated (*Vigna unguiculata*), evolved from perennial wild species *dekinditiana* to annual type through selection which led to the loss in seed dormancy, pod dehiscence and increase in pod and seed size. The cultivated cowpea is divided into four culti-groups that comprises of *V. unguiculata*, *V. biflora*, *V. sesquipedalis* and *V. textilis* based on seed and pod characteristics (Ng, 1995).

Cowpea is native to Africa as one of the oldest food source for humans according to the oldest archeological evidence of cowpea with largest crop morphology in terms of growth habit, pod and seed characteristics (Flight, 1976; Ehlers and hall, 1997). However, the exact center of origin remains a matter of speculation between southern and west Africa particularly due to the existence of different subspecies of the cultivated cowpea.

Southern Africa appears to be the probable center of origin that consist of the greatest genetic diversity of wild types, suggesting West Africa as the center of maximum genetic diversity and highest domestication of the cultivated crop by the local farmers (Padulosi *et al*., 1990; Ba *et al*., 2004). Asia has been reported as the second center of maximum cowpea diversity from which the crop was distributed to other regions of the world. A recent study by Huynh *et al*., (2013) to access the distribution of the cowpea species reported a small genetic difference occurring between cowpea from African and non-African regions, suggesting that Africa is the main source for worldwide distribution of cowpea.

2.2 Plant genetic resources

Plant genetic resource refers to any raw material either in the form of crop wild relatives (CWR), breeding lines, landraces, genetic stocks, weedy types, commercial and obsolete cultivars that can be used by farmers or breeders to improve productivity and quality of the crop (Haussmann *et al*., 2004). It is a genetic pool that buffers against temporal environmental variability thereby providing source of genes that can be utilized to improve food security and local adaptation to changing climate especially in arid and semi-arid regions with low and unpredictable rainfall. Owing to global concern over decline in genetic diversity due to erosion of diverse CWR and landraces particularly through substitution with uniformly improved cultivars by farmers and urbanization of arable land, systematic collection and conservation of accessions has gained priority across the world (FAO, 2010). Until recently, global *ex situ* conservation of accessions to secure the derisible traits, has grown by 20% although collection of CWR and less economic crops is still low and limited to seed storage at low moisture content for storage in low temperature gene banks. This lead to the promotion of *in situ* conservation that aid in continuous evolution, commonly practiced in conservation of CWR and landraces that harbors the source of novel genes (Nwosu *et al*., 2013).

Cowpea germplasm is maintained in many centers and other breeding cowpea programs with considerable collection throughout the world. South Africa, through National Plant Genetic Resource Centre (NPGRC), located at Roodeplaat in South Africa and various botanical gardens and nature reserves distributed across the country, contribute significantly to conservation of enormous plant genetic resource for food and agriculture (DAFF, 2016). The largest accessions of cowpea (15 000 accessions) is maintained by International Institute of Tropical Agriculture (IITA) which is mandated to conduct cowpea research that consist of greater portion of landraces collected from farmers' fields and few wild types (Mahalakshmi *et al*., 2007). This germplasm may only accelerate the success of crop improvement for food, health and nutritional security when it is accessible to breeders for maximum utilization of the existing genetic diversity.

2.3 Utilization of cowpea

Cowpea is the most grown indigenous legumes for home consumptions and income generation through marketing of the surplus (Asiwe, 2009). It is a nutritious crop (Table 2.1) with the potential to improve global food and nutritional security as well as poverty due to its widely distributed production and most edible plant parts that include leaves, immature pods and seeds used for human consumption. The cowpea haulm is commonly used for livestock feeding. Cowpea is used for various dishes depending on the region and plant part. In South Africa, green leaves are consumed as relish, immature pods can be boiled and consumed as snack while dry grains are cooked and consumed alone or in combination with maize or sorghum (Lekunze, 2014).

The existence of diverse crop morphology and edible plant use in cowpea serve an important role of satisfying variable needs of the value chain actors. It is also, known as hunger crop, it presents food for the current season before other crops mature such as cereals to escape hunger during the mid-growing season (Hall, 2012). In South Africa, the crop is underutilized, although it plays a significant role in mixed farming within the smallholder sector. Its ability to tolerate water stress, heat and poor soil conditions makes cowpea a crop of choice to avert the risk of crop failure and reduce the production input cost. On average, cowpea add 40.0 kg/ha of nitrogen to the soil which improve yield of the follow up crop especially in rotation system or the cereal intercrop with additional cultural benefit of disease and weed suppression (Makoi *et al*., 2009; Sauerborn *et al.*, 2000).

Traditionally, it is used for treatment of amenorrhea from extraction of the boiled seeds and treatment of chest pain, epilepsy, and constipation from its root paste particularly in Southern Africa (Van Wyk and Nigel, 2000). The consumption of cowpea leaves is associated with improved vision, blood and immune system (Hallensleben *et al*., 2009). Mamiro *et al*., (2011) reported daily intake of cowpea with consumption *per capita* ranging between 40.0 to 200.0 gm.

Hence, it provides a cheap source of protein, vitamins, and minerals in both rural and peri-urban areas to replace expensive source of proteins obtained from meat (SACG, 2006). Mokoboki *et al*., (2000) has shown that cowpea straws have sufficient protein to retain the live weight of livestock in dry season when crop residues are the primary source of feed. Cowpea improvement programme tailored for South African context will improve the cowpea value chain, thereby contributing significantly to poverty alleviation, malnutrition and crop-livestock farming.

Table 2. 1 Nutritional value of cowpea (%)

Physicochemical traits	Seeds	Hay	Leaves
Carbohydrates	50-67	-	8
Protein	23-25	-	4.7
Crude fiber	5.9-7.3	9.6	2
Fat	1.3-1.5	22.3	0.3
Water	11	18	85
Ash	3.4-3.9	23.3	-
Calcium	0.104-0.076	-	0.256
Iron	0.005	-	0.005
Phosphorus	0.146	2.6	0.063

Source: FAO, 2004

2.4 Production status and constraints of cowpea in South Africa

Cowpea is produced in many parts of the world including Asia, Australia, Brazil, the Caribbean's, India, United States of America and most of African countries (Ba *et al*., 2004). In 2014, the worldwide area under production of cowpea was estimated at 14 million hectares with annual production of 3 million tons of which Africa accounted for about 60% of the global production (IITA, 2015; FAOSTAT, 2014).

In South Africa, the crop is largely planted in a small area by smallholder farmers as a result of cultivation of unimproved varieties which are highly susceptible to both abiotic and biotic stress factors (Asiwe, 2009).

Common biotic factors that limit cowpea production include aphids, weevils, pod sucking buds, viruses and weeds such as grass, *Alectra vogelii* and Striga. Drought constitute the most important constraint in most cowpea production regions where the crop is produced in dry environments with little or no irrigation while most farmers are growing locally adapted varieties (landraces) with low yield potential (Timko and Singh, 2008).

Table 2. 2 Cowpea production in selected countries of the world.

Country	Area harvested (ha)	Yield (kg/ha)	Production (t)
Nigeria	3701500	5776	2137900
Niger	5320780	2982	1586446
Cameroon	257750	6760	174230
Myanmar	132000	8727	115200
Haiti	41080	7118	29240
United States of America	11655	16852	19641
Sri Lanka	11519	13266	15281
China	13000	10385	13500
South Africa	11100	4387	4870
Philippines	440	33409	1470
Total	10940904	124655	4825358

Source: FAOSTAT, 2014

2.5 Morphological characterization of cowpea

Genetic diversity is indispensable for the success of any breeding programs as it provides genetic variability within the traits of interest that can be exploited to adapt crop to the current climatic changes. Collections of germplasm accessions have been assembled adequately in major crops than in indigenous or underutilized crops using morphological markers to study for characterization and evaluation of ideotypic performance and highly heritable traits (Shardendu *et al*., 2011; Olukolu *et al*., 2012; Duran *et al*., 2005). Agro-morphological traits such as pods per plant, plant height and leaves per plant have since been used for taxonomic classification and genetic diversity studies for most crop species including cowpea.

Method of phenotyping for such quantitative traits, is usually reproducible and requires low technology to examine the diversity in large sample size, although the variation found is relatively lower compared to molecular approach which is not affected by environmental effects (Rao, 2004).

Molecular markers are more efficient in revealing polymorphism among cowpea germplasm (Egbadzor *et al*., 2014). However, morphological markers are still important complementary markers that have direct or indirect influences on the biochemical status of the plant and responsible for visual representation of the genotypic make-up of the plant. Mahalakshmi *et al*., (2007), observed high genetic diversity of cowpea from the entire IITA germplasm through characterization of morphological traits. Gerrano *et al*., (2015) also found sufficient genetic variability indicating the potential of selecting breeding parents for economic yield improvement. However, cowpea growers are still experiencing low yield returns from lack of available improved cowpea cultivars despite the available genetic diversity existing among the cowpea germplasm accessions.

2.6 End-use preferred traits

The smallholder farming sector is still realizing a slow adoption of improved cowpea varieties in South Africa as a result of improved varieties with little or no preferred traits by the end-users. This can be improved through indigenous knowledge on traits of preference from participation of farmers in the evaluation and selection of potential cultivars to provide information to guide farmers based breeding objectives (Coulibaly *et al*., 2010; Hoffmann *et al.*, 2007). In South Africa, smallholder farmers select cowpea based on seed colour, size, duration to maturity and yield performance (Asiwe, 2009). For example, some farmers in Limpopo province of South Africa prefer early maturity types in order to escape end-of-season drought stress. Horn *et al*., (2015) reported high adoption of long podded types.

The variation in end-use attributes is partially caused by the differences in environmental and socioeconomic factors affecting specific production areas. Farmer's preferred cultivars consist of a range of characters such as drought tolerance, multi-purpose, higher market value, good taste, fast to cook, early maturity and long storage period as guided by the prevailing environmental conditions (Hallensleben *et al*., 2009). Therefore, a single breeding programme can satisfy various end-users traits in different production areas. However, large seed size is the common traits of preference to most cowpea producers that is exhibited by most of the local varieties (Kwaga, 2014; Gerrano *et al*., 2017).

2.7 Growth and yield performances of cowpea

A considerable number of cowpea farmers is still growing landraces which produce relatively low yield (0.5 t/ha) as compared to the potential yield of 3.0 t/ha (Asiwe, 2009). This is not surprising because the introduced cowpea cultivars developed for a specific region in West Africa but perform poorly under local conditions due to narrow genetic base and environmental variations that result from routine use of improved lines as parents to develop new varieties (Boukar *et al*., 2016).

Aliyu *et al*., (2014) hypothesized that lower yield performance of locally unimproved varieties planted at farmer's field is attributable to less optimization in combination of traits. Generally, the performance of grain yield in cowpea is determined by yield components that include number of pods per plant, seed per plant, number of branches and harvest index. In contrast, traits such as photosensitive and long maturity type lead to significant yield suppression particularly on locally adapted varieties (Kamai *et al*., 2014). Thus, knowledge of direction, magnitude of associations and heritability of the traits is imperative in successful yield improvement of locally adapted varieties to increase the suitability of cowpea for low input production by the resource poor farmers.

Several studies have indicated the production of high fodder yield that increases markedly under high light intensity contribute to low grain yield as a results of low pod number. In contrast, Kamara *et al., (*2012) reported that fodder yield, total dry matter, harvest index, and 100-seed weight were significantly correlated with grain yield further contributing a genetic gain of 3.6 percent achieved as the old varieties are replaced with the new varieties.

Manggoel *et al*., (2012) indicated that duration to 50% flowering and pod length had indirect effect on grain yield implying that their direct selection may lead to low yield returns. Furthermore, Moura *et al*., (2012) using path analysis, has found that superior genotypes can be achieved through direct selection for duration to 50% flowering, pod number and number of branches per plant and through indirect selection for grain size, which simultaneously lead to short cooking time. Nutritionally, the independent nature of association for nutritional components of cowpea allows for improvement of one element without affecting the other.

Mokoboki *et al*., (2000) noted a significant varietal difference for seed traits and straw characters such as green retention and dry matter content which determines the quality and quantity of biomass yield which mitigate the depression of livestock farming. Furthermore, good performance for both grain and fodder yield from a single genotype indicate that selection is widely shifting towards the improvement for dual purpose cultivars (Singh *et al*., 2003). Therefore, it is important to incorporate traits that will improve the balance between grain yield and biomass yield with high response to good agronomic practices and resistance to adverse climatic factors such as drought.

