Heritability and expression of grain yield and nutritional characteristics of cowpea

By

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DECLARATION

“I, Bogaleng Milcah Masemola declare that the doctoral research thesis that I herewith submit for the degree qualification of Philosophy Doctor at the University of the Free State, is my independent work and that I have not previously submitted it for qualification at another institution of higher education. I further cede copyright of the thesis to the University of the Free State.”

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DEDICATION

To my late father Tuys Masemola, late grandfather Frans Mokuwe and late grandmother Ramogohlo Mokuwe. Your teachings will forever be my guide and your prayers will remain my shield.

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LIST OF ABBREVIATIONS AND SI UNITS

AA	Atomic absorption
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
ARC	Agricultural Research Council
ARC-VIMP	Agricultural Research Council-Vegetables, Industrial, and Medicinal Plants
bp	Base pair
BSA	Bovine serum albumin
Ca	Calcium
Cm	Centimetre
DAFF	Department of Agriculture, Forestry and Fisheries
DArT	Diversity Arrays Technology
ddH_2O	Double-distilled water
DNA	Deoxyribonucleic acid
DF	Degrees of freedom
D50F	Days to 50% flowering
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
L	Number of locations
SLI	season by location interaction
Ɛ	Residual error
F_1	First generation
F_2	Second-generation
Fe	Iron
g	Gram
g/mol	Gram per mol
g/m^2	Gram per square metre
GCA	General combining ability
GLI	Genotype by location interaction
GLSI	Genotype by location by season interaction
GRC	Genetic Resources Centre
GSI	Genotype by season interaction
GY	Grain yield
H^2	Broad-sense heritability
h^2	Narrow-sense heritability
HCl	Hydrochloric acid
HSWt	Hundred seed weight

IITA	International Institute of Tropical Agriculture
INERA	Institute of Environment and Agricultural Research
K	Potassium
KALRO	Kenya Agricultural and Livestock Research Organization
KCl	Potassium chloride
LL	Leaf length
LSD	Least significant difference
LW	Leaf width
m	Metre
M	Molarity
MAGIC	Multi-parent advanced generation inter cross
MAS	Marker-assisted selection
$MgCl_2$	Magnesium chloride
Mg	Magnesium
MGDW	Molecular grade distilled water
mL	Millilitre
mg/kg	Milligrams per kilograms
mg/ml	Milligrams per millilitre
Mm	Millimetre
MPH	Mid-parent heterosis
MSƐ	Mean squares for error
MSg	Mean squares for genotypes
MSgs	Mean squares for genotypes by season
Na	Sodium
ng	Nanogram
nm	Nanometre
ng/µl	Nanograms per microliter
NB	Number of branches
NH4Cl	Ammonium Chloride
NIR	Near infrared
NPP	Number of pods per plant
NSPP	Number of seeds per pod
P	Phosphorus
PAGE	Polyacrylamide gel electrophoresis
PAP	3′-phosphoadenosine-5′-phosphate
PCR	Polymerase chain reaction
PH	Plant height
PL	Pod length
PW	Pod width
QTL	Quantitative trait loci

R	Number of replications
r/s	Revolution per second
Rep(Env)	Replication nested within the environment
RAPD	Randomly amplified polymorphic DNA
Rpm	Rotations per minute
S	Number of seasons
SA	South Africa
SCA	Specific combining ability
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
SSRs	Simple sequence repeats
TCA	Trichloroacetic acid
t/ha	Tons per hectare
TEMED	Tetramethyl ethylenediamine
UCR	University of California
USA	United State of America
UV	Ultraviolet
V	Voltage
VIS	Visible
v/v	Volume per volume percentage
w/v	Weight in volume
w/w	weight per weight
x g	Relative centrifugal force
XRF	X-ray fluorescence
Zn	Zinc
µl	Microliter
σ^2Ɛ	Environmental variance/ residual variance
σ^2g	Genotypic variance
σ^2gl	Genotype by location interaction variance
σ^2gls	Genotype by location by season interaction variance
σ^2gs	Genotype by season interaction variance
σ^2p	Phenotypic variance
%	Percentage
ºC	Degrees Celsius

ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) is an autogamous diploid legume crop. It is highly self-pollinating, yielding high levels of homozygosity and contributing to a narrow genetic base. Improving cowpea depends on identifying and selecting parental genotypes based on commercial characteristics of interest, followed by hybridisation and selection to develop new elite lines that can be evaluated in several environments for performance. A study on the combining ability of selected parental genotypes is expected to provide insight into the selection of potential parental genotypes, gene action and heritability for grain yield and nutritional characteristics. The objective of this study was to determine general combining ability (GCA) and specific combining ability (SCA) effect and heritability of economic traits in selected parental genotypes identify the best parents, and new F_1 progenies. A half diallel mating design was used to generate 45 F_1 progenies, after which the 55 genotypes (the 45 F_1 progenies and 10 parental genotypes) were evaluated for grain yield, yield components and nutritional characteristics at two locations for two seasons using a randomised complete block design with three replications.

The results indicated significant genotype, genotype by environment, GCA by environment and SCA by environment interaction effects for all measured characteristics except for GCA and SCA for iron (Fe) and zinc (Zn) contents. Both additive and non-additive gene effects were important for the expression of the grain yield and nutritional characteristics measured. However, non-additive gene effects were predominant for most of the yield characteristics, implying potential for hybrid breeding. The low to medium narrow-sense heritability observed for all measured characteristics indicated the predominance of non-additive genetic effects, which suggests that selection would be effective for some of the characteristics, but not for those with low heritability. The parental genotypes TVU13953 and IT96D-602 were the best general combiners for grain yield and most yield components. Glenda, IT93K-129-4, TVU7778 and 98K-5301 were the best general combiners for amylose content. Kisumi-mix and 98K-5301 were the best general combiners for protein content, IT845-2246 and TVU-14196 for Fe, and Glenda and ITOOK-1060 for Zn content. Six hybrid progenies (Glenda x TVU13953, 98K-5301 x TVU13953, IT96D-602 x 98K-5301, IT96D-602 x TVU13953, IT96D-602 x Glenda and IT845-2246 x TVU13953) had superior performance for grain yield. TVU7778 x Kisumi-mix, IT845-2246 x 98K-5301, Kisumi-mix x IT93K-129-4, and Kisumi-mix x ITOOK-1060 had the highest SCA (≥ 2.80) effects for protein content. TVU-14196 x

TVU13953 and TVU-14196 x IT845-2246 exhibited noteworthy positive SCA effects for Fe content. Significantly positive SCA effects were observed for Zn in four hybrids: ITOOK-1060 x Glenda, Kisumi-mix x ITOOK-1060, IT96D-602 x ITOOK-1060, and IT96D-602 x IT93K-129-4. The crosses had increased genetic variability, indicating that hybridisation and selection can be used to improve grain yield, yield components, and nutritional characteristics in cowpea. The F_1 progenies expressed heterosis, indicating the possibility of hybrid breeding in cowpea. The best general combiners could be used as parental genotypes for future cowpea breeding programmes.

Keywords: cowpea, combining ability, grain yield, heritability, nutritional characteristics, progenies, yield components

CHAPTER 1

GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp, 2n = 2x = 22) is an important annual grain legume and leafy vegetable crop. It is a diploid crop belonging to the order Rosales, family Fabaceae and the genus Vigna (Boukar *et al.* 2020). The crop is widely cultivated in the semi-arid tropics including Africa, Asia, South and Central America (Nkomo et *al*. 2021). Small-scale farmers are the largest producers of cowpea in South Africa under dry land farming conditions (DAFF 2011). Major cowpea production areas in South Africa are Limpopo, Mpumalanga, North-West and KwaZulu-Natal (Mofokeng and Mashingaidze 2019). The Agricultural Research Council-Vegetables, Industrial and Medicinal Plants (ARC-VIMP) does cowpea research in South Africa. They initially focused on pre-breeding activities aimed at importing cowpea collections from abroad, followed by evaluation for adaptation of these accessions in different agro-climatic conditions and then identification of stable parental genotypes for further genetic improvement.

Cowpea genetic resources are held at several research gene banks at both national and international research institutions, across the world. The Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Gerrano *et al*. 2022), holds the most extensive collection of cowpea germplasm (15,371 accessions). The IITA collection contains germplasm from 90 countries and half of the collection (7912 accessions) is from West Africa, the centre of diversity for the crop (Drevon *et al.* 2015; Boukar *et al.* 2019). Despite the extensive germplasm collections available globally, the genetic base of cowpea is narrow for economic characteristics such as grain yield, yield components, drought tolerance and insect pest tolerance (Tshilenge-Lukanda *et al.* 2013). These could be due to the self-pollinating nature of the crop, with a lack of genetic variation that allows no adaptation to the changing environment (Wamalwa *et al.* 2016). Therefore, a systematic breeding approach is required for cowpea genetic improvement for higher grain yield and balanced nutritional value. The knowledge of the mode of inheritance for the expression of grain yield and nutritional characteristics in cowpea would be beneficial for establishing an appropriate breeding programme and identification of good parental genotypes for hybridisation in South Africa.

Parental selection is the most critical stage in a breeding programme, which will determine the possible combinations to improve the genetic variability in cross progeny and ultimately determines the superiority of progenies, which can be exploited during selection (Witcombe *et al*. 2013; Owusu *et al.* 2020). Evaluation of possible parental genotypes for general combining ability (GCA) and specific combining ability (SCA) will enhance crossing and breeding efficiency (Dias *et al*. 2016; Mwale *et al.* 2017). The use of only parental genotypes known to produce progenies with higher characteristic values will enhance breeding and crossing efficiency, which ultimately will increase the genetic gains in cowpea. Hybrid selection depends on the SCA of a cross and high magnitude of heterosis (Sharma *et al.* 2010) and should be investigated as a possible breeding strategy in cowpea.

Information on cowpea combining ability of possible parents would help accelerate improvement of cowpea for grain yield, yield components and nutritional characteristics in South Africa. Studies conducted in other countries such as Nigeria (Olunloyo *et al.* 2019), Ghana (Owusu *et al.* 2020) and Kenya (Jou-Nteufa and Ceyhan 2022) reported significant positive GCA and SCA effects for the most important grain yield characteristics. Other studies also reported significant GCA and SCA for protein content (Tchiagam *et al.* 2011; Wang *et al.* 2021), and for phytate, Fe and Zn content (Maina *et al.* 2015). The information on gene action involved will further help in the identification of parental genotypes that are best general combiners and best specific combiners as well as heterotic progenies to enable adequate breeding gains for cowpea cultivar development.

Developing high yielding and nutritionally balanced cowpea cultivars that are well adapted to South African agro-climatic conditions also relies on the understanding of the relationship between grain yield, yield components and nutritional characteristics through correlation and multivariate analysis to generate an appropriate selection index (Samireddypalle *et al.* 2017). The knowledge of how grain yield relates to other characteristics would be essential in crop improvement and selection effectiveness, as selection of one characteristic could simultaneously enhance the correlated characteristic/s. Therefore, this study investigated the combining ability, heterosis, gene action and correlations of grain yield, yield components and nutritional characteristics in selected parental genotypes and their F1 progeny, to aid cowpea breeders with useful information towards producing superior cultivars with desirable grain yield and improved nutritional characteristics.

1.1 RESEARCH QUESTIONS

1. What are the partitioned variances and heritability for grain yield, yield components, nutritional characteristics in cowpea parental genotypes and their progenies and what are the implications for crop improvement?
2. What is the magnitude of combining ability and heterosis among selected cowpea parental genotypes for yield and nutritional characteristics?
3. What is the mode of gene action involved in the inheritance of grain yield, yield components, and nutritional characteristics?
4. What correlations are there between grain yield, yield components and nutritional characteristics in cowpea parental genotypes and their progenies?
5. Which parental genotypes and progenies are superior for grain yield and nutritional characteristics?

1.2 RESEARCH OBJECTIVES

The general aim of this study was to use a diallel mating design to evaluate the progenies and their parental genotypes for grain yield and nutritional characteristics in two diverse environments and two seasons. The specific objectives of the study were,

1. To partition variance and heritability in the cowpea parental genotypes and their progenies for grain yield, yield components, and nutritional characteristics.
2. To determine the combining ability and heterosis for the selected cowpea parental genotypes and F1 progeny for grain yield and nutritional characteristics.
3. To determine the gene action involved in the inheritance of grain yield, yield components and nutritional characteristics.
4. To determine the correlation between grain yield, yield components and nutritional characteristics in cowpea parental genotypes and their progenies.
5. To identify superior parental genotypes and progenies for grain yield and nutritional characteristics.

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CHAPTER 2

PHENOTYPIC, GENOTYPIC AND NUTRITIONAL DIVERGENCE IN COWPEA AND IMPLICATIONS FOR DROUGHT TOLERANCE BREEDING: A REVIEW

Published: Masemola BM, Gerrano AS, Labuschagne M, Minnaar-Ontong A, Mbuma NW (2023) Phenotypic, genotypic, and nutritional divergence in cowpea and implications for drought tolerance breeding: A review. Crop and Pasture Science 74:1105-1115. https://doi.org/10.1071/cp22295

2.1. ABSTRACT

Genetic diversity is crucial for crop improvement in any breeding programme, as it allows for development of new and improved cultivars with desirable characteristics. Cowpea is an important indigenous grain vegetable legume crop. The crop has a significant potential to improve food and nutritional security in sub-Saharan Africa (SSA), and family income for resource poor famers. The crop is a source of proteins, vitamins (folate, thiamine, and vitamin C), minerals (iron, zinc, manganese, and calcium) and amino acids such as lysine and tryptophan, hence, has a high potential for combating nutrients deficiencies. The SSA countries, including South Africa, have been experiencing many dry seasons, which negatively affected agricultural production and productivity. Cowpea has a long taproot with the ability to grow in arid and semi-arid regions; however, lasting exposure to severe droughts will result in significant grain yield and nutritional characteristics reduction. There has been limited progress in drought tolerance research in cowpea due to the complexity of this parental genotypes, as it involves numerous genes, which the environmental conditions affect. It is therefore important to review research done on the nutritional, phenotypic and genotypic diversity of cowpea and the importance of diversity when breeding for complex quantitative characteristics such as drought tolerance and grain yield. The review will also outline the important omics tools used for drought tolerance breeding, cultivar development and as reference for future cowpea breeding programmes.

Keywords: cowpea, legume, genetic diversity, nutritional diversity, quantitative, breeding, drought, crop improvement

2.2. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an important leafy annual legume crop (Gerrano *et al.* 2015; 2017). It is a diploid (2n=22), belonging to the order Rosales, the family Fabaceae, and the genus Vigna (Boukar *et al.* 2020). Africa, Asia, South and Central America, and other semi-arid tropical regions are the production regions for this crop (Nkhoma *et al.* 2020). Cowpea production in SA is mainly for dryland farming by small-scale farmers, although the size of the area under production and quantities produced are not documented (Mofokeng and Mashingaidze 2019).

Cowpea contains high protein content (20 to 25%), fibre, minerals, vitamins, and high levels of amino acids such as lysine and tryptophan and low-fat content (1.40 to 2%) (Jayathilake *et al.* 2018; Gerrano *et al.* 2019). Due to the high nutritional content, the crop is an outstanding replacement for meat, eggs, and other protein rich foods, as a comparatively cheaper source of quality protein, phosphorus, iron, and vitamins.

The crop is edible in all growth stages from vegetative to reproductive stage. Nearly mature "fresh-shelled" grains are used to make soup and the tender pods and leaves have a high nutritional value (Mbuma *et al.* 2021). In many areas of the world, cowpea foliage is an important source of high-quality hay for livestock feed (Kebede and Bekeko 2020). The crop can withstand arid conditions and environments, due to its phenotypic characteristics such as deep rooting, early flowering, and early maturity (Hayatu *et al.* 2014) and biochemical qualities such as accumulation of nontoxic solutes such as fructans, proline and polyamines (Agbicodo *et al.* 2009).

It is deeply rooted, with thick stomata and early maturity, which enables it to thrive better. The deep roots can draw water deep within the soil profile, and less water is lost through the stomata (Nkomo *et al.* 2021). Although the crop can tolerate arid conditions, exposure to water stress early during plant establishment, flowering and pod formation will result in significant grain yield loss (Mofokeng and Mashingaidze 2019). Drought remains one of the most important abiotic constraints, which hinders food security in SSA and the world. Hence, it is important to initiate a cowpea drought tolerance-breeding programme, to develop cultivars with superior performance even in dry and harsh environments. Understanding the genetic diversity present in the germplasm pool is of utmost importance for any successful breeding programme.

2.3. PRODUCTION OF COWPEA

2.3.1. Status of cowpea production in the world

Global production of cowpea grains ranged between 4,982,598 to 8,907,644 tons in the past ten years (Figure 2.1), with Africa being the largest producer of cowpea grain with approximately 14.50 million ha annually (FAOSTAT 2020).

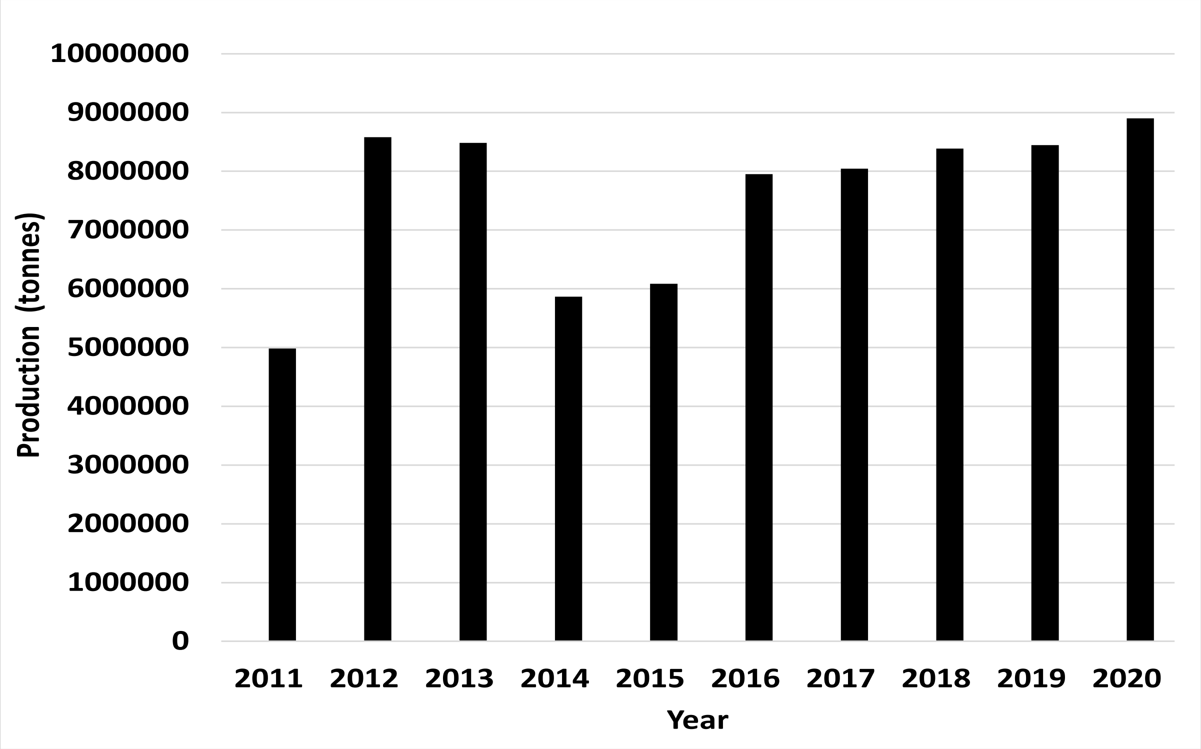

Figure 2.1 World production of cowpea (FAOSTAT 2020)

2.3.2. Status of cowpea production in sub-Saharan Africa

Africa accounts for 85% of the world's cowpea grain production, with approximately 14.50 million ha annually (Figure 2.2). Nigeria is the top producer of cowpea, contributing about 2.50 million tons yearly to global production. The other leading producers include Niger, Burkina Faso, Tanzania, Cameroon, Mali, and Kenya (FAOSTAT 2020).

2.3.3. Cowpea production in southern Africa

Southern Africa produced between 5336 and 5710 tons of cowpea grains in the past ten years (Figure 2.3). South Africa accounted for 4600 to 4867 tons of cowpea grains with a record of 11 154 hectares (ha) of the area under production and a yield of 437 kg per ha in 2020 (FAOSTAT 2020). In South Africa, the areas producing cowpeas are Limpopo, Mpumalanga, North-West, and KwaZulu-Natal Provinces; however, there is no available data on production per Province.

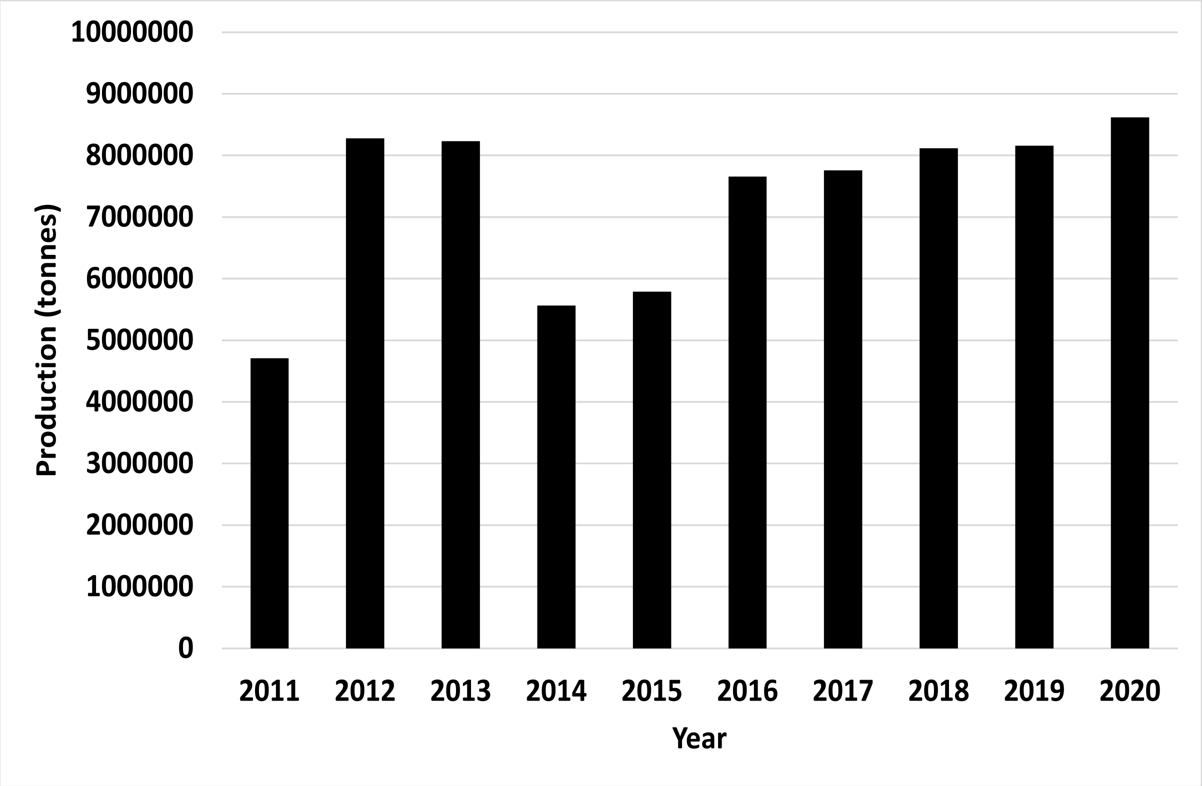

Figure 2.2 Production of cowpea (dry grains) in sub-Saharan Africa (FAOSTAT 2020)

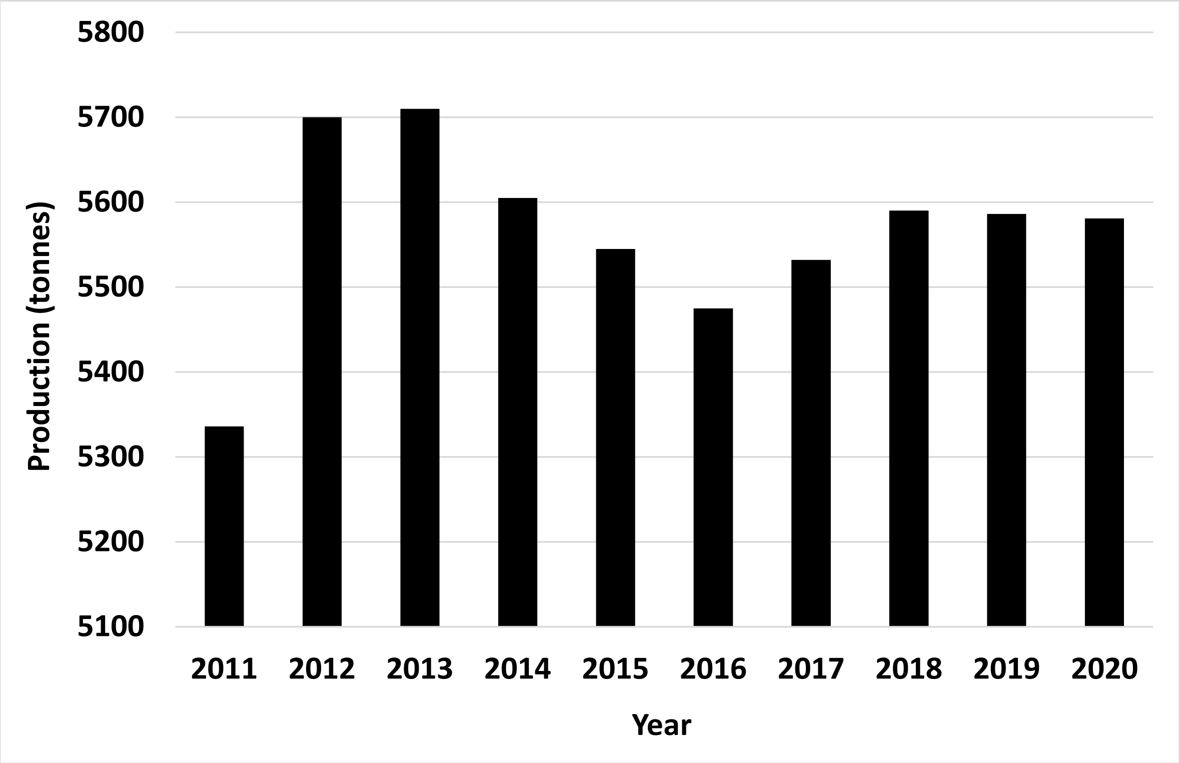

Figure 2.3 Production of cowpea (dry grains) in Southern Africa (FAOSTAT 2020)

2.4. CONSTRAINTS TO COWPEA PRODUCTION

2.4.1. Biotic constraints

Biotic factors, including bacterial diseases (Viswanatha *et al.* 2011), nematodes (Haegeman *et al.* 2012), parasitic weeds (Horn *et al.* 2015), insects (Oliveira *et al.* 2017), viral diseases (Gomes *et al.* 2019), fungal diseases (Horn and Shimelis 2020) and aphids (Mofokeng *et al.* 202l) mainly affect cowpea production and productivity. Insect pests are a major biotic stress, which contributes to significant grain yield losses in both developed and developing countries. Aphids are very problematic in cowpea as they feed on the fruiting stem, resulting in the interruption of nutrient translocation to the developing pods and seeds (Gbaguidi *et al.* 2013; Mofokeng *et al.* 2021). Beetles and other parasitic pests also live and feed on the crop and transmit cowpea viruses (Hema *et al.* 2014). Parasitic weeds attach and grow around the cowpea root surface, thereby absorbing the water and nutrients intended for the growth and development of the crop (Horn *et al.* 2015). Major parasitic weeds of cowpea in SSA include *Striga gesnerioides Vatke* and *Alectra vogelii* (Noubissietchiagam *et al.* 2010).

There are numerous major diseases (Table 2.1) of cowpea, because of bacteria, nematodes, viruses, and fungi, most of which result in drastic grain yield losses (Gonçalves *et al.* 2016).

The symptoms of the diseases vary from discolouration of plants, wilting, death of young plants and poor crop grain yield. The control measures such as the use of resistant cultivars, planting certified seeds of resistant cultivars, treatment with insecticides, fungicides and controlling weeds can mitigate the problem of cowpea diseases. Parasitic weed (*S. gesnerioides*) has a negative effect on the productivity of cowpea, due to its ability to remain dormant in the soil for more than 20 years (Kabambe *et al.* 2013; Phiri *et al.* 2018).

2.4.2. Abiotic constraints

The major abiotic constraints on cowpea production include heat, salinity, and drought stress (Horn and Shimelis 2020). Salinity hampers the ability of cowpea roots to take up water, resulting in changes in metabolites and growth reduction (Khan *et al.* 2007). Exposure to heat for extended time results in irreversible damage to plant metabolic processes. A prolonged period without appreciable rainfall, or when crops do not receive enough moisture for growth and development, is classified as drought (Bodner *et al.* 2015). Water stress during early plant establishment, flowering and pod filling stages severely affects productivity (Nkoana *et al.* 2019). Although cowpea is drought tolerant when compared to other crops, severe drought, especially during pod setting and grain filling stages, can hamper the production of the crop (Singh and Reddy 2011). The current effects of climate change, where rainfall and temperature patterns keep changing, require that efforts be made to enhance the level of drought tolerance, including breeding for improved cowpea cultivars (Fatokun *et al.* 2012).

Table 2.1 The list of major diseases of cowpea and estimated grain yield losses in cowpea producing regions

Pathogen (Scientific name)	Disease	Estimated yield losses	Plant area affected	References
Cercospora canescens	Cercospora leaf spot	40%	Complete matured plants	Omoigui *et al.* (2019)
Uromyces phaseoli var. *vignae*	Cowpea rust	100%	Complete young/mature plants	Honnur *et al.* (2016)
Comovirus	Cowpea mosaic virus	80 to 100%	Leaf and flowers	Damiri *et al.* (2013)
Potyvirus	Cowpea aphid borne mosaic	90%	Complete young/mature plants	Mbeyagala *et al.* (2014)
Cucumovirus	Cucumber mosaic virus	90%	Complete young/mature plants	Mbeyagala *et al.* (2014)
Colletotrichum falcatum	Cowpea severe mosaic virus	90%	Complete young/mature plants	Mbeyagala *et al.* (2014)
Colletotrichum capsici	Brown blotch	75%	Complete young plants	Alabi (1994)
Xanthomonas axonopodis pv. *vignicola*	Cowpea bacterial blight	53 to 71%	Leaves	Viswanatha *et al.* (2011)
Protomycopsis phaseoli	Leaf smut of cowpea	20 to 100%	Leaves	Mbeyagala *et al.* (2014)
Phytophthora vignae	Stem rot of cowpea	50%	Stem	Honnur *et al.* (2016)
Rhizoctonia solani, Macrophomina and Fusarium sp	Root rot of cowpea	10%	Root	Woudenberg *et al.* (2013)

2.5. COWPEA GENETIC RESOURCES AND MANAGEMENT

About 16 203 (Table 2.2) cultivated cowpea genotypes from 88 countries are maintained in the gene bank of the IITA (Gerrano *et al.* 2022). A total of 12 594 accessions were collected from the farmers' fields as landraces, 1422 accessions are superior improved cultivars for grain yield and morphological characters, and 64 accessions are wild cowpea types (Boukar *et al.* 2019). The ARC-VIMP in SA, maintains about 1200 cowpea accessions, from both the national and international field collections.

IITA collected 2608 (Table 2.2) core cowpea genotypes for research/breeding materials, based on geographical, agronomical, and botanical descriptors (Huynh *et al.* 2013). From the complete core collection, 370 accessions make up a reference set known as a mini core collection. The mini-core collection could be a critical resource for scientists to check new adaptive characteristics, conduct comparative genomic studies, and find new favourable alleles and new genotypes for pre-breeding activities (Huynh *et al.* 2013). In addition to the cowpea mini core collection, the primary eight-parent cowpea multi-parent advanced generation inter cross (MAGIC) population was developed recently as a crucial genomic community resource for trait discovery and breeding in the United States and SSA (Table 2.3) (Huynh *et al*. 2018). The eight donor parental genotypes were selected based on agro-morphological trait variability, biotic resistance, and abiotic tolerance (Huynh *et al.* 2018).

Table 2.2 The cowpea collection list based on their biological status in entire and core collections at the International Institute of Tropical Agriculture

Biological status	Total collection	Core collection
Research/breeding materials	2608	223
Advanced cultivars	14	2
Other genotypes	48	2
Traditional landraces	12594	1701
Unknown	875	128
Weedy	38	3
Wild	26	3
Total	16203	2062

Source: Boukar *et al.* (2015; 2019).

Table 2.3 Multi-parent advanced generation inter-cross founder parental genotypes and their characteristics relevant to sub-Saharan Africa

Name	Source	Agronomic trait	Resistance or tolerance trait	Country
SuVita 2	INERA	High yielding under drought, large dark brown seed	Drought tolerant, resistant to Striga, foliar thrips and Macrophomina disease	Senegal, Burkina Faso and Mozambique
CB27	UCR	High yielding under drought, large black eye seed, erect growth habit; early maturity	Heat tolerant, resistant to root-knot nematode, Fusarium wilt and foliar thrips	Mozambique
IT93K-503-1	IITA	High yielding under drought, brown eye seed, stay green under drought.	Drought tolerant, resistant to nematodes, Fusarium wilt and Macrophomina	Senegal
IT89KD-288	IITA	High yielding under drought, brown eye seed; photoperiod sensitive	Resistant to root-knot nematode	Burkina Faso and Nigeria
IT84S-2049	IITA	High yielding under drought, brown eyed seed, and erect growth habit	Resistant to aphid, bacterial blight, viruses, and root-knot nematode	Burkina Faso
IT82E-18	IITA	High yielding under drought, light brown seed, and early maturity	Broadly adapted, resistant to root-knot nematode	Mozambique
IT00K-1263	IITA	High yielding under drought, dark brown seed stay green under drought	Resistant to Striga, aphid, Fusarium wilt and root-knot nematode	Mozambique and Nigeria
IT84S-2246	IITA	High yielding under drought, dark brown seed	Resistant to aphid, bacterial blight, viruses, and root-knot nematode	Burkina Faso and Mozambique

IITA = International Institute of Tropical Agriculture; UCR = University of California, Riverside; INERA= Institute of Environment and Agricultural Research. Source: Huynh *et al.* (2018).

2.6. GENETIC DIVERSITY ANALYSIS

Genetic diversity is essential in crop improvement breeding programmes, which commonly use agro-morphological, biochemical, and molecular markers. Conventional characterisation using agronomic and morphological characteristics are commonly of interest to plant breeders (Gbaguidi *et al.* 2013; Gerrano *et al.* 2020; Mbuma *et al.* 2021). Morphological markers used include seed size, pod size, seed colour and number of pods per plant, and the characteristics directly correlated with the grain yield of cowpea (Gerrano *et al*. 2015; Gerrano *et al.* 2017). Morphological markers are the most used to measure variability. These are relatively cheap, requiring simple equipment and are readily available, yet less reliable, because of environmental factors (Mafakheri *et al.* 2017; Mbuma *et al.* 2021). Countries such as Mozambique assessed their genetic diversity using morphology (Gomes *et al.* 2021) as did South Africa (Gerrano *et al*. 2015; Mbuma *et al.* 2021). The use of morphological and molecular markers was reported on accessions studied in several countries, including Zambia and Malawi (Nkhoma *et al*. 2020), Iran (Mafakheri *et al.* 2017), and Nigeria (Iseghohi *et al.* 2019).

Molecular markers are more robust and reliable to analyse genetic diversity. Molecular markers such as AFLP (Tosti and Negri 2002), SSRs (Siise and Massawe 2013), RAPD (Igwe *et al.* 2017) and SNP have been successfully used to analyse genetic diversity in cowpea (Nkhoma *et al.* 2020; Souleymane *et al.* 2018). Combining molecular and phenotypic marker results is a more robust method to allow the analysis of genetic diversity (Araus and Cairns 2014) for the identification of potential parental genotypes for breeding. Genetic diversity in cowpea has declined due to abiotic and biotic factors, and the artificial selection of preferred superior cultivars from the initial narrow genetic base (Horn *et al.* 2015). Several studies (Fang *et al.* 2007; Boukar *et al.* 2019; Bhadkaria *et al.* 2020; Nwosu and Nwadike 2021) from Asia, North America and Africa revealed a narrow genetic base of cowpea and a strong genetic relatedness among Asia, United State and African cowpea collections.

2.6.1. Genetic diversity for nutritional characteristics breeding

The comprehension of the genetic mechanism for the expression of nutritional properties and genetic control of cowpea grain is vital for the improvement of the nutritional characteristics. The major nutritional components of cowpea and other grain legume crops are proteins, and their functional and nutritional properties depend on their isoelectric point, electrostatic charges; net charge, identity, and location of amino acids (Gonçalves *et al*. 2016). Several studies have reported significant genetic variation in protein content and micronutrient content in cowpea grains, suggesting possible improvement of nutritional value through breeding (Gupta *et al.* 2010; Tchiagam *et al*. 2011; Boukar *et al.* 2015; Mbuma *et al.* 2021). Boukar *et al.* (2011) evaluated 1541 accessions of cowpea for minerals and protein content variability and reported a range of 30.00 to 32.20% protein, 77.00 to 79.50 mg kg^{-1} iron and 54.00 and 58.00 mg kg^{-1} zinc in grains. Cowpea cultivars with high protein content include IT97K-499-35, IT89KD-245 and IT89KD-288 (Boukar *et al.* 2011). Genetic variability among various cowpea genotypes for the concentration of mineral elements and phytochemical composition were previously reported (Gerrano *et al.* 2019; Mbuma *et al.* 2021). These findings indicate a good potential to improve the cowpea genotypes though identification and selection of accessions that have useful genes for crop improvement.

