

**IDENTIFICATION OF COWPEA (*VIGNA UNGUICULATA* (L.) Walp)  
GENOTYPES AND GENETIC IMPROVEMENT FOR ENHANCED  
YIELD AND NUTRITIONAL QUALITY.**

**By**

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## THESIS ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp) is a staple legume crop with potential to address food insecurity and malnutrition in the Sub-Saharan Africa (SSA). It is also among the neglected underutilized legume crop species in the region. The crop's yield production has never met the potential yields of the crop in the SSA. In addition, given its nutritional value, ability to withstand extreme environmental conditions, nitrogen fixation ability and its greater potential to address nutrient deficiencies and food insecurity in the SSA. These attributes make cowpea an ideal crop to sustainable future for the people and environment. There is a need to develop cowpea varieties that are high yielding with high nutritional values to combat food insecurity. Therefore, the objectives of the study were: (1) to assess the genotype by environment interaction effect and select cowpea genotypes with high grain yield and adaptation across selected cowpea growing environments in South Africa, (2) to assess the phenotypic variability and correlation analysis in cowpea based on yield and yield related traits, (3) to evaluate the variations of nutritional content and phytochemical compositions among cowpea genotypes under diverse environments, (4) to assess the genetic diversity among cowpea genotypes using single nucleotide polymorphism (SNP) markers, and to select distinct and complementary genotypes for developing improved cultivars and (5) to determine the combining ability effects and gene action controlling the yield and yield-related traits among selected cowpea parental genotypes and their progenies.

The first study assessed the extent of genotype by environment interaction (GEI) of cowpea genotype to the influence of genotype (G), environment (E) and their interaction (GEI) effects on grain yield in cowpea and to assess the stability of cowpea genotypes to identify stable and high-yielding genotypes for broad or narrow adaptation to improve cowpea productivity in South Africa and identify the identical agro-ecologies using analysis of variance (ANOVA), additive main effects and multiplicative interaction (AMMI) and the genotype-by-environment interaction (GGE) biplot analyses. The AMMI ANOVA showed the significant GEI effect which accounted for 57% variation, whereas genotype and environment main effects accounted for 29% and 13% variation, respectively. The AMMI stability values (ASV) analysis identified genotype Acc-Cowp44 as the most stable genotype recording the lowest ASV of 0.03.

The biplot depicted eight sectors and environments were clustered into three of the eight sector whereby E4 (Brits 2020/2021), E5 (Loskop 2020/2021), E6 (Mafikeng 2020/2021) and E7 (Polokwane 2020/2021) which formed a mega-environment and the second sector which

involved environments E1 (Brits 2019/2020), E2 (Loskop 2019/2020) and E3 (Roodeplaat 2019/2020) formed one mega-environment. The cowpea genotypes Acc-Cowp38, Acc-Cowp2, Acc-Cowp9, Acc-Cowp5 and Acc-Cowp39 were identified as ideal for grain yield, in that order. These genotypes are recommended for production in South Africa or in similar agro-ecologies, and for incorporation in future breeding programs targeting genetic improvement for grain yield.

The second study assessed 50 cowpea genotypes using yield and yield components to determine the phenotypic correlations among them and selection of best performing genotypes among tested genotypes for enhanced cultivar development. The study revealed the significant differences at 5% and 1% level of probability among the assessed grain yield and yield component traits. The study further indicates that number of pods per plant (NPP), pod length (PL), number of seeds per pod (NSP) and hundred seed weight (HSW) had significant and positive correlations with grain yield, therefore these traits can be used as a proxy trait for increased grain yield. Similarly, the principal component analysis (PCA) biplot identified number of branches (NB), number of pods per plant (NPP), pod length (PL), pod width (PW), number of seeds per pod (NSP), and hundred seed weight (HSW) as the important traits in the production of grain yield. Genotypes Acc-Cowp2, 98K\_5301, Acc-Cowp4, Acc-Cowp17 and Acc-Cowp9 were grouped together based on their high exhibition of NPP, PW, NSP, PL, HSW and grain yield (GY). The selected genotypes could be considered as potential sources of gene to improve these traits and could serve as parental genotypes in breeding programs targeting enhanced high-yielding varieties.

The third study assessed the nutritional and phytochemical traits among the 50 cowpea genotypes to select superior lines with high nutritional compositions for cultivar development for nutritional quality. The study highlighted the significant effects for all nutritional and phytochemical traits for genotype, environment, and genotype by environment interaction evaluated except for flavonoids and fat content. Genotypes Acc-Cowp6, Acc-Cowp17, Acc-Cowp14, 98K\_5301, Acc-Cowp9, Acc-Cowp32, Acc-Cowp9, Acc-Cowp4, Acc-Cowp16 and Acc-Cowp21 were selected based on high concentration of Ca, Mg, P, Na and Zn. Genotype Acc-Cowp31 and Acc-Cowp13 were highly associated with protein content while genotype Acc-Cowp39 were in close association with fat content. Further, genotypes Acc-Cowp34, Acc-Cowp18, Acc-Cowp48, Acc-Cowp22, Acc-Cowp26, Acc-Cowp49 and Acc-Cowp28 had low concentrations of total phenolic, flavonoids and condensed tannins.

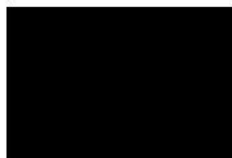
The fourth study used 90 genetically diverse cowpea to assess the magnitude of the genetic diversity and population structure among cowpea genotypes using single nucleotide polymorphisms (SNP) markers. The study revealed that 49% of the selected SNP markers were highly polymorphic and efficiently discriminate the tested cowpea accessions. The low heterozygosity and the high inbreeding coefficients observed among cowpea varieties indicate that the accessions reached an acceptable level of homozygosity. The model-based (structure analysis) and distance-based (UPGM) clustering approaches were used in this study. The model-based analysis revealed the presence of four subpopulations at  $K = 4$  whereas the distance-based cluster analysis classified the cowpea accessions into three distinct clusters. The subpopulations identified exhibited a high level of genetic diversity and were moderately differentiated. This result suggests that the accessions studied are unique and have greater potential to contribute to new varieties for breeding programs in South Africa.

The fifth study determined the combining ability effects and gene action controlling the yield and its related traits among 10 selected parental genotypes and 45 crosses using the half diallel mating design. There were significant genotypic, environmental and their interaction effects for almost all traits except leaf length (LL) and number of seeds per pod (NSP) exhibited by both parental genotypes and their progenies. The GCA effects were significant for LW, PL, NSP and HSW whereas the SCA effects were significant for pod width (PW) only. The GCA x environmental interaction effects were highly significant for all traits while the SCA x environmental interaction effects were significant for all the traits except plant height (PH) and LL. The parents Acc-cowp17, Acc-cowp31, Acc-cowp9, Acc-cowp5, Acc-cowp38 and Acc-cowp19 were identified as good combiners for grain yield and its associated traits productivity. The newly developed progenies Acc-Cowp31 x Acc-Cowp5, Acc-Cowp38 x Acc-Cowp19, Acc-Cowp9 x Acc-Cowp2, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp38, and Acc-Cowp17 x Acc-Cowp38 were found to be the best performing due to their desirable SCA effects for enhanced grain yield. The study revealed that trait expression was controlled by both additive and non-additive effect with the additive gene action shown to be the important in controlling traits including NB, LW, NPP, NSP and HSW.

## **PREFACE**

The research contained in this dissertation was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa in collaboration with the Agricultural Research Council - Vegetable, Industrial and Medicinal Plants, Pretoria, South Africa.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.



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**Supervisor: Prof T. Mabhaudhi**



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**Co-Supervisor: Dr AS Gerrano**

## DECLARATION

I, **Mbali Thembi Gumede**, declare that:

- (i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;
- (ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;
- (iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;
- (iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
  - a) their words have been re-written but the general information attributed to them has been referenced;
  - b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;
- (v) where I have used material for which publications followed, I have indicated in detail my role in the work;
- (vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;
- (vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections



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**Signed:** Miss Mbali Thembi Gumede (*Candidate*)

## DECLARATION 2: PUBLICATIONS

### Publication 1: Chapter 3

Gumede, M.T., Gerrano, A.S., Amelework, A.B. and Modi, A.T., 2022. Analysis of Genetic Diversity and Population Structure of Cowpea (*Vigna unguiculata* (L.) Walp) Genotypes Using Single Nucleotide Polymorphism Markers. *Plants*. 11(24):3480.DOI: <https://doi.org/10.1080/09064710.2022.2069593>.

### Publication 2: Chapter 5

Gumede, M.T., Gerrano, A.S., Modi, A.T. and Thungo, Z., 2022. Influence of genotype and environment on grain yield among cowpea (*Vigna unguiculata* (L.) Walp) genotypes under dry land farming system. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*. 72(1): 709-719.DOI: <https://doi.org/10.3390/plants11243480>.

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## **DEDICATIONS**

This work is dedicated to my mother, Sarah Ncamsile Mabika.

*~Mabika, Dinabantu, Mazalankosi*

Also, to my siblings, family and extended family as a motivation to reach and aim for the greater heights in life.

# TABLE OF CONTENT

THESIS ABSTRACT.....	i
PREFACE.....	iv
DECLARATION.....	v
DECLARATION 2: PUBLICATIONS.....	vi
ACKNOWLEDGEMENTS.....	vii
DEDICATIONS.....	viii
TABLE OF CONTENT.....	ix
LIST OF TABLES.....	xiii
CHAPTER 1: INTRODUCTION TO THESIS.....	1
1.1 Background.....	1
1.2 Justification.....	3
1.3 Aim of the study.....	4
1.4 Specific objections.....	4
1.5 Thesis outline.....	5
1.6 References.....	6
CHAPTER 2: ENHANCEMENT OF PRODUCTIVITY OF COWPEA [ <i>VIGNA UNGUICULATA</i> (L.) WALP] FOR IMPROVED YIELD, AND NUTRITIONAL QUALITY: LITERATURE REVIEW.....	10
Abstract.....	10
2.1 Introduction.....	11
2.2 Production status of cowpea.....	13
2.3 Constraints of cowpea production.....	14
2.4 Breeding progress for enhanced yield and nutrition quality in cowpea.....	17
2.4.1 Grain yield productivity.....	17
2.4.2 Nutritional quality.....	18
2.4.3 Anti-nutritional traits.....	20
2.5 Genetic resources of cowpea.....	20
2.6 Genetic diversity analysis of cowpea.....	22
2.7 Genotype-by environment interaction analysis.....	26
2.8 Combing ability and gene action analysis for yield and yield related traits in cowpea.....	27
2.9 Future prospects.....	28
2.10 Conclusion.....	30
2.11 References.....	30
CHAPTER 3: INFLUENCE OF GENOTYPE AND ENVIRONMENT ON GRAIN YIELD AMONG COWPEA ( <i>VIGNA UNGUICULATA</i> (L.) Walp) GENOTYPES UNDER DRY LAND FARMING SYSTEMS.....	43

Abstract.....	43
3.1 Introduction .....	44
3.2 Materials and methods.....	46
3.2.1 Plant materials .....	46
3.2.2 Study environments.....	47
3.2.3 Experimental design and data collection.....	47
3.2.4 Data Analysis.....	48
3.2.4.1 Analysis of variance.....	48
3.3 Results.....	49
3.3.1 Genotype, environment, and genotype-by-environment interaction effects.....	49
3.3.2 Performance of cowpea genotypes for grain yield.....	49
3.3.3 GGE biplot analysis of which genotypes were superior in which environment .....	54
3.3.4 GGE biplot analysis for the studied environments .....	55
3.3.5 Comparison view of GGE biplot analysis of ideal cowpea genotype.....	56
3.3.6 Comparison view of GGE biplot analysis of ideal environment.....	57
3.4 Discussion.....	58
3.5 Conclusion.....	61
3.6 References .....	62
CHAPTER 4: PHENOTYPIC VARIABILITY AND CORRELATION ANALYSIS IN COWPEA [ <i>VIGNA</i> <i>UNGUICULATA</i> (L.) Walp] BASED ON YIELD AND YIELD RELATED TRAITS.....	
Abstract.....	67
4.1 Introduction .....	68
4.2 Materials and Methods.....	69
4.2.1 Plant materials and description of study environments.....	69
4.2.2 Experiment design and management.....	70
4.2.3 Data Collection.....	72
4.2.4 Data analysis .....	73
4.3 Results.....	74
4.3.1 Genotype, environment and genotype-by-environment interaction effects on evaluated traits .....	74
4.3.2 Mean performances of cowpea genotypes for agronomic traits .....	74
4.3.3 Correlation among agronomic traits.....	80
4.3.5 Principal component analysis of studied agronomic traits.....	80
4.4 Discussion.....	82
4.5 Conclusion.....	85
4.6 References .....	86

CHAPTER 5: VARIATION IN GRAIN NUTRITIONAL AND PHYTOCHEMICAL COMPOSITIONS AMONG COWPEA [ <i>VIGNA UNGUICULATA</i> (L) Walp] GENOTYPES GROWN UNDER DRYLAND FARMING SYSTEM IN SOUTH AFRICA.....	89
Abstract.....	89
5.1 Introduction .....	90
5.2 Materials and Methods.....	93
5.2.1 Plant materials .....	93
3.2.2 Description of study sites.....	95
3.2.3 Experimental design.....	95
3.2.4 Data collection .....	96
3.2.4.1 Quantification of nutritional quality traits.....	96
3.4.4.2 Quantification of phytochemical traits .....	96
3.2.5 Data analysis .....	97
5.3 Results.....	97
5.3.1 Genotype, environment, and genotype-by-environment interaction effects on nutritional and phytochemical traits .....	97
5.3.2 Mean concentrations of nutritional and phytochemical traits.....	100
5.3.3 The correlation analysis among the nutritional and phytochemical traits.....	104
5.3.4 PCA of studied nutritional and phytochemical traits.....	106
5.3.5 Cluster analysis .....	108
5.4 Discussion.....	110
5.5 Conclusion.....	114
5.6 References .....	114
CHAPTER 6: ANALYSIS OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF COWPEA [ <i>VIGNA UNGUICULATA</i> (L.) Walp] GENOTYPES USING SINGLE NUCLEOTIDE POLYMORPHISM MARKERS. 120	
Abstract.....	120
6.1 Introduction .....	121
6.2 Materials and Methods.....	123
6.2.1 Plant materials .....	123
6.2.2 DNA extraction and sequencing .....	123
6.2.3 Data analysis .....	124
6.3 Results.....	127
6.3.1 Allele polymorphism .....	127
6.3.2 Population structure and clustering .....	129
6.3.3 Genetic diversity among subpopulations .....	132
6.3.4 Analysis of Molecular variance (AMOVA).....	133
6.4 Discussion.....	134

6.5 Conclusions .....	138
6.6 References .....	138
CHAPTER 7: COMBINING ABILITY AND GENE ACTION STUDIES FOR GRAIN YIELD AND ITS RELATED TRAITS AMONG SELECTED COWPEA [ <i>VIGNA UNGUICULATA</i> (L.) Walp] GENOTYPES IN SOUTH AFRICA.....	146
Abstract.....	146
7.1 Introduction .....	147
7.2 Materials and Methods.....	149
7.2.1 Plant Materials and generation of crosses .....	149
7.2.2 Description of the study site for progeny evaluation .....	150
7.2.3 Trial design and management.....	150
7.2.4 Data Collection.....	151
7.2.5 Statistical analysis .....	151
7.2.5.1 Analysis of variance.....	151
7.2.5.2 Combining ability analysis.....	151
7.2.5.3 Gene Action and Heritability Estimates .....	152
7.2.5.4 Correlation analysis.....	152
7.3 Results.....	152
7.3.1 Genotype, environmental and their interaction effects.....	152
7.3.2 The mean performance of cowpea parents and their hybrids for assessed traits.....	153
7.3.3 The analysis of variance of GCA and SCA effects of parents and F <sub>1</sub> progenies.....	160
7.3.4 General combining effects of parental genotypes. ....	162
7.3.5 Specific combining ability effects for the F <sub>1</sub> progenies.....	165
7.3.6 Gene action and heritability estimates.....	170
7.3.7 The associations of the assessed yield and yield-related traits.....	171
7.4 Discussion.....	172
7.5 Conclusion.....	175
7.6 References .....	176
CHAPTER 8: OVERVIEW OF RESEARCH FINDINGS.....	181
8.1 Introduction .....	181
8.2 Summary and Implications of research findings.....	181
8.3 Conclusion and recommendations .....	187

## LIST OF TABLES

<b>Table 2.1:</b> Trait specific genotypes reported globally.....	19
<b>Table 2.2:</b> List of cowpea varieties released from 2005 to 2016 in Sub-Saharan Africa by IITA.....	22
<b>Table 2.3:</b> Reported marker systems and genetic analysis in cowpea in different countries...24	
<b>Table 3.1:</b> List of 50 cowpea genotypes used in the study and their origin during 2019/2020 and 2020/2021 cropping season.....	46
<b>Table 3.2:</b> Description of environments evaluated in the study.....	47
<b>Table 3.3:</b> AMMI analysis of variance showing SS, MS, percentage explained variance by IPCA1, IPCA2, IPCA3, IPCA4 and IPCA5 for grain yield among 50 cowpea genotypes across test environments.....	49
<b>Table 3.4:</b> Mean grain yield (t ha <sup>-1</sup> ) of 50 cowpea genotypes under seven test environments during the 2019/2020 and 2020/2021 planting seasons.....	51
<b>Table 4.1:</b> List of cowpea genotypes used in the study with their origin and growth habit...71	
<b>Table 4.2:</b> Morphological descriptions and data collection for agro-morphological characterization of 50 cowpea genotypes evaluated.....	73
<b>Table 4.3:</b> Analysis of variance showing mean square values and significant test for assessed traits among studies cowpea genotypes.....	76
<b>Table 4.4:</b> Mean performance of all traits among 50 genotypes evaluated across all test environments during 2019 and 2020 cropping system.....	76
<b>Table 4.5:</b> Correlation coefficient analysis showing the association between the assessed agronomic traits in cowpea genotypes.....	80
<b>Table 4.6:</b> Summary of rotated principal loading scores, eigen values, percentage and cumulative variation for agronomic traits among 50 cowpea genotypes across test environments.....	81
<b>Table 5.1:</b> List of cowpea genotypes evaluated in the present study Source: (Gumede et al., 2022a; 2022b).....	93
<b>Table 5.2:</b> Soil characteristics, altitude and mean annual weather data for average rainfall, and minimum (Tmin) and maximum (Tmax) air temperature information for the studied environments. Source: SA Weather Services (weathersa.co.za).....	95
<b>Table 5.3:</b> Combined analysis of variance showing mean square values and significant tests for nutritional and phytochemical traits among the 50 assessed genotypes across all test environments.....	99
<b>Table 5.4:</b> The mean concentrations of nutrient elements and phytochemical traits of 50 cowpea genotypes across all locations.....	101
<b>Table 5.5:</b> Pearson correlation coefficients among 50 cowpea mineral and phytochemical contents across all locations.....	105

<b>Table 5.6:</b> Principal components, Eigen values, the proportion of variance and their percentage contributions of different mineral elements and phytochemical traits among 50 cowpea genotypes.....	106
<b>Table 6.1:</b> List of cowpea genotypes used in the study and their origin.....	125
<b>Table 6.2:</b> Genetic diversity within and among 90 cowpea accessions genotypes based on 5864 SNPs markers.....	128
<b>Table 6.3:</b> Genetic diversity within and among the 90-cowpea accession classified by growth habit.....	132
<b>Table 6.4:</b> Analysis of molecular variance (AMOVA) among 90-cowpea accessions classified based on SNP markers.....	133
<b>Table 6.5:</b> Pair-wise estimates of gene flow (above diagonal, within the brackets), genetic differentiation ( $F_{ST}$ ) (above diagonal off brackets); genetic distance (GD) (lower diagonal off brackets) and genetic identity (GI) (lower diagonal within the brackets).....	134
<b>Table 7.1:</b> The geographical region, seed coat colour and growth habit for the parental genotypes used for the study.....	149
<b>Table 7.2:</b> Mean squares and significance tests for the assessed yield and yield related traits among the 10 parental cowpea genotypes and their 45 progenies evaluated in two South African locations.....	153
<b>Table 7.3:</b> Combined means for grain yield and yield related traits evaluated among 10 parents and their 45 progenies of cowpea tested under two South African agro-ecologies.....	156
<b>Table 7.4:</b> Mean squares and significant tests of general and specific combining ability effects for yield and yield relates traits across two sites in South Africa.....	161
<b>Table 7.5:</b> General combining ability effects for yield and yield related traits among 10 parental cowpea genotypes evaluated across two sites.....	164
<b>Table 7.6:</b> Specific combining ability effects for yield and yield related traits among 45 progenies of cowpea assessed under two sites.....	167
<b>Table 7.7:</b> Gene action and heritability estimates for yield and yield related traits among 10 cowpea parents and 45 F1 progenies evaluated across two sites in South Africa.....	170
<b>Table 7.8:</b> Pearson correlation coefficients of grain yield and yield components among 10 cowpea parental lines and their 45 progenies evaluated across two locations in South Africa.....	171

## LIST OF FIGURES

- Figure 2.1:** Total annual production over harvested area of cowpea from 2011 to 2020 in Africa (FAOSTAT,2022) .....13
- Figure 2.2:** Cowpea grains infested by bruchids weevils at the genebank in Agricultural research council harvested during 2019/2020 planting season.....16
- Figure 3.1:** The “Which-won-where” view of GGE biplot showing which genotypes performed best in which environment and delineation of mega-environments. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng 2020/2021; E7, Polokwane 2020/2021.....54
- Figure 3.2:** The “discriminating power vs. representativeness” view of GGE biplot showing the relationship between the studied environments. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng.....56
- Figure 3.3:** The comparison view of GGE biplot showing an ideal genotype based on grain yield performance across seven environments. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng 2020/2021.....57
- Figure 3.4:** The comparison view of GGE biplot showing the ideal environment for grain yield. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng 2020/2021; E7, Polokwane 2020/2021.....58
- Figure 4.1:** Biplot demonstrating the relationship among agronomic traits among 50 cowpea genotypes evaluated across Brits, Loskop, Roodeplaat, Mafikeng and Polokwane during 2019 and 2020 cropping season. (See genotype codes in Table 4.2) .....82
- Figure 5.1:** Biplot analysis for principal component analysis of nutritional and phytochemical traits for the evaluated cowpea genotypes. Note: See genotype codes in Table 5.1.....107
- Figure 5.2:** Dendrogram depicting the interrelatedness of 50 cowpea genotypes evaluated using single linkage and Euclidean distance methods based on nutritional and phytochemical traits.....109
- Figure 6.1:** Population structure analysis using a Bayesian-based approach. (A). Estimation of hypothetical subpopulations using K-values showing the highest Delta k value was observed at the number of populations (K) = 4. (B). Population structure analysis of 90 cowpea accessions.....129
- Figure 6.2:** Neighbor-joining analysis showing the genetic relationships among 90 cowpea accessions tested using 5864 SNP markers. The different colours indicate different clusters.131

# CHAPTER 1: INTRODUCTION TO THESIS

## 1.1 Background

Food legumes play a crucial role in food and nutrition, being the constituent in the diets of the people in developing countries, especially in the Sub-Saharan African countries. They have greater potential to alleviate malnutrition among resource-poor communities and farmers as they are a good complementary food crop to cereal crops as a major source of proteins and minerals. Most of the nutritional requirements of the rural community are mostly met by legume crops, which constitute an important source of plant protein. The diverse contribution of legume crops to the diets and the agricultural farming systems makes them the best crops of choice to help achieve the sustainable developmental goals (SDG) of reducing hunger (SDG 2), poverty (SDG 1), enhancing human health (SDG 3) and nutrition and improving ecosystem resilience. Hence there is a need to enhance the production and nutritional status of legume crops such as cowpea.

