CHARACTERISATION OF COWPEA ACCESSIONS BASED ON AGRO-MORPHOLOGICAL TRAITS, NUTRITIONAL QUALITY AND MOLECULAR MARKERS

By

Goodluck Douglass Ringo

BSc. Agric. General (Sokoine University of Agriculture, Tanzania)

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School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal (UKZN)

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GENERAL ABSTRACT

Cowpea is a food and animal feed crop grown in the semi-arid tropics worldwide. Genetic diversity in crops allows the exploitation of different genotypes to breed new cultivars. Very little work has been undertaken in understanding and exploitation of the genetic variation that exists among cultivated varieties and local landraces of cowpea in South Africa. The objectives of the study were: 1) to assess the level of diversity among selected cowpea accessions using agro-morphological traits, 2) to assess the level of genetic diversity among selected cowpea accessions using single nucleotide polymorphisms (SNP) markers, and c) to determine the crude protein, iron and zinc contents in the grains of selected cowpea accessions grown under two planting dates at the Agricultural Research Council - Potchefstroom campus. Two planting dates were used, the first on 10th December 2015 and the second on 10th January 2016. Data were collected on 14 quantitative and qualitative traits.

The analysis of variance for 10 quantitative traits revealed highly significant (P<0.001) differences among the accessions. The mean performance of accessions showed nine cowpea accessions that yielded higher than the two checks. Grain yield and some of the other agronomic and phenological traits showed a wide phenotypic variability. Among these were: days to 50% flowering, weight of mature pods, fodder yield, and phenotypic coefficient of variation (PCV) for number of main branches, pod length, pod width, number of seeds per pod and hundred seeds weight. Days to 50% flowering, weight of mature pods, grain yield and fodder yield had high genotypic coefficients of variation (GCV), and moderate to high heritability and genetic advance. This indicated that selection based on the mean would be successful in improving these traits. High heritability estimates along with high genetic advance implied additive gene action was more important in the inheritance of these traits. Grain yield was affected by planting date, where early planting date gave the highest mean value of 5725.9 kg /ha, while for the late planting date had a mean yield of 3756.9 kg/ha. Traits associations were estimated by simple correlation coefficients. Grain yield showed positive and significant correlation with weight of mature pods, fodder yield and number of seeds per pod. Selection for weight of mature pods, fodder yield and number of seeds per pod would, therefore, be essential for grain yield improvement in cowpea genotypes. Cluster analysis of phenotypic traits grouped the accessions into seven distinct groups and first six principal components showed 76.89% of total variability among genotypes. Number of main branches, days to 50% flowering, days to maturity and fodder yield contributed to PC1 and number of plants per plot, weight of mature pods, grain yield and fodder yield contributed largely to PC2.

Crude protein, iron and zinc contents of 45 cowpea accessions grown under two planting dates were determined using standard Association of Official Analytical Chemists (AOAC) method. Seven accessions that had high protein content at both environmental conditions were: TVU 13932 (27.88%), 98K-476-8 (27.15%), TVU 9443 (27.13%), RV 503 (27.00%), RV 213 (26.80%), 98K-503-1 (26.74%) and 95K-589-2 (26.72%). Nine accessions with high iron content included; TVU 13998 (140.89 mg/kg), 86D-1010 (138.71 mg/kg), 98K-476-8 (132.73 mg/kg), 95K-589-2 (121.69 mg/kg), RV 343 (117.49 mg/kg), RV 204 (115.83 mg/kg), TVU 13932 (115.46 mg/kg) and RV 500 (111.77 mg/kg); and the five accessions identified with high zinc content were: TVU 13932 (203.42 mg/kg), 98K-503-1 (162.38 mg/kg), 90K-284-2 (151.71 mg/kg), TVU 14190 (143.99 mg/kg) and CH47 (116.17 mg/kg). Correlation analysis showed a positive significant degree of association between protein and zinc contents. Results of the phenotypic coefficient of variation for most characters were close to the corresponding genotypic coefficient of variation values indicating little environmental effect on the expression of these characters. The highest estimates of broad sense heritability (H^2) were obtained for protein, iron and zinc. High heritability estimates along with high genetic advance for iron and zinc contents indicate an additive gene action in their inheritance. Based on the nutritional content data, the first two principal components explained over 77.48% of the total variation.

The analysis of cowpea using 60,000 SNPs markers revealed genetic variation among the 47 cowpea accessions. A polymorphic information content (PIC) ranging from 0.02 to 0.38 with a mean 0.27 was observed, reflecting the relatively high discriminating ability of the markers used. The average gene diversity among genotypes ranged from 0.02 to 0.50 with a mean of 0.34. Genetic distance ranged from 0.47 to 0.86 with a mean of 0.61. The genotypes 97K-499-35 and RV 342 were the most distantly related cowpea accessions. The lowest genetic distance was found between TVU 14190 and RV 213 accessions. The within and among individuals differentiation accounted for 23% and 75% of the total variation, respectively. The 47 cowpea genotypes were clustered into three main groups, with some of the genotypes clustered from the same geographical origin and others grouped in the same cluster irrespective of their geographic origin. The observed low genetic differentiation between geographic origins implied the existence of large levels of gene flow among South Africa accessions and introduction (IITA-Nigeria) accessions. Overall, the present study showed the existence of a wide diversity among the cowpea accessions studied based on agro-morphological traits, SNPs markers and nutritional quality traits. Accessions identified with desirable traits can be recommended for direct production by growers and/or used in breeding programme to develop new cultivars with high yield as well as better nutritional quality.

DECLARATION

I, Goodluck Douglass Ringo, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted.

 Then:

     a. Their words have been re-written but the general information attributed to them has been referenced;

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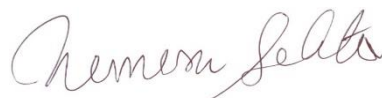
Signed

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Goodluck Douglass Ringo

As the candidate's supervisors, we agree to submission of this dissertation:

_____

Dr. Julia Sibiya (Supervisor)

_____

Dr. Alfred Odindo (Co-Supervisor)

_____

Dr. Nemera Shargie (Co-Supervisor)

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABREVIATIONS

%	Percent
>	Greater than
A	Absorbance
AFLP	Amplified fragment length polymorphism
ALP	Amplicon length polymorphism
Am:Ap	Amylose to amylopectin ratio
ANOVA	Analysis of Variance
AOAC	Association of official analytical chemists
ARC-GCI	Agricultural Research Council-Grain Crops Institute
ATP	Adenosine triphosphate
bp	Base pair
Ca	Calcium
cm	Centimetre
CTAB	Hexadecyl trimethyl ammonium bromide
CV	Coefficient of variation
DFT	Days to 50% flowering
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DTM	Days to maturity
DTT	Dithiotreitol
E	East
EC	Emulsifiable concentrate
EDTA	Ethyl diamine tetra acetic acid
et al	'et alii/alia' (and others)
FAO	Food and Agricultural Organization
Fe	Iron
FY	Fodder yield
g	Grams
GA	Genetic advance
GCV	genotypic coefficients of variation
GH	Growth habit
GOPOD	Glucose oxidase peroxidase 4-aminoantipyrin
GY	Grain yield
h	Hour
H^2	Broad sense heritability
H2O	Water
$HClO_4$	Chloric acid
HNO_3	Nitric acid
HSW	Hundred seed weight
IITA	International Institute of Tropical Agriculture
ISSR	Inter simple sequence repeat
K	Potassium
Kg/ha	Kilogram per hectare

Kj	Kilojoules
LSD	Least significant difference
m	Metre
M	Molar
masl	Meter above sea level
Mg	Magnesium
mg	Milligram
mg·kg^{-1}	Milligram per Kilogram
min	Minute
ml	Milliliter
Mn	Manganese
Mol	Mole
MS	Mean square
MS$_e$	Mean square error
MS$_g$	Mean square genotype
N	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
NFE	Nitrogen Free Extract
nm	Nano meter
NMB	Number of main branches
NSSP	Number of seeds per pod
^{o}C	Degree celsius
p	Probability
P	Phosphorus
PC	Principal component
PCA	principal component analysis
PCR	polymerase chain reaction
PCV	phenotypic coefficient of variation
PH	numeric scale used to specify the acidity or basicity of an aqueous solution
PL	Pod length
pmol	Pico mol
ppm	Part per million
ppm	Part per million
PV	Plant vigour
PW	Pod width
PWt	Weight of mature pods per hectare
QTL	Quantitative trait loci
r	Correlations
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
SNP	Single nucleotide polymorphism
SPLAT	Single polymorphic amplification test
SS	Seed shape
SSR	Simple sequence repeat
UPGMA	Unweighted pair group method using arithmetic averages
USA	United State of America

UV	Ultraviolet
v/v	Volume by volume
W	Watt
w/v	Weight by volume
Zn	Zinc
µg	Microgram
µl	Microlitre
σ^2_g	Genotypic variance
σ^2_p	Phenotypic variance

INTRODUCTION TO DISSERTATION

Background/Justification

Cowpea (*Vigna unguiculata (L.) Walp*) is a leguminous crop belonging to the family Fabaceae (Padulosi and Ng, 1997). The species is of importance in developing countries, especially in sub-Saharan Africa, Asia, Central and South America which are tropic and subtropic areas (Singh et al., 1997). Cowpea originated in Africa, within in west African region which has been identified as its major centre of diversity (Ehlers and Hall, 1997). India, on the other hand, has been indicated as the secondary centre of diversity of the cultivated *Vigna species,* while south-eastern Africa is regarded as the centre of diversity of the wild *Vigna species* (Padulosi and Ng, 1997).

Cowpea grain is a good source of nutrients and contains 23-25% protein, 50-67% starch, vitamin B, C and essential micronutrients such as iron, calcium, and zinc (Mamiro et al., 2011a). Its green pods, green seeds, and young leaves are used as vegetables, whereas dry seeds are used for preparations of different foodstuffs (Nout, 1996; Nielsen et al., 1997). The leaves contain a high content of protein and minerals, such as vitamin B and C, phosphorous, calcium and ß carotene (Maynard, 2008; Mamiro et al., 2011a). The crop is equally important as a nutritious fodder for livestock (Singh and Tarawali, 1997). In addition, cowpea has the capacity to fix atmospheric nitrogen, which helps in maintaining soil fertility for future use, and thus can be used under intercrop systems with cereal crops such as maize (Dakora and Keya, 1997). It also has a mechanism of surviving under drought conditions and still produce grain yield (Singh et al., 2002). Furthermore, cowpea can serve as a cash crop for smallholder farmers (Langyintuo et al., 2003).

Approximately 12 million hectares of world arable land is used for cowpea production. Out of the total world production of 8.3 million tonnes, Africa's contribution was 95.3% in 2015 (FAO, 2015). Cowpea is mostly grown in west and central Africa, where it shows adaptation to semi-arid conditions (Ehlers and Hall, 1997). According to FAO 2016, the top five producers in the world are Nigeria, with annual production per year averaging 3.2 million tonnes, followed by Niger averaging 1.4 million tonnes, Burkina Faso averaging 0.51 million tonnes, Tanzania averaging 0.18 million tonnes and Myanmar averaging 0.16 million tonnes (Ronner and Giller, 2013; FAO, 2015).

In South Africa, cowpea grain yield is very low according to Asiwe (2009) who reported a yield range between 250 and 1000 kg ha^{-1} with an average of 500 kg ha^{-1}. Land size planted to cowpea ranged between 0.25 and 2.0 ha per farmer.The main cowpea producing provinces are Limpopo, KwaZulu-Natal, Mpumalanga and North West. Despite an increase in cowpea production in most parts of Africa, South African cowpea production is still at subsistence level.

Low yields in South Africa are attributed to many factors including biotic and abiotic factors (Asiwe, 2009). Among the biotic constraints, incidences of diseases, insect pests and parasitic weed *Striga spp* cause yield reduction (Asiwe, 2006; Mbwaga et al., 2007; Asiwe, 2009). Abiotic stress factors that limit cowpea production include poor soil fertility, flooding and extreme drought. Poor soil fertility is particularly a problem in fields that are phosphorous and micronutrient deficient, and results in yield reduction (Singh et al., 2003). Although cowpea tolerates drought better than other crops, late- maturing fodder- type varieties planted in low rainfall areas as a sole crop or relay with cereal crops may suffer severe drought stress due to insufficient rainfall (Singh and Tarawali, 1997). Therefore, better strategies or improvement of the existing strategies is required to handle these challenges that impact on production. To strengthen food and nutrition security, the development of more productive and nutritious cowpea varieties is a priority in South Africa.

Although South Africa is the genetic centre of origin and domestication for cowpea and there is a wide genetic diversity available in cowpea (Padulosi and Ng, 1997), little work has been done on exploitation and understanding the genetic diversity that exists in cowpea germplasm collection. Therefore, analysis of genetic diversity will assist in the identification of desirable genes for developing resistant cultivars to biotic and abiotic stresses to tackle these challenges that impact on production levels. Furthermore, knowledge of genetic diversity existing among cowpea accessions can be used as a potential source of novel desirable genes for improvement of yield as well as nutritional quality and for future use in cowpea breeding programmes.

Various methods have been used in estimating the genetic diversity existing among cultivated varieties and local landraces, and these include; morphological analysis, quality analysis and use of molecular markers (Tuinstra et al., 1996; Geleta and Labuschagne, 2005; Mehmood et al., 2008). Both morphological and molecular marker analyses are informative tools for estimating genetic distances and polymorphism (Vieira et al., 2007). Analysis of quality traits

2

is used as a tool for generating information on nutrient composition among different cowpea accessions such as protein, iron and zinc. Protein deficiency has been reported as one of the major nutritional problems in the third world countries (FAO, 1997). Ghaly and Alkoaik (2010), indicated that protein deficiency causes marasmus and kwashiorkor which are majorly prevalent nutritional diseases reported in children. Hence, it is important to characterise the genotypes grown and consumed by farmers, as previous studies have indicated that genetic variability among nutrient composition of various cowpea genotypes is important (Henshaw, 2008; Mamiro et al., 2011b; Odedeji and Oyeleke, 2011). However, little work has been done on local and improved cowpea varieties in relation to their nutrient composition (Animasaun et al., 2015). Knowing information about genetic diversity on cultivated and landrace cowpeas will assist plant breeders in making efficient selections and developing superior cultivars with the aim of addressing the challenges of poor yields as well as poor grain quality (Graham et al., 2001).

There are more than 1000 local cowpea collections and introductions, mainly from IITA-Nigeria that are maintained at the ARC-GCI gene bank that need to be evaluated based on agro-morphological traits, molecular markers and nutritional quality in order to utilise them in cowpea improvement programmes. The overall objective of this study was to assess and describe the level of genetic and nutritional diversity in cowpea accessions maintained at the ARC-GCI gene bank for the benefit of future cowpea improvement programmes in the country and the region.

Specific objectives

The specific objectives of the study were as follows:

I. To assess the level of diversity among selected cowpea accessions using agro-morphological traits

II. To determine grain protein, iron and zinc contents of selected cowpea accessions

III. To assess presence of genetic diversity among selected cowpea accessions using molecular makers

Research hypotheses

1. There is considerable genetic variability existing among cowpea accessions based on phenotypic and molecular markers

2. There is enough variation among cowpea accessions based on nutritional quality traits

Dissertation Outline

This dissertation is made up of literature review, three research chapters and overview of the study as follows:

a) Assessment of genetic diversity in selected cowpea accessions using agro-morphological traits

b) Assessment of grain protein, iron and zinc contents of selected cowpea accessions

c) Assessment of genetic diversity in cowpea accessions using molecular markers and phenotypic traits

d) General overview of the study and future directions

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CHAPTER ONE

LITERATURE REVIEW

This review focuses on the genetic diversity among collections of local and regional cowpea accessions for future cowpea breeding programmes in South Africa. The importance and production of cowpea with a focus on agro-morphology, quality and molecular markers are discussed. This review also covers identification of breeding materials, genetic relatedness, and maximization of genetic resource availability for improvement of cowpea with both desirable characters and high yield and better nutritional quality. Knowledge gaps in the literature review are also highlighted.

1.1 Taxonomy, origin and distribution of cowpea

Cowpea is a leguminous crop species which belongs to *Leguminoseae* family and *Vigna* genus. According to Padulosi and Ng (1997), the genus, *Vigna* is sub grouped into four subgenera based on geographical distribution, morphological appearance and genetic hybridization. Cowpea is a diploid species with 22 chromosomes (Faris, 1964; Padulosi and Ng, 1997). It consists of four subspecies namely: *unguiculata, stenophylla, dekindtiana and tenuis* (Padulosi and Ng, 1997). There are five cultivated cowpea species which are *unguiculata, biflora, melanophthalmus, sesquipedalis and textilis* (Pasquet, 1998).

According to Ehlers and Hall (1997), cowpea originated from Africa. West African region is believed to be the major centre of diversity of cowpea, while India has been identified as the secondary centre of diversity of cowpea due to the presence of several wild relative and cultivated species (Ehlers and Hall, 1997). It is also believed that south-eastern Africa region and Transvaal region in the Republic of South Africa are the centre of diversity of wild *Vigna spp* due the presence of several wild species (Padulosi and Ng, 1997). Molecular studies based on amplified fragment length polymorphism (AFLP) analysis have suggested that domestication of cowpea occurred in north-eastern Africa (Coulibaly et al., 2002). The crop was first introduced to India by the Spanish, and later was taken to the USA and thereafter to South America in the 16th Century (Purseglove, 1968).

1.2   Importance of cowpea

Cowpea is an important food crop and provides essential sources of nutrients to millions of people in Africa and other parts of the developing world (Singh et al., 2003a; Timko and Singh, 2008). Cowpea grains and leaves are an excellent source of high quality protein and vitamins complementing the low protein staple roots, cereals and tuber crops in many African countries (Kitch et al., 1998). The grain seeds contain protein ranging from 21-33%, while the cowpea leaves contain protein ranging from 29-43% (Nielsen et al., 1997). Cowpea leaves have been reported to be an excellent source of micronutrients, especially zinc, iron, calcium and potassium (Imungi and Potter, 1983). The crop is referred to as a poor man's meat because of the high protein content and is used as an excellent source of proteins to poor people living in rural areas who cannot afford animal products (Singh et al., 2003a). The crop fits different cropping systems and intercrops with cereal crops to conserve and improve the nitrogen status in the soil through fixation of atmospheric nitrogen are common (Dahmardeh et al., 2010).

1.3   Cowpea production and productivity in the world

Nigeria is the largest producer of cowpea worldwide. About 2.1 million tonnes of cowpeas are produced yearly in Nigeria followed by Niger (818970.9 tonnes), Burkina Faso (380509.29 tonnes), (Tanzania 133116.14 tonnes), Myanmar (114702.29 tonnes) and Mali (106265.05 tonnes) (FAO, 2016). Globally, cowpea is cultivated on an area of approximately 9.8 million ha with about 91% of this being in West Africa. The average global yield of cowpea was 378 kg ha^{-1}, which is less than 440 kg ha^{-1} in 2003 reported in Nigeria (Mahalakshmi et al., 2007). Cowpea is also grown considerably in countries such as Senegal, Togo, Benin, Ghana, Chad in West Africa; Tanzania, Somalia, Kenya, Zambia, Zimbabwe, Botswana and Mozambique in eastern and southern Africa; India, Pakistan, Sri Lanka, the Philippines, Bangladesh, Indonesia and China in Asia; and Brazil, West Indies, Cuba and southern USA in America (Mahalakshmi et al., 2007).

1.4   Cowpea production in South Africa

The production level of cowpea in South Africa is still very low compared to other countries in Africa. South Africa produced cowpea ranging between 0.25 and 1.0 tonnes per hectare with an average of 0.5 tonnes per hectare per farmer in 2009 reported by Asiwe, (2009b). The main producing provinces are Limpopo, Mpumalanga, North West and KwaZulu-Natal.

Despite an increase in cowpea production in Africa, the trend in South Africa has remained fairly constant. Production level is low due to lack of adequate improved varieties, absence of quality seeds and absence of strong research and production on cowpea (Ndamani, 2015).

1.5 Ecological requirements for cowpea growth

Tropical and subtropical climates characterised by high temperatures, high rainfall and long photoperiods are suitable for production of cowpeas. Cowpea performs well under humid conditions. It is tolerant to dry conditions but susceptible to frost. Germination is promoted by high temperatures and occurs rapidly at temperatures above 18°C, while lower temperatures prolong germination with the risk of seed rot (Davis et al., 1991).

Cowpea can be grown under both irrigated and rain-fed conditions (Davis et al., 1991). Soils with a pH range between 5.5 and 6.5 are suitable for cowpea cultivation. Good soil water drainage is necessary to avoid development of fungal diseases. As a result cowpea grows well in well-drained soils although they are well adapted to a wide range of soils and conditions (Davis et al., 1991).

1.6 Cultural practices

Good agronomic practices must be followed to ensure good growth. Cultural practices such as seed bed preparation, appropriate seeding rate, planting dates, planting of superior cultivars with better yields and weed control are important in cowpea production especially in sub-Saharan Africa where environmental conditions are variable.

1.6.1 Planting dates

Planting date is one of the cultural practices farmers should consider because it contributes to growth and yield of grain legumes without any additional costs to production. For example, it has been shown that early planted groundnuts had no groundnut rosette disease and low *Aphid craccivora* infestations this implies is good to consider planting time to escape diseases and pests (Naidu et al., 1998).

Ezeaku et al. (2015) reported that early planted cowpeas gave significantly higher grain yields than late planted. Similar findings were reported in earlier studies by Javaid et al. (2005) and Akande et al. (2012). Several authors have reported factors which might cause better yield in early planting to include escape of high temperatures during reproduction stage when the crop is sensitive to heat and the crop would mature before the end of rains (Ismail et al., 1997; Ehlers and Hall, 1998).

1.6.2 Weeding control

Weeds are a serious constraint to the crop due to the competition for space, light , and nutrients, consequently leading to a reduction in production quantity and quality (Madukwe et al., 2012). There are several measures used to control weeds, but the most common ones are hand weeding and chemical application. Hand weeding control is a common control practice in cowpea production used by small holder farmers. It is advisable that cowpea fields be weeded twice with hand hoes, first at 2 weeks after sowing and then at 4-5 weeks after sowing to ensure a crop free from weeds. Poor control or delay in weed control can result in extreme reduction of grain yield (Dugje et al., 2009). Chemical control using herbicides normally depends on predominant weed species and availability of herbicides. It is not recommended to use herbicides for weed control if leaves are to be consumed (Dugje et al., 2009).

1.6.3 Irrigation

Although cowpea is considered as a drought tolerant crop, a rainfall of 400-700 mm per annum is required for optimal growth (Ndamani, 2015). Cowpeas are normally grown in dry areas than irrigated areas. According to Ahmed and Suliman (2010), cowpea suffers water deficit in flowering and pod filling stages. This implies that during these stages, supplementary irrigation is required to promote flowering and pod filling to maximize grain yield.

1.7 Major constraints to cowpea production

Despite its importance, cowpea farmers face several detrimental factors in growing the crop. Throughout the tropics, insect pests and diseases are major production constraints (Tarawali et al., 2000; Singh et al., 2003b). Virus diseases, besides other biological agents such as insect pests, bacteria, fungi and nematodes, have long been associated with yield losses ranging from 10-100% in field grown cowpea crops (Shoyinka et al., 1997). Major insect pests

include a complex of pod sucking bugs (*Clavigralla* spp*, Acanthomia* spp), aphids (*Aphid craccivora)*, thrips (*Megalurothrips sjostedti),* mucuna pod borer (*Mucuna vitrata*), and storage weevil (*Callosobruchus maculatus)* (Ehlers and Hall, 1997; Asiwe, 2009a). Major seed borne viruses are the cowpea aphid-borne mosaic potyvirus (CABMV), cucumber mosaic cucumovirus (CMV), cowpea mosaic (CPMV) and cowpea severe mosaic (CPSMV) comoviruses, southern bean mosaic sobemovirus (SBMV) and cowpea mottle carmovirus (CPMoV) (Hampton et al., 1997).