2.6.2. Genetic diversity for drought tolerance breeding

Breeding for a target trait largely relies on the presence of genetic diversity for the trait. It is important to have a diverse population for screening to create adequate genetic variability for a robust selection response (Govindaraj *et al.* 2015). The population should also be large enough to allow selection of superior genotypes for the characteristics of interest (Nkomo *et al.* 2020). Drought is a complex quantitative trait, and breeding for drought tolerance combines selection for grain yield with expression of physiological characteristics correlated with drought tolerance to repeatable controlled stress environments. Several studies (Barrera-Figueroa *et al.* 2011; Farooq *et al.* 2012; Nkoana *et al.* 2019) have indicated the presence of genetic variability for drought tolerance in cowpea, at different growth stages (reproductive, seedling and vegetative stages) using physiological and morphological characteristics. Progress towards cultivar development for drought tolerance is scarce, due to the complexity of this trait. The presence of diversity will aid in germplasm conservation strategies and the development of drought tolerant cultivars for cultivation.

2.7. DROUGHT TOLERANCE BREEDING IN COWPEA

Drought tolerance is a very complex trait as it involves many genetically and physiologically linked genes, being a quantitatively inherited trait, and it is difficult to study (Fang and Xiong 2015; Nkoana *et al.* 2019). Lack of affordable, easy-to-use, and trustworthy screening techniques is one of the numerous obstacles impeding cowpea efforts to breed for drought tolerance (Goufo *et al.* 2017). Carvalho *et al.* (2017) proposed the combination of biochemical, agronomic and physiological characteristics, to integrate the rooting characteristics, cellular water relations, leaf area, and proline accumulation to screen for drought tolerance on cowpeas. Exposure to drought generally results in reduction in grain yield, however, cowpea that performs well under drought is denoted as tolerant (Iseki *et al.* 2018), which is reported to be associated with reduced leaf area, stomatal opening, and increased root biomass (Labuschagne *et al.* 2008; Belko *et al.* 2012). Drought also reduces accumulation of chlorophyll content in cowpea. A decrease in chlorophyll content due to drought is associated with blocked transportation of photosynthetic products (Cui et *al.* 2020). Proline is an amino acid found in plants and it protects the plants from various stresses, hence, its accumulation is a result of plant responses to water-deficit stress, to reduce cell injury (Carvalho *et al.* 2019). A high proline content in cowpea is an indication of drought tolerance (Toscano *et al.* 2016). Delayed leaf senescence plays a vital role as it allows the plants to survive past the midseason drought and recover when it starts raining (Rivero *et al.* 2007). The trait is also easy to measure.

Field evaluation after selection is important when screening for drought tolerance for confirmation of grain yield performance under drought conditions (Batieno *et al.* 2016). Association of deeper root systems and water use efficiency in cowpea is important for adaptation to drought conditions/stress (Hall 2012). Genomic tools or genomic selection is used to best locate the genomic regions or genes that influence drought tolerance, to understand the genes responsible for this complex trait (Jha *et al.* 2020). Molecular markers closely linked to drought tolerance and grain yield are important in marker assisted selection programmes (Khan *et al.* 2016).

Platforms for high throughput genotyping that use marker-assisted selection (MAS) create opportunities for improvement of complex characteristics like drought and grain yield. Candidate genes associated with drought tolerance were mapped (Table 2.4) (Muchero *et al.* 2010). Marker assisted backcrossing was used to transfer quantitative trait loci (QTL) for grain yield under drought and stay-green into Moussa local (Batieno 2016). Pan *et al.* (2017) constructed a genetic map consisting of 34,868 SNPs and used the map with composite interval mapping to detect eleven QTLs of yield-related characteristics. QTLs for yield components have been identified, which include seed size (Kongjaimun *et al.* 2012b), pod length (Kongjaimun *et al.* 2012a) and horticultural characteristics (Xu *et al.* 2013). Recent research used SNPs for genotyping and association mapping for drought tolerance and yield-related characteristics in cowpea (Nkhoma *et al.* 2020). Research on drought tolerance breeding is still underway and the IITA has released about 24 drought tolerant genotypes in 13 different SSA countries (Table 2.5).

Table 2.4 Genes identified which are involved in drought tolerance in cowpea

Gene designation	Accession number	Gene function	References
VuPAP-α	(AF165891)	PAP important for enzymic cascade leading to membrane lipid degradation under environmental stresses or senescence	Mashilo (2013)
VuPAP-β	(AF171230)	PAP important for enzymic cascade leading to membrane lipid degradation under environmental stresses or senescence	Mashilo (2013)
VuPAT1	(AF193067)	Galactolipid acyl hydrolase involves in membrane degradation induced by drought stress	Matos *et al.* (2001)
CPRD22	(D83972)	Cowpea response to dehydration stress	Diouf (2011)
VuPLD1	(U92656)	Putative phospholipase D is a major lipid-degrading enzyme in plant	Diouf (2011)
CPRD8	(D83970)	Cowpea response to dehydration stress	Muchero *et al.* (2009)
CPRD14	(D83971)	Cowpea response to dehydration stress	Muchero *et al*. (2010)
CPRD12	(D88121)	Cowpea response to dehydration stress	Muchero *et al*. (2010)

Source: Mashilo (2013); Agbicodo *et al.* (2009). PAP = 3-phosphoadenosine-5-phosphate.

Table 2.5 List of cowpea cultivars released based on grain yield performance and drought tolerance in sub-Saharan Africa

Cultivar	Country	Release year
IT97K-499-35	Nigeria	2008
T89KD-288, IT89KD-391	Nigeria	2008
IT97K-499-35, IT97K-499-38,	Nigeria	2008
IT98K-205-8	Niger	2009
IT97K-499-35, IT93K-876-30	Mali	2009
T99K-573-1-1	Niger	2010
T82E-16, IT00K-1263, IT97K-1069-6	Mozambique	2010
T99K-494-6	Malawi	2010
T99K-573-1-1, IT99K-573-2-1	Nigeria	2011
T99K-7-21-2-2-1, IT99K-573-1-1	Tanzania	2012
IT99K-573-2-1, IT98K-205-8	Burkina Faso	2012
IT95K-193-12	Benin	2013
IT00K-1263, IT99K-1122	Tanzania	2013
IT07K-292-10, IT07K-318-33	Nigeria	2013
IT05K-321-2, IT97K-390-2, IT82E-16,	Swaziland	2013
IT-82E-18, IT99K-494-4	Swaziland	2013
IT99K-573-2-1, IT99K-573-1-1	Sierra Leone	2015
IT90K-277-2, IT07K- 211-1-8	South Sudan	2016

Source: Boukar *et al.* (2019).

2.7.1. Quantitative trait loci (QTL) for phenotypic and physiological characteristics

Quantitative characteristics are common features of natural variation in eukaryotic populations, including crop plants, which helps partitioning the total phenotypic variance (Campbell *et al.* 2018). Morphological and physiological characteristics, which are quantitatively inherited, influence the mechanisms of drought tolerance (Farooq *et al.* 2012; Nkoana *et al.* 2019). Phenotypic data identified the SNP trait association based on linkage imbalance mapping. They found seven QTLs, five of which suggested pleiotropic effects between delayed senescence, biomass, and grain yield (Muchero *et al.* 2013). Prior research (Pottorff *et al.* 2012) discovered a significant QTL that affects the drought tolerance of cowpeas and determines their leaf shape. Linkage disequilibrium association mapping and bi-parental QTL mapping (consensus genetic linkage maps) were used to determine SNP-trait associations, and identified seven QTL associated with stay-green, and five of the loci were reported to be involved in higher biomass, grain yield and delayed senescence (Muchero *et al*. 2013).

The literature suggests a mapping population of between 100 and 150 genotypes for QTL identification, to get representative estimates of the variance accounted for by a QTL (Lo *et al.* 2018). The accuracy of QTL in estimating genetic gains depends on precise estimation of QTL position, the robustness, and the stability of QTL across accessions and multiple environments.

Research is currently underway to identify and map more QTL, which would be useful in MAS when identifying QTL associated with drought tolerance (Boukar *et al.* 2019).

2.7.2. Application of omics tools to improve drought tolerance, phenotypic and physiological characteristics in cowpea

The use of multi-omics approaches with high throughput techniques has recently gained more focus in cowpea (Kumar *et al.* 2021). The attention on these tools is due to its ability to accelerate the development of genotypes with higher grain yield, good nutritional value, and tolerance to biotic and abiotic stress (Dhaliwal *et al.* 2020). This efficient tool uses genome, and transcriptome, metabolome, and proteome data, to locate the gene of interest and exploit its functions, and ultimately assists in QTL mapping (Kumar *et al.* 2022).

Genomics studies in cowpea breeding are underway, including the use of single-molecule real-time sequencing, and genetic and optical mapping to develop a single-haplotype inbred cowpea genome (Lonardi *et al.* 2019). The construction of annotated pan-genome assemblies representing six subpopulations of domesticated cowpea are underway (Liang *et al.* 2022). The developed reference genome is vital to study cowpea's physiological superiority under abiotic and biotic stresses, and for mining genes of interest (Ferreira-Neto *et al*. 2021). Genetic linkage maps have been constructed using robust high-throughput SNPs (Pan *et al.* 2017; Angira *et al.* 2022) and QTL mapping for drought tolerance and yield-related characteristics (Nkomo *et al.* 2020). The yield-associated trait QTLs were mapped using Diversity Arrays Technology (DArT) markers. Santos *et al.* (2018) conducted a QTL mapping and transcriptome analysis of cowpea for candidate genes for root-knot nematode resistance. Ferreira-Neto *et al.* (2021) developed a cowpea kinome by using genomic and transcriptomic data under biotic and abiotic stresses.

The pathways underlying drought-tolerant and sensitive cowpea genotypes were investigated using proteomic data. (Lima *et al.* 2019). Furthermore, the proteomic data has been used to compare grain protein profiles of four Brazilian cowpea cultivars (Honaiser *et al.* 2022). Metabolomics for characterising legumes adaptive and signalling response to abiotic stresses were reported (Bueno and Lopes 2020). Genetic diversity and linkage disequilibrium of cowpea accessions were reported (Nkhoma *et al.* 2020; Sodedji *et al.* 2021; Gbedevi *et al.* 2021).

Genome editing, transformation, and regeneration systems were developed quickly and effectively using embryonic axis explants (Che *et al.* 2021). Lo *et al.* (2019) studied the genetic basis of variation in seed size of selected cowpea genotypes, using a combination of genome-wide association and meta-analysis and the results revealed regions associated with seed size in cowpea. Paudel *et al*. (2021) undertook a study to understand the genetic basis of flowering time and reported the presence of candidate genes for flowering associated with the significant SNP markers. Integration of conventional breeding with the modern approach of omics techniques could greatly enhance breeding for drought tolerance and yield in cowpea.

2.8. SPEED BREEDING IN COWPEA

Integration of speed breeding with plant improvement technologies, such as MAS, genome editing, and optimized transformation protocols plays an important role in acceleration of improvement of important characteristics and genetic gains in cowpea breeding (Edet and Ishii 2022). The aim of speed breeding is to reduce the length of a cowpea breeding cycle by manipulation of growing conditions including light intensity, photoperiod, soil moisture, temperature, and soil nutrition (Fiyaz *et al.* 2020). Edet and Ishii (2022) used regulated growth chamber conditions and oven-dried seeds for cowpea speed breeding.

Wanga *et al.* (2021), reviewed the opportunities and challenges of speed breeding, which involves manipulation of temperature, soil nutrition, photoperiod, and light intensity, to harness the development of more breeding generations per annum (El-Hashash and El-Absy, 2019). The challenges of speed breeding include its requirements for proper infrastructure, high expertise, effective plant phenomics facilities and funding (Shimelis *et al.* 2019). Speed breeding approaches allow for acceleration of breeding and population evaluations and hence rapid cultivar development and commercialization (Wanga *et al*. 2021).

2.9. CONCLUSIONS

Cowpea is a vital legume crop cultivated globally for its green pods, fresh leaves, and seeds for human consumption. The crop has a significant potential to improve food and nutritional security and it can be cultivated in arid and harsh regions due to its ability to retain water due to its deep root system. Resources-poor farmers prefer the crop due to its minimal resources input requirements; however, prolonged exposure to drought will result in grain yield loss. Research to develop drought tolerant cowpea cultivars is lacking, probably since drought is a complex trait influenced by many genetically and physiologically linked genes, which in turn, react differently across environments. To harness breeding for drought tolerance, it is vital to integrate the use of molecular, physiological, and morphological markers to exploit genetic diversity of cowpea accessions, using characteristics such as stay green, earliness, harvest index, and pod size. Knowledge of genetic diversity will help in selection of parental genotypes for introgression breeding for drought tolerance. Integration of MAS, use of high-throughput omics; and conventional and speed breeding techniques would enhance and accelerate the development of improved cultivars. The climate is continuously changing, and the prolonged effect of this, including drought, would greatly affect the ecosystems. Cowpea breeders have a mandate to work together, to hasten the development of drought tolerant, high grain yielding cultivars with high nutritional characteristics.

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CHAPTER 3

PERFORMANCE OF SELECTED COWPEA GENOTYPES AND F_1 PROGENIES FOR GRAIN YIELD AND YIELD COMPONENTS

3.1. ABSTRACT

Cowpea grain yield in South Africa remains low compared to other cowpea producing countries, hence it is important to embark on research with the aim of developing high-yielding and stable cultivars. The objectives of this study were to determine genetic diversity among selected cowpea genotypes, to determine the genetic variation for grain yield and yield components and to identify superior parental genotypes and F_1 progenies for the measured characteristics. The genetic diversity of the ten selected cowpea parental genotypes was confirmed using 20 SSR molecular markers. The 10 lines were crossed using a half diallel mating design to produce 45 F_1 progenies, and the 55 genotypes (45 F_1 progenies and 10 parents) were evaluated for grain yield and yield components using a randomised complete block design in four environments. The SSR markers detected 56 alleles, which varied from 1 to 6, with an average of 2.8 alleles per locus among the 10 cowpeas parents. Five markers (SSR6265, SSR6217, SSR6451, SSR6277 and SSR6436) were highly informative (with a polymorphic information content (PIC) ≥ 0.5) which indicated their usefulness in determining the genetic structure of cowpea the 10 parents. The study clustered the parents into three genetic groups, which suggested that a new F1 population can be developed using the contrasting lines. Analysis of variance (ANOVA) on phenotypic traits indicated significant genotype and genotype by location interaction for grain yield and yield components. The phenotypic variability confirmed the genetic diversity of the parents assessed through the SSRs. High broad-sense heritability (> 0.80) was observed for all characteristics except for pod width, indicating the potential for a good response to selection. Six F_1 progenies (IT96D-602 x Glenda, IT96D-602 x Kisumi-mix, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953, TVU13953 x Glenda and IT96D-602 x TVU13953) performed better than their parental genotypes for grain yield, showing heterosis, indicating their potential for selection of transgressive segregants. Parental genotypes TVU13953 and IT96D-602 had consistently high yields and could be a foundation for subsequent breeding efforts.

Keywords: broad-sense heritability, cowpea progenies, genetic diversity, grain yield, primers

3.2. INTRODUCTION

Cowpea is an important grain legume that is widely grown in many parts of the world, particularly SSA, for its edible gains, leafy vegetable and forage. The crop is nutrient-dense and has significant potential to mitigate challenges associated with malnutrition (Goncalves *et al.* 2016; Mbuma *et al.* 2021). The crop can tolerate drought, due to its deep rooting, early flowering, and early maturity (Goncalves *et al.* 2016). Cowpea also has the ability to fix atmospheric nitrogen, thus contributing to soil fertility (Kyei-Boahen *et al.* 2017).

The global cowpea production amounted to 8.99 million ton in 2021, with Nigeria being the top producer (3.63 million tons) and Africa contributing 97% of production (FAOSTAT 2021). Globally, the cowpea grain yield productivity for 2021 was 6026 kg/ha, and South Africa produced only 435 kg/ha with only 4689.16 tons of the world's total production (FAOSTAT 2021). Although global cowpea grain production and yield are high, productivity is still hindered by several constraints, such as insect pests, weeds, diseases, heat and drought (Yirzagla *et al*. 2021; Abdou 2022). The low grain yield in South Africa could be attributed to low soil fertility and a lack of improved cultivars (Omomowo and Babalola 2021).

The small-scale producers in South Africa grow cowpea landraces and other imported accessions. These cowpea accessions are poor yielding, unstable and susceptible to major pests and diseases. South Africa also has cowpea germplasm imported from the IITA, Nigeria, which includes wild species, accessions, mutants, and cultivars developed through hybrid breeding. This imported cowpea germplasm is used mainly for pre-breeding purposes to evaluate adaptation and their performance in South African agro-ecological conditions (Gerrano *et al.* 2015; 2019; Mbuma *et al.* 2021; 2022; Gumede *et al.* 2022a).

Studies conducted in South Africa (Asiwe 2009; Gerrano *et al*. 2019; Mbuma *et al.* 2021; Gumede *et al*. 2022a) reported significant variation among the cowpea genotypes for grain yield and yield components. However, the grain yields observed are still below the potential optimum of 1500 to 2500 kg/ha, probably due to the lack of improved cultivars (Kamara *et al.* 2018; Omomowo and Babalola 2021). Therefore, hybridisation should be employed as intervention strategy to improve cowpea productivity for desired yield components and morphology through selection (Kumari and Chauhan 2018; Owusu *et al*. 2018; 2020). A study that produced dual-purpose second-generation (F_2) cowpea is the only evidence of cowpea breeding in South Africa thus far (Moalafi *et al.* 2010).

According to previous studies, cowpea has a narrow genetic base, which results in compromised genetic gains (Boukar *et al.* 2019; Owusu *et al.* 2020). However, a study by Gumede *et al.* (2022b) to assess the genetic diversity and population structure of 90 cowpea accessions in South Africa, using SNP markers, concluded that the accessions evaluated showed genetic variation and a significant potential to contribute to breeding programmes for developing new cultivars. Thus, it would be beneficial to create genetic variation through hybridisation, in efforts to improve the crop for grain yield, yield components and morphology (Kumari and Chauhan 2018; Owusu *et al.* 2018; 2020). Hence, the objectives of this study were to determine the genetic variation among cowpea parental genotypes and their F_1 progenies for grain yield and yield components and to identify superior progenies and parental genotypes for the measured characteristics.

3.3. MATERIALS AND METHODS

3.3.1. Planting materials

Ten cowpea parental genotypes were obtained from the ARC-VIMP pre-breeding programme. The parental genotypes used in this study were selected based on high grain yield, drought tolerance, and good nutritional characteristics (Fe, Zn, and protein) (Table 3.1). Publications of Gerrano et al. (2015; 2019) and Mbuma et al. (2021; 2022), based on research from the ARC legume breeding programme, provided the basis for the selection of ten genotypes. A half diallel was done to minimize labour and expense constraints and enable efficient use of resources required for crossing.

Table 3.1 Description of parental genotypes used to generate the study population

Genotypes	Characteristic of interest	Colour	Origin	Growth habit
IT93K-129-4	Drought tolerant	Cream white	IITA, Nigeria	Erect
TVU7778	Drought susceptible	Speckled purplish	IITA, Nigeria	Semi-erect
98K-5301	Protein	Cream white	IITA, Nigeria	Semi-erect
Glenda	Zinc	Speckled maroon	ARC, South Africa	Winding
TVU-14196	Iron	Black eye-cream	IITA, Nigeria	Semi-erect
IT845-2246	Iron	Dark brown	IITA, Nigeria	Semi-erect
ITOOK-1060	Zinc	Tan	ARC, South Africa	Winding
Kisumi-mix	Protein	Cream brown	KALRO, Kenya	Winding
TVU13953	Yield	Speckled brown	IITA, Nigeria	Erect
IT96D-602	Yield	Brown eye-cream	IITA, Nigeria	Erect

IITA = International Institute of Tropical Agriculture, KALRO = Kenya Agricultural and Livestock Research Organization, ARC = Agricultural Research Council.

3.3.2. DNA isolation

The ten parental genotypes (Table 3.1) were sown (two seeds per parent) in pots filled with sterile soil in the glasshouse at the University of the Free State, Bloemfontein, South Africa in June 2020. Two-week old leaves were sampled on ice and freeze-dried.

Before isolation of total genomic DNA, the freeze-dried leaf material was homogenised using Qiagen's TissueLyser (Haan, Germany). The freeze-dried leaf material was transferred to a 2 ml microcentrifuge tube with two 5 mm stainless steel ball bearings and homogenised for 30 seconds at 30 r/s. Isolation of total genomic DNA was done by using a CTAB (hexadecyltrimethylammonium bromide) DNA isolation method (Saghai-Maroof *et al*. 1984). CTAB extraction buffer with a volume of 750 µl [100 mM Tris-Cl (tris (hydroxymethyl) aminomethane], pH 8.00; 20 mM EDTA (ethylene-diamine tetraacetate), pH 8.0; 1.4 M NaCl; 2% (w/v) CTAB; 0.20% (v/v) β-mercaptho-ethanol) was added to 250 µl of ground leaf tissue and incubated at 65ºC for 1 hour. A volume of 500 µl chloroform:isoamylalcohol (24:1 (v/v) was added and mixed well. Samples were centrifuged at 12000 x *g* for 5 minutes at 5ºC. DNA from the aqueous phase was precipitated with 0.66 volumes isopropanol and incubated at room temperature (20-25ºC) for 20 minutes. After incubation samples were centrifuged at 12000 x *g* for 5 minutes at 5ºC. The supernatant was discarded and tubes drained upside down. The precipitate was washed by adding 500 µl ice-cold 70% (v/v) ethanol and incubated for 20 minutes at room temperature. Samples were centrifuged at 12000 x *g* for 5 minutes at 5ºC.

The supernatant was discarded, and the pellets were air-dried for 1 hour at room temperature (20 - 25ºC). Air-dried DNA samples were re-suspended in TE buffer (10 mM Tris-Cl, pH 8.00; 1 mM EDTA, pH 8.00) overnight at 4ºC. RNase A (0.1 mg/ml) was added and incubated at 37 ºC for 1 - 2 hours. DNA quantity and quality were estimated from a 0.8% (w/v) agarose gel with electrophoresis at 80 V in UNTAN (40 mM Tris-Cl; 2 mM EDTA, pH adjusted to pH 7.4 with acetic acid) buffer. DNA was visualised with ethidium bromide staining under UV light. The concentration of the DNA samples was determined by using a UV spectrophotometer and measuring absorbance at A_{260} and A_{280}. Samples were diluted to a working concentration of 20 ng/µl for subsequent experiments.

3.3.3. SSR analysis

Twenty SSR primers (Table 3.2) were used to confirm genetic diversity of the 10 cowpea parental genotypes following the protocol described by Danso *et al*. (2018). Each PCR amplification reaction contained 40 ng DNA, 2 mM $MgCl_2$, 1x KAPATaq Ready-mix DNA polymerase, 50 ng each of the forward and reverse primer (Integrated DNA technologies) and 0.10 mg/ml Bovine serum albumin (BSA) in a total reaction volume of 10 µl.

Table 3.2 List of molecular markers used to assess the genetic diversity of the cowpea parental genotypes, adopted from Danso *et al.* (2018)

SSR	Forward primer (5'-3')	Reverse primer (3'-5')	Annealing temperature (ºC)
SSR-6265	GCATGTTGCTTTGACAATGG	CAGAAGCGGTGAAAATTCAAC	55.00
SSR-6258	ATTATGCCATGGAGGGTTCA	GGTTTCCTAGTTGGGAAGGAA	55.00
SSR-6243	CAACCGATGTAAAAAGTGGACA	GTAGGGAGTTGGCCACGATA	55.00
SSR-6218	AGGAAATTTGCATTCCCTTGT	GTGGAAGGAATGGGTCCAG	55.00
SSR-6217	TTCCCTATGAACTGGGAGATCTAT	GGGAGTGCTCCGGAAAGT	55.00
SSR-6353	AAACCATGTGGTTGTTGCAC	TCATGGGTTAAATTTGCTTCAA	50.90
SSR-6352	AATTTTGAACCCACCACCAG	GTTGTGAGCTTCCCCAGATG	55.00
SSR-6336	TCAGTCTTAGAATTGAGTTTTCTTCG	TGAAAAACAACGATATGCAGAAG	55.00
SSR-6323	TTTAAGCAGCCAAGCAGTTGT	CAAAGGGTCATCAGGATTGG	55.00
SSR-6277	CACTTAAATTTTCACCAGGCATT	CACCCCCGTACACACACAC	50.90
SSR-6436	TTTCGCAATATGCCCTTTTC	GCAGAATCCTTGTGAACCTG	50.90
SSR-6375	TCAGTGTCAGCACCATACCC	GCTCGGATATGGTCCTGAAA	55.00
SSR-6371	CACTTCAGACTTAGAGCGAAGAAA	TGCTCATCGTGCTTTGTCTT	55.00
SSR-6370	TTGAAGGTATGGCCTTTTGTTT	CAACTTCACAGCCCTCACAA	55.00
SSR-6356	ATGCCCCAACAACAACATTT	TGCAATATGGACCAGAAGAAA	55.00
SSR-6613	CTTTACCTTTATGCAAACCAATTC	CTATTGGAATCTTGCCGTTG	55.00
SSR-6608	GGTTAAGGAAAAGAGGGTAGG	CTAAATTATAATATTCGTCGGTC	55.00
SSR-6503	CGCGGTAGCATGATTGAATTTTG	GAGAACTTCACGCACAATAG	55.00
SSR-6587	GTTGAAAGTTTGATAGTAAAGTGG	GATATAGAATAGCATATTTAACATATTAG	55.00
SSR-6451	GACCAACAGCGACTTTGAGC	AAAGAGATACACATGCCTAACA	55.00

The optimised cycling conditions for the primers were 1 cycle at 94ºC for 2 minutes followed by 35 cycles of denaturation for 30 seconds at 94ºC. The annealing process followed for 30 seconds at 55 or 50.9ºC (depending on the primer), then initial extension for 1 minute at 72ºC and lastly the final extension for 10 minutes at 72ºC.

PCR products for all markers used were separated using the Gel Scan 3000 Real-Time DNA Fragment Analysis system with software version 8.00.01 (Corbett Research, Sydney, Australia). The 5% (w/v) non-denaturing polyacrylamide gel (PAGE) was prepared to a final volume of 25 ml consisting of 1 x TBE (Tris-HCl/Borate/EDTA) buffer (89 mM Trisbase; 89 mM Boric acid; 2 mM EDTA, pH 8.0), 5% AKA (acrylamide:bis-acrylmide; 19:1 w/w), 0.12% (v/v) TEMED (N,N,N',N tetramethyl ethylenediamine) and 0.08% (v/v) APS (ammonium persulfate). Polyacrylamide gels were prepared and left to polymerise overnight at room temperature (20 - 22°C).

The vertical system consisted of an upper and bottom chamber. The upper chamber buffer contained 0.5 x TBE buffer mixed with doubled distilled water (ddH$_2$O). The bottom chamber buffer contained 0.5 x TBE, and 1% (v/v) EtBr mixed with ddH$_2$O. Polymerase chain reaction products were mixed with deionised formamide loading dye (98% (v/v) formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue). Before samples were loaded on the gel, a pre-run was done at 800 V for 45 min at 37°C and 1 µl loading dye was loaded on the gel to track the progress of the pre-run. After the pre-run, 1 µl of the PCR samples mixed with loading dye were loaded on the gel and run at 1 200 V for 45 minutes at 37°C. The 100 bp DNA ladder (Promega, Madison, WI, USA) was loaded on both sides of the gel to determine amplified fragment sizes. The fragments were identified on molecular weight and scored based on presence/absence (1/0). DARwin software (6.0.021) was used to analyse the molecular data and generate a dendrogram.

3.3.4. Development of F$_1$ populations

To synchronize flowering, three seeds of each of the ten selected parental genotypes were planted per pot (filled with sterile loam soil mixed with compost) per week for a total of five pots per genotype per week. To guarantee optimal seed production, crosses were made at the ARC-VIMP and the University of Free State in sterile soil-filled pots, over the course of three weeks. Watering the plants was done twice a day to field capacity and fertilisation with multi-feed was done once every two weeks. A half-diallel mating design was used to cross the parental genotypes in the glasshouse between eight and ten in the morning, the female parental genotypes' flower buds were emasculated and were pollinated by the male parental genotypes' pollen during anthesis. The seeds from the successful F$_1$ progenies (45) were then collected and bulked for evaluation in the field.

3.3.5. Field trail locations

Field experiments were conducted at the ARC research farms, at Loskop and Brits in 2021 and 2022 during the summer cropping season (Table 3.3). Due to low amounts of seed generated from crosses, there was only enough seed for trials in two environments (Loskop and Brits) and two seasons (2021 and 2022). Loskop is situated in the Limpopo Province and is characterised by sandy loam soil and a pH value that ranged from 4.00 to 8.50. Brits is in the Northwest Province, and it is characterised by clay loam soil with a pH ranging from 5.90 to 8.08.

Table 3.3 Description of environmental conditions of the study locations

Descriptions	Loskop: Limpopo	Brits: North-West
Latitude	25°17'59" S	25°9280" S
Longitude	29°39'57" E	27°88'19" E
Altitude (m) above sea level	920	1.118
Average maximum temperature °C	27.38	26.75
Average minimum temperature °C	18.90	18.60
Soil characteristics		
Soil type	Sandy loam	Clay loam
pH (H_2O) 1:2.5	7.31	7.20
P (Bray1)	17.00	26.00
Mg%	39.90	46.80
Ca%	58.30	48.30
K%	3.10	5.60
Na%	0.60	0.80

P = Phosphorus, Mg = Magnesium (%), Ca = Calcium (%), K = Potassium (%), Na = Sodium (%).

3.3.6. Experimental design, trial establishment and management

Fifty-five genotypes (45 F_1 progenies and 10 parents) were planted in a randomised complete block design with three replications, planted using one seed per hole of a 3 m plot spaced at 1 m between rows and 0.50 m between plants. Manual weeding and other normal cultural practices were used when needed to ensure optimal growing conditions. Supplementary irrigation was applied using sprinklers for two hours to give an amount of 12.50 mm when needed to prevent drought stress.

3.3.7. Data collection

Quantitative characteristics were recorded according to the cowpea descriptors (Alves and Bettencourt 2007). The data was collected on five randomly selected plants in each replication. The parameters recorded were plant height (PH, cm), number of branches (NB), leaf length (LL, cm), leaf width (LW, cm), days to 50% flowering (D50F), number of pods per plant (NPP), hundred seed weight (HSWt, g), and grain yield (GY, t/ha). Pod length (PL, mm), pod width (PW, mm) and number of seeds per pod (NSPP) were measured from 10 randomly selected pods. Grain yield was converted to t/ha using the equation:

$$\text{Grain yield} = \frac{\text{Plot weight}}{\text{Plot area}} \text{ x } \frac{(100-14)}{100-\text{mc}} \; x \; 10\,000$$

Where mc was the moisture content of grain at harvest, 14% was the standard constant for legume moisture content, and 10,000 is the conversion factor used for hectare (Nkhoma 2020).

3.3.8. Data analysis

3.3.8.1. Confirmation of genetic diversity

The alleles were scored as present (1) or absent (0) based on the size of the amplified product. Scored data were used to construct a binary data matrix for statistical analysis. Total allele number (Na) and average number of alleles at each locus were calculated manually. Allelic polymorphic information content (PIC) was calculated from the binary data of the 20 SSR markers using an iMEC online marker efficiency calculator developed by Amiryousef *et al.* (2018). PIC evaluates polymorphism of a marker by characterizing the efficiency of each primer for detecting polymorphic loci (Shete *et al.* 2000). A PIC of > 0.50 indicates high diversity, a PIC < 0.25 low diversity and a PIC between 0.25 and 0.50 intermediate diversity (Botstein *et al.* 2004). Cluster analysis based on the unweighted neighbour-joining (NJ) with 30 000 bootstrap repetitions was conducted with DARwin 6.0.19 (Perrier and Jacquemoud-Collet 2006) to construct a rooted dendrogram. Cluster analysis information is sensitive to closely related individuals; therefore, the constructed dendrogram illustrates the relationship of the cowpea parental genotypes based on SSR allele variation. Genetic similarities between the cowpea parental genotypes were compared by using the Jaccard similarity coefficient (Jaccard 1908).

3.3.8.2. Analysis of variance

ANOVA was done on data of all yield components using AGROBASE generation II SQL-version 38 (2019) statistical software. The least significant difference (LSD) at $P \leq 0.001$ and $P \leq 0.05$ was used for means separation. The following statistical linear mixed model was used for genotype analysis across environments.

$$Y_{ijk} = \mu + G_i + L_l + R_k(L_l) + \varepsilon_{ljk}$$

Where Y_{ijk} = Yield components in the k^{th} replication of the i^{th} genotype recorded at the l^{th} location, L_l = Random effect of the l^{th} location, G_i = Random effect of the i^{th} genotype, $R_k(E_i)$ = Effect of k^{th} replication in the l^{th} location, ε_{ljk} = Residual error or random experimental error, μ = Grand mean. The combined ANOVA used the statistical linear mixed model:

$$Y_{ijkl} = \mu + G_i + L_l + R(L)_{k(l)} + GL_{il} + GR(L)_{ik(l)} + S_j + SL_{lj} + SR(L)_{jk(l)} + GS_{ij} + GLS_{ijl} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = Observation for ith genotype, in jth season, in kth replication nested within lth location, μ = Overall mean, G_i = The random effect of the ith genotype, L_l = The random effect of the lth location, $R(L)_{k(l)}$ = The random effect of the kth replication nested within the lth location t and was the error term for the environment effect. Where, GL_{il} = Random interaction effect between the ith genotype and the lth location, $GR(L)_{ik(l)}$ = The random interaction effect between the ith genotype and the kth replication nested in the lth location and was the error term for the genotype and genotype by environment interaction effect. Where, S_j = Random effect of the jth season, SL_{jl} = The random interaction effect between the jth season and the lth location. Where, $SR(L)_{jk(l)}$ = Random interaction effect between the jth season and the kth replication nested within the lth location and was the error term for the season and location by season interaction effect. Where, GS_{ij} = Random interaction effect between the ith genotype and the jth season, GLS_{ijl} = Random interaction effect between the ith genotype, lth location and jth season, and $\mathcal{E}_{ijkl}$ = Residual error.

Variability in performance amongst genotypes was further ascertained by the genotypic (σ^2_g) and phenotypic variance (σ^2_p) which were determined by the formula:

$$\sigma^2 g = \frac{MSg - MS\mathcal{E}}{r}$$

Genotypic variance across environments was determined by the formula:

$$\sigma^2 g = \frac{MSg - MS\mathcal{E}}{re}$$

Genotypic variance for combined analysis was determined by the formula:

$$\sigma^2 g = \frac{MSg + (MSgls - MSgl - MSgs)}{rls}$$

The phenotypic variance for individual environments was determined by the formula:

$$\sigma^2 p = \sigma^2 g + \sigma^2 \mathcal{E}$$

The phenotypic variance across environments was determined by the formula:

$$\sigma^2 p = \sigma^2 g + \sigma^2 gl/l + \sigma^2 \mathcal{E}/\mathrm{gl}$$

The phenotypic variance for combined environments was determined by the formula:

$$\sigma^2 p = \sigma^2 g + \sigma^2 gl/l + \sigma^2 gs/s + \sigma^2 gsl/ls + \sigma^2 \mathcal{E}/\mathrm{rls}$$

Where $\sigma^2 g$ = Genotypic variance, $\sigma^2 p$ = Phenotypic variance, $\sigma^2 ge$ = Genotype by environment variance, $\sigma^2 gs$ = Genotype by season variance, $\sigma^2 gsl$ = Genotype by location by season variance, environmental error ($\sigma^2 \mathcal{E}$) = Error mean squares, MSg = Mean squares for genotype, $MSgsl$ = Mean squares for genotype by season by location interaction, $MSgl$ = Mean squares for genotype by l^{th} location, $MSgs$ = Mean squares for genotype by season, MS_{ε} = Mean squares for error, r = Number of replication, l = Number of l^{th} locations and s = Number of seasons.

Broad-sense heritability (H^2) was determined for each trait using the formula:

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

Where $\sigma^2 g$ = Genotypic variance, $\sigma^2 p$ = Phenotypic variance.

ANOVA was done on data of all yield components using AGROBASE generation II SQL-version 38 (2019) statistical software. The least significant difference (LSD) at $P \leq 0.001$ and $P \leq 0.05$ was used for means separation. The following statistical linear mixed model was used for genotype analysis across environments.

$$Y_{ijk} = \mu + G_i + L_l + R_k(L_l) + \varepsilon_{ljk}$$

Where Y_{ijk} = Yield components in the k^{th} replication of the i^{th} genotype recorded at the l^{th} location, L_l = Random effect of the l^{th} location, G_i = Random effect of the i^{th} genotype, $R_k(E_i)$ = Effect of k^{th} replication in the l^{th} location, ε_{ljk} = Residual error or random experimental error, μ = Grand mean. The combined ANOVA used the statistical linear mixed model:

$$Y_{ijkl} = \mu + G_i + L_l + R(L)_{k(l)} + GL_{il} + GR(L)_{ik(l)} + S_j + SL_{lj} + SR(L)_{jk(l)} + GS_{ij} + GLS_{ijl} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = Observation for i^{th} genotype, in j^{th} season, in k^{th} replication nested within l^{th} location, μ = Overall mean, G_i = The random effect of the i^{th} genotype, L_l = The random effect of the l^{th} location, $R(L)_{k(l)}$ = The random effect of the k^{th} replication nested within the l^{th} location t and was the error term for the environment effect. Where, GL_{il} = Random interaction effect between the i^{th} genotype and the l^{th} location, $GR(L)_{ik(l)}$ = The random interaction effect between the i^{th} genotype and the k^{th} replication nested in the l^{th} location and was the error term for the genotype and genotype by environment interaction effect. Where, S_j = Random effect of the j^{th} season, SL_{jl} = The random interaction effect between the j^{th} season and the l^{th} location. Where, $SR(L)_{jk(l)}$ = Random interaction effect between the j^{th} season and the k^{th} replication nested within the l^{th} location and was the error term for the season and location by season interaction effect. Where, GS_{ij} = Random interaction effect between the i^{th} genotype and the j^{th} season,

GLS_{ijl} = Random interaction effect between the i^{th} genotype, l^{th} location and j^{th} season, and $\mathcal{E}_{ijkl}$ = Residual error.