2.8 Drought and drought tolerance

Drought tolerance is a complex trait and in the advent of changing climate, average yield of most crops is constantly reducing due to the global increase in temperatures and severe drought occurrence, which is expected to worsen in future thereby by exposing crops to novel environments (Shao *et al*., 2009).

Globally, Lobell *et al*., (2011) has shown that maize and wheat yield have decreased from 3.8% and 5.5%, respectively, from 1980 to 2008, indicating the adverse effect of climate change on crop production.

In South Africa, more than half of national water is used in agriculture to irrigate 1.5% of the total arable land, implying that the water will be insufficient to cover the remaining portion allocated for dry land farming (Baleta and Pegram, 2014).
Drought stress affects the crop performance due low and erratic rainfall that may coexist with other abiotic stress such as heat and salinity (Azam-Ali *et al*., 2004). Blum (2011) defines drought as water deficit that results when the capacity of roots fails to extract adequate moisture to meet the evaporative demand of the atmosphere. Drought is the most important constraint in crop production that can cause famine, as recovery from long drought spells is difficult and sometimes drive farmers out of agriculture due to delayed drought relief from government (Ngaka, 2012).

Genotypic variability in response to drought stress exist between and among plant species that enable plant to show variable response to moisture stress. Drought tolerance is the degree in which a plant adapts to water deficits though morphological, biochemical and physiological changes experienced at any plant developmental stage to produce an acceptable economic yield (Cattivelli *et al*., 2008; Turner *et al*., 2001). Drought stress is detrimental to plant growth, it disturbs the normal process of metabolism through reduced plant water status and may lead to a complete wilting and death in most plants species leading to total loss yield and planting materials (Boubacar, 2012).

The severity of drought depends on timing, period and intensity with different effects on specific developmental stages. Hence, it complicates breeding for tolerance to moisture deficits. Cowpea has been shown to tolerate water deficits more than several exotic and indigenous crop species (Table 2.3) (Singh *et al*., 1999).

However, drought and unavailability of improved cowpea varieties are the major constraints that limit cowpea production and productivity in South Africa, where cowpea is largely produced, particularly in Limpopo and Mpumalanga (Asiwe, 2009). Intensifying the cultivation of improved indigenous crop for drought tolerance and high yield is a promising strategy for adaptation to changing climatic conditions by enabling resistance of crop to the damning effect of low and erratic rainfall.

Table 2. 3 Proportion of dead plant during progressive water deficits

	Days from last irrigation					
Crop	7	9	11	15	19	23
Cowpea: IT90-K59-2	0	0	0	0	29	100
Cowpea: TVU11979	0	0	0	13	53	100
Cowpea: TVU7778	0	0	0	27	54	100
Lablab bean	0	0	0	17	94	100
Bambara groundnut	0	0	6	33	66	100
Groundnut	14	59	100	100	44	100
Pearl millet	14	28	68	100	100	100
Sorghum	0	0	93	100	100	100
Greengram	8	17	86	100	100	100
Blackgram	14	75	100	100	100	100
Maize	17	50	100	100	100	100
Soybean	63	100	100	100	100	100
LSD 5%	46	56	23	31	50	NS

Source: Singh *et al*., (1999)

2.8.1 Breeding for drought tolerance

Drought stress affect the whole plant even though the tolerance is usually determined based on the ability of the plant to produce highest economic yield despite moisture stress. Nonetheless, heritability of yield is low as compared to that of physiological traits related to drought tolerance. It is important to understand drought tolerance mechanisms and association of physiological traits underlying the response of contrasting genotypes to water stress to avoid the tradeoffs.

Acquaah (2012) described two approaches used for breeding for drought tolerance as indirect breeding that involves evaluation in the non-target environment and direct breeding that involves evaluation in controlled environments that represent the environmental conditions in target cowpea production areas.

Success in crop improvement for drought tolerance is less pronounced when compared to other traits particularly due to complexity of drought tolerance mechanism involved in response to water deficits and lack of simple, cost effective and reliable evaluation tools (Sinclair, 2011).

In fact, drought tolerance appears to be a less utilized trait according to various reviewers as new knowledge in breeding for drought tolerance is constantly been unrevealed through synergistic approach (Cattivelli *et al*., 2008; Fang and Xiong, 2015). Further progress may be limited by evaluation in non-representative environments that misled the selection criteria in the field where drought is variable in intensity and timing and likely to occur with other stress leading to considerable genotype by environment interaction. Improvement for cowpea drought tolerance is an important goal to serve a niche in cowpea production areas of South Africa where cowpea is largely produced in dry land environments. Most cowpea breeding efforts in South Africa involves the indirect approach of evaluating the introduced drought tolerant genotypes with no controlled breeding programme for drought tolerance within countries especially for underutilized crops such as cowpea (Aliyu *et al*., 2014).

2.8.2 Mechanisms of drought tolerance

Cowpea is commonly cultivated in mixed cropping systems to avert the risk of crop failure in arid and semi-arid regions attributed by its drought tolerance which evolved over time for adaptation to the confined growing conditions. Drought tolerance is considered a complex trait since, different plant species under drought stress undergo different morphological, biochemical and physiological modifications as drought tolerance mechanisms, which occur separately or jointly in response to moisture stress (Blum, 2005). These, mechanisms are categorized as drought escape, dehydration avoidance and tolerance (Mitra, 2001; Turner *et al*., 2001).

Mai-Kodomi *et al*., (1999) described drought tolerance response in cowpea involving the combination of different mechanisms at whole plant level into stage 1 and stage 2. In stage 1, the plant start wilting at whole plant level and stage 2 is characterized by yellowing from the lower leaves.

Although, flowering is the most sensitive stage during drought, cowpea deploys the same phenomenon for drought tolerance at both vegetative and reproductive stage (Singh *et al*., 1999) implying that drought experienced during the early stage of development may enhance the tolerance to late season drought. Understanding the mechanism of drought tolerance will lead to successful breeding efforts geared at improving the drought tolerance in cowpea (Chandler and Bartels, 2008) with a potential to encourage the intensification of cowpea production in dry land farming.

a) Drought escape

In the environment that experience a terminal drought stress, growing of early varieties has been intensively adopted strategy for short completion of life cycle through the utilization of available soil moisture (Abua *et al*., 2014). Hence, early flowering is a useful drought adaptation trait that has been naturally selected over time in the environments characterized by terminal drought stress where cowpea reaches maturity 60 to 70 DAP (Singh *et al*., 2003). Earliness is a genetically controlled traits in neutral day varieties to enable early flowing despite the day length. Hence, ideotypic drought tolerance plant tends to exhibit erect trait with small canopy size and leaf area index to reduce transpiration rate without taxing yield. Although variation in root length may occur, drought tolerant genotypes in this case rely on shallow and profuse root system to utilize the available soil moisture for vigorous growth rather than expressing a slow crop growth rate moisture saving habits (Krishnamurthy *et al*., 2013). Rashwan and Helaly (2015) observed a higher yield potential in early flowering lines which respond well in the selection. Early peanut showed a better adaptation to terminal stress by producing yield higher than in late maturing cultivars (Junjittakarn *et al*., 2014).

Although, selection for earliness has shown substantial progress in adaptation to terminal moisture stress with better yield compared to late maturity type, any exposure to mid-season drought to early cultivars can potentially reduce crop yield (Hall, 2012). As such, a single trait is not reliable in breeding for erratic environmental factors such as drought stress.

b) Dehydration avoidance

Under drought stress, plant species undergo different morphological changes where water absorption become optimized while water loss minimized to maintain turgor despite water deficits to avoid the effect of drought stress (Blum, 2005; Turner *et al*., 2001). Of all the parts involved in response to moisture stress, roots are the major detection and regulatory site for soil water shortage which cause a direct effect in shoot response (Wasson *et al*., 2012). Drought stress induces the further development of prolific root system in the expense of shoot growth for efficient absorption of water and reduced water loss. Differences in root sizes has been reported in various crops where shallow root system is highly associated with drought susceptible genotypes under recurring stress conditions. Moreover, drought stress induces development of deep and dense root system that increases the tolerance to subsequent drought stress (Hayatu and Mukhtar, 2010).

In a study to discern the root anatomy of different crops, moderate xylem passage in crops such as cowpea is responsible for efficient water absorption, thus, better adapted for variable water supply (Purushothaman *et al*., 2013). Root architecture is a major extraction site for water and nutrient for plant growth and development. As a result, roots may have a certain influential aspect on the response to water stress at a whole plant level. Evaporative demand determines the water requirements of the plants to facilitate the transpiration process through stomata in the leaves (Shardendu *et al*., 2011).

Plant commonly reduce water loss through leaf orientation, leaf shedding, stomatal closure, reduction in plant height and leaf area and thick cuticles which disturb the photosynthesis process required for growth and development but the rate is reduced in genotypes adapted for dry environment through interception of large fraction of photosynthetically active radiation (Hamidou *et al*., 2007; Kamai *et al*., 2014). While, leaf rolling manifest as the early signs of low plant water status, leaf wilting index (LWI) and leaf senescence are the most noticeable indication of crop experiencing progressive drought stress in cowpea (Pungulani *et al*., 2012).

Plants that exhibited higher transpiration efficiency and canopy temperature due to limited transpiration and low stomatal conductance that consequently lead to lower relative water content, osmotic potential and higher water use efficiency produced relatively higher yields in faba bean (Khan *et al*., 2007). Canavar *et al*., (2014) reported a highly significant difference between genotypes for water use efficiency in crops with high relative water content and biomass that is negatively correlated with carbon isotope discrimination attributable to limited photosynthetic process and carbon intake. Drought tolerant genotypes are associated with lower carbon isotope discrimination and increased carbohydrates assimilation rate during reproductive phase.

c) Dehydration tolerance

Dehydration tolerance is the last defense induced mechanisms in response to drought stress for maintenance of physiological process under low water potential (Fang and Xiong, 2015). Plant tolerance to dehydration is through osmotic adjustment (accumulation of compatible cell solutes) and eliminate damage due to moisture stress to thereby increasing water retention in the tissue for continuous plant growth under severe drought stress.

Osmotic adjustment is considered an important tolerance mechanism that enable functioning of the intracellular activities in dehydrated plant where the osmotic potential is reduced through the accumulation of compatible solutes (Turner *et al*., 2001). These solutes include organic such as proline, glycine butaine and sugars and inorganic solutes such as Na^+, K^+, and Cl^-. These solutes occur in high non-toxic concentration to forming a bond with water molecules for maintenance of plant turgor under limited water supply and enhancing extraction of water from dry soil. For instance, accumulation of K^+ regulate the closure of stomata.

In the early event of drought stress, ROS such as H_2O_2, OH and O^{2-} radicals are formed in the cell to signal other plant defense to the stress but potentially damaging to the cell under higher concentrations (Tekle and Alemu, 2016). Plant mitigate the effect of harmful ROS through enzymatic (ascorbate peroxidase and catalase) as well as non-enzymatic antioxidants (ascorbic acid, carotenoids and proline) as a defense mechanism to maintain the integrity of the cell. During drought stress, Proline which is the prime osmolyte accumulate higher in drought tolerant plants to reduce cell injury during stress that increases with the progression of stress and peak at flowering stage (Ashraf and Foolad, 2007; Shardendu *et al*., 2011). Additionally, dehydration tolerance in cowpea can be achieved through high cell membrane stability. Dehydration tolerance is mainly linked to survival under drought stress. Hall *et al*., (2000) found that cowpea genotypes that consist of delayed leaf senescence trait could compensate for effect of drought by inducing the second flush of flowers. Thus, delayed leaf senescence is an important selection criterion responsible for survival and recovery (Muchero *et al*., 2008).

2.8.3 Evaluation methods for moisture stress

Drought tolerance is a quantitatively inherited trait that respond considerably to various environmental and edaphic factors. Therefore, evaluation for drought tolerance requires a well-defined testing environment since drought stress is variable in timing and occurrence in difference locations and may occur with other environmental stress such as temperature and salinity (Acquaah, 2012).