Other diseases that affect cowpea are fungal infections such as anthracnose, ascochyta blight, black leaf spot, powdery mildew, brown rust, brown blotch, pythium soft stem rot and septoria leaf spot, while the most common bacterial disease is cowpea bacteria pustule caused by *Xanthomonas axonopodis pv.vignicola (Xav)* (Ehlers and Hall, 1997; Ronner and Giller, 2013; Edema et al., 1997; Singh et al., 2003b).

Other limiting factors in cowpea production are parasitic weeds such as *Alectra vogellii* (Benth) and *Striga gesneriodes* (Wild). Parasitic weeds can result in yield losses as they parasitize cowpeas during flowering in the field (Ehlers and Hall, 1997; Ronner and Giller, 2013). Therefore, the timing of flowering and weeding is important in order to ensure a weed free period so that yield is not compromised.

Drought is a major economic constraint that negatively affects cowpea production worldwide. Yield is severely reduced when drought coincides with critical phenological stages such as pod and seed development, flower development or establishment (Singh et al., 1999; Hall, 2004; Padi, 2004). Poor soil fertility is another limiting factor of cowpea production. Sub-saharan Africa is characterised by low soil fertility especially phosphorous deficiency. Low soil fertility reduces the yield potential of cowpeas due to stunted growth. It occurs in the soil which has a serious problem of soil nutrient deficiency which affects growth and development of cowpea especially phosphorous (Saidou et al., 2012).

1.8   Genetic diversity analysis in cowpea

Genetic diversity analysis is a key aspect for the improvement of cowpea and other crops (Frankham, 2010; Govindaraj et al., 2015). Despite cowpea being the most widely grown legume in Africa, it has low genetic diversity which presents a challenge in breeding efforts as

it reduces the chances for developing new cultivars or varieties (Govindaraj et al., 2015). A wider genetic diversity provides a high chance of getting desirable genes for improvement accompanied with better agronomic characters. Genetic diversity assessment is most applicable to plant breeders as it facilitates the selection of good parents for crossing and minimizes unnecessary crosses (Carpentieri-Pípolo et al., 2003; Nkongolo, 2003). It has been reported that genetic diversity which is present in different populations occurred because of mutations and or migration. In populations, the genetic diversity can be determined by estimating genetic distances among populations and number of alleles per locus using molecular tools (Nkongolo, 2003). Considerable genetic variability in different varieties of cowpea has been reported by other researchers (Animasaun et al., 2015). However, according to Doebley (1989), cultivated cowpea accessions have a low genetic diversity compared to common bean .

The understanding of the level of genetic diversity is very useful for germplasm improvement (Hoxha et al., 2004; Shin et al., 2006; Asare et al., 2010; Adewale et al., 2011; Huynh et al., 2013; Egbadzor et al., 2014b; Ali et al., 2015; Gerrano et al., 2015). Wide genetic base of the crop is useful in developing superior genotypes such as high yield and best grain quality (Singh, 2001; Kuruma et al., 2008; Tignegre et al., 2011), while a low genetic base results in susceptibility to several stresses. Moreover, maximising genetic resources could be a useful guide for introgression efforts to widen the genetic diversity within breeding materials and greater genetic gain in future breeding programmes (Huynh et al., 2013). In breeding programmes, knowing genetic diversity is very important in order to develop more efficient strategies for germplasm management, utilization, fingerprinting and parent selection (Frankel, 1989; Blakeney, 2002; Bucheyeki et al., 2009). It is, therefore, essential to characterize cowpea accessions in order to explore the genetic diversity among the accessions for desirable parental selections (Al-Doss et al., 2013). These will form the basis for future improvement to support development of high yielding, better nutritional quality cowpea.

1.8.1 Morphological characterization

Morphological evaluation is often used by plant breeders for characterization of cowpea accessions. Morphological studies in cowpea have indicated that cowpeas have a low genetic base (Li et al., 2001; Asare et al., 2010). Several researchers reported variability in genetic diversity and genetic distance among some cowpea accessions based on characters such as

the number of pods per plant, and days to the first flower and plant height. Omoigui et al. (2006) reported variability among nine accessions using quantitative traits. The morphological analysis was used for assessment of genetic distance and diversity among cowpea accessions utilizing traits such as number of pods per plant, plant height and days to flowering (Adewale et al., 2011). Morphological analysis was important in generating information for identification of duplicates, variety protection and selection of parents for genetic improvement studies (Vural and Karasu, 2007; Hegde and Mishra, 2009).

Evaluation of crop species is mainly based on morphological traits that include quantitative and qualitative traits (Schut et al., 1997). Qualitative characterization usually does not require special facilities or procedures, they are inexpensive, cheap and easy to score. As a result classification based on qualitative traits is an important tool for classification of genotypes and taxonomy level. Moalafi et al. (2010) conducted an evaluation of cowpea germplasm aimed at improvement of cowpea by crossing a set of parental lines from ARC germplasm and then assessing the performances of F_2 generations which were targeted for developing dual-purpose cowpea types. The evaluations were based on traits such as 100 seed weight, pod length, number of days to flowering, days to 50% flowering, days to pod maturity, number of days to maturity, and grain yield (kg/ha). Similar traits have been used by Imran et al. (2010) to assess cowpea germplasm for characterization of fodder production potential in local cowpea germplasm. In addition to qualitative traits, morphological characterisation can be done based on quantitative traits. Quantitative traits are influenced by polygenes and have a tendency to vary according to change in environmental conditions due to the effect of genotype by environment interaction (Liu and Furnier, 1993). The quantitative traits are thus crucial in a breeding programme for genetic improvement of important traits such as high yield and grain quality.

1.8.1.1 Interactions among morphological traits and yields

Yield is a complex trait that is controlled by polygenes but their expression and interaction are influenced by the environment factors. Several factors including phenological development, planting date, genotypic differences and the environment affect yield and yield components (Ezeaku et al., 2015). Positive correlations among yield and yield components are important in yield improvement as they imply that it would be possible for both traits to be improved simultaneously. Leleji (1981) and Uguru (1996) reported significant positive relationships between grain yield and number of flowers per plant, number of pods per plant and pod length

and number of peduncles per plant. Ajayi et al. (2014) observed a strong negative phenotypic and genotypic correlation between number of pods per plant, pod length, seed per pod, seed weight, plant height.

1.8.2   Nutritional quality traits

Improvement of nutritional quality traits has been ignored by plant breeders in many cowpea breeding programmes. The main focus was on development of high yielding cultivars that have resistance to biotic and abiotic stresses (Wang et al., 2006; Padi, 2007). These efforts have resulted in the improvement of cowpea varieties that are resistant to *Striga* spp, some insects and diseases (Singh et al., 2007). The cowpea grains nutritional qualities such as protein, iron and zinc determination have been studied by very few researchers (Ajeigbe et al., 2008; Anele et al., 2011). Lack of protein, vitamin and minerals in many African diets has resulted in stunted growth of children which disrupts their physical development and cause poor brain development and abnormal growth (Labadarios, 2005; Faber and Wenhold, 2007). Improvement and promotion of orphan crops such as cowpea could provide essential nutrients that are required by poor households. Therefore, there is a need to develop cowpea varieties with good grain nutritional quality to increase food availability and eradicate malnutrition for the people living in rural areas and poor communities living in informal settlements in urban and peri-urban areas in the third world countries.

1.8.2.1   Protein evaluation

Legumes area major source of protein. Proteins are characterised based on the functional characteristics of their soluble fractions (Vasconcelos et al., 2010). Cowpea is used as a source of protein in South Africa. Cowpea is also used as a dual purpose crop for vegetables and grain seeds for preparations of different food stuffs (Mamiro et al., 2011). The protein content is influenced by environmental factors. Matthews and Arthur (1985) reported that environmental factors had huge impacts on protein content than genetic impacts among 225 garden pea accessions. Moreover, protein content ranging from 18.1 to 27.8% for pea genotypes depending on the environment was reported by Gueguen and Barbot (1988). It was also hypothesized that protein content is controlled by many genes and is thus influenced by environmental factors such as nitrogen and sulphur availability (Tabe et al., 2002). Genetic variability for cowpea grain protein content has been reported by several authors (Nielsen et al., 1993; Fatokun, 2002; Animasaun et al., 2015). Ajeigbe et al. (2008) reported significant differences in protein content in cowpea grains. Therefore, there is a need to generate

information on genetic diversity of protein content and gene action involved in protein synthesis in order to improve cowpea protein content.

1.8.2.2   Iron and zinc evaluation

Iron and zinc have been shown to have significant variations in cowpea (Boukar et al., 2011). Differences have also been reported in cereal grain crops (Rengel et al., 1999). In wheat grains a 3-4 fold increase in iron and zinc content in wild landraces compared to popular variety was reported (Chhuneja et al., 2006; Rawat et al., 2009). Similarly, genetic variation of zinc and iron has been reported in maize crop with ranges from 9.6 to 63.2 mg/kg for grain Fe and 12.9 to 57.6 mg/kg of grain zinc (Bänziger and Long, 2000). There was also variation in other legumes such as common bean seeds. Silva et al. (2010) showed a wide variation in iron and zinc contents among 100 diverse common bean lines with ranges from 54.20 to 161.50 mg kg^{-1} and 29.33 to 65.50 mg kg^{-1}, iron and zinc, respectively. Similarly, another study also revealed a variation in iron and iron content from 34 to >100 mg kg^{-1} and 21 to 54 mg kg^{-1}, respectively among 2000 common bean accessions of CIAT (Beebe et al., 2000). Another report involving 117 genotypes of common bean collected from Uganda showed variation in iron and iron contents ranging from 45 to 87 mg kg^{-1} and 22 to 40 mg kg^{-1}, respectively (Mukamuhirwa et al., 2012). Mamiro et al. (2011) observed a wide genetic variability on iron and zinc contents among cowpea accessions. These accessions provide potential parents for improvement of cowpea cultivars with good nutritional quality. Since there is a wide variation in iron and zinc contents in different accessions, accessions with the highest iron and zinc can be used as potential sources for improving nutritional quality (Mahajan et al., 2015).

1.8.2.3   Association between nutritional quality traits

Hussain and Basahy (1998) reported that protein content and ash were positively correlated while carbohydrate content and protein content were negatively correlated. Other researchers found protein content and iron content (0.63) to be positively and significantly correlated, while hundred seed weight was significant but negatively correlated with protein (-0.72) and iron content (-0.61) (Moura et al., 2012). A negative correlation between protein content and 100 seed weight was also reported by Asante et al. (2004). Oluwatosin (1997) and Dwivedi and Cormack (1990) observed that seed protein and oil content were negatively correlated in cowpea. Other researchers reported that protein and oil content were negatively correlated in soya bean (Wilcox, 1998;Cober et al., 2000; Hyten et al., 2004). Other findings observed

negative correlations among protein and starch contents in chickpea and pea (Bastianelli et al., 1998; Frimpong et al., 2009). This indicates a possibility of indirect selection of these nutritional quality traits to develop superior nutritional quality genotypes of cowpea.

1.8.2.4 Physiological processes of iron and zinc

The complex phenomenon of mineral accumulation in grains is normally influenced by many genes. Mobilization is a process which transports mineral elements through roots and translocate to the shoot, redistributing in the whole plant and grains from soils (Grusak and DellaPenna, 1999; Broadley and White, 2009). Various mechanisms for soil mineral uptake and distribution in plants are a result of homeostatic mechanisms which control mineral uptake and distribution in whole plant parts (Maathuis, 2009). Several researchers found that the aleurone layer and the embryo for cereals are the major depositories for grain zinc (Mazzolini et al., 1985; Choi et al., 2007; Borg et al., 2009), while Ozturk et al. (2006) reported that high amounts of protein were present in the outer part of the endosperm.

1.8.3 Molecular marker characterization

Molecular markers are a technology that is used in cowpea programmes to accelerate generation of information on genetic diversity levels among local and regional cowpea accession collections (Hall, 2004; Hegde and Mishra, 2009). The utilization of various types of molecular markers has been useful in breeding programmes contributing to the shortening of the breeding process. Molecular markers are a powerful approach to assessment of genetic diversity and genetic variation among and within many crops species. The use of DNA amplification fingerprinting has been used to reveal genetic relatedness and genetic variation among cowpea accessions (Badiane et al., 2004). Also molecular markers are a useful technique to identify and differentiate homozygous and heterozygous individuals without any progeny test in a population (Collard et al., 2005). Quantitative Trait Loci (QTL) analysis in molecular marker characterization was applied to the identification of desirable traits using marker assisted selection (MAS) (Collard et al., 2005). Moreover, molecular markers enable the determination of the degree of genetic relatedness among cowpea accessions which is crucial for better characterization and identification of gene flow among cowpea accessions (Nkongolo, 2003).

1.8.3.1 Types of molecular markers

There are different kinds of molecular markers used in quantification of genetic diversity in crop species. The simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers are the most commonly used by molecular breeders because they have high output in laboratories and their cost per assay is low. SNPs in particular, are the latest markers which save time, are low cost, multi-locus, and highly reproducible, making them especially efficient for analysing many samples with narrow genetic variation (Tan et al., 2012).

Other markers used to study the genetic diversity in various crops but are now obsolete are; the restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and amplified fragment length polymorphisms (AFLPs). These markers can also identify genes of novelty for purpose of improvement in the crop breeding process (Collard et al., 2005). RAPD markers have been used to characterise the genetic diversity among accessions in various crops such as common beans and cowpea (Tiwari et al., 2005), pea (Mignouna et al., 1998; Asare et al., 2010), and soybean (Simioniuc et al., 2002; Brown-Guedira et al., 2000). Also in recent years, different types of molecular markers have been used to estimate genetic diversity in cowpea accessions including RAPD (Nkongolo, 2003; Sarutayophat et al., 2007), AFLP (Menéndez et al., 1997; Ouédraogo et al., 2002), SSR (Mccouch et al., 2002; Gillaspie Jr et al., 2005; Badiane et al., 2012) and inter-simple sequence repeats (ISSR) (Ajibade et al., 2000). Several types of DNA molecular markers which have been applied in fingerprinting of cultivars and clones in plants include SSR or microsatellites (Djè et al., 1999; Gilbert et al., 1999), sequence tagged sites (Liu et al., 1999) and SNP (Germano and Klein, 1999), RFLPs (Federici et al., 1998; Desplanque et al., 1999), and RAPD (Moeller and Schaal, 1999; Rodriguez et al., 1999).

1.8.3.2 Single nucleotide polymorphism (SNP) as markers for genetic diversity studies

Single nucleotide polymorphism (SNP) markers are one of the best tools used to characterize and identify crop species. They reveal the variation of individuals within species or populations and identify differences in a particular DNA sequence (Horst and Wenzel, 2007). Single nucleotide polymorphism markers are increasingly cost-effective, more abundant, stable, amenable to automation, and efficient (Rafalski, 2002; Duran et al., 2009; Edwards and Batley, 2010). They also commonly appear at different frequencies and thus the frequencies can be determined on genome size and crop species. DNA segments which have SNPs are first amplified by the polymerase chain reaction, a restriction enzyme in used when the product is

incubated, SNP which has enable the enzymes breakdown the molecule to create the cognition site (Collard et al., 2005).

According to Tan et al. (2012) not much has been done to analyse the genetic diversity of cowpea accessions using SNPs markers since 2012. Four-hundred and fifty-eight (458) SNP markers have been used to study genetic diversity among and within cowpea accessions (Egbadzor et al., 2014a) and to reveal duplicates in cowpea accessions (Lucas et al., 2013a). Another study by Oppong-Konadu et al. (2006) assessed genetic diversity with fewer number of SNP markers and could not discriminate all cowpea accessions. This implies that the number of markers should be considered during characterization of genetic diversity to discriminate all cowpea accessions (Varshney et al. (2007).

Furthermore, molecular markers have been used in multiple traits screening, selection of genotype with resistance to pest and disease, variety identification, evaluation of segregating populations, characterization of accessions and identification of genetic relatedness among accessions (Stuber et al., 1999).

1.8.4 Heritability and genetic advance

Heritability as explained by Sesardic (2005) is the proportion of phenotypic variation that is due to genetic differences. Normally the estimation is used by plant breeders for selection of desirable traits as key for improvement of these traits. On the other hand, Dudley and Moll (1969), defined heritability as the total genetic variance which is part of the phenotypic variance that can be influenced by genotypic factors and this is broad sense heritability. Heritability estimates are applicable to quantitative traits such as yield that are controlled by many genes, with small additive, dominant or epistatic effects, and interact with the environment to determine the expected response to selection for improvement of the traits in breeding programmes (Holland et al., 2003). Thus broad sense heritability measures the ratio of total genetic variance (additive, dominance and epistatic variance) to the phenotypic variance (Riaz and Chowdhry, 2003).

Breeders pay more attention to improving the grain yield of a crop by estimation the heritability of yield and yield contributing components and variability. For cowpea, yield components are pod length, pod width, pod number, seed per pod and hundred seed weight. Depending on environmental factors, yield can change as a result of changes in one or more of these yield

components. The heritability value of a trait is useful for effectiveness of selection based on phenotypic expression. Estimation of narrow sense heritability is not easy, but by estimating broad sense heritability along with genetic gain one can assess whether it would be effective or not to select the best individuals based on the phenotype (Holland et al., 2003).

High heritability was obtained in genotypes which had a high genetic diversity background (Ogunniyan and Olakojo, 2014). Also, minimal environmental variation resulted in high heritability. Quantitative traits such as grain yield which are polygenic in nature and are often influenced by environment have low heritability (Ogunniyan and Olakojo, 2014). Low heritability can also be caused by inbreeding individuals resulting in a decrease in the genetic variance or by individuals reared in very diverse environments which increases the environmental variance. A better understanding of heritability and genetic advance guides plant breeders to which selection method would be applicable for improvement of the traits and to predict genetic gain from selection and the relative importance of genetic effects on yield and its components for crop improvement (Poehlman and Sleper, 1995). Moreover, high genetic advance coupled with high heritability estimates offers the most effective condition for selection for a particular character (Malek et al., 2014).

Various authors reported broad sense heritability values for days to 50% flowering at 61% (Allen and Allen, 1981), 86% (Ishiyaku et al., 2005), 78% (Omoigui et al., 2006) in cowpea. Also Ishiyaku et al., (2005) reported that days to flowering is a quantitative character and its inheritance is controlled by many genes. Broad sense heritability values for number of pods per plant of 20% (Omoigui et al., 2006), 23% (Shimelis and Shiringani, 2010), 53% (Singh and Rachie, 1985), and 86% (Damarany, 1994) were reported in cowpea. Days to maturity was estimated to have a relatively higher heritability at 66% (Shimelis and Shiringani, 2010) and 79% (Omoigui et al., 2006). Hundred seed weight has been observed to have a wide diverse and relatively high broad sense heritability ranging from 68% to 97% (Allen and Allen, 1981; ; Singh and Rachie, 1985;Omoigui et al., 2006) and the lowest was 11% due to contribution of genotype by environment interaction as suggested by Shimelis and Shiringani, (2010).

Moreover, high heritability of hundred seed weight implies that a significant increase in seed yield can be observed from selection for this character within the germplasm. There is need to understand more the genetic relationship between seed yield and nineteen yield components. Characters such as hundred seed weight may serve as a criterion for indirect selection for yield. Seed size, usually measured as hundred seed weight, is moderately to highly heritable, with published estimates averaging 67.8% in cowpea. Number of seeds per

pod is has moderate heritability estimates (52.8%) and are influenced by environmental conditions. The yields of both the reproductive and the vegetative portions of the cowpea plant are moderately heritable under different environmental conditions. Heritability estimates for pod number (53.1%), seed yield (45.0%), and fresh fodder yield (54.7%) (Singh and Rachie, 1985) have been reported.

1.9 Characterization of cowpea accessions

Characterization of cowpea accessions is crucial to speed up the breeding process, and facilitate selection of breeding materials for future breeding programme (Cilliers and Swanevelder, 2003; Sarutayophat et al., 2007). Also, characterization can be done using agro-morphological, quality traits and molecular markers to generate information which plant breeders can use in improvement and other many uses apart from breeding activities. This includes effective management of gene bank, removal of duplicate copies, correction of mislabelled accessions, monitoring of contamination through seed or pollen and determining future handling procedures (Reed, 2004).

It is also useful to study genetic variability within accessions, screen accessions for desirable traits which may be considered for improvement in breeding programmes in a given region, country or geographical area. The information about interrelationships among characteristics, from a plant breeder's view helps in the selection of superior genotypes from the breeding population thus important in planning and evaluating breeding programmes (Sheela and Gopalan, 2006).

1.10 Summary of literature review

In conclusion, cowpea is one of the most important legume crops in semi-arid and arid areas, which contributes both food and fodder. This review of the literature established that:

- there is little work on characterization and genetic diversity assessment of cowpea accessions for future cowpea breeding programme in South Africa,

- absence of good genetic resources especially high grain yield, better nutritional quality traits varieties and other agronomic traits,

- the relationship between high grain yield components and better nutritional quality in varieties merging both traits has not been developed conclusively,

- there are no good accession management and planning to maximize genetic resources capacity for future use in cowpea breeding programme,

- Farmers' situation regarding crop yield and better nutritive quality of the production has not been appraised or reported on.

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CHAPTER TWO

ASSESSMENT OF GENETIC DIVERSITY IN SELECTED COWPEA ACCESSIONS USING AGRO-MORPHOLOGICAL TRAITS

Abstract

Assessment of genetic diversity in crop species allows the exploitation of different genotypes in order to develop new and improved varieties for the traits of interest. The objective of the study was, therefore, to estimate genetic diversity among 45 cowpea accessions maintained at the Agricultural Research Council -Grain Crops Institute (ARC-GCI) gene bank using agro-morphological traits. The accessions were grown at ARC-Potchefstroom research farm under two planting dates during 2015/2016 summer cropping season. Data were collected on 11 quantitative and three qualitative traits including grain yield. The analysis of variance for 10 traits revealed highly significant ($P<0.001$) differences among the accessions. Grain yield and other agronomic traits showed a wide phenotypic variability. Among these were; weight of mature pods, fodder yield and days to 50% flowering, while medium phenotypic coefficient of variation (PCV) was observed for number of main branches, pod length, pod width, number of seeds per pod and hundred seed weight. Days to 50% flowering, weight of mature pods, grain yield and fodder yield had high genotypic coefficients of variation (GCV), moderate to high heritability and genetic advance as percent of the mean. This indicated that selection based on the mean would be successful in improving these traits. High heritability estimates along with high genetic advance implied additive gene action for the inheritance of the traits. Grain yield and fodder yield were affected by planting date; whereas optimum planting date gave the highest mean values (3051.5 kg /ha and 5725.9 kg/ha, respectively); while late planting date had lower means (1781.4 kg/ha and 3756.9 kg/ha, respectively). Grain yield showed a positive and significant correlation with weight of mature pods, fodder yield and number of seeds per pod. This implies that selection for weight of mature pods and number of seed per pod would be essential for grain yield improvement. Principal component analysis resulted in the first four principal components explaining 76.89% of total variability among accessions. The number of main branches, days to 50% flowering and days to maturity mainly contributed to PC1. Number of plants per plot, weight of mature pods per hectare and grain yield contributed mainly to PC2. Overall, the studied traits showed huge genetic variation that can be considered in the improvement of these traits through breeding.