Variability in performance amongst genotypes was further ascertained by the genotypic (σ^2_g) and phenotypic variance (σ^2_p) which were determined by the formula:

$$\sigma^2 g = \frac{MSg - MS_{\mathcal{E}}}{r}$$

Genotypic variance across environments was determined by the formula:

$$\sigma^2 g = \frac{MSg - MS_{\mathcal{E}}}{re}$$

Genotypic variance for combined analysis was determined by the formula:

$$\sigma^2 g = \frac{MSg + (MSgls - MSgl - MSgs)}{rls}$$

The phenotypic variance for individual environments was determined by the formula:

$$\sigma^2 p = \sigma^2 g + \sigma^2 \mathcal{E}$$

The phenotypic variance across environments was determined by the formula:

$$\sigma^2 p = \sigma^2 g + \sigma^2 gl/l + \sigma^2 \mathcal{E}/\mathrm{gl}$$

The phenotypic variance for combined environments was determined by the formula:

$$\sigma^2 p = \sigma^2 g + \sigma^2 gl/l + \sigma^2 gs/s + \sigma^2 gsl/ls + \sigma^2 \mathcal{E}/\mathrm{rls}$$

Where $\sigma^2 g$ = Genotypic variance, $\sigma^2 p$ = Phenotypic variance, $\sigma^2 ge$ = Genotype by environment variance, $\sigma^2 gs$ = Genotype by season variance, $\sigma^2 gsl$ = Genotype by location by season variance, environmental error ($\sigma^2 \mathcal{E}$) = Error mean squares, MSg = Mean squares for genotype, $MSgsl$ = Mean squares for genotype by season by location interaction, $MSg\mathrm{l}$ = Mean squares for genotype by l^{th} location, $MSg\mathrm{s}$ = Mean squares for genotype by season, $MS_{\mathcal{E}}$ = Mean squares for error, r = Number of replication, l = Number of l^{th} locations and s = Number of seasons.

Broad-sense heritability (H^2) was determined for each trait using the formula:

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

Where $\sigma^2 g$ = Genotypic variance, $\sigma^2 p$ = Phenotypic variance.

3.4. RESULTS

3.4.1. SSR marker analysis

All the markers produce all expected SSR allele size fragments and can be used for confirmation of genetic diversity among the 10 cowpea parental genotypes. Three of the markers (SSR6336, SSR6370, and SSR6603) were excluded because they were monomorphic for all alleles produced at those loci. In total 56 alleles were detected in the10 cowpea parental genotypes. The number of alleles per locus ranged from one to six, with an average of 2.8. Markers SSR6451 and SSR6436 had the most alleles (six each), while three markers, SSR6336, SSR6370, and SSR6603, had only one allele each (Table 3.4).

The PIC ranged from 0.16 to 0.79 with an average value of 0.43. Out of 17 polymorphic markers, five (29.40%) markers were highly informative (PIC ≥ 0.50), ten markers (58.80%) were intermediate informative (PIC = 0.25 - 0.50), and two markers (11.80%) had low informativeness (PIC ≤ 0.25). The cowpea parental genotypes had a mean gene diversity of 0.49, with values of individual markers ranging from 0.18 (SSR6323 and SSR6356) to 0.81 (SSR6451) (Table 3.4).

Table 3.4 SSR marker analysis showing the number of alleles detected, gene diversity and the polymorphic information content values

Marker	**Number of alleles**	**Gene diversity**	**PIC**
SSR6265	5	0.74	0.70
SSR6258	2	0.44	0.34
SSR6243	2	0.32	0.27
SSR6218	3	0.34	0.31
SSR6217	3	0.64	0.56
SSR6352	3	0.58	0.49
SSR6323	2	0.18	0.16
SSR6375	2	0.32	0.26
SSR6371	2	0.48	0.36
SSR6356	2	0.18	0.16
SSR6613	3	0.54	0.44
SSR6608	4	0.51	0.48
SSR6587	2	0.32	0.26
SSR6451	6	0.81	0.78
SSR6353	2	0.32	0.26
SSR6277	5	0.78	0.74
SSR6436	6	6356	0.71
Mean		0.49	0.42

3.4.2. Genetic relationships amongst cowpea parental genotypes

The ten cowpea parental genotypes used in this study were divided into three main clusters (Figure 3.1). Cluster I had two genotypes (TVU-14196 and IT96D-602) from Nigeria, and they showed 50% genetic similarity. Cluster II contained three genotypes (IT845-2246, TVU13953, and Kisumi-mix), each with a different shade of brown seed. Cluster III included five genotypes (Glenda, ITOOK-1060, 98K-5301, TVU7778, and IT93K-129-4). Two of the genotypes within the cluster are cream-white and one is tan. The other two genotypes are speckled, possibly indicating a cross between the cream white and tan genotypes. Three genotypes are from Nigeria, and two from South Africa. Table 3.5 shows the genetic distances between parental genotypes, ranging from 0.29 (for ITOOK-1060 and TVU7778) to 0.61 (for TVU13953 and Glenda, TVU-14196 and Glenda).

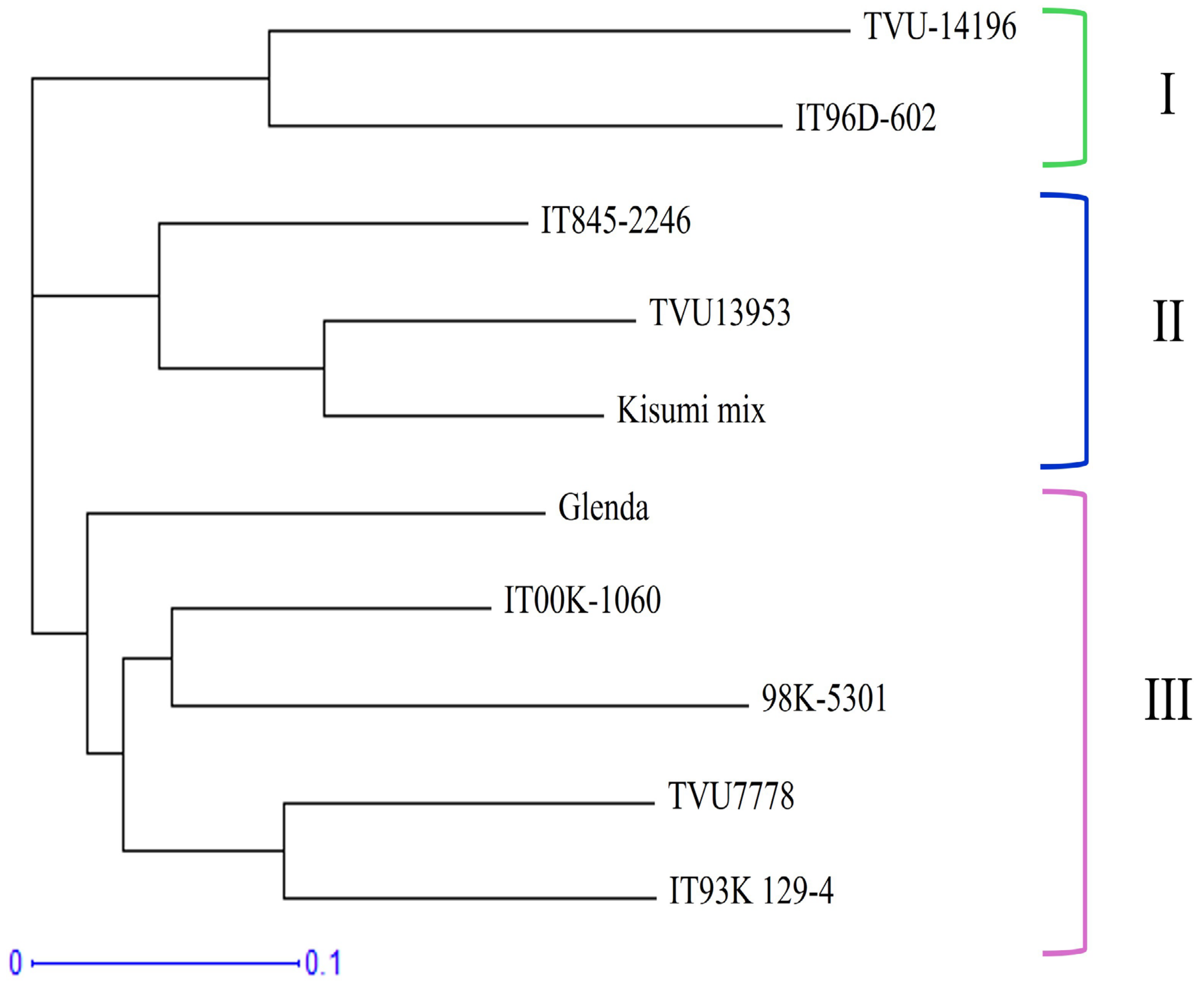

Figure 3.1 Dendrogram constructed based on genetic distance from simple sequence repeat molecular markers of the ten selected cowpea parental genotypes

Table 3.5 Genetic distance among the ten selected parental genotypes

Parental genotypes	Glenda	Kisumi-mix	IT845-2246	IT96D-602	IT93K-129-4	ITOOK-1060	TVU7778	TVU13953
Kisumi-mix	0.53							
IT845-2246	0.45	0.44						
IT96D-602	0.47	0.44	0.50					
IT93K-129-4	0.45	0.42	0.50	0.39				
ITOOK-1060	0.36	0.38	0.42	0.31	0.53			
TVU7778	0.47	0.38	0.47	0.42	0.50	0.29		
TVU13953	0.61	0.39	0.24	0.26	0.53	0.50	0.42	
TVU-14196	0.61	0.58	0.45	0.53	0.42	0.55	0.47	0.58

3.4.3. Analysis of variance for grain yield and yield components

Significant genotype effects ($P \leq 0.05$) were observed for all the characteristics measured at all locations (Table 3.6). The effect of the environment (Brits and Loskop 2021) was highly significant ($P \leq 0.001$) for GY, NPP, NSPP, HSWt and PH. For Brits and Loskop 2022, the location effect was highly significant ($P \leq 0.001$) for GY, NSPP, HSWt and PH. Genotype by location interaction effect was highly significant ($P \leq 0.001$) for all characteristics, except for season 2021 (HSWt, LL and LW) and season 2022 (NPP, PW, LL, LW and NB).

From the combined ANOVA, the genotype effect was highly significant ($P \leq 0.001$) for all characteristics (Tables 3.7 and 3.8). The location effect was highly significant ($P \leq 0.001$) for GY, NPP, NSPP and HSWt. Season effect was highly significant ($P \leq 0.001$) for NPP, PW, PH and D50F. Genotype by location interaction effect was highly significant ($P \leq 0.001$) for all the characteristics measured, except for PL, LL and LW. Genotype by season and genotype by location by season interaction effects were highly significant ($P \leq 0.001$) for GY, NPP, NB, PW, PH and D50F. Season by location interaction effect was highly significant ($P \leq 0.001$) for GY, NPP and PW.

The H^2 ranged from 0.61 (LW) to 0.98 (PH) at Brits 2021 (Table 3.6) whereas it ranged between 0.07 (PL) to 0.97 (PH and HSWt) at Brits 2022. The H^2 ranged from 0.50 (LL) to 0.98 (PH) at Loskop 2021, whereas it ranged from 0.77 (D50F) to 0.98 (PH) at Loskop 2022. The H^2 ranged from 0.71 (NPP) to 0.99 (D50F) in season 2021 (Table 3.7), whereas it ranged from 0.51 (PW) to 0.99 (LW, and PH) in season 2022. The H^2 ranged from 0.79 (GY) to 0.99 (PH and D50F), for combined analysis (Table 3.8).

3.4.4. Mean performance of parental genotypes and their F_1 progenies from combined analysis of variance

Mean performance for GY for combined ANOVA ranged from 0.27 to 2.95 t/ha, with an overall mean of 1.34 t/ha (Table 3.9). The six progenies that were superior for GY (2.70 to 2.95 t/ha) had either Glenda, TVU13953 or IT96D-602 as a common parent. The three progenies which had poor GY (0.27 to 0.55 t/ha) had TVU7778 as a common parent. Loskop 2022 had the highest GY (3.01 t/ha, Table 3.10) compared to other environments.

The mean value of NPP ranged from 19.08 to 87.08, with an overall mean of 44.57 (Table 3.9). The seven progenies with the highest NPP (70.00 to 91.00) had either TVU13953, Glenda or IT96D-602 as a common parent. Brits 2022 had the highest NPP (128.01) compared to other locations (Table 3.10). The mean value of NSPP ranged from 9.11 to 21.19, with an overall mean of 14.20 (Table 3.9). The 10 progenies with the highest NSPP (17.00 to 21.00) had either TVU13953 or IT96D-602 as a common parent. Brits 2021 had the highest NSPP (21.87) compared to other environments (Table 3.10).

The mean of NB ranged from 4.37 to 10.33, with an overall mean of 6.54 (Table 3.9). The three progenies with the highest NB (9.00 to 10.50) had Glenda as a common parent. Brits 2021 had the highest NB (11.47) compared to other environments (Table 3.10).

Mean performance for HSWt ranged from 11.22 to 38.87 g, and an overall mean of 26.96 g (Table 3.9). The eight progenies with the highest HSWt (35.29 to 39.00 g) had either IT96D-602, TVU13953, Glenda or ITOOK-1060 as a common parent. Loskop 2021 had the highest HSWt (40.73 g) compared to other environments (Table 3.10).

The mean value of PL ranged from 110.01 to 214.03 mm (Table 3.9), with an overall mean of 167.99 mm. The eight progenies with the highest PL (207.00 to 230.00 mm) had either TVU13953 or IT96D-602 as a common parent. Loskop 2022 had the highest PL (258.01 mm) compared to other environments (Table 3.10).

The mean value of PW ranged from 5.83 to 12.27 mm, with an overall mean of 8.64 mm (Table 3.9). The 14 progenies that had the highest PW (10.00 to 12.27 mm) had either Glenda, ITOOK-1060 or Kisumi-mix as a common parent. Brits 2022 had the highest PW (17.85 mm) compared to other environments (Table 3.10).

The mean value of LL ranged from 6.27 to 12.14 cm, with an overall mean of 9.08 cm (Table 3.9). The 10 progenies with the highest LL (10.00 to 12.65 cm) had either TVU7778, IT93K-129-4 or Glenda as a common parent. Loskop 2022 had the highest LL (12.08 cm) compared to other environments (Table 3.10).

The mean of LW ranged from 1.71 to 4.94 cm, with an overall mean of 3.18 cm (Table 3.9). The five progenies with the highest LW (4.00 to 4.94 cm) had Glenda as a common parent. Brits 2021 had the highest LW (5.66 cm) compared to other environments (Table 3.10).

The mean of PH ranged from 37.65 to 215.39 cm, with an overall mean of 108.62 cm (Table 3.9). The 10 progenies with the highest PH (130.00 to 216.00 cm) had either IT96D-602, TVU13953, IT93K-129-4 and 98K-5301 as a common parent. Loskop 2021 had the highest PH (220.40 cm) compared to other environments (Table 3.10).

The mean of D50F ranged from 34.75 to 54.50 days, and an overall mean of 44.20 days (Table 3.9). The seven progenies with the lowest D50F (34.00 to 36.50 days) had either IT93K-129-4, IT845-2246 or TVU13953 as a common parent. Loskop 2021 had the highest D50F (55 days) compared to other environments (Table 3.10).

Table 3.6 Mean squares and broad sense heritability for grain yield and yield components for individual environments

Source	DF	GY	NPP	NSPP	NB	HSWt	PL	PW	LL	LW	PH	D50F
Brits 2021												
Rep	2	0.04	45.35	2.97	1.37	89.68	28.93	0.06	0.89	1.31	2.44	0.01
Genotypes	54	1.24**	356.88**	23.72**	10.70**	2310.06**	2548.60**	6.74**	9.05**	1.81**	19415.37**	114.85**
Residual	108	0.02	43.53	2.63	0.64	106.48	90.96	0.42	0.5	0.31	162.14	0.01
σ^2_G		0.40	104.45	7.02	3.35	734.53	819.4	2.11	2.87	0.5	6417.74	0.38
σ^2_P		0.46	147.98	9.66	3.99	841	910.17	2.52	3.35	0.81	6579.88	0.39
H^2		0.87	0.71	0.73	0.84	0.87	0.9	0.84	0.85	0.61	0.98	0.97
Brits 2022												
Rep	2	0.11**	239.41	3.08	0.2	32.29	48.61	24.89	0.49	0.28	192.09	0.1
Genotypes	54	1.12**	1418.41**	19.87**	6.92**	2351.33**	2629.73**	28.74**	9.12**	1.50**	13960.20**	105.69**
Residual	108	0.01	141.93	1.25	0.32	24.96	131.69	23.66	2.83	0.17	133.77	1.39
σ^2_G		0.35	425.49	6.21	2.2	775.46	832.68	1.69	2.1	0.44	4608.81	34.77
σ^2_P		0.4	567.42	7.45	2.52	800.42	964.37	25.36	4.93	0.61	4742.58	36.16
H^2		0.88	0.75	0.83	0.87	0.97	0.86	0.067	0.43	0.72	0.97	0.96
Loskop 2021												
Rep	2	0.12	40.55	0.08	0.52	201.53**	135	0.16	2.38	0.15	27.29	0.11
Genotypes	54	1.40**	1037.48**	26.19**	6.76**	4679.86**	1582.65**	7.88**	9.51**	1.53**	15391.93**	104.20*
Residual	108	0.01	20.42	0.69	0.36	20.68	90.33	0.11	2.43	0.17	83.85	1.2
σ^2_G		0.46	339.02	8.5	2.13	1553.06	497.44	2.59	2.36	0.46	5102.69	0.18
σ^2_P		0.53	359.44	7.18	2.49	1710.74	587.77	2.7	4.79	0.62	5186.54	0.19
H^2		0.87	0.94	0.92	0.86	0.9	0.85	0.96	0.5	0.73	0.98	0.57
Loskop 2022												
Rep	2	0.18**	17.12	0.18	0.1	310.93*	47.54	0.12	0.28	0.12	18.8	0.13
Genotypes	54	0.83**	1139.94**	26.85**	6.25**	4759.71**	2853.03**	8.62**	6.77**	1.54**	15296.55**	106.58**
Residual	108	0.02	19.08	0.49	0.24	55.73	82.12	0.17	0.27	0.13	91.81	1.4
σ^2_G		0.27	373.62	8.79	2.06	1567.99	923.64	2.81	2.18	0.47	5068.25	0.38
σ^2_P		0.29			2.24	1625.72	1005.76	2.99	2.45	0.6	5160.06	0.39
H^2		0.93			0.89	0.97	0.91	0.94	0.89	0.78	0.98	0.77

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, Loc = Location, DF = Degrees of freedom, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering. σ^2p = Phenotypic variance, σ^2g = Genotypic variance, H^2 = Broad-sense heritability.

Table 3.7 Mean squares and broad sense heritability for grain yield and yield components, evaluated in 2021 and 2022 seasons

Source	DF	GY	NPP	NSPP	NB	HSWt	NPP	NSPP	PL	PW	LL	LW	PH	D50F
2021														
Season	1	1.68**	14663.33**	40.49**	0.46	13521.66**	14663.33**	40.49**	0.04	0.02	3.96	1.41	3801.89**	3.71
Genotypes	54	2.56**	1081.36**	45.11**	15.10**	6205.74**	1081.36**	45.11**	3669.19**	13.72**	16.98**	3.05**	32714.12**	222.94**
Genotypes x Season	54	0.08**	313.00**	4.79**	2.36**	784.18	313.00**	4.79**	462.05**	0.89**	1.58	0.28	2093.18**	1.10**
Rep (Season)	4	0.08**	42.95	1.53	0.95	145.61**	42.95	1.53	81.97	0.11	1.63	0.73	14.86	0.01
Residual	216	0.01	31.98	1.66	0.50	63.58	31.98	1.66	90.65	0.26	1.47	0.24	123.00	0.50
σ^2_G		0.41	1.17	1.48	1.39	903.97	1.17	1.48	534.48	2.14	2.56	1.50	5103.40	36.97
σ^2_P		0.43	1.65	1.65	1.65	1034.29	1.65	1.65	611.53	2.28	2.83	1.65	5452.35	37.16
H^2		0.97	0.71	0.89	0.91	0.87	0.71	0.89	0.87	0.94	0.91	0.93	0.94	0.99
2022														
Season	1	0.14*	67.72	10.98**	1.35	9113.48**	67.72	10.98**	46.73	9.83	0.83	0.03	2331.28**	1.21
Genotypes	54	1.89**	2489.89**	44.09**	13.01**	6129.33**	2489.89**	44.09**	5266.94**	25.03**	14.65**	3.00**	29017.86**	208.12**
Genotypes x Season	54	0.05**	68.47	2.63**	0.16	981.70**	68.47	2.63**	215.82**	12.33	1.25	0.03	238.89**	4.14**
Rep (Season)	4	0.14**	128.26	1.63	0.15	171.61*	128.26	1.63	48.08	12.5	0.39	0.20	105.44	0.11
Residual	216	0.02	80.5	0.87	0.28	40.35	80.5	0.87	106.91	11.92	1.55	0.15	112.79	1.40
σ^2_G		0.31	403.57	6.91	2.14	857.94	403.57	6.91	841.85	2.11	2.23	0.49	4796.50	33.40
σ^2_P		0.32	414.98	7.35	2.17	1021.56	414.98	7.35	877.82	4.17	1.65	0.50	4836.31	34.69
H^2		0.97	0.97	0.94	0.99	0.84	0.97	0.94	0.96	0.51	0.92	0.99	0.99	0.93

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, Loc = Location, DF = Degrees of freedom, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering. σ^2p = Phenotypic variance, σ^2g = Genotypic variance, H^2 = Broad-sense heritability.

Table 3.8 Mean squares and broad sense heritability for grain yield and yield components evaluated at Brits and Loskop during the 2021 and 2022 seasons

Source	DF	GY	NPP	NSPP	NB	HSWt	PL	PW	LL	LW	PH	D50F
Loc	1	1.38**	8362.01**	46.82**	1.69	22418.45**	4.52	89.46	0.58	0.92	22.07	0.34
Genotypes	54	4.36**	3312.88**	87.67**	26.78**	12291.46**	31.50**	60814.13**	29.45**	5.93**	8483.58**	425.80**
Rep (Loc) x Season	8	0.11**	85.61	1.58	0.55	158.61*	6.31	60.15	1.01	0.46	65.02	0.06
Season	1	0.05	11432.02**	1.86	0.12	115.4	43.64	4900.31**	2.48	0.51	4220.71**	51.86**
Genotypes x Loc	54	0.09**	192.90**	5.95**	1.42**	1719.55**	7.41	1470.47**	0.98	0.17	408.22**	2.56**
Loc x Season	1	0.43**	6369.04**	4.65	0.12	216.7	5.33	6043.71**	4.21	0.52	24.7	4.58
Genotypes x Season	54	0.09**	258.36**	1.53	1.34**	43.62	7.25	917.85**	2.18	0.13	452.56**	5.25**
Genotypes x Loc x Season	54	0.05**	188.57**	1.47	1.10**	46.33	5.81	861.60**	1.84	0.14	269.65**	2.69**
Residual	432	0.01	56.24	1.27	0.39	51.96	6.09	117.89	1.51	0.2	98.78	0.95
σ^2_G		4.35	3290.89	87.17	26.64	12148.39	8434.32	30.76	29.34	5.91	60686.91	425.38
σ^2_P		5.53	3370.66	89.19	28.24	12425.15	8574.49	32.27	30.57	7.04	61170.77	427.80
H^2		0.79	0.98	0.98	0.94	0.98	0.98	0.95	0.96	0.84	0.99	0.99

$^{**}P \leq 0.001$, $^{*}P \leq 0.05$, Values without asterisk are not significant, Loc = Location, DF = Degrees of freedom, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering. σ^2p = Phenotypic variance, σ^2g = Genotypic variance, H^2 = Broad-sense heritability.

Table 3.9 Mean performance of genotypes evaluated at four environments in South Africa

Genotypes	GY (t/ha)	NPP	NSPP	NB	HSWt (g)	PL (mm)	PW (cm)	LL (cm)	LW (cm)	PH (cm)	D50F
TVU13953 x Glenda	2.84	75.83	17.92	10.33	38.44	214.03	9.80	8.36	2.86	119.95	34.75
ITOOK-1060 x Glenda	1.81	65.66	15.82	9.30	26.10	174.06	10.59	12.13	4.42	127.12	42.00
IIT93K-129-4 x Glenda	1.08	57.54	15.80	9.76	35.29	171.62	10.53	8.46	4.94	201.54	52.50
Kisumi-mix x Glenda	1.07	33.02	11.27	8.30	22.03	171.45	10.37	12.65	2.99	124.19	51.00
TVU7778 x Glenda	0.53	43.00	10.33	7.83	25.04	170.10	10.36	11.56	4.12	114.38	41.08
98K-5301 x Glenda	0.74	37.69	11.27	8.19	20.71	168.83	10.17	7.78	4.13	99.61	51.75
IT96D-602 x Glenda	2.47	43.93	17.39	7.92	20.20	163.60	10.03	9.42	1.80	107.93	50.50
IT845-2246 x Glenda	1.28	43.31	13.45	7.84	38.60	161.70	8.06	8.75	3.29	135.47	51.00
TVU-14196 x Glenda	1.08	41.23	11.58	5.77	21.24	161.45	8.02	9.32	3.37	125.60	47.50
ITOOK-1060 x TVU13953	2.82	86.73	18.19	5.34	31.97	210.55	9.51	8.66	3.72	126.95	51.00
IT93K-129-4 x TVU13953	1.62	74.71	18.50	5.62	27.86	210.03	9.40	10.22	3.13	127.93	50.00
Kisumi-mix x TVU13953	1.57	45.59	19.82	7.84	27.63	209.19	9.23	9.66	3.13	116.32	50.00
TVU7778 x TVU13953	1.32	27.10	18.43	4.97	24.16	192.37	8.28	10.25	3.39	148.35	38.75
98K-5301 x TVU13953	1.00	87.08	20.92	5.70	36.89	215.21	9.93	9.62	2.98	136.32	50.00
IT96D-602 x TVU13953	2.95	90.93	17.80	5.03	38.87	229.62	9.95	9.33	1.72	148.18	34.00
IT845-2246 x ITOOK-1060	1.20	31.63	12.15	4.85	35.82	186.66	8.11	9.93	3.13	74.59	49.17
TVU-14196 x TVU13953	0.66	31.55	21.19	5.25	23.44	190.44	8.21	9.83	3.08	113.36	46.92
IT93K-129-4 x ITOOK-1060	0.72	41.65	11.64	5.60	37.08	175.92	10.63	9.16	3.23	76.83	51.08
Kisumi-mix x ITOOK-1060	0.63	38.45	11.31	5.72	29.37	184.23	11.73	9.39	3.10	121.78	49.00
TVU7778 x ITOOK-1060	0.47	23.69	11.88	6.28	28.75	159.94	8.02	9.78	3.37	107.89	48.25
98K-5301 x ITOOK-1060	0.99	41.46	12.15	4.91	27.70	159.04	7.96	9.78	2.87	131.79	44.75
IT96D-602 x ITOOK-1060	0.94	78.56	17.91	6.89	26.96	157.22	7.95	9.26	3.43	107.89	39.00
IT845-2246 x TVU13953	1.88	70.61	14.48	5.13	24.73	197.21	8.38	9.33	1.81	121.06	36.50
TVU-14196 x ITOOK-1060	1.03	46.12	12.31	5.37	22.78	157.20	7.88	9.41	3.16	107.81	42.75
Kisumi-mix x IT93K-129-4	0.91	45.47	12.06	5.80	23.54	184.90	12.14	9.18	3.60	108.12	38.58
TVU7778 x IT93K-129-4	1.01	23.98	10.41	6.87	31.15	156.29	7.85	10.40	3.12	108.58	34.50
98K-5301 x IT93K-129-4	1.61	46.00	11.79	8.63	23.22	155.41	7.84	9.24	3.11	129.97	36.00
IT96D-602 x IT93K-129-4	0.94	41.88	11.52	6.85	21.16	207.97	8.68	9.21	1.86	171.81	40.00
IT845-2246 x IT93K-129-4	1.48	47.64	15.02	4.37	20.52	155.10	6.92	10.48	3.06	215.39	54.50
TVU-14196 x IT93K-129-4	1.51	60.35	15.49	5.07	19.70	154.54	6.87	8.91	3.88	115.47	42.00
TVU7778 x Kisumi-mix	1.80	25.62	15.30	8.64	29.28	181.54	11.18	10.27	3.28	134.38	53.50
98K-5301 x Kisumi-mix	1.55	50.42	15.00	8.01	29.14	179.06	11.05	9.91	3.62	145.81	37.00
IT96D-602 x Kisumi-mix	2.65	49.22	14.57	7.27	26.49	185.48	12.27	9.93	1.77	121.86	42.00
IT845-2246 x Kisumi-mix	1.54	42.25	14.18	7.57	25.10	177.27	10.89	8.38	3.53	121.16	53.50

Table 3.9 Mean performance of genotypes evaluated at four environments in South Africa (continued)

Genotypes	GY (t/ha)	NPP	NSPP	NB	HSWt (g)	PL (mm)	PW (cm)	LL (cm)	LW (cm)	PH (cm)	D50F
TVU-14196 x Kisumi-mix	1.49	52.48	14.85	8.05	22.64	177.16	10.69	8.31	3.25	120.18	54.75
98K-5301 x TVU7778	1.11	40.02	14.76	8.39	12.41	153.35	6.84	10.34	3.01	84.94	42.00
IT96D-602 x TVU7778	1.69	38.34	13.75	4.74	15.44	152.07	6.55	9.41	1.82	84.15	34.50
IT845-2246 x TVU7778	1.69	38.83	13.58	4.89	14.52	151.39	6.36	8.49	3.54	83.86	39.50
TVU-14196 x TVU7778	1.17	40.54	13.48	8.27	14.27	151.17	5.96	10.13	3.96	83.20	41.33
IT96D-602 x 98K-5301	2.69	36.64	13.34	4.89	30.57	208.07	8.91	8.65	1.71	82.30	41.00
IT845-2246 x 98K-5301	1.36	38.03	13.45	4.93	30.27	149.88	5.83	9.49	3.39	80.73	45.50
TVU-14196 x 98K-5301	1.35	34.95	11.92	5.26	31.60	149.03	7.82	8.33	3.52	76.94	46.00
IT845-2246 x IT96D-602	1.09	32.42	13.13	5.18	30.51	147.78	7.81	8.67	3.55	124.03	47.00
TVU-14196 x IT96D-602	1.61	34.45	16.14	5.03	38.83	144.81	7.76	7.90	3.49	122.73	38.50
TVU-14196 x IT845-2246	0.84	35.69	12.60	8.59	20.61	144.79	7.75	5.47	3.26	70.66	34.50
Glenda	1.18	34.44	14.25	6.15	32.27	144.68	7.72	6.27	4.16	69.79	41.50
98K-5301	0.94	33.71	12.97	5.98	27.98	143.42	8.15	9.32	2.97	66.08	45.00
IT96D-602	1.51	39.15	13.70	6.53	31.41	190.42	7.57	8.22	3.28	126.81	36.50
IT93K-129-4	1.01	38.16	12.45	6.18	34.46	141.60	7.53	4.97	3.54	66.91	39.58
Kisumi-mix	1.07	29.89	14.10	6.83	35.04	141.38	7.35	8.71	3.74	60.39	46.00
TVU7778	0.27	19.08	9.11	5.09	13.22	110.01	7.05	7.38	3.05	37.65	48.50
TVU13953	1.45	48.85	14.79	6.23	22.27	137.28	7.29	9.69	3.75	73.70	39.50
ITOOK-1060	1.09	31.83	14.01	5.43	25.46	117.06	7.21	5.72	1.77	52.85	44.25
IT845-2246	1.02	27.43	14.33	7.18	30.83	116.18	7.10	7.73	3.41	42.65	39.25
TVU-14196	1.10	36.40	14.46	6.94	11.22	139.44	7.30	6.14	3.33	68.30	43.00
Grand mean	1.34	44.57	14.20	6.54	26.67	167.99	8.64	9.08	3.18	108.62	44.20
LSD (*P* = 0.05)	0.08	1.66	0.83	6.69	4.85	0.42	7.31	5.05	0.76	0.30	41.33

First parent on the progenies represent female, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering.

Table 3.10 Grand mean values for grain yield and yield components for four individual environments

Characteristics	GY (t/ha)	NPP	NSPP	NB	HSWt (g)	PL (mm)	PW (cm)	LL (cm)	LW (cm)	PH (cm)	D50F
Brits 2021											
Minimum	0.12	18.47	8.87	4.50	21.53	104.00	5.46	7.30	1.75	36.45	34.00
Maximum	2.10	83.07	21.87	11.47	38.15	223.77	11.06	10.87	5.66	195.32	55.00
Grand mean	1.28	33.74	13.80	6.51	24.06	165.48	8.38	9.03	3.38	109.35	44.37
Brits 2022											
Minimum	0.25	20.05	9.90	4.10	21.58	111.45	6.00	6.47	1.67	37.73	34.00
Maximum	2.57	128.01	20.17	9.67	38.89	243.60	12.66	11.99	4.81	212.13	54.00
Grand mean	1.32	48.28	14.07	6.46	24.05	170.15	9.07	9.07	3.61	105.60	43.98
Loskop 2021											
Minimum mean	0.36	18.69	9.27	4.20	21.93	114.14	6.02	6.28	1.72	36.13	34.75
Maximum	2.20	102.52	21.28	9.74	40.73	210.91	11.74	11.87	4.59	220.40	54.50
Grand mean	1.42	47.07	14.50	6.59	27.05	165.41	8.39	9.25	3.14	107.31	44.20
Loskop 2022											
Minimum	0.36	19.11	8.97	4.24	21.73	110.43	5.82	6.28	1.67	39.31	34.00
Maximum	3.01	106.02	21.30	9.75	39.72	258.05	12.76	12.08	4.84	199.79	51.00
Grand mean	1.36	49.18	14.44	6.59	27.01	170.90	8.73	8.97	3.15	108.45	43.86

GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering.

3.5. DISCUSSION

3.5.1. Genetic diversity analysis of ten selected cowpea parental genotypes

Genetic diversity in a population is essential for selecting diverse genotypes and broadening a population's genetic basis. When analysing the genetic diversity, the PIC value represents the relative informativeness of each marker and in the current study, 29.4% of the markers (five) were highly informative (PIC ≥ 0.50), indicating that the selected primers, particularly SSR6265, SSR6217, SSR6451, SSR6277, and SSR6436, are useful genetic tools for determining the genetic variation of cowpea parental genotypes. Other studies also reported the informativeness of SSR markers in discrimination of cowpea genotypes for analysis of genetic diversity (Ali *et al.* 2015; Sarr *et al.* 2020; Dagnon *et al.* 2022). One to six alleles per marker were observed, which corroborated diversity studies conducted in Nigeria and Ghana (Diouf and Hilu 2005; Adetiloye *et al.* 2013). The number of markers and parental genotypes used on the current studies could have resulted in fewer alleles observed.

The expected heterozygosity (0.10 to 0.81, with an average of 0.49) observed, indicated a wide genetic diversity between the cowpea parental genotypes used in this study, making these genotypes good candidates for gene specific association studies of complex characteristics such as yield and nutritional characteristics to be used as parental genotypes in a breeding programme. The average heterozygosity observed in the current study corroborate a study on Sudanese cowpea germplasm which reported heterozygosity average of 0.49 (Ali *et al.* 2015). The findings are in contrast with a lower heterozygosity average (0.23) and a higher heterozygosity average (0.54) previously reported on cowpea (Seo *et al.* 2020; Dagnon *et al.* 2022).

The genotypes used in this study originated from different geographical regions. Genetic distances between genotypes in each cluster show close relationships or similarities. As a result, it is preferable to avoid crossing individual genotypes within a cluster. However, cluster I and III had genotypes with an almost 50% similarity. This data suggests that crosses between these genotypes should be considered if the goal is to improve only one or two characteristics, or to combine the characteristics into a single genotype. The genotypes with the highest genetic diversity, particularly among the three different clusters, may serve as sources of novel alleles for cowpea breeding.

3.5.2. Analysis of variance and variance components for grain yield and yield components

The significant differences observed between genotypes for individual and combined environments for grain yield and yield components measured, indicated significant genetic variation amongst progenies and their parental genotypes. Differences in genetic makeup of the parental genotypes is necessary to generate variability in progenies (Seo *et al.* 2020), allowing selection for desirable characteristics. The findings agree with Mbuma *et al.* (2021), who evaluated the Southern African cowpea germplasm collection for grain yield and yield components and reported highly significant genotype effects for seed yield, number of pods, and number of seeds per plant. The results also corroborate findings reported by Stoilova and Pereira (2013), who evaluated genetic diversity of 48 cowpea accessions consisting of 18 landraces from Bulgaria and Portugal and 30 advanced breeding genotypes of different origins and reported significant differences in number of pods and seeds per plant. A study by Mofokeng *et al.* (2020), conducted on 100 cowpea genotypes, also reported significant differences among genotypes for grain yield, number of branches per plant, number of pods per plant, pod weight per plant and hundred seed weight. Genetic variation within a population plays an important role in crop improvement, as it allows effective selection.