Cowpea (*Vigna unguiculata* [L.] Walp) is a leguminous leafy and grain crop that originated and domesticated in the African continent with immense economic and social importance in the developing world (da Silva et al., 2018). Cowpea is a diploid member of the Fabaceae family with a chromosome number ( $2n=22$ ) and a genome of 613 to 640.6 Mb (Lornardi et al., 2019; Arumugathan and Earle, 1991). It is extensively cultivated in Asia, Africa, and Latin America which they are planted in more than 10 million hectares of land (Nedumaran et al., 2013). Moreover, Badiane et al. (2012) have reported that the crop is used as the staple crop in > 65 countries in tropical and subtropical regions; the crop is also a significant source of proteins, dietary fibre, vitamins, carbohydrates, essential minerals and phytochemicals in human diets.

Cowpea is among the most important indigenous and underutilized legume crop species which greatly contributes to the livelihoods of people in the developing countries especially the SSA regions (Akibode, 2011). The crop is mainly recognized as a potential source of protein and other important nutrients consumed by people in SSA especially in the Southern Africa (Hall et al., 2003; Hazra et al., 2007). It is a significant cheap source of plant-derived protein in the developing countries where the crop contributes about 83% of total dietary protein (Carvalho et al., 2017; Nkhoma et al., 2020). The protein content in cowpea seed grain ranges from 21 to 33% and 27 to 43% in the leaves (Akibode, 2011). Hence, the crop is regarded as a poor man's food or vegetable meat due to high amount of protein in grain with better biological value on dry weight basis (Raut et al., 2017). The cowpea grains and leaves are rich in carbohydrates

and essential amino acids such as lysine, tryptophan, valine, leucine and phenylalanine (Iqbal *et al.*, 2006; Carvalho *et al.*, 2017; Raizada *et al.*, 2023). Due to its high protein, cowpea efficiently acts as the best substitute protein obtained from meat and dairy for individuals who cannot afford because of poverty (Sharma *et al.*, 2013). Additionally, the crop is consumed as young leaves, succulent immature pods and dry seeds are consumed and thus providing the key macro-and-micro-nutrients such as iron, zinc, calcium, magnesium, sulphur, copper, manganese and sodium, copper, potassium and phosphorus required by the human body for growth and development (Gerrano *et al.*, 2015; Sombié *et al.*, 2018; Jayathilake *et al.*, 2018; Boukar *et al.*, 2019).

Furthermore, the crop is rich in carbohydrates, vitamins including provitamin A, carotenoids, great fiber content and low-fat content, making it an ideal food component in several countries. Cooked cowpea fresh seeds and immature pods are relished by many consumers in different parts of the world and are sometimes preferred than the cooked dry seeds (Nielsen *et al.*, 1997; Ahenkora *et al.*, 1998; Timko *et al.*, 2008). The pods are best when young and slender and are eaten fresh or cooked, often cut into short sections and used in stir-fries or cooked salads. Green, immature cowpea pods and fresh leaves are harvested and sold in local markets for consumption as a vegetable in different parts of the regions in South Africa towards income generation. Nevertheless, cowpea contains anti-nutritional factors (ANFs) such as protease inhibitors, lectin, phytic acid, tannin, oxalic acid, oxalate, polyphenols, flavonoids among others, which can cause adverse physiological effects when ingested by humans and domestic animals (Maia *et al.*, 2000). The ANFs or antinutrients are plant's secondary metabolites that act to reduce food nutrient utilization (Welch and Graham, 2004; Soetan, 2008). Furthermore, the presence of anti-nutritional factors in legumes including cowpea has been recognized as a major factor limiting their wider food use (Liener and Kakade, 1980; Ogunsin and Radha, 2010). For instance, it is an important source of protein in many developing countries including South Africa. However, this protein may not be readily bio-available because of the presence of anti-nutritional factors. Anti-nutrients are substances that lowers the nutritional value of the food by inhibiting the mineral bioavailability as well as the digestibility of vital nutrients (Thakur *et al.*, 2019).

Besides its nutritional benefits food and forage, cowpea is also an important crop to include in cropping systems as it can fix atmospheric nitrogen, thus replenish soil nitrogen. The crop can thrive in drought prone areas and improves the fertility of soils in marginal lands by providing ground cover and crop residues, fixing atmospheric nitrogen and suppressing weeds. The crop's nutritional qualities and its potential to improve the agricultural systems makes it the

best crop to alleviate food insecurity and malnutrition. Cowpea, thus, has the potential to be an ideal crop for production in drier regions in Africa, particularly in Southern Africa.

The cowpea yield production and productivity has been on the increase, but it has never met the potential yields of the crop in Sub-Saharan Africa. A considerable gap remains the challenge between the potential and the actual yield. The reported mean grain yields in Sub-Saharan Africa is between 0.1 to 0.6 ton ha<sup>-1</sup>, which is way less than the expected potential yield of the crop accomplishing 3 ton ha<sup>-1</sup> (Gbaye and Holloway, 2011). The review studies by Horn and Shimelis (2020) has reported that the yield gap is associated to a lack of improved varieties, poor agronomic managements and abiotic and biotic stresses. In addition, Gerrano et al. (2018) further stated that the major constraints that limit the production of cowpea in South Africa are low productivity of the crop available at the farmer's level, current moisture stress, absence of improved high yielding and nutritional value genotypes, low fertility of the soil, losses of yield due to insect pests and disease. Cowpeas are vulnerable to extensive pests and pathogens that attacks the crop at growth stages. These include bacteria, fungi, and viruses (Horn *et al.*, 2015). These may then result to the yield losses between 10-100 % depending on the host-vector relationship as well as the prevailing epidemiological factors (Kareem and Taiwo, 2007). As a result, there is a need for production of improved cowpea varieties with enhanced yield quality and quantity to meet local and regional productivity and market demands.

## **1.2 Justification**

The rapid demand for food production with enhanced nutritional quality and ever-increasing world population growth is still the top major issue that needs to be addressed urgently. The attempts to address hunger, food insecurity and malnutrition in developing countries like Sub-Saharan Africa has been of major interests. The critical shortage of food in SSA countries has drastically escalated. According to the report by Wang *et al.* (2022), during the year 2022, at least 12 million people which makes 12% of SSA's population are estimated to be subjected to acute food insecurity. Consequently, the people suffer from severe malnutrition, with Zn, Fe, I, and Vitamin A deficiencies the most prevalent (Kangas *et al.*, 2020) and inability to meet the dietary needs for growth. Food insecurity and malnutrition remain a significant concern as it contributes weakened immune system and the death of children and women especially the pregnant women. Evidently, more than five million childhood deaths occur from micronutrient malnutrition every year (WHO, 2003). At least 22 mineral elements are required for the wellbeing of humans (Graham *et al.*, 2007). Agriculture has been the main source of food and

the largest source of income for the people in the rural and poor families. Improvement of agricultural food systems by targeting the nutritional and yield potentials in the staple crops will significantly improve the diet of the people, thus reducing the malnutrition and simultaneously eradicate the hunger and food insecurity. Therefore, there is a need to improve the food nutrition and productivity to combat insecurity in the Sub-Saharan Africa.

### **1.3 Aim of the study**

The aim of the study was to improve cowpea yield production and its nutritional quality for sustainable production and to contribute to alleviating hunger, poverty and malnutrition in South Africa.

### **1.4 Specific objections**

- I. Assess the genotype by environment interaction effect and select cowpea genotypes with high grain yield and adaptation across selected cowpea growing environments in South Africa.
- II. Assessing the phenotypic variability and correlation analysis in cowpea based on yield and yield related traits.
- III. Evaluate the variations of nutritional content and phytochemical compositions among tested cowpea genotypes under diverse environments.
- IV. Assess the genetic diversity among cowpea genotypes using single nucleotide polymorphism (SNP) markers and select distinct and complementary genotypes for developing improved cultivars.
- V. Determine the combining ability effects and gene action controlling the yield and yield-related traits among selected cowpea parental genotypes and their progenies.

### **1.5 Hypotheses**

- The selected ideal genotypes and environments will be highly influenced by the interaction of genotype and environments.
- The cowpea genotype will have significant variations for yield and yield related traits assessed.
- The compositions of nutritional and phytochemical traits will vary based on the effects on genotype, environments and genotype x environment interactions.
- The cowpea genotypes assessed will have significant genetic diversity that will allow efficient selection for development of improved varieties.

- The selected parental genotypes will be good combiners to produce best the performing progenies for yield and yield related traits.

### **1.5 Thesis outline**

This thesis is comprised of five experimental chapters that are distinct but linked to each other by objectives, one literature review chapter and the introductory chapter providing the background, problem statement, aims and objectives and the hypotheses of the study. The thesis chapter are in the form of research chapters, chapter following the format of a stand-alone research paper, where a chapter has been published or submitted to a journal for publication, a foot note is presented providing the details. Lastly, a general discussion of research findings and provides the implications of the study on cowpea production and its potential improvement.

**Chapter 1:** This chapter provides the context of the background of the study, rationale, aims and objectives of the study and the structure of the thesis.

**Chapter 2:** Enhancement of productivity of cowpea [*Vigna unguiculata* (L.) walp] for improved yield, and nutritional quality: A review.

**Chapter 3:** Influence of genotype and environment on grain yield among cowpea (*Vigna unguiculata* (L.) walp) genotypes under dry land farming system.

**Chapter 4:** Phenotypic variability and correlation analysis in cowpea [*Vigna unguiculata* (L.) Walp] based on yield and yield related traits.

**Chapter 5:** Variation in grain nutritional and phytochemical compositions among cowpea [*Vigna unguiculata* (L.) Walp] genotypes grown under dryland farming systems in South Africa.

**Chapter 6:** Analysis of genetic diversity and population structure of cowpea [*Vigna unguiculata* (L.) Walp] genotypes using single nucleotide polymorphism markers.

**Chapter 7:** Combining ability and gene action studies for grain yield and its related traits among selected cowpea [*Vigna unguiculata* (L.) Walp] genotypes.

**Chapter 8:** General discussion and implications of the study

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## CHAPTER 2: ENHANCEMENT OF PRODUCTIVITY OF COWPEA [*Vigna unguiculata* (L.) WALP] FOR IMPROVED YIELD, NUTRITIONAL QUALITY: LITERATURE REVIEW.

### **Abstract**

Cowpea is an important grain legume crop widely grown in Sub-Saharan Africa due to its high-quality plant-derived protein. The proteins obtained from cowpea serve as the best substitute for meat and dairy proteins for poor rural households hence it is referred to as poor man's meat. Its grains contain high protein and carbohydrates content with relatively low-fat content, amino acids and essential minerals, thus is referred to a highly nutritious food crop in human diets. The cowpea yield production has been relatively low due to biotic and abiotic constraints, use of unimproved varieties and poor agronomic management. There are several breeding techniques such as conventional breeding, molecular markers and genomic selection has been proven to be efficient in enhancement of cowpea. The plant genetic diversity is extremely important on the basis of cowpea breeding as it's provided the knowledge of genetic variation and genetic relationship among cowpea genotypes. Single nucleotide polymorphism has emerged as a powerful tool in determining genetic diversity of cowpea as compared to other markers such as amplified fragment length polymorphism (AFLP), simple nucleotide sequence (SSR) and single sequence repeats (SSR). The review aims to highlight the aspects of cowpea production, constraints and explore possible breeding methods for developing enhanced cowpea cultivars for improved yield and nutritional quality in Sub-Saharan Africa.

**Keywords:** Cowpea, yield components, nutritional status, genetic diversity, Breeding, cultivar development.

## 2.1 Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is an important grain legume crop widely cultivated in semi and arid areas around the world. The crop is a herbaceous legume with good adaptability to warm regions with adequate rainfall and is grown all over Africa, Asia, Southern United States and Latin America (Jayathilake *et al.*, 2018; Goncalves *et al.*, 2016). In the Sub-Saharan Africa, the crop is a major staple crop which is essential for food and nourishment for both humans and animals and also the income generation for farmers and food traders (Boukar *et al.*, 2016). Over 90% of smallholder farmers in semi-arid areas grow cowpea for their own consumption and or for sale in local markets (Fatokun *et al.*, 2002). The crop is highly adapted to low-input farming systems and is one of the most preferred legumes by various resource limited farmers. Cowpea is also a nitrogen fixing crop resulting in soil fertility improvement and is an effective crop for breaking disease cycles in relay farming, mixed farming, and crop rotations (Fatokun *et al.*, 2002).

The ever-increasing world population growth and rapid demand for increased production of food with enhanced nutritional quality are still the top major global issue that needs to be addressed urgently. The human nutritional health status is characterized as unbalanced and inadequate making macronutrients and micronutrients deficiency a significant global issue. More than two billion people are affected by malnutrition due to micronutrients deficiency, mostly among disadvantaged, low-income families in under and developing countries characterised with zinc, iron, and vitamin A deficiencies (Sah, 2016). The dietary deficiencies of trace minerals such as iron and zinc contribute to major public health issues globally amongst women and children at the SSA (Wessels *et al.*, 2012). Sah (2016) further stated that more than five million children die due to micronutrient malnutrition every year.

Global cowpea production is hampered by a wide array of biotic and abiotic production stress factors including heat and drought stress, pests and diseases (Horn and Shimelis, 2020), with adverse effect on yield and quality. Globally, up to 60-100% losses in yield and quality traits are attributable to environmental stress. Nevertheless, the crop has high tolerance to environmental challenges including low soil fertility, dryland growing conditions, pest and diseases where other crops cannot survive growth (Nkhoma *et al.*, 2020). In the SSA, cowpea is one of the preferred crops and an important component in production systems for the poor-resource rural communities. The contribution of cowpea to food security in Sub-Saharan Africa is limited by poor productivity in terms of yield and quality. According to Horn and Shimelis (2020), the low cowpea yield production is associated with the lack of improved varieties with

high susceptibility to extreme environments, poor soil fertility and other biotic and abiotic stresses.

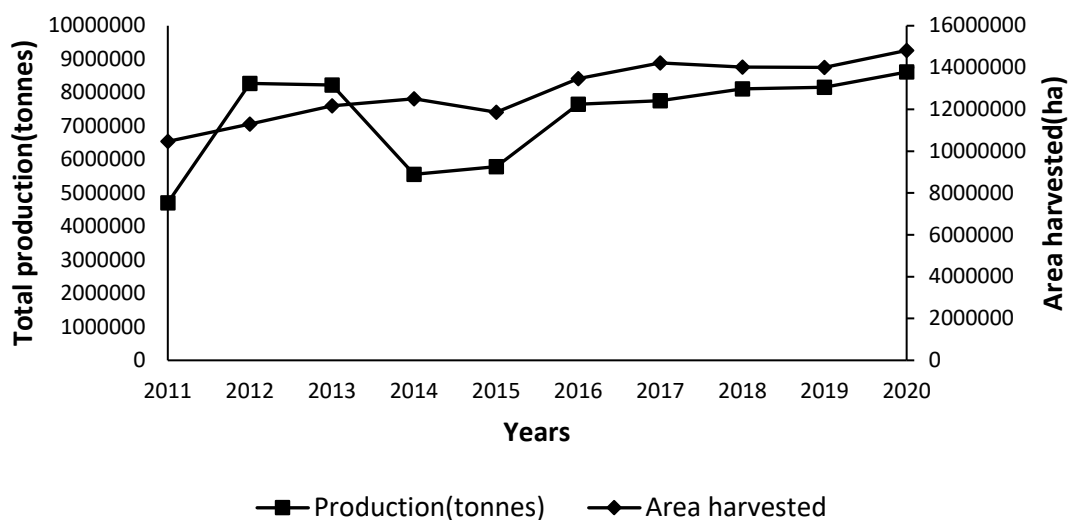
A significant inverse association between nutritional traits and grain yield was reported in cowpea breeding programmes, hindering simultaneous improvement of the crop for yield and quality (Asiwe, 2022). As a result, cowpea breeding programmes targeting jointly genetic improvement of yield and quality in cowpea are important to address food and nutrition security. Further, cowpea grains contain a proportion of anti-nutritional traits such as tannins, oxalic acid, protease inhibitor, alkaloids and polysaccharides (Ojwang *et al.*, 2013). The presence of anti-nutritional traits has been stressed out as a major challenge for the available nutrient's intake in cowpea (Onwuka, 2006). The anti-nutritional factors in cowpea are believed to reduce essential mineral bioavailability and food nutrient utilization, raising concerns about the possible effects on human health as they may lead to mineral deficiencies diseases such as anemia (Goncalves *et al.*, 2016; Simion, 2018). Breeding for low anti-nutritional quality traits is key in cowpea breeding programmes (Alghamdi *et al.*, 2019). Several approaches such as supplementation, fortification and more recently biofortification have been used to address malnutrition due to micronutrient and macronutrient deficiencies in people and food products (Bouis *et al.*, 2011). Biofortification is the plant breeding strategy implemented to improve food nutritional quality through increased nutrient density and availability (Singh and Pratap, 2016; Coelho *et al.*, 2021).

The development of high-yielding cultivars with superior agronomic yield and nutritional quality attributes in the face of biotic and abiotic production challenges is among key breeding objectives in cowpea improvement programmes. Major cowpea yield traits include grain yield, number of branches per plant, leaf length, leaf width, pod length, pod width, number of pods per plant, number of seeds per pod, grain yield and hundred-seed weight (Mofokeng *et al.*, 2020). Essential nutrients including iron, manganese, potassium, phosphorus, magnesium, sodium, iron, manganese, sulphur, zinc, copper, chloride, selenium and iodine and protein content are key to influence cowpea grain nutrition (Giami, 2005). Further, reduced concentration of key anti-nutritional traits such as tannins, protease inhibitors, polyphenols and phytates is important to enhance nutritional quality in cowpea (Alghamdi *et al.*, 2019). It is therefore necessary to advance cowpea productivity by targeting improved locally adapted and best performing cowpea varieties in terms of yield and nutritional status, for improved and sustainable production to ensure food and nutritional security. Therefore, the objective of this

review was to highlight breeding progress for improved yield and its component traits, nutritional quality and low anti-nutritional traits for bio-fortification in cowpea.

## 2.2 Production status of cowpea

Cowpea production worldwide is estimated at about 5.9 million tons annual production rate at harvested area of 15.1 million hectares in Africa, Asia, Southern United States and Latin America (FAOSTAT, 2020). The crop contributes about 95% of the world production and Africa produces over 83% of the world's cowpeas, with West Africa accounting for approximately 80% of total production (Boukar *et al.*, 2016; Barry, 2016). West and Central Africa are the leading cowpea producing regions in the world. Furthermore, FAOSTAT (2020) reported that the cowpea production in Africa is most dominant in Nigeria, Niger, Burkina Faso, Kenya, Mali, Cameroon and United Republic of Tanzania, with a total production of 3.6, 2.4, 0.65, 0.25, 0.25, 0.22 and 0.13 tonnes per hectare, respectively. Over the previous three decades, global cowpea production has increased by an average of 5% annually, with annual area growth of 3.5% and yield growth of 1.5 percent, with area expansion accounting for 70% of total growth (Fatokun *et al.*, 2012; Boukar *et al.*, 2016). The highest production of cowpea in Africa was obtained in 2012 and 2013 with 8 277 360 and 8 230 574 tonnes, respectively, and the decline was observed during the year 2014 and 2016, then in 2016 the production increased until 2020 where the highest total production of 8 616 095 was obtained over a cultivated area of 14 818 944 hectares of land (Figure 2.1).



**Figure 2.1:** Total annual production over harvested area of cowpea from 2011 to 2020 in Africa (FAOSTAT, 2022)

In SSA, the subsistence and smallholder farmers are the main cowpea consumers and producers. In Sub-Saharan Africa, the crop is cultivated over 14.2 million hectares of land with an annual production rate of 8.6 million tonnes under rainfed conditions. The crop is a major cultivated legume crop of commercial importance in Sub-Saharan Africa especially in West-Africa regarding the area harvested and the production proportion. While the crop is cultivated worldwide, but the production attributes vary by agro ecological zone within the producing countries (Kormawa *et al.*, 2002). Korwama *et al.* (2000) further stated that among the aspects that determine the production of cowpea at national levels at West Africa are the cost input (price/unit) for cowpea production and technology level agro ecological adaptation and government policies. The increase in production largely depends on the area cultivated. The increase in yield harvested also determines the production for the subsequent season.

Cowpea production greatly boost the local trade in various developing countries where it is produced, hence it is a source of economic strength and employment in many families. The crop is a staple element of the diet in several under-developed and developing areas and a major source of proteins, alternately for costly animal protein (Gbaye and Holloway, 2011). In the past four decades, cowpea production has been on the increase, but it has never met the potential yields of the crop in Sub-Saharan Africa. A considerable gap remains the challenge between the actual and potential yield. The cowpea mean grain yields in the SSA is between 100 to 599 Kg/ha, which is way less than the expected potential yield of the crop accomplishing 3tons/ha (1500 to 3000 kg/ha) (Gerrano *et al.*, 2019). Horn and Shimelis (2020) states that the yield gap is associate to a lack of improved varieties, poor agronomic managements and other abiotic and biotic stresses. In addition, Gerrano *et al.* (2019) further stated that the major constraints that limit the production of cowpea in South Africa are low productivity of the crop available at the farmer's level, current moisture stress, absence of improved high yielding and nutritional value genotypes, low fertility of the soil, losses of yield due to insect pests and disease.

### **2.3 Constraints of cowpea production**

There are wide arrays of biotic and abiotic stresses that have major contribution to the reduction of cowpea yield in Sub-Saharan Africa. The biotic stresses includes fungal, viral, and bacterial diseases, parasitic weeds and insects pests (Gomes *et al.*, 2019) while the abiotic stresses includes poor soil fertility, heat and drought stress. There are more than 20 viruses that have reported to have a major contribution to viral diseases that had been identified as having negative impacts on cowpea productivity (Horn and Shimelis, 2020). The common viral

diseases in cowpea includes cowpea aphid-borne mosaic virus (CABMV), cowpea severe mosaic virus (CSMV) and cowpea mild mottle virus (CPMMV) (Mbeyangala et al., 2014; Orawu et al., 2005). Amayo et al. (2012) reported that these virus can contribute about 90% yield losses and in some cases, to the loss of total canopy.

The main fungal disease of cowpea includes leaf smut caused by *Protomyces phaseoli* (Bailey et al., 1990), anthracnose caused by *Collectrichum* and stem blight caused by *Macrophomina phaseolina* (Boukar et al., 2016). Mbeyangala et al. (2014) reported that fungal diseases can contribute to 20-100% yield losses. The most prevalent bacterial diseases include bacterial blight caused by *Xanthomonas campestris* pv. *vignicola* and bacterial blight caused by *Xanthomonas campestris* pv. *vignaeguilalae* (Horn and Shimelis, 2020; Boukar et al., 2016). These bacterial pathogens can cause yield losses of up to 71%, 68% and 53% in pods, seed and fodders, respectively, in varieties that are susceptible (Viswanatha et al., 2011). The plant-parasitic nematode that is most prevalent in cowpea field is root-knot nematode (*Meloidogone* spp.) (Oliviera et al., 2012). These nematodes cause major loss in cowpea productivity as they damage root systems thereby hindering water and nutrients uptake (Boukar et al., 2016; Haegeman et al., 2012).

Parasitic weeds including *Striga gesneroides* and *Alectra vogelli* are two main parasitic weeds that can cause a significant damage in cowpea production in Sub-Saharan Africa (Horn and Shimelis, 2020). These weeds spread and attack the host's root surface where they absorb nutrients and causing noticeable losses of yield. In the case of insect pests, aphids (*Aphis craccivora*) in seedling stage, bruchids weevils (*Callosobruchus maculatus*) in grains, maruca (*Maruca vitrata*) as pod and shoot borers, beetles (*Oothecha mutabilis*), leaf hoppers and foliage beetles have been reported to be the major field pest causing damage in cowpea plant (Boukar et al., 2016; Ngakou et al., 2008; Gbaguidi et al., 2013). Horn and Shimelis (2020) stated that the damage caused by weevils in cowpea grains may lead to grain losses of up to 100% if not controlled. Poor soil fertility, heat and drought stress are the major abiotic constraints affecting cowpea productivity in Sub-Saharan Africa.