Evaluation for water stress is fundamental in most drought tolerance studies of cowpea. Several methods reported for drought tolerance evaluation in cowpea include pot evaluation (Watanebe *et al*., 1997), wooden box screening (Slabbert *et al*., 2013) and field evaluation (Ishiyaku and Aliyu, 2013). Among these methods, field evaluation is commonly used for yield evaluation over a wide range of environments conditions while the other methods are used for evaluation at seedling state for physiological traits related to yield in controlled environments. Although, Voltas *et al*., (2005) reported a significant progress in discriminating drought tolerant genotypes from susceptible genotypes through field screening, the method is expensive, labor intensive and often produce inconsistent results due to existing complementary stresses in the testing locations. Thus, pot evaluation and wooden box methods are effective for pre-screening in the greenhouse to select for drought tolerant cultivars that can be evaluated further in the field. Such methods are highly useful in the modern high throughput drought evaluations experiments. Root traits such as root depth, density and number of whorls are also traits for adaptation in moisture stress which can be easily evaluated using root box–pinboard method (Lynch and Brown, 2012).

3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1 Experiment # 1: Field study

The specific objective of this experiment was to determine the agronomic performance of cowpea germplasm in South Africa.

3.1.1 Site selection

The field experiment was conducted at ARC-Roodeplaat Research Farm, Pretoria (25.604° S, 28.345° E) in 2015/2016 summer cropping season. The altitude of Roodeplaat is 1168 m above sea level. The experimental site is characterized by a clay loam soil with 30% clay which receives an annual rainfall of 700 mm particularly during the summer with maximum temperature of 30 ^{o}C. The climatic conditions during the trial is presented in Table 3.1.

Table 3. 1 Meteorological data for 2015/2016 cropping season at Roodeplaat, Pretoria

	2015							2016				
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Maximum temp (^{o}C)	21.26	22.27	27.26	28.10	32.58	31.77	33.88	31.67	32.46	29.35	28.41	23.43
Minimum temp (^{o}C)	2.94	3.17	5.97	11.08	14.16	13.95	18.09	17.63	17.82	15.61	11.79	7.92
Total rainfall (mm)	1.02	0.00	0.00	56.64	9.40	29.72	60.20	135.13	49.53	204.47	3.30	54.61
Relative humidity (%)	84.52	83.09	76.57	79.43	77.00	68.24	80.00	81.24	84.76	87.21	88.47	90.59

Temp=temperature

3.1.2 Planting materials

Twenty eight cowpea genotypes were used in the study comprising of local and exotic genotypes. The growth habit of the germplasm included semi-prostrate, prostate and erect types (Table 3.2). Six of the genotypes were exotic and the seed consisted of diverse testa colour.

Table 3. 2 Cowpea genotypes that were used in the study

No	Genotype	Growth habit	Seed colour	Origin
1	Acc1121	Semi–prostrate	Brown	Local
2	Acc1165	Prostrate	Brown	Local
3	Acc1168	Semi–prostrate	Black	Local
4	Acc1179	Prostrate	Purplish grey	Local
5	Acc1257	Semi–prostrate	Brown	Local
6	Acc1958	Semi–prostrate	Purple	Local
7	Acc1985	Semi–prostrate	Black	Local
8	Acc1998	Prostrate	Dark grey	Local
9	Acc2024	Semi–prostrate	Dark purple	Local
10	Acc2355	Semi–prostrate	Brown	Local
11	Acc2438	Semi–prostrate	Grey	Local
12	Acc3361	Semi–prostrate	Purplish grey	Local
13	Acc3916	Semi–prostrate	Purple	Local
14	Acc4294	Semi–prostrate	Red	Local
15	Acc4565	Semi–prostrate	Cream	Local
16	Acc4663	Semi–prostrate	Grey	Local
17	Acc4670	Semi–prostrate	Dark grey	Local
18	Acc4749	Semi–prostrate	Dark grey	Local
19	Acc4893	Semi–prostrate	Purplish grey	Local
20	Acc5352	Intermediate	Cream	Local
21	Cwp1	Prostrate	Brown	Exotic
22	Cwp2	Prostrate	Cream	Exotic
23	Cwp4	Prostrate	Brown	Exotic
24	Cwp5	Prostrate	Light brown	Exotic
25	Glenda (Check)	Semi-erect	Red	Local
26	L-cwp2	Semi–prostrate	Light grey	Exotic
27	L-cwp3	Semi–prostrate	Brown	Exotic
28	Phokwane1	Semi–prostrate	Green	Local

3.1.3 Experimental design, layout and management

Twenty eight cowpea genotypes (Table 3.2) including Glenda as a standard check were planted in the field for characterization and evaluation of agronomic performances. The experiment was arranged as a randomized complete block design replicated three times. Each plot consisted of three rows 3 m long spaced at 0.7 m apart. The intra-row spacing was 0.3 m. Two seeds were sown per each hill which were thinned to one seedling per hill 15 days after planting (DAP). The experiment was irrigated to supplement rainfall for an ideal crop growth. No fertilizers were applied to ensure optimum genetic potential of the genotypes under low-input conditions for the growth and development of the crop (Gerrano *et al.*, 2015). Weeds were controlled manually using a hand hoe.

3.1.4 Data collection

Quantitative morphological data were collected using the IBPGR (1983) descriptor for cowpea from a middle row which were used as a net plot and sampling unit from five randomly selected plants that were tagged on each plot during the experiment. The following traits were measured on a plot basis: days to 50% flowering (D50), days to 95% maturity (D95) and grain yield (GY). The number of primary branches (NB), leaf area index (LAI), number of internode per plant (NIP), number of pods per plant (NPP), plant height (PH), 100 seed weight (HSWT) and seed yield per plant (GYP) were measured from five randomly selected plants. The fresh weight of the five randomly selected plants were placed in an oven at a temperature of 75 ^{o}C for 7 days for the measurement of dry weight per plant (PWT). Ten pods were randomly selected from each plot to determine pod length (PL) and the number of seed per pod (NSPP). Leaf area index (LAI) was measured using Ceptometer AccuPAR model LP-80® at 50% flowering stage.

Table 3. 3 List of morphological quantitative traits measured in the experiment

Traits	Unit
D50 = 50% flowering	count
D95 = days to 95 % maturity	count
HSWT = hundred seed weight	g
LAI = leaf area index	-
NB = number of branches	count
NIP = number of internode per plant	count
PH = plant height	m
PL = pod length	cm
NPP = number of pods per plant	count
PWT = dry weight per plant	g
GYP = grain yield per plant	g
GY = total grain yield	kg/ha

3.1.5 Data analysis

The data collected from the quantitative traits were subjected to analysis of variance (ANOVA) using Agrobase Generation II statistical package (Agrobase, 2008) followed by least significant difference (LSD) at 5% level. The data were further subjected to principal component analysis and path analysis. Path analysis was computed through portioning of correlation coefficients into the indirect and direct effects of the yield attributes on yield according to Alege and Mustapha (2007) using SPSS AMOS 24 statistical package (Arbuckle, 2016).

A procedure by Uguru (1995) was used to compare genetic variability and heritability effects among the traits as follows:

$$\text{Genetic variance } (\sigma 2g) = \text{MSg} - \frac{\text{MSe}}{\text{r}} \text{ ....Equation no 1}$$

$$\text{Phenotypic variance } (\sigma\ 2\text{p}) = \text{MSg} + \text{MSe} \text{ ....Equation no 2}$$

Where: MS_g = mean squares of genotype, MS_e = mean

squares of error and *r* = number of replications

Phenotypic variance (σ^2p) = MS_g + MS_e……………………………….......Equation no 3

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\text{Vg}}}{\text{X}} \times 100$$ ……………..Equation no 4

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\text{Vp}}}{\text{X}} \times 100$$ ……………………….Equation no 5

Where: *X* = grand mean for the phenotypic traits, *V*g = genotypic variance, and *V*p = phenotypic variance, and Broad-sense heritability (h^2bs).

$$\text{h2bs} = \frac{\sigma\,2\text{g}}{\sigma\,2\text{p}} \times 100$$ ………………………………………………………….Equation no 6

3.2 Experiment # 2: Greenhouse study

The specific objective of this experiment was to determine the variability in response to moisture stress among the cowpea germplasm.

3.2.1 Experimental site

The experiment was conducted in drought screening greenhouse using plastic box screening method at Agricultural Research Council – Vegetable and Ornamental Plants, South Africa, (Latitude 25.604˚S and Longitude 28.345˚E). The plastic box used for screening during the experiment is a modification of wooden box developed by Watanabe *et al*., (1997). Greenhouse temperatures were kept at 30 ^{0}C during the day and 15 ^{0}C during the night.

3.2.2 Planting materials

Twenty eight cowpea genotypes were used in the study including two controls (drought tolerant - IT96D-602 and drought sensitive - TVU7778) genotypes that were designated as controls (De Ronde and Spreeth, 2007). The names of the genotypes are given in Table 3.2.

3.2.3 Experimental design and management

The experiment was arranged in a randomized complete block design (RCBD) with three replications consisting of a total of 12 boxes. Each box with a size of 155 cm × 77 cm × 23 cm was filled with a mixture of red topsoil and vermiculite mix (1:1). Initially, the boxes were irrigated to field capacity and the excess water was allowed to drain before planting.

Seeds of each genotype was planted in the boxes at the spacing of 15 cm between rows and 10 cm within rows for each box to form 10 rows which is represented by 5 plants of a genotype per row. Two seeds were planted per hill at a depth of 2 cm. The plants were thinned to one plant per hill 7 days after planting (DAP). Boxes were uniformly irrigated using watering can for a period of 3 weeks to allow the plant to develop the first trifoliate before induction of moisture stress.

3.2.4 Drought treatment procedure

The plants were allowed to grow until the appearance of first trifoliate (3 weeks after planting). The last irrigation was applied on the 6th March 2017 after which irrigation was withheld for a period of five weeks to allow for permanent wilting of the susceptible plants followed by re-watering for a period of two weeks to identify the genotype with high tolerance and ability to recover from moisture stress (Muchero *et al*., 2008).

3.2.5 Measurements and scoring procedure

Wilting and status of the plants were monitored and recorded. Measurements were conducted on the leaf wilting index (LWI) and relative water content (RWC) and leaf proline content (LPC) during the drought stress. Stem greenness and plant regrowth were scored after two weeks of re-watering. Measurements on leaf wilting index (LWI) was recorded at week two of moisture stress (LWI2) and week four of moisture stress (LWI4) by dividing the number of wilted leaves with the total number of leaves. Stem greenness (STG2) was scored on a scale of 1 to 5 with 1 representing yellow to 5 representing completely green.

A scale of 1 to 5 was also used to assess plant regrowth (RG2) as follows: 1- no regrowth, 3 - regrowth from auxiliary buds and 5 - regrowth from the apical meristems. Relative water content (RWC) was determined on the second week (RWC2) and fourth week (RWC4) of moisture stress by the method described by Bogale *et al*., (2011) according to the following formula:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation no 7}$$

Where FW - fresh weight, DW - dry weight, TW - turgid weight.

Leaves from the greenhouse drought screening experiment were collected for proline content determination at week five of drought stress. Proline content was determined following the procedure by Bates *et al*., (1973). Initially, 0.05g dry weight of leaves was homogenized in 1.25 ml of 30% aqueous sulfosalicylic acid followed by centrifugation of the mixture at 15000 rpm for 15 minutes to collect the supernant. 0.5 ml of the supernant was then reacted with 1.0 ml acid-ninhdrin and 0.5 ml of glacial acetic acid in a test tube for 1 hour at 100 ^{o}C. The reaction was terminated by an ice bath followed by the extraction of the mixture with 1.0 ml of toluene which was mixed vigorously using a test tube stirrer for 20 seconds. The chromophore contacting toluene was then aspirated from aqueous phase and warmed to a room temperature followed by reading of three absorbance samples of each genotype at 520 mn using toluene for blank. Lastly, the proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$$\mu\text{mole proline/g of fresh leaves} = \frac{\mu\text{g proline/ml} \times \text{ml toluene}/115.55\ \mu\text{g}/\ \mu\text{mole}}{(\text{g sample})\ /5} \ldots\ldots\ldots$$

……………………………………………………………………………………Equation no 8

3.2.6 Data analysis

Data collected was subjected to analysis of variance using Statistical Analysis System (SAS) program version 9.3 (SAS, 2000) followed by mean separation (Duncan, 1955) and pearson correlation.

4.0 CHAPTER FOUR: RESULTS

4.1 Experiment # 1: Field experiment

4.1.1 Genetic variation

The analysis of variance showed highly significant ($p \leq 0.01$) differences among the cowpea genotypes for all the traits studied (Table 4.1). Glenda and Acc5352 were the earliest genotypes which attained 50% flowering at 48 and 53 days respectively. The majority of the genotypes was late in maturity. The earliest flowering genotypes also showed lowest mean values for duration to 95% maturity. The grain yield varied significantly among the genotypes. Genotype Cwp4 and L-cwp3 achieved the highest grain yield of 2624.89 kg/ha and 2528.46 kg/ha, respectively. In contrast, Cwp1 (264.18 kg/ha) and Acc2024 (263.76 kg/ha) attained the lowest grain yield (Table 4.1). Glenda and Acc5352 produced moderate grain yield.