The significant genotype by location interaction for all the yield components measured, except for leaf length and leaf width, indicated that the locations influenced the performance of cowpea parental genotypes and progenies significantly, causing variable genotype performance in different environments. The significant genotype by season and genotype by season by location interaction for grain yield, number of pods per plant, pod width, number of branches, plant height and days to 50% flowering, indicated changes in genotype performance ranking across seasons and locations.

Previous studies also reported significant genotype by location interaction for grain yield and yield components (Ishiyaku *et al.* 2017; Odeseye *et al.* 2018; Gerrano *et al.* 2020; Mbuma *et al.* 2021). The location affects the genotype performance, thus reducing the relationship between genotype and their corresponding phenotype. The difference in the performance of genotypes in different environments and seasons observed in this study could be due to varying climatic condition such as the difference in temperature and rainfall received during the cropping seasons. The significant interaction between genotype and the location could imply that the selection for superior yielding ability in one location might not lead to good yielding ability in another location due to instability of the genotype. Thus, breeding for stable yielding genotypes for the targeted production areas is important. Phenotypic variances were consistently only slightly higher than the genotypic variances for all the characteristics measured, showing that the phenotype was largely representative of the genotype. This indicates that characteristics were largely determined genetically. High genetic variance is necessary to obtain a good response to selection, as this indicates a limited effect of location on the desired characteristic.

3.5.3. Broad-sense heritability estimates for grain yield and yield components

The broad-sense heritability was higher than 0.80 from combined ANOVA for all characteristics measured, except for pod width. The heritability estimates for all the locations were more than 0.60 for all the yield components measured, except for pod length and leaf length (Brits 2022) and leaf length (Loskop 2021). These findings indicated a considerable proportion of genetic variance to the total variance in the population and could result in good response to selection during breeding. The results corroborated a study by Mofokeng *et al.* (2020), on 100 cowpea genotypes where high heritability (0.97) was reported for hundred seed weight. The study of grain yield and yield components in cowpea gave high estimates of broad-sense heritability (0.91) for grain size (Aliyu and Makinde 2016). Ajayi *et al.* (2014) also reported high broad-sense heritability (≤ 0.99) for seed length and seed weight. In contrast, other studies reported < 0.50 of H^2 for seed yield and related characteristics (Aliyu and Makinde 2016; Mbuma *et al.* 2021). The high broad-sense heritability observed for this study suggests that the effects of environmental conditions in the tested locations were low; hence, and enhanced selection efficiency.

3.5.4. Performance of parental genotypes and their F_1 progenies

The genotype mean performances for the current study was categorised into low yielding (below average, 0.27 t/ha), and high yielding (above average, ≥ 2.50 t/ha), which indicated the presence of large variation which could be exploited for selection, advancement, and future breeding for improved grain yield. The results agree with findings by several authors (Iseki *et al.* 2021; Owusu *et al.* 2021) that reported grain yield performance of more than 2 t/ha for parental genotypes and progenies.

The progenies that had higher than average grain yield had either TVU13953, IT96D-602 or Glenda in common as parents, suggesting that these parental genotypes had contributed significantly to grain yield enhancement observed in the progenies. The finding suggests the need for combining ability studies to determine the nature of gene action involved in expression of grain yield and yield components. The progenies with common parental genotypes (either TVU13953, IT96D-602 or Glenda) requires further evaluation for nutritional characteristics, as well as resistance and tolerance to biotic and abiotic stresses. Furthermore, the potential parental genotypes identified could be basic material for the genetic improvement of cowpea grain yield and its components. The parental genotypes TVU13953 and IT96D-602 also had above average mean performance for grain yield in previous studies (Mbuma *et al*. 2021; Gerrano *et al*. 2022). However, the parental line Glenda led to lower grain yield than the average performance of the population studied (Gerrano *et al.* 2019).

The superior progenies (IT96D-602 x Glenda, IT96D-602 x Kisumi-mix, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953, TVU13953 x Glenda and IT96D-602 x TVU13953) identified for grain yield suggests there might heterosis in the F_1 generation, which could be exploited in hybrid breeding. The parental genotypes require further evaluation for general and specific combining ability, for better use in strategic breeding for grain yield enhancement. Superior progenies over parental genotypes were also reported in other studies (Kumari and Chauhan 2018; Owusu *et al.* 2018; 2020). This emphasises the potential of hybrid cowpea in South Africa and further implies the possibility of effective heterosis breeding for improving grain yield and yield components.

The parental genotypes generally had below average (≤ 1.34 t/ha) mean grain yield compared to progenies, with only two parental genotypes (TVU13953 and IT96D-602) having above average (≥ 1.34 t/ha) mean grain yield. The parental genotypes TVU13953 and IT96D-602 were also superior for hundred seed weight, number of pods per plant and height compared to the other parental genotypes, and the superiority of these parental genotypes for the noted yield components was evident in the progenies that had the highest mean for these characteristics. Previous studies reported a positive correlation of grain yield with number of seeds per pod and plant height (Walle *et al.* 2018; Mbuma *et al.* 2021), indicating that genotypes with high number of pods are most likely to have high grain yield. This indicates the importance of determining the interrelationship between the primary and secondary characteristics in cowpea to enable indirect selection. Parental line TVU7778 generally performed poorly and produced inferior progenies for most of the important characteristics, which could be because of its disability to pass on good genes to its progeny. TVU7778 could have a low breeding value for grain yield and yield components; however, a diallel analysis can confirm this possibility.

Two progenies namely ITOOK-1060 x TVU13953 and IT96D-602 x TVU13953 had above average values for grain yield, number of pods per plant, number of seeds per pod, plant height, pod length, and pod width. The improvement of cowpea for grain yield can be done through indirect selection of yield components, as grain yield is a polygenic characteristic (Aliyu and Makinde 2016; Mofokeng *et al.* 2020). Previous studies reported cowpea cultivars with above average plant height (Owusu *et al.* 2021) and pod length (Jayasingha and Fernando 2020). Studies on other legumes reported strong correlation between grain yield and number of branches per stem, number of leaves and plant height (Upadhyaya *et al.* 2012; Gerrano *et al.* 2013; Siwale *et al.* 2022).

Earliness in flowering is also important, particularly in the ever-changing climatic conditions, hence resource poor farmers will benefit from early flowering to mitigate the short rainy seasons. In the current study four progenies (IT96D-602 x TVU13953, IT96D-602 x 98K-5301, TVU-14196 x IT845-2246 and TVU13953 x Glenda) indicated early flowering (34 to 35 days). However, early flowering lead to a yield penalty. The current study corroborated findings of the negative correlation of days to 50% flowering with seed yield (Thorat and Gadewar 2013; Shanko *et al.* 2014). The advantages of early flowering should therefore be weighed against possible reduced yield compared to later flowering genotypes.

3.6. CONCLUSIONS

The presence of parental genetic variation in this study confirmed that crossing of these genotypes with the aim of improving yield characteristics could improve selection efficiency. The results further indicated significant genetic variation amongst the F_1 progenies. Two parental genotypes (TVU13953 and IT96D-602) displayed superior performance compared to other parental genotypes for grain yield, thus they could be selected for future breeding activities for the improvement of grain yield, if they also have good breeding values. Broad-sense heritability was high for almost all measured characteristics, indicating that there should be a positive response to selection.

There was significant genotype by season by location interaction, indicating the importance of testing genotypes for stability of desired characteristics in different locations and seasons. Six progenies (IT96D-602 x Glenda, IT96D-602 x Kisumi-mix, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953, TVU13953 x Glenda and IT96D-602 x TVU13953) with above-average grain yield could be evaluated for potential hybrid production. To determine the mode of inheritance for grain yield and yield components, there should be more research done on the ability of parental genotypes to combine to produce progenies with high vigour.

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CHAPTER 4

COMBINING ABILITY OF SELECTED COWPEA GENOTYPES AND HETEROSIS AND HERITABILITY OF YIELD AND YIELD COMPONENTS

4.1. ABSTRACT

Crop improvement depends on the choice of parental genotypes and an understanding of the mechanisms governing the genetic combination of those parental genotypes. The objectives of this study were to estimate the combining ability of selected cowpea parental genotypes, determine the magnitude of heterosis in the F_1 progenies and identify best combiners for the measured grain yield and yield components. Following a half-diallel design crossing of ten selected parental genotypes, 45 F_1 progenies were developed and planted together with the parental genotypes in two distinct field locations in the growing seasons of 2021 and 2022. Significant general combining ability (GCA) and specific combining ability (SCA) effects were observed, which indicated that both additive and non-additive gene action were involved in the expression of all measured characteristics. Genotypes TV13953 and IT96D-602 were the best combiners for grain yield, hundred seed weight, number of pods per plant and number of seeds per plant. The combination of Glenda x TVU13953 had positive significant SCA effects for all characteristics, except for days to 50% flowering (D50F). Six combinations (TVU13953 x Glenda, ITOOK-1060 x TVU13953, 98K-5301 x Glenda, ITOOK-1060 x TVU13953, IT96D-602 x Glenda, and IT96D-602 x TVU13953) had superior SCA effects for grain yield and most yield components. The positive and significant mid-parent heterosis for grain yield observed in 60% of the F_1 progenies suggests the importance of either dominant or partial dominant genes in expression of grain yield in F_1 progenies. Cowpea is a self-pollinated crop that is typically improved through pedigree breeding, but from the best progenies, there is a possibility of selecting the best-performing segregants for genetic line cultivar development.

Keywords: cowpea progenies, variance components, narrow-sense heritability, combining ability, gene action, heterosis

4.2. INTRODUCTION

Grain yield is an important characteristic for both farmers and breeders. Since grain yield is a complex quantitative and polygenic characteristic, direct selection based only on grain yield is challenging (Zaki and Radwan 2022). Grain yield depends on yield components such as pod length, hundred seed weight, plant height, all of which are susceptible to environmental influences, thus reducing selection efficiency. Cowpea is an autogamous diploid (2n = 22 chromosomes) and self-pollinating crop with a narrow genetic base (Rashwan 2010; Zaki and Radwan 2022). The crop has a high level of homozygosity and often lacks genetic diversity, leading to compromised genetic gains from selection (Owusu *et al*. 2020). Another challenge is associated with incompatibility between parental genotypes (Verma *et al.* 2021).

There are two types of combining ability, namely GCA and SCA. The GCA is the average contribution of parental genotype to the progeny or hybrid performance in comparison to other parental genotypes, due to additive gene action (Ayo-Vaughan *et al.* 2011). Additive genes are heritable and characteristics with high additive gene action will show a good response to selection (Owusu *et al.* 2018). The SCA is the contribution of one parent to progeny performance in a cross with another specific parent (Dias *et al*. 2016; Mwale *et al.* 2017). Dominant and interaction gene effects, which are not heritable, primarily govern the SCA. Breeding advances is attainable through characteristics controlled primarily by additive genetic effects rather than by dominant or interacting genes. Hybrid development as a breeding strategy for improvement grain yield and yield components requires good SCA as well as a certain level of heterosis. Thus, it is important to exploit hybrid vigour or the superiority of the crossbreeds over the parent's average performance if hybrid breeding is a focus of the breeding programme (Sharma *et al.* 2010).

Heritability is the genetic proportion of the phenotype of the measured characteristics (Owusu *et al*. 2020). There are two types of heritability: narrow-sense and broad-sense. The ratio of additive genetic variance to total phenotypic variance in a population determines the narrow-sense heritability. The narrow-sense heritability is vital in a breeding programme because the additive effects of the genetic variance determine the potential for genetic enhancement in a population through selection (Owusu *et al*. 2020). Broad-sense heritability is the ratio of genetic variance to a total phenotypic variance (Shimelis and Shiringani 2010), and it is used to determine the genetic variability of a characteristic in a breeding population, without considering the underlying gene action. Therefore, narrow-sense heritability is more useful as it refers to the actual genetic control of the measured characteristics.

Research on cowpea in South Africa has traditionally concentrated on pre-breeding operations, which entail the introduction of accessions from other countries and assessing these accessions throughout the primary cowpea-growing regions of the country. Previous studies on cowpea diversity have reported significant variation in grain yield for most accessions. However, most of these accessions are low-yielding and unstable under South African climatic conditions (Gerrano *et al.* 2015; 2019; Mbuma *et al.* 2021; Gumede *et al.* 2022).

Studies on combining ability in cowpea were conducted in India (Kumari and Chauhan 2018), Nigeria (Olunloyo *et al.* 2019), Ghana (Owusu *et al.* 2020), Zambia (Nkhoma 2020), Kenya (Jou-Nteufa and Ceyhan 2022) and Iraq (Al-Obeidi *et al.* 2022), which reported a significant positive GCA and SCA for the most important yield characteristics. Thus, knowledge on the nature of combining ability in selected cowpea parental genotypes and the success of cross combinations could potentially improve the grain yield and yield components.

Information regarding cowpea combining ability for grain yield and yield components is of utmost importance in developing newly improved cowpea cultivars that are well adapted in South African conditions and beyond. Therefore, the objectives of the current study were to partition variance components, determine heritability for grain yield and yield components, estimate the combining ability of selected cowpea parental genotypes, determine the magnitude of heterosis in the progenies and identify best combiners for the measured characteristics through a diallel mating design.

4.3. MATERIALS AND METHODS

4.3.1. Planting materials

Section 3.3.1 of Chapter 3 contains a detailed description of the materials used in this study.

4.3.2. Experimental locations

Section 3.3.3 of Chapter 3 describes the experimental locations in detail.

4.3.3. Experimental design, trial establishment and management

The details of the trial setup, management, and experimental design are outlined in Chapter 3, section 3.3.4.

4.3.4. Data collection

Section 3.3.5 of Chapter 3 provided details on data collection.

4.3.5. Data analysis

Data was subjected to a half-diallel analyses following method 2 as described by Griffing (1956) using AGD-R software version 4.0 (Rodríguez *et al.* 2015; Kamweru *et al.* 2023). The total sum of squares was partitioned into main effects due to replication, environment, genotypes, and genotype by environment interaction. The sum of squares of genotypes was further split into GCA and SCA effects using the following models:

$$X_{ijk}=\mu + g_i + g_j + s_{ij} + \sum eijk$$

The Baker ratio determined the contribution of GCA towards the total sum of squares following the formula below (Owusu *et al.* 2020):

$$\text{Baker ratio} = \frac{2\sigma^2 GCA}{2\sigma^2 GCA + \sigma^2 SCA}$$

The adjusted means of the F_1 progenies and parental genotypes were used to calculate the mid-parent heterosis using the formula below (Al-Obeidi *et al.* 2022):

$$\text{MPH} = \frac{F_1 - MP}{MP}\ x\ 100$$

Where MPH is the mid-parent heterosis, F_1 is the mean of the first-generation progenies and MP = the average of the two parental genotypes.

4.4. RESULTS

4.4.1. Diallel analysis for grain yield and yield components

For Loskop in season 2021 and 2022, genotype, GCA, and SCA effects were significant ($P \leq 0.05$) for all characteristics measured (Table 4.1). For Brits in season 2021, the genotype, GCA and SCA effects were significant ($P \leq 0.05$) for most characteristics except for LW. The genotype, GCA and SCA effects were highly significant ($P \leq 0.001$) for most of the characteristics except for genotype (PW), GCA (PW) and SCA (LL and PW) in Brits 2022.

The genotype, GCA and SCA effects were significant ($P \leq 0.05$), for all the characteristics measured in Brits and Loskop across seasons 2021 and 2022 (Table 4.2). The genotype by season, GCA by season and SCA by season interaction effects were significant ($P \leq 0.05$) for GY in Brits and Loskop across season 2021 and 2022 and for NB and PH in Brits across seasons 2021 and 2022. The combined diallel analysis revealed highly significant ($P \leq 0.001$) genotype, GCA and SCA effects for all the characteristics recorded (Table 4.3). The genotype by environment, GCA by environment and SCA by environment interaction effects were significant ($P \leq 0.05$) for GY, HSWt, NSPP, NPP, PL, NB, and PH.

4.4.2. Variance components and narrow-sense heritability of yield and yield components

Variance components for GCA were highly significant ($P \leq 0.001$) for all the characteristics and significant ($P \leq 0.05$) for NB (Table 4.4). The SCA, GCA by environment and SCA by environment interaction variance components were highly significant ($P \leq 0.001$) for all the measured characteristics. The SCA variance was higher than the GCA variance for all the characteristics measured. The ratio of GCA to SCA variance for characteristics ranged from 0.12 (PL) to 0.92 (LW). The ratio of GCA to SCA variance was less than unity (≤ 1) and phenotypic variance was higher than genotypic variance for all characteristics measured. Narrow-sense heritability for characteristics ranged from 0.12 to 0.58 for PL and PH, respectively.

4.4.3. Estimates of general combining ability for grain yield and yield components

The parental genotypes TVU13953 and IT96D-602 had significant ($P \leq 0.05$) and positive GCA effects for all characteristics except for D50M and LW for IT96D-602 parental line (Table 4.5). The parental genotypes IT96D-602, TVU13953 and Glenda had positive GCA effects (from highest to the lowest, respectively) for grain yield at all the tested environments. There were significant ($P \leq 0.05$) positive GCA effects for Glenda (GY, HSWt, NPP and PH), IT845-2246 (LL, LW and PW), TVU-14196 (PL). The parental genotypes IT93K-129-4, Kisumi-mix and TVU7778 revealed significant ($P \leq 0.05$) positive GCA effects for D50F. The parental line 98K-5301 had significant ($P \leq 0.05$) and positive GCA effects for PH, NPP, PL and NB. IT96D-602 (0.66) had the highest significant ($P \leq 0.05$) and positive GCA effect for GY and Glenda the lowest (0.16).

4.4.4. Estimates of specific combining ability for grain yield and yield components

Combination Glenda x TVU13953 had significant (*P* ≤ 0.05) and positive SCA effects for all characteristics measured except for D50F (Table 4.6). The six progenies with significant (*P* ≤ 0.05) and positive SCA effects for GY had either parent TVU13953, IT96D-602 or Glenda in common. TVU13953 x IT96D-602 (0.88) recorded the highest significant (*P* ≤ 0.05) positive SCA for GY, whereas the lowest SCA (0.02) for the same trait was shown by ITOOK-1060 x 98K-5301.

The 14 progenies with significant (*P* ≤ 0.05) and positive SCA effects for HSWt had either parent Glenda, Kisumi-mix, 98K-5301, IT93K-129-4, IT96D-602 or TVU-14196 in common (Table 4.6). The nine progenies with significant (*P* ≤ 0.05) and positive SCA effects for NSSP had either parent TVU13953, 98K-5301, Glenda or IT96D-602 in common. The 11 progenies with significant (*P* ≤ 0.05) and positive SCA effects for NPP had either parent TVU13953, 98K-5301, Glenda, ITOOK-1060, Kisumi-mix or IT96D-602 in common.

The 11 progenies with significant (*P* ≤ 0.05) and positive SCA effects for PH had either parent TVU13953, IT96D-602, IT845-2246 or IT93K-129-4 in common (Table 4.6). The 10 progenies with significant *(P* ≤ 0.05) and positive SCA effects for NB had either parent Glenda, ITOOK-1060, Kisumi-mix or IT93K-129-4 in common. The seven progenies with significant (*P* ≤ 0.05) and positive SCA effects for D50F had either parent TVU13953, ITOOK-1060 or Glenda in common. Progeny Itook-1060 x TVU14196 had significant (*P* ≤ 0.05) and positive SCA effects for HSWt, NSPP, NPP, NB and LL. Progeny TVU13953 x IT96D-602 had significant (*P* ≤ 0.05) and positive SCA for GY, HSWt, NPP and PH.

4.4.5. Mid-parent heterosis for grain yield and yield components

The mid-parent heterosis for GY ranged from -48.24% (IT845-2246 x TVU7778) to 179.34% (IT96D-602 x Glenda). Of the 45 progenies, 60% had positive and significant (*P* ≤ 0.05) mid-parent heterosis (Table 4.7). Mid-parent heterosis for HSWt ranged from -71.95% (Glenda x Kisumi-mix) to 132.11% (TVU13953 x IT96D-602), and 37.8% of the progenies had positive estimates. Mid-parent heterosis ranging from -54.82% (ITOOK-1060 x Kisumi-mix) to 76.09% (IT93K-129-4 x Glenda) was seen for NPP. Of the 45 progenies, 20% showed positive mid-parent heterosis. Mid-parent heterosis for NSPP ranged from -35.16% (TVU-14196 x ITOOK-1060) to 25.78% (98K-5301 x ITOOK-1060), and 44.4% of the progenies had positive estimates. The mid-parent heterosis for NB ranged from

-40.08% (IT845-2246 x TVU7778) to 52.86% (IT845-2246 x TVU13953). Of the 45 progenies, 51.1% had positive heterosis, and the remaining 48.9% had negative mid-parent heterosis. PH mid-parent heterosis ranged from -71.90% (TVU-14196 x TVU7778) to 34.60% (ITOOK-1060 x TVU13953), and 15.6% of the progenies showed positive mid-parent heterosis (Table 4.6). Mid-parent heterosis for D50F ranged from -23.36% (98K-5301 x Kisumi-mix) to 35.51% (TVU13953 x Glenda), and 48.9% of the progenies had positive mid-parent heterosis.

Table 4.1 Diallel analysis mean squares for grain yield and yield components for individual environments

Sources	DF	GY	NPP	NSPP	NB	HSWt	PL	PW	PH	LL	LW	D50F
Brits 2021												
Rep	2	0.09	77.30	2.36	1.72	48.62	105.83	0.50	196.71	1.58	2.17	87.23
Genotype	54	1.25**	352.69**	25.21**	10.11**	2331.35**	2863.03**	7.33**	19478.49**	9.65**	1.78**	161.02**
GCA	9	4.59**	1174.71**	85.55**	24.38**	8633.07**	8313.12**	25.37**	80635.92**	36.23**	7.29**	333.56**
SCA	45	0.59**	192.99**	13.41**	7.41**	1079.08**	1753.44**	3.82**	7545.44**	4.39**	0.69	127.33**
Residual	105	0.02	55.01	3.57	1.29	116.45	171.08	0.71	384.53	0.94	0.41	46.87
Brits 2022												
Rep	2	0.13**	260.30	3.60	0.07	39.43	50.85	26.66	125.10	0.51	0.28	0.57
Genotype	54	1.12**	1411.53**	19.84**	6.48**	2338.32**	2626.52**	28.66	13927.94**	8.60**	1.49**	105.15**
GCA	9	3.91**	4776.33**	81.97**	17.91**	8997.26**	10387.43**	33.62	53614.53**	30.11**	6.13**	290.15**
SCA	45	0.56**	746.84**	7.43**	4.26**	1022.40**	1076.84**	27.76	6012.25**	4.92	0.57**	68.80**
Residual	105	0.01	145.58	1.28	0.53	25.43	136.00	24.31	146.19	2.91	0.17	1.42
Loskop 2021												
Rep	2	0.12**	43.59	0.05	0.31	183.00	158.66	0.15	28.52	2.34	0.14	6.12
Genotype	54	1.39**	1015.90**	25.82**	6.31**	4670.42**	1536.13**	7.70**	15208.49**	9.24**	1.52**	128.78**
GCA	9	4.70**	3197.85**	93.00**	17.56**	13281.41**	4292.78**	27.57**	57578.65**	31.33**	6.05**	373.54**
SCA	45	0.74**	605.39**	12.81**	4.12**	2958.92**	1040.27**	3.93**	6945.36**	5.14**	0.62**	92.41**
Residual	105	0.01	20.95	0.72	0.58	21.89	92.61	0.11	90.17	2.5	0.17	9.31
Loskop 2022												
Rep	2	0.17**	18.80	0.20	0.0	286.30	52.48	0.12	15.49	0.26	0.12	0.59
Genotype	54	0.83**	1139.95**	26.84**	5.83**	4759.18**	2855.71**	8.62**	15284.52**	6.77**	1.53**	106.58**
GCA	9	2.69**	3668.07**	101.61**	15.65**	13088.53**	10117.24**	33.2**	56603.09**	22.59**	6.02**	375.7**
SCA	45	0.46**	634.33**	11.89**	3.87**	3093.31**	1403.41**	3.70**	7020.81**	3.60**	0.64**	52.75**
Residual	105	0.02	19.04	0.50	0.45	56.45	80.68	0.17	97.89	0.27	0.13	1.39

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, DF = Degrees of freedom, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering, GCA = General combining ability, SCA = Specific combining ability.

Table 4.2 Diallel analysis mean squares for grain yield and yield components evaluated at Brits and Loskop during the 2021 and 2022 cropping seasons

Sources	DF	GY	NPP	NSPP	HSWt	NB	PL	PW	LL	LW	PH	D50F
Brits 2021 and 2022												
Season	1	0.10	17134.44**	3.82	296.81	1.22	21.27	37.28	0.41	0.87	13295.57**	15.70
Rep (Season)	4	0.07*	151.91	3.27	64.19	0.42	0.45	13.44	0.72	0.83	64.23	54.50
Genotype	54	2.26**	1335.13**	40.53**	4560.91**	13.96**	3.83*	25.23*	15.17*	3.02*	31307.36**	233.61**
GCA	9	8.18**	5058.80**	160.80**	17418.46**	37.75**	7.86**	47.40**	58.13**	13.00**	128267.66**	603.16**
SCA	45	1.09*	600.54*	16.79**	2024.10**	9.33*	3.06*	23.92*	7.41*	1.05*	12173.52**	163.95**
Genotype x Season	54	0.06*	423.84*	2.50	74.44	2.26*	0.74*	10.00	2.43*	0.23	1729.53**	18.40
GCA x Season	9	0.12*	847.97**	3.65	129.71	3.10*	1.38	9.14	3.96	0.45	3503.80**	9.05
SCA x Season	45	0.05*	342.18*	2.41	61.01	2.20*	0.61	7.31	2.00	0.20	1460.07**	17.56
Residual	213	0.01	96.85	2.03	67.68	0.81	0.38	12.42	1.72	0.25	166.89	18.69
Loskop 2021 and 2022												
Season	1	0.37**	336.48*	0.55	19.85	0.02	0.01	8.3*	7.82	0.00	42.79	22.80
Rep (Season)	4	0.12**	31.53	0.12	234.66**	0.15	0.82*	0.14	1.28	0.13	22.06	3.35
Genotype	54	2.14**	2139.23**	52.29**	9415.32**	11.98**	3.94**	15.54*	14.38*	2.99*	30460.32**	219.14**
GCA	9	7.21**	6854.37**	194.38**	26376.50**	32.78**	7.11**	58.98**	51.06**	11.92**	114065.92**	711.08**
SCA	45	1.14**	1227.22**	24.22**	6037.29**	7.86*	3.38**	6.91*	7.34*	1.22*	13934.53**	124.10**
Genotype x Season	54	0.07*	17.07	0.41	14.63	0.13	0.04	0.76	1.59	0.04	31.15	16.22
GCA x Season	9	0.19**	13.37	0.39	5.03	0.33	0.08	1.51	2.81	0.05	110.46	38.16**
SCA x Season	45	0.05**	12.63	0.48	15.06	0.11	0.02	0.75	1.36	0.04	30.71	21.06
Residual	213	0.01	19.98	0.61	39.41	0.52	0.19	0.15	1.37	0.15	94.09	5.30

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, DF = Degrees of freedom, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering, Env = Environment, GCA = General combining ability, SCA = Specific combining ability.

Table 4.3 Diallel analysis mean squares for grain yield and yield components evaluated at four environments in South Africa

Sources	DF	GY	NPP	NSPP	NB	HSWt	PL	PW	LL	LW	PH	D50F
Environment	3	0.63**	8769.87**	19.52**	0.76	7452.91**	13.13**	18.61**	3.23	0.38	0.76	33.57
Rep (Env)	8	0.13**	100.00	1.55	0.52	139.34	0.76	6.86**	1.18	0.68	0.52	23.623
Genotype	54	4.35**	3293.31**	87.84**	24.74**	12300.90**	6.78**	32.90**	28.98**	5.83**	24.74**	444.68**
GCA	9	15.42**	11746.32**	348.66**	69.34**	40886.84**	13.26**	102.54**	110.68**	24.51**	69.34**	1290.70**
SCA	45	2.15**	1631.79**	36.22**	16.04**	6604.64**	5.63**	20.17**	13.68**	2.14**	16.04**	279.94**
Genotype x Env	162	0.08**	208.92**	3.29**	1.33**	599.46**	0.64**	6.47	1.76	0.16	1.33**	18.95
GCA x Env	27	0.15**	356.88**	4.49**	2.06**	1037.81**	1.10**	5.74	3.19	0.33	2.06**	27.42
SCA x Env	135	0.06**	182.58**	3.10**	1.21**	516.36**	0.53**	6.35	1.46	0.13	1.21**	20.4
Residual	423	0.02	59.86	1.51	0.71	55.07	0.31	6.28	1.65	0.22	0.71	14.66

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, DF = Degrees of freedom, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering, Env = Environment, GCA = General combining ability, SCA = Specific combining ability.

Table 4.4 Variance components and heritability estimates for grain yield and yield components evaluated at four environments in South Africa

Sources	GY	NPP	NSPP	NB	HSWt	PL	PW	PH	LL	LW	D50F
σ^2GCA	0.09**	69.03**	2.16**	0.36**	234.45**	0.05**	0.58**	1498.84**	0.66**	0.15**	6.97**
σ^2SCA	0.17**	120.77**	2.76**	1.24**	507.36**	0.42**	1.15**	1985.28**	1.02**	0.17**	21.62**
σ^2GCA x Env	0.02**	4.84**	0.04**	0.02**	14.49**	0.02**	0.00	35.24**	0.05**	0.01**	0.19**
σ^2SCA x Env	0.02**	40.93**	0.53**	0.17**	153.78**	0.08**	0.00	249.22**	0.00	0.00	1.82**
σ^2Error	0.02	59.80	1.51	0.71	55.03	0.31	6.34	177.48	1.63	0.21	15.00
σ^2Additive	0.18	138.06	4.32	0.73	468.90	0.10	1.15	2997.69	1.32	0.31	13.94
σ^2Dominance	0.17	120.77	2.76	1.24	507.36	0.42	1.15	1985.28	1.02	0.17	21.62
σ^2GCA:SCA	0.53	0.57	0.78	0.30	0.46	0.12	0.50	0.75	0.65	0.88	0.32
Baker ratio	0.51	0.53	0.61	0.37	0.48	0.19	0.50	0.60	0.57	0.65	0.39
σ^2p	0.37	318.62	8.59	2.67	1031.28	0.83	8.64	5160.44	3.97	0.68	50.57
h^2	0.49	0.43	0.50	0.27	0.45	0.12	0.13	0.58	0.33	0.45	0.28

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering, GCA = General combining ability, SCA = Specific combining ability, σ^2 = Variance, Env = Environment, σ^2GCA = General combining ability variance, σ^2SCA = Specific combining ability variance, σ^2p = Phenotypic variance, h^2 = Narrow-sense heritability.

Table 4.5 Estimates of general combining ability for grain yield and yield components evaluated at four environments in South Africa

Parental genotypes	GY	NPP	NSSP	NB	HSWt	PL	PW	PH	LL	LW	D50F
Glenda	0.16**	1.39*	0.130	-0.24*	5.44**	-0.07	-0.400	24.85**	-0.16	-0.04	-0.41
TVU13953	0.45**	14.36**	2.16**	0.90**	25.95**	0.61**	0.67**	69.46**	1.49**	0.43**	-1.52
ITOOK-1060	-0.20	-1.44	-0.97	-0.27**	-1.60	-0.10	-0.36	-10.96	0.14	0.09	4.05**
IT93K-129-4	-0.22	-0.46	-0.87	-0.38*	-16.89**	-0.09	-0.46	-23.55	0.17	0.06	2.01**
Kisumi-mix	-0.05	-5.37**	0.16	-0.40**	-5.55	-0.04**	-0.53	-3.19**	0.17	-0.04	3.11**
TVU7778	-0.38**	-12.00	-2.31**	-0.12	-8.22	0.09	-1.27	-54.59**	0.25	-0.06	3.42**
98K-5301	-0.12	3.38**	-1.04**	0.63**	-14.37**	0.11*	0.16	20.98	0.14	-0.06	-3.17
IT96D-602	0.66**	15.02**	2.98**	1.15**	33.15**	0.52**	1.62**	66.57**	0.35**	-1.04	-4.72
IT845-2246	-0.10	-6.16**	-0.22	-1.17**	-9.22	-0.28**	0.90**	-22.42	0.54**	0.45**	-1.05
TVU-14196	-0.20	-8.72**	-0.02	-0.09	-8.74	0.18**	-0.35	-25.19**	-2.02	0.21	-1.73*

$^{**}P \leq 0.001$, $^{*}P \leq 0.05$, Values without asterisk are not significant, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering.

Table 4.6 Estimates of specific combining ability for grain yield and yield components evaluated at four environments in South Africa

F_1 progenies	GY	NPP	NSPP	NB	HSWt	PL	PW	PH	LL	LW	D50F
TVU13953 x Glenda	0.37**	30.65**	4.70**	2.11**	75.02**	0.82**	2.80**	81.65**	1.73**	0.85**	-7.42**
ITOOK-1060 x Glenda	-0.23**	-11.46	-2.08**	-1.04**	6.09**	-0.32	-0.16	-30.56**	-0.59	-0.24	3.51**
IIT93K-129-4 x Glenda	-0.20*	-7.12*	-1.16*	-0.11	-9.75**	-0.09	0.16	-9.23	-0.33	0.06	5.05**
Kisumi-mix x Glenda	-0.10**	-1.72	-0.24	-0.29	-18.99	0.08**	-0.63	9.56	0.58	0.03	3.45**
TVU7778 x Glenda	-0.59	-2.29	-1.68**	-0.75	-16.30	0.11**	-1.03	-30.74	0.01	0.05	2.31
98K-5301 x Glenda	0.44*	-6.31**	1.50*	0.26	-23.48**	0.34**	-0.71	-56.02	0.13	0.35	-1.27
IT96D-602 x Glenda	0.79**	-3.41	1.12*	0.58	9.45	-0.15	0.07	-1.31	0.65	-0.23	-2.22
IT845-2246 x Glenda	-0.28**	7.88**	0.37	1.06**	-9.99*	-0.01	0.23	-11.17**	0.02	-0.04	-3.63**
TVU-14196 x Glenda	-0.13**	-2.77	0.72	0.03	-1.90	1.09**	-1.05	-36.66**	-1.42**	-0.37**	-3.21**
ITOOK-1060 x TVU13953	0.70*	-11.99	-2.94**	-1.89**	-0.15	-0.88	-1.11	-59.98**	0.87	-0.41*	7.11**
IT93K-129-4 x TVU13953	-0.30**	-12.31**	-1.48	-0.90**	-29.36**	-1.12**	-0.80	-56.16**	-1.41**	-0.37**	6.65**
Kisumi-mix x TVU13953	-0.18**	-4.30	-0.71	0.89*	-26.95	-1.38**	-0.95	-6.52	-0.48	-0.44**	4.56**
TVU7778 x TVU13953	-0.71	-21.24**	-2.19**	-0.77	-38.91	-0.19	-1.49	-91.02	-1.10**	-0.39**	4.75**
98K-5301 x TVU13953	0.19	8.35**	2.11**	0.27	-14.94**	-0.42**	-0.26	59.31	-0.28	-0.49**	0.02
IT96D-602 x TVU13953	0.88**	13.16**	2.33**	0.02	8.90**	-0.80	1.20	83.50**	-0.57	-0.79**	-3.62**
IT845-2246 x TVU13953	0.00	-12.72**	-1.96	-1.23**	-33.20**	-0.63**	0.37	-66.84**	-1.71**	-0.09	-1.29
TVU-14196 x TVU13953	-0.67	-16.57**	-2.82	-1.37**	-24.72	1.03**	-1.04	-77.46**	-2.26**	0.32	1.39
IT93K-129-4 x ITOOK-1060	0.16	-1.188.50*	-0.78	-0.11	11.94**	-0.40	-0.08	16.38**	-0.72	0.05	3.58**
Kisumi-mix x ITOOK-1060	0.23	2.81	-1.39**	3.03**	-10.58**	-0.21	-0.24	-4.85	0.24	0.16	3.74**
TVU7778 x ITOOK-1060	-0.09**	-5.65	0.48	-1.01	-9.54**	1.02**	-0.21	8.06	-0.01	-0.06	-3.82
98K-5301 x ITOOK-1060	0.02	5.99**	0.78	0.96	-4.73	0.54**	-0.31	-3.98	-0.11	-0.05	-1.98
IT96D-602 x ITOOK-1060	0.00	16.59**	2.30**	1.24**	12.12**	-0.23	0.96	52.70**	0.57	-0.46**	-7.19**
IT845-2246 x ITOOK-1060	0.13	4.29	-1.10*	-0.18	-2.95	0.09	0.73	-4.28	0.74	0.16	-4.86**
TVU-14196 x ITOOK-1060	-0.58**	-1.18	-1.57**	0.81**	18.74**	-0.40	0.26	16.54	2.17**	0.06	-1.18
Kisumi-mix x IT93K-129-4	0.44	-7.16*	-0.14	-0.06	-3.31	-0.28	-0.46	-49.24**	0.52	-0.22	-3.14**
TVU7778 x IT93K-129-4	-0.11**	6.37**	0.29	-0.31	-6.82**	1.15**	-0.58	-19.87*	0.29	-0.08	-0.28
98K-5301 x IT93K-129-4	-0.09	1.39	2.65**	1.27	-9.72**	0.43**	-0.79	-5.66	-0.17	0.43**	-4.12**
IT96D-602 x IT93K-129-4	-0.23**	16.73**	1.88**	0.89**	30.06	0.12**	0.87	37.06**	0.10	-0.37**	-4.15**
IT845-2246 x IT93K-129-4	0.15	-1.28	0.47	0.64**	8.00**	0.43**	0.93	12.99**	-0.21	-0.30	-3.31**
TVU-14196 x IT93K-129-4	0.00	-0.22	-1.12**	-0.26	-9.47**	-0.43**	-1.12	13.19**	0.32	-0.04	-6.55**
TVU7778 x Kisumi-mix	-0.45	2.72	-0.12	0.18	12.93*	0.99**	0.05	-27.01**	-0.23	0.29	-2.13
98K-5301 x Kisumi-mix	-0.16**	-0.90	-2.04**	0.82**	5.60**	0.59**	-0.99	-19.86**	-0.05	0.04	-2.46
IT96D-602 x Kisumi-mix	-0.41*	11.47**	0.47	0.55	22.76	-0.27	1.30	45.10	0.89	-0.39**	-7.74**
IT845-2246 x Kisumi-mix	0.17	14.50**	-0.38	-0.23	12.60	-0.21	1.34	-14.53**	-0.04	-0.06	-0.40
TVU-14196 x Kisumi-mix	-0.01	11.66**	0.59	0.82**	-22.24**	0.05	0.17	53.67	-2.27**	-0.29	0.77
98K-5301 x TVU7778	0.77	5.02	-0.95**	-1.59**	6.37**	-0.19	0.82	-31.65*	-0.04	0.04	-4.60**
IT96D-602 x TVU7778	0.84	-3.63	0.14	0.29	19.11**	-0.46**	1.52	33.25	-0.77	-0.27	-6.55**
IT845-2246 x TVU7778	0.76	-8.57**	0.40	-0.06	22.94	-0.50**	1.22	49.50**	0.71	-0.01	-0.22
TVU-14196 x TVU7778	0.24	3.58	0.80	-1.39**	11.46**	-0.43**	0.30	44.25**	1.45**	0.81**	2.92**
IT96D-602 x 98K-5301	-0.39	15.61**	1.78**	-0.04	25.98**	-0.03	0.74	-21.28**	0.71	-0.35**	0.54
IT845-2246 x 98K-5301	-0.02	-9.35**	0.54	-0.97**	34.41	-0.25	0.45	10.66*	-0.34	-0.08	7.87**
TVU-14196 x 98K-5301	0.42	-13.32**	-1.29**	-1.69**	-1.17	-1.06**	4.92**	13.80	0.20	-0.32	-1.15
IT845-2246 x IT96D-602	-0.29**	-18.97**	-1.14*	-1.62**	-37.70	0.06**	-0.82	-76.44**	-0.21	0.67**	0.42
TVU-14196 x IT96D-602	-0.72*	-19.11**	-2.54**	-1.04**	-43.01**	0.39	-1.62	-60.18**	-1.67**	0.78**	6.76**
TVU-14196 x IT845-2246	-0.02	9.62**	-1.40**	-2.25**	5.13**	-0.10	-1.33	38.60**	1.22**	-0.43	-5.07**

$^{**}P \leq 0.001$, $^{*}P \leq 0.05$, Values without asterisk are not significant, First parent on the progenies represent female, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering.