**Figure 2.2:** Cowpea grains infested by bruchid weevils harvested during the 2019/2020 planting season.

Currently, extreme temperature and drought are the most prominent abiotic stresses restricting the cowpea to meet their potential production, resulting to low grain yield and quality of up to 60% (Hempton *et al.*, 2016). Although cowpea is known to have good drought tolerance, but yield can be significantly reduced when subjected during vegetative and flowering stage (Boukar *et al.*, 2016). The agricultural systems in Sub-Saharan Africa which are rainfed are highly susceptible to rainfall variability. As a results, Fatokun *et al.* (2012) elaborated the importance of breeders and farmers to work jointly to identify and use early maturing cowpea varieties with the improved potential to escape terminal drought stress.

Heat stress beyond a threshold temperature of 16 °C can reduce the setting of pods, thus reducing the grain yield by 4-14%, depending on the varieties (Hall, 2004). According to Boukar *et al.* (2016), high temperatures at night may lead to abortion of flowers resulting in failure for pod formation, consequently reducing grain yield. Poor soil fertility is also a major challenging in cowpea production in Sub-Saharan Africa. Boukar *et al.* (2016) alluded that soils that are deficient in phosphorus which is an element for nitrogen fixation in root nodules of legumes may drastically lower the production of cowpea. Despite that cowpea is a nitrogen fixing crop, it may be supplemented by applying organic matter such as manure and use of synthetic fertilisers as per the recommendations (Kimiti and Odee, 2010). It is important to

promote cowpea varieties with enhanced tolerance to poor nutrient availability to improve productivity in Sub-Saharan Africa. There is a need for an exploitation of genetic diversity for development of varieties that are resistant to abiotic and biotic stresses (Lusser *et al.*, 2012).

## **2.4 Breeding progress for enhanced yield and nutrition quality in cowpea**

### **2.4.1 Grain yield productivity**

To mitigate the devastation caused by wide arrays of cowpea production constraints, breeding programs are using both molecular and conventional breeding to develop improved lines with high grain yield potential, improved nutrition and adaptation to major production agro-ecologies to meet the consumer and producer-preferred traits (Boukar *et al.*, 2016). The International Institute of Tropical Agriculture (IITA) has been the leading breeding program by providing germplasm for genetic variation used in developing varieties with enhanced nutrition and increased yield.

The improvement in yield is the most significant traits in cowpea breeding programs. Grain yield is a complex quantitative trait controlled by genotype, environment and their interactions and various genetic factors (Sail *et al.*, 2007; Owusu *et al.*, 2021). During the evaluation of genotypes for improved yield productions, the yield related traits including number of days to flowering, number of days to maturity, number of productive branches, plant height, leaf length, leaf width, number of pods per plant, pod length and width, number of seeds per pod, hundred seed weight and grain yield are considered in selection for yield improvement (Gerrano *et al.*, 2022; Edematie *et al.*, 2021). These yield components are often correlated and selecting for one can lead to either negative or positive response on the other. For instance, Positive and strong correlation have been reported between grain yield and pod length in the studies by Manngoel *et al.* (2012). Similarly, Owusu *et al.* (2021) also reported a positive correlation between number of pods per plant, number of seeds per plant and pod weight. This suggests that selection for one of the positively correlated traits indirectly results to the improvement of other traits.

In the study by Ezeaku *et al.* (2015) evaluating the effects of planting date and cultivar on growth and yield of cowpea, the results showed the significant differences in the performances of the cultivars when planted in diverse environments. The results showed that cultivar IT-98K-131-2 had the mean grain yield 1220 kg ha<sup>-1</sup> in early planting date and 732 kg ha<sup>-1</sup> in late season planting in Ishiagu, where as in Mgbakwu, the cultivar IT-98K-131-2 produced 921 and 326 kg ha<sup>-1</sup> in early and late planting dates, respectively. In the same study, cultivar IT-97K-556-4 produced the mean grain yield of 1154 and 424 kg ha<sup>-1</sup> in early and late planting dates,

respectively, in Ishiagu, whereas Mgbakwu, the mean grain yield produced were 1594 and 251 kg ha<sup>-1</sup> for early and late planting dates (Ezeaku *et al.*, 2015). Further, a brief list of varieties that has been reported by different researchers based on their performance on certain trait is presented in Table 2.

#### **2.4.2 Nutritional quality**

Cowpea is considered as a superior source of health-promoting components such as soluble and insoluble dietary fiber, proteins essential minerals and many other functional compounds which includes vitamin B (Mudryj *et al.*, 2014). Nutrients in the human diets provided by cowpea can be classified as macronutrients (regarded as the primary energy intake including carbohydrates, total fats, and proteins), micronutrients including essential minerals, vitamins and dietary fibre (Awika and Duodu, 2017) which are required for perform normal physiological function (Badiane *et al.*, 2012). The nutritionally improved varieties provide significant amounts of bioavailable nutrients with greater potential to mitigate the deficiencies in nutrients among developing and developed communities. Therefore, plant derived mineral and protein nutrition is the cheapest alternative to fight malnutrition that is prevalent in Sub Saharan Africa.

Cowpea is a good source of essential minerals including calcium (Ca), copper (Cu), iron(Fe), potassium(K), magnesium(Mg), manganese(Mn), sodium(Na), phosphorus(P) and zinc(Zn) which are very essential for human health, growth and development (Mbuma et al., 2021). The intake of micro-essential minerals is mandatory for humans to meet the metabolic needs and hence avoid an array health problems associated with deficiencies of essential minerals (Santos and Boiteux, 2013) . Cowpea seeds and leaves are rich in potassium with content ranging from 1.9 to 28.9 kg<sup>-1</sup> (Goncalves et al., 2016) with good amount of magnesium, calcium, phosphorus and contains a small amount of iron, sodium, selenium, copper, zinc and manganese (da Silva et al., 2018). In the evaluation of proteins and mineral content (Fe, Zn, Mg and K) in grains by (Boukar *et al.*,2011) whereby a set of 1542 cowpea germplasm lines were evaluated. The results showed that the varieties rich in grain proteins included TVu 10425, TVu 2822, TVu 16531, TVu 450 and TVu 16616 with the grain protein of 32.2 %, 31.8 %, 31.3 %, 31.1 % and 31.0 % respectively. The following lines shown the high levels of iron (Fe); TVu 2723, TVu 14878, TVu 2852, TVu 526, TVu 10342 with the iron content of 79.5 mg/kg, 79.5 mg/kg, 78.7 mg/kg, 78.1 mg/kg,77.0 mg/kg respectively, and lastly the lines which showed the high content of zinc included TVu 10342, TVu1732, TVu 9576, TVu2651 and TVu 14877 with the zinc content of 58.0 mg/kg, 56.1 mg/kg, 55.3 mg/kg, 54.5 mg/kg, 54.0 mg/kg. It is highly noticeable

that the breeding lines which showed the high content of iron also showed the high content of protein and zinc. In addition, a total of 240 genotypes were screened by Muranaka *et al.* (2016) and reported a positive correlation between crude protein and iron and zinc in cowpea grains. Furthermore, Gondwe *et al.* (2019) reported a positive correlation relationship between protein and calcium. This indicates that the protein content increases as the iron, zinc and calcium content increases and they are all essential minerals for human health. Therefore, these minerals could be selected for breeding programmes at the same time in order to generate nutrient dense enhanced cultivars of cowpea. Table 2.1 shows some of the genotypes with improved nutrition, yield and yield related traits released globally.

**Table 2.1:** Traits specific genotypes reported globally.

Traits	Genotype name	Country	References
High grain yield	IT99K-494-6	Malawi	Kabambe et al.,2014
High Protein	Tvu2506	Nigeria	Boukar et al.,2011
High number of pods per plant	EC725164, EC723741, EC723971, EC725180	India	Rana et al., 2016
High pod length	EC723681, EC725162, EC724327, EC724045, EC724536	India	Rana et al.,2016
High number of seeds per pod	EC725164, EC723741, EC723971, EC725180	India	Rana et al., 2016
High protein and mineral content	IT97K-1042-3 IT99K-216-1 IT97K-499	Brazil	Singh,2007
Early maturing, High seed yield, High protein	Sakha-1	Egypt	Metwally et al.,2021
High seed yield and quality	Kafr El Sheikh-1 Kaha-1	Egypt	Metwally et al.,2021
High grain yield	IT98K-205-81 KVx421-25, KVx442-3-25 KVx771-10, KVx735-33-2	Burkina Faso	INERA,2012

### 2.4.3 Anti-nutritional traits

Cowpea provides a significant amount of proteins, calories, vitamins and essential minerals to the daily diets of many countries. However, there are anti-nutritional factors present in cowpea such as phenolic compounds, tannins, protease inhibitors and phytates which are the major limiting factors to the consumption of cowpea (Ileke, 2004; Towo and kamala, 2003). Anti-nutrients are secondary metabolites produced by plants that antagonize and lowers the nutritional quality of food by inhibiting mineral bioavailability and digestibility of key essential minerals, rendering them unavailable to cells when consumed (Maina *et al.*, 2015). Preet and Punia (2000) reported that the crop had high amounts of phenolic compounds which plays a major role in lowering the protein and starch digestibility. Further, phytic and oxalic acids both found in cowpea also minimise the bioavailability of essential minerals resulting in mineral deficiency illnesses such as anaemia or either form detrimental complexes with metal ions such as calcium-oxalate which causes kidney failure in humans (Salawu *et al.*, 2014; Simion,2018). Additionally, (Seerama *et al.*, 2012; Towo and Kamala, 2003) also stated that condensed tannins have also been disclosed to reduced iron availability and the hard-to-cook phenomena. Nevertheless, anti-nutrients have been reported to have pharmacological benefits (Simion.2018). For instance, tannins possess anti-cancer properties and phytic acids are known to prevent colon cancer (Simion, 2018).

Breeding for the reduction of these inhibitors is important to the level where the nutrients are readily available for absorption in the body. There are many techniques that have been reported to be effective in elimination or minimising the chemicals including autoclaving, fermentation, toasting, soaking, dehulling and cooking (Jayathilake *et al.*, 2018; Alghamdi *et al.*, 2019). The study by Alghamdi *et al.* (2019) reported the total phenols, trypsin inhibitor, tannins and phytic content to range between 0.32- 0.81 mg g<sup>-1</sup>, 2.07- 3.13 mg g<sup>-1</sup>, 0.19- 0.43 mg g<sup>-1</sup> and 2.33- 3.27 mg g<sup>-1</sup>, respectively. In addition, Ojwang *et al.* (2013) also reported the proanthocyanidin content of cowpea to range between 2.2 – 6.3 mg g<sup>-1</sup> which is believed to be similar to other legumes such as beans, peas and lentil. There is a possibility in future of breeding for anti-nutrients to be completely eliminated through genetic engineering, but because there are molecules may also have good beneficial, so such genetic alterations could make food crops more nutritious without improving other aspects of human health (Simion, 2018).

### 2.5 Genetic resources of cowpea

The IITA (International Institute of Tropical Agriculture) has been the driving force for most breeding programs by providing cowpea germplasm collection for genetic variation for various

national and international institutions. It maintains about 15 000 accessions of cultivated cowpeas and more than 2000 wild relatives in its gene bank (Boukar *et al.*,2016).There are more than 20 breeding lines which were released by IITA in 10 countries from 2005 to 2016 (Table 3).In South Africa, Gerrano *et al.* (2022) reported that Agricultural Research council has been the major source of cowpea germplasm collection. It maintains about 1200 collections which are sourced from the various international and national field collections and some imported from the IITA and World Vegetable center for research purposes and development for increased genetic variations in the country (Gerrano *et al.*, 2022). Additionally, the first eight-parent cowpea multi-parent advanced generation intercross (MAGIC) population selected based on the abiotic and biotic tolerance was created recently as an additional resource for trait discovery and breeding within the genomic community as the cowpea minicore collection (Huynh *et al.*, 2016; Boukar *et al.*, 2019).

**Table 2.2:** List of cowpea varieties released from 2005 to 2016 in Sub-Saharan Africa by IITA

Year of Release	Variety	Country
2005	IT93-452-1, IT90K-277-2	Nigeria
2008	IT97K-499-5	Nigeria
2009	IT89KD-288, IT89KD-391	Nigeria
	IT97K-499-35, IT97K-499-38, IT98K-205-8	Niger
2010	IT97K-499-35, IT93K-876-30	Mali
	IT99K-573-1-1	Niger
2011	IT82E-16, IT00K-1263, IT97K-1069-6	Mozambique
	IT99K-494-6	Malawi
	IT99K-573-1-1, IT99K-573-1-1	Nigeria
2012	IT99K-7-21-2-2-1, IT99K-573-1-1	Tanzania
2013	IT99K-573-2-1, IT98K-205-8	Burkina Faso
	IT95K-193-12	Benin
2015	IT00-1263, IT99K-1122	Tanzania
	IT07K-292-10, IT07K-318-33	Nigeria
	IT83E-16, IT-82E-18, IT05K-321-2, IT97K-390-2	Swaziland
	IT82E-16, IT82E-18, IT99K-494-4	
	IT99K-573-1-1, IT99K-573-2-1	Sierra Leone
2016	IT90K-277-2, IT07K-211-1-8	South Sudan
	IT99K-573-2-1, IT99K-573-1-1	Ghana

Source: Boukar *et al.* (2016).

## 2.6 Genetic diversity analysis of cowpea

Genetic diversity can be assessed using different methods that indicates the level of diversity among genotypes. It can be carried out using agro-morphological or phenotypic markers and

molecular markers (Govindaraj *et al.*, 2015) which can be used to identify agro-morphological traits and molecular diversity analysis, respectively (Kassa *et al.*, 2012; Beyene *et al.*, 2005). The use of phenotypic markers is a common approach as it is easy and inexpensive, but it requires more labour and land as it requires the trial establishment. The major challenge about this method is that the traits are highly influenced by environment in the field and that may hinder the real genetic variation among genotypes (Horn and Shimelis, 2020) and consequently, reduces the accuracy of the results obtained. Hence, the emergence of molecular markers for effective genetic diversity analysis (Sejake *et al.*, 2021). Molecular markers are not affected by the effects exerted by environments because the genetic diversity is reviewed at the genomic DNA level which makes it possible to visualise the precise genetic variations among genotypes (Cui *et al.*, 2017). Nevertheless, they may not be associated with any agronomic traits, therefore needs to be supplemented with morphological markers to presume a meaningful conclusion (Gumede *et al.*, 2022). For instance, Nkhoma *et al.* (2020) in Zambia used both morphological characters and SNP markers for genetic diversity analysis of 90 cowpea genotypes.

The molecular marker techniques including restriction fragment length polymorphism (RFLP), Simple sequence repeats (SSR; Dagnon *et al.*, 2022), single nucleotide polymorphism (SNP; Gumede *et al.*, 2022), random amplified polymorphic DNA (RAPD; Ghodake *et al.*, 2023) and amplified fragment length polymorphism (AFLP; Fang *et al.*, 2007) has been successfully applied in plant genetic analysis of cowpea (Horn *et al.*, 2018). However, the modern biotechnology has opted for SNPs mainly because the SNPs have high genomic abundance, locus-specific and lower genotyping error rates (Kassa *et al.*, 2012). Single nucleotide polymorphism (SNP) has appeared as the powerful tool in the studies of genetic diversity as compared to other molecular markers such as AFLP and SSR (Varshney and Tuberosa, 2007). Deulvot *et al.* (2010) states that this is because the SNPs are abundant in the genomes of plants and other organisms. There are various studies of genetic diversity analysis that have been done on cowpea using different molecular markers in different countries (Table 2.3)

**Table 2.3:** Reported marker systems and genetic analysis in cowpea in different countries.

Marker type	No. of markers	No. of genotypes	Country	References
SNP	14116	90	Zambia	Nkhoma <i>et al.</i> ,2020
SNP	5864	90	South Africa	Gumede <i>et al.</i> , 2022
SNP	6498	357	Ethiopia	Ketema <i>et al.</i> , 2020
SSR	28	70	Togo	Dagnon <i>et al.</i> , 2022
SSR	8	32	India	Vinay <i>et al.</i> , 2022
RAPD	10	8	India	Ghodake <i>et al.</i> , 2023
RAPD	28	56	Kenya	Ba <i>et al.</i> , 2004
SNP	5828	768	United States of America	Xiong <i>et al.</i> , 2016
SSR	22	32	Iran	Mafakheri <i>et al.</i> , 2017
SSR	23	81	Ethiopia	Desalegne <i>et al.</i> , 2016
RAPD	18	17	India	Malviya <i>et al.</i> , 2012
SSR	20	87	Greece	Zafeiriou <i>et al.</i> , 2023
SNP	5147	188	Niger	Abdoulaye <i>et al.</i> , 2023

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RAPD	20	87	United States of America	Patil <i>et al.</i> , 2013
SSR	20	141	Ghana	Asare <i>et al.</i> , 2010

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Notes; SSR= simple sequence repeat, AFLP= amplified fragment length polymorphism, SNP= single nucleotide polymorphism

## 2.7 Genotype-by environment interaction analysis

The use of appropriate statistical methods is essential for plant breeding programs with the aid to select superior varieties with good performance under different environmental conditions. Although there are several factors that are known to influence genotype, selection for adoption but the yield potential and nutritional composition are the most important factors (Mbeyangala *et al.*, 2021). The varieties that display stability and good performance from year to year when subjected under certain environmental are desirable to farmers as opposed to varieties with consistent performance location to location (Kang, 2002). To assess the stability of genotypes, multi-environmental trials (MET) are conducted over the years to detect and explain the interaction mechanism between genotype, environment enabling the selection of high performing genotypes with good stability and well adapted from cultivar development (Alvarado *et al.*, 2022).

The genotype-by-environment interaction (GEI) refers to distinctive response of different genotypes across various environmental conditions (Kang, 2004). The analysis of interaction of genotype with environment is important as it allows the selection and recommendation of superior genotypes for large-scale production. There are several statistical techniques that are used to assess the GEI which includes the additive main effects and multiplicative interaction (AMMI) analysis and genotypes plus genotypes by environments interaction (GGE) biplot analysis (Yan *et al.*, 2007; Yan and Tinker, 2006). The AMMI analysis is used on breeding programs with the purpose of determining genotype (G), environment (E) and their interaction on key traits for effective selection whereas the GGE biplot is useful identifying and selecting the superior and stable genotypes and depict mega-environments to allow selection for better breeding strategies for cultivar development and improvement programmes (Yan *et al.*, 2000; Yan and Kang, 2003; Yan and Tinker, 2004; Yan *et al.*, 2007). In cowpea, the large effects of genotypes by environment were reported in recent studies (Gumede *et al.*, 2022; Iseki *et al.*, 2021; Gerrano *et al.*, 2020) and further used the analysis to identify high-yielding genotypes with narrow and wide adaptation for yield production. Further, the effect of genotype by environment interaction was reported on the concentration of mineral elements in cowpea (Tulu *et al.*, 2023; Guldiken *et al.*, 2021; Gerrano *et al.*, 2019). For instance, in the study by Gerrano *et al.* (2019) whereby there was an assessment of grain minerals and total protein content on selected cowpea genotypes planted Gauteng and Limpopo, it was reported that the concentration of calcium was significantly influenced by genotype, location and its interaction and varied from 0.07 to 0.16 mg kg<sup>-1</sup> with an average of 0.11 mg kg<sup>-1</sup>. This was due to the

variation of the genotypic make up as well as the environmental conditions in Gauteng and Limpopo.

## **2.8 Combining ability and gene action analysis for yield and yield related traits in cowpea.**

The successful advancement of varieties that have potentials to produce maximum yields requires the screening of available genetic materials to select suitable genetic resources for improved yield productivity (Abaza *et al.*, 2020; Mansour *et al.*, 2018). Hence the need to understand the nature of inheritance for economically important traits such as grain yield and yield components for the selection of suitable strategies during breeding. Combining ability is described as the potential of the parental lines to produce the hybrids possessing superior traits (Romanus *et al.*, 2008; Owusu *et al.*, 2018). It is broadly categorized into two categories namely the general combining ability (GCA) which refers to the additive gene effects used to determine the average performance of a parent in hybrid combinations while specific combining ability (SCA) refers to the dominant gene effects used to identify the performance of certain cross combinations (hybrid) under specific terms (Deepak, 2014; Kamara *et al.*, 2021). The combining ability analysis plays a vital role in identification of parents with high GCA and parental combinations with high SCA (Owusu *et al.*, 2018).

The proportions of SCA and GCA effects obtained by combining ability analysis are used to identify the gene action responsible in expression of agronomic characters (Dholariya *et al.*, 2014). The higher GCA effects indicates a greater role of additive gene effects controlling these characters whereas SCA indicate the high dominance gene effects (Mwadzingeni *et al.*, 2018). The progenies that exhibit good SCA effects are recommended for genetic enhancement to generate stable pure line varieties in autogamous crops such as cowpea (Nkhoma *et al.*, 2020). The estimation of additive and non-additive gene action could be useful in determination of the possibility of commercial exploitation of heterosis and isolation of pure line among to progenies of the good hybrid.

In cowpea, previous studies by (Romanus *et al.*, 2008; Mukati *et al.*, 2014; Wankhande *et al.*, 2016; Pallavi *et al.*, 2018; Owusu *et al.*, 2018) used GCA and SCA gene effects to identify the parents and promising progenies to recommend in the improvements of cowpea grain yield and yield related traits. For instance, Owusu *et al.* (2018) reported the importance of both additive and non-additive gene effects in the expression of traits with the non-additive gene effects being the most important in expression of traits such as grain yield, number of seeds per pod and pod weight. Similarly, Romanus *et al.* (2008) also reported both additive and non-additive

gene effects being important in the expression of characters, however, additive gene effects was reported to be important in the expression of all the characters except pod number. The knowledge of combining ability and gene action would assist cowpea breeders to identify potential parents, breeding strategies suitable for certain cowpea varieties and the selection of superior genotypes from the segregating populations to enhance cowpea productivity. It is very important to understand the gene action of different cowpea genotypes in several agronomic characteristics as influenced by different environmental conditions.

## **2.9 Future prospects**

Cowpea being a highly nutritious staple crop with good adaptability to adverse environmental conditions, its ability to improve soil fertility, makes it a noble economic crop in Sub-Saharan Africa and globally. With the drastic increase in human population in the world which leads to a high demand for increased agricultural productivity. Although, cowpea production is increasing in Africa because of its nutritional value and its importance in agricultural systems, but the yields of cowpea are quite below that the potential yield expected due to biotic and abiotic stresses which are considered the major limiting factors to the crop's production potential (Horn and Shimelis, 2020). Further, the use of cowpea genotypes with poor adaptability and low yields potentials also contributes to yield losses (Horn and Shimelis, 2020). Significant breeding efforts have obtained exceptional achievements in cowpea improvement to address limitations in cowpea production (Asiwe, 2022). There is still a need to develop and select available germplasm for enhanced yield and nutrition in cowpea. Kebede *et al.* (2020) alluded that researchers and plant breeders should make efforts in identifying cowpea varieties with enhanced nutritional content and yield potentials, tolerance to drought and heat stress and other desirable traits.

Improvement of grain yield and nutritional quality is the main goal for breeding programs in cowpea improvement. Biofortification has now been recognized as a viable tool in plant breeding for improving nutritional quality of food by elimination anti-nutritional traits with the aid of increasing the nutrient content availability and accessibility (Singh *et al.*, 2016; Iqbal *et al.*, 2006). The cowpea breeding program in Brazilian Agricultural Research Corporation produced biofortified cowpea seeds for iron and zinc. The results showed that the biofortified cowpea varieties have exhibited a high content of iron and zinc compared to conventional varieties for both micronutrients (Freire-Filho, 2011). Similarly, Coelho *et al.* (2021) also examined the bioaccessibility and bioavailability of iron and zinc in biofortified cowpea seeds. The results also revealed biofortified cowpea had high iron and zinc levels reaching 50%

bioaccessibility and 44.2% bioavailability. Biofortification has been proven to be inexpensive, sustainable and efficient in providing micronutrients for disadvantaged communities complementing conventional interventions (Bouis *et al.*, 2011; Haas *et al.*, 2016). Therefore, adoption of biofortification to enhance nutritional quality in cowpea for poor communities in Africa could be an economic and convenient strategy.