Keywords: Cowpea, genetic variation, heritability, genetic advance, phenotypic correlation and principal component analysis

2.1 Introduction

Cowpea is the most important legume crop grown sub-Saharan Africa, Asia, Central and South America which are tropic and subtropics areas (Singh et al., 1997), with an annual production of 6 million tons in worldwide (FAO, 2016). Major cowpea producing areas in Africa are central, east and West Africa (Ehlers and Hall, 1997). The grain, pod and leaves are commonly used as human food. After harvesting, the dry leaves and stalks are cut and stored for use as fodder. Cowpea, as a nitrogen-fixing legume, can do well in poor soils (Dube and Fanadzo, 2013) and produce potential yield compared to other legume crops. It has been reported that cowpeas have a wide genetic diversity consisting of wild perennials, wild annuals and cultivated forms (Nagalakshmi et al., 2010). High genetic diversity is associated with several advantages which are successfully used in breeding programmes (Nagalakshmi et al., 2010). Genotypes with wider genetic variations can be exploited for crop improvement due to excess of genepool. Furthermore, knowledge of the genetic variation within the same species can be used as a guiding map for selection in any breeding programme (Adewale et al., 2011). Understanding of genetic diversity enables plant breeders to select the best genes that can adapt well to an environment.

In any successful breeding programme, the parental selection is normally the important factor to consider (Manggoel et al., 2012). Even though, environmental factors greatly influence morphological variation, morphological traits can still be precise and efficient for selection and is the pre-requisite for the breeding programme. So, morphological assessment of genotypes to understand the genetic diversity with and within a population is still applicable for high precision diversity analysis (Adewale et al., 2011). Previous researchers used morphological traits to evaluate genetic diversity within and among the accessions. Many previous findings showed that genetic diversity patterns existed among accessions of various crops using different techniques. Examples include sorghum (Bucheyeki et al., 2009; Gerrano et al., 2014; Amelework et al., 2016), rice (Nascimento et al., 2011; Onaga et al., 2013; Thenmozhi and Rajasekaran, 2013), barley (Drikvand et al., 2012; Ebrahim et al., 2015), wheat (Salem et al., 2008), maize (Thakur et al.; Beyene et al., 2005; Hartings et al., 2008), sunflower (Masvodza et al., 2015), groundnut (Molosiwa et al., 2011; G et al., 2015), common bean (Lima et al., 2012; Hegay et al., 2014), chickpea (Naghavi et al., 2012; Ghaffari et al., 2014) and cowpea (Doumbia et al., 2013; Stoilova and Pereira, 2013; Animasaun et al., 2015; Gerrano et al., 2015), Bambara groungnut (Shegro et al., 2013; Amara et al., 2016).

Morphological characters and agronomic parameters were used successfully for selection of desirable traits; for example, plant morphology, seed coat colour and pod characteristics in cowpea (Doumbia et al., 2013). They also contributed to the understanding of the association between yield and its component traits in order to facilitate effective selection for yield improvement (Umaharan et al., 1997; Espósito et al., 2009; Al-Tabbal and Al-Fraihat, 2012; Manggoel et al., 2012). In cowpea, grain yield has been reported to be highly directly associated with number of branches and days to 50% flowering (Ekpo et al., 2012), number of seeds and hundred seed weight (Hemavathy et al., 2015) in mung bean, days to 50% flowering, days to maturity and number of main branches (Malik et al., 2007) in soya bean. Obute (2001) used morphological traits which included plant height, number of leaves, leaf length, the number of pods per plant, pod length and number of seeds per pod to describe an aneuploidy *Vigna unguiculata* from the other cytotypes. Other authors used morphological cowpea descriptors for parameters such as days to 50% flowering, growth habit, flower colour, pod placement, 100 grain weight, grain coat colour, grain coat pattern and grain coat texture for characterisation of cowpeas (Mahalakshmi et al., 2007).

Agro morphological characterization in the existing cowpea accessions would provide genetic information. This is important for genetic resources conservation in the ARC as well as in the national plant genetic resources centres in South Africa. Cowpea production is faced with several challenges including biotic and abiotic that cause grain and fodder to be low in South Africa. The problem of lower yields is further compounded by the absence of improved varieties, absence availability of good seeds for planting and lack of knowledge of good agronomic practices (Asiwe, 2009).This study sought to contribute information on genetic diversity which could be useful to accelerate crop breeding schemes through the use of agro-morphological traits. This would be an important preliminary step in the adoption of new varieties and important in the development a sustainable management strategy for plant genetic resources. In addition this would enable plant breeders to use particular accessions for parental selection and widening the genetic base of the crop. Therefore, the objective of the study was to estimate the genetic diversity among cowpea accessions.

2.2   Materials and methods

2.2.1   Experimental site

The study was conducted at the research farm of the Agricultural Research Council (ARC) at Potchefstroom research farm (26°74''S; 27°8'E) during the 2015/16 growing season. Potchefstroom is located at an altitude of 1344 m above sea level and the average minimum and maximum temperature is 9.61˚C and 25.48˚C, respectively with an average annual total rainfall of 618.88 mm.

2.2.2   Experimental material

A total of 45 cowpea accessions (Table 2.1) maintained at the ARC-GCI gene bank, Potchefstroom were used.

Table 2.1: List of cowpea accessions used for the study

Entry No.	Accessions	Origin/Locations
1	RV 194	South Africa
2	RV 202	South Africa
3	RV 204	South Africa
4	RV 207	South Africa
5	RV 213	South Africa
6	RV 321	South Africa
7	RV 342	South Africa
8	RV 343	South Africa
9	RV 344	South Africa
10	RV 351	South Africa
11	RV 500	South Africa
12	RV 503	South Africa
13	RV 553	South Africa
14	RV 554	South Africa
15	RV 555	South Africa
16	RV 558	South Africa
17	RV 568	South Africa
18	CH47	South Africa
19	Bechuana white	South Africa
20	Dr Saunders	South Africa
21	Glenda	South Africa
22	TVU 9443	Introduction (IITA-Nigeria)
23	TVU 9620	Introduction (IITA-Nigeria)
24	95K-589-2	Introduction (IITA-Nigeria)

Entry No.	Accessions	Origin/Locations
25	86D-1010	Introduction (IITA-Nigeria)
26	83S-911	Introduction (IITA-Nigeria)
27	TVU 12637	Introduction (IITA-Nigeria)
28	TVU 13998	Introduction (IITA-Nigeria)
29	90K-284-2	Introduction (IITA-Nigeria)
30	ITOOK-1263	Introduction (IITA-Nigeria)
31	TVU 13778	Introduction (IITA-Nigeria)
32	99K-494-6	Introduction (IITA-Nigeria)
33	TVU 12746	Introduction (IITA-Nigeria)
34	98K-476-8	Introduction (IITA-Nigeria)
35	TVU 2095	Introduction (IITA-Nigeria)
36	TVU 9596	Introduction (IITA-Nigeria)
37	98D-1399	Introduction (IITA-Nigeria)
38	TVU 14190	Introduction (IITA-Nigeria)
39	TVU 13004	Introduction (IITA-Nigeria)
40	98K-503-1	Introduction (IITA-Nigeria)
41	TVU 11986	Introduction (IITA-Nigeria)
42	97K-499-35	Introduction (IITA-Nigeria)
43	TVU 3416	Introduction (IITA-Nigeria)
44	TVU 13932	Introduction (IITA-Nigeria
45	PAN 311	South Africa

IITA=International Institute of Tropical Agriculture

2.2.3 Experimental design

The experimental trials were established under two environments viz. optimum and late planting dates. Forty-five cowpea accessions were planted in a 9x5 alpha lattice design with three replications. Each accession was planted in two row plots of 5 m long using a spacing of 0.20 m within a row, and 0.75 m between rows.

2.2.4 Management practices

Both trials were similar with regards to management practices, such as thinning, supplementary irrigation, weeding and chemical spraying. Planting was done after land preparation where two seeds were sown per hill and two weeks after full emergence, seedlings were thinned to one plant per hill. The trials were irrigated using a sprinkler irrigation system. Weed control was done using dual gold herbicide, applied before emergence to control annual grasses and broadleaf weeds, and hand weeding was done after two weeks of planting and the second hand weeding was done four weeks of planting. Trials were also sprayed with

40

seizer EC 100 insecticide every two weeks starting from flowering to control insect pests. Pods were harvested manually after they reached physiological maturity.

2.3 Data collection

The quantitative and qualitative traits measured are indicated in Tables 2.2 and 2.3 below.

Table 2.2: List of quantitative traits collected in present study

Traits	Code	Measurement
Number of plant per plot	NPP	Counted number of plants per plot
Number of main branches	NMB	Counted the branches whose origin is in the leaf axils on the main stem in the 8th week after sowing
Days to flowering	DTF	Counted from planting to the date that 50% the plants have started flowering
Number days to 50% maturity	DTM	Counted number of days from planting date to the stage when 50% of plants have produced mature pods in the plot
Weight of mature pods per plot	PWt	Weight of mature pods measured from the net plot of the experimental trial
Pod length (cm)	PL	Mean length of 10 pods from 10 randomly selected plants in net plot
Pod width (cm)	PW	Mean width of 10 pods taken measured for length
Number of seeds per pod	NSSP	Was determined by the average number of seeds per five pods counted on ten plants
Hundred (100) seed weight (g)	HSW	Weight of 100 seed at 12% moisture
Grain yield per plot (g/plot)	GY	Weight of grain from net plot of experimental trial at maturity stage
Fodder yield (g/plot)	FY	Weight of dry fodder taken from 2 m^2 area in the middle part of net plot of experimental trial

Table 2.3: List of qualitative traits measured in present study

Traits	Code	Observation
Plant vigour	PV	Rated based on plant width and height 3-4 weeks after sowing, on a scale of 3, 5, 7 or 9, where non vigorous (3), intermediate (5), vigorous (7) and very vigorous (9)
Growth habit	GH	Rated 6-7 weeks after sowing, as Acute erect (1), Erect (2), Semi-erect (3), Intermediate (4), Semi-prostrate (5), Prostrate (6) and Climbing (7)
Seed shape	SS	Rated as Kidney (1), Ovoid (2), Crowder (3), Globose (4) and Rhomboid (5)

2.4   Data analysis

Data from all the variables measured were subjected to analysis of variance (ANOVA), using GenStat, 17th edition software (Payne, 2014). Principal component analysis (PCA) was also analyzed based on the correlation matrix using SPSS to identify influential traits for selection. PCA biplot was plotted to show the relationship and differences between variables and among studied accessions using GenStat, 17th edition.

2.4.1   Phenotypic correlation coefficients

Phenotypic correlation was estimated among the agronomic traits. The estimation of correlation coefficients was done using GenStat 17th edition. The correlation coefficient was calculated as:

$$r_{xy} = \frac{Cov\,(x,\,y)}{\sqrt{(s_x^2)(s_y^2)}}$$

Where r_{xy} = the phenotypic correlation between *x* and *y*

$Cov\,(x,\,y)$ = the covariance between *x* and *y*

s_x^2 = the variance of *x*

s_y^2 = the variance of *y*

In this experiment, the genetic correlation was not calculated as the phenotypic correlation was fairly equivalent to the genotypic correlation (Kearsey and Farquhar, 1998).

2.4.2   Estimation of genetic parameters

Genetic parameters were estimated for agro-morphological traits on cowpea accessions as follows:

2.4.2.1   Phenotypic and genotypic variability

The phenotypic variation for each trait was partitioned into genetic and non-genetic factors and estimated according to Johnson et al. (1955) and Uguru (2005). Variability present in the

population was estimated by simple measures, namely, mean, range, standard error, phenotypic and genotypic variances and coefficient of variations. Phenotypic and genotypic variances and coefficient of variations were calculated according to the method suggested by (Singh and Chaudhary, 1985) as follows:

$$\text{Genotypic variance} = \delta_g^2 = \frac{[\text{MSg} - \text{MSe}]}{r}$$

$$\delta_e^2 = \text{MS}_e$$

$$\text{Phenotypic variance} = \delta_p^2 = \delta_g^2 + \delta_e^2$$

Where

$\delta_g^2 = $ Genotypic variance

$\delta_e^2 = $ Environmental variance

$\delta_p^2 = $ Phenotypic variance

$\text{MS}_g = $ Genotypic Mean Squares

$\text{MS}_e = $ Residual Mean Squares

$r = $ Number of replications

- Phenotypic coefficient of variation (PCV) $= \left[\frac{\sqrt{\sigma_p^2}}{\chi}\right] * 100$

Where

X = population mean

- Genotypic coefficient of variation (GCV) $= \left[\frac{\sqrt{\sigma_p^2}}{\chi}\right] * 100$

2.4.2.2 Heritability

Broad-sense heritability (H^2) for each variate was calculated based on the formula suggested by Hanson et al. (1956) as follows:

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where, H^2 = heritability in the broad sense

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

The expected Genetic Advance for each trait was calculated as

$$GA = K\sqrt{V_P}H^2$$

Where, K = 1.40 at 20% selection intensity for trait; V_p = Phenotypic variance for trait; H^2 = Broad sense heritability of the trait; Genetic advance as percentage of mean is calculated as,

$$GA\ (\%\ of\ Mean) = \frac{GA}{\pi} \times 100$$

Where, GA=Genetic advance; π =Mean

2.5 Results

2.5.1 Quantitative traits variation under optimum planting date

ANOVA for optimum planting date revealed highly significant (P≤0.001) differences among the cowpea accessions for all traits studied (Table 2.4) indicating the existence of high genetic variation. The mean number of main branches per plant ranged from 4-9 with a mean of 6. The highest number of main branches was recorded in accessions 99K-494-6 and TVU 13932; while the lowest was obtained in accession RV 344 compared to the other accessions. The mean value for days to 50% flowering ranged from 59-79 days, with a mean of 66. The early flowering dates obtained for the top 8 genotypes namely 98D-1399, RV 343, RV 344,RV 351, 83S-911, TVU 13932, RV 503 and 99K-494-6 were above the check 1. Mean days to maturity values ranged from 79-109, with a mean value of 90. The early maturing genotypes obtained in this present study were; RV 343, 98D-1399, RV 351, RV 500, RV 503, 83S-911, CH47 and 99K-494-6. Grain yield and yield-related traits showed highly significant (P≤0.001) differences among the accessions. Number of seeds per pod ranged from 9 to 19, and accessions with the highest number of seeds per pod were found in accessions; 83S-911, 99K-494-6, RV 555, RV 554, RV 321, TVU 12637, TVU 12746, RV 500, 98D-1399, RV 558, TVU 13004 and RV 553. Pod length ranged from 12.7 to 22.6 cm. Accessions 83S-911, TVU 12637, 98D-1399, TVU 12746 and TVU 3416 had the longest pod length compared to the other accessions. Hundred seed weight ranged from 9.1 g in Dr Saunders to 24.2 g in 90K-284-2. Grain yield per hectare ranged from 640 to 6227 kg/ha. In the present study, the accessions 99K-494-6, RV 213, RV 204, RV 207, RV 202, RV 321, RV 503, RV 558, 86D-1010, Glenda, TVU 11986, TVU 13998, TVU 12746 and RV 194 yielded higher than the check. Fodder yield per hectare ranged from 1 800 to 9 867 kg/ha. Accessions that had higher fodder yield than checks were; TVU 13932, RV 204, 95K-589-2, 90K-284-2, TVU 2095, TVU 11986, Glenda, Dr Saunders, RV 207, 98K-503-1 and RV 342 (Table 2.4).

45

Table 2.4: Mean, Mean square, standard error (SE), coefficient of variation (CV), range, least significant difference (LSD) values of 10 quantitative traits of cowpea accessions evaluated under optimum planting date at Potchefstroom

No.	Accessions	Quantitative traits[a]									
		NMB	DTF	DTM	PWt	PL	PW	NSPP	HSW	GY	FY
1	RV 194	7	70	93	5231	13.47	9.47	15	16.47	3358	5467
2	RV 202	6	68	94	5320	16.41	8.27	15	17.67	4339	6789
3	RV 204	8	66	91	6600	18.25	7.27	16	15.30	4524	8944
4	RV 207	5	72	108	6116	14.07	9.40	16	17.40	4373	7056
5	RV 213	6	69	94	5364	17.37	8.73	15	17.13	4542	4433
6	RV 321	6	61	83	6964	18.35	7.73	18	10.77	4289	4489
7	RV 342	6	60	82	4324	16.17	7.60	14	13.50	3204	6822
8	RV 343	6	57	79	4818	17.02	8.87	14	13.50	3071	4667
9	RV 344	4	58	82	3760	16.51	9.20	14	21.07	2747	3267
10	RV 351	5	58	81	2364	16.19	8.47	14	19.13	1689	3778
11	RV 500	6	63	81	4116	18.02	6.93	17	9.47	2116	3933
12	RV 503	6	60	81	5204	15.88	6.97	15	12.13	4196	6178
13	RV 553	6	65	84	4036	19.28	8.87	17	18.23	3280	3289
14	RV 554	6	67	100	4484	18.53	8.47	18	13.00	2547	6800
15	RV 555	7	79	109	4609	17.02	8.90	18	15.03	2960	5311
16	RV 558	6	67	87	5702	17.43	8.00	17	15.17	4062	4158
17	RV 568	6	63	88	4071	13.07	7.33	10	18.30	2671	5109
18	CH47	7	62	82	2191	16.14	8.73	13	18.27	1907	4711
19	Bechuana white	7	67	93	4996	18.57	8.00	16	17.53	3453	6822
20	Dr Saunders	6	65	93	3307	13.27	6.80	15	9.43	2493	7333
21	Glenda	7	64	88	5227	16.21	7.00	15	13.13	3742	8222
22	TVU 9443	7	67	92	2164	16.11	7.93	12	14.67	1418	4622
23	TVU 9620	7	67	82	2253	19.45	9.43	16	18.47	1342	4511
24	95K-589-2	7	74	106	4076	19.01	7.93	16	18.40	3036	8578
25	86D-1010	6	66	84	5329	19.13	8.93	14	17.57	4004	4867
26	83S-911	5	59	81	4484	21.37	8.27	19	15.27	3120	4311
27	TVU 12637	6	68	91	3422	21.35	7.80	18	15.60	1987	4644
28	TVU 13998	6	67	92	5169	17.55	8.13	16	16.87	3551	6111

No.	Accessions	Quantitative traits[a]									
		NMB	DTF	DTM	PWt	PL	PW	NSPP	HSW	GY	FY
29	90K-284-2	7	66	92	5284	18.75	9.20	14	24.17	3102	8578
30	ITOOK-1263	6	70	101	4231	19.02	9.60	15	20.67	2636	5822
31	TVU 13778	6	67	92	3924	18.19	8.87	16	18.67	2689	5111
32	99K-494-6	8	60	82	7222	19.11	9.47	19	16.23	5284	6004
33	TVU 12746	6	63	89	3053	20.53	7.40	18	16.10	3440	4511
34	98K-476-8	7	69	93	4440	17.75	8.27	16	19.60	2920	6267
35	TVU 2095	6	75	95	3133	19.35	11.27	16	19.23	2333	8489
36	TVU 9596	7	70	91	1836	15.49	6.73	14	15.50	1320	5163
37	98D-1399	5	57	79	4640	20.68	7.60	17	15.13	3289	3244
38	TVU 14190	6	68	88	3498	15.99	7.73	16	18.33	2533	4311
39	TVU 13004	6	75	101	2876	19.00	10.13	17	16.47	1724	5067
40	98K-503-1	7	75	106	3013	18.67	9.27	16	18.63	2569	6989
41	TVU 11986	6	67	83	5369	17.39	8.13	15	13.90	3667	8361
42	97K-499-35	6	67	95	4027	17.81	8.93	15	22.40	2911	4956
43	TVU 3416	6	72	96	4249	20.53	9.00	16	16.60	3173	6778
44	TVU 13932	8	59	96	3824	19.50	7.90	13	17.60	2413	9244
45	PAN 311	6	60	81	5173	18.39	8.53	17	15.37	3293	3551
	Genotype MS	1.7**	85.3**	199.3**	4628257**	12.1**	2.7**	9.3**	27.7**	2548824**	8345929**
	Residual	0.36	0.25	0.40	807411	0.70	0.20	0.30	0.10	470576	338585
	SED	0.90	5.30	8.10	1446	2.10	1.00	1.80	3.00	1088	1722
	Range	3.6-8.2	56-79	79-109	1173-7760	12.7-22.6	6.2-11.6	10.2-19	9.1-24.2	640-6227	1800-9867
	Mean	6	66	90	4368.10	17.72	8.39	15.54	16.51	3051.50	5725.90
	LSD (5%)	1	0.80	1.03	1458.02	1.32	0.68	0.822	0.43	1113.09	944.17
	CV (%)	9.60	0.80	0.70	20.90	4.60	5	3.30	1.60	22.50	10.20

NS, *, **Non-significant; significant at 5% and highly significant at 1% level of probability, respectively

[a]NMB - number of main branches, DTF-days to 50% flowering, DTM-days to maturity, PWt- weight of mature pods per hectare, PL-pod length, PW- pod width, NSPP-number of seeds per pod, HSW-hundred seed weight, GY-grain yield and FY-fodder yield

2.5.2   Quantitative traits variation under late planting date

ANOVA for late planting date revealed highly significant (P≤0.001) differences for all quantitative traits among the accessions (Table 2.5) indicating the existence of high genetic variation. The number of main branches ranged from 2 to 8 with a mean of 6. RV 344 was the only one with a lower value compared to other genotypes; while the highest values were recorded in 22% of the entire accessions with mean values of 7. The days to 50% flowering ranged from 54-95 with mean 69. The early flowering dates obtained for the top 18 genotypes RV 351, 98D-1399, RV 342, RV 568, RV 344, RV 500, 83S-911, RV 343, RV 503,TVU 12746, 90K-284-2, CH47, RV 321, Glenda 99K-494-6, RV 553 RV 558 and Dr Saunders were above the check. Days to maturity ranged from 79 to 109 with a mean value of 90. The early maturing genotypes obtained in this present study were; 98D-1399, RV 500, RV 351, 83S-911, CH47, RV 503, RV 344, TVU 13778, RV 342 and RV 204. Grain yield and yield-related traits had a highly significant (P≤0.001) difference among all the characters investigated. The number of seeds per pod ranged from 6 to 17 and the cowpea accession with the highest number of seeds per pod were found in accessions RV 500, TVU 13004, RV 554, RV 321, 98D-1399, 83S-911, RV 207, 99K-494-6, RV 558 and TVU 12637. Pod length ranged from 12.7 to 22.6. The cowpea accessions 83S-911, TVU 12637, 98D-1399, TVU 12746 and TVU 3416 had the longest pod length compared to all the other accessions. Hundred seed weight ranged from 8.8 g in Dr Saunders to 24.4 g in RV 344. Grain yield per hectare ranged from 346.7 to 3853 kg/ha. Cowpea accessions that yielded better than checks were; RV 202, RV 207, RV 503, TVU 13998, 86D-1010, RV 55'8, RV 213, 90K-284-2, 98D-1399, 99K-494-6 and RV 194. Fodder yield per hectare ranged from 1467 to 7000 kg/ha and the following accessions had higher fodder yield compared the checks; ITOOK-1263, TVU 11986, TVU 13998,98K-503-1, RV 554, TVU 9596, RV 204, 98K-476-8, 97K-499-35 and TVU 3416.