Table 4.7 Mid-parent heterosis for yield and yield components evaluated at four environments in South Africa

F$_1$ progenies	GY	NPP	NSPP	NB	HSWt	PL	PW	LL	LW	PH	D50F
TVU13953 x Glenda	115.97*	-28.03*	-18.97*	-40.08*	-67.41*	-34.13*	-23.42	-4.55	38.29*	-46.82*	16.55*
ITOOK-1060 x Glenda	59.47*	-49.54*	-10.68*	-37.74*	-31.81*	-24.81*	-29.51*	-50.58*	-32.92*	-31.92*	2.08
IIT93K-129-4 x Glenda	-1.37	-36.91*	-15.51**	-36.83*	-69.32*	-16.59*	-27.59*	-33.57*	-22.06*	-23.39*	-22.78*
Kisumi-mix x Glenda	-4.89	-2.59	25.78*	-21.81*	-26.41*	-16.58*	-27.34	-40.79*	32.11*	-25.07*	-14.22*
TVU7778 x Glenda	-26.90*	-37.77*	13.07*	-28.22*	-52.92*	-25.14*	-28.72*	-40.96*	-12.50	-37.38*	9.54*
98K-5301 x Glenda	99.32*	-9.59	20.76*	-25.95*	-5.18	-14.68*	-21.98	0.19	-13.68	-29.01*	-16.43*
IT96D-602 x Glenda	179.34*	-4.03	-19.64*	-19.95*	-57.56*	2.41	-23.78	-23.09*	106.67*	19.31	-23.27*
IT845-2246 x Glenda	16.36*	-28.57*	6.25	-14.99*	-4.40	-19.34*	-8.06	-20.00*	15.05	-32.23*	-20.83*
TVU-14196 x Glenda	-5.26	-14.09	23.96*	13.43	-30.09*	-12.01*	-6.36	-33.42*	11.13	-25.54*	-11.05*
ITOOK-1060 x TVU13953	162.02*	-38.58*	-20.84*	9.18	-10.64	-39.60*	-23.76	-11.03	-25.81*	-57.32*	-22.55*
IT93K-129-4 x TVU13953	9.76	45.34*	-26.38*	10.41	13.14	-33.61*	-21.17	-28.28*	16.45*	-52.23*	-16.25*
Kisumi-mix x TVU13953	24.60*	14.72	-27.12*	-16.71*	103.43*	-33.40*	-20.69	-4.76	19.65*	-52.94*	-20.92*
TVU7778 x TVU13953	53.49*	73.04*	-35.16*	13.88*	15.70	-35.73*	-13.41	-16.73*	0.29	-58.94	10.32***
98K-5301 x TVU13953	135.98*	-37.75*	-33.65*	7.11	-59.11*	-34.78*	-22.26	-1.20	12.75	-67.38*	-12.00*
IT96D-602 x TVU13953	168.66*	-37.39*	-19.97*	26.84*	88.56*	-28.64*	-25.33	-4.02	104.36*	-48.06*	24.26*
IT845-2246 x ITOOK-1060	13.74*	-6.32	16.63*	30.00*	35.25*	-37.52*	-11.78	-32.28*	45.85*	-71.90*	-18.40*
TVU-14196 x TVU13953	-30.19*	76.09*	-30.98*	25.43*	25.66*	-27.35*	-11.14	-19.48*	14.94	-53.04*	-16.08*
IT93K-129-4 x ITOOK-1060	-31.43*	-15.98	13.66*	3.66	56.97*	-26.48*	-30.67*	-41.65*	-17.80*	-34.92*	-14.59*
Kisumi-mix x ITOOK-1060	-41.67*	-19.74	24.27*	7.17	64.56*	-29.86*	-37.94*	-23.16*	-11.13	-45.74*	-9.69*
TVU7778 x ITOOK-1060	-30.88*	7.45	-2.69	-16.24*	42.85*	-29.01*	-11.10	-33.03*	-28.49*	-43.82*	-13.13*
98K-5301 x ITOOK-1060	-2.46	-20.96	11.03*	16.19*	62.23*	-18.11*	-3.52	-23.11*	-17.42*	-36.31*	0.84
IT96D-602 x ITOOK-1060	-27.69*	-54.82*	-22.64*	-13.21	21.73*	-2.21	-7.04	-24.73*	-26.38*	11.86	28.82*
IT845-2246 x TVU13953	52.23*	-27.67*	0.55	30.70*	132.11*	-35.74*	-14.14	-6.65	97.79*	-29.63*	15.75*
TVU-14196 x ITOOK-1060	-5.94	-26.03*	15.64*	15.18*	-6.28	-18.42*	-7.93	-36.98*	-19.30*	-31.41*	-6.14
Kisumi-mix x IT93K-129-4	-12.50	-25.17*	10.07*	12.16	-16.37	-23.48*	-38.71	-25.49*	1.11	-42.49*	2.16
TVU7778 x IT93K-129-4	57.81*	19.35	3.55	-17.98*	-8.44	-19.51*	-7.13	-40.63*	5.61	-29.83*	0.71
98K-5301 x IT93K-129-4	65.13*	-21.88*	7.80	-29.55*	31.84*	-8.30*	0.00	-22.67*	4.66	-21.77*	1.49
IT96D-602 x IT93K-129-4	-25.40*	-7.70	13.50*	-7.23	18.47	-20.18*	-13.02	-28.39*	83.33	30.37*	6.98
IT845-2246 x IT93K-129-4	45.81*	-31.16*	-10.85*	52.86*	11.44	-16.90*	5.71	-39.41*	13.56	-26.40*	-19.19*
TVU-14196 x IT93K-129-4	43.13*	-38.23*	-13.14*	29.39*	-5.27	-9.07*	7.93	-37.65*	-11.47	-19.60	0.69
TVU7778 x Kisumi-mix	83.62*	-4.43	-24.15*	-31.02*	-57.87*	-30.76*	-35.60*	-21.67*	3.51	-49.36*	-23.36*
98K-5301 x Kisumi-mix	54.23*	-36.93*	-9.77	-20.04*	-52.96*	-20.47*	-29.86*	-9.03	-7.32	-43.52*	15.20*
IT96D-602 x Kisumi-mix	105.43*	-29.87*	-4.60	-8.12	-46.82*	-10.56*	-39.20*	-14.75*	98.31*	-18.20	5.95
IT845-2246 x Kisumi-mix	47.37*	-32.17*	0.25	-7.46	-4.58	-27.35*	-33.65*	-1.91	1.27	-40.60*	-14.02*
TVU-14196 x Kisumi-mix	37.33*	-36.84*	-3.84	-14.47*	-61.71*	-20.74*	-31.48*	-10.65	8.77	-34.70*	-13.70*
98K-5301 x TVU7778	-21.26*	-45.97*	-25.20*	-34.03*	-60.93*	-17.37*	11.11	-19.25*	0.00	-37.18*	11.31*
IT96D-602 x TVU7778	24.72*	-33.72*	-17.05*	22.57*	-35.99*	-1.20	11.60	-17.11*	73.90*	16.39	15.75*
IT845-2246 x TVU7778	-48.24*	-41.89*	-13.70*	25.46*	-62.29*	-25.30*	11.24	-11.01	-8.76	-41.62*	11.08*
TVU-14196 x TVU7778	70.80*	-31.57**	-12.57*	-27.27*	-38.25*	-17.49*	20.39	-33.27*	-19.44*	-34.41*	10.69*

Table 4.7 Mid-parent heterosis for yield and yield components evaluated at four environments in South Africa (continued)

IT96D-602 x 98K-5301	119.59*	-0.57	-0.04	27.91*	11.92	-19.78*	-11.78	1.39	82.75*	25.91*	35.51*
IT845-2246 x 98K-5301	38.78*	-19.62	1.49	33.47*	-6.62	-13.40*	30.79*	-10.17	-5.90	-32.21*	-1.10
TVU-14196 x 98K-5301	58.82*	0.30	15.06*	22.81*	-37.82*	-5.10	-1.21	-7.20	-10.51	-23.26*	-11.96*
IT845-2246 x IT96D-602	-13.83*	2.68	6.74	32.34*	1.63	3.74	-6.08	-8.02	-5.77	25.36*	-19.95*
TVU-14196 x IT96D-602	23.37*	9.65	-12.76*	33.90*	-33.13*	13.89*	-4.19	-9.11	-5.30	34.60*	2.60
TVU-14196 x IT845-2246	-20.75*	-10.58	14.25*	-17.81*	-71.95*	-11.73*	-7.10	26.78*	3.37	-25.26*	19.20*
Mean heterosis	35.92	-15.47	-4.17	0.34	-6.33	-19.83	-13.75	-19.85	13.19	-28.70	-2.54

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, First parent on the progenies represent female, GY = Grain yield, NPP = Number of pods per plant, NSPP = Number of seeds per plant, NB = Number of branches, HSWt = Hundred seed weight, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, PH = Plant height, D50F = Days to 50% flowering.

4.5. DISCUSSION

4.5.1. Diallel analysis of variance and heritability for grain yield and yield components

The significant effects of genotype, GCA and SCA for measured grain yield and yield components, highlighted the presence of large variation among parental genotypes and the F_1 progenies, which could contribute to selection efficiency within the population. The significant SCA and GCA effects indicated that both additive and non-additive (dominance and epistasis) gene action played an important role in the expression of cowpea grain yield. The results agree with Ayo-Vaughan *et al.* (2013), who studied gene action and combining ability for eight parental cowpea genotypes and 28 F_1 progenies, and reported significant GCA and SCA for grain yield, pod width, pod length and hundred seed weight. Owusu *et al.* (2018) investigated the combining ability of five parental genotypes and 20 F_1 progenies. They found significant GCA and SCA for all characteristics examined, except for SCA for pod length and GCA for pod weight and number of pods per plant. Additionally, the same study revealed higher SCA than GCA effects for grain yield, days to 50% flowering, number of branches, and number of pods and pod weight. Therefore, the results from the current study indicated the importance of both additive and non-additive genetic effects in the improvement of cowpea grain yield and yield components.

The significant GCA by environment, SCA by environment interaction, GCA by season and SCA by season interaction indicated that both the location and season played a role in the expression of GCA and SCA for all the measured characteristics. Previous studies using a full diallel mating design to study combining ability in cowpea for grain yield and yield components and reported significant GCA by environment and SCA by environment interaction for grain yield and most of the measured characteristics (Ayo-Vaughan *et al.* 2013; Kumari and Chauhan 2018; Owusu *et al*. 2018). Therefore, the results from the current and previous studies suggests that there is a need for evaluation of cowpea in multiple environments to enhance selection efficiency of stable and superior genotypes for advancement.

The predominance of SCA over GCA variance, ratio of GCA to SCA variance and Baker ratio less than unity (≤ 1) observed for grain yield and yield components indicated that dominance gene action was predominant over additive gene action, which shows that there is potential for hybrid breeding in cowpea. Because non-additive gene action predominates, selection in early generations may not be effective, therefore, when there is homogeneity, selection should be done in later generations. Recurrent selection modified for biparental progenies to develop breeding populations could exploit both additive and non-additive genetic variance for genetic improvement of grain yield and yield components.

The results in this study correspond with findings by Owusu *et al.* (2020) who determined combining ability, heritability, and genetic advance on five parental genotypes and 20 F_1 progenies and found significant GCA and SCA effects for pod yield and maturity characteristics and higher SCA over GCA variance. The results did not corroborate findings of Azam *et al.* (2022), who studied combining ability and heterosis for grain yield and its components on six mung bean parental genotypes and their 15 F_1 progenies from a half-diallel mating design and reported significantly higher mean square values for GCA than SCA for all the yield components measured. The results are also in contrast with Mukati *et al.* (2014), who studied combining ability and gene action for eight parental genotypes and 28 F_1 progenies and reported higher GCA variance than SCA variance. Additive genetic variation is required for an effective response to selection and cultivar improvement for grain yield and yield components.

The estimates of narrow-sense heritability of important characteristics play an important role in selection of elite genotypes in a breeding programme, as it shows the ability of parental genotypes to transfer these characteristics to their progeny. The narrow-sense heritability estimates ranged from 0.12 (pod length) to 0.58 (plant height) indicating that selection could be effective for some of the characteristics (such as plant height), but not for those with low heritability (such as pod length). Non-additive genetic effects are exploitable in hybrid breeding. The study found low narrow-sense heritability for pod length and pod width, indicating that non-additive genetic effects primarily control these characteristics, making genetic improvement of difficult. Low to moderate narrow-sense heritability for yield components was reported in a previous study (Mwale *et al.* 2017), which was undertaken to estimate combining ability and heritability for five drought tolerant cowpea genotypes using a North Carolina II mating design. Purnamasari *et al.* (2019) studied the inheritance and diversity though diallel mating of five cowpea genotypes and reported low narrow-sense heritability for yield components.

The results also corroborated the findings by Jou-Nteufa and Ceyhan (2022) who determined combining ability and heritability in F_2 populations of cowpea through diallel analysis and reported moderate to low narrow-sense heritability. On the other hand, Chiulele (2010) conducted a combining ability study under drought stress and reported high narrow-sense heritability for grain yield. The findings from these studies indicates that the narrow-sense heritability of grain yield and yield components is affected by the specific set of genotypes tested, as well as the environment in which they are grown.

4.5.2. Estimates of general combining ability for grain yield and yield components

The positive significant GCA estimates for all the characteristics measured was an indication of gene flow from parental genotypes to progenies. Parental genotypes TVU13953 and IT96D-602, with high positive GCA values translate to the best combiners for desirable grain yield, hundred seed weight, pod width, number of branches, leaf length, leaf width, number of pods per plant and number of seeds per plant. The results confirm the contribution of these parental genotypes (TVU13953 and IT96D-602) as favourable donors for the improvement of grain yield, as was evident from superior progenies observed for grain yield in Chapter 3. The parent Glenda was also a good general combiner for grain yield, hundred seed weight, number of seeds per plant and plant height. As a result, these parental genotypes are better than other parental genotypes for use to increase grain yield.

The yield components are usually positively associated with grain yield, thus parental line 98K-5301 could also contribute positively as a donor for grain yield as this parent was the best combiner for number of branches, number of pods per plant, pod length and plant height. The parental genotypes IT93K-129-4, Kisumi-mix and TVU7778 were the best combiners for days to 50% flowering and could be beneficial parental genotypes for breeding for early maturity to escape drought stress in drought prone areas. Romanus *et al.* (2008), who studied combining ability on 21 F_1 progenies derived from diallel and their seven parental genotypes, also reported two best general combiners for grain yield. Pethe *et al.* (2018) used line by tester analysis for determining of combining ability in 11 cowpea parental genotypes and their 24 F_1 progenies and identified two good general combiners for grain yield per plant.

Parental genotypes TVU1395, IT96D-602 and Glenda could be useful in a breeding programme, as these parental genotypes carry favourable genes for grain yield and yield components. Hence, these genotypes could be useful as basic parental material to create a segregating population. Indirect selection based on associated yield components could also result in effective genetic gains and resource use efficiency in grain yield improvement programmes. In addition, the exclusion of poor combiners might further reduce the time and resources needed to develop a high yielding cultivar. The poor combiners for grain yield could be good parental genotypes for other breeding goals. For instance, the Kisumi genotype has a high protein content, according to research by Gerrano *et al.* (2015), indicating that cowpeas descended from this parental genotype could be bred for nutritional value. Hence, evaluating the combining ability of this parent for nutritional characteristics, and for protein content, might be beneficial. The findings from this study indicated the importance of evaluating parental genotypes for combining ability for all-important characteristics to ensure successfully breeding and reduction in time for releasing superior cultivars.

4.5.3. Estimates of specific combining ability for grain yield and yield components

The significant and positive SCA indicated an opportunity for improvement of grain yield through hybrid breeding. The six progenies with positive significant SCA for grain yield had either one or two of the three best general combiners in common (TVU13953, IT96D-602 and Glenda), thus these progenies could be used for further breeding of improved cultivars for yield. Magar *et al.* (2016) also recorded significant positive SCA effects for seed yield per plant and number of seeds per pod from a study using diallel analysis in cowpea for seven parental genotypes and their 21 progenies. Best specific combinations for grain yield and yield components measured was reported in a diallel analysis of 10 parental genotypes, 21 F_1, and 21 reciprocal F_1 (Olajide and Olawale 2021). The progenies Glenda x TVU13953 and TVU7778 x IT96D-602, involved parental genotypes TVU13953 and IT96D-602, respectively with higher GCA for grain yield, and best SCA for grain yield and could also be used as parental material for producing desirable segregants in a breeding programme. The progeny Glenda x 98K-5301 for instance, had a significant SCA, whereas one of the parental genotypes (98K-5301) was a poor combiner for grain yield. This could be because the parental genotypes involved might have the same genes (additive gene action) controlling grain yield. Several researchers reported progenies with best SCA for grain yield and yield components (Ayo-Vaughan *et al.* 2013; Owusu *et al.* 2018).

4.5.4. Magnitude of mid-parent heterosis for grain yield and yield components

The magnitude and direction of mid-parent heterosis is important for improvement of grain yield and yield components through hybrid breeding. The positive and significant mid-parent heterosis for grain yield observed for 60% of the progenies in the current study indicated the superiority of progenies over their mid parental genotypes and further suggests the importance of either dominant or partial dominant genes in expression of grain yield. Thus, the best approach for improving grain yield could be through hybrid breeding due to the presence of significant heterosis. The results corroborate findings by Risha *et al.* (2017), who reported positive significant heterosis (68.46%) for seed yield per plant in a study that evaluated standard heterosis and heterobeltiosis in vegetable cowpea, using three genotypes, eight testers and their 24 F_1 progenies produced by using the line by tester design. Singh (2014) also determined heterotic response, combining ability and gene action for yield and yield contributing characteristics in cowpea, and reported positive significant heterosis for grain yield.

Although significant amounts of positive heterosis was seen for grain yield, this was not the case for yield components except for leaf width (57.80%), while for the rest of the characteristics half of the values indicated negative mid-parent heterosis, suggesting lack of either dominant or partial dominant genes in expression of these characteristics. Several researchers (Patil *et al.* 2005; Meena *et al.* 2009; Kamai *et al.* 2014) reported the varied magnitude of heterosis for plant height, 100 seed weight, and pod length in cowpea. The progenies with negative mid-parent heterosis for D50F could suggest earlier flowering and maturity dates compared to their parental genotypes, and selection of these progenies will benefit the drought prone arears. Previous studies indicated earlier flowering in progenies than the better parent on cowpea (Patel 2009) and chickpea (Gadekar and Dodiya 2013). When breeding for cultivar release it is important to base selection and ranking on standard check heterosis as selection based on the mid parent, heterosis might be misleading, without consideration of commercial checks.

4.6. CONCLUSIONS

Both additive and non-additive gene effects were involved in the inheritance of grain yield and yield components measured, but non-additive genetic effects were predominant. The low to medium narrow-sense heritability observed further confirmed the involvement of both additive and non-additive genetic effects in the inheritance of grain yield and yield components. The environment also played a major role in expression of both GCA and SCA. Parental genotypes Glenda, TVU13953 and IT96D-602 were the best general combiners for grain yield, number of pods per plant, number of seeds per pod, number of branches and plant height. The selection of the best general combiners could improve cowpea grain yield, as the characteristic/s will be transferred from the parental genotypes to offspring. The progenies with the best SCA and significant positive mid-parent heterosis for grain yield were TVU13953 x Glenda, ITOOK-1060 x TVU13953, 98K-5301 x Glenda, ITOOK-1060 x TVU13953, IT96D-602 x Glenda and IT96D-602 x TVU13953. These progenies could be tested in multiple environments to determine yield stability and stable hybrids could be considered for commercial release.

Although heterosis for grain yield was high, considering hybrid breeding could still be a challenge as cowpea yield few seeds per cross thus breeders need to weigh other better methods such as pure line selection.

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CHAPTER 5

VARIATION IN PERFORMANCE OF COWPEA F_1 PROGENIES AND PARENTAL GENOTYPES FOR NUTRITIONAL CHARACTERISTICS

5.1. ABSTRACT

Cowpea is a nutrient dense grain and vegetable legume crop that has a significant potential to reduce malnutrition. The purpose of this study was to determine variation among selected cowpea parental genotypes and progenies for nutritional characteristics and to identify superior parental genotypes for future breeding. In the cropping seasons of 2021 and 2022, the population was assessed at Brits and Loskop using a randomized complete block design with three replications. The parental genotypes and their F_1 progenies (45) were analysed for nutritional characteristics at two locations in two seasons. Significant genotype and genotype by environment interaction effects were found for all nutritional characteristics measured, except for Zn content, suggesting the possibility of a response to selection. Broad-sense heritability was high (0.96 to 0.99) for protein, starch, amylose and phytic acid contents. Fe and Zn content indicated low broad-sense heritability of 0.14 and 0.22, respectively. The findings indicated that selection efficiency could be realised for characteristics such as protein as compared to characteristics with low heritability (Fe). The progenies 98K-5301 x ITOOK-1060 and IT845-2246 x 98K-5301 were superior for protein content, Fe, and Zn contents, while the cross 98K-5301 x Glenda was superior for both protein and Zn content. Parental genotype Kisumi-mix was superior for protein, IT845-2246 for Fe, Glenda for Zn, and 98K-5301 for protein content. Sufficient genetic variability was evident for measured nutritional characteristics in this genotype set for improvement of cowpea through direct and indirect selection.

Keywords: cowpea progenies, broad-sense heritability, nutritional characteristics, malnutrition

5.2. INTRODUCTION

Plant based protein is a cheaper alternative to animal protein to combat nutritional insecurity in SSA. Hidden hunger due to deficiencies in Fe, Zn, and vitamin A in the diets of the majority of the South African population, results in malnutrition, which causes serious constraints to human health (Kehoe et *al.* 2021). Malnutrition refers to imbalances or deficiencies of essential nutrients, resulting in stunted growth, obesity and compromised human health (Statistics South Africa 2017; National Department of Health *et al.* 2016). The most common deficiencies in the diets of poor South African communities are Zn, Fe, and vitamin A, which cause health problems such as impaired mental development and anaemia, caused by Zn and Fe deficiency, and blindness because of vitamin A deficiency (Stevens *et al.* 2015). The problem of malnutrition in South Africa is serious, as 27% of children are stunted, and 44% have vitamin A deficiency (May *et al.* 2020). In addition to that, about 67.6% of females and 31.3% of males older than 15 years are obese or overweight, and 33.3% of women aged 15 to 49 years are anaemic, hence these calls for a robust approach to mitigate the problem of malnutrition that the country is facing (National Department of Health *et al.* 2016).

Cowpea is a rich source of plant protein and contains considerable amounts of carbohydrates, fatty acids, vitamins, and minerals (Mekonnen *et al.* 2022). The crop is a cheap alternative to replace expensive animal proteins and thus relieve the strain on the lower income families (Gerrano *et al.* 2017). Starch is the fundamental component of carbohydrates and constitutes amylose and amylopectin (Perera *et al.* 2010). Starch is the main source of energy, contributing to growth and development, and control of insulin, glucose, and cholesterol metabolism (Perera *et al.* 2010). Essential mineral elements such as Fe and Zn in cowpea play an important role in human bodily processes. Fe plays a vital role in DNA synthesis and haemoglobin formation (Ayeni *et al.* 2018). Zn is a trace element that is responsible for structural, catalyst, and ion regulation in humans to reduce prevalence of diseases (Da Rosa *et al.* 2010).

Cowpea is consumed as a vegetable in South Africa (tender green pods or leaves) or as grains (soup or snack). The primary producers of cowpea in South Africa are small-scale farmers, who depend on the local collections of accessions, which are low yielding, and poorly adapted to the country's climatic conditions, with limited information on nutritional characteristics. Hence, it is important to embark on the genetic improvement of cowpea for nutritional characteristics in addition to grain yield. Some studies have reported the presence of variability for nutritional characteristics in South African cowpea collections (Gerrano *et al.* 2015; 2019; 2022; Mbuma *et al.* 2021).

The genetic improvement of cowpea for grain yield has received the most attention worldwide (Ayo-Vaughan *et al.* 2013; Owusu *et al.* 2018; Gerrano *et al.* 2019; 2022; Owusu *et al.* 2020; Mbuma *et al.* 2021), however there are no targeted programmes aimed at improvement of cowpea nutritional characteristics in South Africa.

Hybridisation is a vital method of combining characteristics of different parental genotypes to produce new gene combinations, with an aim of creating variability in a population for selection, to combine desirable characteristics into a single individual and finally to utilize and exploit newly developed cultivars. The aim of this study was to evaluate performance of selected cowpea parental genotypes and their F_1 progenies for nutritional characteristics and to identify superior parental genotypes for future breeding advancement and the best progenies for improvement of nutritional characteristics in cowpea.

5.3. MATERIALS AND METHODS

5.3.1. Planting materials

In Chapter 3, Section 3.3.1, the details of the planting materials used in this investigation were covered.

5.3.2. Experimental locations

Section 3.3.3 of Chapter 3 contains a description of the experimental locations in detail.

5.3.3. Experimental design, trial establishment and management

The details of the trial setup, management, and experimental design are outlined in Chapter 3, Section 3.3.4.

5.3.4. Data collection

The harvested cowpea seeds from Loskop and Brits locations for 2021 and 2022 growing seasons were ground using an A10 Yellowline, 1KA analysis grinder (Merck Chemicals Pty Ltd) and then sieved to fine flour using a 1 mm sieve. The flour was used for analysis of Fe (mg kg^{-1}), Zn (mg kg^{-1}), phytic acid (mg kg^{-1}), starch (%), and amylose (%).

5.3.4.1. Determination of protein content

The cowpea grain was analysed for crude protein using a near infrared (NIR) spectroscope (Model DA 7250, Perten, Instruments AB, Sweden) in the 900-1700 nm wavelength range.

The samples were left at room temperature (21ºC) before use to avoid temperature interference (Anderson *et al*. 2019). To gather reflectance spectra, the samples were placed in a 90 mm petri dish and scanned using Labspec pro-portables from ASDinc (Malvern Panalytical Pty Ltd, United Kingdom). Each sample was scanned three times, and the values were averaged. Wet chemistry analysis was performed on 100 randomly selected cowpea samples using nitrogen combustion, to pre-calibrate the apparatus and benchmark the results.

5.3.4.2. Determination of Fe and Zn content

Direct X-ray fluorescence (XRF) (Malvern Panalytical Pty Ltd, United Kingdom) was used for determination of Fe and Zn content (Borgese *et al.* 2020). The spectrometer was calibrated using the results of wet chemistry analysis of 100 samples with Atomic Absorption Spectroscopy (AA). For XRF analysis, each sample was placed in the spectrometer, which was aligned with the X-ray source and detector. The spectrometer then generates X-rays and measures the fluorescent X-rays from the samples.

5.3.4.3. Determination of phytic acid

Phytic acid was measured according to the colorimetric method (Dragicevic *et al.* 2011). The finely ground cowpea grain samples were weighed (0.2500 g ± 0.0003) into 15 mL tubes with screw lids. Extraction of phytic acid was done on each sample for one hour using an extraction solution of 5% trichloroacetic acid (TCA) and a horizontal mechanical shaker. After centrifuging the extracts for 20 minutes, 1.50 mL of Wade reagent was added, and 0.5 mL of the supernatant was transferred to 15 mL tubes. To get a clear supernatant, the samples were centrifuged for 10 000 rpm for 10 minutes.

The absorbance was measured at 500 nm using a UV VIS spectrophotometer (Jenway 7315, Mettler Toledo). The spectrophotometer was first zeroed with double distilled water, then the Wade reagent absorbance was measured, and finally the sample absorbance was measured by filling the micro cuvette to a quarter of the volume with supernatant. Different intensities of pink in the supernatant were evident (representing different phytic acid content due to the bond between Fe and phosphate). The formula below was subsequently used to calculate the phytic acid concentration of a sample in micromoles per 100 mL (µm/100 mL).

$$\text{Phytic acid} = \frac{(\Delta\text{A}-\text{a})}{b}$$

Where ΔA = Change (reduction) in absorbance, a = Constant from the standard curve equation, b = Slope of the standard curve.

5.3.4.4. Mineral bioavailability estimation

The molar ratios of phytic acid/minerals were estimated for prediction of bioavailability of the Fe and Zn. The ratios were computed following the method described by Akomo *et al.* (2016). The method involved dividing the concentration of phytic acid (millimoles) with that of the minerals, either Fe or Zn. The molar mass of phytic acid (660.04 g/mol), Fe (55.85 g/mol) and Zn (65.38 g/mol) were first used for converting the concentration units to moles, followed by calculation of the molar ratio of phytic acid to Fe or Zn by dividing the number of moles of phytic acid by the number of moles of Fe or Zn.

5.3.4.5. Determination of starch content

A polarimetric method (or Ewers' method) was used (Farcas *et al.* 2013) to measure starch content. A digital scale was used to weigh the milled flour samples to the nearest 0.001 g, and duplicates were placed in 100 mL Erlenmeyer flasks, then 50 mL of 1M hydrochloric acid (HCl) solution was added. This was done by pipette and gently swirling of the mixture, followed by stirring of the sample suspensions in flasks every five minutes to prevent sample coagulation, using a glass rod. After that, the flasks were arranged in three wooden racks and placed in a water bath that had been heated to 95ºC, after which the samples were placed on the bench to cool to about 20ºC. After cooling, the samples were transferred quantitatively to 100 mL volumetric flasks by using a 55 mm funnel. The leftover solution was rinsed out of the Erlenmeyer flasks with 10 mL distilled water before being moved to volumetric flasks.

Tungstophosphoric acid [$H_3P(W3O10)_4$] (10 mL) was added to the volumetric flasks using an automatic pipette, and distilled water was added to fill the volume up to the mark. The volumetric flasks were sealed with a stopper, gently shaken, and placed on the stand. A double filtration (84 g/m^2 filter) of samples was done into 150 mL beakers to collect 60 mL of the filtrate for analysis. Distilled water was used to rinse the polarimeter sample tube (100 mm), after which the filtrate was added to the polarimeter (AP-300 automatic polarimeter, ATAGO Co. Ltd. Japan). The reading was done at 589 nm (λ) wavelength and a polarimeter temperature of about 20ºC. A polarimeter gives two readings, called optical rotation, and computes the average.

Pure maize (accepted specific rotation of pure maize starch is +184.6) starch was used as the standard and starch content of the samples was calculated using the formula:

$$\text{Starch (\%)} = \frac{10000 \ x \ p}{\text{x } (a)_D^{20^\circ} \text{ x S}}$$

Where P = Measured angle of optical rotation in degrees, L = Length (dm) of the sample tube, $(a)_D^{20°}$= Specific rotation of pure starch, S = Exact mass of the sample.

5.3.4.6. Determination of amylose

The iodine-based method was used for determination of amylose content (Cruz and Khush 2000). Using a digital scale, 100 mg of finely ground samples were weighed before being placed in 15 mL screw-cap tubes. One mL of 95% ethanol and 9 mL of 1M NaOH were then added to each tube, which were closed with screw lids and mixed well by vortexing. The tubes were placed in a pre-heated water bath (95°C) for 15 to 30 minutes to allow them to gelatinise. The samples were left at room temperature (21°C) for an hour to cool, during which time they were gently mixed at 10 minutes interval. The cooled samples were centrifuged for 5 minutes at 3000 rpm and distilled water added to the mark and shaken well. The second set of volumetric flasks (15 mL) was used to transfer (using a pipette) 0.50 mL of starch solution to each flask, and 1mL of 1M acetic acid was added and then mixed by vortexing.

Iodine solution (0.20 mL) was added and the volume in the flask adjusted to 10 mL with distilled water. The blank sample containing 1 mL ethanol and 9 mL of sodium hydroxide solutions was used to zero the spectrophotometer and the positive control containing 64% maize amylose was used to draw the standard curve. The spectrophotometer (UV VIS, Jenway 7315, Mettler Toledo) set at a wavelength 620 nm was used to measure the absorbance of all samples against the blank.

The amount of amylose was calculated from the calibration curve, obtained through a general linear formula: $Y = MX + C$

Where: Y = Concentration of amylose in mg, M = Slope of the curve, X = Sample absorbance at 620 nm, C = Constant. The regression equation for the study was as follows:

$$Y = 0.1853x + 0.0815$$

The following formula was used to calculate the percentage of amylose:

$$\text{Amylose (\%)} = \frac{\text{Total amylose in sample solution (mg) x 20}}{\text{Sample mass (mg)}} \text{ x } 100$$

Where: 20 = Dilution factor, 100 = Conversion factor of amylose concentration from mg to percentage.

5.3.5. Data analysis

ANOVA was done on all measured characteristics using Agrobase generation II SQL-version 38 (2019) statistical software. The least significant difference (LSD) test at $P \leq 0.001$ and $P \leq 0.05$ was used for means separation. The statistical linear mixed model used for genotype analysis at multi-location environments was described in Chapter 3, section 3.3.6.

5.4. RESULTS

5.4.1. Analysis of variance for nutritional characteristics

The genotype effects were highly significant ($P \leq 0.001$) at Brits and Loskop (2021 and 2022) for all the nutritional characteristics measured, except for Fe and Zn content (Table 5.1). The genotype by location interaction effect was highly significant ($P \leq 0.001$) for all the nutritional characteristics, except for Fe, Zn and phytic acid content (Table 5.2).

From combined ANOVA, highly significant ($P \leq 0.001$) genotype effects were observed for all measured nutritional characteristics except for Zn content (Table 5.2 and 5.3). The effects of location were significant for all the characteristics measured except for Fe and Zn content. Genotype by season interaction effect was highly significant ($P \leq 0.001$) for all measured nutritional characteristics except for amylose and molar ratio of phytic acid to Zn content. The effects of genotype by season by location interaction were highly significant ($P \leq 0.001$) for all the characteristics measured except for Zn content.

Broad-sense heritability (H^2) ranged from 0.02 (Fe and Zn) to 0.95 (protein) in Brits 2021 (Table 5.1). The H^2 ranged between 0.13 (Zn) to 0.89 (molar ratio of phytic acid to Fe) for Loskop 2021. For Brits 2022, the H^2 ranged from 0.03 (Zn) to 0.89 (phytic acid:Fe), whereas it ranged between 0.01 (Zn) to 0.98 (phytic acid), in Loskop 2022. The H^2 ranged from 0.05 (Zn) to 0.96 (phytic acid) in 2021 (Table 5.2), whereas it ranged from 0.09 (Fe) to 0.96 (phytic acid) in 2022. The H^2 ranged from 0.22 (Zn) to 0.99 (protein) for combined environments (Table 5.3).