The highest seed yield per plant (85.0 g) was observed for Acc2355. Glenda, which produced relatively small seeds (11.4 g per 100 seeds) also attained a low seed load per plant. The mean hundred seed weight (HSWT) ranged from 10.25 g for Acc1998 to 24.08 g for L-cwp3.The longest pod (19.32 cm) was attained by genotype Cwp2.

Despite a relatively low HSWT (10.25 g), genotype Acc1998 achieved the highest mean number of pod per plant (NPP). There was variation in phonological development (Figure 4.1), pod length (Figure 4.2) and seeds traits (Figure 4.3) among the genotypes.

Table 4. 1 Mean squares and mean values of cowpea genotypes for selected morphological quantitative traits

No	Genotype	D50	D95	HSWT	LAI	NB	NIP	NSPP	PH	NPP	PL	PWT	GYP	GY
1	Acc1168	61	109	14.03	3.88	7.67	12.67	14.12	1.66	33.73	15.70	868.60	64.83	2151.84
2	Acc3361	68	131	13.11	4.77	7.20	12.33	11.12	1.53	21.47	14.34	227.80	17.74	480.06
3	Acc3916	62	131	17.20	3.72	7.07	13.20	11.13	1.64	14.02	15.39	419.50	24.09	598.30
4	Acc4565	68	135	13.67	1.07	7.27	12.60	11.14	1.67	23.33	15.93	481.50	31.43	831.60
5	Acc1985	68	108	14.30	4.14	7.47	13.53	12.92	0.82	41.75	15.76	897.60	41.39	1345.77
6	Acc4749	66	112	11.48	4.39	8.00	13.00	12.05	0.91	33.00	13.48	593.50	42.08	1462.71
7	Acc2438	68	118	14.26	4.61	7.40	12.60	10.99	1.78	13.79	14.69	232.40	20.62	476.28
8	Acc2024	68	113	11.90	4.73	8.40	13.20	12.22	1.26	11.59	12.05	310.92	10.26	263.76
9	Acc4670	63	114	12.53	4.31	6.80	12.33	11.46	1.12	20.20	15.82	314.30	40.53	533.16
10	Acc4294	68	130	14.47	4.49	7.47	13.80	10.97	1.21	17.60	15.40	364.40	23.58	552.70
11	Acc4663	68	113	14.54	4.24	6.93	13.80	10.27	1.76	14.78	14.78	211.60	11.91	346.71
12	Acc1257	68	122	12.57	3.91	6.93	12.07	11.80	1.17	26.23	14.03	538.80	34.90	789.76
13	Acc1998	68	116	10.25	4.28	8.60	13.47	13.58	1.40	50.07	12.94	635.50	65.51	2190.04
14	Acc4893	68	117	15.66	3.95	8.40	13.13	11.92	1.07	35.07	15.49	350.90	40.65	1799.24
15	Acc1179	63	116	15.20	4.02	7.93	13.47	13.39	1.36	37.40	16.17	819.80	54.52	2147.40
16	Acc2355	58	106	17.54	3.47	8.00	13.07	13.41	1.42	53.49	17.03	1247.30	85.66	1936.03
17	Acc1121	68	116	14.75	4.02	6.77	12.93	12.32	1.74	39.83	15.63	851.40	58.16	1949.20
18	Acc5352	53	89	14.17	2.16	6.87	11.93	10.72	0.66	39.17	16.32	745.30	54.75	1836.00
19	Acc1958	65	113	14.34	4.39	8.27	13.53	11.49	0.70	41.67	10.61	576.40	47.67	1578.36
20	Acc1165	68	129	12.61	4.31	9.77	15.01	13.42	1.47	48.97	15.76	872.30	53.31	1578.36
21	Phokwane1	60	116	14.63	3.82	7.33	13.27	11.33	1.45	37.27	15.03	588.70	39.88	1787.66
22	Glenda	48	89	11.40	2.07	5.80	12.20	8.88	0.40	25.97	11.70	307.50	20.88	1366.19
23	L-cwp2	64	107	16.96	3.77	6.73	14.87	13.25	1.30	45.40	17.61	1396.00	75.57	591.90
24	L-cwp3	67	136	24.08	1.91	6.00	1.80	12.65	1.48	25.71	15.83	680.80	38.67	2528.46
25	Cwp1	61	100	12.97	3.97	3.67	6.60	12.84	1.51	40.53	16.61	2383.30	55.81	264.18
26	Cwp2	68	125	21.84	3.85	6.33	13.53	10.97	1.82	40.03	19.23	939.40	65.11	2168.18
27	Cwp4	68	140	14.08	4.06	7.60	12.27	13.41	1.72	34.37	16.57	1123.70	78.75	2624.89
28	Cwp5	68	132	14.08	4.31	7.20	13.93	11.49	1.41	44.90	18.09	1000.90	61.90	1723.58
	GM	64.73	117.28	14.61	3.92	7.10	12.51	11.97	1.34	32.55	15.29	1025.90	45.03	1387.568
	CV%	5.18	6.5	9.74	16.16	11.91	7.95	10.53	21.09	14.34	11.91	14.00	13.39	17.96
	LSD	4.58	10.4	1.95	0.09	1.16	1.36	1.72	0.39	6.38	2.49	235.17	8.24	340.619
	MS_e	11.24	57.0	2.03	0.40	0.72	0.99	1.59	0.08	21.78	3.32	20639.00	36.35	62136.166
	MS_g	75.78**	465.18**	25.57**	1.58**	7.69**	19.47**	4.41**	0.41**	437.57**	10.67**	825745.00**	1256.24**	1829331.36**

** represent significance at P ≤ 0.01; LSD = least significant difference; CV = coefficient of variation; MSe = mean square for error; MSg = mean square for genotype; GM = grand mean; D50 = days to 50% flowering; D95 = days to 95% maturity; HSWT = hundred seed weight; LAI = leaf area index; NB = number of primary branches; NIP = number of internode per plant; NSPP = number of seed per pod; PH = plant height; NPP = number of pod per plant; PL = pod length; PWT = dry weight per plant; GYP = grain yield per plant; GY = total grain yield.

The results showed that plant height ranged from 0.3 m for Glenda to 1.82 m for Cwp2. Several other genotypes, which exhibited a tallness trait in the descending order were Acc2438, Acc4663 and Cwp4. The highest mean value for dry weight per plant was recorded in Cwp1 (2383.30 g) and Acc2355 (1247.30 g); while the lowest value of 211.60 g was recorded in Acc4663. In general, genotypes that showed significant performance for fodder yield were Cwp1>Acc2355>Cwp4>Cwp5>Cwp2. Similarly, Cwp4>L-cwp3>Acc1998>Cwp2>Acc1168 genotypes were top five genotypes that showed superiority for grain yield performance.

Figure 4. 1 Variation in phenological development of cowpea in the field

Figure 4. 2 Variation in pod length, width and shape

Figure 4. 3 Variation in seed, colour and shape

4.1.2 Genetic parameters and path coefficient analysis

The results showed a small difference between genotypic and phenotypic coefficient of variation. The highest genotypic coefficient of variation was estimated for grain yield (130344.55) and the lowest was observed for days to 50% flowering (13.11) (Table 4.2). Broad sense heritability was generally higher for most of the traits. The highest broad sense heritability (98.57%) was observed for seed yield per plant while the lowest (84.54%) was observed for the number of seed per plant (NSPP).

Grain yield showed a significant and positive correlation with the number of pod per plant and seed yield per plant. Days to 50% flowering exhibited a negative and non-significant correlation with gain yield (-0.09), seed yield per plant (-0.09) and dry weight per plant (-0.13). Inversely, number of pod per plant exhibited a highly significant and positive association with number of seed per pod, gain yield, seed yield per plant and dry weight per plant. The results showed that days to 50% flowering correlated positively and highly significantly with days to 95% maturity (0.72) and plant height (0.50) as well as significantly with leaf area index (0.46). The association results revealed a negative and non-significant correlation between grain yield and leaf area index, number of primary branches per plant and number of internode per plant. However, number of primary branches per plant and number of internode per plant had a significant and positive correlation with each other and highly significant and negative correlation with hundred seed weight.

There was a highly significant ($p \leq 0.01$) positive association (0.53) between pod length and seed yield per plant. as well as dry weight per plant (Table 4.3). In addition, the number of pod per plant showed a highly significant ($p \leq 0.01$) positive correlation with the seed yield per plant. Path coefficient analysis showed that number of pod per plant (0.93) had the highest positive and direct effect on yield (Figure 4.4). Hundred seed weight (0.19), number of seed per pod (0.21) and number of primary branches (0.24) also exerted positive but low direct effect on yield. The duration to 50% flowering, dry weight per plant, number of internode per plant exhibited a negative effect on yield.

The highest negative effect (-0.73) was observed for dry weight per plant. Despite the high negative effect of dry weight per plant and number of internode per plant, the traits showed the highest positive indirect effect on grain yield via pod number per plant and number of internode per plant, respectively. Similarly, neutralization of negative direct effect was observed for days to 50% flowering through plant height (0.50) and leaf area index through number of primary branches per plant through (0.47).

Table 4. 2 Genetic variability and heritability parameters for selected quantitative traits of cowpea

	Genetic parameters							
Traits	GM	MS_g	MS_e	σ^2g	σ^2p	GCV	PCV	h^2bs (%)
D50	64.73	75.78	11.24	72.03	83.27	13.11	14.1	93.01
D95	117.28	465.18	57	446.18	503.18	18.01	19.13	94.17
HSWT	14.61	25.57	2.03	24.9	26.92	34.15	35.51	96.16
LAI	3.92	1.58	0.4	1.45	1.85	30.76	34.75	88.54
NB	7.1	7.69	0.72	7.45	8.16	38.43	40.23	95.52
NIP	12.51	19.47	0.99	19.14	20.13	34.99	35.88	97.51
NSPP	11.97	4.41	1.59	3.88	5.47	16.46	19.54	84.24
PH	1.34	0.41	0.08	0.39	0.47	46.39	50.98	91.00
NPP	32.55	437.57	21.78	430.31	452.09	63.73	65.33	97.56
PL	15.29	10.67	3.32	9.57	12.88	20.23	23.48	86.18
PWT	1025.9	825745.00	20639.00	818865.33	846384.00	88.21	89.69	96.75
GYP	45.03	1256.24	36.35	1244.12	1280.47	78.33	79.46	98.57
GY	1387.57	1829331.36	62136.17	1808619.3	1870755.47	130344.55	134822.62	96.68

GM = grand mean; MS_g = genotype mean squares; MS_e = Error mean squares; σ^2g = genotypic variance; σ^2p = phenotypic variance; GCV = genotypic coefficient of Variation; PCV = phenotypic coefficient of variation; h^2bs (%) = broad sense heritability.

Table 4. 3 Pearson correlation coefficients among quantitative traits of selected cowpea genotypes

Traits	D50	D95	HSWT	LAI	NB	NIP	NSPP	PH	NPP	PL	PWT	GYP	GY
D50	1.00												
D95	0.72**	1.00											
HSWT	0.09ns	0.29ns	1.00										
LAI	0.46*	0.12ns	-0.30ns	1.00									
NB	0.18ns	0.01ns	-0.54**	0.47*	1.00								
NIP	0.09ns	-0.07ns	-0.43**	0.42*	0.89*	1.00							
NSPP	0.28ns	0.13ns	0.06ns	0.24ns	0.15ns	-0.07ns	1.00ns						
PH	0.50**	0.59**	0.33ns	0.17ns	-0.08ns	-0.06ns	0.28ns	1.00					
NPP	-0.12ns	-0.22ns	0.05ns	0.00ns	0.17ns	0.14ns	0.54**	-0.12ns	1.00				
PL	0.11ns	0.24ns	0.53*	-0.12ns	-0.21ns	-0.05ns	0.26ns	0.49*	0.32ns	1.00			
PWT	-0.13ns	-0.20ns	0.15ns	-0.02ns	-0.28ns	-0.26ns	0.55*	0.15	0.64**	0.51**	1.00		
GYP	-0.09ns	-0.07ns	0.21ns	-0.02ns	0.07ns	0.06ns	0.66*	0.13*	0.85**	0.53**	0.70*	1.00	
GY	-0.09ns	0.10ns	0.34ns	-0.23ns	-0.02ns	-0.13ns	0.39ns	0.01	0.60**	0.21ns	0.17ns	0.64*	1.00

*, **, ns represent significance at P ≤ 0.05, P ≤ 0.01, not significant, respectively; D50 = days to 50% flowering; D95 = days to 95% maturity; HSWT = hundred seed weight; LAI = leaf area index; NB = number of primary branches; NIP = number of internode per plant, NSPP = number of seed per pod; PH = plant height; NPP = number of pod per plant; PL = pod length; PWT = dry weight per plant, GYP = grain yield per plant, GY = total grain yield.