Table 2.5: Mean, mean squares, standard error (SE), coefficient of variation (CV), range, least significant difference (LSD) values of 10 quantitative traits of accessions evaluated under late planting s date at Potchefstroom

No.	Accessions	Quantitative traits[a]									
		NMB	DTF	DTM	PWt	PL	PW	NSPP	HSW	GY	FY
1	RV 194	6	89	114	3484	13.65	9.47	11	16.40	2207	3711
2	RV 202	6	67	98	4144	16.86	8.27	12	17.20	3398	3470
3	RV 204	6	67	97	2538	18.58	7.27	12	18.57	2131	4689
4	RV 207	4	65	103	4284	14.07	9.40	14	18.27	3221	2733
5	RV 213	6	87	116	3191	17.37	8.73	13	17.57	2447	2978
6	RV 321	6	59	98	3084	18.95	8.07	14	10.40	2002	3178
7	RV 342	6	56	96	2109	14.97	7.60	11	16.43	1669	2356
8	RV 343	6	57	98	2604	17.02	8.87	10	15.40	2096	2089
9	RV 344	3	57	96	2218	16.51	9.20	11	24.40	1576	3089
10	RV 351	6	54	83	1898	16.19	8.20	11	19.27	1349	3311
11	RV 500	7	57	82	1744	17.69	6.80	15	9.87	1196	3089
12	RV 503	6	57	95	3529	15.88	7.13	13	12.67	3131	4078
13	RV 553	6	59	98	2000	19.28	8.07	11	21.27	1400	2378
14	RV 554	6	90	115	2862	18.53	8.47	15	15.67	2145	5089
15	RV 555	6	89	103	2044	17.02	8.77	13	14.27	1236	3467
16	RV 558	6	59	100	3284	17.43	8.00	13	14.90	2449	3789
17	RV 568	7	56	98	2062	13.23	7.33	9	19.60	1338	2044
18	CH47	6	59	95	1996	16.14	8.73	10	20.40	1258	4044
19	Bechuana white	7	60	108	3369	18.57	8.00	12	16.03	2196	3844
20	Dr Saunders	6	59	102	1773	13.27	6.80	12	8.77	1382	3344
21	Glenda	6	59	103	1913	16.21	7.00	11	14.30	1567	3944
22	TVU 9443	7	89	118	1142	16.11	7.93	10	13.63	798	4278
23	TVU 9620	6	87	120	1253	19.45	9.43	8	17.40	880	4200
24	95K-589-2	7	87	116	2071	19.01	7.93	11	16.77	1258	4533
25	86D-1010	6	62	102	4182	19.13	8.93	12	19.37	2707	4356
26	83S-911	5	57	83	3004	21.37	8.27	14	16.17	2132	2867
27	TVU 12637	7	86	112	1938	21.35	7.80	13	15.40	1529	4178
28	TVU 13998	6	61	97	3906	17.55	8.47	11	16.33	2791	5533

No.	Accessions	Quantitative traits[a]									
		NMB	DTF	DTM	PWt	PL	PW	NSPP	HSW	GY	FY
29	90K-284-2	6	58	101	3724	18.75	9.20	10	24.23	2431	3867
30	ITOOK-1263	6	73	113	2624	19.02	9.60	10	17.50	1872	6644
31	TVU 13778	6	63	96	2796	18.19	8.87	12	20.30	1716	2933
32	99K-494-6	7	59	98	2951	19.11	9.47	14	15.67	2244	3333
33	TVU 12746	6	58	101	2111	20.56	7.43	13	15.53	1338	3111
34	98K-476-8	6	90	118	1342	17.75	8.27	11	16.53	1182	4644
35	TVU 2095	6	92	116	1240	19.35	11.27	13	16.07	704	3356
36	TVU 9596	7	87	107	764	15.49	6.73	11	16.17	636	4822
37	98D-1399	5	54	80	2938	20.68	7.60	14	15.70	2271	3022
38	TVU 14190	6	62	108	1747	15.99	7.73	13	17.27	1409	3556
39	TVU 13004	7	87	117	2324	19.91	10.27	15	16.50	1413	2767
40	98K-503-1	7	62	110	2918	18.71	9.27	11	22.27	2038	5147
41	TVU 11986	6	88	112	1591	17.39	8.13	13	13.37	929	5599
42	97K-499-35	6	62	107	2400	17.81	8.93	11	21.37	1376	4644
43	TVU 3416	6	87	118	1569	20.43	9.00	10	14.60	853	4622
44	TVU 13932	7	95	121	2776	19.37	7.87	12	17.30	1680	3667
45	PAN 311	6	54	79	4316	18.39	8.53	14	16.63	2587	2667
	Genotype MS	1.8**	610.7**	373.8**	2927356**	12.4**	2.7**	8.4**	31.5**	1686080**	2964451**
	Residual	0.30	0.50	0.30	247183	0.70	0.30	2.00	0.10	116883	212511
	SED	2.00	14.20	11.10	1065	2.2	1	2.00	3.20	799.10	1058
	Range	6.4-17.4	54-95	79-121	547-5693	12.70-22.60	6.20-11.60	6.4-17.4	8.6-24.8	347-3853	1267-7000
	Mean	6	69.36	103	2528	17.74	8.38	12.01	16.75	1781.40	3756.90
	LSD (5%)	0.9	1.09	0.83	1165.10	1.40	0.85	2.29	0.41	767.57	740.94
	CV%	9	1	0.5	28.4	4.9	6.2	11.8	1.5	26.6	12.2

NS, *, **Non-significant or significant at 5% and highly significant at 1% level of probability, respectively

[a]NMB - number of main branches, DTF-days to 50% flowering, DTM-days to maturity, PWt- weight of mature pods per hectare, PL-pod length, PW- pod width, NSPP-number of seeds per pod, HSW-hundred seed weight, GY-grain yield and FY-fodder yield

2.5.3 Quantitative traits variation under combined planting dates

A combined ANOVA revealed highly significant (P≤0.001) differences for all quantitative traits among the accessions studied (Table 2.6) indicating the presence of significant genetic variation. The number of main branches per plant ranged from 3 to 7, with an average of 6. The days to 50% flowering ranged from 54 to 95 with a mean value of 68. Early flowering accessions were; 98D-1399, RV 351, RV 343, RV 344, 83S-911, RV 342, RV 503, 99K-494-6, RV 568, RV 500, RV 321, CH47 and TVU 12746. Days to maturity ranged from 79 to121 days, with a mean of 97. Early maturing accessions under both planting dates were 98D-1399, RV 500, RV 351, 83S-911, RV 503, CH47, RV 343, RV 344, RV 342 and 99K-494-6. Number of seeds per pod ranged from 10 to 16, and the highest number of seeds per pod were obtained from RV 554, RV 500, RV 321, 99K-494-6, TVU 13004, 83S-911, TVU 12637, RV 555 and 98D-1399. Pod length ranged from 13.15 to 21.37 cm. Accessions 83S-911, TVU 12637, 98D-1399 and TVU 12746 had the longest pod length compared to all the other accessions. Hundred seed weight ranged from 9.10 g in Dr Saunders to 24.2 g in 90K-284-2. For combined data, grain yield per hectare ranged from 978 to 3797 kg/ha. The top five highest yielding accessions were RV 207, 99K-494-6, RV 202, RV 503 and 86D-1010. Fodder yield per hectare ranged from 2833 to 6980 Kg/ha. Over the two planting dates, accessions gave higher fodder yield per hectare than checks were TVU 11986, RV 204, 95K-589-2, TVU 13932 and ITOOK-1263.

Table 2.6: Mean, mean squares, standard error (SE), coefficient of variation (CV), range, least significant difference (LSD) values of 10 quantitative traits of accessions evaluated at two planting dates

No.	Accessions	Quantitative traits[a]									
		NMB	DTF	DTM	PWt	PL	PW	NSPP	HSW	GY	FY
1	RV 194	6	79	104	4191	13.56	9.47	13	16.43	2732	4589
2	RV 202	6	67	96	4732	16.64	8.27	13	17.43	3732	5028
3	RV 204	7	67	94	4402	18.41	7.27	14	16.93	3328	6817
4	RV 207	5	69	106	5200	14.07	9.40	14	17.83	3797	4894
5	RV 213	6	78	105	3944	17.37	8.73	14	17.35	2661	3706
6	RV 321	6	60	91	5191	18.65	7.90	16	10.58	3146	3833
7	RV 342	6	58	89	3050	15.57	7.60	12	14.97	2303	4589
8	RV 343	6	57	89	3438	17.02	8.87	12	14.45	2417	3378
9	RV 344	3	57	89	2989	16.51	9.20	13	22.73	2161	3178
10	RV 351	5	56	82	2330	16.19	8.33	12	19.20	1686	3544
11	RV 500	7	60	81	3030	17.85	6.87	16	9.67	1689	3511
12	RV 503	6	59	88	4367	15.88	7.05	14	12.40	3713	5128
13	RV 553	6	62	91	3101	19.28	8.47	13	19.75	2173	2833
14	RV 554	6	78	107	3673	18.53	8.47	16	14.33	2341	5944
15	RV 555	6	84	106	3327	17.02	8.83	15	14.65	2098	4389
16	RV 558	6	63	94	4327	17.43	8.00	15	15.03	3249	3973
17	RV 568	7	60	93	3067	13.15	7.33	10	18.95	2171	3577
18	CH47	6	60	88	2093	16.14	8.73	11	19.33	1582	4378
19	Bechuana white	7	63	101	4182	18.57	8.00	14	16.78	2824	5333
20	Dr Saunders	6	62	98	2540	13.27	6.80	14	9.10	1938	5339
21	Glenda	7	62	96	3570	16.21	7.00	13	13.72	2654	6083
22	TVU 9443	7	78	105	1653	16.11	7.93	11	14.15	1108	4450
23	TVU 9620	6	77	101	1753	19.45	9.43	12	17.93	1111	4356
24	95K-589-2	7	81	111	3073	19.01	7.93	13	17.58	2147	6556
25	86D-1010	6	64	93	4756	19.13	8.93	13	18.47	3522	4611
26	83S-911	5	58	82	3911	21.37	8.27	16	15.72	2626	3589
27	TVU 12637	7	77	102	2680	21.35	7.80	15	15.50	1758	4411
28	TVU 13998	6	64	95	4538	17.55	8.30	13	16.60	3171	5822

		Quantitative traits[a]									
No.	Accessions	NMB	DTF	DTM	PWt	PL	PW	NSPP	HSW	GY	FY
29	90K-284-2	6	62	97	4504	18.75	9.20	12	24.20	2749	6222
30	ITOOK-1263	6	72	107	3411	19.02	9.60	12	19.08	2487	6233
31	TVU 13778	6	65	94	3360	18.19	8.87	14	19.48	2202	4022
32	99K-494-6	7	60	90	5087	19.11	9.47	16	15.95	3764	4668
33	TVU 12746	6	60	95	3346	20.54	7.42	15	15.82	2166	3811
34	98K-476-8	6	79	106	2891	17.75	8.27	13	18.07	2034	5456
35	TVU 2095	6	84	106	2353	19.35	11.27	15	17.65	1612	5922
36	TVU 9596	7	78	99	1300	15.49	6.73	12	15.83	978	4992
37	98D-1399	5	55	80	3789	20.68	7.60	15	15.42	2780	3133
38	TVU 14190	6	65	98	2622	15.99	7.73	14	17.80	1971	3933
39	TVU 13004	7	81	109	2600	19.46	10.20	16	16.48	1569	3833
40	98K-503-1	7	69	108	3132	18.69	9.27	13	20.45	2303	6068
41	TVU 11986	6	78	98	3480	17.39	8.13	14	13.63	2298	6980
42	97K-499-35	6	64	101	3213	17.81	8.93	13	21.88	2127	4800
43	TVU 3416	6	80	107	3076	20.48	9.00	13	15.60	2013	5700
44	TVU 13932	7	77	109	3300	19.44	7.88	12	17.45	2047	6456
45	PAN 311	6	57	80	4744	18.39	8.53	15	16.00	2940	3109
	Genotype MS	0.5*	210.7**	126.8**	1652949**	0.1ns	0.03ns	3.3**	4.3**	762973**	3809790**
	Residual	0.4	0.3	0.3	528981	0.7	0.22	1.4	0.1	269922	273340
	SED	0.9	10.8	11.7	1588.0	2.1	1.00	3	3.1	1117	1736
	Range	2.2-8.2	54-95	79-121	546.7-8760	12.7-22.6	6.2-11.6	6.4-19	8.6-24.8	346.7-6227	1267-9867
	Mean	6	68	97	3451.5	17.729	8.384	14	17	2393	4731
	LSD (5%)	1	0.95	0.93	1357.22	1.35	0.76	1.88	0.42	937.09	843.90
	CV%	10.3	0.9	0.6	24.4	4.7	5.6	8.6	1.6	24.3	11.1

NS, *, **Non-significant or significant at 5% and highly significant at 1% level of probability, respectively

[a]NMB - number of main branches, DTF-days to 50% flowering, DTM-days to maturity, PWt- weight of mature pods, PL-pod length, PW- pod width, NSPP-number of seeds per pod, HSW-hundred seed weight, GY-grain yield and FY-fodder yield

2.5.4 Phenotypic and genotypic coefficients of variation

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were highest for combined data in PWt, GY, and FY (Table 2.7 Under combined data, low GCV values were recorded for PL and PW (Table 2.7

Table 2.7: Estimates of components of variance, PCV and GCV for 10 traits of cowpea accessions under two planting dates at Potchefstroom, 2015/16 cropping season

Trait	Estimates of Components of Variance					PCV %	GCV%
	Grand mean	MS_g	MS_e	$\sigma^2 g$	$\sigma^2 p$		
NMB	6.00	0.54	0.39	0.41	0.93	16.06	10.68
DTF	67.68	210.75	0.35	210.63	211.09	21.47	21.44
DTM	96.82	124.612	0.34	124.50	124.95	11.55	11.52
PWt	3447	1352103	717381	1112976	2069484	41.73	30.61
PL	17.71	0.11	0.71	-0.13	0.81	5.09	0.00
PW	8.39	0.04	0.23	-0.04	0.26	6.09	0.00
NSPP	13.62	3.03	1.38	2.573	4.409	15.42	11.78
HSW	16.62	4.34	0.07	4.32	4.41	12.63	12.5
GY	2397	666711	341894	552746.3	1008605	41.9	31.02
FY	4737.3	3818247	274318	3726808	4092565	42.7	40.75

[1] $\sigma^2 g$=genotypic variance; $\sigma^2 p$= phenotypic variance; $\sigma^2 e$= environmental variance

NMB-number of main branches, DTF-days to 50% flowering, DTM-days to maturity, PWt-weight of mature pods, PL-pod length, PW- pod width, NSPP-number of seeds per pod, HSW-hundred seed weight, GY-grain yield, FY-fodder yield.

2.5.5 Heritability

According to Robinson et al. (1949), broad sense heritability (%) can be described as high, moderate or low based on the percentage as follows: >60% (high), 30-60% (moderate), and 0-30% (low). In this study, moderate to high heritability estimates were observed for different traits under the planting dates.

For the combined results over different planting dates, broad sense heritability ranged from -15.26 to 99.78% (Table 2.8). High broad sense heritability estimates were observed for days to 50% flowering, days to maturity,100 seeds weight and fodder yield. Moderate heritability estimates were observed for number of main branches, the weight of mature pods per hectare, number of seeds per pod and grain yield. Pod length and pod width had low broad sense heritability. The expected genetic advance values for the 10 quantitative traits across planting dates showed a wide range of genetic advances, ranging from -0.29 to 3194.95, with FY recording the highest genetic advance, followed by PWt, GY, DTF and DTM (Table 2.8). Low genetic advance was obtained for HSW, NSPP, PW and PL.

Table 2.8: Estimates of heritability (broad-sense), GA and GA as per cent of the mean for 10 quantitative traits of 45 cowpea accessions grown across two planting dates at Potchefstroom, 2015/16

Trait	Heritability	Genetic advance	Genetic advance (% of mean)
No. of main branches	44.19	0.88	14.62
Days to 50% flowering	99.78	29.86	44.13
Days to maturity	99.64	22.94	23.7
Weight of mature pods	53.78	1593.76	46.24
Pod length	-15.65	-0.29	-1.64
Pod width	-15.29	-0.16	-1.92
No. of seed per pod	58.36	2.52	18.53
100-seed weight	97.95	4.24	25.49
Grain yield	54.80	1133.79	47.30
Fodder yield	91.06	3794.95	80.11

2.5.6 Correlations between grain yield and its component traits across planting dates.

There were significant correlations among phenotypic traits under different planting dates in combined data. The phenotypic, genotypic and environmental correlation coefficient between pairs of 10 traits for the combined data over two environments are presented in Table 2.9Positive and significant correlations were observed between grain yield and weight of mature pods number of seeds per pod while the number of main branches had a negative correlation with grain yield. Genotypic correlations for these traits with grain yield per plant were similar to the observed phenotypic correlations. Day to 50% flowering was positively correlated with days to maturity, fodder yield, number of main branches, pod width and hundred seed weight. Positive and significant correlations were observed between days to maturity with hundred seed weight and pod width. Negative and significant correlations were

observed between days to maturity with the weight of mature pods, grain yield and number of seeds per pods. Fodder yield had a significant positive correlation with number of main branches, grain yield and a number of seeds per pod. Hundred seed weight had a positive and significant correlation with pod width. Weight of mature pods had a significant negative correlation with number of main branches. Also, number of main branches were significant and negatively correlated with pod width. Number of seeds per pod was positive and significantly correlated with pod length. Pod length had a significant, positive correlation with pod width. All other studied traits had non-significant correlations.

Table 2.9: Phenotypic Correlation coefficient among various pairs of 10 traits in cowpea accessions across different dates planting at Potchefstroom, 2015/2016

Trait	DTF	DTM	FY	GY	HSW	PWt	NMB	NSPP	PL	PW
DTF	-									
DTM	`0.73**	-								
FY	0.13	-0.05	-							
GY	-0.28**	-0.44**	0.41**	-						
HSW	-0.05	0.11	-0.00	-0.03	-					
PWt	-0.26**	-0.44**	0.41**	0.95**	-0.04	-				
NMB	0.23**	0.19*	0.13	-0.12	-0.14	-0.11	-			
NSPP	-0.08	-0.41**	0.28**	0.49**	-0.27**	0.54**	-0.06	-		
PL	0.09	0.02	0.02	-0.03	0.11	0.04	0.10	0.28**	-	
PW	0.26**	0.24**	0.01	0.00	0.44**	0.04	-0.20**	0.02	0.22**	-

*, **, *** Indicates significance at 0.05, 0.01, 0.001 probability levels, respectively

[a]DTF-days to 50% flowering, DTM-days to maturity, FY-fodder yield, GY-grain yield, HSW-hundred seed weight, PWt- weight of mature pods, NMB-number of main branches, NSPP-number of seeds per pod, PL-pod length, PW- pod width

2.5.7 Principal component analysis across both environmental

The genetic diversity of 45 cowpea accessions was observed from a principal component analysis. The principal component analysis revealed four eigenvectors larger than one. These cumulatively explained 76.86% of total variation, among the 11 phenotypic traits describing the accessions (Table 2.10). The first principal component (PC1) alone had an eigenvalue of 3.13 and observed 28.47% of total variation mainly due to variation in fodder yield, days to maturity, days to 50% flowering and a number of main branches were essential variables for variation among the cowpea accessions. The traits which contributed to the second principal component (PC2) and accounted for 18.90% of the total variation were; grain yield, weight of mature pods, and number of plants per plot. Third principal component (PC3) alone explained 15.68% of the total variation, and this was due to the number of main branches, hundred seed weight and pod width. The fourth principal component (PC4) explained 13.84% of the variation and this was contributed by the number of seeds per pod and pod length. However, PC1 and PC2 explained most of the variation among the cowpea accessions, revealing a high degree of association among the traits studied.

Table 2.10: Principal component analysis of quantitative traits in cowpea accessions showing eigenvectors, eigenvalues, individual, and cumulative percentage of variation explained by the first four principal components (PC) axes across different planting dates

Trait	Eigenvector			
	PC1	PC2	PC3	PC4
NPP	0.09	0.32	-0.05	-0.15
NMB	0.26	-0.01	-0.28	-0.03
DTF	0.30	-0.07	0.04	0.12
DTM	0.34	0.01	0.10	0.02
PWt	0.03	0.37	0.05	0.09
PL	0.01	-0.05	0.08	0.45
PW	0.01	-0.02	0.47	0.18
NSPP	-0.03	0.01	-0.13	0.55
HSW	-0.02	0.04	0.48	-0.19
GY	0.02	0.38	0.05	0.01
FY	0.37	0.19	-0.04	-0.11
Eigenvalue	3.13	2.08	1.73	1.52
Individual %	28.47	18.90	15.68	13.84
Cumulative %	28.47	47.37	63.05	76.89

NPP=number plants per plants, NMB=number main branches, DTF=days to 50% flowering, DTM=days to maturity, PWt=weight of mature pods, PL=pod length, PW=pod width, NSPP=number of seeds per pod, HSW=hundred seeds weight, GY=grain yield and FY=fodder yield

2.5.8   Principal component biplot.

The relationships between the different variables and accessions with respective to principal components are further illustrated on the first two principal component biplots in Figure 2.1 across the different planting dates. Smaller angles between dimension vectors in the same direction indicated a high correlation of the variable traits in terms of discriminating accessions. Accessions excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull of the first principal component, with genotypes such as 99K-494-6, RV 207, 86D-1010, RV 202, RV 2O4, RV 202, RV 204 and TVU 13998 excelling in yield which was contributed mostly by high number of seeds per pod, weight of mature pods, number of plant per plot and pod length across both planting dates.

Figure 2.1: Principal component analysis score plot of PC1 and PC2 decribing the overall variation among cowpea accessions estimated using quantitative traits for combined data

2.6   Discussion

2.6.1   Genetic variation under optimum planting date, late planting date and combined data

Analysis of variance revealed high significant (P≤0.01) differences for all phenotypic traits among the 45 cowpea accessions studied at optimum planting date and late planting date under combined data (Table 2.6) indicating the existence of high genetic variation. The mean number of main branches per plant in the present study was 6. The mean values obtained in this study were higher than the values reported in previous studies (Basaran et al., 2011; Cobbinah et al., 2011; Kamai et al., 2014). The higher number of main branches might be due to the inherent genetic variation including environmental factors. Days to 50% flowering ranged from 54 to 95 with a mean of 68 days, and accessions 98D-1399, RV 351, RV 343, PAN 311, RV 344, 83S-911, RV 342, RV 503, 99K-494-6, RV 568, RV 500, RV 321, CH47 and TVU 12746 were early flowering. This finding is similar to that observed in the studies reported by Oyekanmi and Sangodoyin (2007), Cobbinah et al. (2011) and Manggoel and Uguru (2011). Different results were reported on days to flowering depending on the cowpea accessions used and region of the study. Early flowering or late flowering might be attributed to inherent genetic variation and environmental factors such as temperature, altitude and soil conditions (Hadley et al., 1983; Gerrano et al., 2015). Manggoel and Uguru (2011) reported days to 50% flowering ranging from 58 to 72 days in two photoperiodic groups of cowpea. Ishiyaku and Singh (2003) also reported days to flowering ranging from 36 to 42 days among two cowpea genotypes that were influenced by a single dominant gene. Cowpea under controlled conditions had 22 to 35 days to 50% flowering among the shortest and longest duration of juvenility as reported by Ehlers and Hall (1997).