5.4.2. Nutritional content of parental genotypes and their F_1 progenies

The protein content from combined ANOVA ranged from 19.32 to 32.19%, with an overall mean of 24.44% (Table 5.4). The nine progenies with the highest protein content (28.31 to 32.19%) had either 98K-5301or Kisumi-mix as a common parent. Brits 2021 showed the highest (30.98%) protein content compared to other environments (Table 5.5).

The Fe content ranged from 47.29 to 61.20 mg kg^{-1}, and over all mean of 55.39 mg kg^{-1} (Table 5.4). The eight progenies with the highest Fe concentration (58.28 to 61.20 mg kg^{-1}) had either TVU-14196 or IT845-2246 as a common parent. Brits 2022 showed higher (72.80 mg kg^{-1}) Fe content compared to the other environments (Table 5.5).

The Zn content ranged from 35.02 to 39.52 mg kg^{-1}, with an overall mean of 37.94 mg kg^{-1} (Table 5.4). The nine progenies with the highest Zn content (39.06 to 39.52 mg kg^{-1}) had either ITOOK-1060 or Glenda as a common parent. Loskop 2022 had higher (42.06 mg kg^{-1}) content of Zn than the other environments (Table 5.5).

The starch content ranged from 38.93 to 58.66% with an overall mean of 49.56% (Table 5.4). The 12 progenies with the highest starch content (54.17 to 58.66%) had either TVU-14196, IT93K-129-4, IT 845-2246 or TVU7778 as a common parent. Loskop 2022 had highest (59.49%) starch content compared to other environments (Table 5.5).

The amylose content ranged from 13.26 to 24.76% and an overall mean of 14.20%, (Table 5.4). The nine progenies with the highest amylose content (20.62 to 24.76 %) had either TVU-14196, IT96D-602, IT 845-2246 or 98K-5301 as a common parent. Loskop 2022 recorded the highest (24.63%) amylose content compared to other environments (Table 5.5).

The mean of phytic acid content ranged from 3037.80 to 7766.17 mg kg^{-1}, and an overall mean of 5201.63 mg kg^{-1} (Table 5.4). The five progenies that had low phytic acid content (3037.80 to 3335.60 mg kg^{-1}) had either TVU-14196, ITOOK-1060 or IT845-2246 as a common parent. Brits 2021 had lower (3011.88 mg kg^{-1}) phytic acid content than the other environments (Table 5.5).

The mean of phytic acid to Fe molar ratio ranged from 14.41 to 36.90, and an overall mean of 23.34 (Table 5.4). Cross TVU-14196 x IT845-2246 had molar ratios of < 10 for phytic acid to Fe. Loskop 2021 had a lower (7.51) phytic acid to Fe molar ratio compared to other environments (Table 5.5).

The phytic acid to Zn molar ratio ranged from 11.13 to 28.68 with an overall mean of 20.16 (Table 5.4). The three progenies with phytic acid to Zn molar ratios that are lower (more acceptable, that is ≤ 15) had either ITOOK-1060, Glenda or TVU-14196 as a common parent. Brits 2021 recorded lower (8.51) molar ratios of phytic acid to Zn compared to other environments (Table 5.5).

Table 5.1 Mean squares for nutritional characteristics for individual environments

Source	DF	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
Brits 2021									
Rep	2	5.30	66.64	7.94	72.91	36.57	2.24	0.11	0.15
Genotypes	54	122.60**	120.70	9.07	12197.36**	433.20**	97.14**	26.20**	17.77**
Residual	108	6.46	66.57	8.52	1527.50	85.95	5.32	0.50	0.85
σ^2g		8.57	1.53	0.18	3556.62	115.75	30.61	38.71	5.64
σ^2p		9.06	66.59	8.70	5084.12	201.70	35.92	45.17	6.49
H^2		0.95	0.02	0.02	0.70	0.57	0.85	0.86	0.87
Brits 2022									
Rep	2	2.02	33.06	26.52	465.81	4.13	0.43	0.17	0.13
Genotypes	54	97.45**	54.54	10.65	12642.72*	236.50**	110.83**	21.12**	19.38**
Residual	108	10.13	37.80	9.65	1408.94	9.29	6.94	1.73	2.59
σ^2g		6.46	5.58	0.33	3744.59	75.74	34.63	29.11	5.59
σ^2p		8.20	43.38	9.98	5153.54	85.02	41.57	39.23	8.19
H^2		0.79	0.13	0.03	0.73	0.89	0.83	0.74	0.68
Loskop 2021									
Rep	2	0.18	38.40	0.20	220.84	0.68	22.01	15.52	4.03
Genotypes	54	22.58**	80.70	5.47	14796.11**	261.45**	179.68**	99.77**	21.68**
Residual	108	0.21	55.59	4.24	87.97	10.35	22.00	2.58	0.65
σ^2g		7.46	8.37	0.41	4902.71	83.70	52.56	32.40	7.01
σ^2p		7.66	63.96	4.65	4990.68	94.05	74.56	34.98	7.66
H^2		0.97	0.13	0.09	0.98	0.89	0.71	0.93	0.92
Loskop 2022									
Rep	2	1.05	28.89	11.80	110.26	0.07	36.99	5.25	0.39
Genotypes	54	24.89**	74.82	17.14	15641.39**	25.47**	572.25**	119.03**	25.59**
Residual	108	0.75	60.22	16.52	122.02	1.98	22.42	3.06	0.52
σ^2g		8.04	4.87	0.21	5173.13	7.83	183.28	38.66	8.36
σ^2p		8.79	65.09	16.72	5295.14	9.81	2053.70	41.72	8.87
H^2		0.91	0.08	0.01	0.98	0.80	0.89	0.93	0.94

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, H^2= Broad-sense heritability, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 5.2 Mean squares for nutritional characteristics evaluated in 2021 and 2022 cropping seasons

Source	DF	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
2021									
Season	1	6.48**	268.74	3.72	298.81	9715.58**	2655.89**	44.89*	5.3
Genotype	54	46.38**	83.79	6.86	25859.21**	435.74**	204.27**	210.42**	34.54**
Genotype x Season	54	2.40**	63.56	6.55	1134.26	258.91**	72.55**	11.95**	4.91**
Rep (season)	4	0.14	79.55	4.64	146.88	18.63	12.13	10.41	2.09
Residual	216	0.36	13193.24	6.38	807.74	48.15	13.66	4.52	0.75
σ^2g		7.33	13.96	1.14	4120.83	29.47	21.95	33.08	4.94
σ^2p		7.73	3.37	0.05	4309.87	72.62	34.15	35.07	5.76
H^2		0.95	0.24	0.05	0.96	0.41	0.65	0.94	0.86
2022									
Season	1	0.92	837.14	25.09	573.75	12391.18**	12668.36**	119.73**	3.92
Genotype	54	43.69**	61.79	11.62	27185.47**	119.38**	417.48**	194.28**	36.93**
Genotype x Season	54	2.32**	67.57	13.17	1098.65	142.58**	265.60**	22.21**	8.04**
Rep (season)	4	0.61	30.98	19.16	288.04	2.10	18.71	3.63	0.26
Residual	216	1.24	49.01	13.09	765.48	5.63	14.68	6.59	1.56
σ^2g		6.89	0.96	1.00	4530.91	15.68	25.31	28.68	4.82
σ^{2p}		7.28	11.26	3.19	4347.8	19.9	69.58	32.38	6.15
H^2		0.95	0.09	0.31	0.96	0.79	0.36	0.89	0.78

$^{**}P \leq 0.001$, $^{*}P \leq 0.05$, Values without asterisk are not significant, Loc = Location, σ^2p = Genotypic variance, σ^2g = Phenotypic variance, H^2= Broad-sense heritability, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 5.3 Mean squares for nutritional characteristics evaluated at Brits and Loskop during the 2021 and 2022 seasons

Source	DF	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
2021 and 2022									
Loc	1	155.62**	78.63	24.07	850.34	22025.51	13462.61**	9.17*	6.14
Genotype	54	399.44**	94.41*	11.80	51591.78**	210.13**	238.42**	69.73**	88.63**
Rep (loc) x Season	8	7.02	55.26	11.9	217.46	10.36	15.42	1.18	0.38
Season	1	10.01	1021.27**	1.00	48509.46**	10724.31	5847.40**	180.18**	0.45
Genotype x Loc	54	20.39**	53.35	7.10	1115.76	188.77**	164.88**	10.87**	2.33**
Loc x Season	1	9.00	1027.25**	4.74	22.22	81.26	1861.63	0.05	1.26
Genotype x Season	54	5.26	51.16	6.68	1452.89	168.17	383.33**	1.74	1.44**
Genotype x Loc x Season	54	13.77**	77.78*	12.62	1117.15**	389.55**	173.27**	2.08**	2.39**
Residual	432	5.56	35.04	0.73	786.61	26.89	14.17	1.15	0.80
σ^2G		87.25	5.64	0.89	50140.28	20.23	12.78	387.56	59.2
σ^2P		88.35	14.47	3.99	50954.52	103.81	88.71	390.88	61.87
H^2		0.99	0.39	0.22	0.98	0.19	0.14	0.99	0.96

$^{**}P \leq 0.001$, $^{*}P \leq 0.05$, Values without asterisk are not significant, Loc = Location, σ^2p = Genotypic variance, σ^2g = Phenotypic variance, H^2= Broad-sense heritability, Fe = Iron ,Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 5.4 Mean performance of genotypes across environments and seasons

Genotypes	Protein (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Phytic acid (mg kg^{-1})	Phytic acid:Fe	Phytic acid:Zn	Starch (%)	Amylose (%)
TVU13953 x Glenda	22.97	54.40	36.97	4703.51	18.59	12.67	58.37	19.03
ITOOK-1060 x Glenda	24.26	55.36	39.26	4778.10	23.80	11.13	43.50	17.09
IT93K-129-4 x Glenda	23.21	56.60	39.13	6976.88	31.75	28.68	50.91	18.41
Kisumi-mix x Glenda	29.19	55.42	39.08	5390.51	26.22	22.53	38.93	15.40
TVU7778 x Glenda	24.19	53.88	38.00	4391.28	23.02	17.78	39.64	19.78
98K-5301 x Glenda	28.31	52.80	39.08	6710.72	25.27	20.49	42.00	18.82
IT96D-602 x Glenda	20.68	57.12	38.10	6834.46	26.15	24.31	50.69	15.74
IT845-2246 x Glenda	22.93	58.64	38.39	3335.60	21.51	15.89	46.89	15.22
TVU-14196 x Glenda	23.82	57.48	38.10	3163.41	19.72	14.21	52.91	15.91
ITOOK-1060 x TVU13953	24.43	50.08	37.76	4433.95	28.70	13.60	43.42	13.26
IT93K-129-4 x TVU13953	23.35	54.28	37.76	7591.51	29.24	26.80	52.51	18.51
Kisumi-mix x TVU13953	27.77	56.48	37.90	7253.15	26.10	21.85	40.89	14.38
TVU7778 x TVU13953	23.99	55.00	37.37	5215.80	23.37	18.76	53.57	18.47
98K-5301 x TVU13953	22.86	54.96	37.07	7393.77	18.54	20.90	49.66	22.11
IT96D-602 x TVU13953	19.32	51.84	36.68	5677.90	27.96	26.92	53.69	15.03
IT845-2246 x TVU13953	24.87	52.08	37.90	3499.57	17.95	15.60	42.37	16.34
TVU-14196 x TVU13953	25.07	52.63	37.07	3509.14	20.50	11.46	54.47	20.74
IT93K-129-4 x ITOOK-1060	26.04	57.80	38.30	4734.35	30.52	24.88	52.69	17.09
Kisumi-mix x ITOOK-1060	28.84	56.64	39.52	4374.52	27.94	19.82	42.02	17.16
TVU7778 x ITOOK-1060	23.36	55.72	39.42	5935.93	23.67	20.01	50.81	19.82
98K-5301 x ITOOK-1060	28.37	57.74	39.06	6642.24	22.08	23.76	42.02	14.02
IT96D-602 x ITOOK-1060	19.90	54.32	39.37	6327.94	26.00	22.66	49.52	20.90
IT845-2246 x ITOOK-1060	24.07	56.12	38.93	3053.38	17.46	15.79	42.04	16.39
TVU-14196 x ITOOK-1060	23.32	55.72	39.18	3037.80	21.47	13.52	53.55	15.35
Kisumi-mix x IT93K-129-4	28.36	55.28	38.69	7355.72	20.28	17.03	40.87	15.38
TVU7778 x IT93K-129-4	23.31	55.80	38.10	4634.59	25.79	21.58	54.26	18.04
98K-5301 x IT93K-129-4	28.65	47.29	37.46	7765.21	23.87	25.73	40.51	16.23
IT96D-602 x IT93K-129-4	20.16	52.84	38.13	7185.29	25.06	25.33	56.90	16.26
IT845-2246 x IT93K-129-4	23.97	53.84	38.05	3761.79	24.69	20.82	52.91	16.48
TVU-14196 x IT93K-129-4	23.28	56.96	38.20	3686.72	22.55	12.62	58.66	21.05
TVU7778 x Kisumi-mix	23.26	53.00	38.03	5507.18	23.60	20.84	51.31	15.74
98K-5301 x Kisumi-mix	32.19	53.36	35.75	7601.22	22.41	24.76	43.33	16.58
IT96D-602 x Kisumi-mix	19.84	54.96	37.71	6735.51	24.14	24.52	51.26	17.78
IT845-2246 x Kisumi-mix	24.61	54.96	38.34	3515.92	24.78	22.04	48.08	17.59
TVU-14196 x Kisumi-mix	25.62	58.28	36.68	3834.09	22.93	16.21	53.66	15.66
98K-5301 x TVU7778	28.45	59.44	38.83	6650.70	22.28	22.49	45.41	14.29
IT96D-602 x TVU7778	19.78	50.88	37.56	5457.95	28.99	20.39	57.84	22.33
IT845-2246 x TVU7778	23.96	57.61	38.22	3813.17	23.44	24.59	55.14	18.07
TVU-14196 x TVU7778	24.00	53.48	37.56	3435.88	18.76	20.22	54.67	20.88
IT96D-602 x 98K-5301	20.12	58.88	38.59	6703.89	25.61	21.74	52.36	17.47
IT845-2246 x 98K-5301	27.86	59.12	36.58	3428.97	23.67	26.10	56.44	24.76
TVU-14196 x 98K-5301	28.52	58.58	38.59	3679.37	15.96	24.21	43.56	21.00
IT845-2246 x IT96D-602	23.86	60.12	36.24	3851.91	18.36	16.94	58.04	20.62
TVU-14196 x IT96D-602	23.50	61.20	36.68	3930.82	17.57	18.42	53.59	14.01
TVU-14196 x IT 845-2246	23.43	56.84	37.88	3174.76	9.10	21.81	54.17	19.41
Glenda	24.87	54.20	38.93	4294.91	14.41	15.39	53.71	14.36
98K-5301	27.69	57.20	37.27	6864.22	36.90	22.74	57.84	15.29
IT96D-602	19.67	57.88	37.56	7766.17	21.62	17.55	54.09	20.05
IT93K-129-4	23.67	57.64	38.08	7208.53	31.56	26.79	53.22	17.39
Kisumi-mix	28.00	55.04	38.59	7303.76	22.98	17.96	42.88	17.46
TVU7778	24.78	55.20	36.68	5417.47	25.75	20.63	51.19	18.00
TVU13953	22.86	52.14	36.24	4926.70	21.01	21.99	47.53	18.25
ITOOK-1060	24.14	53.40	38.59	3347.61	33.54	14.51	44.12	14.27
IT845-2246	26.70	53.98	38.05	3538.39	16.15	15.81	45.20	14.16
TVU-14196	23.50	57.84	38.44	4746.00	20.10	25.27	46.17	16.18
Grand mean	24.50	55.39	37.94	5201.63	23.34	20.16	49.56	14.20
LSD	0.60	4.99	2.09	262.90	3.49	2.53	1.59	0.72

First parent on the progenies represent female, Protein, Fe = Iron, Zn = Zinc, Phytic acid, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn, Starch and Amylose.

Table 5.5 Comparisons of grand mean values for separate environments

	Protein (%)	Fe (mg kg-1)	Zn (mg kg-1)	Phytic acid (mg kg-1)	Phytic acid:Fe	Phytic acid:Zn	Starch (%)	Amylose (%)
Brits 2021								
Minimum	18.63	46.72	34.63	3011.88	12.23	8.51	36.23	12.2
Maximum	30.98	72.32	42.26	7814.42	49.53	33.8	58.91	22.77
Grand mean	24.07	55.73	38	5187.95	32.79	14.35	49.32	16.77
Brits 2022								
Minimum	19.6	49.28	35.02	3044.47	14.89	10.53	38.04	13.92
Maximum	29.12	72.8	42.26	7746.08	47.46	34.95	58.04	23.86
Grand mean	24.41	55.74	38.26	5200.41	25.43	16.94	38.84	17.83
Loskop 2021								
Minimum	19.69	35.87	35.02	3013.3	7.51	9.81	39.13	11.8
Maximum	29.57	68.48	40.69	7836.78	41.57	41.46	59.42	23.17
Grand mean	24.55	57.54	37.8	5201.04	21.94	20.02	50.06	17.02
Loskop 2022								
Minimum	17.33	32.16	24.65	3040.01	7.66	9.78	36.73	13.52
Maximum	29.72	63.84	42.06	7832.24	18.45	46.62	59.49	24.63
Grand mean	24.52	52.56	37.7	5217.14	13.18	29.34	50.04	18.05

Protein, Fe = Iron, Zn = Zinc, Phytic acid, Phytic acid: Fe = Molar ratio of phytic acid to Fe, phytic acid: Zn = Molar ratio of phytic acid to Zn, Starch and Amylose.

5.5. DISCUSSION

5.5.1. Analysis of variance for nutritional characteristics

The genotypes exhibited significant differences for all the measured nutritional characteristics, except for Zn, which indicated the existence of genetic variability among the cowpea parental genotypes and the F_1 progenies. There was deviation of some F_1 progenies toward higher nutritional characteristics values over the parental genotypes, which suggest a possible transfer of favourable alleles from the parental genotypes to the progenies. The findings further confirm the contribution of the parental genotypes to genetic variability in the progenies, which could be due to gene diversity of the parental genotypes observed in Chapter 3.

The significant differences between genotypes also suggested the opportunity to identify genotypes with better nutritional content for selection and further enhancement of cowpea nutritional characteristics through breeding (Alidu *et al.* 2020; Mbuma *et al.* 2021). Developing a cowpea cultivar with enhanced nutritional value would contribute to alleviation of malnutrition in South Africa, particularly for Fe, Zn and protein content.

Significant genotype by location interactions observed among the parental genotypes and the F_1 progenies for all the measured nutritional characteristics except Zn, suggested a significant effect of different environmental conditions in accumulation of nutritional characteristics. Differences in environmental conditions have previously been reported to influence mineral uptake, translocation, and distribution of nutrients in plants, which affects their growth, health, and thus productivity (Dakora and Belane 2019). Soil nitrogen (N) was also shown to influence nutrient accumulation in cowpea seeds, particularly protein content and micronutrient levels (Gage *et al.* 2017; Gerrano *et al.* 2022). This could be a result of the potential of cowpea to fix the atmospheric nitrogen, during the symbiotic interaction with rhizobia. Cowpea genotypes differ in their ability to fix N due to the differences in roots and growth patterns, as well as their response to soil conditions (fertility, water content , salinity, and pH) (Dias *et al.* 2018). The organic matter also influences the availability and uptake of micronutrients such as Fe and Zn by plants, which soil type, pH, salinity and microorganisms affect their decomposition. Therefore, it would be beneficial to incorporate proper agronomic practices to enable sufficient nutrient uptake and accumulation for well keeping of cowpea and thus their nutritional productivity.

The significant variation in amylose content observed implies differences in amylose impact on the viscosity and crystallinity of starch, among other physicochemical characteristics (Petroni *et al.* 2017). A study by Zia-ud- Din *et al.* (2017) reported that despite the high amylose content in legume starch, the protein contents of genotypes are lower when the starch contents are higher, and this was evident in the current results.

The significant genotype by season and genotype by location by season interaction for all the measured nutritional characteristics indicated change in ranking of genotypes for measured characteristics across seasons and locations. Therefore, it will be beneficial to do further multi-environment evaluation to improve selection efficiency and genetic gains (Gage *et al.* 2017; Dias *et al.* 2018).

5.5.2. Variance components and broad-sense heritability estimates

The high broad-sense heritability (0.96 to 0.99) for protein, starch, amylose and phytic acid contents indicated less environmental effects and opportunity for response selection for the characteristics. Some of the nutritional characteristics had very low genetic variances compared to phenotypic variances (such as Fe, Zn, and phytic acid to Zn and Fe ratios) indicating a large environmental influence, and low heritability. Previous studies (Buratto and Moda-Cirino 2017; Dias *et al.* 2021; Pessoa *et al.* 2023) reported higher phenotypic variance over genotypic variance for the Fe, Zn, and protein content in legumes. The broad-sense heritability for combined environments in this study ranged from 0.14 to 0.99.

The high broad-sense heritability (> 0.96) for protein, starch, amylose and phytic acid, indicated that the variation observed was largely due to genotypic effects, suggesting potential increased selection efficiency for these characteristics. Previous studies also reported high broad-sense heritability (0.60 - 0.99) for amylose and total starch in chickpea (Parameshwarappa *et al.* 2012; Mallu *et al.* 2014). On the contrary a study conducted by Manu *et al.* (2017), who evaluated 237 chickpea genotypes reported low heritability estimates for amylose (> 0.10), and total starch (> 0.12) contents. The results are also in contrast with previous studies that reported high heritability for Fe and Zn in cowpea (Coelho *et al.* 2021; Pessoa *et al.* 2023), pea (Powers 2021), and chickpea (Samineni *et al*. 2022).

5.5.3. Nutritional characteristics of parental genotypes and their F$_1$ progenies

The values of measured nutritional characteristics of some progenies were higher than that of their respective parental genotypes. For instance, nine progenies (Kisumi-mix x Glenda, 98K-5301 x Glenda, Kisumi-mix x ITOOK-1060, 98K-5301 x ITOOK-1060, Kisumi-mix x IT93K-129-4, 98K-5301 x IT93K-129-4, 98K-5301 x Kisumi-mix, 98K-5301 x TVU7778 and TVU-14196 x 98K-5301) indicated superiority for protein content compared to all the parental genotypes. These progenies were of either Kisumi-mix or 98K-5301 parentage, suggesting that these two parental genotypes could be potential donors for increasing protein content.

The potential of these two genotypes for high protein content were also reported by Gerrano *et al.* (2015), confirming the potential of these genotypes for the genetic improvement of protein content in cowpea. Three progenies with high protein content also had high Fe and Zn content (98K-5301 x ITOOK-1060, IT845-2246 x 98K-5301 and 98K-5301 x Glenda). This indicates that selection of multiple characteristics (protein content, Fe and Zn content) in cowpea could potentially accelerate the release of a nutritionally improved cowpea cultivars, which will ultimately be beneficial to combat malnutrition, particularly for the poor rural communities. The progenies which indicated superiority for Fe was of TVU-14196 or IT845-2246 parentage, which suggest that these parental genotypes could have favourable alleles for Fe enhancement when in combination with other parental genotypes, which will be further investigated in the following Chapter.

Cowpea cultivars with high protein content coupled with high levels of Fe and Zn are desirable to mitigate the issues associated with malnutrition. Starch is the main source of carbohydrates and maintains a healthy digestive system and lowers the chance of developing diabetes and cardiovascular illnesses, and high amylose content helps in maintaining the physiological properties of starch (Gonçalves *et al.* 2016; Padhi *et al.* 2022). Therefore, five progenies from the current study (TVU13953 x Glenda, TVU-14196 x TVU13953, TVU-14196 x IT93K-129-

4, IT96D-602 x TVU7778 and IT845-2246 x IT96D-602) might be an excellent source of starch because the starch in legumes digests more slowly than starch in cereals (Padhi *et al.* 2022), thus assisting those who suffer from diabetes by halting the rise in blood sugar levels that occurs immediately after eating.

Generally, the parental genotypes had lower nutritional contents values than their progenies, which indicated an improvement in the characteristics, possibly due to heterosis in the progenies. Thus, it would be beneficial to study the combining ability and heterosis for the nutritional characteristics, to further understand the mode of inheritance, the magnitude of heterosis and to identify best combiners for the measured nutritional characteristics. This would further guide effective selection of parental genotypes for future breeding purposes and breeding strategy for nutritional improvement in cowpea.

5.5.4. Phytate to mineral molar ratios: bioavailability of Fe and Zn

Molar ratios of phytic acid to Fe and Zn are an indicator of the bioavailability of Fe and Zn. The molar ratio for phytic acid to Fe ratio should be below 10 whereas that for phytic acid to Zn should be below 15 to make the minerals bioavailable (Diouf *et al.* 2020; Siwale *et al.* 2023). Only cross TVU-14196 x IT845-2246 had phytic acid to Fe molar ratio less than 10, which indicated a good Fe bioavailability. These findings also suggest that there is a need for breeding for reduced phytic acid in cowpea genotypes.

Six progenies (TVU13953 x Glenda, ITOOK-1060 x Glenda, TVU-14196 x Glenda, ITOOK-1060 x TVU13953, TVU-14196 x TVU13953, TVU-14196 x ITOOK-1060) and two parental genotypes (ITOOK-1060 and Glenda), recorded below threshold phytic acid to Zn ratio. Zn bioavailability in these genotypes is therefore quite good, thus confirming their potential to contribute to meeting South Africa's daily dietary Zn requirements. The results from the current study suggest that although the population studied had high content of Fe and Zn, this does not necessarily translate to high bioavailability, thus this warrants targeted breeding for increasing the bioavailability of Fe and Zn, as strategic intervention to breed for Fe and Zn biofortified cowpea (Abizari *et al.* 2012).

Other studies reported phytic acid to Fe molar ratios greater than 2.50, 3.00 or 3.30 for biofortified cowpea cultivars and 4.40 or 5.80 for non-biofortified, respectively (Abizari *et al.* 2012; Pereira *et al.* 2014; Coelho *et al.* 2021). To realise an increase in Fe and Zn bioavailability in cowpea, phytic acid reduction processes are important (Kumari and Roy 2023). These processes include soaking, which was reported to reduce the phytic acid to different extents depending on whether water or vinegar was used and also the time of soaking (Abizari et *al.* 2012; Pereira *et al.* 2014). Heat and moisture treatment was also reported to reduce the phytic acid, when combining steaming and cooking (Matos *et al.* 2013). Germination and fermentation reduce the phytic acid content through leaching of phytic acid in dripping water (Nkhata *et al.* 2018). The overall findings emphasised the potential of improving cowpea nutritional value in South Africa and further implies the possibility for broadening the genetic base for improving nutritional characteristics in cowpea.

5.6. CONCLUSIONS

The current study reported wide variation for grain nutritional characteristics (protein, Fe, starch amylose, phytic acid, phytic acid to Fe and phytic acid to Zn molar ratios) for the F_1 progenies and their parental genotypes. Broad-sense heritability was high (0.96 to 0.99) for protein, starch amylose and phytic acid, suggesting possible response to selection for improvement of these characteristics. Broad-sense heritably for Fe and Zn was lower than 0.50, suggesting that the environmental effects contributed more than genetic effects to the expression of Fe and Zn content of these cowpea genotypes. Three superior progenies for high protein content coupled with high levels of Fe and Zn were identified, namely 98K-5301 x ITOOK-1060, IT845-2246 x 98K-5301, and 98K-5301 x Glenda. However, it would be beneficial to conduct further evaluations, and to determine correlations between the nutritional characteristics, and grain yield and yield components. A better understanding of the mode of inheritance, the degree of heterosis, and combining ability of parental genotypes for the measured nutritional characteristics is needed, which will be reported in the following chapter.

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CHAPTER 6

DIALLEL ANALYSIS AND HETEROSIS OF SELECTED COWPEA GENOTYPES FOR NUTRITIONAL CHARACTERISTICS

6.1. ABSTRACT

Nutritional enhancement of cowpea through breeding is important for sustainable intervention to combat malnutrition. The objectives of this study were to estimate combining ability for nutritional characteristics, mid-parent heterosis and to identify best combiners for measured nutritional characteristics. A half-diallel mating design was used to produce 45 F_1 progenies. The progenies and the ten parental genotypes were evaluated over the course of two growing seasons (2021 and 2022) in two different locations. The seeds harvested were used for nutritional analysis and the data collected were subjected to diallel analysis using AGD-R software. Significant genotype, GCA and SCA effects were evident for all the nutritional characteristics studied except for Zn (SCA and GCA). The results also indicated predominance of non-additive over additive gene action, as confirmed by a higher magnitude of SCA than GCA variances. Parental genotypes Glenda, IT93K-129-4, TVU7778 and 98K-5301 were the best general combiners for amylose content. Parental genotypes IT93K-129-4, 98K-5301, IT96D-602 and TVU-14196 were the best general combiners for starch content, Kisumi-mix and 98K-5301 for protein content, IT845-2246 and TVU-14196 for Fe, and Glenda and ITOOK-1060 for Zn. These parental genotypes could be used for breeding of improved nutritional content. Four progenies (Kisumi-mix x ITOOK-1060, Kisumi-mix x IT93K-129-4, TVU7778 x Kisumi-mix and IT845-2246 x 98K-5301) had high SCA ($\geq$ 2.80) effects for protein content, which can be useful in breeding, on condition that they also have good grain yield.

Keywords: cowpea progenies, heterosis, combining ability, malnutrition, gene action

6.2. INTRODUCTION

The population of South Africa is rapidly increasing, hence, there is a need to keep up with the demand for nutritious food (De la Hey and Beinart 2017). The world is also striving to achieve the United Nations Sustainable Development Goal two of having a world free from hunger and malnutrition by the year 2030 (Mugiyo *et al.* 2021). Malnutrition contributes to more than one third of all child deaths worldwide, due to a lack of essential minerals and nutrients in their diets (Adepoju and Allen 2019).

The interventions that are underway to address malnutrition include supplementation of diets and fortified foods, however, this approach is not practical, and it is costly to distribute supplements to large populations. Hence, crops such as cowpea have a significant potential to contribute to nutritional food security globally. Cowpea is a legume crop which is grown in many parts of the world for its edible seeds and forage. Cowpea has good nutritional value that could meet the requirements of resource poor communities, by providing plant proteins and minerals (Santos and Boiteux 2013; Mbuma *et al.* 2021). Legume crops such as cowpea are a rich source of plant protein, which is healthier and cheaper compared to animal protein (Redjeki *et al*. 2013).

The grains of cowpea contain 23 to 32% protein (Jayathilake *et al.* 2018), about 1 to 1.5% fat (Kirse and Karklina 2015) and 50 to 60% carbohydrate (Khalid and Elharadallou 2013). The grain also contains minerals such as Fe, Zn, magnesium and calcium, vitamin A, and vitamin B6 (Gerrano *et al.* 2019). Amylose and amylopectin are the two main components of starch, and their proportion plays an important role in starch quality for different applications (Maaran *et al.* 2014). Vitamins aid in fighting infections and contribute to regulation of hormones. Fe plays a vital role in DNA synthesis and haemoglobin formation (Ayeni *et al.* 2018). Zn is a trace element that is responsible for structural, catalyst, and ion regulation in organisms to reduce prevalence of diseases (Da Rosa *et al.* 2010).

Breeding for crops such as cowpea is important in an effort to fight food insecurity, due to their nutritional superiority over cereals crops. The nutritional potential of cowpea, with regards to protein, micronutrients (particularly Zn and Fe), other minerals and carbohydrates is significant, however the cowpea accessions that are available in South African collections were only evaluated in pre-breeding efforts for genetic diversity of nutritional values (Gerrano *et al.* 2015; 2017; 2019; Mbuma *et al.* 2021). These studies identified and selected parental genotypes based on nutritional variability. However, there are no reports on breeding for nutritional characteristics in cowpea in South Africa up to date. Therefore, it is important to initiate breeding for nutritious cowpea in South Africa.

In breeding, it is important to understand how parental genotypes combine for important characteristics. Knowledge on the mechanisms that govern the inheritance of important nutritional characteristics, could contribute to effective breeding efforts to harness the nutritional value of cowpea. Thus, understanding of genetic mechanisms involved in the expression of nutritional characteristics will help in formulating an efficient breeding strategy for cowpea (Maina *et al.* 2015). Therefore, the objectives of this study were to estimate combining ability of selected parental genotypes for measured nutritional characteristics in order to establish their genetic control, to determine possible heterosis and identify the best general and specific combiners for measured nutritional characteristics.

6.3. MATERIALS AND METHODS

6.3.1. Planting materials

The details of the materials used in this study are as described in Chapter 3, Section 3.3.1.

6.3.2. Experimental locations

The details of the experimental locations are as described in Chapter 3, Section 3.3.3.

6.3.3. Experimental design, trial establishment and management

The details of the experimental design, trial establishment and management are as described in Chapter 3, Section 3.3.4.

6.3.4. Data collection

The details on data collection are as described in Chapter 5, Section 5.3.4, and sub-section 5.3.4.1 to 5.3.5.6.

6.3.5. Data analysis

The details on data analysis are as described in Chapter 4, Section 4.3.6.

6.4. RESULTS

6.4.1. Analysis of variance for nutritional characteristics of cowpea grains

At Brits and Loskop (2021 and 2022) genotype, GCA and SCA effects were highly significant ($P \leq 0.001$) for protein, starch, amylose, phytic acid: Fe and phytic acid: Zn (Table 6.1). The genotype effect was also highly significant ($P \leq 0.001$) for Fe at Brits and Loskop (2021 and 2022).

Across season 2021 and 2022 the genotype, GCA and SCA effects were significant ($P \leq 0.05$), for all the nutritional characteristics measured except genotypes (phytic acid) and SCA (phytic acid, Fe, phytic acid: Fe and Zn) in Brits (Table 6.2). For Loskop in season 2021 and 2022 the genotype, GCA and SCA effects were significant ($P \leq 0.05$), for all the nutritional characteristics measured except genotypes (Zn) and SCA (Fe and phytic acid: Fe and phytic acid: Zn). The genotype by season and SCA by season interaction effects were highly significant ($P \leq 0.001$) for protein, whereas the GCA by season interaction effects were significant ($P \leq 0.05$) for Fe and Zn in Brits across season 2021 and 2022. The genotype by season, GCA by season and SCA by season interaction effects were significant ($P \leq 0.05$) for all the nutritional characteristics except genotype (Zn), SCA (Zn, Fe and phytic acid: Fe and phytic acid: Zn) in Loskop across season 2021 and 2022.

In the combined diallel analysis the effect of genotype, GCA and SCA was highly significant ($P \leq 0.001$) for amylose, starch, protein, Fe, Zn, phytic acid: Fe and phytic acid: Zn (Table 6.3). The genotype by environment, GCA by environment and SCA by environment interaction effects were highly significant ($P \leq 0.001$) for all characteristics measured except for Fe and Zn.

6.4.2. Variance components and heritability for nutritional characteristics of cowpea grains

Variance components for GCA, SCA and SCA by environment interaction were significant ($P \leq 0.05$) for protein, starch and amylose and for SCA (Fe) (Table 6.4). Variance components for GCA by environment interaction was significant ($P \leq 0.05$) for starch and amylose.

The SCA variance was higher than the GCA variance for all the measured characteristics. Except for protein the dominance variance was greater than the additive variance for all nutritional characteristics measured. (Table 6.4). The ratio of GCA to SCA variance ranged from 0.07 (Fe.) to 0.99 (protein) and the Baker ratio for the characteristics ranged from 0.12

(Fe) to 0.67 for protein content. The narrow-sense heritability ranged between 0.01 (Fe and phytic acid: Zn) and 0.63 for protein content.

6.4.3. Estimates of general combining ability for nutritional characteristics

The parental genotypes Kisumi-mix and 98K-5301 had significant ($P \leq 0.05$) and positive GCA effects for protein content. Significant ($P \leq 0.05$) and positive GCA effects were recorded for Fe (IT845-2246 and TVU-14196) and Zn (Glenda, TVU-14196 and ITOOK-1060). Two parental genotypes namely, Glenda and TVU7778 recorded significant ($P \leq 0.05$) and positive GCA effects for amylose (Table 6.5). Parental genotypes IT96D-602, TVU7778 and TVU-14196 had significant ($P \leq 0.05$) and positive GCA effects for starch (Table 6.5).

Three parental genotypes TVU-14196, ITOOK-1060 and IT93K-129-4 recorded significant ($P \leq 0.05$) and negative GCA effects for phytic acid. Two parental genotypes namely IT845-2246 and TVU-14196 had significant ($P \leq 0.05$) and negative GCA effects for phytic acid: Fe. Two parental genotypes namely, Glenda and TVU-14196 had significant ($P \leq 0.05$) and negative GCA effects for phytic acid: Zn (Table 6.5).

6.4.4. Estimates of specific combining ability for nutritional characteristics of cowpea grains

The 10 progenies with significant ($P \leq 0.05$) positive SCA effects for protein content had either Kisumi-mix or 98K-5301 as a common parental genotype. Four progenies (Kisumi-mix x ITOOK-1060, Kisumi-mix x IT93K-129-4, TVU7778 x Kisumi-mix and IT845-2246 x 98K-5301) had high SCA (≥ 2.80) effects for protein. Two progenies namely, TVU-14196 x TVU13953 and TVU-14196 x IT845-2246 had significant ($P \leq 0.05$) positive SCA effects for Fe. Four progenies namely, ITOOK-1060 x Glenda, Kisumi-mix x ITOOK-1060, IT96D-602 x ITOOK-1060 and IT96D-602 x IT93K-129-4 had significant ($P \leq 0.05$) positive SCA effects for Zn (Table 6.6).