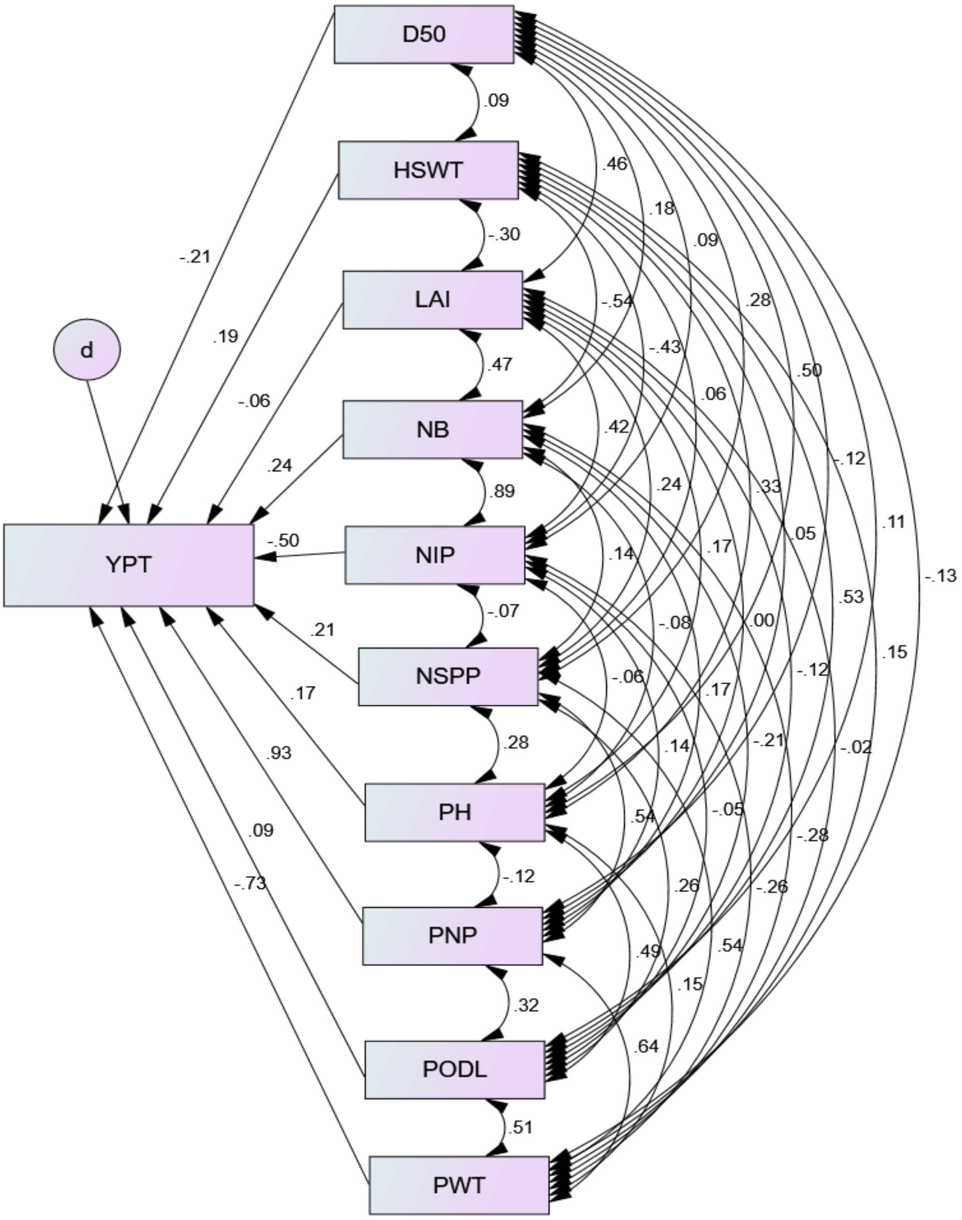

Figure 4.4 The direct and indirect effect of yield contributing traits on the grain yield

D50 = days to 50% flowering; D95 = days to 95% maturity; HSWT = hundred seed weight; LAI = leaf area index; NB = number of primary branches; NIP = number of internode per plant, NSPP = number of seed per pod; PH = plant height; NPP = number of pod per plant; PL = pod length; PWT = dry weight per plant, GYP = grain yield per plant

4.1.3 Principal component analysis and principal component biplot

The principal component (PC) analysis are revealed that the first four principal components accounted for 78.2% of the variation among the genotypes and had the eigenvalues that were greater than one (Table 4.4). However, PC1 and PC2 explained more than 50% of the total variation, which was predominately attributed to grain yield per plant, dry weight per plant, pod length, number of seed per pod and hundred seed weight for PC1 and pod number per plant, days to 95% maturity, plant height and days to 50% flowering. Results also showed that the cowpea genotypes were scattered randomly across the entire quadrant but genotype Acc4893, Phokwane1 and Cwp4 were grouped close to the origin (Figure 4.5). Conversely, genotype Glenda, Acc2014, L-cwp3 and Acc2355 were grouped in the extreme margins of the cartersian plane. The principal component biplot showed that Cwp1, L-cwp2 and Acc2355 showed a superior performance for traits, which include number of pods per plant, number of seed per plant, grain yield per plant and total yield per plot. These traits revealed a negative and significant association with the other traits as indicated by a large angle between the traits. Genotype in quadrant three, performed poorly for all the traits studied.

Table 4. 4 Principal component analysis of 28 cowpea genotypes showing the eigenvector, eigenvalue and cumulative percentage of the first four principal component axes

	Eigenvector			
	PC1	PC2	PC3	PC4
D50	0.099	-0.292	0.423	-0.03
D95	0.157	-0.39	0.279	0.261
HSWT	0.301	-0.235	-0.189	0.242
LAI	-0.077	0.048	0.45	-0.345
NB	-0.164	0.243	0.46	0.232
NIP	-0.166	0.216	0.407	0.211
NSPP	0.307	0.188	0.223	-0.186
PH	0.247	-0.304	0.246	-0.117
NPP	0.283	0.423	0.049	0.085
PODL	0.365	-0.049	0.023	-0.042
PWT	0.343	0.219	-0.079	-0.501
GYP	0.379	0.333	0.061	0.059
GY	0.271	0.202	-0.049	0.586
Eigenvalue	4.139	2.968	2.705	1.136
Variability (%)	29.567	21.201	19.319	8.113
Cumulative %	29.567	50.768	70.087	78.2

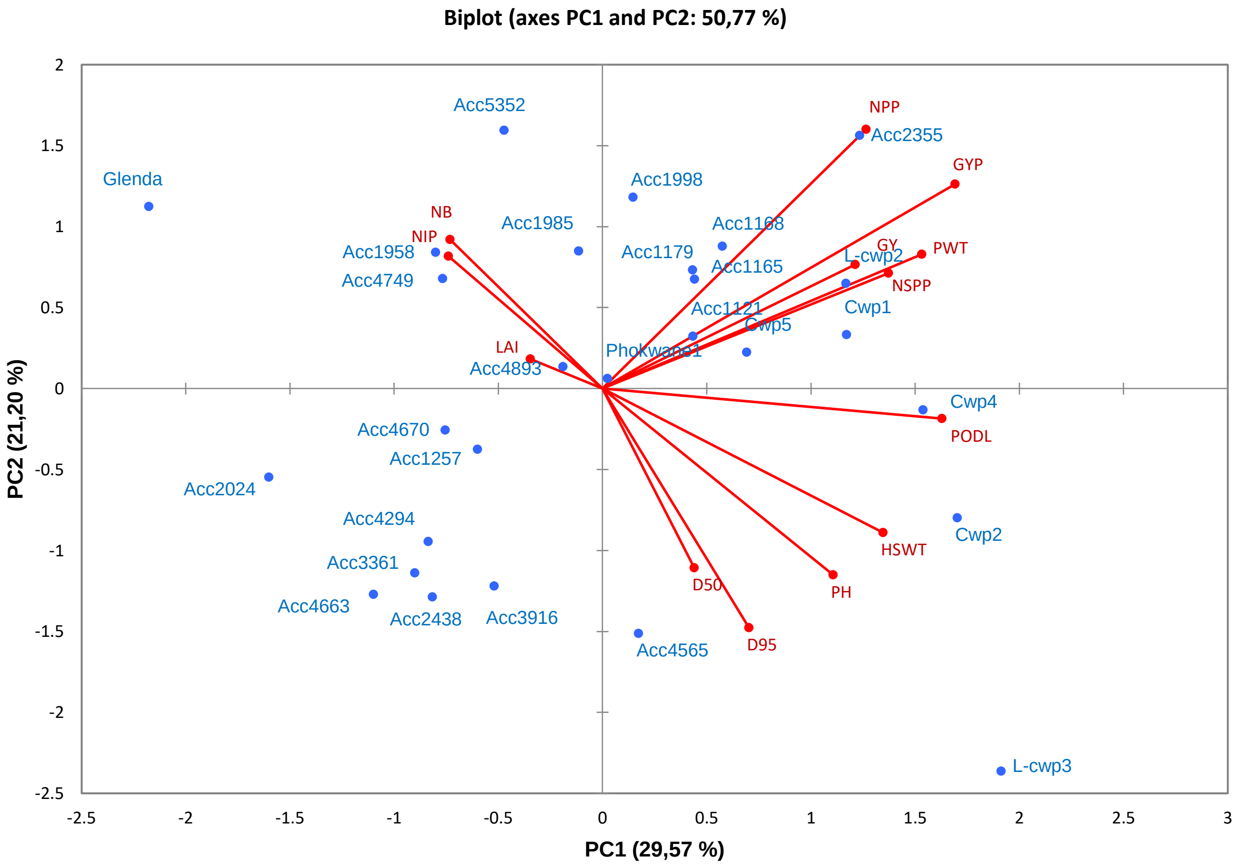

Figure 4. 5 Biplot showing the dissimilarity and similarity of cowpea genotypes and interrelationship of yield and yield related traits through PC1 and PC2

4.2 Experiment # 2: Greenhouse study

4.2.1 Relative water content and leaf wilting index

Genotypic response of cowpea to moisture deficits was highly significant for relative water content at both week two (W2DS) and week four (W4DS) of drought stress (Table 4.5). The genotypes exhibited a higher relative water content (RWC) in week two than in week four of drought stress (W4DS). The RWC measured in W2DS ranged from 79.77% (Acc1257) to 46.43% (TVU7778). In W4DS, genotype Acc1257, Acc2355, Acc1168, IT96D-602 and Acc5352 maintained a RWC > 40.0% (Table 4.5) attained by genotype.

The results showed no significant differences among the genotypes for leaf wilting index (LWI) at W2DS but highly significant ($p \leq 0.01$) differences were observed at W4DS. The majority of the genotypes exhibited a low LWI at W2DS. Genotypes TVU7778, Cwp2 and Acc1121 were markedly sensitive to moisture due to high leaf wilting index at both W2DS and W4DS (Figure 4.6). Mean values for leaf wilting index at W4DS varied from 0.11 for Acc3916 to 1.00 for Cwp2, L-cwp3 and Pokhwane1.