Days to maturity ranged from 79 to 121 days, and early maturing genotypes obtained in the present study were; 98D-1399, PAN 311, RV 500, RV 351, 83S-911, RV 503, CH47, RV 343, RV 344, RV 342 and 99K-494-6. Ayo-Vaughan et al. (2011), Adeyanju and Ishiyaku (2007), Mak and Yap (1980), Hadley et al. (1983) and Gerrano et al. (2015) reported that early maturity indicated the scope of opportunity for earliness selection. Mak and Yap (1980) also reported that early maturity was dominant over late maturity among the cowpea accessions. Therefore, these accessions which are early maturing are desirable for drought escape especially in

drought stress conditions (Singh et al., 1997), hence they can be utilised in drought-prone areas of South Africa (Gerrano et al., 2015).

Grain yield and yield-related traits showed a highly significant (P≤0.001) difference among all the characters investigated. Number of seeds per pod ranged from 6 to 19, and cowpea accessions with the highest number of seeds per pod were obtained in RV 554, RV 500, RV 321, 99K-494-6, TVU 13004, 83S-911, TVU 12637, RV 555 and 98D-1399. Number of seeds per pod is an important agronomic trait for cowpea that contributes to grain yield (Ogunbodede, 1989; Okeleye et al., 1999). Those accessions with high number of seeds per pod could be considered in selection to increase grain yield as yield-related traits for improvement. Pod length ranged from 12.7-22.6 cm. Accessions 83S-911, TVU 12637, 98D-1399 and TVU 12746 had the longest pod compared to all other accessions. Other investigators reported pod length that ranged from 18.5-24.5 cm (Musvosvi et al., 2009), 15.75- 18.05 cm (Cobbinah et al., 2011) and 8.95 to 20.17 cm (Egbe et al., 2010). Differences in pod length between the present study and previous works might be due to genetic, environmental and/or genetic and environmental interaction, depending on the particular location. Pod length should also be considered in selection as it contributes to increased yield in cowpea (Ogunbodede, 1989; Okeleye et al., 1999).

Hundred seed weight ranged from 8.6 g in Dr Saunders to 24.8 g in 90K-284-2. In this study, higher hundred-seed weight than previous findings was obtained. Idahosa et al. (2010) reported HSW ranging from 8.97 to 13.40 g, from 7.79 to 18.67g (Gerrano et al., 2015). Hundred seed weight can be used for indirect selection to maximise grain yield in cowpea. Also, Berhe et al. (1998) reported that hundred seed weight was the highest contributor to grain yield in faba bean. Grain yield per hectare ranged from 346.7 to 6227 kg/ha. In this current study, the top five highest yielding accessions were; RV 207, 99K-494-6, RV 202, RV 503 and 86D-1010. Fodder yield per hectare ranged from 1467 to 9867 kg/ha. Accessions TVU 11986, RV 204, 95K-589-2, TVU 13932 and ITOOK-1263 gave higher fodder yield. These accessions should be considered as useful to improve for both human food as well as livestock feed.

2.6.2 Phenotypic and genotypic coefficients of variation

The estimates of the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) would assist to estimate genetic variability in particular traits. PCV values for most of the traits were near or close to the corresponding GCV indicating little environmental effects on the expression of the traits. Narrow differences between PCV and GCV implied minimal environmental influence and the role of additive gene effects indicating the traits could be improved through selection. According to Kaushik et al. (2007), higher GCV: PCV ratio suggested that the trait was much under the influence of genetic rather than environmental. In the present study, high PCV values were recorded for the weight of mature pods, grain yield, fodder yield, number main branches, and pod width. Similar findings were reported by Adewale et al. (2010) for pod yield, grain yield and fodder yield in cowpea. High PCV indicates the high influence of environmental effects indicating low heritability for these traits. However, there were relatively closer estimates of PCV and GCV in days to 50% flowering, days to maturity and hundred seeds weight. Adewale et al. (2010), found similar results for relatively closer estimates of PCV and GCV in days to 50% flowering, days to maturity and hundred seed weight in cowpea. This is an indication of low environmental effects for this trait thus high heritability. The possibility of improving these traits through selection is high.

2.6.3 Estimation of broad-sense heritability and genetic advance

The heritability estimates are important genetic parameters that play a significant role in the selection of different cowpea genotypes from a population (Rashwan, 2010). As reported by Manggoel et al. (2012) and Rashwan (2010), high broad-sense heritability values usually show the predominance of additive gene action in the expression of the traits. In this study, the highest estimates of broad sense heritability were observed for days to 50% flowering, days to maturity, hundred seed weight, and fodder yield under different planting dates in combined data. These findings are similar to those reported by Oladejo et al. (2011), Aliyu and Makinde (2016) and Adewale et al. (2010). Moderate heritability values were observed for weight of mature pods, number of main branches, and number of seeds per pod. Broad sense heritability could be high, medium or low in different crops under different environments, depending on the particular genotypes (Shahrokhi et al., 2013; Ullah et al., 2013). Selection for traits with 71-96% or more heritability could be easier, as they indicate a higher correlation between genotype and phenotype, and little environmental contribution to the phenotype. For

the traits that have low heritability, less than 45%, it is difficult to make selections due to higher environmental effects. Moreover, higher heritability shows additive effects and more response to selection in the next generation when coupled with high genetic advance (Songsri et al., 2008; Ahmed et al., 2013).

2.6.4 Genetic advance

Genetic advance (GA) under selection is important to consider in the improvement of characters in genotypic value for the development of new population compared with the base population under one cycle of selection at a specified selection intensity (Gashaw et al., 2010). In this study, high heritability coupled with high expected genetic advance was obtained for days to 50% flowering, days to maturity, and fodder yield. Similar results have been reported by Ajayi et al. (2014) for days to 50% flowering, days to maturity and fodder yield in cowpea. Olayiwola and Soremi (2014) reported high heritability and genetic advance in dry fodder yield in cowpea, while Pandey et al. (2015) reported high heritability and genetic advance in days to 50% flowering in red bean. Johnson et al. (1955) reported that relatively high heritability estimates accompanied with the high genetic advance are usually more useful in predicting gain under selection compared to heritability estimates alone. Medium heritability with the relatively higher genetic advance for weight of mature pods and grain yield were also observed. Normally, low or medium heritability and genetic advance give an indication of high environmental effects on the trait. Although a breeder can be able to improve the trait through direct selection, the rate of improvement will be slow when there is high environmental effects. Moreover, combined heritability and genetic advance would help breeders in quick direct parental selection for the traits of interest.

2.6.5 Correlations between grain yield and its components

Grain yield had a positive correlation with the weight of mature pods, number of seeds per pod and fodder yield across planting dates. These results confirm previous findings by Ehlers and Hall (1998), Peksen (2004), Romanus et al. (2008) and Javed et al. (2016). The positive associations indicate that selecting for number of seeds per pod would have a positive effect on pods related traits in improving grain yield. High positive correlation among these traits indicates that they share some common genes and geographical background (Almeida et al., 2014). Some traits showed negative significant correlation and could be utilised in breeding

for indirect selection due to an inherent association of these traits, for example, as reported in cowpea (Moura et al., 2012).Positive significant correlation between days to 50% flowering and days to maturity, fodder yield, the number of main branches, and pod width under different planting dates in combined data was also reported in this study. Days to 50% flowering was negatively but significantly correlated with grain yield and weight of mature pods. These results were similar to those reported by Oladejo et al. (2011), where they recorded positive and significant correlations between grain yield and weight of mature pods. This suggests that selection for weight of mature pods, number of seeds per pod and fodder yield should be considered in improving grain yield. Similar results were reported by Oladejo et al. (2011) who suggested that pod weight can be used to improve the grain yield in cowpea. Baghizadeh et al. (2010) and Romanus et al. (2008), also suggested that number of seeds per pod and pod length can be selected to improve grain yield in cowpea. Similar observation have been reported by Kamara et al. (2008) for fodder yield and it can therefore be selected during grain yield improvement. Days to 50% flowering showed a positive, significant correlation with days to maturity, fodder yield, number of main branches and pod width and can be used in indirect selection for grain yield enhancement as well as fodder yield.

Therefore, these traits should not be ignored when selecting for grain yield enhancement. Positive, significant correlation between days to maturity and hundred seed weight under different planting dates and the combined data agrees with previous results reported by Meena et al. (2015). Negative and significant correlations were observed between days to maturity and weight of mature pods in optimum planting date and combined data. Similar results reported by (Gul et al., 2008). Also negative and significant correlations between grain yield and number of seeds per pod.

Fodder yield had a significant, positive correlation with number of main branches and grain yield. Positive, significant correlations were also observed for fodder yield and number seeds per pod. Udensi et al. (2012) and Gerrano et al. (2015) reported that correlations among desirable traits are useful for identification of breeding potential of the measured traits. This shows that indirect selection using these traits (days to maturity with fodder yield, pod width, number of main branches and hundred seed weight and fodder yield with number of main branches) can be applied to improve the traits as well as grain and fodder yield in cowpea.

2.6.6 Principal component analysis

Principal component analysis (PCA) is the best tool to categorize particular traits of cowpea that revealed the most variance in the data (Manggoel and Uguru, 2011). Žáková and Benková (2006), also reported traits that had high contributions to the source of variation of genetic diversity among 106 Slovakian barley accessions. PCA is the most applicable statistical tool in investigating multivariate analysis with significant correlations (Johnson, 1998). In the present study, the PCA grouped the 11 agro-morphological traits into 11 components, which accounted for the entire (100%) variability among the studied accessions. Chatfield and Collins (1980) indicated that an eigenvalue of less than one should be removed to ensure fewer components are dealt with. In addition, eigenvalues greater than one are considered significant and component loadings greater than ±0.3 are considered to be meaningful (Hair et al., 1998). The implication of PCA enables the plant breeder to identify phenotypic traits that contribute high genetic variation among cowpea accessions and shows the potential for parental selection for crop improvement. Hence, in this study, the first principal component variation was due to the number of main branches, days to 50% flowering and days to maturity and fodder yield. In PC2, variation was contributed by number of plants per plot, weight of mature pods and grain yield. The first and the second principal components explained the most variation among the accessions, revealing a high degree of association among the traits studied. Similar results were found using phenotypic traits in cowpea (Adewale et al., 2011; Gerrano et al., 2015; Sousa et al., 2015; Molosiwa et al., 2016), in common bean (Chiorato et al., 2006; Darkwa et al., 2016), in finger millet (Ulaganathan and Nirmalakumari, 2015), and in rice (Maji and Shaibu, 2012). The principal biplot demarcated the accessions with agro-morphological traits explained by the first two principal components (Ali et al., 2011). Principal component analysis grouped accession with variables in particular distances enabling the breeder to visualize and decide on the best genotypes to be selected based on variables using two principal components (Ali et al., 2011). Smaller angles between dimension vectors in the same direction indicated high correlation of the variable traits in terms of discriminating accessions. Accessions excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull of the first two principal components. These traits with high correlations to particular genotypes should be considered in selecting the parents for breeding programmes in yield improvement of cowpea.

2.7   Conclusion

The present results suggest that there is wide genetic variability among cowpea accessions for exploitation for future improvement in cowpea breeding programme. The ANOVA showed that the optimum planting date had higher yields compared to late planting date, implying that the right time of planting to improve yield is important. Some of the traits had high heritability estimates, constituting high breeding value thus more additive genetic effects which is important for crop improvement in the studied traits. In addition, positive phenotypic correlations among each pair of quantitative traits clearly indicated the possibility that the traits shared some common genes as well as the geographical background. Principal component analysis observed showed first four principal components explaining 76.89% of total variability among accessions. Some accessions showed stability and high grain yield over two planting dates such as RV 202, RV 207 and 86D-1010. Overall, the evaluation of cowpea accessions for genetic diversity is very useful to identify potential accessions for improvement of grain fodder yield as well as related traits in cowpea breeding programme in South Africa.

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CHAPTER THREE

ASSESSMENT OF GENETIC DIVERSITY IN SELECTED COWPEA ACCESSIONS USING GRAIN PROTEIN, IRON AND ZINC CONTENTS

Abstract

Cowpeas are grown and used as food and fodder in South Africa. Currently, little information is available on the nutritional quality of cowpea accessions available at the ARC.The objective of this study was to determine the genetic diversity of grain protein, iron and zinc contents of selected cowpea accessions maintained at the ARC, Potchefstroom gene bank, South Africa. The grain samples of 45 cowpea accessions were harvested from field trials grown under two planting dates. The mineral composition of the accessions were determined using standard AOAC method. The results from first planting, second planting dates and combined data showed a wide genetic diversity in total grain protein, iron and zinc contents. The mean values of total protein content varied from 17.74% to 27.47%, 19.58% to 29.53% and 18.73% to 27.88% for first planting data, second planting date and combined data respectively. Iron content varied from 14.82 mg/kg to 140.52 mg/kg, 25.61 mg/kg to 205.82 mg/kg and 26.48 mg/kg to 140.89 mg/kg for the first planting date, second planting date and combined data, respectively. Zinc content varied from 36.43 mg/kg to 75.22 mg/kg, 32.95 mg/kg to 276.26 mg/kg and 36.83 mg/kg to 203.42 mg/kg in early planting dates, late planting dates and combined data, respectively. The correlation method revealed a positive correlated between protein and zinc contents. Results revealed values of phenotypic coefficient variation for most characters were close to the corresponding genotypic coefficient of variation values indicating little environmental effect on the expression of these characters. The highest estimates of broad sense heritability (H^2) were obtained for protein, iron and zinc. High heritability estimates along with high genetic advance for iron and zinc contents indicating an additive gene action in its inheritance. Based on Principal Component Analysis (PCA), the first two components explained over 77.48% of genetic variation. The current study showed significant diversity among the tested accessions for protein, iron and zinc contents indicating their potential for future cowpea breeding programmes for nutritional quality improvement in South Africa.

Keywords: Cowpea, genetic variation, heritability, principal component analysis.

3.1 Introduction

Cowpea has an advantage of being drought tolerant and many subsistence farmers in different regions of Africa cultivate it as a major source of nutrition as well as a dual purpose viz. used for grain and vegetable (Dube and Fanadzo, 2013). Cowpea contains 16.35 to 27.27% protein, 90.53 to 91.30 ppm sodium, 30.33 to 71.30 ppm potassium, 146.73 to 968.20 ppm magnesium, and 43.33 to 171.20 ppm calcium (Asante et al., 2006). The utilisation of vegetable protein in the developing countries of the world is currently increasing due to affordability compared to animal protein. Animal protein is unaffordable by poor families in the developing and undeveloped countries. As reported by FAO (1997), protein solves the problem of nutrition such as protein deficiency in the developing countries. In addition to being a major source of protein, vitamins, and minerals, cowpea fodder is used for livestock feed as a source of nutrients (Chinma et al., 2008). Cowpea has been reported to be a major nutritious source of macronutrients and micronutrients (Asante et al., 2006; Singh, 2006a; Boukar et al., 2011; Santos and Boiteux, 2015). Timko and Singh (2008) reported that cowpea has fairly high levels of micronutrients than other crops. Moreover, it has been reported that there is vast genetic diversity in seed mineral content in cowpea accessions for potential in breeding for improved better quality seed grain (Nagalakshmi et al., 2010; Animasaun et al., 2015; Santos and Boiteux, 2015).

Genotypes with wider genetic variation may allow exploitation for crop improvement due to excess of genepool. Furthermore, knowledge of the genetic variation within the same species is used as a guiding map for selection in any breeding programme (Adewale et al., 2011). Reports by FAO (1997) and Timko and Singh (2008) indicated a huge variability in protein, zinc and iron contents in cowpea grains. Availability of genetic and nutritional diversity information will contribute to improved cowpea breeding in South Africa. Also, knowledge of nutritional diversity existing within and among cowpea accessions, will broaden the gene pool for parent selection and development of more improved cultivars with good yield as well as better nutritional values and germplasms resources. Hence, the objective of the present study was to assess the level of genetic variability with respect to protein, zinc and iron content among selected cowpea accessions.

3.2   Materials and methods

3.2.1   Genotypes, experimental site and design

The grain samples of 45 selected cowpea accessions were planted during 2015/16 summer cropping season under two planting dates at the research farm of the ARC-GCI, which is located at Potchefstroom (26°74''S; 27°8'E; altitude of 1344 m above sea level). Potchefstroom has an average minimum and maximum temperature of 9.61°C and 25.48°C, respectively and an average annual total rainfall of 618.88 mm. When necessary, the trial fields received supplementary irrigation. Weed control was done using a pre-emergence herbicide, dual gold to control annual grasses and broadleaf weeds, and first hand weeding was done two weeks after emergence, followed by a second weeding four weeks after emergence. Trials were also sprayed with a broad spectrum insecticide seizer EC 100 every two weeks after flowering to control pollen and pod insects. Pods were harvested manually after they reached physiological maturity.

The 45 accessions were planted in a 9 x 5 alpha lattice design with three replications. Each accession was planted in two rows of 5 m long plot, using a 0.20 m within row spacing and 0.75 m between rows spacing.

3.2.2   Proximate composition of cowpea grain

The proximate analysis was done according to the standard procedures of the Association of Official Analytical Chemists (AOAC, 2000) at Döhne Analytical Services, South Africa. The cowpea grain protein content was estimated using the Kjeldahl method described in AOAC (2000). This method involves protein digestion, distillation and determination of % nitrogen content of the distillate by titration and then multiplying the % nitrogen by a factor of 6.25 to obtain the corresponding protein content in %.

3.2.3   Determination of micronutrients in cowpea grain

Micronutrients elements iron and zinc were determined as described in AOAC (2000). A wet digestion procedure was followed by digesting 5 g of sample with 5 ml of nitric acid (HNO_3) and 3 ml of perchloric acid ($HClO_4$) on an aluminum digestion block. Minerals in the digest

were measured by atomic absorption spectrometry (Analytikjena NovAA400) where micro minerals iron (Fe) and zinc (Zn) are analyzed undiluted.

3.2.4   Data analysis

Data for all the variables measured were subjected to analysis of variance (ANOVA), to estimate the nutritional quality level of variability among the cowpea accessions using GenStat 17th edition software (Payne, 2014). Principal component analysis (PCA) based on the correlation matrix was performed using SPSS to identify important quality traits for selection. PCA biplot were plotted to show relationships between variables and studied accessions using GenStat 17th edition.

3.2.5   Correlation coefficient analysis

The Pearson's correlation coefficient, r value was estimated using GenStat 17th edition. In this experiment, the genetic correlation was not calculated as the phenotypic correlation was fairly equivalent to the genotypic correlation (Kearsey and Farquhar, 1998).

3.2.6   Estimation of genetic parameters

Genetic parameters were estimated for nutritional quality traits on cowpea accessions as follows:

3.2.6.1   Phenotypic and genotypic variability

The phenotypic variation for each trait was partitioned into genetic and non-genetic factors and estimated according to Johnson et al. (1955) and Uguru (2005). Variability present in the population was estimated by simple measures, namely; mean, range, standard error, phenotypic and genotypic variances and coefficient of variation. Phenotypic and genotypic variances and coefficient of variation were calculated according to the method suggested by (Singh and Chaudhary, 1985) as follow;

- o   Genotypic variance = $\sigma^2 g$ = $\dfrac{MSt - \sigma^2 e}{r}$

Where, MST = Mean square treatment

σ^2e = environmental variance (error mean square) from ANOVA

r = number of replications;

o   Phenotypic variance = σ^2p = σ^2g + σ^2e

o   Phenotypic coefficient of variation (PCV) = $\dfrac{\sqrt{\sigma^2 p}}{\bar{x}}$ x 100

Where x̄= population mean

o   Genotypic coefficient of variation (GCV) = $\dfrac{\sqrt{\sigma^2 g}}{\bar{x}}$ x 100

3.2.6.2 Heritability

Broad-sense heritability (H^2) for each variate was calculated based on the formula suggested by (Hanson et al., 1956) as follows:

$H^2 = \dfrac{\sigma^2 g}{\sigma^2 p}$ x 100

Where, H^2 = heritability in the broad sense

σ^2g = genotypic variance

σ^2p = phenotypic variance

The heritability estimates were categorised as suggested by Robinson et al. (1949) (0-30% = low; 31-60% = moderate; above 60% = high).

The expected Genetic Advance for each trait was calculated as

$$GA=K\sqrt{V_P}H^2$$

Where, K = 1.40 at 20% selection intensity for trait; V_p = Phenotypic variance for trait; H^2 = Broad sense heritability of the trait; Genetic advance as percentage of mean is calculated as,

$$GA\ (\%\ of\ Mean) = \dfrac{GA}{\pi}x\ 100$$

81

Where, GA=Genetic advance; π =Mean

3.3 Results

3.3.1 Analysis of variance for protein, iron and zinc contents under first planting

The ANOVA revealed that there were highly significant (P≤ 0.001) differences (Table 3.1) among the accessions grown in early planting date, suggesting that there was a high degree of genetic diversity among them for all measured parameters. Diversity was observed for the concentration of the protein, iron and zinc content in seeds of 45 genotypes cowpea. Wide variation was found in protein concentration and ranged from 17.74 to 27.47% with a mean of 24%. Among all accessions, the highest value was obtained in accession TVU 13778 (27.47%), while the lowest was found in RV 343 (17.74%). Protein concentration was low in the accessions from the early planting dates. Other accessions with high protein content were; RV 207, 90K-284-2, TVU 9443, 86D-1010,ITOOK-1263, 99K-494-6, TVU 9620, TVU 12746, RV 213, 98K-503-1, TVU 9596 and CH47. The iron concentration in grain seed varied from 14.82 to 140.52 mg/kg with mean 81.65 mg/kg. Among all accessions, the highest was obtained in accession 86D-1010 (140.52 mg/kg) and lowest in TVU 3416 (14.82 mg/kg). Iron concentration was much higher in accessions from the first planting date. Other accessions with higher iron concentration compared to the check were; 98K-476-8, RV 500, 95K-589-2, RV 343, RV 204 and CH47. The zinc concentration in grain seed varied from 36.43 to 75.22 mg/kg with a mean 47.82 mg/kg. The accession which had the highest zinc concentration was TVU 12637 (75.22 mg/kg) and lowest was RV 574 (36.43 mg/kg). Zinc concentration was much lower in accessions from the first planting date. Other accessions with higher zinc concentration than the check were; TVU 12637, RV 500, ITOOK-1263, RV 551, RV 342, CH47, 86D-1010, TVU 9620, 90K-284-2, RV 343 and RV 213.