The 13 progenies with significant ($P \leq 0.05$) positive SCA effects for amylose content had mostly TVU7778 or Glenda as a common parent. The 12 progenies with significant ($P \leq 0.05$) positive SCA effects for starch content had mostly TVU7778, IT93K-129-4 or ITOOK-1060 as a common parental genotype (Table 6.6).

The seven progenies with significant ($P \leq 0.05$) negative SCA effects for phytic acid had mostly TVU-14196 or ITOOK-1060 as a common parental genotype. The seven progenies with significant ($P \leq 0.05$) negative SCA effects for phytic acid: Fe had mostly TVU-14196 or IT845-2246 as a common parental genotype. The eight progenies with significant ($P \leq 0.05$) negative SCA effects for phytic acid: Zn had mostly Glenda or ITOOK-1060 as a common parental genotype (Table 6.6).

6.4.5. Mid-parent heterosis for nutritional characteristics of cowpea grains

Mid-parent heterosis ranging from -20.31% (IT845-2246 x Kisumi-mix) to 28.21% (98K-5301 x Kisumi-mix) was observed for protein content. Of the 45 progenies, 13.33% had significant ($P \leq 0.05$) and positive mid-parent heterosis (Table 6.7). The mid-parent heterosis for Fe ranged from -12.30% (IT96D-602 x Kisumi-mix) to 11.26% (TVU-14196 x IT845-2246), and 6.67% of the progenies had significant ($P \leq 0.05$) and positive estimates (Table 6.7). Mid-parent heterosis ranging from -7.06% (IT96D-602 x TVU7778) to 5.29% (Kisumi-mix x ITOOK-1060) was observed for Zn content. Of the 45 progenies, 11.11% had significant ($P \leq 0.05$) and positive mid-parent heterosis. Mid-parent heterosis for molar ratio of phytic acid to Fe ranged from -30.29% (TVU-14196 x ITOOK-1060) to 38.89% (IT93K-129-4 x Glenda), and 35.56% of the progenies had significant ($P \leq 0.05$) and positive estimates. Mid-parent heterosis ranging from -21.76% (Kisumi-mix x IT93K-129-4) to 37.56% (IT93K-129-4 x Glenda) was observed for molar ratio of phytic acid to Zn. Of the 45 progenies, 33.33% had significant ($P \leq 0.05$) and positive mid-parent heterosis.

The mid-parent heterosis for starch ranged from -16.38% (Kisumi-mix x IT93K-129-4) to 44.32% (IT845-2246 x Kisumi-mix), and 33.33% of the progenies had significant ($P \leq 0.05$) and positive estimates. Mid-parent heterosis for phytic acid ranged from -45.53% (TVU7778 x Glenda) to 210.55% (TVU-14196 x TVU13953), and 40.00% of the progenies had significant ($P \leq 0.05$) and positive estimates. The mid-parent heterosis for amylose ranged from -22.06% (98K-5301 x Glenda) to 56.10% (ITOOK-1060 x Glenda), and 31.11% of progenies had significant ($P \leq 0.05$) and positive and mid-parent heterosis.

Table 6.1 Diallel analysis mean squares for measured nutritional characteristics in individual environments

Sources	DF	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
Brits 2021									
Rep	2	0.13	121.44	8.88	150.35	28.36	1.89	0.78	2.16
Genotype	54	25.00**	111.65*	8.52	895.23**	460.87**	96.76**	125.42**	18.16**
GCA	9	100.36**	72.77	12.44	1507.6**	768.86**	183.92**	490.35**	57.26**
SCA	45	9.92**	65.00	7.80	1130.53**	393.32**	79.24**	52.43**	10.35**
Residual	108	0.49	63.50	8.23	310.23	72.27	5.51	4.96	0.63
Brits 2022									
Rep	2	0.61	37.58	29.54	125.35	3.52	0.59	0.28	0.01
Genotype	54	22.80**	128.07**	9.63	710.22*	233.81**	105.47**	108.49**	23.62**
GCA	9	90.41**	50.04	13.80	2000.25**	852.03**	149.94**	299.86**	81.03**
SCA	45	9.28**	34.47	8.81	3056.35*	109.64**	97.23**	70.22**	12.14**
Residual	108	0.45	39.96	8.6	405.20	10.64	9.62	4.69	0.39
Loskop 2021									
Rep	2	0.19	38.4	0.20	105.23	22.02	0.20	15.37*	3.54
Genotype	54	22.53**	103.40*	5.62	650.56**	179.69**	5.62	99.74**	21.64**
GCA	9	91.26**	81.04	5.63	1889.53*	525.58**	5.63	328.97**	56.14**
SCA	45	8.78**	76.53	5.62	2045.30**	110.51**	5.62	53.90**	14.74**
Residual	108	0.23	55.43	4.16	235.23	22.00	4.16	2.59	0.68
Loskop 2022									
Rep	2	1.12	5.97	0.07	103.25	36.99	1.79	5.70	0.22
Genotype	54	24.32**	122.43**	25.48	802.32*	572.06**	7.83	119.03**	25.59**
GCA	9	89.38**	53.38	64.46	1989.23*	1304.17**	5.29	430.69**	67.07**
SCA	45	11.31**	60.05	17.68	2397.23**	425.64**	8.36	56.70**	17.3**
Residual	108	0.76	46.06	1.97	315.65	22.51	8.20	3.05	0.52

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, DF = Degree of freedom, GCA = General combining ability, SCA = Specific combing ability, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 6.2 Diallel analysis mean squares for measured nutritional characteristics evaluated at Brits and Loskop in 2021 and 2022 cropping seasons

Sources	DF	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
Brits 2021 and 2022									
Season	1	1.82	425.48**	277.98**	125.35	3.62	6.92	17.22	84.94*
Rep (Season)	4	0.03	37.35	15.62	710.22	13.58	4.39	1.26	0.07
Genotype	54	44.59**	211.99**	142.06*	2000.25**	31.76	36.99*	203.29**	34.67*
GCA	9	177.61**	530.73**	267.86**	3056.35*	41.97*	146.43**	745.17**	126.76**
SCA	45	18.08**	148.70	120.29	405.20	32.73*	15.39*	99.55*	17.16*
Genotype x Season	54	2.71**	185.80	145.25	201.23	19.39	3.28	15.02*	1.71
GCA x Season	9	2.33	517.44**	330.27**	315.25	26.46	3.89	15.37	2.38
SCA x Season	45	2.93**	123.37	114.15	236.52	15.35	3.29	13.04	1.57
Residual	216	1.14	36.18	8.75	102.23	12.42	1.99	8.60	1.77
Loskop 2021 and 2022									
Season	1	0.01	595.86**	678.75**	103.20	11.92*	94.16**	0.40	78.59**
Rep (Season)	4	0.61	17.88	13.94	602.23	0.07	0.96	9.82	1.66
Genotype	54	45.88**	248.84**	177.84	2456.20**	15.12*	26.08*	214.39**	45.02**
GCA	9	180.34**	211.44**	382.92**	1153.56**	46.84**	77.20**	751.02**	122.09**
SCA	45	19.45**	256.58**	144.14	850.21**	8.89	6.84	107.73**	29.63**
Genotype x Season	54	1.10*	156.80	162.83	325.26	7.13	4.94	3.26	2.10
GCA x Season	9	2.30*	64.85	201.71**	215.20	16.08*	32.26*	6.22	1.44
SCA x Season	45	0.99	175.04	147.94	316.20	5.56	5.74	2.84	2.37
Residual	216	0.48	49.52	19.79	103.25	0.39	2.66	2.84	0.60

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, DF = Degree of freedom, GCA = General combining ability, SCA = Specific combing ability, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 6.3 Diallel analysis mean squares for measured nutritional characteristics combined for all environments

Sources	DF	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
Env	3	3.44**	611.04*	6.13	303.2*	1943.69**	757.06**	62.79**	64.84**
Rep (Env)	8	0.51	50.85	10.10	950.21*	8.16	15.37	5.53	1.48
Genotype	54	89.80**	96.91*	10.86	3056.20**	217.93*	235.44*	414.73**	74.93**
GCA	9	365.91**	143.71*	5.38	2153.56*	616.92**	482.32**	1470.95**	228.52**
SCA	45	34.57**	87.39*	11.95*	702.23**	137.90**	186.70**	203.49**	44.21**
Genotype x Env	162	1.62**	53.43	6.91	425.26*	254.57**	239.51**	12.65**	4.70**
GCA x Env	27	1.83**	73.95	10.59	315.20**	602.14**	560.43**	26.31**	10.99**
SCA x Env	135	1.57**	49.55	6.21	216.25*	182.97**	175.31**	9.92**	3.44**
Residual	431	0.48	51.25	7.29	153.25	23.80	14.91	3.82	0.56

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, DF = Degree of freedom, Env = Environment (location and season), GCA = General combining ability, SCA = Specific combing ability, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 6.4 Variance components and narrow-sense heritability for nutritional characteristics combined for all environments

Variance	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
σ^2GCA	2.40**	0.22	0.11	751.11**	1.08	0.03	8.67**	13.74**
σ^2SCA	2.41**	3.15**	0.40	2689.15**	1.47	0.13	15.02**	18.44**
σ^2GCA x Env	0.00	0.68	5.67	0.00	3.99	0.05	0.31*	0.96**
σ^2SCA x Env	0.59**	0.02	49.25	177.67**	0.00	0.09	1.67**	2.19**
σ^2Error	0.36	51.23	15.21	766.74	118.75	8.12	5.69	4.95
σ^2Additive	4.80	0.44	0.11	1502.22	1.08	0.05	15.02	18.44
σ^2Dominance	2.41	3.15	0.80	2689.15	2.94	0.13	17.35	27.48
GCA: SCA	0.99	0.07	0.28	0.27	0.73	0.20	0.57	0.75
Baker ratio	0.67	0.12	0.35	0.35	0.60	0.28	0.53	0.60
σ^2p	7.57	54.82	16.13	4958.12	122.78	8.31	38.07	50.87
h^2	0.63	0.01	0.04	0.30	0.02	0.01	0.45	0.54

**$P \le 0.001$, *$P \le 0.05$, Values without asterisk are not significant, σ^2 = Variance, Env = Environment, σ^2p = Phenotypic variance, h^2= Narrow-sense heritability, GCA = General combining ability, SCA = Specific combing ability, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 6.5 Estimates of general combining ability for measured nutritional characteristics combined for all environments

Parental genotypes	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
Glenda	-0.35	0.06	0.16*	0.28	-0.99	-2.14**	0.04	0.91**
TVU13953	0.96	0.78	-0.10	-0.27	-0.27	-1.41	-1.90	-0.15
ITOOK-1060	0.07	0.77	0.24**	-3.81**	2.81**	0.68	2.20	-1.38**
IT93K-129-4	-0.43	-1.23*	0.07	-3.55 **	3.09 **	0.14	2.56	0.38
Kisumi-mix	1.82**	0.98	-0.31*	-0.86	0.94	0.25	-2.86**	-0.03
TVU7778	-0.38	0.70	0.14	-0.38*	0.57	0.16	3.74**	2.26**
98K-5301	2.35**	0.63	0.07	-0.59	-0.36	2.11**	-1.56	-0.93**
IT96D-602	-3.60**	-0.23	-0.21**	1.21	0.21	1.57	5.27**	-0.20
IT845-2246	0.40	1.49**	-0.22**	1.47**	-2.47**	-1.13	-2.81**	0.04
TVU-14196	-0.60	1.83**	0.17*	-2.09**	-3.55**	-2.76**	4.08**	-2.00**

***P* ≤ 0.001, **P* ≤ 0.05, Values without asterisk are not significant, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 6.6 Estimates of specific combining ability for measured nutritional characteristics combined for all environments

F_1 progenies	Proteins	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
TVU13953 x Glenda	1.09	1.45	-0.55	644.96**	2.75**	-3.80**	-9.43**	-0.10
ITOOK-1060 x Glenda	-1.03	-0.90	1.84*	-603.31**	-1.42	-4.24**	1.14	1.49**
IT93K-129-4 x Glenda	-0.84	1.95	-0.23	58.91	6.07**	7.95**	-4.00**	1.21
Kisumi-mix x Glenda	2.23**	1.89	0.05	134.55**	2.93**	4.26**	-0.47	-2.83**
TVU7778 x Glenda	-0.73	-1.13	0.29	-36.99	0.26	-0.32	-8.61**	1.08
98K-5301 x Glenda	1.50	-4.20*	-1.69*	135.43**	3.32**	0.24	-6.08**	2.64**
IT96D-602 x Glenda	-0.99	-3.05	0.53	1252.14**	3.60**	4.77**	-0.19	-2.63**
IT845-2246 x Glenda	-0.89	2.45	1.48	49.46	1.55	-1.02	-1.09	-3.09**
TVU-14196 x Glenda	0.03	-2.17	-0.82	101.87**	-0.87	-1.12	0.37	-0.47
ITOOK-1060 x TVU13953	-0.85	-1.38	0.65	190.66**	2.91**	2.53*	0.36	-2.56**
IT93K-129-4 x TVU13953	-1.83**	-5.06*	-1.38	125.41**	3.31**	5.39**	-2.24	1.01
Kisumi-mix x TVU13953	0.20	-0.59	0.61	-35.71	1.91	2.86*	-5.34**	-3.37**
TVU7778 x TVU13953	-0.76	2.37	1.19	-34.37	-0.41	-0.33	-4.08**	1.34**
98K-5301 x TVU13953	0.59	-1.41	0.87	48.25	0.57	0.82	2.97**	-0.02
IT96D-602 x TVU13953	-1.85**	3.66	-0.37	123.54**	4.56**	6.59**	4.57**	-2.07**
IT845-2246 x TVU13953	1.31	1.64	0.91	38.25	-2.71**	-1.94	3.83**	-0.85
TVU-14196 x TVU13953	-1.53**	6.35**	0.42	68.05	-4.11**	-4.77**	-2.66	0.63
IT93K-129-4 x ITOOK-1060	-1.45	4.07	0.21	58.33	1.37	1.45	-0.79	1.63**
Kisumi-mix x ITOOK-1060	2.85**	1.26	1.60*	-34.88*	0.72	-1.18	-2.69**	1.20
TVU7778 x ITOOK-1060	-0.28	1.54	-1.74*	-1109.38**	-3.09**	-0.87	-2.13	-1.83**
98K-5301 x ITOOK-1060	1.19	-1.23	0.29	56.92	-3.62**	1.05	-1.75	-1.82**
IT96D-602 x ITOOK-1060	-1.16	-0.29	1.51*	-126.63**	-0.31	0.23	2.17**	-0.23
IT845-2246 x ITOOK-1060	-0.31	2.29	-0.16	-142.31**	-6.52**	-3.64**	6.25**	0.53
TVU-14196 x ITOOK-1060	0.59	-3.20	0.82	38.14	-1.21	-6.24**	-2.58	0.56
Kisumi-mix x IT93K-129-4	2.93**	0.15	-0.03	82.39	6.96**	-6.11**	-1.44	-1.64**
TVU7778 x IT93K-129-4	-0.63	-1.30	-0.10	-67.52	2.74**	-1.78	4.93**	-1.47**
98K-5301 x IT93K-129-4	2.33**	3.63	0.24	176.63**	-2.29	0.79	-6.68**	-2.29**
IT96D-602 x IT93K-129-4	-0.95	0.64	1.61*	321.24**	-1.99	0.70	2.92**	0.02
IT845-2246 x IT93K-129-4	-0.04	-0.38	-0.24	39.22	2.57**	-0.90	5.94**	-0.78
TVU-14196 x IT93K-129-4	0.08	-1.68	0.58	996.38**	0.21	-6.29**	3.58**	0.47
TVU7778 x Kisumi-mix	2.94**	0.57	-1.04	-67.24	-1.13	0.51	-1.80	1.77**
98K-5301 x Kisumi-mix	3.91**	1.24	0.70	-20.46	-2.43	1.99	-2.25	2.53**
IT96D-602 x Kisumi-mix	-2.76**	-5.69**	-0.26	-43.84	-0.44	2.49*	-2.49	0.58
IT845-2246 x Kisumi-mix	2.91**	-0.24	0.61	40.92	3.01**	2.82*	0.70	-0.20
TVU-14196 x Kisumi-mix	2.21**	0.31	-0.05	-347.98**	5.03**	-2.32	1.16	1.55**
98K-5301 x TVU7778	2.17**	1.39	1.47	-49.83	-0.40	0.18	1.39	1.43**
IT96D-602 x TVU7778	-0.98	-3.18	-2.47**	-26.23	4.82**	-1.53	4.19**	3.62**
IT845-2246 x TVU7778	0.18	-0.48	-0.30	-50.22	2.29	5.37**	6.57**	-1.63**
TVU-14196 x TVU7778	1.50	1.16	0.51	668.22**	-2.14	-1.40	2.31**	2.53**
IT96D-602 x 98K-5301	-1.84**	2.43	1.38	-332.40**	2.38	-1.91	-0.73	-0.80
IT845-2246 x 98K-5301	2.91**	-0.33	-1.11	-29.15	2.81**	4.90**	0.47	3.33**
TVU-14196 x 98K-5301	-2.13**	-1.39	0.61	49.55	-1.67	0.15	-5.28**	-1.50**
IT845-2246 x IT96D-602	-1.48	1.94	-1.28	-36.20	-4.53**	2.45	-6.13**	3.77**
TVU-14196 x IT96D-602	-3.10**	2.72	0.39	96.65	-4.16**	5.20**	2.37**	-0.72
TVU-14196 x IT845-2246	0.28	5.15**	-0.80	-792.01**	2.20	-3.99**	1.18	1.54**

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, First parent on the progenies represent female, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

Table 6.7 Mid-parent heterosis for measured nutritional characteristics combined for all environments

F_1 progenies	Protein	Fe	Zn	Phytic acid	Phytic acid:Fe	Phytic acid:Zn	Starch	Amylose
TVU13953 x Glenda	-9.45	1.97	-2.68	98.55*	0.20	12.67*	-6.21	14.26
ITOOK-1060 x Glenda	-14.55*	-0.52	5.27*	103.94*	6.97	24.57*	16.11*	56.10*
IIT93K-129-4 x Glenda	8.72	3.38	-1.90	75.86*	38.89*	37.56*	-14.44	7.35
Kisumi-mix x Glenda	-6.50	2.91	0.25	-4.06	28.19*	29.07*	24.98*	12.71
TVU7778 x Glenda	-16.17*	-2.91	-2.68	-45.53*	16.97*	23.00*	1.88	-2.64
98K-5301 x Glenda	-4.89	-5.54	4.88*	4.38	23.77*	27.37*	-2.47	-22.06*
IT96D-602 x Glenda	-12.61*	-9.03*	4.05*	-0.98	31.65*	28.94*	-14.92*	38.80*
IT845-2246 x Glenda	-3.64	5.81	2.55	29.79	11.47*	19.77*	-16.21*	20.82*
TVU-14196 x Glenda	6.91	-8.57	-3.40	30.73	5.93	15.56*	13.00	26.55*
ITOOK-1060 x TVU13953	-11.58	0.54	1.87	31.68	23.57*	-15.47*	4.63	25.21*
IT93K-129-4 x TVU13953	-6.15	-5.94	-2.34	73.45*	32.67*	-16.38*	16.02*	6.50
Kisumi-mix x TVU13953	26.32*	3.12	3.32	57.76*	23.27*	-10.80*	9.81	5.98
TVU7778 x TVU13953	4.84	3.22	0.97	59.92*	15.95*	5.32	4.96	3.34
98K-5301 x TVU13953	-2.64	0.59	2.21	74.61*	21.16*	-6.25	-0.93	-11.18
IT96D-602 x TVU13953	-5.27	7.97	1.57	130.61*	32.87*	-14.19*	13.75	-7.67
IT845-2246 x TVU13953	-14.69*	10.51*	-0.64	196.78*	6.85	-7.85	33.60*	40.72*
TVU-14196 x TVU13953	-5.18	-0.49	-4.67*	210.55*	-8.54	-1.23	18.87*	4.69
IT93K-129-4 x ITOOK-1060	9.31	8.13	-0.25	60.86*	-0.78	-4.71	17.78*	39.62*
Kisumi-mix x ITOOK-1060	11.64	3.11	5.29*	-25.24	-12.09*	9.11*	35.52*	48.53*
TVU7778 x ITOOK-1060	17.80*	8.24	4.54*	101.24*	-11.62*	17.25*	28.81*	50.17*
98K-5301 x ITOOK-1060	11.92	1.41	3.43	-21.80	-3.08	20.60*	4.80	-4.82
IT96D-602 x ITOOK-1060	11.95	-1.10	0.45	86.39*	-5.45	12.66*	17.08*	37.95*
IT845-2246 x ITOOK-1060	-25.14*	6.62	-1.87	129.98*	-23.09*	31.53*	39.42*	44.12*
TVU-14196 x ITOOK-1060	12.29*	-9.97*	-0.63	78.40*	-30.29*	21.94*	15.72	-9.65
Kisumi-mix x IT93K-129-4	-7.78	-6.29	-6.28*	-7.39	-22.27*	-21.76*	-16.38*	17.04
TVU7778 x IT93K-129-4	1.37	-0.30	-0.31	-1.58	-10.77*	-10.06*	-10.19	15.28
98K-5301 x IT93K-129-4	-6.79	4.44	0.98	-24.84	-2.02	-13.87*	-1.55	16.88
IT96D-602 x IT93K-129-4	-8.05	1.39	4.27	-23.05	-2.80	-11.48*	1.23	45.36*
IT845-2246 x IT93K-129-4	-1.54	1.15	-0.89	33.07	-12.54*	-12.21*	23.49*	-0.40
TVU-14196 x IT93K-129-4	9.33	-2.58	0.25	-6.55	-27.03*	-16.65*	-10.90	-8.79
TVU7778 x Kisumi-mix	6.03	5.36	-3.54	-19.45	7.42	1.33	17.63*	9.01
98K-5301 x Kisumi-mix	28.21*	-3.47	3.20	-44.26	15.92*	-1.26	17.48*	-18.44*
IT96D-602 x Kisumi-mix	-6.13	-12.30*	1.19	24.57	15.44*	2.46	16.25*	2.20
IT845-2246 x Kisumi-mix	-20.31*	4.26	2.29	-33.28	10.20*	3.77	44.32*	19.93*
TVU-14196 x Kisumi-mix	-5.03	9.45*	3.99	-24.33	-5.12	-0.11	21.56*	7.07
98K-5301 x TVU7778	11.94	5.16	2.24	37.05	4.31	-7.22	4.05	14.60
IT96D-602 x TVU7778	1.59	-5.36	-7.06	-16.56	-0.59	5.92	15.02	12.60
IT845-2246 x TVU7778	14.76*	-0.67	-2.97	25.11	8.76	-4.70	6.45	21.81*
TVU-14196 x TVU7778	-9.02	-3.98	-0.19	62.92*	-1.00	-15.70*	31.30*	-5.69
IT96D-602 x 98K-5301	0.93	3.18	3.14	74.17*	2.67	1.69	0.59	-15.03
IT845-2246 x 98K-5301	-14.11*	2.83	-2.87	72.26*	11.75*	-2.25	-6.80	-12.24
TVU-14196 x 98K-5301	20.32*	-0.63	1.94	29.17	8.03	-21.61*	-1.58	-8.64
IT845-2246 x IT96D-602	-0.77	-2.19	-4.14	23.92	-1.77	-8.15	-10.57	-0.98
TVU-14196 x IT96D-602	8.00	-1.72	-3.47	20.38	2.42	-10.33*	-12.06	-3.06
TVU-14196 x IT845-2246	-16.55*	11.26*	-0.95	96.97*	15.95*	8.78	12.35	-6.11
Mean heterosis	9.60	0.59	-0.06	-0.19	0.31	5.39	7.33	9.60

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, First parent on the progenies represent female, Fe = Iron, Zn = Zinc, Phytic acid: Fe = Molar ratio of phytic acid to Fe, Phytic acid: Zn = Molar ratio of phytic acid to Zn.

6.5. DISCUSSION

6.5.1. Diallel analysis of variance and heritability

The significant effects of genotypes, GCA and SCA for individual environments, across environments and seasons, as well as for combined environments, indicated large variations among parental genotypes and their F_1 progenies, and the combining ability of the parental genotypes for all the measured nutritional characteristics. The findings further suggest that the genes involved in expression of the nutritional characteristics measured were mostly non-additive.

The results are in line with a study by Alladoum *et al.* (2018), who explored the genetic architecture for total protein, dietary fibre and vitamin C contents of cowpea through an 8 x 8 half-diallel cross and reported significant and positive GCA and SCA for all the measured characteristics. The results are also in agreement with Tchiagam *et al.* (2011), who investigated combining ability for protein content in cowpea and reported significant GCA and SCA. Other studies also reported significant GCA and SCA for phytate and protein content in cowpea, respectively (Maina *et al.* 2015; Wang *et al.* 2021). In maize, significant effects of genotype, GCA, and SCA were found for Fe, Zn, phytic acid, and the molar ratios of phytic acid to Fe and Zn (Akhtar *et al.* 2023).

The significant genotype by environment, GCA by environment and SCA by environment interaction observed indicated the large role the environment played on the expression of all the nutritional characteristics measured except for Fe (GCA and SCA) and Zn content. This indicated that the environment influenced the GCA and SCA of parent and that this should be taken into consideration when selecting parental genotypes for breeding. The outcomes are consistent with a study that used a 5 x 5 half-diallel to perform genetic analysis of the seed protein contents in cowpea and reported GCA by environment and SCA by environment interaction (Tchiagam *et al.* 2011).

These results also corroborate a report of significant GCA and SCA by environment interaction for phytates in cowpea (Maina *et al.* 2015). The significant variance components for GCA and SCA further confirmed the importance of both additive and non-additive gene action in the expression of the measured nutritional characteristics. Non-additive gene action was more prevalent compared to additive gene action, as confirmed by higher SCA than GCA variances. The non-additive gene action refers to the characteristics that are not transferable from the parental genotypes to their progeny and which are usually only expressed in the F_1 progenies (heterosis). The ratio of GCA to SCA variance and Baker's ratio were less than one for all nutritional characteristics. This further confirms the non-additive genetic nature of the measured characteristics, which is accompanied by low narrow-sense heritability (0.01 to 0.30) values for molar ratio of Phytic acid to Zn, Fe, molar ratio of Phytic acid to Fe, Zn and phytic acid content, respectively, indicating that selection for these characteristics would not lead to genetic gains. Three characteristics (starch, amylose and protein, respectively) had medium narrow-sense heritability (0.45 to 0.63) suggesting that response to selection might be possible for the aforementioned nutritional characteristics.

Although heterosis was low for most of the characteristics (less than 15%), it was medium for molar ratio of phytic acid to Fe (35.56%) and molar ratio of phytic acid to Zn (33.33%). Hybrid production is therefore not a viable option for increasing nutritional value of cowpea, although some success is possible for molar ratios (as an indicator of mineral bioavailability) a target for improvement through hybrid breeding. Again, cowpea hybrid breeding could be challenging and the possible gains should be weighed against this.

Other studies also reported higher SCA over GCA variance for protein in pigeon pea, for phytates in cowpea and for starch in peas, respectively (Beekham and Umaharan 2010; Tchiagam *et al.* 2011; Maina *et al.* 2015; Kumari *et al.* 2015). The current results were in contrast with a study by Akhtar *et al.* (2023), who reported higher GCA over SCA variance for Fe, Zn, and phytic acid molar ratio of Fe to phytic acid and molar ratio of Fe to phytic acid in maize evaluated under low nitrogen conditions.

Efficient transfer of nutritional characteristics from the potential parental genotypes to their progeny is determined by gene action. The low to medium (≤ 0.63) narrow-sense heritability observed suggested that selection could be effective for some characteristics (such as protein) compared to others (such as Fe and Zn). The results are in agreement with that of Maina *et al.* (2015) and Karmakar *et al.* (2013), who reported low narrow-sense heritability (0.12 to 0.27) for phytates in cowpea and ridge gourd, respectively. The results are in contrast with medium narrow-sense heritability (0.34 to 0.56 for Fe and Zn, respectively) previously reported (Mukamuhirwa *et al.* 2015; Noubissié *et al.* 2019).

Protein content was determined mainly by additive gene action and heritability was relatively high and the GCA was important, therefore pedigree breeding would enhance protein content of cowpea genotypes, through selection of superior genotypes from segregating generations. The non-additive genes were predominant for expression of Fe and Zn, which suggest that the genetic improvement of these characteristics would be difficult. Thus, it would be beneficial to exploit recurrent selection and breeding for gradual genetic improvement of Fe and Zn. However, recurrent selection is time consuming and costly, hence involvement of molecular markers would hasten the improvement of Fe and Zn.

6.5.2. Estimates of general combining ability for nutritional characteristics measured

The few positive and significant GCA effects observed for all the nutritional characteristics measured indicated a limited flow of productive genes from the parental genotypes to the progenies. Parental genotypes 98K-5301 and Kisumi-mix were best general combiners for protein content, TVU-14196 and IT845-2246 were best general combiner for Fe. The parental genotypes, ITOOK-1060, TVU-14196 and Glenda were best general combiners for Zn. Two parental genotypes namely, TVU7778 and Glenda were best general combiners for amylose. Three parental genotypes namely IT96D-602, TVU-14196 and TVU7778 were best general combiners for starch content.

Three parental genotypes, namely ITOOK-1060, IT93K-129-4 and TVU-14196 were poor general combiners for grain phytates. For grain phytates, it is important to select parental genotypes with significant negative GCA effects, indicating poor general combiners and suggest that this trait has dominant genetic control to reduce grain phytate content. The best combiners identified contain favourable donor alleles for the nutritional characteristics studied, which are transferable to progeny. Thus, selection of these parental genotypes for specific trait improvement could shorten the time taken to produce improved cowpeas with high nutritional value. Traditional selection methods (recurrent or pedigree selection), which will require time and resources, can lead to homogeneity of the nutritional characteristics on these parental genotypes. Good general combiners for protein and minerals were also reported by Singh *et al.* (2012) and Ceyhan and Kahraman (2013). Fuengtee *et al.* (2020), determined genetic effects of sugars, phytoglycogen, total starch, and amylopectin and estimated GCA of 11 parental genotypes and 24 F_1 progenies, which identified one good general combiner for total starch and amylopectin.

6.5.3. Estimates of specific combining ability for nutritional characteristics measured

Some progenies with significant SCA effects, involved parental genotypes with good GCA effects. For instance, the high significant SCA for combinations IT845-2246 x 98K-5301 and IT845-2246 x Kisumi-mix for protein involved two best general combiners for protein (98K-

5301 and Kisumi-mix), which indicated that favourable genes for protein were passed from the two parental genotypes to their progenies. However, selection in later generations is a useful method for achieving a stable population and preventing non-additive effects from interfering. Sharma and Mehta (2014) conducted a study to obtain information on combining ability for protein content and green pod yield in six cowpea genotypes and four testers and identified two best specific combiners for protein. Fuengtee *et al.* (2020) identified two sweet-waxy corn F_1 progenies with high sugar, phytoglycogen, total starch, and amylopectin content.

6.5.4. Magnitude of heterosis for nutritional characteristics measured

The mid-parent heterosis for the current study was determined to identify best parent combinations with superior progenies for the nutritional characteristics measured. The varied magnitudes and direction of mid-parent heterosis observed suggested that the gene action involved varied due to differences in genetic makeup of the parental genotypes involved. Positive heterosis over the mid-parent were observed in progenies 98K-5301 x Kisumi-mix and Kisumi-mix x TVU13953 for protein (28.21 and 26.32%), TVU-14196 x IT845-2246 for Fe (11.26%), Kisumi-mix x ITOOK-1060 for Zn (5.29%), IT845-2246 x ITOOK-1060 and IT845-2246 x TVU13953 for starch (39.42 and 33.60%) due to SCA of the parental genotypes involved. This suggested the involvement of dominant and partially dominant genes in controlling the expression of those characteristics.

The significant negative heterosis for phytic acid, molar ratios of grain phytates to Fe and phytates to Zn could be used to increase the bioavailability of both Zn and Fe by producing progenies. The dominance effects are the primary cause of the hybrid vigour, which is contingent upon the significance of SCA (Ndhlela *et al.* 2015; Solomon *et al.* 2015; Tian *et al.* 2017; Han *et al.* 2018). Sharma and Mehta (2014) reported significant positive relative heterosis, heterobeltiosis and standard heterosis in cowpea progenies of six genotypes and four testers for protein content. Ceyhan *et al.* (2014), also determined combining ability and heterosis for concentration of mineral elements and protein in common bean and reported very low heterosis for protein (-0.70%) in the F_1 generation.

6.6. CONCLUSIONS

Efficient identification and selection of suitable parental genotypes, strategic breeding methods and selection of potential genotypes within segregants to use for breeding, depends on the knowledge of combining ability and gene action involved in the expression of important characteristics. Integrating combining ability and heterosis helps in identifying suitable progenies and provides valuable insights for improvement of cowpea nutritional value through conventional pedigree breeding. The current study demonstrated that both additive and non-additive were involved in expression of the nutritional characteristics measured, however non-additive genetic effects were predominant, therefore, improvement the nutritional characteristics measured could done by effective recurrent selection.

The narrow-sense heritability values ranged from low to intermediate; but there was some heterosis for characteristics in some progenies, 98K-5301 x Kisumi-mix and Kisumi-mix x TVU13953 for protein (28.21 and 26.32%), TVU-14196 x IT845-2246 for Fe (11.26%), IT845-2246 x ITOOK-1060 and IT845-2246 x TVU13953 for starch (39.42 and 33.60%). Therefore, the most effective way to enhance the contents of these nutritional characteristics for the selected progenies may be through hybrid breeding. Future cowpea breeding programmes for nutritional value could make use of the parental genotypes that performed best as general combiners for the nutritional characteristics measured.

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CHAPTER 7

CORRELATIONS BETWEEN GRAIN YIELD, YIELD COMPONENTS AND NUTRITIONAL CHARACTERISTICS FOR COWPEA F_1 PROGENIES AND THEIR PARENTAL GENOTYPES

7.1. ABSTRACT

Understanding the relationship between grain yield, yield components and nutritional characteristics through correlation studies is important for developing cowpea cultivars with improved grain yield and desired nutritional characteristics. The objective of this study was to determine the phenotypic correlations between grain yield, yield components and nutritional characteristics. The yield components and nutritional data were subjected to correlation coefficient analysis, principal component analysis (PCA), and heat map clustering. The results observed positive correlation between grain yield and all yield components except leaf length, leaf width and days to 50% flowering, for protein with Fe content and negative correlations of phytic acid with Fe and Zn content. The results indicated that indirect selection of yield can be done through selection of characteristics highly related with yield. The first two principal components (PC-1 and PC-2) explained 65.85% of the total variation in the dataset and PCA biplots further grouped the genotypes into four quadrants based on association with measured characteristics. Genotypes IT96D-602 x Glenda, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953 and IT96D-602 x TVU13953 were associated with higher grain yield. Genotypes 98K-5301 x Kisumi-mix, Kisumi-mix x Glenda, 98K-5301 x TVU7778 and Kisumi-mix x TVU13953 were associated with high protein content and Fe concentration. The selection of genotypes with a combination of good yield and desirable nutritional characteristics can be beneficial for future breeding activities in South Africa.

Keywords: cowpea, correlations, cluster, principal components analysis, nutritional characteristics

7.2. INTRODUCTION

Cowpea is a vegetable and grain legume commonly known as black-eyed pea (Agbicodo 2009). The crop is nutrient dense and cultivated for grain, fresh leaves, and green pods (Goncalves *et al.* 2016; Gomes *et al.* 2019). Cowpea has the potential to contribute to the combat of food and nutrition insecurity, particularly in poor communities (Santos and Boiteux 2013). However, the production and productivity of cowpea in South Africa remains low compared to other countries (FAOSTAT 2021). Therefore, it is important to breed cowpea cultivars with improved grain yield and enhanced nutritional characteristics.

In the pursuit of developing improved cowpea cultivars, it is crucial to improve the crop nutritional value alongside grain yield. Grain yield and nutritional characteristics are complex characteristics controlled by numerous genes (Owusu *et al.* 2018). Environmental and genetic factors combine to influence polygenic characteristics, often leading to low broad-sense heritability and ineffective selection (Thangaraj 2018; Horn and Shimelis 2020).

Therefore, understanding correlation and selecting based on the association of grain yield with nutritional characteristics would increase selection efficiency and ultimately increase genetic gains (Khadka *et al.* 2020; Mbuma *et al.* 2022). It is crucial to comprehend the impact of selecting one trait on the expression of other characteristics when selecting multiple characteristics (Shanko *et al.* 2014). Designing appropriate selection criteria for improved grain yield and desired nutritional value in cowpea relies on knowledge of positive correlating characteristics, as this information will enable direct or indirect selection of associated characteristics (Moura *et al.* 2012). The use of multivariate statistical techniques like PCA, in addition to correlation analysis, could help to comprehend the relationships between the tested genotypes and measured characteristics (Singh *et al.* 2016).

Previous studies on correlation analysis in cowpea focused mostly on nutritional characteristics (Boukar *et al.* 2011; Moura *et al.* 2012; Mbuma *et al.* 2021a; 2021b) and morphological characteristics (Gerrano *et al.* 2019; Kalambe *et al.* 2019; Mbuma *et al.* 2022). Few reports are available regarding the relationships between cowpea grain yield and related characteristics and nutritional attributes (Mbuma *et al.* 2022). Therefore, understanding the relationship between grain yield, yield components and nutritional characteristics would help in identifying the best breeding and selection strategies for improving cowpea grain yield and nutritional characteristics. The objectives of this study were to determine the correlation between grain yield, yield components and nutritional characteristics in cowpea parental genotypes and their progenies and to categorise and cluster characteristics and genotypes by visualizing their differences through PCA and heat map clustering.