The development of innovative genetic tools such as DNA molecular markers, dense genetic maps and whole-genome transcription profiling approaches to discover genomic regions and genes has made a remarkable progress in recent years (Millan *et al.*, 2006). The combination of conventional breeding with molecular breeding approaches can accelerate development and deployment of improved varieties thus improving cowpea productivity in Sub-Saharan Africa. Several DNA based markers including simple sequence repeats (SSR) (Xu *et al.*, 2010; Mafakheri *et al.*, 2017), single nucleotide sequence (SNP) (Nkhoma *et al.*, 2020), restriction fragment length polymorphism (RFLP) (Fatokun *et al.*, 1993), amplified fragment length polymorphism (AFLP) (Coulibaly *et al.*, 2002) and diversity array technology (DArT) (Oliviera *et al.*, 2020). The study by Coulibaly *et al.* (2002) used AFLP markers in 117 cowpea accessions to assess the genetic relationship between wild and domesticated cowpeas. The study showed that the wild cowpea was more diverse than the domesticate one.

The assessment by Mafakheri *et al.* (2017) examined genetic diversity in 32 cowpea genotypes using a set of 22 SSR's primer pairs and the results of the study detected 186 alleles with an average of 2 allele for each locus and the genetic distance between genotypes was estimated at 0.0066. Moreover, SNP markers have also been used to study genetic diversity and marker-traits association in cowpea (Nkhoma *et al.*, 2020; Fatokun *et al.*, 2018; Seo *et al.*, 2020). SNP markers have recently gained an increasing importance due to their low cost, higher frequency in the genome than SSR's and other markers, co-dominance and low genotyping error rates (Carvalho *et al.*, 2016). Mapping approaches which include quantitative trait locus (QTL) has enabled marker-assisted breeding programs to accurately dissect functional genes for cultivar development (Pottorff *et al.*, 2012). Evidently, the QTL analysis and physical mapping in leaf morphology of cowpea recombinant inbred lines (RIL) population using SNP markers was reported by Pottorff *et al.* (2012). In addition, mapping of QTL for grain yield components based on DArT markers across cowpea and yard-long bean was reported by Oliviera *et al.* (2020). Specific molecular markers could be used to select target traits with marker assisted selection (Badiane *et al.*, 2014). Therefore, the biotechnology based on genetic maps and the use of DNA markers may be the useful tool for efficient cowpea breeding.

## 2.10 Conclusion

Cowpea as a legume crop is a key crop to overcome food insecurity in SSA due to its high nutrients dense and its ability to withstand harsh environments. The yield gaps in cowpea are associated with the use of poor agronomic practices and unimproved cultivars. It is of high necessity to breed for biofortified cowpea varieties for enhanced nutritional status and improved agronomic practises for improvement of production has been proved to be an important strategy to address malnutrition and food security while improving agricultural practices in Sub-Saharan Africa. Several breeding techniques can be used to boost up the breeding of cowpea such as conventional breeding, genomic selection and molecular markers. These breeding techniques will assist to develop new locally adapted varieties that has the ability to withstand biotic and abiotic stresses and to develop farmers preferred varieties in order to meets the market demands in Africa.

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### **CHAPTER 3: INFLUENCE OF GENOTYPE AND ENVIRONMENT ON GRAIN YIELD AMONG COWPEA (*Vigna unguiculata* (L.) Walp) GENOTYPES UNDER DRY LAND FARMING SYSTEMS.**

#### **Abstract**

The identification of high-yielding and stable genotypes for cultivation across differential production regions is among key breeding objectives in cowpea improvement programs. The objective of this study was to determine the influence of genotype (G), environment (E) and their interaction (GEI) effects on grain yield in cowpea to select ideal genotypes with suitable adaptation for production in South Africa and identical agro-ecologies, and for cultivar design and development. Fifty cowpea genotypes were tested for grain yield across seven environments of South Africa using a 10 x 5 alpha lattice design replicated three times, during the 2019/2020 and 2020/2021 planting seasons. Grain yield data was subjected to analysis of variance (ANOVA), additive main effects and multiplicative interaction (AMMI) and the genotype-by-environment interaction (GGE) biplot analyses. ANOVA and AMMI analyses showed significant GEI effect which accounted for 57 % variation, whereas genotype and environment main effects accounted for 29% and 13% variation, respectively. The significant grain yield mean values were recorded for genotypes G35 (0.47 t ha<sup>-1</sup>), G1 (0.45 t ha<sup>-1</sup>), and G47 (0.43 t ha<sup>-1</sup>) across all test environments. Based on AMMI stability values (ASV), Acc-Cowp44 was identified as the most stable genotype across all sites, recording the lowest ASV of 0.03. The comparison view of GGE biplot revealed Acc-Cowp29, Acc-Cowp38 and Acc-Cowp5 as ideal genotypes, in that order, possessing high grain yield of 0.19 t ha<sup>-1</sup>, 0.47 t ha<sup>-1</sup>, and 0.36 t ha<sup>-1</sup>, respectively. The identified genotypes are recommended for production and inclusion in subsequent breeding activities aiming to enhance genetic gain in cowpea yield.

**Key words:** AMMI, ASV, GEI, GGE biplot, stability, *Vigna unguiculata*, yield

### 3.1 Introduction

Cowpea (*Vigna unguiculata* (L) Walp), a Fabaceae, is a widely cultivated vegetable and forage crop in the tropical and subtropical agro-ecologies of the world (Awurum *et al.*, 2013; Alemu *et al.*, 2016; Odeseye *et al.*, 2018; Owade *et al.* 2020). Globally, ~8.9 million metric tonnes of cowpea grains are produced annually on about 14.4 million hectares of land (FAOSTAT, 2020). In 2019, ~95% of total global cowpea production was in West Africa, with Nigeria as a leading producer (Herniter *et al.*, 2020). The crop is ranked the second after groundnuts (*Arachis hypogaea*) among the most cultivated legume crops in Africa in terms of total production (FAOSTAT, 2020). The vegetable crop is cultivated for its fresh leaves, green pods and grain which are sources of protein, macro and micro-nutrients required for human nutrition (Gerrano *et al.*, 2017; Martos-Fuertes *et al.*, 2017; da Silva *et al.*, 2018; Gerrano *et al.*, 2019; ElMasry *et al.*, 2021). Nutritionally, cowpea seed contains about 50-66% carbohydrate, 9-14% starch, 28.9-35% protein and 0.6-3.7% fat (Giami *et al.*, 2005; Rengadu *et al.*, 2020a, b). Gerrano *et al.* (2017) also reported the significant quantities of micronutrients found in cowpea grain including calcium (0.11 mg kg<sup>-1</sup>), copper (5.85 mg kg<sup>-1</sup>), iron (74.64 mg kg<sup>-1</sup>), potassium (13247 mg kg<sup>-1</sup>), magnesium (2064 mg kg<sup>-1</sup>), manganese (11.68 mg kg<sup>-1</sup>), sodium (361.60 mg kg<sup>-1</sup>), phosphorus (5315.00 mg kg<sup>-1</sup>) and zinc (40.16 mg kg<sup>-1</sup>).

Cowpea shares considerable tolerance to most abiotic stresses including drought and heat stress, salinity, and poor soil nitrogen status (Daryanto *et al.*, 2015; Meena *et al.*, 2015; Sousa *et al.*, 2018; Nkoana *et al.*, 2019; Gerrano *et al.*, 2020). Further, the crop is resistant to major biotic stress factors including weeds (*Striga gesnerioides* (Willd.) Vatke and *Alectra vogelli* Benth), and animal pests such as bruchids, beetles and leaf hoppers (Horn and Shimelis, 2020). In addition, the crop possesses tolerance to viral (for example: cowpea aphid borne mosaic, southern bean mosaic sobemovirus and mottle virus), bacterial (for example: bacterial blight caused by *Xanthomonas campestris* pv. *vignaunguiculatae*) and fungal (for example: leaf smut, stem and root rot) pathogens (Horn and Shimelis, 2020; Mbeyagala *et al.*, 2014; Viswanatha *et al.*, 2011). The nutritional quality and environmental stress tolerance attributes make cowpea a vital food and nutrition security suitable for cultivation using low-input agricultural production systems (Gerrano *et al.*, 2017).

Despite the significant role of cowpea in food and nutrition security, the release of improved cultivars for wide scale-production and breeding remains unsatisfactory, partially attributable to limited breeding effort to identify and select suitable genotypes that possess superior and stable performance. The substantial differences in genotypic responses occur under diverse

environmental conditions (Akinwale *et al.*, 2011). The phenomenon is known as a genotype-by-environment interaction and it occurs frequently in plant breeding programs. Further, GEI effect causes variable crop performance across environmental conditions (Yan and Kang, 2003), limiting the selection gain in genetic improvement programs. As a result, identification and selection of high-yielding and stable breeding parents is key to speed-up genetic improvement for increased yield and productivity in cowpea. Multi-environment trial (MET) analysis to investigate genotype-by-environment interaction (GEI) effect for major selection traits is a key procedure in cultivar design and development (Cooper and DeLacy, 1994).

GEI analysis is a key statistical procedure to aid identification and selection of high-yielding genotypes possessing broad or narrow adaptations (Yan *et al.*, 2000; Yan and Kang, 2003; Yan and Tinker, 2006). Genotypes with broad adaptation are associated with high stability (Yan *et al.*, 2000), suitable for production in different environments and for genetic advancement to aid in product profile and cultivar development. GEI analysis is conducted using the additive main effect and multiplicative interaction (AMMI) for estimation of genotype (G), environment (E) and GEI effects (Yan and Hunt 2001). Further, genotype and genotype-by-environment interaction (GGE) biplot analysis is important to identify and select high-yielding and stable genotypes with specific or wide adaptation to production environments (Yan and Kang, 2003; Yan and Tinker, 2006; Yan *et al.*, 2007). To identify and select high-yielding and stable cowpea genotypes, fifty elite and genetically diverse germplasm accessions sourced from the Agricultural Research Council (ARC), South Africa, were subjected to GEI analysis using METs conducted in South Africa. Other studies used GEI analysis in cowpea to identify high-yielding genotypes with narrow and wide adaption for production and breeding (Ddamulira *et al.*, 2015; Horn *et al.*, 2018, Gerrano *et al.*, 2020; Mbuma *et al.*, 2021; Iseki *et al.*, 2021). The objective of this study was to determine the influence of genotype (G), environment (E) and their interaction (GEI) effects on grain yield in cowpea and to assess the stability of cowpea genotypes to identify stable and high-yielding genotypes for broad or narrow adaptation to improve cowpea productivity in South Africa and identical agro-ecologies.

## 3.2 Materials and methods

### 3.2.1 Plant materials

Fifty cowpea genotypes obtained from the ARC gene bank collections from South Africa and Nigeria were used in this study (Table 3.1).

**Table 3.1:** List of 50 cowpea genotypes used in the study and their origin.

Genotype code	Genotype Name	Origin	Genotype Code	Genotype Name	Origin
G1	Acc-Cowp2	South Africa	G26	Acc-Cowp29	South Africa
G2	Acc-Cowp3	South Africa	G27	Acc-Cowp30	South Africa
G3	Acc-Cowp4	South Africa	G28	Acc-Cowp31	South Africa
G4	Acc-Cowp5	South Africa	G29	Acc-Cowp32	South Africa
G5	Acc-Cowp6	South Africa	G30	Acc-Cowp33	South Africa
G6	Acc-Cowp7	South Africa	G31	Acc-Cowp34	South Africa
G7	Acc-Cowp9	South Africa	G32	Acc-Cowp35	South Africa
G8	Acc-Cowp10	South Africa	G33	Acc-Cowp36	South Africa
G9	Acc-Cowp11	South Africa	G34	Acc-Cowp37	South Africa
G10	Acc-Cowp12	South Africa	G35	Acc-Cowp38	South Africa
G11	Acc-Cowp13	South Africa	G36	Acc-Cowp39	South Africa
G12	Acc-Cowp14	South Africa	G37	Acc-Cowp40	South Africa
G13	Acc-Cowp15	South Africa	G38	Acc-Cowp41	South Africa
G14	Acc-Cowp16	South Africa	G39	Acc-Cowp43	South Africa
G15	Acc-Cowp17	South Africa	G40	Acc-Cowp44	South Africa
G16	Acc-Cowp18	South Africa	G41	Acc-Cowp45	South Africa
G17	Acc-Cowp19	South Africa	G42	Acc-Cowp46	South Africa
G18	Acc-Cowp20	South Africa	G43	Acc-Cowp47	South Africa
G19	Acc-Cowp21	South Africa	G44	Acc-Cowp48	South Africa
G20	Acc-Cowp22	South Africa	G45	Acc-Cowp49	South Africa
G21	Acc-Cowp24	South Africa	G46	Acc-Cowp50	South Africa

G22	Acc-Cowp25	South Africa	G47	98K-5301	Nigeria
G23	Acc-Cowp26	South Africa	G48	Glenda	South Africa
G24	Acc-Cowp27	South Africa	G49	TVU13953	Nigeria
G25	Acc-Cowp28	South Africa	G50	VegCowDakCream	South Africa

Note: VegCowDakCream = Vegetable cowpea Dakama Cream

### 3.2.2 Study environments

The field trials were conducted in the diverse agro-ecological regions of South Africa, including Brits (25.6276° S, 27.7816° E), Loskop (25.1773° S, 29.3936°E), Roodeplaat (25.6080° S, 28.3525° E), Mafikeng (25.8201° S, 25.6298° E) and Polokwane (24.0295° S, 29.7425° E) during the 2019/2020 and 2020/2021 summer cropping seasons. The following seven test environments were defined i.e., E1 = Brits 2019/2020, E2 = Loskop 2019/2020, E3 = Roodeplaat 2019/2020, E4 = Brits 2020/2021, E5 = Loskop 2020/2021, E6 = Mafikeng 2020/2021 and E7 = Polokwane 2020/2021. The environmental conditions of the studied locations are presented in Table 3.2.

**Table 3.2:** Description of environments evaluated in the study.

Location	Soil type	Soil pH	Altitude (masl)	Average rainfall (mm)	Min. Temperature (°C)	Max. Temperature (°C)
Brits	Clay loam	4.5-5.5	1083	629	18.3	28.9
Loskop	Loamy	5.5-6.5	920	497	18.6	30.0
Roodeplaat	Clay loam	5.0 – 6.2	1168	772	10.0	21.0
Mafikeng	Sandy	4.5-5.2	1359	571	17.6	28.8
Polokwane	Clay loam	5.6	1310	495	16.7	27.6

Note: Masl = meters above sea level, Min = Minimum, Max = Maximum

### 3.2.3 Experimental design and data collection

The cowpea genotypes were evaluated using a 10 x 5 alpha-lattice experimental design replicated three times. Each genotype was sown in a 6 m<sup>2</sup> plot comprised of two 3 m length rows. The intra- and inter-row spacing were 0.3 m and 1 m, respectively. Two seeds were hand sown per hole and later thinned to one seedling two weeks after emergence. The plants were

rained, however, supplemental drip irrigation was applied, three times a week, when the rainfall was not enough for optimal growth and development. Data was recorded on three randomly selected plants per plot in each replication. Agronomic practices such as weeding, insects and diseases control were carried out using chemical and cultural practices as per recommendation for the cowpea. To simulate low-input soil conditions, no fertiliser was added (Gerrano *et al.*, 2019). At physiological maturity, when the plants and pods turned brown in colour, grain yield data were recorded in grams plant<sup>-1</sup> for each genotype, and later converted to t ha<sup>-1</sup>.

### 3.2.4 Data Analysis

#### 3.2.4.1 Analysis of variance

Following analysis of variance (ANOVA), the additive main effect and multiplicative interaction (AMMI) analysis was performed to infer the effects due to genotype (G), environment (E) and genotype-by-environment interaction (GEI) (Gauch *et al.*, 2008; Gauch, 2006; Gauch, 2013). The AMMI analysis was done using the following statistical AMMI model:

$$\bar{Y}_{ij} = \mu - G_i + E_j + \sum_{k=1}^m \lambda_k \alpha_{ik} \gamma_{jk} + \rho_{ij}$$

Where  $\bar{Y}_{ij}$  is the yield of genotype  $i^{\text{th}}$  in the  $j^{\text{th}}$  environment,  $\mu$  is the grand mean,  $G_i$  is the mean of the  $i^{\text{th}}$  genotype minus the grand mean,  $E_j$  is the mean of the  $j^{\text{th}}$  environment minus the grand mean,  $\lambda_k$  is the square root of the eigen value of the  $k^{\text{th}}$  interaction principal component axis (IPCA) axis of the  $i^{\text{th}}$  genotype and  $j^{\text{th}}$  environment, and  $\rho_{ij}$  is the deviation from the model. Further, AMMI stability values (ASV's) were computed to rank genotypes based on stability using Genstat® version 20 according to Purchase (1997) using the following formula:

$$ASV = \sqrt{\left[ \left( \frac{SSIPCA1}{SSIPCA1} (IPCA1) \right)^2 + [IPCA1]^2 \right]}$$

Where  $\frac{SSIPCA1}{SSIPCA1}$  denotes the weighted value assigned to the first interaction principal component score due to its high contributions in the GE model. SSIPCA1 and SSIPCA2 are the sum of squares for IPCA1 and IPCA2, respectively.

Further, grain yield data were subjected to genotype and genotype-environment (GGE) biplot analysis to identify high-responsive and stable genotypes (Yan and Tinker, 2006) using Genstat® version 20 (Payne, 2014).

### 3.3 Results

#### 3.3.1 Genotype, environment, and genotype-by-environment interaction effects

AMMI showed significant ( $P \leq 0.01$ ) G, E and GEI effects for grain yield (Table 3.3). The first, second, third, fourth and fifth IPCAs were significant ( $P \leq 0.01$ ) for grain yield, explaining 98.11% total GEI signal. IPCA1, IPCA2, IPCA3, IPCA4 and IPCA5 explained 49.96, 26.54, 11.65, 6.52 and 3.44% GEI signal of the AMMI model, respectively. Further, AMMI revealed high variation of 57.03% due to GEI effect, followed by 29.27 and 13.42% for genotype and environment main effects, respectively.

**Table 3.3:** AMMI analysis of variance showing SS, MS, percentage explained variance by IPCA1, IPCA2, IPCA3, IPCA4 and IPCA5 for grain yield among 50 cowpea genotypes across test environments.

Source of variation	d.f.	SS	MS	Total variation explained (%)	GEI explained (%)
Treatments	349	26.58	0.07		
Genotypes (G)	49	7.78	0.15**	29.27	
Environments (E)	6	3.57	0.59**	13.42	
G x E	294	15.23	0.05**	57.03	
IPCA1	54	7.61	0.14**		49.96
IPCA2	52	4.04	0.08**		26.54
IPCA3	50	1.78	0.04**		11.65
IPCA4	48	0.99	0.02**		6.52
IPCA5	46	0.53	0.01**		3.44
Block	14	2.93	0.01**	0.00	
Residual	188	3.58	0.019**		1.87
Total	1049	26.58	0.03		

Note: \*\* = significant at  $P \leq 0.01$ , d.f. = degrees of freedom, SS = sum of squares, MS = mean squares, IPCA1, IPCA2, IPCA3, IPCA4, IPCA5 = interaction principal component axis one, two, three, four and five, respectively.

#### 3.3.2 Performance of cowpea genotypes for grain yield

Mean performances, IPCA scores and ASVs for grain yield among 50 cowpea genotypes evaluated in seven environments are shown in Table 4. The highest grain yield values were recorded for G47 (0.64 t ha<sup>-1</sup>) under E1, G41 (0.55 t ha<sup>-1</sup>) under E2, G15 (0.61 t ha<sup>-1</sup>) under E3, G46 (0.43 t ha<sup>-1</sup>) under E4, G28 (0.82 t ha<sup>-1</sup>) under E5, G6 (0.51 t ha<sup>-1</sup>) under E6 and G43 (0.79 t ha<sup>-1</sup>) under E7. Across the environments, grain yield ranged from 0.13 t ha<sup>-1</sup> to 0.47 t ha<sup>-1</sup>.

<sup>1</sup> which were recorded for genotypes G10 and G35, respectively. High grain yield values were recorded for genotypes G35, G1, G47, G15 and G7 (i.e., 0.47 t ha<sup>-1</sup>, 0.45 t ha<sup>-1</sup>, 0.43 t ha<sup>-1</sup>, 0.41 t ha<sup>-1</sup> and 0.37 t ha<sup>-1</sup>, in that order). Genotypes G10, G30, G21, G45 and G6 recorded low grain yield values of 0.13 t ha<sup>-1</sup>, 0.14 t ha<sup>-1</sup>, 0.15 t ha<sup>-1</sup>, 0.16 t ha<sup>-1</sup> and 0.17 t ha<sup>-1</sup>, respectively, across test environments. Based on ASV analysis, G40 was the most stable genotype recording the lowest ASV of 0.03, followed by G9 (0.04), G38 (0.05), G26 (0.07) and G48 (0.08). The following genotypes (i.e., G41, G17 and G29) recorded high ASVs of 0.87, 0.74 and 0.70, respectively.

**Table 3.4:** Mean grain yield (t ha<sup>-1</sup>) of 50 cowpea genotypes under seven test environments during the 2019/2020 and 2020/2021 planting seasons.