Table 3.1: Mean protein (%), iron (mg/kg) and zinc (mg/kg) contents in grains of 45 cowpea accessions grown under first planting, at Potchefstroom, 2015/16

Entry	Genotypes	Grain nutrient content		
		Crude protein (%)	Iron (mg/kg)	Zinc (mg/kg)
1	RV 194	22.76	109.17	47.96
2	RV 202	19.72	70.25	46.83
3	RV 204	24.30	118.17	44.62
4	RV 207	27.12	92.80	48.17
5	RV 213	26.16	75.54	49.69
6	RV 321	24.94	85.22	46.27
7	RV 342	24.06	75.72	51.08
8	RV 343	17.74	120.23	50.31
9	RV 344	25.65	97.04	47.81
10	RV 351	23.13	75.74	46.5
11	RV 500	18.69	129.10	61.76
13	RV 503	25.51	50.14	45.41
14	RV 553	24.75	82.75	44.26
15	RV 554	22.08	71.61	45.33
16	RV 555	25.31	75.67	45.32
17	RV 558	24.49	86.48	45.87
18	RV 568	22.74	57.94	42.9
19	CH47	26.01	118.13	50.88
20	Bechuana white	25.91	115.11	49.63
21	Dr Saunders	21.14	58.02	45.13
22	Glenda	23.48	71.09	42.92
23	TVU 9443	26.90	95.16	48.03
24	TVU 9620	26.43	90.36	50.46
25	95K-589-2	25.77	125.50	48.41
26	86D-1010	26.79	140.52	50.54
27	83S-911	19.32	76.68	48.59
28	TVU 12637	23.75	83.42	75.22
29	TVU 13998	22.30	74.55	46.46
30	90K-284-2	27.03	87.20	50.46
31	ITOOK-1263	26.53	106.79	52.67
32	TVU 13778	27.47	84.22	45.97
33	99K-494-6	26.44	87.53	49.52
34	TVU 12746	26.25	88.20	48.16
35	98K-476-8	24.72	133.57	43.27
36	TVU 2095	22.17	17.24	41.41
37	TVU 9596	26.06	79.43	48.19
38	98D-1399	20.73	42.03	43.96

Entry	Genotypes	Grain nutrient content		
		Crude protein (%)	Iron (mg/kg)	Zinc (mg/kg)
39	TVU 14190	25.25	17.45	48.95
40	TVU 13004	22.16	82.91	42.9
41	98K-503-1	26.11	53.31	46.03
42	TVU 11986	25.90	75.49	41.89
43	97K-499-35	17.86	75.92	45.87
44	TVU 3416	24.83	14.82	46.17
45	PAN 311	24.21	24.57	49.09
	Mean squares	13.49**	1787.41**	63.90**
	LSD	3.53	14.32	5.39
	Mean	24.00	82.50	47.82
	Range	17.74-27.47	14.82-140.52	36.43-75.22
	SE±	2.87	30.15	5.93
	CV (%)	7.30	8.60	5.60

3.3.2   Analysis of variance of protein, iron and zinc contents under second planting

The ANOVA revealed highly significant (P≤ 0.001) differences (Table 3.2) among the accessions studied, suggesting that there was a high degree of genetic diversity among them for all measured parameters. Wide genetic variation was found in protein concentration ranging from 19.58 to 29.53% with a mean value of 25.95%. Among all accessions, the highest total protein content value was obtained in accession 98K-476-8 (29.53%); while the lowest was found in 97K-499-35 (19.58%). Protein concentration was much higher in the accessions from the second planting date. Cowpea accessions with high protein content above the check were RV 568, Dr Saunders, RV 503, TVU 2095, TVU 13932, RV 321, 95K-589-2, TVU 3416, RV 213, RV 500, TVU 9443, 98K-503-1, RV 558, RV 202, RV 204, TVU 14190 and RV 351.

The iron concentration in grain varied from 25.61 to 205.82 mg/kg with mean of 78.75 mg/kg. Among all accessions, the highest value was obtained in accession TVU 13998 (205.82 mg/kg) and lowest was found in TVU 3416 (25.61 mg/kg). The iron concentration was lower for accessions in the second planting date. Cowpea accessions which exhibited highest iron content were 86D-1010, 98K-476-8, 95K-589-2, TVU 13932, RV 342, RV 343, TVU 2095, RV 204 and CH47.

The zinc concentration in grain samples varied from 32.97 to 276.26 mg/kg with a mean 88.19 mg/kg. Accessions which had highest zinc concentration were 98K-503-1 (276.26 mg/kg), followed by 90K-284-2, TVU 14190, TVU 13932, CH47 and Glenda, and the lowest was found in accession RV 202 (32.97mg/kg).

Table 3.2: Mean protein (%), iron (mg/kg) and zinc (mg/kg) contents in grains of 45 cowpea accessions grown under second planting, at Potchefstroom, 2015/16

| Entry | Genotypes | Grain nutrients content | | |
		Crude Protein (%)	Iron (mg/kg)	Zinc (mg/kg)
1	RV 194	23.99	94.67	41.64
2	RV 202	26.99	48.06	32.97
3	RV 204	26.97	113.54	39.67
4	RV 207	24.87	70.13	38.92
5	RV 213	27.42	51.01	50.69
6	RV 321	27.69	47.56	76.81
7	RV 342	22.98	115.26	47.61
8	RV 343	24.51	114.81	43.77
9	RV 344	25.25	83.29	46.22
10	RV 351	26.33	82.58	81.78
11	RV 500	27.41	94.81	47.39
12	RV 503	28.45	38.1	40.07
13	RV 553	25.67	53.83	42.16
14	RV 554	25.33	45.23	40.31
15	RV 555	25.18	68.26	47.16
16	RV 558	27.20	57.69	122.61
17	RV 568	29.09	38.24	126.43
18	CH47	25.81	101.45	180.06
19	Bechuana white	26.00	100.82	69.83
20	Dr Saunders	29.04	25.61	42.28
21	Glenda	25.25	29.03	175.29
22	TVU 9443	27.36	47.37	49.33
23	TVU 9620	24.10	82.57	115.32
24	95K-589-2	27.65	117.97	44.07
25	86D-1010	25.99	136.93	44.03
26	83S-911	25.47	58.02	39.63
27	TVU 12637	22.30	63.95	95.39
28	TVU 13998	24.63	205.82	136.11
29	90K-284-2	24.67	53.44	250.81
30	ITOOK-1263	25.84	87.92	41.5

Entry	Genotypes	Grain nutrients content		
		Crude Protein (%)	Iron (mg/kg)	Zinc (mg/kg)
31	TVU 13778	24.50	66.16	36.92
32	99K-494-6	26.04	76.35	125.83
33	TVU 12746	25.67	64.21	90.1
34	98K-476-8	29.53	131.91	134.72
35	TVU 2095	28.36	114.06	48.49
36	TVU 9596	25.43	75.21	44.5
37	98D-1399	25.29	66.8	74.51
38	TVU 14190	26.38	65.77	237.02
39	TVU 13004	22.95	66.65	43.2
40	98K-503-1	27.36	82.87	276.26
41	TVU 11986	25.83	68.2	141.92
42	97K-499-35	19.58	86.58	45.79
43	TVU 3416	27.64	37.89	70.31
44	TVU 13932	27.88	115.46	203.42
45	PAN 311	26.08	97.62	145.51
	Mean squares	7.01*	2414.07**	7928.60**
	LSD	3.82	13.44	7.78
	Mean	25.95	78.75	88.19
	Range	19.58- 29.53	25.61-205.82	32.97-276.26
	SE±	2.32	34.87	62.68
	CV (%)	7.30	8.50	4.40

3.3.3 Analysis of variance of protein, iron and zinc contents for combined data

The combined ANOVA revealed highly significant (P≤ 0.001) differences (Table 3.3) among the accessions grown across the two planting dates, suggesting that there was a high degree of genetic diversity among them for all measured parameters. Wide variation was found in protein content ranging from 18.73 to 27.88% with a mean of 25.95%. Among all accessions, the highest value was obtained in accession TVU 13932 (27.88%), while the lowest was found in 97K-499-35 (18.73%). Other accessions with high protein content above the check were 98K-476-8, TVU 9443, RV 503, RV 213, 98K-503-1, 95K-589-2, 86D-1010, RV 321, TVU 3416, 99K-494-6, ITOOK-1263, RV 207 and TVU 13778.

The iron concentration in the grain varied from 26.48 to 140.89 mg/kg with mean value of 80.56 mg/kg. Among all accessions, the highest was obtained in accession TVU 13998 (140.89 mg/kg) and lowest in TVU 3416 (26.48 mg/kg). Others with highest iron concentration

were 86D-1010, 98K-476-8, 95K-589-2, RV 343, RV 204, TVU 13932, RV 500 and CH47. The zinc concentration in grain varied from 39.83 to 203.42 mg/kg with a mean 70.01 mg/kg. Accession which had highest zinc concentration was TVU 13932 (203.42mg/kg) and lowest was found in RV 202 (39.83 mg/kg). Other accessions with higher zinc content were 98K-503-1, 90K-284-2, TVU 14190, CH47 and Glenda.

Table 3.3: Mean of protein (%), iron (mg/kg) and zinc (mg/kg) contents for combined data

Entry	Genotypes	Grain nutrients content		
		Crude Protein (%)	Iron (mg/kg)	Zinc (mg/kg)
1	RV 194	23.39	101.84	44.77
2	RV 202	23.40	59.04	39.83
3	RV 204	25.65	115.83	42.12
4	RV 207	25.98	81.34	43.49
5	RV 213	26.80	63.15	50.20
6	RV 321	26.33	66.19	61.70
7	RV 342	23.51	95.7	49.32
8	RV 343	21.16	117.49	47.01
9	RV 344	25.44	90.09	47.01
10	RV 351	24.75	79.2	64.33
11	RV 500	23.10	111.77	54.50
12	RV 503	27.00	44.06	42.71
13	RV 553	25.21	68.13	43.20
14	RV 554	23.72	58.27	42.80
15	RV 555	25.24	71.92	46.25
16	RV 558	25.86	71.93	84.65
17	RV 568	25.95	47.99	85.11
18	CH47	25.91	109.7	116.17
19	Bechuana white	25.96	107.89	59.84
20	Dr Saunders	25.14	41.64	43.69
21	Glenda	24.37	49.84	109.82
22	TVU 9443	27.13	71.01	48.69
23	TVU 9620	25.25	86.43	83.24
24	95K-589-2	26.72	121.69	46.22
25	86D-1010	26.39	138.71	47.25
26	83S-911	22.42	67.25	44.07
27	TVU 12637	23.02	73.58	85.41
28	TVU 13998	23.48	140.89	91.77
29	90K-284-2	25.84	70.14	151.71
30	ITOOK-1263	26.18	97.25	47.03

Entry	Genotypes	Grain nutrients content		
		Crude Protein (%)	Iron (mg/kg)	Zinc (mg/kg)
31	TVU 13778	25.97	75.09	41.40
32	99K-494-6	26.24	81.88	88.08
33	TVU 12746	25.95	76.08	69.35
34	98K-476-8	27.15	132.73	89.49
35	TVU 2095	25.30	66.17	44.99
36	TVU 9596	25.74	77.30	46.33
37	98D-1399	23.03	54.55	59.40
38	TVU 14190	25.82	41.87	143.99
39	TVU 13004	22.56	74.69	43.05
40	98K-503-1	26.74	68.25	162.38
41	TVU 11986	25.87	71.81	92.44
42	97K-499-35	18.73	81.31	45.83
43	TVU 3416	26.25	26.48	58.37
44	TVU 13932	27.88	115.46	203.42
45	PAN 311	25.15	61.49	97.82
	Mean squares	8.45**	1279.18**	3821.65**
	LSD	0.54	2.00	0.97
	Mean	25.08	80.56	70.01
	Range	18.73-27.88	26.48-140.89	39.83-203.42
	SE±	2.77	36.6	49.07
	CV (%)	7.38	8.53	4.86

3.3.4 Relationships of protein, iron and zinc in cowpea seeds

Table 3.4 shows correlations between protein, iron and zinc contents under combined planting dates. The study showed positive and significant correlations among crude protein and zinc, but iron was not correlated with zinc (Table 3.4). Crude protein was also not correlated with iron.

Table 3.4: Simple correlation coefficients among protein, iron and zinc content in cowpea grains accession grown across early planting dates and late planting dates Potchefstroom, 2015/2016

Traits	Crude Protein%	Fe (mg/kg)	Zn (mg/kg)
Crude Protein%	-		
Fe (mg/kg)	-0.05	-	
Zn (mg/kg)	0.21**	-0.00	-

** Level of significance at 0.05 and 0.001, respectively

The phenotypic coefficient of variation (PVC) and genotypic coefficient of variation (GCV) were highest for iron (44.91 and 44.10) and for zinc (90.48 and 90.35%, respectively) (Table 3.5).

Low PVCs and GVCs for crude protein were obtained (12.76 and 10.40%, respectively) and are presented in Table 3.5.

Table 3.5: Estimates of components of variance, PCV and GCV of protein, iron and zinc contents among the cowpea accessions grown across early planting and late planting dates, Potchefstroom, 2015/2016

Traits	Estimates of Component of Variance					PCV (%)	GCV (%)
	Grand mean	MS_g	MS_e	σ^2_g	$\sigma^2 p$		
Crude protein	24.97	8.45	3.40	6.75	10.14	12.76	10.40
Iron	80.36	1279.18	47.03	1255.67	1302.70	44.91	44.10
Zinc	68.37	3821.65	11.02	3816.14	3827.16	90.48	90.35

According to Robinson et al. (1949), broad sense heritability (%) can be classified as follows: >60% = high, moderate = 30-60%, and low = 0-30%. In the present study, heritability estimates ranged from moderate to high.

For combined data over planting dates, broad sense heritability estimates ranged from 66.52 to 99.71% (Table 3.6). High broad sense heritablity were observed for zinc,iron and crude protein contents.

Table 3.6: Estimates of heritability (broad sense), GA and GA as per cent of the mean of protein, iron and zinc of 45 cowpea accessions grown across first planting dates and second planting dates at Potchefstroom, 2015/2016

Traits	Heritability estimate (%)	Genetic advance	Genetic advance (% of mean)
Crude protein	66.52	4.36	17.48
Iron	96.39	71.67	89.18
Zinc	99.71	127.07	185.86

In the present study, the expected genetic advance values for the three traits for combined data across different planting dates indicated a wide range. The genetic advance ranged from 4.36 to 127.07, with zinc having the highest genetic advance (Table 3.6). Low genetic advance was obtained for crude protein.

3.3.5 Principal component analysis for combined data

The first two principal components explained 77.48% of the total variation among the cowpea accessions at first planting dates (Table 3.7). PC1 had an eigenvalue of 1.32 and accounted for 44.06% of total variation. This component was associated with protein and zinc contents which were the main contributers to the variability in PC1. PC2 had an eigenvalue of 1.00, contributing 33.42% of genetic variation, with iron contributing mainly to the variability.

Table 3.7: Principal component analysis for total protein, iron and zinc content in cowpea grain revealing eigenvalue, total variance, and eigenvector and contribution to total variation explained by the first two PC axes for combined data

Trait	Eigenvectors		
	PC1	PC2	PC3
Protein	0.71	-0.07	-0.70
Iron	0.01	1.00	-0.09
Zinc	0.71	0.06	0.70
Eigenvalue	1.32	1.00	0.68
Individual (%)	44.06	33.42	22.52
Cumulative %	44.06	77.48	100.00

3.3.5.1   Principal component biplot for combined data

The relationships between the different variables and accessions with respective principal components are further illustrated on the first two principal component biplots (Figure 3.2) for combined data. Smaller angles between dimension vectors in the same direction indicated high correlation of the variable traits in terms of discriminating accessions. Accessions excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull of the first two principal component, with genotypes such as TVU 13932, 98K-503-1, 98K-589-2 and 86D-1010 contain high concentrations of protein contents. Genotypes contain high concentration of iron and zinc. High iron content were found TVU 13998, 98K-476-8, RV 343, 95K-589-2 and 86D-1010 while high zinc content were found TVU 13932, 98K-503-1, 90K-284-2 and TVU 14190 for combined data.

Figure 3.2: Principal component score plot of PC1 and PC2 describing the overall varition among cowpea accessions using grain quality traits for combined data

3.4 Discussion

3.4.1 Genetic variation

Analysis of variance revealed highly significant (P≤0.001) differences for protein, iron and zinc contents in grain seeds among the 45 cowpea accessions studied in first planting date, second planting date and across the environments indicating a high level of genetic diversity. This high genetic variation gives an opportunity for improving the traits and provides important genetic information for parental selection for improvement of nutritional quality in cowpea. According to Bidinger (1991), presence of wide genetic variability allows for effective selection for traits of interest in breeding programmes. A highly significant interaction between different planting dates and genotypes was observed for protein, iron and zinc, which indicated that genotypes reacted differently to different planting dates. This might probably be due to the seasonal variation of the environmental conditions as well as the differences in the mineral mining and use abilities of the genotypes.

Protein contents ranged from 18.73 to 27.88% with overall mean 25.08%. Cowpea accessions TVU 13778, RV 207, ITOOK-1263, 99K-494-6,TVU 3416, RV 321, 86D-1010, 95K-589-2, 98K-503-1, RV 213, RV 503, TVU 9443, 98K-476-8 and TVU 13932 had the highest protein content within a range of 25.95 to 27.88%. These values were relatively higher than what was reported by Odedeji and Oyeleke (2011), and Animasaun et al.(2015) in cowpea, but lower than the values reported by Santos and Boiteux (2013). Protein concentration was lower in the first planting date compared to the second planting date. This could probably be due to variation genotypes, environmental factors and climatic conditions (Ali-Khan and Youngs, 1973). Other findings by Singh et al. (2003), Nielsen et al. (1993) and Giami (2005), also reported the existence of the variation in crude protein content for cowpea grain planted in different seasons. Previous work by Tabe et al. (2002), demonstrated that variation in protein content can be influenced by genetic difference as well as environmental factors in the soil and climatic conditions and the ability of the individual genotypes to develop a symbiotic relationship with the nitrogen-fixing bacteria in the root nodules.

In the present study, iron content ranged from 26.48 to140.89 mg/kg with a mean value of 80.56 mg/kg. Cowpea accession TVU 13998 (140.89mg/kg) had the highest iron concentrations while the lowest was reported in TVU 3416 (26.48 mg/kg). These values were

higher than the values reported by Mamiro et al. (2011), and Umar (2014) on cowpea grain but lower than values reported by Okonya and Maass (2014) and Gerrano et al. (2015) in cowpea leaves. However, iron concentration was higher in the early planting dates compared to late planting dates. In the present study zinc content ranged from 39.83-203.42 mg/kg with a mean of 70.01 mg/kg. Cowpea accession TVU13932 (203.42 mg/kg) had the highest concentration, while the lowest concentrations was in RV 202 (39.83 mg/kg). These values were higher than the values reported by Mamiro et al. (2011) and Umar (2014) in cowpea grain seed, but lower than values reported by Gerrano et al. (2015) in cowpea leaves. The variation in iron content and zinc content might be due to variation in mineral mining, genotype characteristics, environmental conditions and climatic conditions (Gerrano et al., 2015). Mamiro et al. (2011) also reported a wide range of variation in mineral concentrations such as iron, zinc and calcium and protein content in cowpea grain. Gregorio (2002) reported that interaction effects of environmental conditions (soil nutrients and soil types) and genotypes contributed to variation in iron and zinc contents in seeds.

There was genetic diversity or variation in the nutritional content among the tested genotypes in cowpea accessions. These findings are in agreement with those reported by Boukar et al. (2011) and Umar (2014). These large variations in protein, iron and zinc concentration observed in this study are similar to those reported in other different crops, for example, in common beans by Mahajan et al. (2015),and in sorghum by Badigannavar et al. (2016). The wide variation in protein, iron and zinc content among the cowpea genotypes is an indication that there is potential for improvement of nutritional quality in development of new cultivars (Timko and Singh, 2008).

3.4.2 Relationships of protein, iron and zinc in cowpea seeds

There was significant correlation among nutritional quality traits. Iron showed a positive correlation with zinc in early planting dates. These results are in agreement with previous findings by Singh (2006b) and Tryphone and Nchimbi-Msolla (2010) in common beans, Morgounov et al. (2007) in wheat, and Ng'uni et al. (2012) in sorghum, while other findings (Umar, 2014) contrast these. Crude protein showed a positive correlation with zinc content. This confirms the findings reported by Badigannavar et al. (2016) and Ng'uni et al. (2012) in

sorghum. A significant correlation among protein and zinc concentration implies it is possible to improve the two traits simultaneously in cowpea accessions.

3.4.3 Phenotypic and genotypic coefficients of variation

The estimates of phenotypic coefficients of variation (PCV) and genotypic coefficient of variation (GCV) showed genetic variability in particular traits. PCV values for most of the traits were close to the corresponding genotypic coefficient of variation (GCV) indicating little environmental effects on the expression of these characters. This implies the influence of additive gene effects indicating that the traits could be improved through selection. Kaushik et al. (2007), indicated that a higher GCV: PCV suggested that the trait was under the influence of genetic rather than environmental influence. In the present study, a high PCV was observed for iron and zinc content. Similar findings were reported by Gerrano et al. (2015) for zinc content and iron content in cowpea. A high PCV and low GCV indicates much environmental effects resulting in low heritability for these traits. However, the relatively close estimates of PCV and GCV in iron and zinc contents indicate less environmental influence. Gerrano et al. (2015) reported similar results of relatively closer estimates of PCV and GCV in iron and zinc in cowpea. The low environmental effects for this trait results in high heritability. The possibility of improving these traits are thus better through selection.

3.4.4 Estimation of broad-sense heritability and genetic advance

Heritability estimates are important genetic parameters that play a significant role in selection of different cowpea genotypes from a population (Rashwan, 2010). As reported by Manggoel et al. (2012) and Rashwan (2010), high broad-sense heritability values usually show the predominance of additive gene action in the expression of the traits. The present study revealed high heritability for crude protein, iron and zinc. High heritability values for protein, iron and zinc contents in cowpea were reported by other researchers (Gerrano et al., 2015), in common beans (Silva et al., 2010), in sorghum (Badigannavar et al., 2016), in rice (Samak et al., 2011; Sala et al., 2015), and pearl millet (Govindaraj et al., 2011). When heritability values are high (from 71% -96% or more), phenotypic selection for traits would be easier. This is because there could be a high correlation between the genotype and phenotype and little environmental contribution to the phenotype. For traits with low heritability less than 45% it is

difficult to make selections due to a higher effect of the environment. Moreover, higher heritability shows that additive gene effects are more important and results in more gain to selection in the next generation.

3.4.5 Genetic advance

Genetic advance under selection (GA) is important to consider in improvement of characters as it shows the genotypic value for the new population compared with the base population under one cycle of selection at a specified selection intensity (Gashaw et al., 2010). The maximum genetic advance as percentage of mean was observed for zinc and iron for the late planting dates and combined data, respectively and zinc content in early planting dates. Similar results of high heritability estimates and genetic advance for iron and zinc in cowpea have been reported by Gerrano et al. (2015) High heritability estimates and genetic advance for iron and zinc content were also reported in rice (Samak et al., 2011; Sala et al., 2015), and pearl millet by Govindaraj et al. (2011). Furthermore, heritability estimates play an important role in selection based on phenotypic performance. According to Johnson et al. (1955), relatively high heritability estimates accompanied with high genetic advance are usually more useful in predicting gain under selection compared to heritability estimates alone. Normally, medium and low heritability coupled with low genetic advance is an indication of high environmental effects on the traits during the growth period. Although, the breeder can improve the traits, the rate of improvement will be slow.

3.4.6 Principal component analysis

In the present study, only the first two eigenvectors which had eigenvalues greater than one and cumulatively explained about 77.48% were considered. Protein, iron and zinc traits were important in contributing to variation among accessions. The PC1 and PC2 explained most of the variation among the cowpea accessions. Similarly, findings reported by Gerrano et al.(2015) observed genetic variation among 25 cowpea accessions for nutritional quality using multivariate analysis. Principal component analysis groups accessions with variables in particular distances enabling a breeder to visualize and decide on the best genotypes to be selected based on variables using two principal components. Smaller angles between dimension vectors in the same direction indicate high correlation of the variable traits in terms

of discriminating accessions. Accessions excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull of the first two principal component. In the present study, the following top three accessions were discriminated; TVU 13778, RV 207, RV 213 with high protein RV 568, Dr Saunders, 98K-476-8 in early planting dates, high protein in late planting dates and TVU 13932,98K-503-1,98K-589-2 high protein in combined data. High iron 86D-1010,98K-476-8,RV 500 in early planting dates, high iron TVU 13998, 86D-1010 AND 98K-476-8 in late planting dates and TVU 13998,98K-476-8, RV 343 high iron in combined data. Khodadadi et al. (2011) reported that the principal component analysis is the most efficient tool for selecting the best genotypes for future breeding programmes.