Table 4. 5 Variability among 28 cowpea genotypes for selected physiological traits in response to drought stress

No	Genotype	RWC2	RWC4	RG2	STG2	LPC (µmol/g dry weight)	LWI2	LWI4
1	Acc1121	56.90	20.66	1.00	1.56	2.03	0.54	0.96
2	Acc1165	58.34	32.87	1.67	1.78	1.93	0.00	0.78
3	Acc1168	63.18	45.85	5.00	4.33	4.89	0.04	0.28
4	Acc1179	60.09	30.47	2.33	2.44	2.68	0.17	0.69
5	Acc1257	79.77	58.73	4.78	4.67	8.04	0.06	0.17
6	Acc1958	64.26	36.37	2.27	3.47	3.34	0.15	0.84
7	Acc1985	68.66	33.10	1.44	2.00	3.61	0.15	0.91
8	Acc1998	66.88	25.96	2.56	2.56	0.56	0.22	0.78
9	Acc2024	70.13	22.21	1.00	1.36	0.39	0.17	0.89
10	Acc2355	62.96	52.09	3.22	4.78	5.48	0.09	0.19
11	Acc2438	57.69	29.26	1.80	2.20	1.38	0.17	0.44
12	Acc3361	65.21	35.87	1.67	1.78	0.46	0.39	0.96
13	Acc3916	59.69	38.97	3.00	4.33	4.55	0.04	0.11
14	Acc4294	65.16	30.40	1.42	1.53	3.29	0.00	0.46
15	Acc4565	59.11	33.65	2.60	2.60	1.87	0.50	0.67
16	Acc4663	63.13	39.23	2.20	2.33	1.38	0.33	0.61
17	Acc4670	64.04	35.78	1.00	1.00	3.88	0.00	0.94
18	Acc4749	59.03	31.43	2.33	2.33	2.48	0.33	0.67
19	Acc4893	59.12	33.66	1.93	1.93	1.16	0.17	0.94
20	Acc5352	57.02	40.57	2.07	2.20	2.35	0.00	0.81
21	Cwp2	54.27	13.19	1.00	1.00	1.92	0.66	1.00
22	Cwp4	60.12	31.88	1.93	1.73	0.48	0.28	0.96
23	Cwp5	59.34	41.76	1.00	1.00	2.64	0.24	0.94
24	Glenda	59.70	27.52	2.33	2.64	1.73	0.00	0.78
25	IT96D-602	61.67	42.46	2.94	4.69	8.81	0.09	0.78
26	L-cwp3	63.89	28.71	2.00	2.00	0.44	0.33	1.00
27	Phokwane1	59.63	28.54	1.07	1.20	3.63	0.24	1.00
28	TVU7778	46.43	13.54	1.33	1.42	2.32	0.93	0.99
	LSD	6.51	8.79	2.10	2.21	1.71	0.50	0.45
	MS$_g$	102.62**	300.275**	3.127*	4.16**	13.329**	0.15ns	0.23**

**, ns represent significance at ≤ 0.01, not significant, respectively; RWC2 = relative water content at week two of drought stress; RWC4 = relative water content at week four of drought stress; LWI2 = leaf wilting index at week two of drought stress; LWI4 = leaf wilting index at week four of drought stress; LPC = leaf proline content; RG2 = regrowth and STG2 = stem greenness.

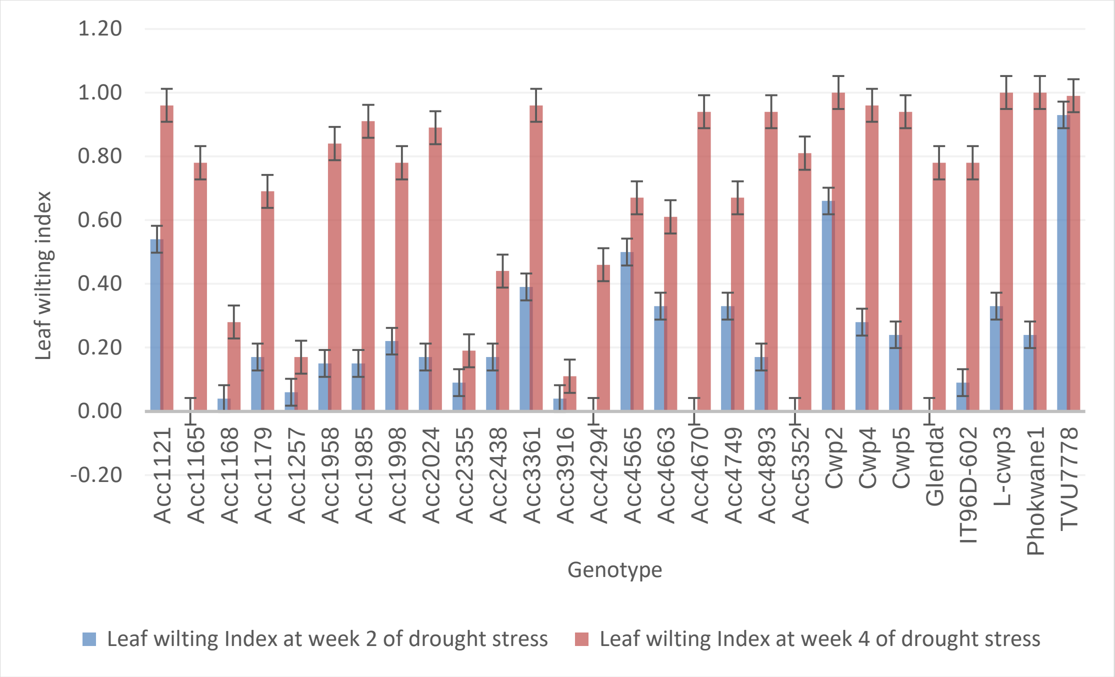

Figure 4. 6 Leaf wilting index of 28 cowpea genotypes in response to two and four weeks of drought stress

4.2.2 Leaf proline content

The response of genotypes varied significantly through accumulation of proline under serve drought stress at week five of drought stress (Figure 4.7). The accumulation of proline content among the genotypes peaked at 8.81 µmol/g dry weight for genotype IT96D-602 (drought tolerant control), followed by Acc1257 (8.04 µmol/g dry weight) and Acc2355 (5.48 µmol/g dry weight). The drought susceptible control (TVU7778) accumulated a 2.32 µmol/g dry weight of proline content, which was higher than some of the genotypes. Genotype Acc2438 recorded the minimum value (0.39 µmol/g dry weight) of proline content.

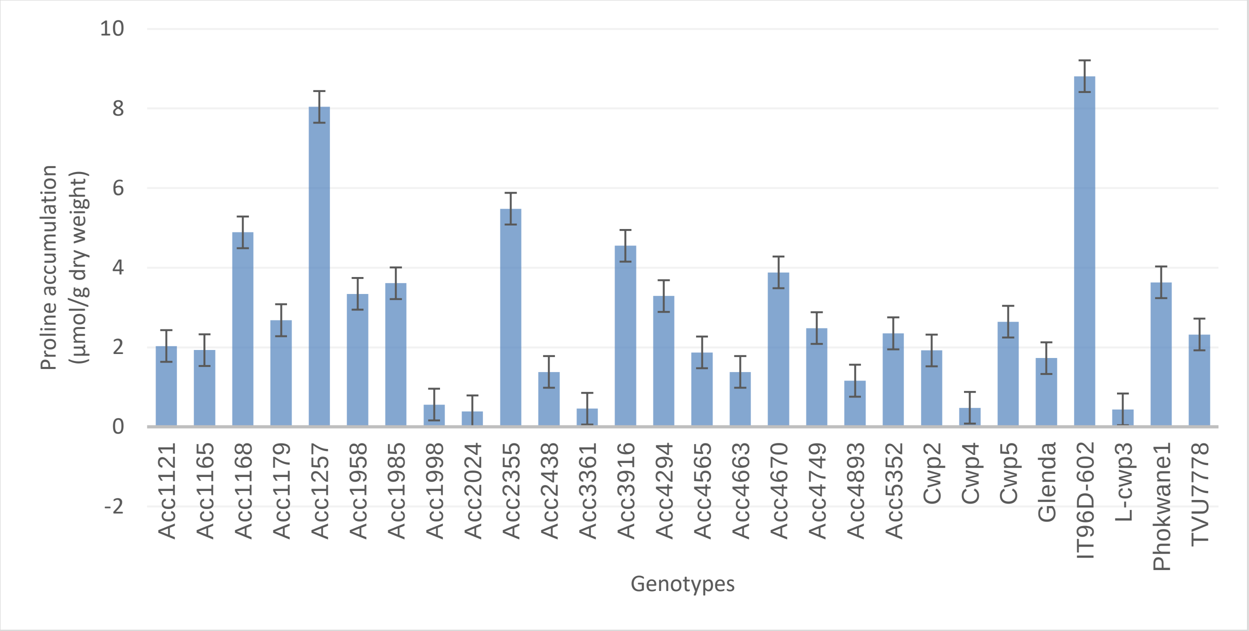

Figure 4. 7 Proline content of 28 cowpea genotypes in response to five weeks of drought stress

4.2.3 Regrowth and stem greenness

Regrowth and stem greenness were scored after terminating the drought treatment to determine the genotypes with ability to recover from five weeks of drought stress. There were highly significant ($p \leq 0.01$) differences in regrowth and stem greenness traits among the genotypes (Table 4.5).

Genotypes Cwp5 and Acc4670 were completely dry and dead as indicated by stem greenness value of 1.00. The high mean values for stem greenness (> 4.00) were scored for genotypes Acc1257, Acc2355, Acc1168, Acc3916 and IT96D-602. Similarly, genotype Acc1257, Acc2355, Acc1168, Acc3916 and IT96D-602 also scored high values for regrowth. However, the highest value was observed for genotype Acc1168 (5.00) and Acc1257 (4.78) indicating that these genotypes had a potential to recover from apical meristem compared to genotypes Acc1257, Acc2355 and IT96D-602 which recovered from auxiliary buds (Figure 4.8). Other genotypes died under moisture stress as indicated by brown stems (Figure 4.8).

Figure 4. 8 Phenotypic response to drought: A - regrowth from apical meristem vs. B - regrowth from auxiliary buds; C - maintenance of stem greenness vs. D - brown and dead stem of drought susceptible genotypes; E - leaf orientation and F - early flowering in drought tolerant control

4.2.4 Correlation among the traits

There were high significant (p ≤ 0.01) positive correlations between the physiological traits among the cowpea genotypes (Table 4.6). Leaf wilting index at week two and week four of drought stress correlated negatively with the rest of physiological traits but showed positive and highly significant correlation between leaf wilting indexes at both week two and week four. Stem greenness had positive (0.924) and highly significant (p ≤ 0.01) correlation with regrowth. However, stem greenness at week 2 showed a negative (-0.699) but highly significant (p ≤ 0.01) correlation with leaf wilting index at week four of drought stress.

Table 4.6 Pearson correlation coefficients among physiological traits of 28 selected cowpea genotypes

Variables	RWC2	RWC4	RG2	STG2	LPC	LWI2	LWI4
RWC2	**1**						
RWC4	**0.412****	**1**					
RG2	**0.339****	**0.469****	**1**				
STG2	**0.311****	**0.496****	**0.924****	**1**			
LPC	**0.238***	**0.545****	**0.311****	**0.425****	**1**		
LWI2	**-0.437****	**-0.409****	**-0.401****	**-0.428****	-0.177	**1**	
LWI4	**-0.258***	**-0.492****	**-0.691****	**-0.699****	**-0.339****	**0.405****	**1**

*, **, ns significant at P ≤ 0.05, P ≤ 0.01, not significant, respectively; RWC2 = relative water content at week two of drought stress; RWC4 = relative water content at week four of drought stress; LWI2 = leaf wilting index at week two of drought stress; LWI4 = leaf wilting index at week four of drought stress; LPC = leaf proline content; RG2 = regrowth and STG2 = stem greenness

5.0 CHAPTER FIVE: DISCUSSION

Cowpea is an important crop for cultivation in hot and dry regions of South Africa. However, the production and productivity of the crop is reportedly low making cowpea one of the underutilized crop species with the potential to improve the food and nutritional security in the country. Analysis of variance showed highly significant ($P \leq 0.01$) differences for all phenotypic traits among the genotypes used in the current study, indicating the potential to select parental line(s) for improving cowpea for the traits of interest. Similar results were also reported in cowpea (Nwosu *et al*., 2013; Nkoana *et al*., 2017), Soybean (Akram *et al*., 2011) and bambara groundnut (Shegro *et al*., 2013; Unigwe *et al*., 2016). Glenda (local check) and Acc5352 were the earliest genotypes which reached 50% flowering in 48 and 53 days respectively after planting, while most genotypes were late flowering types. Early genotypes had short plant height and reached 95% maturity than the rest of the genotypes. The present results agree with Khan *et al*., (2010) who reported that earliness in cowpea is associated with short plant height. Similarly, high dry weight per plant observed for Cwp1 and Acc2355 signifies that the genotypes had long duration to 50% flowering and days to physiological maturity. Acc5352 is desirable for its earliness, moderate grain and dry weight per plant as a dual-purpose genotype for escaping the late season drought while proving food and fodder for the current cropping season (Timko and Singh, 2008). Mean values for number of pod per plant found in this study were consistent with the findings from Badawi *et al*., (2015) but greater than values reported by Manggoel *et al*., (2012). Masenya and Asiwe (2016) observed significant varietal differences of 97 cowpea genotypes under two agro-ecological conditions. As a result, highly significant differences were observed for grain yield in the study may be attributable to varietal response to the environment factors. Although, Cwp1 showed superior performance for dry weight per plant, it recorded the lowest grain yield. The wide range observed for dry weight per plant and grain yield revealed the presence of sufficient genetic variability that is prerequisite in cowpea development for dual-purpose variety with improved grain and fodder yield.