Genotype*	Environments							Mean	IPCA1	IPCA2	ASV
	Brits 2019/2020	Loskop 2019/2020	Roodeplaat 2019/2020	Brits 2020/2021	Loskop 2020/2021	Mafikeng 2020/2021	Polokwane 2020/2021				
G1	0.58	0.45	0.50	0.20	0.52	0.23	0.67	0.45	-0.14	0.26	0.38
G2	0.12	0.15	0.11	0.35	0.36	0.16	0.33	0.23	0.12	-0.05	0.23
G3	0.17	0.20	0.45	0.25	0.55	0.37	0.61	0.37	0.14	0.12	0.29
G4	0.55	0.15	0.50	0.22	0.27	0.14	0.70	0.36	-0.15	0.32	0.43
G5	0.11	0.21	0.17	0.26	0.37	0.08	0.45	0.24	0.09	0.09	0.19
G6	0.09	0.16	0.09	0.05	0.11	0.51	0.17	0.17	0.07	-0.21	0.25
G7	0.50	0.50	0.53	0.13	0.26	0.11	0.59	0.37	-0.26	0.27	0.57
G8	0.50	0.43	0.48	0.25	0.34	0.07	0.41	0.36	-0.26	0.10	0.50
G9	0.11	0.14	0.17	0.20	0.23	0.09	0.32	0.18	0.02	0.02	0.04
G10	0.18	0.10	0.10	0.09	0.28	0.11	0.03	0.13	-0.06	-0.16	0.19
G11	0.09	0.13	0.10	0.15	0.36	0.20	0.54	0.23	0.18	0.15	0.37
G12	0.13	0.25	0.23	0.20	0.76	0.13	0.44	0.31	0.16	0.09	0.31
G13	0.18	0.17	0.10	0.23	0.33	0.24	0.19	0.20	0.05	-0.14	0.17
G14	0.53	0.47	0.50	0.16	0.23	0.19	0.45	0.36	-0.29	0.12	0.55
G15	0.58	0.50	0.61	0.25	0.31	0.28	0.32	0.41	-0.33	-0.04	0.63
G16	0.09	0.19	0.10	0.20	0.28	0.17	0.28	0.19	0.07	-0.05	0.14
G17	0.62	0.45	0.50	0.27	0.33	0.21	0.09	0.35	-0.38	-0.18	0.74
G18	0.10	0.19	0.09	0.15	0.46	0.24	0.52	0.25	0.20	0.12	0.39
G19	0.15	0.10	0.24	0.26	0.21	0.38	0.17	0.22	0.03	-0.21	0.22
G20	0.10	0.17	0.11	0.17	0.26	0.15	0.36	0.19	0.07	0.03	0.14
G21	0.08	0.12	0.08	0.20	0.35	0.05	0.18	0.15	0.04	-0.07	0.10

Table 4 continued

Genotype*	Environments							Mean	IPCA1	IPCA2	ASV
	Brits 2019/2020	Loskop 2019/2020	Roodeplaat 2019/2020	Brits 2020/2021	Loskop 2020/2021	Mafikeng 2020/2021	Polokwane 2020/2021				
G22	0.12	0.17	0.10	0.34	0.24	0.15	0.18	0.19	0.03	-0.17	0.18
G23	0.09	0.23	0.17	0.18	0.42	0.35	0.10	0.22	0.07	-0.24	0.28
G24	0.13	0.16	0.11	0.24	0.28	0.20	0.30	0.20	0.07	-0.06	0.15
G25	0.19	0.18	0.16	0.30	0.32	0.30	0.09	0.22	0.01	-0.27	0.27
G26	0.13	0.11	0.13	0.25	0.25	0.07	0.32	0.18	0.04	0.02	0.07
G27	0.18	0.24	0.14	0.41	0.38	0.32	0.43	0.30	0.15	-0.08	0.29
G28	0.11	0.27	0.09	0.23	0.82	0.36	0.66	0.36	0.36	0.14	0.70
G29	0.61	0.48	0.47	0.15	0.35	0.09	0.25	0.34	-0.37	0.02	0.70
G30	0.11	0.13	0.15	0.19	0.26	0.10	0.05	0.14	-0.04	-0.17	0.18
G31	0.16	0.20	0.18	0.20	0.41	0.23	0.69	0.30	0.18	0.23	0.42
G32	0.12	0.12	0.11	0.16	0.23	0.17	0.32	0.18	0.06	0.00	0.11
G33	0.16	0.16	0.09	0.32	0.29	0.14	0.24	0.20	0.05	-0.11	0.14
G34	0.12	0.21	0.12	0.25	0.40	0.16	0.28	0.22	0.08	-0.05	0.17
G35	0.61	0.45	0.55	0.36	0.72	0.10	0.49	0.47	-0.19	0.13	0.38
G36	0.56	0.25	0.45	0.19	0.51	0.06	0.52	0.36	-0.17	0.23	0.40
G37	0.12	0.24	0.10	0.24	0.38	0.23	0.49	0.26	0.16	0.06	0.31
G38	0.16	0.12	0.23	0.26	0.27	0.11	0.25	0.20	-0.01	-0.05	0.05
G39	0.09	0.17	0.08	0.35	0.31	0.22	0.29	0.22	0.13	-0.11	0.26
G40	0.19	0.23	0.23	0.27	0.35	0.15	0.36	0.25	0.02	0.01	0.03
G41	0.55	0.55	0.58	0.24	0.28	0.06	0.10	0.34	-0.46	-0.10	0.87
G42	0.15	0.17	0.25	0.19	0.30	0.13	0.46	0.24	0.04	0.12	0.14
G43	0.11	0.27	0.12	0.17	0.50	0.30	0.78	0.32	0.27	0.27	0.58
G44	0.16	0.20	0.18	0.26	0.41	0.28	0.08	0.22	0.02	-0.25	0.25
G45	0.10	0.13	0.09	0.16	0.29	0.13	0.20	0.16	0.04	-0.07	0.11

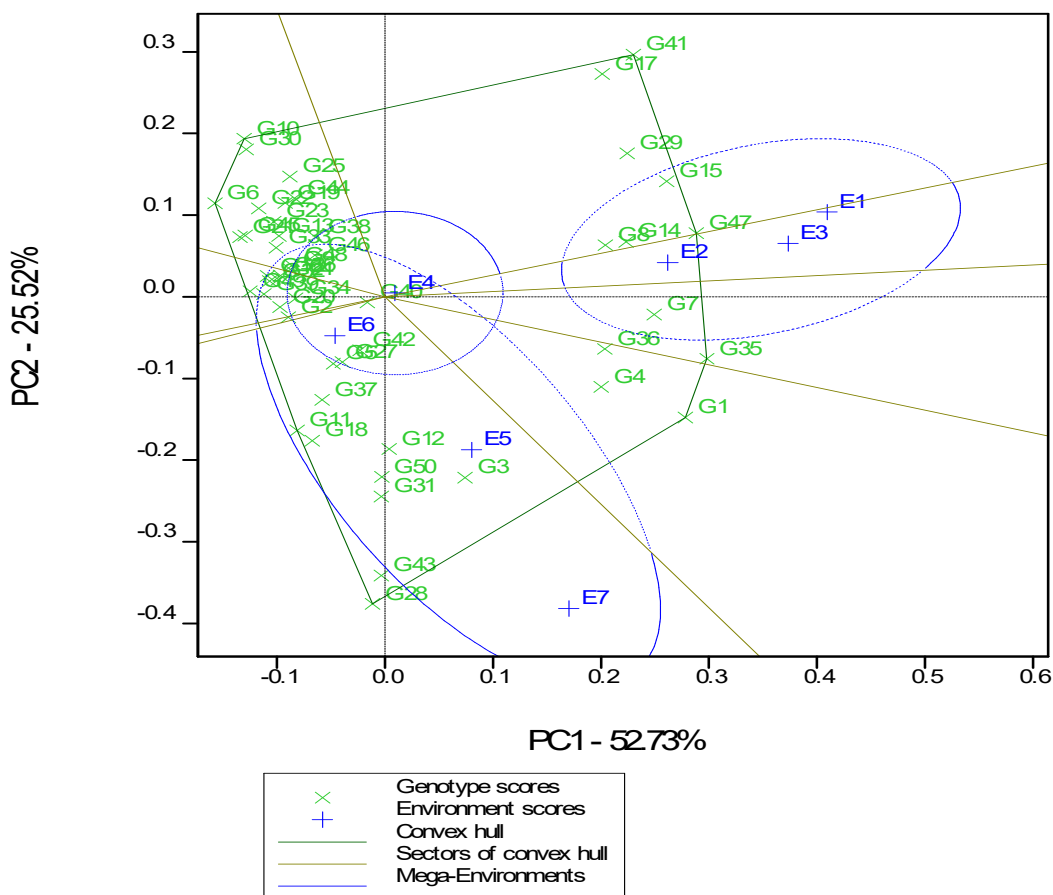
Table 4 continued

Genotype*	Environments							Mean	IPCA1	IPCA2	ASV
	Brits 2019/2020	Loskop 2019/2020	Roodeplaat 2019/2020	Brits 2020/2021	Loskop 2020/2021	Mafikeng 2020/2021	Polokwane 2020/2021				
G46	0.20	0.18	0.15	0.43	0.34	0.26	0.24	0.26	0.07	-0.19	0.23
G47	0.64	0.54	0.55	0.31	0.31	0.21	0.44	0.43	-0.32	0.05	0.60
G48	0.14	0.22	0.10	0.23	0.28	0.13	0.28	0.20	0.04	-0.04	0.08
G49	0.11	0.11	0.10	0.18	0.25	0.42	0.30	0.21	0.14	-0.13	0.29
G50	0.17	0.20	0.19	0.27	0.31	0.25	0.70	0.30	0.17	0.21	0.38
G.M	0.24	0.24	0.24	0.24	0.35	0.20	0.35	-	-	-	-
Min	0.08	0.10	0.08	0.05	0.11	0.05	0.03	0.13	-0.46	-0.27	0.03
Max	0.64	0.55	0.61	0.43	0.82	0.51	0.78	0.47	0.36	0.32	0.87
CV%	17.9	11.9	19.1	11.2	18.9	23.7	20.9	-	-	-	-
LSD	0.98	0.70	0.54	0.84	0.54	0.62	0.74	-	-	-	-

Note: \* = refer to Table 1. G.M = grand mean. Min = minimum. Max = maximum. CV% = percentage coefficient of variation. LSD = least significant differences

### 3.3.3 GGE biplot analysis of which genotypes were superior in which environment.

A “which-won-where” GGE biplot showing which genotypes won in which environment is presented in Figure 3.1. The biplot consists of an irregular polygon and a set of lines drawn from the origin to perpendicularly dissect each side of the polygon. The set of lines divide the biplot into sectors. Identifying winning genotypes for each sector (Yan et al. 2007). The “which-won-where” biplot explained 78.26% total variation of which PC1 and PC2 accounted for 52.73% and 25.52% of the total variation, respectively. The biplot depicted eight sectors and environments were clustered into three of the eight sectors. Genotypes such as G28, G43, G31, G3, G50, G12, G18, G11, G37, G27 and G42 constituted the largest sector and were associated with environments E4, E5, E6 and E7 which formed a mega-environment. Genotypes G47, G15, G14, G7 and G8 formed the second sector which involved environments E1, E2 and E3. The genotypes which were located at the vertices of the polygon were G1, G35, G47, G41, G10, G6 and G28.

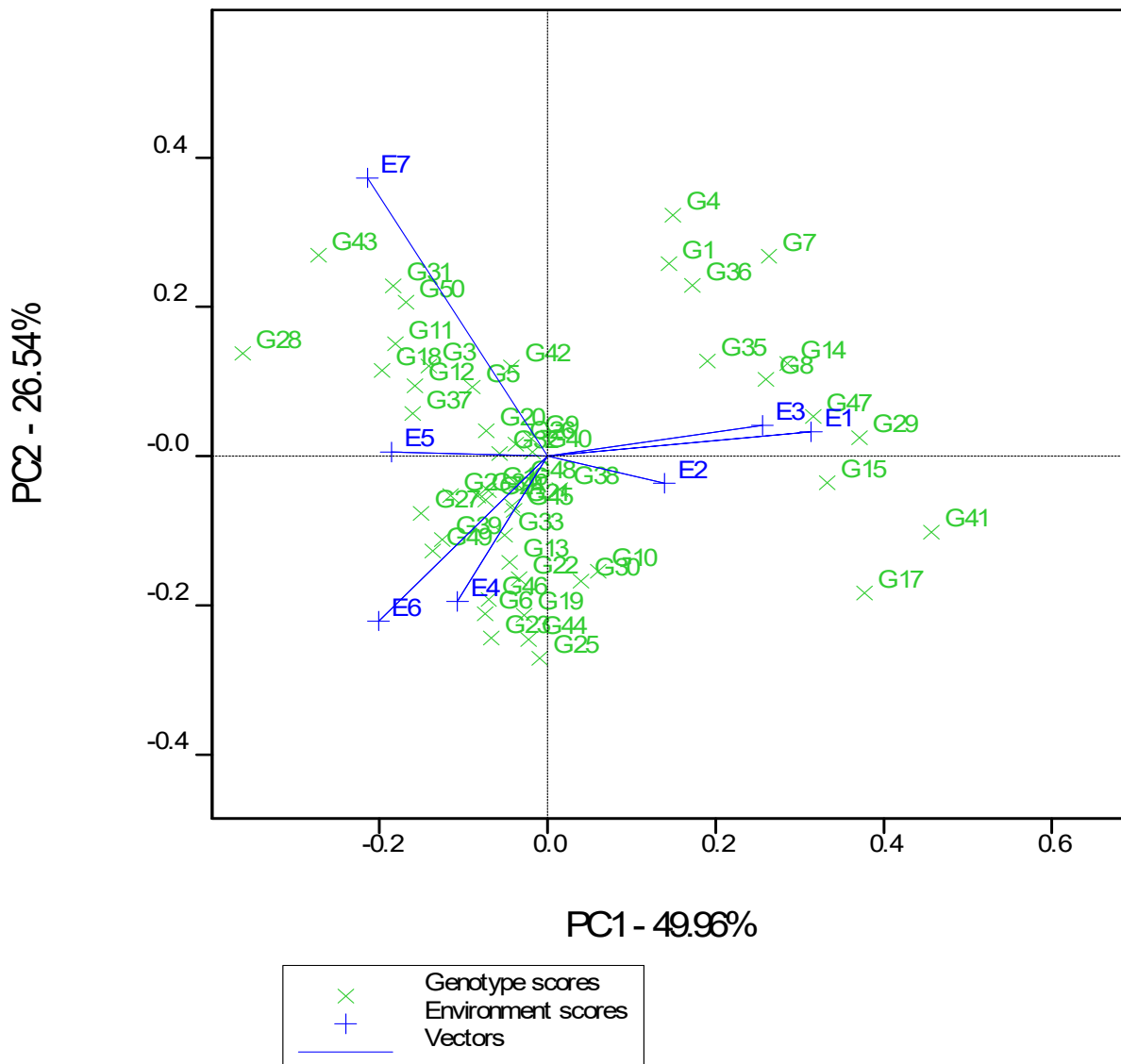


**Figure 3.1:** The “Which-won-where” view of GGE biplot showing which genotypes performed best in which environment and delineation of mega-environments. E1, Brits

2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng 2020/2021; E7, Polokwane 2020/2021.

### **3.3.4 GGE biplot analysis for the studied environments**

GGE biplot analysis revealing the test environments' discriminating power and representativeness of the target environment is presented in Figure 3.2. Vector lines are drawn from the biplot origin to each test environment marker, measuring the discriminative power of the environment. Long vectors indicate test environments with more discriminating power. Further, the average environment axis (AEA) are the dotted vertical and horizontal lines indicating points where the PC1 and PC2 axes had respective values of zero. Test environments with small angles with the AEA are more representative of the mega-environment (Yan *et al.*, 2007). AMMI revealed a total variation of 76.51% contributed by IPCA1 (49.96%) and IPCA2 (26.54%). Environment E7 showed the longest vector line, suggesting high discriminating ability for this environment. The other test environments were plotted closer to the origin, signifying that most genotypes performed similarly in these environments. Environment E5 showed the smallest angle with the AEA, signifying high representativeness of the mega-environment involving E4, E5, E6 and E7. Also, environment E1 showed the most representativeness in the mega-environment involving E1, E2 and E3.

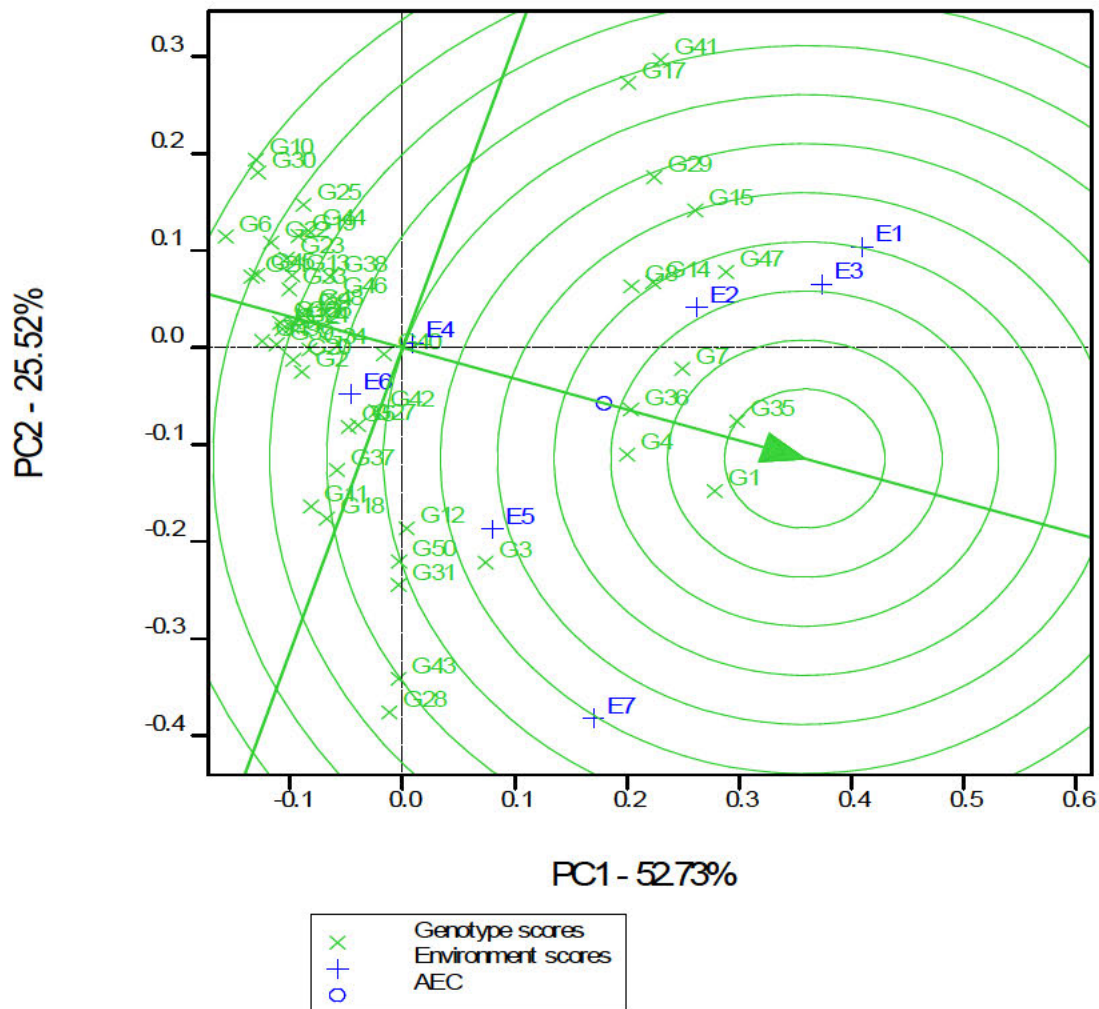


**Figure 3.2:** The “discriminating power vs. representativeness” view of GGE biplot showing the relationship between the studied environments. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng.

### 3.3.5 Comparison view of GGE biplot analysis of ideal cowpea genotype

The GGE biplot clustering the studied genotypes relative to the ideal genotype is presented in Figure 3.3. The ideal genotype is located at the innermost concentric circle, indicated by an arrowhead in the biplot (Yan and Tinker, 2006). The two principal components accounted for 78.26% total variation among genotypes, contributed by PC1 (52.73%) and PC2 (25.52%), respectively. G35 was identified as an ideal genotype for grain yield, given its position at the epicentre of the concentric circles. G1, G35, G4 and G36 were the next genotypes next to the

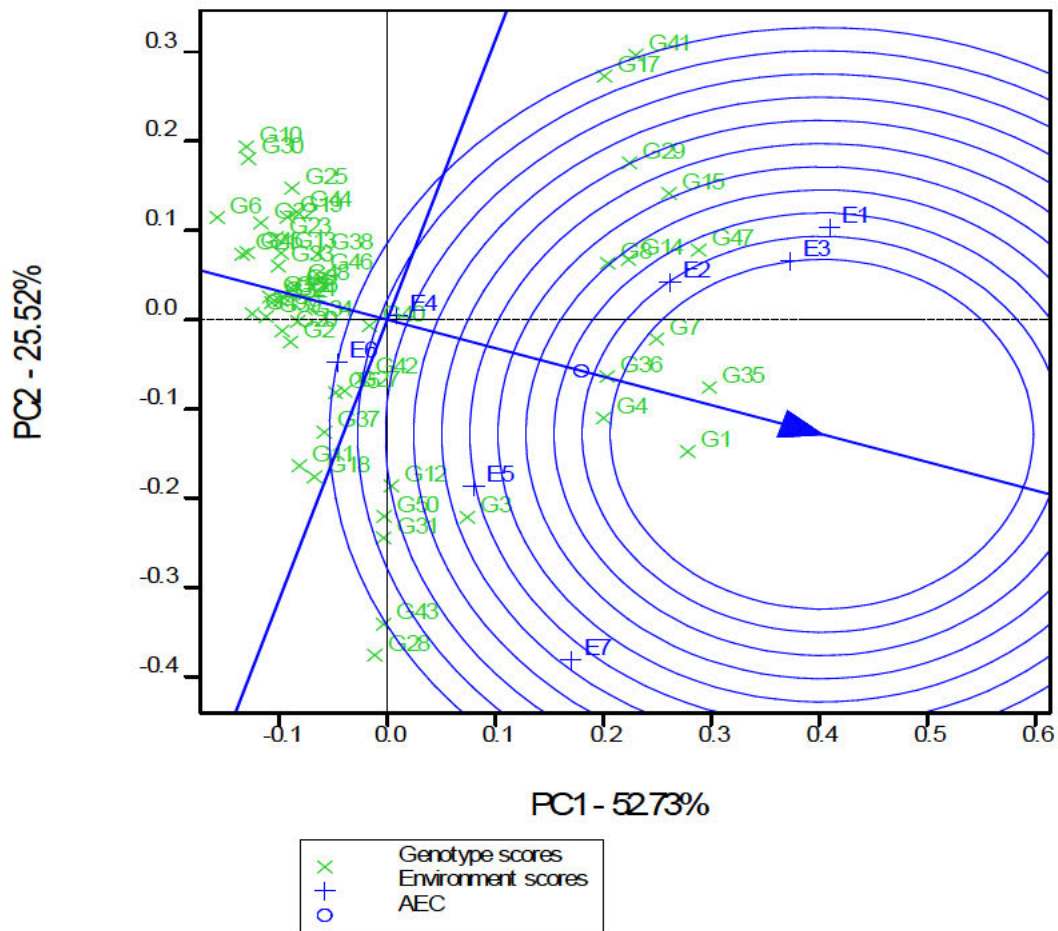
ideal genotype, in that order. G6, G10 and G30 were the furthest from the ideal genotype, signifying low stability.



**Figure 3.3:** The comparison view of GGE biplot showing an ideal genotype based on grain yield performance across seven environments. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng 2020/2021.

### 3.3.6 Comparison view of GGE biplot analysis of ideal environment

GGE biplot visually compares test environments relative to the ideal environment is presented in Figure 3.4. Environments E3, E1 and E2 were plotted closest to the epicentre of the concentric circles, in that order, providing the most ideal production conditions for grain yield. Environments E6, E4, E7 and E5 were plotted farthest from the epicentre, in that order, considered the worst discriminatory environments.



**Figure 3.4:** The comparison view of GGE biplot showing the ideal environment for grain yield. E1, Brits 2019/2020; E2, Loskop 2019/2020; E3, Roodeplaat 2019/2020; E4, Brits 2020/2021; E5, Loskop 2020/2021; E6, Mafikeng 2020/2021; E7, Polokwane 2020/2021.

### 3.4 Discussion

The development and evaluation of stability in cowpea genotypes across a variety of environments is an important aspect for the selection of stable and high-yielding genotypes. This study determined genotype-by-environment interaction effect for grain yield among cowpea genotypes across seven environments of South Africa to identify and select high-yielding and stable genotypes for production, and for use as breeding parents in cultivar design and development. The AMMI analysis of variance revealed significant genotype (29%), environment (13%), and genotype-by-environment interaction (57%) effects for grain yield (Tables 3). The high GEI effect of 57% indicates the larger influence of environment-specific effects on cowpea genotypes allowing identification of genotypes with specific and wide

adaptation. Significant GEI effect for grain yield in cowpea was reported by other studies (Mbuma *et al.*, 2020; Shiringani and Shimelis, 2011; Martos-Fuentes *et al.*, 2017), signifying the importance of GEI analysis to select high-yielding and stable cowpea genotypes. Contrary to the present findings, Simion *et al.* (2018) reported genotype effect as the largest contributor to total phenotypic variation, recording 78% variance. Furthermore, Mohammed *et al.* (2016) reported the highest phenotypic variance of 75% due to environment effect in cowpea grain yield. Nevertheless, the significant genotype effect of 29% observed in the present study is useful to identify and select high-yielding cowpea genotypes for production, and cultivar design and development.