3.5   Conclusion

Knowledge of the genetic diversity of cowpea accessions is essential for selection. Availability of genetic diversity and heritability estimates for nutritional quality would be useful to the plant breeder to estimate genetic advance and predict percentage genetic advance among the accessions under study. The traits had high to very high broad-sense heritability values and genetic advance, which indicates that the evaluated traits were under additive gene action. High crude protein content recorded at both environmental were in the TVU 13932,98K-476-8, TVU 9443, RV 503, RV 213, 98K-503-1, 95K-589-2, 86D-1010, RV 321, TVU 3416, 99K-494-6, ITOOK-1263, RV 207 and TVU 13778. The candidate accessions with highest levels of iron were TVU 13998,86D-1010, 98K-476-8, 95K-589-2, RV 343, RV 204, TVU 13932, RV 500 and CH47. The candidate accessions with highest levels of zinc content were TVU 13932 (203.42mg/kg), 98K-503-1, 90K-284-2, TVU 14190, CH47 and Glenda. Hence, these accessions can be recommended for further quality improvement in cowpea breeding or direct production. The existence of a wide range of genetic diversity for protein, iron and zinc content in selected cowpea accessions indicates their potential for future cowpea breeding programmes in South Africa.

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CHAPTER FOUR

ASSESSMENT OF GENETIC DIVERSITY IN COWPEA ACCESSIONS USING SINGLE NUCLEOTIDE POLYMORPHISM MARKERS (SNPS) ANALYSIS

Abstract

The knowledge of genetic diversity among cowpea accessions is important for genetic improvement. This study was conducted to estimate the genetic diversity of 47 cowpea accessions collected from two environmental conditions and maintained in the Agricultural Research Council – Grain Crops Institute, Potchefstroom, South Africa. The accessions were evaluated for genetic diversity using infinitum micro array containing 60000 single nucleotide polymorphisms (SNPs) markers. The polymorphic information content (PIC) ranging from 0.02 to 0.38 with a mean 0.27 was observed, showing the relatively high discriminating ability of the markers used. The average gene diversity among accessions ranged from 0.02 to 0.50 with a mean of 0.34. Genetic distance ranged from 0.47 to 0.86 with a mean of 0.61. Accessions 97K-499-35 and RV 342 were the most distant cowpea accessions separated from the rest of the accessions. The lowest genetic distance was found between TVU 14190 and RV 213 accessions and it was 0.45. The within and among individuals differentiation accounted for 23% and 75% of the total variation, respectively. The 47 cowpea accessions were clustered into three main groups, with some of the accessions clustered together based on their geographical origin. The observed low genetic differentiation between geographic origins implied the existence of large levels of gene flow among South African accessions and introductions (IITA-Nigeria). This study provides information on the existence of genetic variation among the cowpea accessions which can be exploited by plant breeders to utilize the unique alleles/genes and facilitate introgression for particular crossing strategies in future cowpea breeding programmes to achieve genetic gain for improvement and developing superior cultivars in South Africa.

Keywords: Single nucleotide polymorphism markers, genetic diversity, cowpea

4.1   Introduction

Cowpea is believed to have originated in Africa within the west African region which has been identified as its major centre of diversity (Ehlers and Hall, 1997). While India is indicated as the secondary centre of diversity of the cultivated *Vigna species*, south-eastern Africa is regarded as the centre of diversity of the wild *Vigna species* (Padulosi and Ng, 1997). Kouam et al. (2012), reported on the presence of high levels of genetic variability within the domesticated species and their wild relatives. High degree of genetic diversity in the germplasm is important in crop conservation and for successful crop improvement programmes (Varshney et al., 2007).

Knowledge of the genetic diversity of cowpea is the key for cowpea improvement programmes (Varshney et al., 2007;Doumbia, 2012; Egbadzor et al., 2014). Cowpea germplasm which have high genetic diversity will facilitate the development of new cultivars (Doumbia, 2012).

Molecular markers are tools used for genetic diversity analysis in a wide range of crops (Ba et al., 2004). Moreover, molecular markers provide a means to determine the degree of genetic relatedness and the level of gene flow among cowpea genotypes (Nkongolo, 2003). In addition, molecular markers are genetic markers based on individual nucleotide sequence variation, which are the direct selection of genetic polymorphisms at the DNA level (Tan et al., 2012). DNA markers have peculiar features such as polymorphism, high reproducibility, and simplicity which makes them an attractive technique for the analysis of genetic diversity in plant species with narrow genetic variation (Tan et al., 2012). Different types of molecular markers have been used in assessing genetic diversity of cowpea, for example, Amplified Fragment Length Polymorphism (AFLP) markers (Coulibaly et al., 2002; Tosti and Negri, 2002; Fang et al., 2007), Random Amplified Polymorphic DNA (RAPDs) (Mignouna et al., 1998; Nkongolo, 2003; Ba et al., 2004), Restriction Fragment Length Polymorphism (RFLP) markers (Fatokun et al., 1993), Simple Sequence Repeat (SSR) markers (Kuruma et al., 2008; Asare et al., 2010; Ali et al., 2015; Desalegne et al., 2016), and Single Nucleotide Polymorphism (SNP) markers (Egbadzor et al., 2014). These molecular markers have been used successfully to assess and estimate the genetic diversity in cowpea accessions. However, there is little information on SNP markers in cowpea. SNPs are the best and more efficient markers for estimation of genetic diversity (Varshney et al., 2007; Deulvot et al., 2010). Therefore, the objective of this study was to assess the genetic diversity of cowpea

accessions collected from South Africa and International Institute of Tropical Agriculture (IITA-Nigeria) that are maintained in the gene bank of the Agricultural Research Council (ARC) in South Africa using SNP markers.

4.2 Materials and methods

4.2.1 Experimental material

A total of 47 cowpea accessions were used (Table 4.1)

Table 4.1: Cowpea accessions maintained at the Agricultural Research Council-Grain
Crops Institute (ARC-GCI), Potchefstroom and used in the study

Entry No.	Accessions	Origin/Locations
1	RV 194	South Africa
2	RV 202	South Africa
3	RV 204	South Africa
4	RV 207	South Africa
5	RV 213	South Africa
6	RV 321	South Africa
7	RV 342	South Africa
8	RV 343	South Africa
9	RV 344	South Africa
10	RV 351	South Africa
11	RV 500	South Africa
12	RV 503	South Africa
13	RV 551	South Africa
14	RV 553	South Africa
15	RV 554	South Africa
16	RV 555	South Africa
17	RV 558	South Africa
18	RV 568	South Africa
19	RV 574	South Africa
20	CH47	South Africa
21	Bechuana white	South Africa
22	Dr Saunders	South Africa
23	Glenda	South Africa
24	TVU 9443	Introduction (IITA-Nigeria)
25	TVU 9620	Introduction(IITA-Nigeria)
26	95K-589-2	Introduction(IITA-Nigeria)

Entry No.	Accessions	Origin/Locations
27	86D-1010	Introduction(IITA-Nigeria)
28	83S-911	Introduction(IITA-Nigeria)
29	TVU 12637	Introduction(IITA-Nigeria)
30	TVU 13998	Introduction(IITA-Nigeria)
31	90K-284-2	Introduction(IITA-Nigeria)
32	ITOOK-1263	Introduction(IITA-Nigeria)
33	TVU 13778	Introduction(IITA-Nigeria)
34	99K-494-6	Introduction(IITA-Nigeria)
35	TVU 12746	Introduction(IITA-Nigeria)
36	98K-476-8	Introduction(IITA-Nigeria)
37	TVU 2095	Introduction(IITA-Nigeria)
38	TVU 9596	Introduction(IITA-Nigeria)
39	98D-1399	Introduction(IITA-Nigeria)
40	TVU 14190	Introduction(IITA-Nigeria)
41	TVU 13004	Introduction(IITA-Nigeria)
42	98K-503-1	Introduction(IITA-Nigeria)
43	TVU 11986	Introduction(IITA-Nigeria)
44	97K-499-35	Introduction(IITA-Nigeria)
45	TVU 3416	Introduction(IITA-Nigeria)
46	TVU 13932	Introduction(IITA-Nigeria)
47	PAN 311	South Africa

4.2.2 Experimental site

The study was conducted at the research farm of the Agricultural Research Council, ARC-GCI located at Potchefstroom (26°74''S; 27°8'E) during the 2015 and 2016 growing seasons. Potchefstroom is located at an altitude of 1344 m above sea level and the average minimum and maximum temperature is 9.61˚C and 25.48˚C respectively with an average annual total rainfall of 618.88 mm

4.2.3 Experimental design

Trials were established in two environments; early and late season planting dates at Potchefstroom and laid out in an 8 x 6 alpha lattice design with three replications. Each replication was planted with 48 cowpea accessions one was not germinated, giving a total of 144 cowpea accessions for three replications. Each accession was planted in two row-plots,

which were 5 m long using a spacing of 0.20 m within a row, with 0.75 m spacing between rows.

4.2.4   Management practices

Both trials were similar with regards to management practices, such as thinning, irrigation, weeding and chemical spraying. Planting was done after land preparation where two seeds were sown per hill and two weeks after emergence plants were thinned to one plant per hill. The trials were both under rain-fed conditions with supplemental irrigation applied in case of absence of rain for a couple of days. Weeding was done using dual gold herbicide applied before planting to control annual grasses and broadleaf weeds. Hand weeding was done after 2 weeks of planting, and the second weeding was done 4 weeks after planting. Trials were also sprayed with seizer EC 100 insecticide every two weeks to control insects which cause damage on growing parts of cowpea.

4.2.5   DNA isolation

Forty seven random samples of cowpea leaves were harvested from each accessions after three weeks. The leaf samples were dried for 3-days using a freeze dryer and then grinded to a fine powder using a tissue lyzer. The DNA was isolated from the tissue lysed material using the CTAB (hexadecyltrimethylammonium bromide) method. A volume of 750 ml CTAB buffer (50 ml tris hydroxymethyl amino methane, pH 8.0), 20 mM EDTA (ethylene-diaminetetra acetate, pH 8.0), 5 M NaCl, 2% (w/v) CTAB, 0.2% (v/v) b-mercaptho-ethanol was added to approximately 250 mg fine leaf powder in a 1.5 ml microfuge tube and incubated in a water bath at 65°C for 1 h (Saghai-Maroof et al., 1984). The suspension was extracted with 500 ml chloroform: isoamylalcohol [24:1 (v/v)] mix with repeated hand shaking and the phases separated by centrifugation at 10000 rpm for 10 min. The upper layer was removed and transferred into a new 1.5 ml eppendorf containing 500 µl of ice-cold isopropanol (500-600 µl of upper phase and repeat the chloroform step if the interphase is not clean). One µl of 10 mg/ml RNAse A was added and incubated for 30 minutes at 37°C. The suspension was stored for 20-30 minutes at -20 °C and then centrifuged at 12000 rpm for 25 min to pellet the DNA. The supernatant (isopropanol) was removed and all the liquid drained. 500 µl of 70% ethanol was added to wash the pellet and spinned at 4000 rpm for 5 minutes. Ethanol was removed

and the pellet allowed to air dry for 1 hour. The pellet was then resuspended in 200 µl TE buffer. DNA for all samples was quantified using a Qubit® 2.0 Fluorometer and was quantified with Nanodrop spectrophotometer (Nanodrop ND-1000). Agarose gel electrophoresis was also performed to quantify the DNA.

4.2.6 SNPs analysis

Genotyping was conducted at the ARC - Biotechnology Platform in Pretoria, South Africa. A total of 60k SNPs were used based on Illumina protocols. Approximately 12 µl of DNA was loaded in each well of a Bead Chip for each genotype. Samples were processed according to the Illumina Infinium–II assay protocol (Illumina, Inc. San Diego, CA, 92122, USA). A total of 250 ng of genomic DNA from each accession was used for amplification, after which PCR products were hybridized to bead chips via the address sequence for detection on an Illumina iSCAN Reader.

4.2.7 Genetic diversity analysis

Genotypic data were subjected to analyses with various measures of genetic diversity within and among accessions using GenAlex software version 6.5 (Goudet, 2001; Peakall and Smouse, 2012). Genetic diversity parameters such as total number of alleles per locus (N_a), number of effective alleles per locus (N_e), observed heterozygosity (H_o), Shannon's Information Index (I), gene diversity (H_e) and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). To examine the degree of population differentiation, other genetic parameters such as differentiation (F_{ST}), gene flow (N_m), Nei's unbiased genetic distance (GD) and identity (GI) were estimated using GenAlex. The partitioning of total genetic variation into within and among populations was done with a molecular analysis of variance (AMOVA) procedure using GenAlex.

4.2.8 Cluster analysis

The genotypic data were used to obtain a dissimilarity matrix using the Jaccard index. The matrix was used to run a cluster analysis. Cluster analysis was done based on neighbor-joining algorithm using the un-weighted pair group method using arithmetic average (UPGMA) in

DARwin 5.0 software (Perrier and Jacquemoud-Collet, 2006). A dendrogram was then generated on the dissimilarity matrix. To investigate the genetic relationships among accessions, genetic distances between all pairs of individual accessions were estimated to draw a dendrogram. Bootstrap analysis was performed for node construction using 10,000 bootstrap values.

4.3 Results

The characteristics of 60k SNP markers analyzed are shown in Figures 4.1 and 4.2. The PIC ranged from 0.02 (2_05157, 2_00015, 2_05243 and 2_04218) to 0.38 (recorded from 1% of the markers), with an average of 0.27. Approximately 52% of the markers used had PIC values exceeding 0.30 demonstrating the high discriminatory power of the markers, suggesting that considerable variation between accessions is detectable with SNP markers (Figure 4.3). SNPs diversity data ranged from 0.02 to 0.50 with an average of 0.34, and the vast majority (75%) between 0.30 and 0.50 (Figure 4.4). There was wide genetic distance among accessions, indicating a wide diversity amongst them. The highest genetic distance between the accessions was 0.86 which was recorded between the accessions 97K-499-35 and RV 342 and the lowest was 0.45 which was found between the accessions TVU 14190 and RV 213 with an average of 0.60 (Figure 4.5).
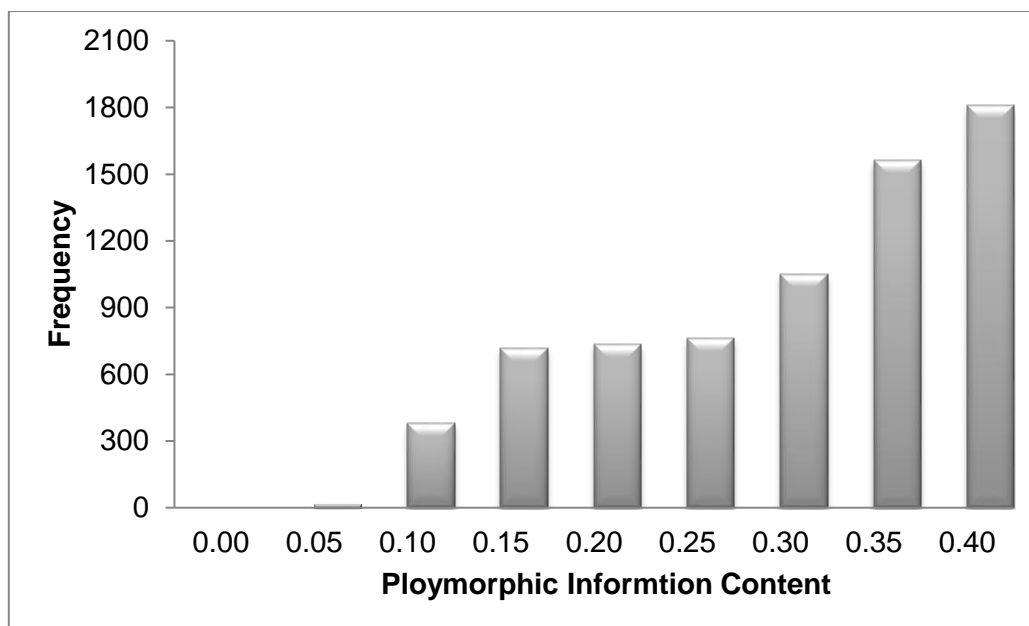
Figure 4.3: Distribution of the 7032 SNP markers for Polymorphic Information Content

The highest number of accessions tested per population was 24.00 observed in IITA-Nigeria while the lowest was 23.00 was observed in South Africa with an average 23.50 (Table 4.2). The highest average number of effective alleles per locus per population observed was 0.558 in IITA-Nigerian accessions; while the lowest value observed was 0.556 in South African accessions with a mean of 1.557. The highest average observed gene diversity within accessions per population was 0.107 revealed in IITA-Nigerian accessions while 0.089 was observed in South African accessions with an average of 0.098. The highest average gene diversity within accessions per population of 0.337 was observed in accessions from IITA-Nigeria and the lowest 0.336 was observed in accessions from South Africa with a mean of 0.337. The high inbreeding coefficient of 0.727 was observed in South African accessions while the lowest was 0. 675 from IITA-Nigerian accessions with a mean of 0.701. The highest percentage of polymorphic loci was 0.9792 in accessions from IITA-Nigeria, while the lowest was 0.969 in South African accessions with a mean of 0.974.
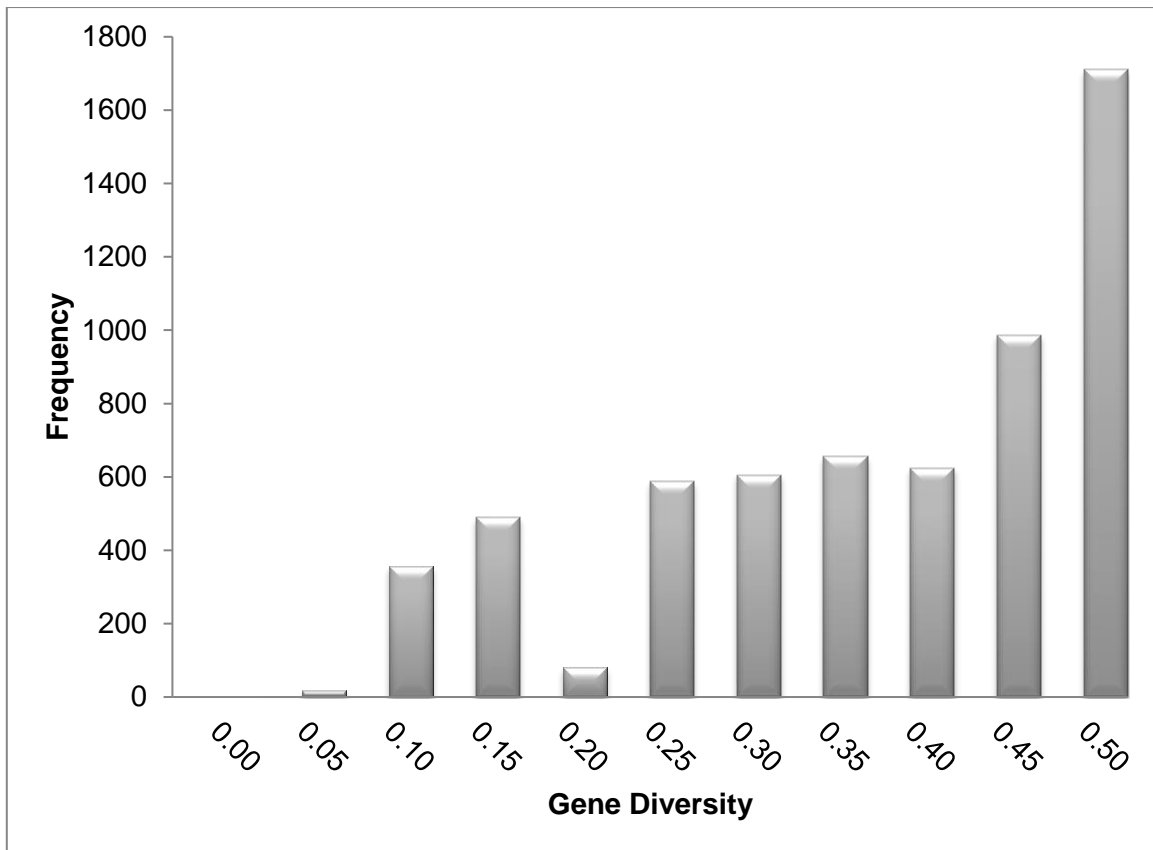
.

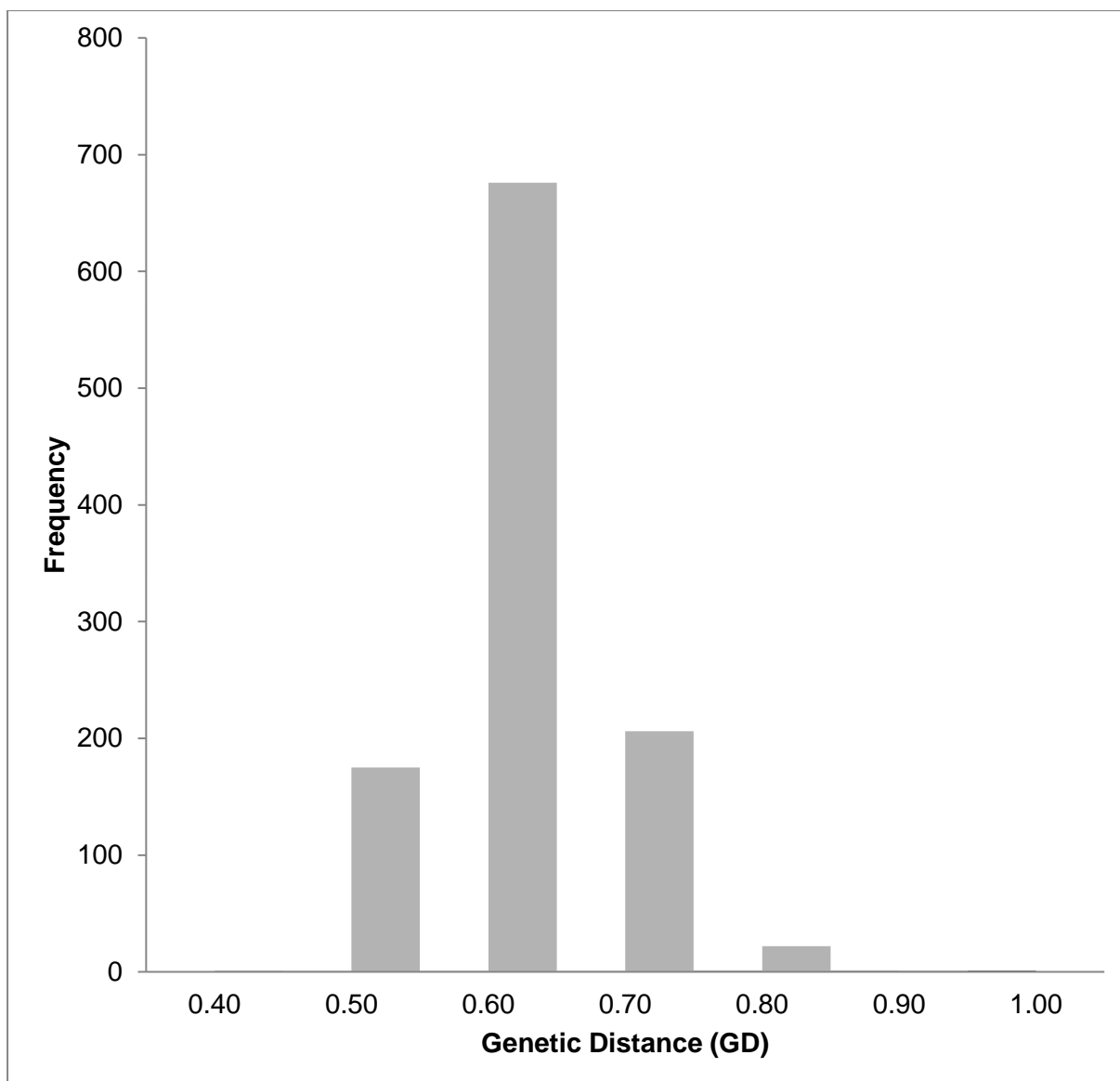Figure 4.4: Distribution of the 7032 SNP markers for gene diversity

Figure 4.5: Summary of genetic distance data of 47 cowpea accessions using SNP molecular markers

The average observed (Ho) and expected (He) heterozygosity among selected cowpea accessions varied among the source of origins; The highest average of (Ho) 0.107 was recorded by Nigeria while the lowest 0.089 observed by South Africa. Highest average of (He) was 0.337 detected by Nigeria whereas the lowest 0.336 observed by South Africa revealed 98% polymorphic loci (Table 4.2).