The large seed size (> 24.0 g per 100 seeds) was of interest to the local current cowpea breeding efforts. Large and pod length are the most important traits of preference by most cowpea growers and consumers (Singh, 2005; Asiwe, 2009; Nkonkgolo *et al*., 2003; Horn *et al*., 2015). Furthermore, selection of large seed size can be achieved in early stages of breeding programme (De Souza *et al*., 2003). Gerrano *et al*., (2017) reported that cowpea seed size is an important quantitative trait for consumer acceptability and commercialization in South Africa. They also reported that genotypes with large seed was of interest to cowpea breeders although the trait may be confounded by low germination rate compared to small seeds. Pod length varied considerably among the genotypes which was consistent with previous observations (Gerrano *et al*., 2015). Genotypes that are discriminated with small seed size share similar trait with the wild cowpea. In general, most genotypes outperformed the local check (Glenda) for most of the phenotypic traits that were measured.

The principal component biplot also revealed the presence of sufficient phenotypic variability for the genotypes used in the study indicated by random scattering of the genotypes across the four quadrants. The principal component analysis showed that the first four principal components with eigenvalue greater than one explained the cumulative variation of 78.2%. Traits that contributed highly to the total variation were yield per plant, dry weight per plant, pod length, number of seed per pod and hundred seed weight for first principal component and pod number per plant, days to 95% maturity, plant height and days to 50% flowering for second principal component. Molosiwa *et al*., (2016) also found that seed parameters were most reliable for discriminating the accessions collected different agro-ecological zones of Botswana. Genotype Acc4893 and Phokwane1 were scattered close to the origin implying that these genotypes were mostly genetically similar compared to genetically dissimilar genotypes that were scattered away from the origin. This was in agreement with previous (Nkoana *et al*., 2017 – Appendix I). As such, genotypes Acc2024, Acc5352, Acc2355 and L-cwp3 can be considered the most genetically distinct genotypes that can serve as the potential parental lines for improvement of quantitative traits of interest.

Crossing of genetically diverse cultivars may lead to heterotic group that create the potential of identifying the superior genotypes within a segregating population (Singh, 2005).

The estimate of broad sense heritability and genetic parameters revealed a small difference between the phenotypic variance and genotypic variance, thus genetic makeup had a considerable contribution to the observed variation than environmental factors. Similar results were reported by Aliyu and Makinde (2016) who found the lowest environmental variance. The broad sense heritability estimates were also higher among the genotypes studied. The high heritability in broad sense indicate that additive gene action dominated the expression of the traits. Nwosu *et al*., (2013) also reported a relatively higher heritability values in most phenotypic traits. As a result, success in cowpea improvement can be achieved through phenotypic selection.
Karama *et al., (*2012) reported higher yield returns in improved varieties; while Asiwe (2009) reported an average yield of 500 kg/ha for most cowpea growers due to the cultivation of unimproved cowpea varieties in South Africa. Moreover, cowpea is predominately cultivated for grain yield which is mainly dependent on various yield related traits. Therefore, understanding and knowledge of correlation of traits is necessary for discerning the opportunity for simultaneous selection of different traits that influence the grain yield. The significant and positive correlation between grain yield with pod number per plant and grain yield per plant imply that selection for these traits may lead to higher yield returns. Pod length correlated positively and significantly with plant height (0.49) and hundred seed weight (0.53). Therefore, these justify the low grain yield obtained in most late maturing types as attributable to negative correlation (-0.12) that existed between plant height and pod number per plant (Nwosu *et al*., 2013). Although days to 50% flowering is a highly heritable trait, its negative correlation with both grain yield and fodder yield, implied that direct selection for days to 50% flowering may slow down genetic gain in grain yield improvement programs. However, Manggoel *et al*., (2012) has shown that the significance of traits which are negatively associated with grain yield can be exploited through indirect selection of other yield contributing traits using path analysis.

As such, path analysis is an important complimentary tool for correlation which indicates the cause and effect of other traits on grain yield since, the magnitude of association for a particular trait with low heritability may be higher due to the effect of other traits. The direct and indirect effect of yield related traits on grain yield are shown with the aid of a path analysis diagram (Figure 4.2). Path analysis showed that the highest magnitude of correlation (0.89) that existed between number of primary branches and number of internode per plant had small direct and positive effect on grain yield through number of primary branches. Number of pod per plant had a high and positive direct effect on grain yield. Almeida *et al*., (2014) reported a high positive and direct effect of number of pod per plant at four different agro-ecological conditions in Brazil. By contrast, direct selection based on days to 50% flowering, dry weight per plant and number of internode per plant may slow down the progress in grain yield improvement programmes due to the negative direct effect of these traits on grain yield. The present results are slightly different from Mahbub *et al*., (2015) who reported a negative and direct effect of pod number per plant on yield. Path coefficient analysis provide an additional information on the direct and indirect effect of yield component traits on grain yield. As such, path analysis is an invaluable complementary tool for correlation studies which shows the magnitude and nature of association between any two traits (Silva *et al*., 2007). Days to 50% of flowering, dry weight per plant, pod number per plant, number of internode per plant and seed yield per plant are important selection indices that can inform the breeding objectives for trait of interest. The wide genetic variability revealed among the genotypes for the quantitative traits measured in the study such as pod length, seed size and plant height provides a valuable prerequisite resource for breeding of the traits of interest.

In conclusion, this study affirmed that collection, characterization, and evaluation of germplasm for agro-morphological traits is important in discerning any genetic variability that can be exploited in plant breeding. The significant differences ($p \leq 0.01$) found among phenotypic traits from genotypes used in the study, indicate the potential for selecting parents for future breeding program to improve cowpea value chain in South Africa.

Most genotypes were the late maturing types and performed better than the local check for most of the traits However, Glenda was the earliest genotypes to reach maturity. The genotypes (Cwp4, L-cwp3 and Acc1998) achieved > 2000 kg/ha of grain yield. These genotypes could be evaluated further over more testing locations and seasons in order to validate their usefulness in future cowpea breeding efforts. The principal component analysis further verified the wide genetic variability within the genotypes indicating the scope for cowpea improvement for both fodder and grain yield. Phenotypic variation among the genotypes were primarily attributed to grain yield per plant, dry weight per plant, pod length, number of seed per pod and hundred seed weight, number of pod per plant, days to 95% maturity, plant height and days to 50% flowering.

Among the above ground plant parts, leaf is the most important plant organ that house most traits which interact with the environment for regulation of plant water relations under both water deficits and adequate water supply. Leaf wilting index represents the overall response of leaf components associated with drought tolerance in crop plant. The present study revealed no significant differences among the genotypes for leaf wilting index at week 2 of drought stress (W2DS) that was followed by highly significant difference among cowpea genotypes for leaf wilting index at week four of drought stress (W4DS). This implies that leaf wilting index is not an ideal discriminating trait between drought tolerant and susceptible genotypes during the early stage of water stress conditions. Nonetheless, several studies have shown that wilting signs in drought susceptible cowpea genotypes are apparent from the first week of drought stress (Muchero *et al*., 2008; Chiulele and Agenbag, 2004). The differential response of genotypes was clear in W4DS which ranged from 0.11 for genotype Acc3916 to 1.00 for genotype Phokwane1, L-cwp3 and Cwp2. Other genotypes, which exhibited low values for leaf wilting index were Acc1257, Acc2355 and Acc1168. According to Pungulani *et al*., (2013), lowest values for leaf wilting index signify the drought tolerant trait that is significantly correlated with stay green or delayed leaf senescence trait under drought stress. The results showed that leaf wilting index is negatively correlated with stem greenness and recovery. Muchero *et al*., (2008) also found that drought induced senescence was correlated with stem greenness and regrowth.

As such, it was found from the present results, that plants from genotypes with low leaf wilting index tolerated the vegetative drought stress by maintaining green stems and recovered from drought stress after re-watering. Evaluation of genotype based on vegetative drought tolerance has been emphasised as a reliable and cost effective pre-screening approach to screen for large number of genotypes according to Muchero *et al., (*2008) who found similar genotypic response of cowpea genotypes at both vegetative and reproductive stage.

Leaf wilting index scoring is limited to seedling stage drought evaluation to avoid the effect of leaf senescence due to aging of lower leaves. Furthermore, leaf wilting index may also be misleading in type 2 drought tolerance mechanism which allows a slow growth of the tips with moisture remobilization from lower leaves to the young leaves in response to limited water supply (Mai-Kodomi *et al*., 1999).

The **r**elative water content of genotypes decreased with progression of drought stress which also showed the existence of varietal differences in response to moisture stress due to inconsistent level of reduction among the genotypes. Genotypes that were drought susceptible undergone a sharp decrease in relative water content including the drought susceptible control (TVU7778). Several studies reported similar results in cowpea (Verbree *et al*., 2015), tepary bean (Narina *et al*., 2014) and wheat (Arjenaki *et al*., 2012) who found that drought tolerant cultivars had high relative water content. The additive gene effects control the inheritance of relative water content (Farshadfar and Amiri, 2015). Genotype TVU778 and Cwp2 were found to be the most drought susceptible with the lowest relative water content on W4DS among the cowpea genotypes. The significant decrease in relative water content at W4DS was mainly observed in the genotypes, which failed to recover from drought stress implying that relative water content is an important indicator of drought tolerance in cowpea. High relative water content in Acc1168, Acc3916, Acc1257 and Acc2355 could imply that such genotypes exhibited sensitive stomata closure or enhanced water absorption through maintenance of high leaf water potential and dense root system in deeper soil layers of the soil.

De Ronde and Spreeth (2007) reported a significant difference in root architecture of the two controls used in the study where the drought tolerant control (IT96D-602) had accumulated more root system in the deeper soils than the drought susceptible control. The study also found that both genotypes had low grain yield under drought stress. Higher relative water content prevents the disruption of photosynthetic process and absorption of carbon dioxide into the leaves under drought stress for cell elongation which lead to increased roots and shoot.

As a result, photosynthetic rate was shown to reduce linearly with field capacity and yield signifying that drought stress impairs the photosynthetic process mainly due stomata closure, leaf senescence and reduced carbon dioxide in the chloroplast (Mafakheri *et al*., 2010; Goufu *et al*., 2017). Batieno *et al*., (2016) observed that, despite varietal’s unique response to drought stress, yield reduction under drought stress treatment were significantly higher in genotypes with high yielding potential in non-stressed conditions. Furthermore, Farouk *et al*., (2013) and Hayatu *et al*., (2014) reported that high relative water content is associated with increased mean yield under drought stressed environments with no quantifiable effects under full irrigation. The unique varietal response to drought stress is partly attributed by achievement of high relative water content as a drought tolerance trait. Therefore, low grain yield experienced by smallholder farmers could either be due to low yield potential of landraces since, landraces are broadly characterized by inherent resistance to abiotic stresses. Effort to incorporate trait that reduce sensitivity of high yielding varieties under limited water supply would maximise the simultaneous exploitation of genetic potential for both high yield and drought tolerance.

Osmotic adjustment is a common dehydration tolerance mechanism in many crops for maintenance of leaf turgor under low tissue water potential. Accumulation of compatible and concentrated osmolytes such as proline in cytosol are considered the last dehydration tolerance mechanism which is optimized at the latest stage of drought stress for survival purpose rather than the enhancement of grain yield (Kholova *et al*., 2010).

In addition, drought intensity, growth stage and crop species affect the accumulation of proline but occur in significant amount in cowpea especially at flowering more than at vegetative stage. The genotypes evaluated for proline in the study showed highly significant variability in proline content after five weeks of drought stress implying that genotypes with the highest proline content continued with physiological processes better than other genotypes, which accumulated relatively lower proline content.