In the present study, cowpea genotypes such as Acc-Cowp38, Acc-Cowp2 and 98K\_5301 recorded high grain yield values of 0.47 t ha<sup>-1</sup>, 0.45 t ha<sup>-1</sup> and 0.43 t ha<sup>-1</sup>, respectively, across the test environments (Table 3.4). Grain yield ranged between 0.13 and 0.47 t ha<sup>-1</sup>, which is higher than the range of 0.048 t ha<sup>-1</sup> to 0.23 t ha<sup>-1</sup> reported by Mbuma *et al.* (2020), which could be attributable to favorable environmental conditions in the test locations. Andrade *et al.* (2013) also reported a grain yield range of between 0.6 to 1.1 t ha<sup>-1</sup> in cowpea, which was associated with the application of biofertilizers. The cowpea genotypes identified with superior grain yield could be important to enhance genetic improvement for grain yield. ASV analysis is a salient statistical procedure to estimate genotype stability across test locations, whereby a low ASV value indicates high stability (Purchase, 1997). Smaller ASV indicates wide adaptation of genotype across environments, whereas high ASV indicates narrow adaptation of genotype to a certain environment (Purchase, 1997; Mahmodi *et al.*, 2011). Based on AMMI stability values (ASV), this study identified G40 (0.03), G9 (0.04), G38 (0.05), G26 (0.07) and G48 (0.08) as the stable genotypes. Furthermore, IPCA scores are also used to determine the genotype stability. The highest IPCA scores, whether negative or positive, is the more specifically adapted as a genotype to a specific environment (Gerrano *et al.*, 2020). Horn *et al.* (2018) further explained that the closer the IPCA approach to zero is the more stable the genotype across all test environments and identified Genotype 40 as the most stable genotype with an ASV of 0.08, which is significantly higher than the ASV of the most stable genotype (0.03) obtained from the current study. The ASV values and IPCA scores have been used by numerous studies (Horn *et al.*, 2018; Gerrano *et al.*, 2020; Goa *et al.*, 2022). The genotypes identified as stable can be recommended for production under diverse environments of South Africa or in identical agro-ecologies to enhance cowpea productivity and to enhance selection gain for increased yield.

Visualization of the “which-won-where” GGE biplot analysis is an important statistical technique to identify mega-environments and their corresponding winner genotypes (Gauch and Zobel, 1997; Yan *et al.*, 2000; Yan and Hunt, 2001). The GGE biplot revealed two mega-environments of which the first mega-environment involved environments E4 (Brits 2020/2021), E5 (Loskop 2020/2021), E6 (Mafikeng 2020/2021) and E7 (Polokwane 2020/2021), within which there is an overlapping mega-environment involving environment E4 (Brits 2020/2021) and E6 (Mafikeng 2020/2021) (Figure 3.1). This indicates that environment E4 and E6 have close association based on the environmental conditions such as rainfall and temperature prevailing in these locations, and they were planted in the same cropping season. In agreement, Gerrano *et al.* (2020) also asserted that rainfall and temperature differences played a major role in depiction of mega-environments. In addition, Iseki *et al.* (2021) also stated that both climate and soil type should be considered in genotype selection. The soil pH across all test environments varied from 4.5 to 6.5 (Table 3.3), which is suitable for cowpea growth. In agreement to this, Singh (2003) states that cowpea grows well in soils where soil pH ranges from 5.5 to 6.5. The high yield was obtained in Loskop (E5) and Polokwane (E7) characterized by loam and clay loam, respectively, which is well drained soil types. On the contrary, the study by Mfeka *et al.* 2019 identified sandy soil as the best soil in comparison to sandy loam clay soil for cowpea to show better overall total yield. The primary cause of yield variation among different soil types under same precipitation is caused by the difference in soil chemical properties.

Based on the “discriminating power vs. representativeness” view of GGE biplot, environments E7 (Polokwane 2020/2021) possessed the most discriminating power for grain yield, whereby environments E5 (Loskop 2020/2021) and E1 (Brits 2019/2020) were the most representative in the first and second mega-environments, respectively (Figure 3.2). These environments (i.e., E1 and E5) could substitute the other environments in subsequent breeding activities. Further, genotypes such as G1, G6, G10, G28, G35, G41 and G47 were located at the vertices of the polygon in the “which-won-where” GGE biplot, indicating poor stability and specific narrow adaptation for these genotypes (Figure 3.1). The genotypes G1, G5, G10, G28, G35, G41 and G47 showed high response to the influence of the environment as they were located farthest away from the origin and can be further recommended for specific adaptation. Evidently, G6 recorded high grain yield of 0.50 t ha<sup>-1</sup> under E6 whereas the same genotypes recorded the low yield in E1, E3 and E4 of 0.09, 0.09 and 0.05 t ha<sup>-1</sup>, respectively. In contrast, G40, G34 and G2 were located close to the origin which identifies that these genotypes were among the least

environmentally responsive and therefore can be used in breeding for wide adaptation. According to Yan and Rajcan (2002), the genotypes with shorted vectors located closer to the origin are less susceptible to the GEI effects exerted by a specific environment.

Based on the comparison view of GGE biplot, the following ideal genotypes were identified: G35 (Acc-Cowp38), G1 (Acc-Cowp2), G7 (Acc-Cowp9), G4 (Acc-Cowp5) and G36 (Acc-Cowp39), in that order (Figure 3). These genotypes (i.e., G35, G1, G7, G4 and G36) possessed grain yield of 0.47 t ha<sup>-1</sup>, 0.45 t ha<sup>-1</sup>, 0.37 t ha<sup>-1</sup>, 0.36 t ha<sup>-1</sup> and 0.36 t ha<sup>-1</sup>, respectively, across the test environments (Table 3.4). The cowpea genotypes identified as ideal could be released for production or subjected to further genetic advancement to increase selection gain for grain yield in breeding programs. The comparison view of GGE biplot analysis revealed environment E1 (Brits 2019/2020) and E3 (Roodeplaat 2019/2020) as ideal environments, possessing high discriminatory power for grain yield among the studied cowpea genotypes (Figure 3.4). For example, wide ranges of grain yield of 0.09-0.64 t ha<sup>-1</sup> and 0.08-0.55 t ha<sup>-1</sup> were observed under environments E1 (Brits 2019/2020) and E3 (Roodeplaat 2019/2020), in that order (Table 3.4), suggesting the presence of wide phenotypic variation for grain yield among test genotypes in these environments.

### **3.5 Conclusion**

The present study determined genotype-by-environment interaction for grain yield in cowpea to select ideal genotypes with suitable adaptation for production in South Africa and identical agro-ecologies, and to recommend breeding parents for cultivar design and development. Significant genotype-by-environment interaction effect was detected for grain yield among the test genotypes. The polygon view of biplot depicted three possible mega-environments. The study also revealed that environment E1 and E3 (Brits and Roodeplaat) were the most ideal environments and genotypes Acc-cowp38 was identified as the high yielding genotype across all test sites. The AMMI model was used to measure the genotype stability using the AMMI stability values (ASV) and genotype Acc-cowp44 was identified as the best genotype for breeding for stability. The cowpea genotypes Acc-Cowp38, Acc-Cowp2, Acc-Cowp9, Acc-Cowp5 and Acc-Cowp39 were identified as ideal for grain yield, in that order. These genotypes are recommended for production in South Africa or in similar agro-ecologies, and for incorporation in future breeding programs targeting genetic improvement for grain yield.

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## CHAPTER 4: PHENOTYPIC VARIABILITY AND CORRELATION RELATIONSHIP ANALYSIS IN COWPEA [*VIGNA UNGUICULATA* (L.) Walp] BASED ON YIELD AND YIELD RELATED TRAITS.

### Abstract

Assessing phenotypic variations for yield and yield related traits is the fundamental aspects for crop improvement in crop species. The objective of this study was to determine the phenotypic variations and correlations among the grain yield and its components for identification and selection of promising genotypes for enhanced cultivar development. Fifty cowpea genotypes were evaluated under five diverse environments using a 5x10 alpha lattice design with three replications. The results revealed significant differences among genotype (G), environment (E) and genotype-by-environment interaction (GEI) for the studied agronomic traits. The overall mean performances showed that there were significantly high variations for all the traits assessed with the ranges of 6.01 - 8.87 (NB), 84.21-168.22 cm (PH), 7.15 - 16.49 cm (LL) , 6.15 - 10.83 cm (LW), 82.04 - 106.17 (PL), 15.12 - 22.36 cm, 8.45 - 9.89 mm (PW), 11.98 - 17.07 (NSP), 19.01 - 26.59 g (HSW) and 0.13 - 0.47 (GY) t ha<sup>-1</sup>. The correlation analysis revealed there were significant and positive correlations ( $P \leq 0.05$ ) between grain yield (GY) and number of seeds per pod (NSP), pod length (PL), pod width (PW) and hundred seed weight (HSW) suggested that the selection should be focused on traits such as NPP, PL, PW, NSP and HSW for the improvement of yield in cowpea. The principal component analysis identified three PC's which accounted for a total variation of 73.62% amongst the tested genotypes and identified NB, NPP, PL, PW, NSP, and HSW as the important traits because of their high contribution on PC1 and PC2. The PCA biplot depicted three groups of cowpea genotypes based on their associations with assessed traits. Genotypes Acc-Cowp2, 98K\_5301, Acc-Cowp4, Acc-Cowp17 and Acc-Cowp9 were grouped together based on their high values of NPP, PW, NSP, PL, HSW and GY. Cowpea genotypes such as Acc-Cowp2, Acc-Cowp10, Acc-Cowp9, Acc-Cowp38, Acc-Cowp47, Acc-Cowp40 and 98K\_5301 were selected for recommendation in breeding programs for increased yield and yield traits.

**Keywords:** cowpea, correlations, cluster analysis, crop improvement, grain yield, phenotypic variations, principal components

## 4.1 Introduction

Agricultural food systems are under increasing threat due to vast increase in global human population, extreme climate variability and economic contraction, threatening food security. According to the report by FAO *et al.*, (2023), in 2022, about 2.4 billion of people mainly dominated by women and people from the rural areas had limited or no access to sufficient, affordable, and nutritious food for all year round. The recent Covid 19 pandemic crisis was also a major driver of food insecurity, affecting people's incomes, contributing to the rise of inflation and the rise of the cost of healthy food which has left billions of people with no access to affordable healthy diet (Bundervoet *et al.*, 2021). Sustaining and maximizing the production of staple foods in developing countries which are affordable is vital as it would feed more people nutritiously with efficient use of resources, improve long term soil fertility, and create economic opportunities. Legume crops are staple crops that play an important and diverse role in the diets of poor people worldwide. They are ideal crops for simultaneously achieving three SDG goals in the targeted population which are breaking poverty (SDG 1), improving human health and nutrition (SDG 3) and enhancing ecosystem resilience in a changing climate (SDG 13).

Cowpea is a multi-purpose legume crop, ranking the second to groundnuts among the most cultivated legumes in the world (Gomes *et al.*, 2021; FAOSTAT, 2020). Cowpea is grown worldwide, particularly in Sub-Saharan Africa, Asia, Central and South American, the Mediterranean region and Southern United States (Lazaride *et al.*, 2017, Timko *et al.*, 2007). Globally, Cowpea production is estimated to be 5.9 million tonnes per annum on 15.1 million hectares of land (FAOSTAT, 2020). It is mostly cultivated for its dry seeds and immature seeds, leaves and fresh pods that are consumed as green vegetables (Timko and Singh, 2008). The crop plays a major nutritional role for both humans and animals in the developing countries throughout the world's tropical and subtropical regions (Fang *et al.*, 2007). It is well adapted to agro-climatic conditions and has good tolerance to extreme heat and drought conditions (Gomes *et al.*, 2019). In Sub-Saharan Africa, the average grain yield of cowpea ranges between 1 and 6 tons per hectare which is significantly less than the potential yield of 3 tons per ha reported in other regions (Horn and Shimelis, 2020). The principal constraints to cowpea yield productions include insect pests and diseases, poor soil fertility, and use of unimproved cultivars. Hence, the need for breeding programs to develop high yielding varieties to boost cowpea production.

The knowledge of direct and indirect influence of different traits on dependent traits such as grain yield and the relationship between components such as yield and yield related traits is critical for selection of superior genotypes in cowpea breeding programs (Vijayabharathi *et al.*, 2009). Cowpea grain yield is a quantitative trait that is controlled by a variety of genetic factors and several contributing characters as well as the environmental factors. The knowledge on the influence of yield contributing characters to yield is a pre-requisite to effective selection strategy (Rashid *et al.*, 2013). Understanding the relationship on agronomic traits effects on complex traits such as grain yield could be attained through determining the correlation relationships among the traits (Manggoel *et al.*, 2012). The correlation analysis is important as it provides the magnitude of association between characters and the viability of indirect selection which may possibly lead to an effective and faster selection progress for the desired character (Santos *et al.*, 2014). Understanding the association between yield components and identifying traits that are more influential in yield productivity would increase the efficacy of selecting superior genotypes possessing high yield components. Therefore, the objective of this study was to assess cowpea genotypes using yield components to determine the phenotypic correlations among the grain yield components for identification and selection of superior genotypes among cowpea genotypes for enhanced cultivar development.

## **4.2 Materials and Methods**

### **4.2.1 Plant materials and description of study environments**

The study evaluated 50 cowpea genotypes sourced from ARC Genebank, collected from South Africa and Nigeria. The genotypes were selected based on their growth habits (Table 4.1). The 50 cowpea genotypes were field evaluated during 2020 and 2021 cropping seasons. The field experiments were evaluated in Brits (25.6276° S, 27.7816° E, 1083 m above sea level), Loskop (25.1773° S, 29.3936° E, 920 m above sea level), Roodeplaat (25.6080° S, 28.3525° E, 1168 m above sea level), Mafikeng (25.8201° S, 25.6298° E, 1359 m above sea level) and Polokwane (24.0295° S, 29.7425° E, 495 m above sea level) during the 2019/2020 and 2020/2021 summer cropping seasons. The Brits site is characterized by clay loam soil with a soil pH of 4.5 – 5.5 with an annual rainfall of 629 mm and mean daily minimum and maximum temperatures of 18.3 °C and 28.9 °C. Loskop comprises of loamy clay soil with a pH of 5.5-6.5 with an annual rainfall of 497 mm and overall daily temperature ranges of 18.6 °C-30.0 °C. The soils in Loskop are loam and a soil pH of 5.0-6.2 with an annual rainfall of 772 mm and a daily minimum and maximum temperature of 10.0 °C and 21.0 °C, respectively. The Mafikeng site in comprised of sandy clay soil with a soil pH of 4.5- 5.2 and an annual rainfall of 571 mm with a daily

temperature minimum and maximum of 17.6 °C and 28.8 °C. Polokwane is defined by loam soils with a soil pH of 5.6 and an annual rainfall of 495 mm with mean daily minimum and maximum temperature of 16.7 °C and 27.6 °C.

#### **4.2.2 Experiment design and management**

The cowpea genotypes were planted in a field trial laid out using a 10 x 5 alpha-lattice experimental design replicated three times. Each genotype was sown in a 6-m<sup>2</sup> plot comprised of two 3 m rows. The intra- and inter-row spacing were 0.3 m and 1 m, respectively. Two seeds were hand sown per hole and later thinned to one seedling two weeks after emergence. The plants were rainfed, however, supplemental drip irrigation was applied, three times a week, when the rainfall was not enough for optimal growth and development. Three plants per plot in each replicate were randomly selected plants to record data. The trial management practices such as weeding, insects and diseases control were carried out using chemical and cultural practices based on the recommendation for cowpea. There was no fertiliser application performed.

**Table 4.1:** List of cowpea genotypes used in the study with their origin and growth habit.

<b>Genotype code</b>	<b>Genotype name</b>	<b>Origin</b>	<b>Growth habit</b>	<b>Seed shape</b>	<b>Seed coat colour</b>
<b>G1</b>	Acc-Cowp2	South Africa	Prostrate	Kidney	Red
<b>G2</b>	Acc-Cowp3	South Africa	Prostrate	Kidney	Brown
<b>G3</b>	Acc-Cowp4	South Africa	Prostrate	Kidney	Red
<b>G4</b>	Acc-Cowp5	South Africa	Prostrate	Rhomboid	Black
<b>G5</b>	Acc-Cowp6	South Africa	Prostrate	Kidney	Brown
<b>G6</b>	Acc-Cowp7	South Africa	Prostrate	Ovoid	Purple
<b>G7</b>	Acc-Cowp9	South Africa	Semi-erect	Rhomboid	Cream
<b>G8</b>	Acc-Cowp10	South Africa	Prostrate	Rhomboid	Purple
<b>G9</b>	Acc-Cowp11	South Africa	Climbing	Kidney	Light brown
<b>G10</b>	Acc-Cowp12	South Africa	Prostrate	Rhomboid	Brown
<b>G11</b>	Acc-Cowp13	South Africa	Prostrate	Kidney	Brown
<b>G12</b>	Acc-Cowp14	South Africa	Erect	Ovoid	Red
<b>G13</b>	Acc-Cowp15	South Africa	Prostrate	Ovoid	Red
<b>G14</b>	Acc-Cowp16	South Africa	Prostrate	Rhomboid	Black
<b>G15</b>	Acc-Cowp17	South Africa	Erect	Ovoid	Cream
<b>G16</b>	Acc-Cowp18	South Africa	Erect	Rhomboid	Red-speckled
<b>G17</b>	Acc-Cowp19	South Africa	Erect	Globose	Light brown
<b>G18</b>	Acc-Cowp20	South Africa	Erect	Rhomboid	Red
<b>G19</b>	Acc-Cowp21	South Africa	Erect	Kidney	Cream
<b>G20</b>	Acc-Cowp22	South Africa	Prostrate	Rhomboid	Brown
<b>G21</b>	Acc-Cowp24	South Africa	Climbing	Rhomboid	Black
<b>G22</b>	Acc-Cowp25	South Africa	Climbing	Rhomboid	Red
<b>G23</b>	Acc-Cowp26	South Africa	Semi-erect	Kidney	Grey-speckled
<b>G24</b>	Acc-Cowp27	South Africa	Semi-erect	Kidney	Red-speckled
<b>G25</b>	Acc-Cowp28	South Africa	Prostrate	Kidney	Red
<b>G26</b>	Acc-Cowp29	South Africa	Prostrate	Kidney	Brown
<b>G27</b>	Acc-Cowp30	South Africa	Semi-erect	Globose	Black

<b>G28</b>	Acc-Cowp31	South Africa	Prostrate	Globose	Cream
<b>G29</b>	Acc-Cowp32	South Africa	Prostrate	Kidney	Red
<b>G30</b>	Acc-Cowp33	South Africa	Prostrate	Kidney	Red
<b>G31</b>	Acc-Cowp34	South Africa	Semi-erect	Rhomboid	Light red
<b>G32</b>	Acc-Cowp35	South Africa	Climbing	Kidney	Light brown
<b>G33</b>	Acc-Cowp36	South Africa	Climbing	Kidney	Light brown
<b>G34</b>	Acc-Cowp37	South Africa	Prostrate	Kidney	Light brown
<b>G35</b>	Acc-Cowp38	South Africa	Climbing	Globose	Black
<b>G36</b>	Acc-Cowp39	South Africa	Semi-erect	Kidney	Red
<b>G37</b>	Acc-Cowp40	South Africa	Semi-erect	Kidney	Black
<b>G38</b>	Acc-Cowp41	South Africa	Erect	Ovoid	Cream
<b>G39</b>	Acc-Cowp43	South Africa	Semi-erect	Kidney	Brown
<b>G40</b>	Acc-Cowp44	South Africa	Erect	Kidney	Light brown
<b>G41</b>	Acc-Cowp45	South Africa	Prostrate	Ovoid	Cream
<b>G42</b>	Acc-Cowp46	South Africa	Erect	Kidney	White
<b>G43</b>	Acc-Cowp47	South Africa	Erect	Rhomboid	Brown
<b>G44</b>	Acc-Cowp48	South Africa	Prostrate	Kidney	Cream
<b>G45</b>	Acc-Cowp49	South Africa	Prostrate	Rhomboid	Brown
<b>G46</b>	Acc-Cowp50	South Africa	Prostrate	Kidney	Black
<b>G47</b>	98K-5301	South Africa	Erect	Kidney	White
<b>G48</b>	Glenda	South Africa	Semi-erect	Kidney	Brown
<b>G49</b>	TVU13953	South Africa	Prostrate	Kidney	Cream
<b>G50</b>	VegCowDakCream	South Africa	Erect	Kidney	White

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Note: VegCowDakCream = Vegetable cowpea Dakama Cream

### 4.2.3 Data Collection

Data was collected from thirteen quantitative traits following the descriptors of IBPGR (1983). The list of traits and details of data collection and units are provided in Table 4.2. Grain yield was determined in g and later converted to t/ha.

**Table 4.2:** Morphological descriptions and data collection for agro-morphological characterization of 50 cowpea genotypes evaluated.

<b>Descriptor</b>	<b>Code</b>	<b>Unit</b>	<b>Data collection</b>
Plant height	PH	cm	Measured on ten plants from cotyledon scar to tip of plant
Number of branches	NB	count	Counted the number of productive branches on three randomly selected plants
Leaflet length	LL	cm	Distance between the leaflet tip and the pulvin measured on the third fully opened leaf from the tip
Leaflet width	LW	cm	Width of the broadest portion of the third fully opened leaf from tip measured
No. of pods/plant	NPP	count	Counted number of pods developed per plant
Pod length	PL	cm	Measured on 10 matured pods
Pod width	PW	mm	Measured on 10 matured pods
Number of seeds per pod	NSP	count	Counted number of seeds developed per pod
Hundred seed weight	HSW	g	Weighed from 100 randomly sampled seeds
Grain yield	GY	t/ha	Product of average grain yield per plant at 10.5 to 11.5% moisture content

#### 4.2.4 Data analysis

Data collected from all test sites were subjected to analysis of variance (ANOVA) using Genstat 20<sup>th</sup> edition (VSN International, Hemphstead, UK) to check whether there were significant differences among genotypes and test environments. Pearson's correlation coefficients ( $r$ ) were used to magnify the traits relationship was determined using Genstat 20<sup>th</sup> edition. Principal component analysis and biplot (PCA) were performed using the same software to determine traits associations and delineate the associations among the evaluated genotypes and traits.

## 4.3 Results

### 4.3.1 Genotype, environment and genotype-by-environment interaction effects on evaluated traits

Analysis of variance revealed significant effects ( $P \leq 0.05$ ) on genotype, environment and genotype-by-environment interactions for assessed agronomic traits (Table 4.3). Significant ( $P \leq 0.05$ ) effects for genotype, environment and genotype-by-environments interactions were recorded for NB. Further, ANOVA revealed high significant differences ( $P \leq 0.01$ ) effects on genotype, environments and genotype-by-environment interactions for PH, LL, LW, NPP, PL, PW, NSP, HSW and GY.

### 4.3.2 Mean performances of cowpea genotypes for agronomic traits

The mean values of agronomic traits among 50 cowpea genotypes evaluated are presented in Table 4.4: Significant differences at  $P \leq 0.005$  for genotype, environment and GEI were observed for evaluated agronomic traits across all test environments. The high NB  $> 8$  was recorded for genotypes G43 (8.87) and G28 (8.41) while the lowest NB  $\leq 7$  was recorded for genotypes G34 (6.01). For PH, high significant differences ( $P \leq 0.01$ ) were observed across all test environments and the highest was recorded  $\leq 150$  cm for genotypes G8 (168.40 cm), G41 compared to genotypes G34 (84.2 cm) which recorded the lowest PH. Highly significant ( $P \leq 0.01$ ) leaf length was observed and the highest LL  $> 11$  were recorded for genotypes G43 (16.49 cm) and the lowest LL  $\leq 9$  was recorded for genotypes G19 (7.15 cm). The highest leaf width, LW  $> 7$  was recorded for genotypes G43 (16.49 cm) while the lowest LW  $\leq 5$  was recorded for genotype G18 (4.15 cm).