Table 4.2: Genetic diversity within and among the 47 among cowpea accessions studied.

Population	Genetic parameter						
	N	Ne	I	Ho	He	F	%P
South Africa	23.00	1.556	0.495	0.089	0.336	0.727	0.9694
Nigeria	24.00	1.558	0.496	0.107	0.337	0.675	0.9792
Overall mean	23.50	1.557	0.496	0.098	0.337	0.701	0.9743
SE	0.50	0.003	0.002	0.001	0.001	0.003	0.0049

N= number of accessions tested per population; N_e= average number of effective alleles per locus per population; I = Shannon information index; H_o= average observed gene diversity within accessions per population; H_e= average gene diversity within accessions per population; F_{IS}= inbreeding coefficient; %P= percentage of polymorphic loci; SE= Standard error.

Pair-wise estimates of genetic differentiation (F_{ST}) between the two population was 0.035 (s), gene flow (N_m) was 6.89, genetic distance GD was 0.022 and genetic identity (GI) was 0.979.

The analysis of molecular variances (AMOVA) based on 60k SNPs marker is presented in Table 4.3.

The F-statistic observed for the total accessions among the geographical backgrounds was 2%. The variation within individuals of sub population was 23% and variation among individuals of total population was 75%. According to these results observed that there was low differentiation among population in studied involved great diversity among individuals of cowpea accessions

Based on their molecular profiles using 60k SNP markers, the generated dendrograms showed genetic relationship among 47 cowpea accessions are presented in Figure 4.4. SNP markers were effective in discriminating the accessions into different clusters. The accessions

were grouped into three major clusters and these are indicated by using colours: red, black and blue. Most of the accessions shown in red colour were from South Africa and few from IITA-Nigeria. Black colour had almost half from South Africa and another half from IITA-Nigeria and the last group with blue colour were mostly from IITA- (Figure 4.7). In most cases, cowpea accessions collected from the same locality showed a higher genetic similarity as compared to those collected from different localities, but some accessions belonged from different origins. This was confirmed by high genetic differentiation between certain accessions.

Table 4.3: Summary of analysis of molecular variance (AMOVA) among 47 cowpea accessions based on 60k SNPs markers

Source	df	SS	MS	Est. Var.	%	F-statistics
Among Populations	1	3909.50	3909.50	30.40	2%	0.001
Among Individuals	45	111658.48	2481.30	1076.58	75%	0.001
Within Individual	47	15422.50	328.14	328.14	23%	0.001
Total	93	130990.48	-	1435.12	100%	-

DF= Degree of freedom, SS= sum of squares, MS= mean sum of squares, Est. var. = estimated variance, % = Percentage contribution to total variation
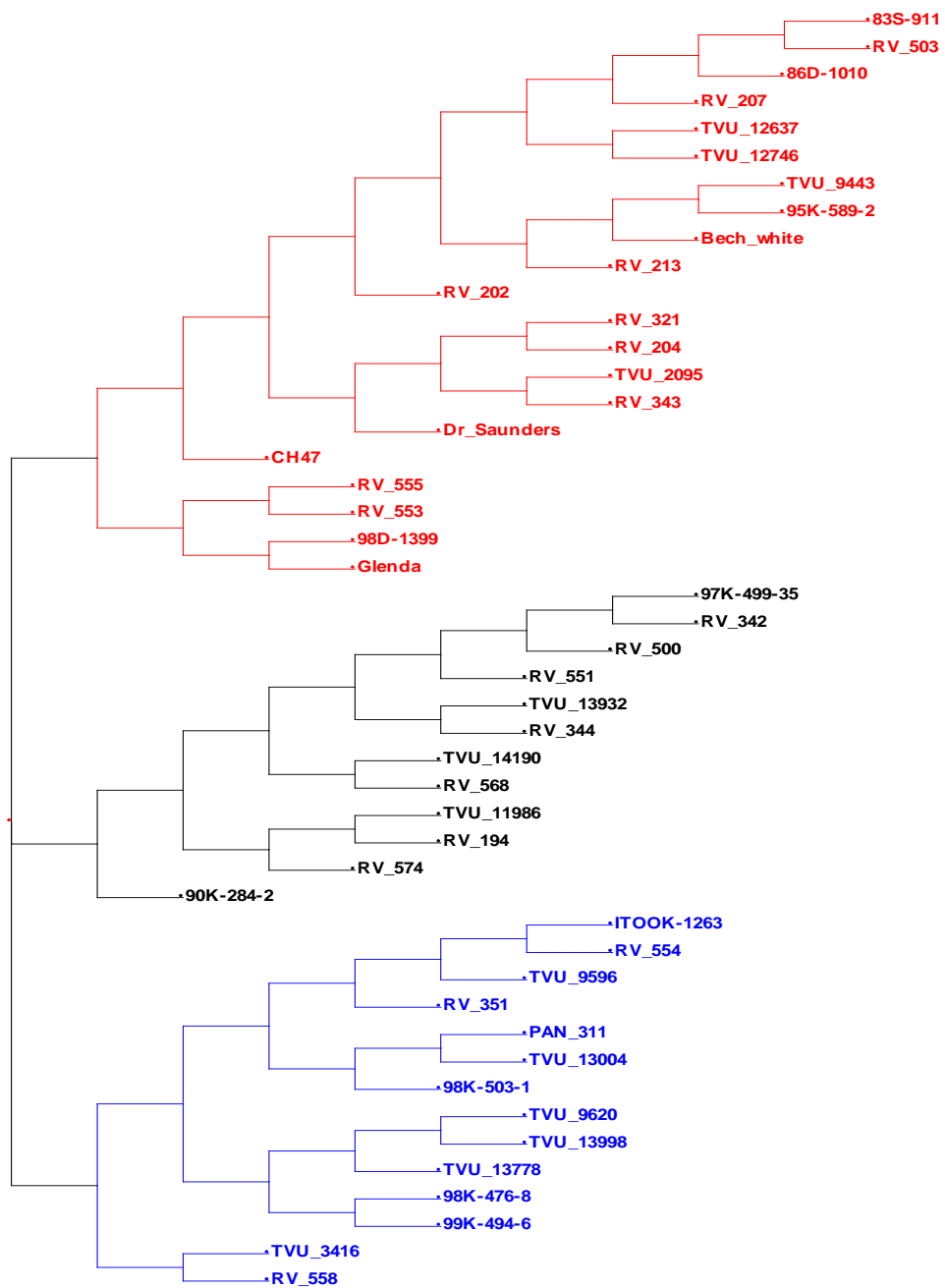
Figure 4.6: Dendrogram showing genetic relationship among 47 cowpea accessions performed 60k SNP markers. The different colours indicate the clustering patterns among the accessions
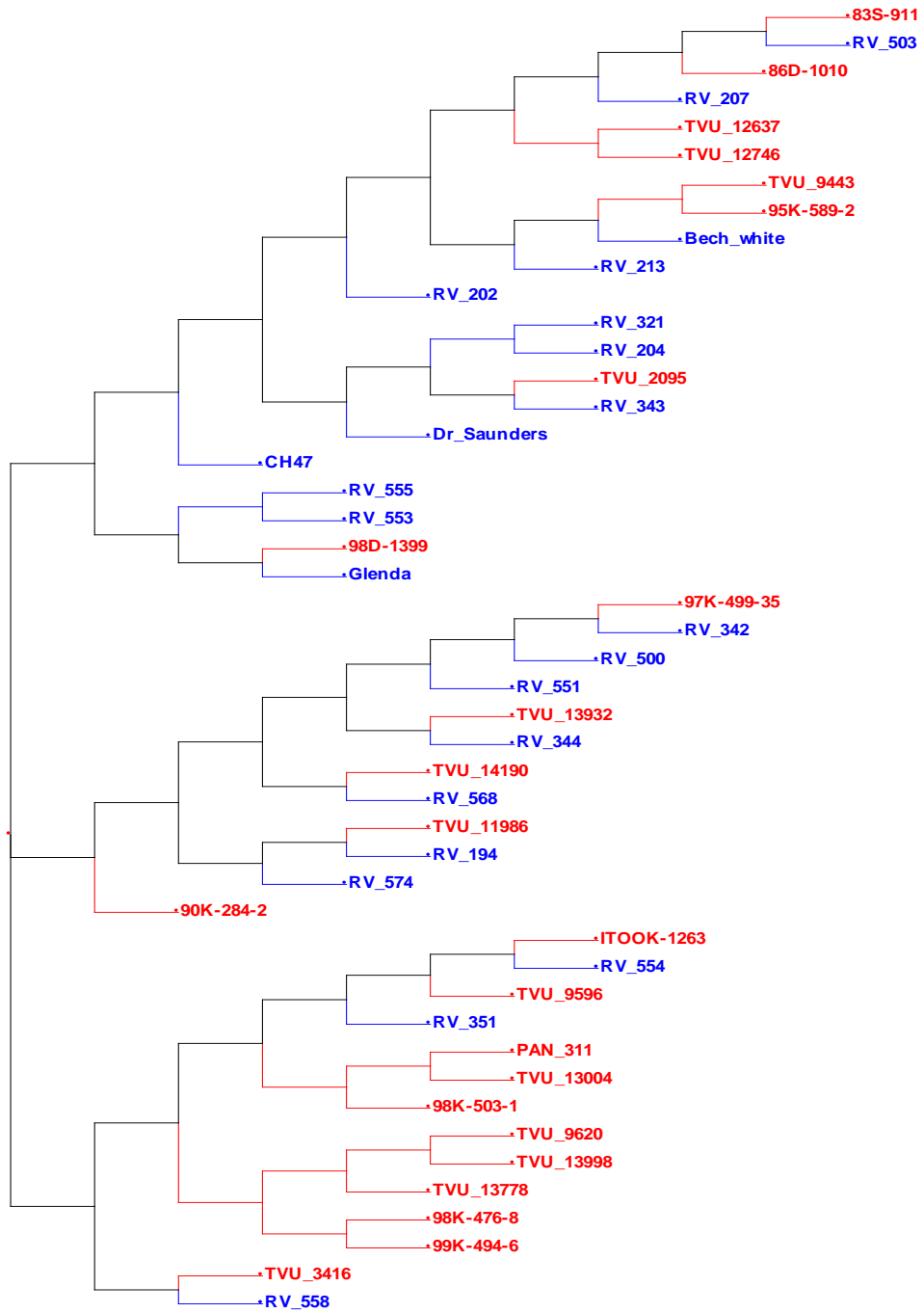
Figure 4.7: Dendrogram showing genetic relationship among cowpea accessions performed using 60k SNP markers. The different colours indicate the clustering patterns among the accessons. Figure based on geographic origin Blue = accessions collected from South Africa.

4.4   Discussion

Molecular marker application enable the identification of   genetic relatedness amongst accessions and   this information is useful in breeding programmes for management and utilization of germplasm (Cieslarová et al., 2011; Huynh et al., 2013). Genetic variation is very important for breeders in improvement of traits in developing new superior cultivars to address farmers' challenges as well as contributing to increased cowpea production.

In this study, the polymorphic information content (PIC) ranged from 0.02 to 0.38 with a mean value of 0.27. The highest PIC value was 0.38 and this is almost similar to a PIC value of 0.37 that was reported by Umar (2014) performed in 119 SNP markers. A PIC value of 0.41 was reported by Xiong et al. (2016) in cowpea accessions using SNPs markers , while a value of 0.33 was reported by Badiane et al. (2012) in cowpea accessions using SSR markers. These results are in contrast to the high PIC value of 0.83 reported by Doumbia (2012) using 20 SSR markers. According to the scale indicated by Botstein et al. (1980), mean PIC value ≥ 0.5 is highly informative, 0.25~0.50 is reasonably informative and < 0.25 is slightly informative, and loci (marker) with many alleles and a PIC value near 1 are most desirable. It seems the origin of the accessions did not influence the PIC values and the genetic distances among the cowpea accessions were also low due to the inherent self-pollination mechanism of the cowpea. The accuracy of SNP markers used in the current study can be confirmed by the fact that they were carefully selected at a ratio of 10 per chromosome with a good coverage of the genome. Hence, the diversity data can be considered reliable. This set of SNP markers will be useful for future cowpea breeding programmes.

The gene diversity among accessions ranged from 0.02 to 0.50 with a mean of 0.34. Xiong et al. (2016) reported the highest value of gene diversity of 0.68 using SNP markers, and Egbadzor et al. (2014) reported a value of 0.63 using 458 SNPs markers, both of which are higher than that observed in this study. The variation in the gene diversity values might be due to the number of markers used, as well as the origin and nature of accessions used.

4.4.1   Genetic distance

The highest genetic distance was between 97K-499-35 and RV 342 (0.86) cowpea accessions and lowest between TVU 14190 and RV 213 (0.45). Those accessions that revealed the

highest genetic distance indicate that they belong to different genetic clusters. Also, accessions observed with high genetic distance can be used as potential parents in developing superior cultivars as they are expected to have greater genetic variations (Fang et al., 2007; Asare et al., 2010; Xiong et al., 2016). However, the genetic distances among cowpea accessions are low, corresponding to the initial hindrance during domestication, which was maintained by the inherent self-pollination mechanism in the crops (Asare et al., 2010). Furthermore, this low genetic distance among accessions might have been due to gene flow resulting from seed exchange practices by farmers within and between regions. Hence, the accessions with greater genetic divergence have potential for conservation, and parental selection in cowpea breeding programmes in South Africa.

The AMOVA clearly indicated a significant difference among populations and individuals and within individuals. The variance among populations was significantly low (2% of the total variation), while the variance among individuals was significantly high (75% of the total variation). The variance within individuals was significant and contributed 23% of the total variation. The significant F statistic indicated genetic differences among cowpea accessions. According to Wright (1965), the F-statistics quantify the scope of genetic differentiation among and within populations. This allows plant breeders to select and fix unique alleles among cowpea accessions (Hartl et al., 1997). The results of this study were low compared to estimates of genetic variation among accessions that have been reported by Ali et al. (2015) using SSR markers.

The SNP markers were effective in discriminating cowpea accessions based on genetic backgrounds. Accessions indicated by red colour and clustered together were from South Africa and few introductions from IITA-Nigeria are derived from the same genetic backgrounds. The same applies for the accessions indicated by black colour. Almost half of them are from South Africa and the other half from IITA-Nigeria. The last group indicated by blue colour were mostly from IITA-Nigeria and very few from South Africa which had the same background. This might be due to gene flow, and seed exchange among farmers within and between regions, thus causing clustering. The clustering of accessions based on their genetic similarity in this study would help in selection of genetically diverse parental lines to get superior recombinants for future cowpea breeding programmes (Asare et al., 2010). The use of molecular markers is a modern technology which provides reliable information among

accessions for conservation and identification of parental lines to use for developing new cultivars. The results from this study will contribute to initiatives aimed at the effort of improving cowpea accessions in South Africa.

4.5 Conclusion

Knowledge of genetic diversity among cowpea accessions is important for effective genetic improvement and management of germplasm resources as applicable to the identification of diverse germplasm for parental selection in breeding programmes. The SNP markers have potential application in precise estimation of genetic diversity among cowpea accessions used. This study revealed wide genetic diversity among cowpea accessions. This implies there is a genetic variation can be used by plant breeders among cowpea accession for improvement of traits through crossing of genetically diverse accessions.

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CHAPTER FIVE

GENERAL OVERVIEW OF THE STUDY CONCLUSIONS AND FUTURE DIRECTIONS

Cowpea is a food and animal feed crop that is grown worldwide. The narrow genetic base of cowpea has been a serious challenge to plant breeders. Knowledge of genetic diversity is very important to breeders for developing new varieties and can also contribute to widening of the genetic base of cowpea during parental selection. The objectives of the study were to: (i) to assess the level of genetic diversity present in selected cowpea accessions using agro-morphological traits, (ii) to determine the genetic diversity of grain protein, iron and zinc content of selected cowpea accessions and (iii) to assess the presence of genetic diversity among selected cowpea accessions using molecular makers. This chapter outlines the findings of the study conducted and gives the breeding implications of the findings and its challenges, future directions in cowpea breeding.

5.1 Summary of research findings

Assessment of genetic diversity among selected cowpea accessions using agro-morphological traits

The analysis of variance for 10 traits revealed a large genetic variation among the accessions. Grain yield and fodder yield were affected by planting date, with optimum planting date giving the highest mean values of 3051.5 kg/ha and 5725.9 kg/ha respectively, while late planting date produced 1781.4 kg/ha and 3756.9 kg/ha grain and fodder yield, respectively. A wide phenotypic variability in grain yield weight of mature pods, fodder yield and days to 50% flowering was observed. The study also observed high genotypic coefficients of variation (GCV), moderate to high heritability estimates and genetic advance as percent of the mean in days to 50% flowering, the weight of mature pods, grain yield and fodder yield. The grain yield showed positive and significant correlation with the weight of mature pods, fodder yield and a number of seeds per pod. Principal component analysis (PCA) resulted in four PCs contributing to 76.89% of the total variation. The traits that contributed most to the variation

were number of main branches, days to 50% flowering, days to maturity, number of plants per plot, weight of mature pods and grain yield.

Estimation of genetic diversity among selected cowpea accessions using nutritional quality traits

Generally, the cowpea accessions had wide genetic variation for crude protein, iron and zinc contents. The crude protein content of the accessions ranged from 18.73 to 27.88% across planting dates with a mean of 25.08%.The iron content of the accessions varied from 26.48 to 140.89 mg/kg across planting dates with a mean value 80.56 56 mg/kg. The zinc contents of the accessions varied from 39.83 to 203.42 mg/kg across planting dates with a mean 70.01 mg/kg. The accessions that had highest crude protein content in the combined data over planting dates were; TVU 13932 (27.88%), 98K-476-8 (27.15%), TVU 9443 (27.13%), RV 503 (27.00%), RV 213 (26.80%), 98K-503-1 (26.74%) and 95K-589-2 (26.72).The accessions observed with high iron content were; TVU 13998(140.89 mg/kg), 86D-1010 (138.71 mg/kg), 98K-476-8 (132.73 mg/kg), 95K-589-2 (121.69 mg/kg), RV 343 (117.49 mg/kg), RV 204 (115.83 mg/kg), TVU 13932 (115.46 mg/kg) and RV 500 (111.77 mg/kg).The accessions identified with high zinc content were: TVU 13932 (203.42 mg/kg), 98K-503-1 (162.38 mg/kg), 90K-284-2 (151.71 mg/kg), and TVU 14190 (143.99 mg/kg) and CH47 (116.17 mg/kg).Positive and significant correlations were observed for crude protein and zinc, indicating the possibility of simultaneously improving crude protein and zinc content. The first two principal components explained 77.48% of the genetic variation. Most traits were crude protein, iron and zinc contents in contributed to variation among accessions.

Genetic diversity analysis of selected cowpea accessions using SNP markers

The polymorphic information content (PIC) ranged from 0.02 to 0.38 with a mean 0.27, reflecting the high discriminating ability of the markers used. The average gene diversity among genotypes ranged from 0.02 to 0.050 with a mean of 0.34. Genetic distance ranged from 0.47 to 0.86 with a mean of 0.61. The 97K-499-35 and RV 342 accessions were the most distantly related. The lowest genetic distance was found between TVU 14190 and RV 213.

The inbreeding coefficient ranged from 0.693 to 0.733 with a mean of 0.713. The within individuals and among individuals differentiation accounted for 23% and 75% of the total variation, respectively. The pair-wise estimates of genetic differentiation between the two

populations was 0.035, gene flow was 6.89, genetic distance was 0.022 and genetic identity was 0.979.The dendrogram clustered cowpea accessions into three main groups, some of accessions clustered based on geographical origin and other group had collections from different sites implying the existence of a large level of gene flow among geographical origins.

5.2 Implications of the research findings

➢ Due to the existence of a wide range of genetic diversity among the selected cowpea accessions; there is an opportunity for parent selection among those accessions that can be utilized in cowpea improvement for increased yield production as well as nutritional quality.

➢ The positive relationship observed between grain yield and weight of mature pods, fodder yield and number of seeds per pod shows the possibility of simultaneously improving grain yield and other related traits via selection.

➢ The ANOVA for crude protein, iron and zinc content among the accessions revealed a highly significant variation among them.

➢ The PCA clustered accessions based on their similarity or differences in four quadrants which confirmed the variation among them for these nutrition traits. Therefore, it would be possible to select for specific mineral and protein content for improvement in these accessions.

Accessions with highest genetic distances can be used for crossing with each other to develop new superior cultivars.

5.3 General challenges in cowpea production and breeding

➢ Cowpea production is hindered by many factors which result in low production, but a major concern is the lack of information on genetic diversity in cowpea accessions as well as absence of cultivars with desirable agronomic traits.

➢ Cowpea production was constrained by insect pests such as blister beetle that feeds on cowpea flowers causing serious damage to cowpea thus reducing yield production.

> Cowpea production was challenged during dry periods whereby pods of some of the accessions had a tendency to shatter and scatter the grain making it very difficult to harvest. This also reduced yield.

5.4 Recommendations

The following recommendations resulted from this study:
> Cowpea accessions with wide genetic diversity should be considered in the selection and development of new cowpea cultivars that are well adapted to local conditions, with high grain yield as well as nutritional quality.
> Cowpea accessions observed with highest genetic distance can be considered as parents for crossing in developing superior varieties.
> Cowpea breeding programme in South Africa to develop cowpea varieties which are tolerant and resistant to insects pests or use of regular sprays of insecticide in the field
> Cowpea programme to develop cowpea varieties with pods that do not shatter and scatter the grain. This will contribute to increased cowpea production levels in South Africa.
> Use of good seeds with high germination percentages to increase production of cowpea in South Africa.

5.5 Conclusion

The overall study was to estimation of genetic diversity among cowpea accessions based on agro-morphological traits, nutritional quality traits and molecular markers. The study was successful to characterize the levels of genetic diversity among selected cowpea accessions. The accessions revealed wide genetic variation and good desirable traits that can be selected in breeding for superior cultivars with better nutritional quality and also be used to maximise the genetic resource availability for future use. Above all, the findings will contribute to increased production levels of cowpea with better nutritional quality to communities in South Africa.