7.3. MATERIALS AND METHODS

7.3.1. Planting materials

Section 3.3.1 of Chapter 3 contains a detailed description of the materials used in this study.

7.3.2. Experimental locations

Section 3.3.3 of Chapter 3 contains a description of the experimental locations in detail.

7.3.3. Experimental design, trial establishment and management

The details of the trial setup, management, and experimental design are described in Chapter 3, Section 3.3.4.

7.3.4. Data collection

Section 3.3.5 of Chapter 3 and Section 5.3.4, Sub-section 5.3.4.1 to 5.3.5.6 of Chapter 5 provided details on data collection.

7.3.5. Data analysis

The association between pairs of variables was determined using Pearson’s correlation coefficient, at 5% confidence level for all the phenotypic and nutritional characteristics measured using AGROBASE Generation II SQL version 38 (2019).

The PCA was performed using GenStat version 23 (2023), and then PCA biplots categorising numerous variables by visualizing their differences and heat maps were generated using R-statistical software version 4.1.0 (R Core Team 2021) and using ggplot2 version 3.3.5 (Wickham 2016).

Table 7.1 List of the parental genotypes and their F_1 progenies used for the study

Entry	Genotypes	Entry	Genotypes
1	TVU13953 x Glenda	31	TVU7778 x Kisumi-mix
2	ITOOK-1060 x Glenda	32	98K-5301 x Kisumi-mix
3	IIT93K-129-4 x Glenda	33	IT96D-602 x Kisumi-mix
4	Kisumi-mix x Glenda	34	IT845-2246 x Kisumi-mix
5	TVU7778 x Glenda	35	TVU-14196 x Kisumi-mix
6	98K-5301 x Glenda	36	98K-5301 x TVU7778
7	IT96D-602 x Glenda	37	IT96D-602 x TVU7778
8	IT845-2246 x Glenda	38	IT845-2246 x TVU7778
9	TVU-14196 x Glenda	39	TVU-14196 x TVU7778
10	ITOOK-1060 x TVU13953	40	IT96D-602 x 98K-5301
11	IT93K-129-4 x TVU13953	41	IT845-2246 x 98K-5301
12	Kisumi-mix x TVU13953	42	TVU-14196 x 98K-5301
13	TVU7778 x TVU13953	43	IT845-2246 x IT96D-602
14	98K-5301 x TVU13953	44	TVU-14196 x IT96D-602
15	IT96D-602 x TVU13953	45	TVU-14196 x IT845-2246
16	IT845-2246 x ITOOK-1060	46	Glenda
17	TVU-14196 x TVU13953	47	98K-5301
18	IT93K-129-4 x ITOOK-1060	48	IT96D-602
19	TVU7778 x ITOOK-1060	49	Kisumi-mix
20	Kisumi-mix x ITOOK-1060	50	IT93K-129-4
21	98K-5301 x ITOOK-1060	51	TVU7778
22	IT96D-602 x ITOOK-1060	52	TVU13953
23	IT845-2246 x TVU13953	53	ITOOK-1060
24	TVU-14196 x ITOOK-1060	54	IT845-2246
25	Kisumi-mix x IT93K-129-4	55	TVU-14196
26	TVU7778 x IT93K-129-4		
27	98K-5301 x IT93K-129-4		
28	IT96D-602 x IT93K-129-4		
29	IT845-2246 x IT93K-129-4		
30	TVU-14196 x IT93K-129-4		

First parent on the progenies represent female.

7.4. RESULTS

7.4.1. Correlation of grain yield, yield components and nutritional characteristics

Tables 7.2 to 7.4 present phenotypic correlations between all possible pairs of measured nutritional characteristics, yield components, and grain yield. Significant ($P \leq 0.05$) positive correlations (r = 0.38 to r = 0.71) were found between GY and all measured yield components except LL (Table 7.2). GY was also significantly ($P \leq 0.05$) positively correlated (r = 0.35) with starch content (Table 7.4). Significant ($P \leq 0.05$) positive correlations (r = 0.37 to r = 0.53) were also found for NB with all yield components except PW (Table 7.2). Significant ($P \leq 0.05$) positive correlations (r = 0.33 to r = 0.75) were found for NB and NPP with all yield components except PW, for PW with NSPP (r = 0.73), HSWt (r = 0.61) and GY (r = 0.38). Significant ($P \leq 0.05$) negative correlations (r = 0.36 to r = 0.53) were found for D50F with all yield component except LL and PL.

Significant ($P \leq 0.05$) positive correlations were found for Zn with Fe (r = 0.45), for starch with amylose (r = 0.20) and for phytic acid with PL and PW (r = 0.24, r = 0.24, Table 7.2 and

7.3). Significant ($P \leq 0.05$) negative correlations were found for phytic acid and protein (r = -0.20), and for protein content with PH, PL, PW, NSPP, HSWt and D50F (r = -0.20 to r = -0.34).

7.4.2. Principal component analysis of grain yield, yield components and nutritional characteristics

The first two principal components (PC-1 and PC-2) explained 65.85% of the total variation among the cowpea F_1 progenies and their parental genotypes (Table 7.5). PC-1 accounted for 51.84% of the total variation and had an eigenvalue of 5.70. Starch and amylose content contributed the most to variability and associations with the first principal component. PC-2 had an eigenvalue of 1.55 and accounted for 14.05% of the total variation, with protein, Fe and Zn content contributing most of the variation.

The biplot of PC-1 and PC-2 showed that genotypes IT96D-602 x Glenda, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953 and IT96D-602 x TVU13953 were associated with high GY. Genotypes 98K-5301 x Kisumi-mix, Kisumi-mix x Glenda, 98K-5301 x TVU7778 and Kisumi-mix x TVU13953 were associated with high protein and Fe content.

7.4.3. Clustered heat map of grain yield, yield components and nutritional characteristics

A clustered heat map illustrated the hierarchical clustering of genotypes based on yield components and nutritional values (Figure 7.2). Different colours on the heat map represent the strength of relationships between the genotypes and the measured characteristics. According to the colour scale, shades of purple represent positive relationships between the characteristics and above average mean values. The columns represent the measured grain yield, yield components and nutritional characteristics and the rows designate the genotype entry numbers (list of genotype names are given in Table 7.1).

The heat map clustered the genotypes into one main cluster, and three sub clusters namely, A (IT96D-602 x Kisumi-mix, IT96D-602 x TVU7778, TVU-14196 x TVU13953, IT96D-602 x ITOOK-1060 and IT96D-602 x IT93K-129-4) which were associated with GY, PL, PH, NSSP, starch, and Zn. Protein, Fe, and Zn were associated with sub-cluster B (98K-5301 x Kisumi-mix, Kisumi-mix x Glenda, 98K-5301 x Glenda, 98K-5301 x TVU7778, TVU-14196 x 98K-5301, TVU7778 x ITOOK-1060, 98K-5301 x ITOOK-1060, Kisumi-mix x IT93K-129-4 and 98K-5301 x IT93K-129-4). Sub-cluster C (IT96D-602 x Glenda, ITOOK-1060 x TVU13953, IT96D-602 x 98K-5301 and IT96D-602 x TVU13953) was associated with GY, NB, PL and NSSP.

Table 7.2 Phenotypic correlation between the grain yield and yield components for combined environments

	GY	NB	NPP	PH	LL	LW	PL	PW	NSPP	HSWt
NB	0.38**									
NPP	0.58**	0.53**								
PH	0.75**	-0.49**	0.67**							
LL	0.13	0.27**	0.39**	0.36**						
LW	-0.25*	-0.23*	-0.22*	-0.22*	-0.02					
PL	0.65**	0.37**	0.58**	0.67**	0. 0.1	-0.32**				
PW	0.38**	0.15	0.30**	0.33**	0.10	-0.09	-0.12			
NSPP	0.71**	0.50**	0.63**	0.73**	0.26*	-0.22*	0. 27*	0.73**		
HSWt	0.71**	0.46**	0.70**	0.75**	0.35**	-0.24*	0.03	0.61**	0.37**	
D50F	-0.52**	-0.53**	-0.53**	-0.50**	-0.11	0.21*	-0.06	-0.44**	-0.36**	-0.51**

**$P \le 0.001$, *$P \le 0.05$, Values without asterisk are not significant, GY = Grain yield, HSWt = Hundred seed weight, NPP = Number of pods per plant, NSPP = Number of seeds per plant, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, NB = Number of branches, PH = Plant height, D50F = Days to 50% flowering.

Table 7.3 Phenotypic correlation between the nutritional characteristics for combined environments

	Fe	Zn	Phytic acid	Starch	Amylose
Zn	0.45**				
Phytic acid	-0.03	-0.02			
Starch	-0.03	-0.02	-0.20*		
Amylose	-0.01	-0.04	-0.04	0.20*	
Protein	0.01	-0.01	-0.20*	-0.35**	0.10

**$P \le 0.001$, *$P \le 0.05$, Values without asterisk are not significant, Fe = Iron, Zn = Zinc.

Table 7.4 Phenotypic correlation between the grain yield, yield components and nutritional characteristics for combined environments

	GY	Protein	Fe	Zn	Phytic acid	Starch	Amylose	NB	NPP	PH	LL	LW	PL	PW	NSPP	HSWt
Protein	-0.15															
Fe	-0.01	0.01														
Zn	-0.04	0.01	0.45**													
Phytic acid	0.19	-0.20*	-0.03	-0.02												
Starch	0.35**	-0.35**	-0.03	-0.02	0.20*											
Amylose	-0.10	0.10	-0.01	-0.04	-0.04	-0.20*										
NB	0.38**	-0.10	0.01	-0.01	0.16	0.29*	0.03									
NPP	0.58**	-0.14	0.02	0.02	0.17	0.32**	0.01	0.53**								
PH	0.75**	-0.22*	0.02	-0.01	0.14	0.32**	-0.10	-0.49**	0.67**							
LL	0.33**	0.11	0.01	-0.08	0.00	-0.12	0.16	0.27*	0.39**	0.36*						
LW	-0.25*	0.46**	-0.01	0.02	-0.40**	-0.35**	0.02	-0.23*	-0.22*	-0.22*	-0.02					
PL	0.65**	-0.27*	0.01	-0.06	0.24*	0.30**	-0.14	0.37**	0.58**	0.67**	0.27*	-0.32**				
PW	0.38**	-0.20*	-0.04	-0.02	0.24*	0.21*	-0.06	0.15	0.30**	0.33**	0.10	-0.09	0.35**			
NSPP	0.71**	-0.21*	0.00	-0.06	0.19	0.33**	-0.14	0.50**	0.63**	0.73**	0.26*	-0.22*	0.73**	0.33**		
HSWt	0.71**	-0.34**	-0.05	-0.02	0.10	0.35**	0.02	0.46**	0.70**	0.75**	0.35**	-0.24*	0.61**	0.37**	0.69***	
D50F	-0.52**	0.20*	-0.05	0.02	-0.31**	-0.47**	0.01	-0.53**	-0.53**	-0.50**	-0.11	0.21*	-0.44**	-0.36**	-0.55**	-0.51**

**$P \leq 0.001$, *$P \leq 0.05$, Values without asterisk are not significant, GY = Grain yield, HSWt = Hundred seed weight, NPP = Number of pods per plant, NSPP = Number of seeds per plant, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, NB = Number of branches, PH = Plant height, D50F = Days to 50% flowering, Fe = Iron, Zn = Zinc.

Table 7.5 Eigenvectors from principal component analysis for grain yield, yield components and nutritional characteristics for combined environments

Characteristics	PC-1	PC-2	PC-3
GY	0.35	-0.11	-0.15
HSW	0.37	-0.12	0.05
LL	0.23	0.35	0.13
LW	-0.21	0.42	-0.14
NB	0.28	0.29	0.31
NP	0.37	0.18	-0.05
NSPP	0.38	0.08	-0.02
PH	0.37	0.13	-0.02
Protein	-0.12	0.66	0.24
D50F	-0.01	-0.28	0.81
Amylose	0.57	0.14	0.09
PL	0.45	-0.44	0.25
Fe	-0.14	-0.55	0.13
Zn	-0.36	-0.50	0.39
Phytic acid	0.21	0.28	0.80
Starch	-0.52	0.39	0.34
Eigenvalue	5.70	1.55	1.00
Variability (%)	51.84	14.05	9.09
Cumulative (%)	51.84	65.89	74.98

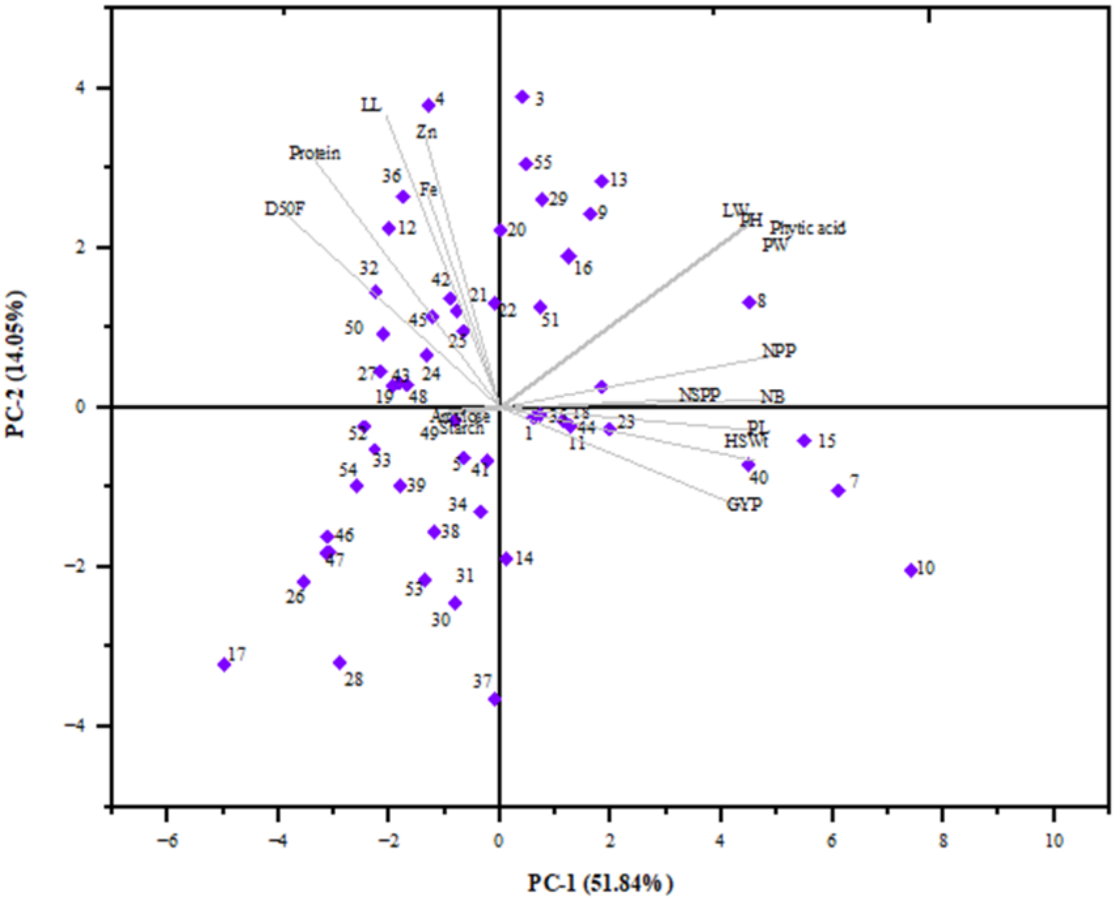

Figure 7.1 Principal component analysis biplot displaying the performance of genotypes and their associated characteristics.

Numbers 1 to 55 = Genotype entry numbers (genotype names are listed in Table 7.1). GY = Grain yield, HSWt = Hundred seed weight, NPP = Number of pods per plant, NSPP = Number of seeds per plant, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, NB = Number of branches, PH = Plant height, D50F = Days to 50% flowering, Fe = Iron, Zn = Zinc.

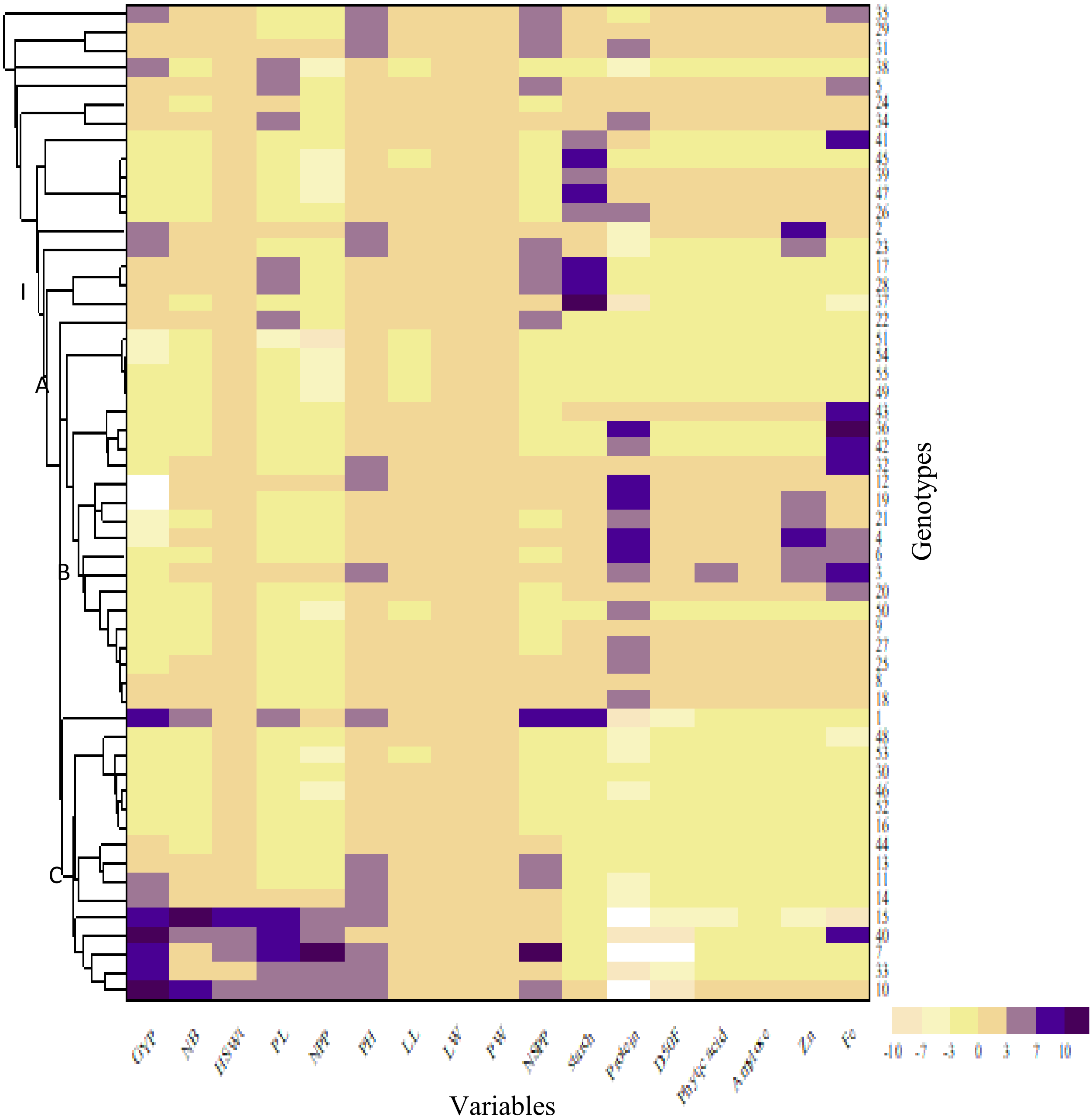

Figure 7.2 Clustered heat map for grain yield, yield components, nutritional characteristics measured on cowpea genotypes.

The numbers 1 to 55 = Genotypes entry numbers (genotype names are listed in table 7.1). GY = Grain yield, HSWt = Hundred seeds weight, NPP = Number of pods per plant, NSPP = Number of seeds per plant, PL = Pod length, PW = Pod width, LL = Leaf length, LW = Leaf width, NB = Number of branches, PH = Plant height, D50F = Days to 50% flowering, Fe = Iron, Zn = Zinc.

7.5. DISCUSSION

7.5.1. Pearson correlation between the grain yield and yield components

All the measured yield components had significant positive correlations with grain yield, apart from leaf length, leaf width, plant height, and days to 50% flowering; these correlations suggested that enhancing these yield components could lead to increased grain yield. The strong positive correlation between grain yield and plant height suggests the capacity of the tallest plants to efficiently accumulate photo-assimilates for optimum growth and development of plants, thus resulting in improved grain yields. Previous studies also reported similar results (Shanko *et al.* 2014; Gerrano *et al.* 2019; Kalambe *et al.* 2019). Previous reports have suggested that grain yield and number of pods per plant share genes, which explains the strong correlations found in this study (Almeida *et al.* 2014; Mubai *et al.* 2020). Selecting for robust and positively correlating yield components will therefore have a direct impact on increasing cowpea grain yield.

Significant positive correlations between number of branches and number of pods per plant with all yield components except leaf width and days to 50% flowering indicated that selection for these characteristics could improve other yield components, thus multiple trait selection could result in improvement of the positively associated characteristics. The significant positive correlation between the number of branches and number of pods per plant indicated that an increase in number of branches would positively influence the number of pods per plant and could ultimately improve the grain yield. Previous studies also reported positive correlations between number of branches and number of pods per plant, with grain yield (Sabale *et al*. 2019; Snehal *et al.* 2021; Mbuma *et al.* 2022).

The negative correlation observed between grain yield and days to 50% flowering indicated that variation in flowering date of cowpea genotypes had a negative impact on grain yield. Previous studies also reported variation in grain yield in cowpea genotypes because of the timing and rate of flowering (Kalambe *et al.* 2019; Sharma *et al.* 2019). The results indicate that selecting only for days to 50% flowering could result in reduced grain yield, therefore it would be beneficial to select for multiple characteristics.

7.5.2. Pearson correlation between the nutritional characteristics

The primary nutritional characteristics considered for correlation analysis was protein content because it is essential in the human diet as it helps to build and repair body tissues

(Okareh *et al.* 2015). Although the correlation was not statistically significant, the positive correlation between protein and Fe content suggested that improving cowpea for Fe could also lead to improvements in protein content. The results agree with several reports (Moura *et al.* 2012; Santos and Boiteux 2013; Goncalves *et al.* 2020; Dhanasekar *et al.* 2021; Mbuma *et al.* 2021a). Significant positive correlation found between the Fe and Zn content, suggested simultaneous improvement of the two characteristics is possible. Selection for increased Fe content can cause a related increase in protein and Zn content.

The significant negative correlation of protein with phytic acid and starch content indicated that improvement of either phytic acid or starch could result in reduced protein content of the tested cowpea genotypes. The results agree with previous findings (Teka *et al.* 2020; Sivakumaran *et al.* 2018), who reported negative significant correlation of protein with phytic acid and starch content (r = -0.67 and r = -0.63, respectively). The results are in contrast with findings of other studies (Sivakumaran *et al.* 2018; Nkhata *et al.* 2018), that reported significant positive correlation between protein and phytic acid content (r = 0.22). The results imply that improvement in phytic acid or starch content would result in reduced protein content.

Phytic acid also correlated negatively with both Fe and Zn. Due to the ability of phytic acid to bind with these minerals, the results indicated that improvement of cowpea genotypes for Fe and Zn could result in reduction in phytic acid content. The results are in agreement with findings by Akond *et al.* (2011), who reported significant negative correlation between phytic acid and Fe. The reduction in phytic acid content could potentially increase bioavailability of Fe and Zn.

7.5.3. Pearson correlation between the grain yield, yield components and nutritional characteristics

Incorporation of grain yield, yield components and nutritional characteristics for an integrated selection index could accelerate the development of high yielding and nutritious cowpea cultivars. A positive correlation of leaf length, leaf width and days to 50% flowering with protein content, for number of branches, number of pods per plant, plant height and leaf length with Fe, and for number of pods per plant and days to 50% flowering with Zn was evident. This indicated that selection for these yield components could ultimately result in indirect selection for increased protein, Fe and Zn content. A study by Mbuma *et al.* (2022), reported positive correlation of grain yield components with Fe and Zn. The significant negative correlation of phytic acid with days to 50% flowering, indicated the possibility of reducing phytic acid when selecting for days to 50% flowering. It is possible to improve both nutritional characteristics (except for phytic acid) and grain yield components simultaneously, based on the strength and direction of the correlation between them.

7.5.4. Principal component analysis of grain yield, yield components and nutritional characteristics

The first two PCs explained almost two thirds of the variation in this dataset, with PC-1 contributing by far the most of this variation (52%), with amylose, starch and pod length having the highest loads on this PC. Various authors (Molosiwa *et al.* 2016; Gerrano *et al.* 2019; Mbuma *et al.* 2021a; 2021b) reported similar results. Kisumi-mix was associated with high protein content, suggesting that this parent might be selected for superior protein in future breeding efforts. Additionally, in Chapter 6, the Kisumi-mix was the second-best parent for overall protein combining ability. Its high protein content as previously reported (Gerrano *et al.* 2015), indicates that this parent may serve as prospective breeding material to enhance protein content.

The progenies IT96D-602 x Glenda, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953 and IT96D-602 x TVU13953were associated with higher grain yield, which indicated that these progenies are superior for grain yield. The progenies associated with high grain yield, had two parental genotypes (TVU13953 and IT96D-602) which were reported in Chapter 4 to be best general combiners for grain yield, which further indicated the importance of these two parental genotypes for improvement of grain yield.

The results indicated parental variability and breeding potential for higher grain yield and nutritional quality in future cowpea breeding. Studies by Gerrano *et al.* (2019), also reported association of specific genotypes with Fe and Zn. A study by Mbuma *et al.* (2021b) reported four clusters of cowpea genotypes based on associated yield components and nutritional characteristics. To create better cowpea cultivars, the parental genotypes identified in this study could serve as the genetic resources for breeding.

7.5.5. Clustered heat map displaying groups of genotypes based on associated characteristics

The clustered heat map visually demonstrated the association of genotypes with the measured grain yield, yield components and nutritional characteristics. The results indicated one cluster, with three-sub cluster. The first sub-cluster (A) discriminated five progenies, which were associated with high, pod length, plant height, number of seeds per plot and starch content. The second sub-cluster (B) contained seven progenies, which were associated with high protein, Fe and Zn values. The next sub-cluster (C) contained four progenies, which were also associated with high grain yield, number of branches, hundred seed weight and pod length. Nevertheless, more than three characteristics distinguished the genotypes in each of the sub-clusters, indicating that variation may be moderate to narrow for effective selection of both grain yield and nutritional characteristics (Shanko et *al.* 2014). Introduction of other breeding methods such as recurrent selection, marker assisted breeding and mutation breeding could be beneficial for developing a well-balanced cowpea cultivar (Wanga *et al.* 2021; Mbuma *et al.* 2022). The results further indicated that the characteristics grain yield and protein, as well as starch and protein were grouped in opposite directions on the biplot, further confirming that these characteristics are strongly negatively correlated and simultaneous selection for the characteristics would be difficult.

A study by Mbuma *et al.* (2022), who studied interrelationship between grain yield components and nutritional characteristics in cowpea genotypes, reported association between the yield components and nutritional characteristics and cowpea genotypes by five distinctive clusters. A study, which evaluated association between nutritional characteristics of 59 bambara groundnut genotypes, also reported the expected negative correlation between starch and protein content (Siwale *et al.* 2023). Strategic recombination of genotypes on the bases of the trait clusters could potentially improve gains for grain yield and nutritional characteristics through directed crossing, in efforts to produce cultivars with high grain yield and balanced nutritional value.

7.6. CONCLUSIONS

The current study revealed positive significant correlation among some measured characteristics which suggest simultaneous improvement of characteristics through multiple and indirect selection. The genotypes IT96D-602 x Glenda, IT96D-602 x 98K-5301, ITOOK-1060 x TVU13953 and IT96D-602 x TVU13953 were associated with high grain yield. Genotypes 98K-5301 x Kisumi-mix, Kisumi-mix x Glenda, 98K-5301 x TVU7778 and Kisumi-mix x TVU13953 were associated with high protein and Fe content. A well-designed multiple trait selection index should be used to maximise selection efficiency for improved grain yield, yield components and nutritional characteristics in an attempt to develop superior cowpea cultivars.

7.7. REFERENCES

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CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1. GENERAL DISCUSSION

Cowpea is a self-pollinating diploid crop often having a narrow genetic base, making it difficult to genetically improve the crop (Kumari and Chauhan 2018). The lack of variation in cowpea contributes to low productivity and limited adaptation of imported accessions to changing environmental conditions (Deepa *et al.* 2018). To address the problem of low production and productivity of cowpea in South Africa, diverse parental genotypes were selected based on grain yield, drought tolerance and nutritional characteristics. Hybridisation followed as a vital method of combining characteristics possessed by the different parental genotypes, in efforts to heterosis in the progenies for selection (Kumari and Chauhan 2018; Magar *et al.* 2016). Understanding combining ability and the nature of gene action involved in expression of grain yield and nutritional characteristics would help in identifying superior parental genotypes and progenies (Mwale *et al.* 2017). The development of a better cowpea cultivars with all the desired characteristics in one individual is possible with efficient selection in populations with sufficient genetic variation.

The objectives of the study were 1) to partition variance and heritability among cowpea parental genotypes and progenies for grain yield, yield components, nutritional characteristics, and their implications in crop improvement, 2) to determine the combining ability and heterosis of selected cowpea parental genotypes for yield and nutritional characteristics, 3) to determine the gene action involved in inheritance of grain yield, yield components and nutritional quality, 4) to determine the correlation between grain yield, yield components and nutritional characteristics in cowpea parental genotypes and their progenies and, 5) to identify superior parental genotypes and progenies for grain yield and nutritional characteristics.

The study found significant effects of genotype, GCA and SCA for grain yield, yield components and nutritional characteristics measured except for Zn. The findings indicated the presence of variation amongst progenies and their parental genotypes. The study also found significant genotype by environment, GCA by environment and SCA by environment interaction for all measured characteristics except for Fe (GCA and SCA) and Zn, which indicated differences in environmental influence on the performance of cowpea parental genotypes and progenies. The results also indicated a change in genotype performance ranking across seasons and environments. The heterogeneity of soils at Brits and Loskop could be a contributing factor to the changes in performances of genotypes. However, it would be beneficial to breed and select for targeted production areas to reduce the effect of genotype by environment interaction and to obtain a good response to selection.

High broad-sense heritability was found for grain yield, yield components and nutritional characteristics measured, except for Fe and Zn, which indicated that all the measured characteristics except for Fe and Zn were mainly genetically determined. The finding implies the possibility of high selection efficiency for these characteristics. The low to medium narrow-sense heritability observed for grain yield, yield components and nutritional characteristics indicated the predominance of non-additive genetic effects, which controlled the expression of grain yield, yield components and nutritional characteristics, resulting in difficulty of genetic improvement of these characteristics.

The SCA variance was higher than GCA variance for grain yield, yield components and nutritional characteristics, indicating that although both additive and non-additive gene action played an important role in the expression of measured characteristics, non-additive gene action was predominant. Selection should be carried out in later generations after homogeneity is achieved, as the predominance of non-additive genes suggests that selection in earlier generations may not be effective (Kumari and Chauhan 2018).

The positive significant GCA estimates observed, indicated the transfer of characteristics from parental genotypes to their progeny. The parental genotypes TVU13953 and IT96D-602 were the best general combiners for grain yield, hundred seed weight, pod width, number of branches, plant height, leaf length, leaf width, number of pods per plant and number of seeds per pod. Glenda was a good general combiner for grain yield, hundred seed weight, number of pods per plant and plant height. The parental genotypes Kisumi-mix and 98K-5301 were the best general combiners for protein content. Parental genotypes IT845-2246 and TVU-14196 were the best general combiners for Fe content, and Glenda and ITOOK-1060 were best general combiners for Zn content.

Six progenies (TVU13953 x Glenda, ITOOK-1060 x TVU13953, 98K-5301 x Glenda, ITOOK-1060 x TVU13953, IT96D-602 x Glenda, and IT96D-602 x TVU13953) indicated the best SCA and superior performance for grain yield. The positive and significant mid-parent heterosis (60%) for grain yield indicated the superiority of progenies over their mid parental genotypes and importance of either dominant or partial dominant genes in expression of grain yield. The results highlighted the potential of hybrid breeding, however, cowpea cannot compete with other high yielding crops such as tomato, thus it will be beneficial to explore pedigree selection or pure line selection for cowpea cultivar development.

Two progenies, namely 98K-5301 x TVU13953 and IT96D-602 x TVU13953 were superior for grain yield, number of pods per plant, number of seeds per plant, plant height, pod length and pod width. Four progenies (IT96D-602 x TVU13953, IT96D-602 x 98K-5301, TVU-14196 x IT845-2246 and TVU13953 x Glenda) were early flowering (34 to 35 days).

Four progenies (Kisumi-mix x ITOOK-1060, Kisumi-mix x IT93K-129-4, TVU7778 x Kisumi-mix and IT845-2246 x 98K-5301) expressed high SCA (≥ 2.80) effects for protein content, as did progenies TVU-14196 x TVU13953 and TVU-14196 x IT845-2246 for Fe. Four progenies (ITOOK-1060 x Glenda, Kisumi-mix x ITOOK-1060, IT96D-602 x ITOOK-1060 and IT96D-602 x IT93K-129-4) indicated significant positive SCA effects for Zn content. However, heterosis for nutritional characteristics was observed only in some progenies, such as 98K-5301 x Kisumi-mix and Kisumi-mix x TVU13953 for protein (28.21 and 26.32%), TVU-14196 x IT845-2246 for Fe (11.26%), Kisumi-mix x ITOOK-1060 for Zn (5.29%), IT845-2246 x ITOOK-1060 and IT845-2246 x TVU13953 for starch (39.42 and 33.60%). This suggested that dominant and partially dominant genes regulate the expression of these characteristics in the specific crosses, and that the most effective way to enhance the contents of these nutritional characteristics may be through hybrid breeding. The parental genotypes generally showed below average mean performance compared to progenies, indicating heterosis in the progenies for the nutritional characteristics.

The significant positive correlations found between grain yield and all measured yield components except for leaf length, leaf width, plant height, and days to 50% flowering, indicated that improving these yield components could result in higher grain yield. The positive correlation between protein and Fe indicated that improvement of cowpea for Fe could result in simultaneous improvement of protein content; however, the correlation was not significant. The Fe content was significantly positively correlated with Zn content. The study further observed positive correlations of yield components leaf length, leaf width and days to 50% flowering with protein content, for number of branches, number of pods per plant, plant height and leaf length with Fe, and for number of pods per plant and days to 50% flowering with Zn content. This indicated that selection for these yield components could lead to indirect selection for protein, Fe and Zn content (Shanko *et al.* 2014).

Four progenies namely, IT96D-602 x Glenda, ITOOK-1060 x TVU13953, IT96D-602 x TVU13953 and IT96D-602 x 98K-5301) were associated with higher grain yield, number of branches, pod length, and number of seeds per plant. Seven progenies namely, Kisumi-mix x Glenda, 98K-5301 x Glenda, Kisumi-mix x TVU13953, 98K-5301 x ITOOK-1060, 98K-5301 x Kisumi-mix, 98K-5301 x TVU7778 and TVU-14196 x 98K-5301 were associated with important yield components, protein, Fe and Zn content. The results suggested these genotypes could possibly possess favourable characteristics for improving the grain yield and nutritional value of cowpea.

8.2. CONCLUSIONS

In the current study genetically diverse parental cowpea genotypes were crossed using a half-diallel design, in order to determine gene action involved in important characteristics for cowpea improvement of grain yield and nutritional value. The GCA and SCA of parental genotypes were determined for these characteristics, and the possibility for hybrid breeding was investigated. The findings indicated that the environment played a significant role in genotype performance and further suggested that the ranking of GCA and SCA effects changed from one environment to the other. The findings emphasize the importance of multi-environment testing in cowpea breeding and research.

The high broad-sense heritability observed for all characteristics except for Fe and Zn, indicated that these characteristics were largely genetically determined and the possibility of accurate prediction of genetic gains and high selection efficiency. Although the broad sense heritability values were relatively high, the diallel analysis indicated that both additive and non-additive gene effects controlled grain yield, yield components and nutritional characteristics, with non-additive gene action being predominant. This indicated that good performance for certain characteristics will not necessarily be transferred from parental genotype to progeny. The high levels of heterosis expressed for grain yield and yield components in the F_1 progeny indicated the potential for hybrid breeding in cowpea, something that should be seriously considered in future breeding efforts. Very limited heterosis was expressed for the nutritional characteristics, and narrow-sense heritability of Fe and Zn, for example, was low, therefore a different breeding approach, such as marker assisted breeding, could be followed to improve nutritional characteristics of the parental genotypes.

8.3. IMPLICATIONS FOR FUTURE BREEDING

1. Heterosis breeding for yield should be seriously considered in cowpea breeding in South Africa.
2. Good F_1 progenies that were identified from this study can already be tested in various environments to determine their value as commercial hybrids.
3. The identified parental genotypes with good values for important characteristics, and good GCA and SCA for the same characteristics should be included in breeding efforts.
4. A different approach should be considered for the improvement of nutritional characteristics especially Fe and Zn content in cowpea, as the heritability of these minerals was low, making improvement through selection difficult.

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