The highest NPP value was observed for genotypes G35 (106.21), while the lowest NPP was recorded in the genotypes G33 (13.07). Highly significant differences ( $P \leq 0.01$ ) were observed among studied genotypes for PL and genotypes G1 recorded the highest PL of 18.03 cm compared to the rest of the genotypes, while genotypes G31 a lowest NPP of 13.07 cm, 13.18 cm, 13.43 cm, 13.47 cm and 13.47 cm respectively. Significant ( $P \leq 0.01$ ) genotypic differences were recorded for PW and genotypes G35 recorded a high PW  $> 9$  of 9.8 mm while G33 recorded lowest PW of 7.1 mm. The high NSP for studied genotypes were recorded for genotypes G35 (17.07) and the lowest NSP were recorded for genotypes G20 with low NSP of 10.63. High significant ( $P \leq 0.01$ ) differences were observed for HSW among the studies cowpea genotypes across all test environments. Genotypes G35 recorded a high HSW of 26.59g while genotypes G20 recorded a lowest HSW of 14.79 g. High significant ( $P \leq 0.01$ )

differences were observed for GY among studied cowpea genotypes and genotypes G35 recorded a high GY of 0.47 t ha<sup>-1</sup> while genotypes G45 recorded a low GY of 0.09 t ha<sup>-1</sup>.

**Table 4.3:** Analysis of variance showing mean square values and significant test for assessed traits among studies cowpea genotypes.

Source of variation	d.f	NB	PH	LL	LW	NPP	PL	PW	NSP	HSW	GY
Rep	2	8.13*	403.02*	5.67 <sup>ns</sup>	883.81 <sup>ns</sup>	3.53 <sup>ns</sup>	19.98 <sup>ns</sup>	0.33 <sup>ns</sup>	4.24 <sup>ns</sup>	15.59 <sup>ns</sup>	0.003 <sup>ns</sup>
Genotype (G)	49	12.09*	6335.56**	37.87**	1859.0**	3023.98	47.56**	9.43**	30.61**	125.62**	0.16**
Environment(E)	6	249.03*	4781.88**	444.66**	448797.20**	365047.63**	2189.91**	1083.21**	1410.38**	6854.71**	0.59**
G x E	294	8.32*	3653.92**	21.21**	1723.53**	1863.56**	54.60**	14.24**	18.22**	65.17**	0.05**
Error	698	1.58	68.64	12.94	3698.81	2.89	19.81	0.39	1.73	8.82	0.003
Total	1049	5.39	4101.38	18.88		2753.45	43.27	10.88	15.76	69.24	0.03

NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*: significant at 5% probability level of significance, \*\*: significant at 1% probability level of significance

**Table 4.4:** Mean performances of all traits among 50 genotypes evaluated across all test environments during 2019 and 2020 cropping system.

Genotype code	NB	PH	LL	LW	NPP	PL	PW	NSP	HSW	GY(t/ha)
G1	8.34	130.91	9.75	6.42	105.03	18.03	9.51	15.10	24.41	0.45
G2	6.83	138.55	8.67	5.13	87.13	15.46	8.61	10.50	17.71	0.23
G3	8.21	132.90	9.93	6.91	98.17	16.67	9.38	13.33	24.41	0.37
G4	7.81	141.94	9.33	5.71	94.83	15.83	9.18	13.23	20.98	0.36
G5	8.84	137.93	9.98	6.78	85.33	13.58	7.68	11.23	20.05	0.24
G6	7.04	112.33	9.02	6.51	63.23	14.04	8.34	12.77	16.13	0.17
G7	7.98	132.13	10.26	7.71	98.57	16.75	9.37	12.40	21.85	0.37
G8	7.76	168.40	9.27	6.46	93.27	15.78	9.54	13.93	20.83	0.36

G9	6.62	131.31	9.93	6.75	73.33	13.99	8.63	11.70	15.85	0.18
G10	7.09	153.95	11.08	10.85	68.33	13.47	7.45	11.03	17.73	0.13
G11	6.76	147.81	9.61	6.21	71.67	13.18	8.56	11.71	18.55	0.23
G12	7.70	144.45	9.80	6.09	66.11	14.84	7.25	10.96	19.62	0.31
G13	7.00	116.60	9.10	6.09	87.37	13.76	8.43	11.71	16.78	0.20
G14	7.67	145.51	8.81	6.31	94.27	15.98	9.16	13.47	21.14	0.36
G15	7.55	117.18	9.51	5.97	99.67	16.88	9.25	13.41	22.43	0.41
G16	6.37	122.56	8.56	5.67	72.67	14.32	8.29	11.47	18.45	0.19
G17	7.40	157.91	9.68	5.96	93.53	15.64	9.23	13.83	20.75	0.35
G18	7.87	120.47	9.60	4.15	87.43	13.60	7.95	11.53	18.47	0.25
G19	8.75	103.45	7.15	4.61	84.43	14.62	8.12	11.50	20.17	0.22
G20	6.49	131.17	10.93	6.76	66.17	14.97	8.36	10.63	14.79	0.19
G21	7.87	157.19	10.16	6.78	77.36	14.96	7.66	12.71	17.92	0.15
G22	7.03	114.60	9.43	5.70	80.10	14.77	7.86	11.47	17.91	0.19
G23	6.42	104.19	8.27	4.68	80.23	15.36	8.85	12.43	15.86	0.22
G24	7.67	115.80	9.89	6.04	86.07	14.42	8.32	12.81	16.31	0.20
G25	6.90	103.36	9.38	5.38	85.17	15.34	8.35	12.31	17.62	0.22
G26	6.04	108.87	11.72	4.44	68.07	13.43	8.25	12.00	20.06	0.18
G27	7.61	144.41	9.72	9.10	75.41	14.42	8.97	13.96	20.11	0.30
G28	8.41	152.47	10.18	6.34	95.41	16.32	9.47	13.33	21.14	0.36
G29	6.36	151.13	10.07	6.43	90.23	15.54	8.94	13.53	20.56	0.34
G30	8.56	145.19	10.15	5.19	84.26	14.95	8.66	12.90	19.16	0.14
G31	8.15	144.19	9.69	5.77	73.43	13.07	8.34	11.40	18.03	0.30

G32	7.26	112.60	8.69	5.71	87.21	15.21	8.13	12.07	17.39	0.18
G33	6.10	101.75	9.25	5.48	56.83	14.64	7.11	11.17	16.19	0.20
G34	6.01	84.27	7.88	5.06	68.26	14.64	7.74	11.40	15.95	0.22
G35	6.84	161.17	9.41	5.38	106.17	22.36	9.81	17.07	26.59	0.47
G36	6.90	139.15	9.02	5.24	96.21	16.67	9.43	13.91	21.53	0.36
G37	7.76	139.47	9.38	5.74	65.27	13.99	8.66	11.13	18.02	0.26
G38	7.35	147.48	9.01	5.47	85.73	14.79	7.96	11.81	19.15	0.20
G39	7.10	123.37	9.48	6.19	81.13	15.27	7.94	11.57	19.15	0.22
G40	6.48	143.20	10.14	6.34	76.33	14.53	8.13	12.33	20.08	0.25
G41	7.34	162.18	12.63	8.03	89.20	15.54	8.96	13.23	20.51	0.34
G42	7.67	138.67	9.89	6.16	79.43	15.23	7.37	11.37	16.22	0.24
G43	8.87	158.59	16.49	6.59	88.30	15.49	8.94	13.70	20.19	0.32
G44	7.82	137.54	9.97	6.48	86.81	13.47	8.55	12.57	17.73	0.18
G45	6.27	111.26	11.54	4.89	82.03	15.14	8.74	11.53	18.22	0.09
G46	6.90	113.88	8.67	5.43	78.17	13.93	7.22	11.11	15.67	0.26
G47	6.60	123.42	9.26	6.18	100.13	17.14	9.54	14.45	22.69	0.43
G48	8.22	139.0	9.32	6.51	71.30	14.67	8.49	12.63	16.71	0.20
G49	7.20	140.92	10.85	7.04	73.46	14.84	8.95	12.41	19.16	0.21
G50	7.78	159.59	9.88	6.50	60.33	15.18	8.23	13.32	15.77	0.30
<b>Mean</b>	<b>7.35</b>	<b>261.25</b>	<b>9.79</b>	<b>6.15</b>	<b>82.04</b>	<b>15.12</b>	<b>8.45</b>	<b>11.98</b>	<b>19.01</b>	<b>0.13</b>
<b>Minimum</b>	<b>6.01</b>	<b>84.21</b>	<b>7.15</b>	<b>4.15</b>	<b>56.83</b>	<b>13.07</b>	<b>7.11</b>	<b>10.50</b>	<b>14.79</b>	<b>0.09</b>
<b>Maximum</b>	<b>8.87</b>	<b>168.22</b>	<b>16.49</b>	<b>10.85</b>	<b>106.17</b>	<b>22.36</b>	<b>9.81</b>	<b>17.07</b>	<b>26.59</b>	<b>0.47</b>
<b>CV%</b>	<b>17.11</b>	<b>7.31</b>	<b>36.76</b>	<b>6.92</b>	<b>2.08</b>	<b>9.41</b>	<b>7.42</b>	<b>10.94</b>	<b>15.7</b>	<b>1.78</b>

<b>LSD</b>	<b>0.76</b>	<b>17.82</b>	<b>2.18</b>	<b>2.18</b>	<b>1.03</b>	<b>2.75</b>	<b>0.38</b>	<b>1.80</b>	<b>1.8</b>	<b>0.34</b>
<b>SED</b>	<b>0.39</b>	<b>9.07</b>	<b>1.11</b>	<b>1.11</b>	<b>0.52</b>	<b>0.51</b>	<b>0.19</b>	<b>0.41</b>	<b>0.92</b>	<b>0.22</b>

Note: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*: significant at 5% probability level of significance, \*\*: significant at 1% probability level of significance. See genotypes (G) in Table 2.

### 4.3.3 Correlation coefficient among agronomic traits

Pearson correlations coefficients showing the relationships among the studies agronomic traits evaluated among cowpea genotypes across test environments is presented in Table 4.5. NB revealed significant and positive correlations with PH ( $r = 0.35$ ;  $p \leq 0.05$ ), NPP ( $r = 0.31$ ;  $p \leq 0.05$ ) and HSW ( $r = 0.25$ ;  $p \leq 0.05$ ). Further, significant, and positive correlations were observed for PH with LL ( $r = 0.29$ ;  $p \leq 0.05$ ) and PL ( $r = 0.29$ ;  $p \leq 0.05$ ) and between LL and LW ( $r = 0.34$ ;  $p \leq 0.05$ ). High significant differences and positive correlations were observed for NPP and PL ( $r = 0.68$ ;  $p \leq 0.01$ ), PW ( $r = 0.69$ ;  $p \leq 0.01$ ), NSP ( $r = 0.75$ ;  $p \leq 0.01$ ) and GY ( $r = 0.66$ ;  $p \leq 0.01$ ). Significant correlations observed between PL and PW ( $r = 0.65$ ;  $p \leq 0.01$ ), NSP ( $r = 0.79$ ;  $p \leq 0.01$ ), HSW ( $r = 0.72$ ;  $p \leq 0.01$ ) and GY ( $r = 0.71$ ;  $p \leq 0.01$ ). Significant and positive correlations observed between PW and NSP ( $r = 0.73$ ;  $p \leq 0.01$ ), HSW ( $r = 0.68$ ;  $p \leq 0.01$ ) and GY ( $r = 0.66$ ;  $p \leq 0.01$ ). Further, high significant and positive correlations between NSP and HSW ( $r = 0.74$ ;  $p \leq 0.01$ ) and GY ( $r = 0.73$ ;  $p \leq 0.01$ ) and between HSW and GY ( $r = 0.77$ ;  $p \leq 0.01$ ).

**Table 4.5:** Correlation coefficient analysis showing the association between the assessed agronomic traits in cowpea genotypes.

Traits	NB	PH	LL	LW	NPP	PL	PW	NSP	HSW	GY
NB	-									
PH	0.35*	-								
LL	0.18 <sup>ns</sup>	0.29*	-							
LW	0.18 <sup>ns</sup>	-0.05 <sup>ns</sup>	0.34*	-						
NPP	0.31*	0.23 <sup>ns</sup>	0.01 <sup>ns</sup>	-0.04 <sup>ns</sup>	-					
PL	0.04 <sup>ns</sup>	0.29*	-0.01 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.68**	-				
PW	0.14 <sup>ns</sup>	0.25 <sup>ns</sup>	0.13 <sup>ns</sup>	0.12 <sup>ns</sup>	0.69**	0.65**	-			
NSP	0.16 <sup>ns</sup>	0.38 <sup>ns</sup>	0.13 <sup>ns</sup>	0.02 <sup>ns</sup>	0.63**	0.79**	0.73**	-		
HSW	0.27*	0.38 <sup>ns</sup>	0.12 <sup>ns</sup>	0.07 <sup>ns</sup>	0.75**	0.72**	0.68**	0.74**	-	
GY	0.25 <sup>ns</sup>	0.38 <sup>ns</sup>	0.02 <sup>ns</sup>	0.04 <sup>ns</sup>	0.66**	0.71**	0.66**	0.73**	0.77**	-

NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*: significant at 5% level of significance, \*\*: significant at 1% level of significance

### 4.3.5 Principal component analysis (PCA) of studied agronomic traits.

Table 4.6 shows the PC scores with Eigen values, percent, and cumulative variance for the evaluated traits among the 50 studies cowpea genotypes across all test environments. There were three principal components (PCs) identified for the assessed agronomic traits with eigen values greater than 1 which cumulatively accounted for 73.63%. PC1 was positively influenced by NPP, PL, PW, NSP, HSW and GY accounting for 47.88% total variation while PC2

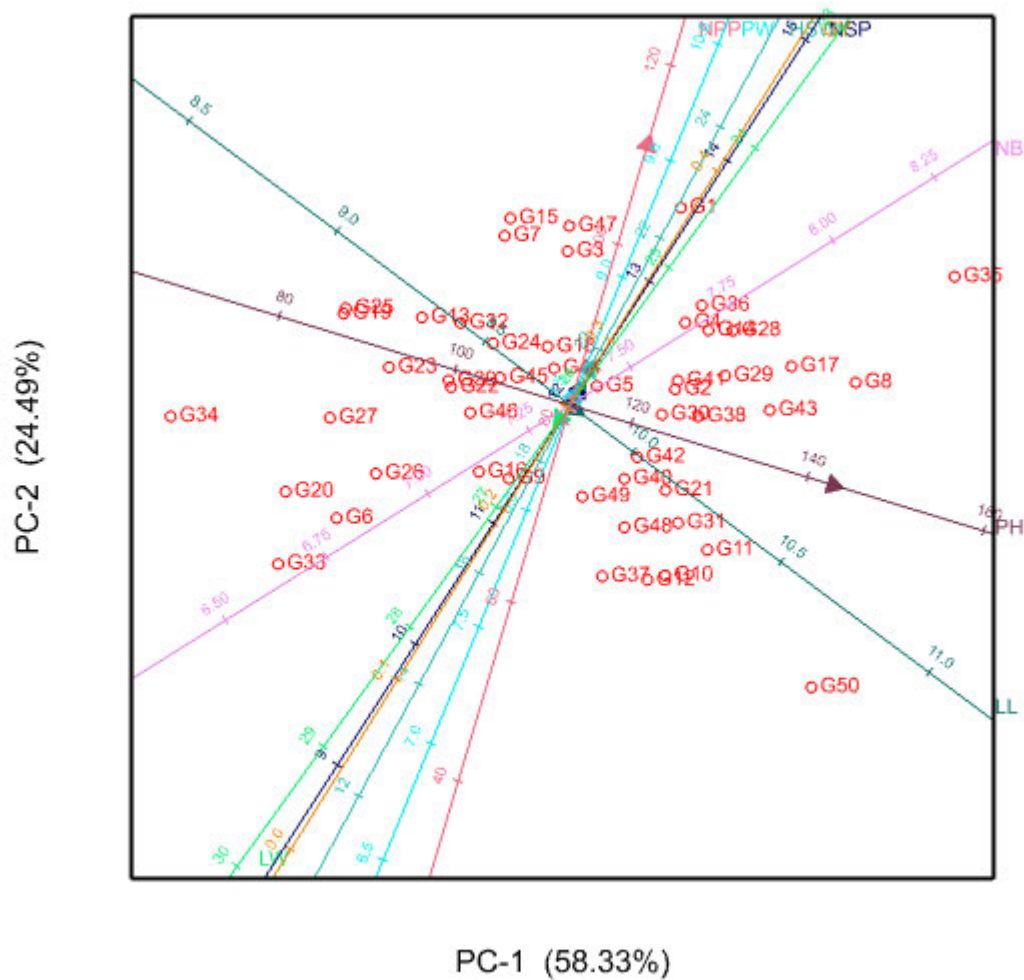
positively correlated with LL, LW and NB which accounted for 63.35 % of total variation. PC3 was positively influenced by NB and negatively correlated with LW with a total variation of 73.62 %.

The principal component biplot based on PCA analysis was used to visualize the relationship between agronomic traits and to determine the association of genotypes based on these traits (Figure 4.1). The angles below 45° between the dimensions of two variables reveals the strong positive traits associations, while the longer vector shows the discriminating ability of a particular trait. The PCA biplot distinguished three groups of genotypes with their trait associations. Therefore, genotypes such as G47, G3, G15, G1 and G7 were grouped together based on high NPP, PW, NSP, PL, HSW and GY. Genotype G36, G16, G28, G35, G17,G29,G5, G8, G4, G2, G30, G38, G42, G48,G21,G31,G11 were further grouped together based on high NB, PH and LL. Lastly, genotypes G16 and G19 were grouped together based on superiority on LW.

**Table 4.6:** Summary of rotated principal loading scores, eigenvalue, percentage and cumulative variation for agronomic traits among 50 cowpea genotypes across test environments.

<b>Traits</b>	<b>PC1</b>	<b>PC2</b>	<b>PC2</b>
NB	0.32	0.55	-0.44
PH	0.48	0.37	-0.60
LL	0.15	0.76	0.13
LW	0.05	0.66	0.62
NPP	0.83	-0.13	0.02
PL	0.85	-0.27	0.08
PW	0.83	-0.04	0.26
NSP	0.88	-0.07	0.08
HSW	0.89	-0.01	0.03
GY	0.87	-0.07	-0.02
<b>Eigen value</b>	4.79	1.55	1.03
<b>Variability (%)</b>	47.88	15.47	10.27
<b>Cumulative (%)</b>	47.88	63.35	73.62

NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*: significant at 5% level of significance, \*\*: significant at 1% level of significance



**Figure 4.1:** Biplot demonstrating the relationship among agronomic traits among 50 cowpea genotypes evaluated across Brits, Loskop, Roodeplaat, Mafikeng and Polokwane during 2019 and 2020 cropping season. (See genotype codes in Table 4.2).

#### 4.4 Discussion

Understanding the degree of the relationship between yield and yield related traits is the prerequisite research for the improvement of any neglected crop such as cowpea for maintaining sustainable genetic advancement. The analysis of phenotypic variations among genotypes for different traits and their associations to contribute to yield production would be of great importance for any successful breeding programs. The current study determined the phenotypic diversity/variation and correlation relationship for yield and yield related traits in cowpea genotypes evaluated in different regions of South Africa. The wide variation recorded among the assessed traits in this study will provides a better opportunity for improvement and would

allow the selection of varieties with better quality for grain yield for maximizing cowpea production.

The analysis of variance indicated the existence of variability among the genotypes evaluated since the effect of genotypes, environment and genotype-by-environment was significant for all the traits evaluated. The results showed the significant differences at 5% and 1% level of probability among all studied agronomic traits. The significant genotypic differences observed for yield and its related traits evaluated under different environments shows the degree of genetic variation which allows the further development of cowpea genotypes for yield improvement. The significant effect on environments reveals that there was a considerable impact of environments on yield and yield components for all evaluated genotypes (Khan *et al.*, 2022; Masindeni *et al.*, 2006). The significant effect on the interaction of genotype and environments signifies that the genotypes assessed had a different response in the test environments which allows the identification and selection of cowpea genotypes with specific adaptations to improve cowpea productivity. Similarly, the studies by Adewale *et al.* (2010) reported significant variation among cowpea genotypes for assessed grain yield components in Nigeria and Owusu *et al.* (2021) also reported significant variation among cowpea genotypes for maturity and yield traits assessed in the Northern Ghana. The studied genotypes revealed a considerable diversity among yield and yield related traits.

The overall mean and comparison of each evaluated yield and yield components among cowpea genotypes across the five sites are presented in Table 4.3. The overall mean performances showed that there were greater variations for all the traits assessed with the ranges of 6.01 - 8.87, 84.21-168.22 cm, 7.15 - 16.49 cm , 6.15 - 10.83 cm, 82.04 - 106.17, 15.12 - 22.36 cm, 8.45 - 9.89 mm, 11.98 - 17.07, 19.01 - 26.59 g and 0.13 - 0.47 t ha<sup>-1</sup> for NB, PH, LL, LW, NPP, PL, PW, NSP, HSW and GY . Genotypes such as G43 were selected for superior for traits such as NB, LL, and LW, then G8 was selected based its superiority on plant height. Further genotype G35 was selected as the superior genotypes in traits including NPP, PW, NSP, HSW and GY while G1 was selected as the best performer for PL. The selected genotypes could be considered as potential sources of gene to improve these traits and could serve as parental genotypes in breeding programs targeting enhanced high-yielding varieties. The superior performance of these genotypes is very essential as it allows indirect selection to improve cowpea yield productivity. Similarly, other studies have selected genotypes of cowpea with superior performances in agronomic traits to enhance grain yield production (Siwale *et al.*, 2022; Mbuma *et al.*, 2022; Gerrano *et al.*, 2022).

The analysis of association of agronomic traits contributing to yield performances and their variability among genotypes would be of great importance for successful crop improvement programs. The knowledge of correlation coefficients analysis among the traits is beneficial to breeding programs as it indicates which traits should be targeted for selection to increase productivity of yield (Mashilo *et al.*, 2016). The positive and significant association of characters to improve grain yield in cowpea is important as it suggests that high possibility of simultaneous selection of these traits. In this study, the correlation coefficients results revealed the production of NB had significant and positive correlations with PH, NPP and HSW. These findings concur with the study by Gerrano *et al.* (2022) which also reported a positive and significant correlations of NB with NPP and HSW. This results therefore indicates that NB can be indirectly used as a sole criterion in selecting for enhancement of NPP and HSW. In addition, Ddamulira *et al.* (2017) has stressed out that plant height could be a good determinant for the number of branches produced on main stem.

Further, PH revealed a positive and significant correlation with LL and PL. Likewise, LL was positively and significantly correlated to LW, however, these traits did not have any significant correlations with GY which indicates that selection for traits such as PH, LL and LW may not have influence the productivity of grain yield. The positive correlations indicated by most traits shows that multiple traits selection would be possible as they have direct proportional influence against each other. The study further revealed that NPP positively and significantly correlated with PL, PW, NSP and GY. Similarly, Lazard *et al.* (2017) also reported positive and significant correlation between NPP with NSP and GY. PL, PW, NSP and HSW also revealed strong, positive and significant correlation relationship with GY. However, Gerrano *et al.* (2015) reported a negative correlation between NSP and GY in cowpea which contradict with the current findings.

Nevertheless, the present findings indicates that NPP, PL, NSP and HSW can be used as a proxy trait for increased grain yield. For instance, genotype G37 and G1 which were among the top yielding genotypes with 0.45 and 0.41 t ha<sup>-1</sup> grain yield had the highest NPP value of 105 and 106, respectively. In addition, the positive and significant correlation of NPP and NSP with GY may be expected in cowpea because the increased production of pod number significantly increases the grain yield productivity. Therefore, the concentration should be focused on traits such as NPP, PL, PW, NSP and HSW for the improvement of yield in cowpea. The selection of cowpea varieties which are characterized with high NPP, PL, PW, and NSP should be considered to maximize yield production in cowpea.

The principal component analysis was further done to measure the distinct variations and interrelationships between the traits by grouping them. Mofokeng *et al.* (2020) has also alluded that the use of PCA is among on the approach that breeders has adopted to selects characters with high influence for meaningful selection in cultivar development. This study identified NB, NPP, PL, PW, NSP, and HSW as the important traits because of their high contribution on the PCs (Table 3), which suggests that they could be as sole or simultaneous selection criteria in the aid of cowpea production improvement programs. Therefore, the genotypes of cowpea that reveals high mean performances in these traits would be selected for improvement. Evidently, the studies by Mofokeng *et al.* (2020) also reported number of branches, number of pods per plant, number of seeds per pod, hundred seed weight and seed weight as the influential traits in cowpea production improvement.