Since, proline content is responsible for preventing cell damage and reduction in relative water content, several studies have associated increased proline content with maintenance of high relative water content under stress (Goufo *et al*., 2017; Zegaoui *et al*., 2017; Cattevelli *et al*., 2008). As expected, genotype IT96D-602 accumulated the highest proline content followed by Acc2355, Acc3916 and Acc1168; while the lowest proline content was found in Acc2024. The highest proline content in IT96D-602 could be because of its earliness, which was further reduced by limited moisture to escape the event of drought stress (Figure 6F). Genotypes with low proline content are more sensitive to drought stress and has low ability to retain high relative water content under drought stress (Gazendam and Oelofse, 2007). Chiulele and Agenbag (2004) found that proline content in cowpea genotypes is not statistically different among the genotypes before the induction of drought stress. Goufo *et al*., (2017) reported that greater amount of proline is biosynthesized in leaves than in roots which correlated with yield under stress from two cowpea cultivars with contrasting tolerance to drought stress. Furthermore, the accumulation of proline exists in larger quantity in the growing tips to optimize the survival chance of the drought tolerant genotypes, thus cowpea response to drought is variable at whole plant level (Zegaoui *et al*., 2017). Proline content is a desirable trait for enhancing the yield stability in high yielding genotypes under marginal environments due to its consistent response under drought worsening factors such as high temperatures. It can be inferred that the aerial part of the plant plays a significant role in drought tolerance although, the signaling is originated from the roots where abscisic acid is synthesized and transported to the leaves to induce stomatal closure and proline accumulation. The proline content reported in this study was lower than that reported by Gazendam and Oelofse (2007) as the genotypes were subjected to longer period of drought stress, implying that accumulation of proline increase with drought stress, reach a peak and reduce with the further progression of drought stress. Proline content is highly adaptable trait that is synthesized by pyrroline-5-carboxylate synthetase (P5CS) enzyme under drought stress which is subjected to degradation by proline dehydrogenase (PROHD) in response to relief from drought stress (Liang *et al*., 2013).

Genotypes with the ability to recover from moisture stress are desirable in most cowpea production areas with low and poorly distributed rain, which results in drought stress that is often worsened by high temperatures. In the present study, there was significant genotypic differences that existed among the genotypes studied. The highest values for stem greenness were recorded for genotype IT96D-602, Acc1168, Acc3916, Acc1257 and Acc2355. The same genotypes also recorded high regrowth values. Similar results were also reported by Muchero *et al*., (2013) who concluded that stem greenness was the reliable predicator for drought tolerance under both field and greenhouse conditions. Additionally, these traits are also readily visualized after the reintroduction of moisture as explained by positive and highly significant correlation which exist between the traits. Selection based on stem greenness and regrowth has the potential to speed up the improvement for drought tolerance in crop plant which has been hindered by lack of simple and reliable traits that shows a holistic plant response to drought stress. Fatokun *et al*., (2012) found that cowpea genotypes which possess a stem greenness and low wilting index were able tolerate six weeks of drought stress which also reduced days to 50% flowering with average of 12 days.

Stem greenness, a trait that can be found in all maturity types could enhance the acclimation of cowpea to environments prone to both terminal and intermittent drought. According to Singh *et al*., (1999), the regrowth value close to three and five indicate that such genotypes had tolerated a period of water deficits and recovered from auxiliary buds and apical meristem, respectively. The drought evaluation results therefore, imply that genotype IT96D-602, Acc3916 and Acc2355 exhibited a recovery from auxiliary buds while genotype Acc1168 and Acc1257 recovered from apical meristem.

Muchero *et al*., (2008) classified the genotype, which showed recovery from auxiliary buds as medium drought tolerant genotypes and highly drought tolerant genotypes for genotypes which recovered from the apical meristem. The box screening method was able to discriminate between drought tolerant and drought susceptible cowpea genotypes. However, the method needs to be combined with field evaluation of the genotypes in order to determine their yield performance. Nonetheless, the method is cheaper than the proline analysis and will be useful in cowpea breeding programs that receive limited funding. Since, the water content in the crop is dependent on the balance between the water transpired by the shoots and absorbed by the roots, better understanding of root architectural traits related to drought stress will improve the progress in breeding for drought tolerance.

6.0 CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

Adoption rate exotic varieties by cowpea growers in South Africa has been low due to the lack of locally preferred traits. Furthermore, only few studies have reported the performance of cowpea in South Africa particularly the landraces. Therefore, there is a need to collect and evaluate more landraces for their drought tolerance and yield performance. This study showed that there is a wide genetic variability that is a prerequisite for breeding for high yield and drought tolerance. Grain yield ranged from 264.18 Kg/ha to 2624.89 kg/ha among the genotypes. As expected most of the genotypes (which were mainly landraces) showed relatively long duration to 50.0% flowering and 95.0% maturity. However, Genotype Acc5352 was the earliest in terms of flowering after Glenda, which is desirable for escaping drought stress in production regions that experience particularly terminal drought stress. Genotypes Cwp4 (2624.89 kg/ha), L-cwp3 (2528.46 kg/ha) and Acc1998 (2190.04 kg/ha) were high grain yielding and can be evaluated further over more seasons and locations in order to validate their stability and suitability for distribution to the growers.

The genotypes used in the study were distantly related according to the principal component analysis. The most distinct genotypes were Acc2355, Acc5352 and L-cwp3. Such genotypes are desirable potential parents for improving the drought tolerance, earliness and grain yield due to their likelihood of producing transgressive progenies that can be selected for the traits of interest. Improved locally adapted cowpea germplasm will enhance the adoption rate by the growers. Drought stress affected the performance of all cowpea genotypes due to diminished relative water content at week 4 of drought stress. Nonetheless, the genotypes Acc1257, Acc2355, Acc1168, and Acc5352 were considered highly drought tolerant with the ability to recover from drought stress were comparable to the drought tolerant control (IT96D-602). Stem greenness and regrowth appeared to be reliable indicators of drought tolerance in cowpea while other traits aided in showing the level of drought tolerance. All the traits studied were highly correlated with the stem greenness and regrowth indicating the importance of these traits in discriminating between the drought tolerance and susceptibility. However, exotic genotypes generally performed well and gave better performance for yield and other yield related traits, but they were susceptible to drought. Drought tolerant genotypes should possess high yield potential trait in nature for suitability in the smallholder sector where production is largely under rainfed conditions and is characterised by high temperatures as well as low, erratic rainfall. Hence, further studies should evaluate the yield potential of the drought tolerant genotypes under field drought conditions. However, the study was limited to a single location, hence the conclusions that were drawn from the results should be approached with considerable caution.

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APPENDIX

Preliminary Evaluation of Diverse Cowpea (*Vigna unguiculata*) Germplasm for Agronomic Performance

K.D. Nkoana[1,2], A.S. Gerrano[1] and E. T. Gwata[2]
[1]Agricultural Research Council, Vegetable and Ornamental Plant (ARC-VOP) Private Bag X 293, Pretoria 0001, South Africa
[2]University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

Corresponding author: nkoanek@arc.agric.za

INTRODUCTION

Cultivation of indigenous crops such as Cowpea (*Vigna unguiculata*) is one of the potential adaptation strategy to climate change which is currently threatening food security especially in sub-Saharan Africa (Massawe *et al.*, 2015). Cowpea is a relatively nutritious food legume with most edible plant parts (leaves, immature pods and dry grains) and better tolerance to moisture stress and poor soil conditions than most of the crops. In South Africa, however, production is low due unavailability of improved varieties limiting its value to subsistence purposes (Asiwe, 2009). Therefore, it is important to evaluate more cowpea germplasm to identify source of genes that can be used in yield improvement programs.

OBJECTIVE

The objective of the study was to evaluate cowpea genotypes for yield and yield related traits

METHODOLODY

A field experiment was conducted at ARC-VOP experimental farm in 2015/2016 summer growing season. The experiment consisted of twenty-eight genotypes arranged in a 7×4 rectangular lattice design replicated three times.

Data was analyzed using Genstat 12th Ed. (Payne *et al.*, 2009).

RESULTS AND DISCUSION

Analysis of variance showed a significant differences for most traits studied indicating sufficient genetic variability required for successful cowpea improvement (Table 1). The principal component analysis also showed the total variability among the genotypes (Figure 1).

Genotype 4565 produced the highest yield with the mean value of 3293 kg/ha which prove that there is a potential of yield improvement as compared to the 500 kg/ha yield return of cowpea in smallholder sector.

Table 1. Mean values of cowpea genotypes at Roodeplaat

Genotype	50% Flowering	Plant Height (m)	Leaf Area Index	Seed Number Per Pod	Pod Length (mm)	Yield Per Plant (g)	Harvest Index	Yield Per Plot (kg/ha)
1168	62.7	1.37	3.67	10.59	16.25	44.71	0.31	1332
1257	68.0	1.329	3.73	11.51	14.60	29.74	0.51	963
1329	64.7	1.499	3.78	13.76	16.46	91.99	0.62	2283
1974	63.0	1.459	4.11	11.82	15.63	29.55	0.26	985
1985	68.0	1.394	5.04	11.25	9.45	23.49	0.29	638
1998	68.0	1.176	3.90	13.03	16.78	50.15	0.63	1672
2013	63.0	1.56	4.25	11.38	15.16	39.13	0.38	1304
2024	68.0	1.71	4.43	10.49	15.53	28.86	0.20	947
2261	61.3	1.169	3.06	11.62	14.10	37.97	0.70	1216
2273	48.0	0.355	2.05	9.05	13.98	22.02	0.71	734
2338	63.3	1.944	3.91	7.13	10.25	13.61	0.17	323
2438	66.3	1.289	4.01	12.92	14.63	36.84	0.46	1228
3361	58.0	0.97	3.32	9.56	13.77	22.72	0.50	757
3916	64.7	1.223	4.27	10.33	14.96	23.49	0.41	610
4294	68.0	1.417	4.13	13.25	15.75	63.25	0.54	2108
4439	56.7	0.987	3.26	10.43	13.27	29.47	0.48	453
4565	66.3	1.179	3.81	13.55	17.41	98.77	0.84	3292
4663	68.0	1.415	3.91	10.56	14.42	27.80	0.33	909
4670	68.0	1.429	4.62	12.67	15.95	74.90	0.58	2497
4749	61.3	1.399	4.75	13.80	16.29	66.36	0.70	2212
4893	60.0	1.242	3.25	13.30	16.83	58.39	0.51	1873
Cwp Acc# 1	66.3	1.15	4.97	12.06	13.46	28.26	0.28	847
Cwp Acc# 2	65.0	1.28	3.90	10.98	13.63	41.51	0.36	1384
Cwp Acc# 4	64.7	1.133	3.51	9.47	13.36	17.26	0.16	455
Cwp Acc# 5	68.0	1.761	3.73	11.48	16.81	41.86	0.20	1042
Glenda	64.7	1.299	3.68	11.87	15.32	40.23	0.34	1341
L-cwp Acc# 2	66.0	1.215	4.26	13.33	14.50	66.58	0.69	2219
L-cwp Acc# 3	65.0	1.344	3.83	13.23	16.45	54.39	0.42	1813
CV (%)	10.1	32.2	20.20	16.00	17.70	60.80	65.91	60.30
LSD(0.05)	10.6	5.3	1.30	3.00	4.20	42.77	33.00	1320.40

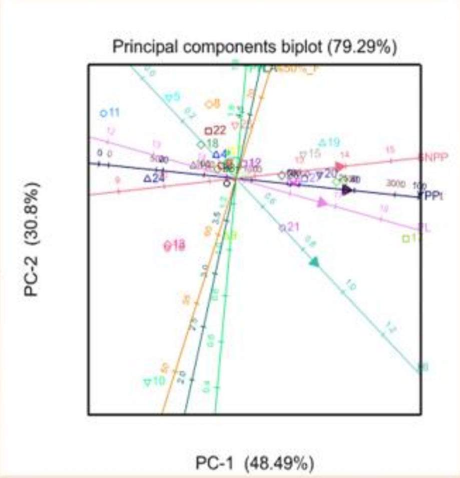

Figure 1. Biplot showing phenotypic traits and the cowpea genotypes.

CONCLUSSION

The quantitative traits provided a useful measure of genetic differences among the genotypes and would enable the identification of potential parental lines for future cowpea breeding program in the ARC for the traits of interest.

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