Concurrently, the PCA biplot was also used to distinguish the variations among the genotypes based on the assessed traits. The biplot depicted three groups of cowpea genotypes based on their associations with assessed traits. Genotypes G47, G3, G15, G1 and G7 were grouped together based on their high exhibition of NPP, PW, NSP, PL, HSW and GY. Further Genotypes G36, G16, G28, G35, G17, G29, G5, G8, G4, G2, G30, G38, G42, G48, G21, G31, G11 were grouped together due to their high performance for NB, PH and LL. Lastly, genotypes G16 and G9 were grouped together based on their excellent performance on LW. These genotypes could be of great genetic material for breeding for improvement and conservation of genetic resources for improved cowpea productivity. The genotypes showed clear distinctions amongst them which indicates that the genotypes were genetically different and possessed significant variation. The extensive variety reported in the study more especially for the NB, PH, NPP, NSP, HSW and GY indicates the diversity among the cowpea genotypes and further implying that there is a possibility of genetically enhancing these traits through selection.

#### **4.5 Conclusion**

The study revealed significant variability among the cowpea genotypes for the yield components and further indicated the potential for simultaneous selection. Cowpea genotypes such as Acc-Cowp2, Acc-Cowp10, Acc-Cowp9, Acc-Cowp38, Acc-Cowp47, Acc-Cowp40 and 98K\_5301 were selected for recommendation in breeding programs for increased yield and yield traits. The PCA identified NB, NPP, PL, PW, NSP, and HSW as the important traits because of their high contributions which suggests that they could be as sole or simultaneous selection criteria in the aid of cowpea production improvement programs. The cluster analysis

distinguished genotypes into four distinct groups which were associated high possession of grain yield traits which could be listed in selection criteria for good parental combinations in future breeding programs.

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## CHAPTER 5: VARIATIONS IN GRAIN NUTRITIONAL AND PHYTOCHEMICAL COMPOSITIONS AMONG COWPEA [*VIGNA UNGUICULATA* (L) Walp] GENOTYPES GROWN UNDER DRYLAND FARMING SYSTEM IN SOUTH AFRICA.

### Abstract

Cowpea (*Vigna unguiculata* (L) Walp) is an important legume crop with greater potential to address food and nutritional insecurity in Sub-Saharan Africa. The objectives of this study were to quantify grain nutritional and phytochemical traits among the 50 cowpea genotypes to select superior lines with high nutritional compositions for cultivar development for nutritional quality. Fifty cowpea genotypes were planted at field conditions in a 10 x 5 alpha lattice design replicated three times at four different locations. Cowpea grain samples were analysed for Ca, Cu, Fe, K, Mg, Na, P, Zn, protein, fat, flavonoids, phenolic and condensed tannins. Genotype, environment and genotype-by-environment interaction effects were significant ( $P \leq 0.01$ ) for all traits except for fat and flavonoids contents. The significant and positive correlations recorded between the essential mineral elements indicate that the minerals can be improved simultaneously while the significant but different negative correlations between mineral and phytochemicals indicates that selecting the genotypes with high concentration of phytochemical traits might unintentionally reduce the contents of essential minerals. The result in this study will contribute to cowpea breeding programs targeting high nutritional value in order to assist with alleviating malnutrition among poor communities who require cheaper sources of plant-based nutrients.

**Key words:** Cowpea, correlations, food security, minerals, phytochemical, principal component analysis, variations.

## 5.1 Introduction

A majority of the world's population consume food with lowered concentrations of essential nutrients, including vitamins, minerals and most importantly protein, than their daily minimal requirements (Masuda *et al.*, 2020). As a result, about 2 billion people globally are affected by nutrient deficiencies known as “hidden hunger” which affect all groups of communities with the infants, babies and pregnant women being the most vulnerable (Silva *et al.*, 2021; Kumar and Pandey, 2020). This has worsened the world's food insecurity challenges and contributes to the decline in human health status. This may be due to the fact that the diet of people from developing countries is dominated with cereal-based food which has high energy but less nutritious. The deficiencies in nutrients in the body can result in a vast increase in the prevalence of diseases and disorders that afflicts billions of people, more especially the pregnant women, infants and children in developing countries.

Some of the diseases that are related to nutrient deficiencies include iron and zinc deficiencies which has regarded as a global challenge affecting mostly pregnant women and infants. Iron and phosphorus deficiencies can cause diseases such as anaemia, abnormal pregnancies, poor resistance to infections and impaired neural motor development while zinc deficiencies can cause diseases such as growth retardation, delayed skeletal and sexual maturity, alopecia, dermatitis and diarrhoea (WHO, 2005). Furthermore, diseases such as rickets, osteoporosis and osteopenia can be caused by calcium deficiencies in the body and then diseases such as hyponatremia and hypokalaemia are associated with the low concentration of sodium, magnesium and potassium in the body, respectively.

Grain legumes are an important constituent element of the diets for majority of the people in the world and they play an important role in food and nutritional security (Mudryj *et al.*, 2014; Jha *et al.*, 2020). Cowpea is an important indigenous and underutilised legume crop which is recognized as an excellent and inexpensive source of plant based protein, fatty acids, vitamins, and minerals, important to meet the dietary needs of food insecure households. These mineral nutrients can be distinguished into macro- and micronutrients. The macro nutrients includes phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) whilst the micro or trace minerals includes boron (B), chlorine (Cl), chromium (Cr), fluoride (F), iodine (I), iron (Fe), molybdenum (Mo), sodium (Na), manganese (Mn), nickel (Ni), selenium (Se), vanadium (V) and zinc (Zn) (Gupta and Gupta., 2014; Sombié *et al.*, 2018). The crop is widely grown in the tropical and subtropical agro ecologies of the world. According to the global estimates by the FAOSTAT (2020), about 8.9 million metric tonnes of cowpea grains are

produced annually on about 14.4 million hectares of land. Cowpea thrives under a wide array of environmental stress factors, including biotic (i.e. fungal, viral, bacterial, root-knot nematodes, parasitic and insect pests diseases) and abiotic (i.e. drought, extreme heat stresses and poor soil fertility), making the crop ideal crop for production under resource-poor production conditions.

Significant ranges of nutrient quantities were reported in cowpea grains, including 22.5- 25.6 % protein, 28.3-36.2% starch, 1.3 -1.9 % fat, 1.7- 3% insoluble fiber and 3.2- 3.7 % essential minerals (Antova *et al.*, 2014). The recent study by Gerrano *et al.* (2022b) assessed the nutritional traits in cowpea grown under various conditions and reported mean ranges of 33.11 - 69.03 mg.100g<sup>-1</sup>, 4.00 - 4.70 mg.100g<sup>-1</sup>, 14.40 -19.63 mg.100g<sup>-1</sup> and 27.98- 31.98% for Fe, Zn, Mn and protein content, respectively. Comparably, Sombié *et al.* (2018) also studied the genotypic variation of mineral elements of cowpea cultivated in Burkina Faso and reported the mean ranges of 2.97- 7.07 mg.100g<sup>-1</sup>, 1.81-4.425 mg.100g<sup>-1</sup>, 118.78-239.80 mg.100g<sup>-1</sup>, 57.06-123.39 mg.100g<sup>-1</sup>, 1.19- 5.41 mg.100g<sup>-1</sup> and 719.08 – 1201.97 mg.100g<sup>-1</sup> for Fe, Zn, Mg, Ca, Na and K, respectively. Furthermore, the crop contains bioactive compounds such as polyphenols, tannins, protease inhibitors, phytates and flavonoids, which are beneficial for human health (Nassourou *et al.*, 2016; Mbuma *et al.*, 2020). However, the presence of these compounds, which are referred to as anti-nutritional factors, are a major factor limiting the bioavailability of nutrients (Ileke, 2004; Maina *et al.*, 2015). These anti-nutrients antagonize and reduce the nutritional value of food restricting the mineral bioavailability and digestibility of essential nutrients (Alghamdi *et al.*, 2019). For instance, the high concentrations of [polyphenols in cowpea play a major role in reduction of protein digestibility and starch bioavailability while condensed tannins have been reported to reduce the iron availability (Jayathilake *et al.*, 2018; Owade *et al.*, 2020).

There are studies that have reported the quantities of anti-nutritional factors in cowpea. Maina *et al.* (2015) assessed the polyphenols and phytates content in cowpea and reported the means ranges of 27.02- 323.02 mg GAE.100g<sup>-1</sup>, 3.77- 277.98 mg.100g<sup>-1</sup> and 60.90- 1497.31 mg.100g<sup>-1</sup> for total phenols, tannins and phytic acid content, respectively. Comparably, Alghamdi *et al.* (2019) also reported anti-nutritional factors quantities in cowpea with the mean ranges of 0.32 – 0.81 mg.g<sup>-1</sup>, 0.7 – 4.1 mg.g<sup>-1</sup>, 2.20 – 3.30 mg.g<sup>-1</sup>, 0.19 – 0.43 mg.100g<sup>-1</sup> and 2.33 – 3.27 mg.g<sup>-1</sup> for total phenols, flavonoids, trypsin inhibitor, tannins and phytic acids, respectively. The epidemiological studies have reported that consuming foods with the concentration of phenolics and antioxidants can help prevent human chronic non-communicable diseases such

as cancer, ageing, diabetes and cardiovascular diseases (Zhao *et al.*, 2014). These nutritional and phytochemical qualities make cowpea grain a key food resource to mitigate global nutritional deficiencies and to improve human health status. Therefore, genetic crop improvement targeting to enhance the concentration of nutritional and phytochemical compositions in cowpea grains is the key to alleviating hidden hunger. This therefore explains the need for further identification of superior varieties of cowpea with the enhanced nutritional and phytochemical compositions.

Significant genetic variation exists in cowpea grain nutritional and phytochemical compositions (Alidu *et al.*, 2020; Mbuma *et al.*, 2020; Nassourou *et al.*, 2016). Therefore, it is very important to allow the genetic improvement of the attributes such as nutritional and phytochemical compositions in current breeding programs. Key breeding programs such as the International Institute of Tropical Agriculture (IITA) in Africa is one of the largest cowpea core collections holding more than 5000 cowpea accessions released. The IITA plays a major role in sharing the germplasm with the national agricultural institutions including the South Africa. Some of the lines that were sourced in the IITA were recorded as improved lines in terms of nutrition such as protein, iron and zinc and some genotypes sourced from the IITA are recorded as the high yielding genotypes. However, there is very little or not much research has been done to assess nutritional quality including the anti-nutritional compounds available in cowpea in diverse environments in South Africa. Therefore, it is important to further evaluate the available germplasm for nutritional and phytochemical content in order to identify the superior genotypes which can be used as parents in breeding for nutrient-dense varieties.

There is currently very few research that has been carried out to assess genetic variation, the interaction of genotype-by-environment and the association of the genotypes with traits or mineral element content and phytochemical compounds in cowpea. The magnitude of genotype-by-environment interaction in this study will be quantified through conducting Mult environment trials (MET's). The MET's are useful in recommending the genotypes with narrow or broad adaptations regarding the nutritional content in cowpea (Yan *et al.*, 2000; Yan and Kang 2003; Yan and Tinker 2006). Genotypes with broad adaptation are associated with high stability (Yan *et al.*, 2000), suitable for production in different environments and for genetic advancement. It is stated that the availability of some nutrients elements in cowpea grains varies widely due to the genotype (Mwenye *et al.*, 2011) and environmental conditions in which the genotypes are planted (Kamara *et al.*, 2010). Therefore, it is important to carry out the MET's to account for genotype-by-environment interactions and its influences for

effective selection. Further, the multivariate analysis was conducted to assess the interrelationships between the test genotypes and the variables which improve selection strategies of cowpea improvements for food and nutritional security. Therefore, the objective of this study was to quantify the nutritional and phytochemical traits among the 50 cowpea genotypes to select superior lines with high nutritional compositions for cultivar development for nutritional quality.

## 5.2 Materials and Methods

### 5.2.1 Plant materials

Fifty cowpea genotypes obtained from the Agricultural Research Council germplasm collections from South Africa and Nigeria were used in this study (Table 5.1). The genotypes used were selected based on suitable agronomic attributes and origin.

**Table 5.1:** List of cowpea genotypes evaluated in the present study Source: (Gumede et al., 2022a; 2022b).

Genotype code	Genotype name	Origin	Growth habit	Seed shape	Seed color	coat	Grain yield (t ha <sup>-1</sup> )
G1	Acc-Cowp2	South Africa	Prostrate	Kidney	Red		0.45
G2	Acc-Cowp3	South Africa	Prostrate	Kidney	Brown		0.23
G3	Acc-Cowp4	South Africa	Prostrate	Kidney	Red		0.37
G4	Acc-Cowp5	South Africa	Prostrate	Rhomboid	Black		0.36
G5	Acc-Cowp6	South Africa	Prostrate	Kidney	Brown		0.24
G6	Acc-Cowp7	South Africa	Prostrate	Ovoid	Purple		0.17
G7	Acc-Cowp9	South Africa	Semi-erect	Rhomboid	Cream		0.37
G8	Acc-Cowp10	South Africa	Prostrate	Rhomboid	Purple		0.36
G9	Acc-Cowp11	South Africa	Climbing	Kidney	Light brown		0.18
G10	Acc-Cowp12	South Africa	Prostrate	Rhomboid	Brown		0.13
G11	Acc-Cowp13	South Africa	Prostrate	Kidney	Brown		0.23
G12	Acc-Cowp14	South Africa	Erect	Ovoid	Red		0.31
G13	Acc-Cowp15	South Africa	Prostrate	Ovoid	Red		0.20
G14	Acc-Cowp16	South Africa	Prostrate	Rhomboid	Black		0.36
G15	Acc-Cowp17	South Africa	Erect	Ovoid	Cream		0.41

<b>G16</b>	Acc-Cowp18	South Africa	Erect	Rhomboid	Red-speckled	0.19
<b>G17</b>	Acc-Cowp19	South Africa	Erect	Globose	Light brown	0.35
<b>G18</b>	Acc-Cowp20	South Africa	Erect	Rhomboid	Red	0.25
<b>G19</b>	Acc-Cowp21	South Africa	Erect	Kidney	Cream	0.22
<b>G20</b>	Acc-Cowp22	South Africa	Prostrate	Rhomboid	Brown	0.19
<b>G21</b>	Acc-Cowp24	South Africa	Climbing	Rhomboid	Black	0.15
<b>G22</b>	Acc-Cowp25	South Africa	Climbing	Rhomboid	Red	0.19
<b>G23</b>	Acc-Cowp26	South Africa	Semi-erect	Kidney	Grey-speckled	0.22
<b>G24</b>	Acc-Cowp27	South Africa	Semi-erect	Kidney	Red-speckled	0.20
<b>G25</b>	Acc-Cowp28	South Africa	Prostrate	Kidney	Red	0.22
<b>G26</b>	Acc-Cowp29	South Africa	Prostrate	Kidney	Brown	0.18
<b>G27</b>	Acc-Cowp30	South Africa	Semi-erect	Globose	Black	0.30
<b>G28</b>	Acc-Cowp31	South Africa	Prostrate	Globose	Cream	0.36
<b>G29</b>	Acc-Cowp32	South Africa	Prostrate	Kidney	Red	0.34
<b>G30</b>	Acc-Cowp33	South Africa	Prostrate	Kidney	Red	0.14
<b>G31</b>	Acc-Cowp34	South Africa	Semi-erect	Rhomboid	Light red	0.30
<b>G32</b>	Acc-Cowp35	South Africa	Climbing	Kidney	Light brown	0.18
<b>G33</b>	Acc-Cowp36	South Africa	Climbing	Kidney	Light brown	0.20
<b>G34</b>	Acc-Cowp37	South Africa	Prostrate	Kidney	Light brown	0.22
<b>G35</b>	Acc-Cowp38	South Africa	Climbing	Globose	Black	0.47
<b>G36</b>	Acc-Cowp39	South Africa	Semi-erect	Kidney	Red	0.36
<b>G37</b>	Acc-Cowp40	South Africa	Semi-erect	Kidney	Black	0.26
<b>G38</b>	Acc-Cowp41	South Africa	Erect	Ovoid	Cream	0.20
<b>G39</b>	Acc-Cowp43	South Africa	Semi-erect	Kidney	Brown	0.22
<b>G40</b>	Acc-Cowp44	South Africa	Erect	Kidney	Light brown	0.25
<b>G41</b>	Acc-Cowp45	South Africa	Prostrate	Ovoid	Cream	0.34
<b>G42</b>	Acc-Cowp46	South Africa	Erect	Kidney	White	0.24
<b>G43</b>	Acc-Cowp47	South Africa	Erect	Rhomboid	Brown	0.32
<b>G44</b>	Acc-Cowp48	South Africa	Prostrate	Kidney	Cream	0.22

<b>G45</b>	Acc-Cowp49	South Africa	Prostrate	Rhomboid	Brown	0.16
<b>G46</b>	Acc-Cowp50	South Africa	Prostrate	Kidney	Black	0.26
<b>G47</b>	98K-5301	South Africa	Erect	Kidney	White	0.43
<b>G48</b>	Glenda	South Africa	Semi-erect	Kidney	Brown	0.20
<b>G49</b>	TVU13953	South Africa	Prostrate	Kidney	Cream	0.21
<b>G50</b>	VegCowDakCream	South Africa	Erect	Kidney	White	0.30

Note: VegCowDakCream = Vegetable cowpea Dakama Cream

### 3.2.2 Description of study sites

The field experiments were evaluated at Brits (25.6276° S, 27.7816° E, 1083 m above sea level), Loskop, Groblersdal experimental farm (25.1773° S, 29.3936° E, 920 m above sea level), Roodeplaat (25.6080° S, 28.3525° E, 1168 m above sea level), Mafikeng, University of North-West experimental farm (25.8201° S, 25.6298° E, 1359 m above sea level) and Polokwane, Ga-Molepo (24.0295° S, 29.7425° E, 495 m above sea level). The description of climatic and soil conditions for the studied sites is presented in Table 5.2.

**Table 5.2:** Soil characteristics, altitude and mean annual weather data for average rainfall, and minimum (T<sub>min</sub>) and maximum (T<sub>max</sub>) air temperature information for the studied environments. Source: SA Weather Services (weathersa.co.za).

Environment	Soil type	Altitude (masl)	Average rainfall (mm)	T <sub>min</sub> (°C)	T <sub>max</sub> (°C)
Brits	Clay loam	1083	629	18.3	28.9
Loskop	Loamy	920	497	18.6	30.0
Roodeplaat	Clay loam	1168	772	10.0	21.0
Mafikeng	Sandy	1359	571	17.6	28.8
Polokwane	Clay loam	1310	495	16.7	27.6

Note: Masl = meters above sea level, Min = Minimum, Max = Maximum.

### 3.2.3 Experimental design

The cowpea genotypes were evaluated using a 10 x 5 alpha-lattice experimental design replicated three times. Each genotype was sown in a **6 m<sup>2</sup>-plot comprised of two 3 m-rows**. The intra- and inter-row spacings were 0.3 m and 1 m, respectively. Two seeds were hand sown per hole and later thinned to one seedling two weeks after emergence. The plants were cultivated under rainfed conditions, with supplemental moisture supplied using sprinkler irrigation system when the rainfall was not enough for optimal growth and development.

Agronomic practices such as weeding, insects and diseases control were carried out using chemical and cultural practices as per recommendation for cowpea. To simulate low-input soil conditions, no fertiliser was applied.

### **3.2.4 Data collection**

#### **3.2.4.1 Quantification of nutritional quality traits**

The nutritional analyses were conducted in three replicates for each genotype after oven drying the mature seeds in a forced-air oven for 24 hours at 80°C to reduce moisture content to less than 12.5%. The dried seeds were ground to flour using the laboratory electric seed blender (MiniMix 100 W CC, Interscience) and sieved using 0.5 mm sieve. The quantification of mineral elements was done as described by Ang and Lee (2005) at the Agricultural Research Council analytical laboratory in Pretoria. Briefly, an amount of 0.5 g of flour samples for each genotype under each site were weighed and then digested with 10 ml of 1:3 nitric acid: hydrochloric acid. The mixture was therefore boiled at 95°C until the sample was dissolved. The concentrations of mineral elements namely Ca, Fe, K, Mg, P, Na, Zn, Cu were quantified using the inductively coupled plasma atomic emission spectroscopy (ICPE-9820, Shimadzu Corporation, Kyoto, Japan) and were expressed in mg.100 g<sup>-1</sup>. The determination of fat content was done using the Soxhlet method using diethyl ether (AOAC Method 920.39) as described by Norhayati et al. (2018). Crude protein content (N × 6.25) was determined by the combustion method (Leco ®model, FP-528, St. Joseph, MI).

#### **3.4.4.2 Quantification of phytochemical traits**

For the determination of phenolic and flavonoid content, the procedure was described by Amoo et al. (2012). An amount of 0.2 g of cowpea sample was extracted in an ultrasonic bath containing ice-cold water by sonication for 30 minutes using 10 mL of 50% aqueous methanol (MeOH). The total phenolic content was determined as described by Makkar (2003) using the Folin-Ciocalteu method. The calibration curve was determined using gallic acid as a standard and the results were expressed in mg gallic acid equivalent (GAE) per gram. The flavonoid content was further determined using an aluminium chloride method as described by Marinova et al. (2005). A reaction mixture which contains 250 µL sample extract, 75 µL of aluminum chloride (10% w/v), 1.6 mL of distilled water, 75 µL (5% w/v) sodium nitrite, and 0.5 mL (1 M) NaOH was briefly vortexed and absorbance measured at 510 nm. The calibration curve was done using catechin as a standard and the flavonoids content results were expressed in mg catechin equivalent (CE) per gram.

The condensed tannins content was determined using the butanol-HCl method as described by Makkar (2003). Three millilitres of butanol-HCl (95:5 v/v) were added to 500 µl of each sample, followed by 100 µl of ferric reagent (2% w/v ferric ammonium sulphate in 2 N HCl). The mixtures were placed in a boiling water bath for 60 min. The absorbance was then read at 550 nm using a UV–visible spectrophotometer (Varian Cary 50, Australia) against a blank prepared in a similar way but without heating. Each sample had three replicates. Condensed tannins (% per dry matter) as leucocyanidin equivalents were calculated using the formula described by Porter et al. (1986):

$$\text{Condensed tannins \% per dry matter} = A_{550 \text{ nm}} \times 78.26 \times \text{Dilution factor \% dry matter}$$

Where  $A_{550 \text{ nm}}$  is the absorbance value at 550 nm.

### **3.2.5 Data analysis**

The data collected on nutritional and phytochemical traits were subjected to analysis of variance (ANOVA) across all locations using Genstat 20<sup>th</sup> edition (VSN International, Hemstead, UK). The mean values were separated at 5% probability level using Fischer's unprotected least significant difference (LSD). Subsequently, the Pearson correlation analysis was conducted to deduce trait and genotype association using Genstat 20<sup>th</sup> (VSN International, Hemstead, UK) software. Principal component analysis (PCA) was conducted using Genstat among the 50 tested genotypes. Further the dendrogram analysis was done on SPSS using the single linkage and Euclidean distance method using SPSS version 25 (SPSS Inc., Chicago, IL, USA 2008).

## **5.3 Results**

### **5.3.1 Genotype, environment, and genotype-by-environment interaction effects on nutritional and phytochemical traits**

The combined analysis of variance across all test environments highlighted the significant effects for all nutritional and phytochemical traits for genotype, environment, and genotype by environment interaction evaluated except for flavonoids and fat content (Table 5.3). The effects of genotypes revealed high significance ( $p \leq 0.001$ ) for all mineral elements evaluated, protein, total phenolics and condensed tannins except for flavonoids and fat contents which showed no significant effects. This results therefore suggests the existence of genetic variation among the tested genotypes. Furthermore, the environmental effects showed high significance at  $p \leq 0.001$  for Ca, Cu, K, Mg, Na, P, Zn and protein. It then showed significant effects at  $p \leq 0.05$  for Fe and showed no significant differences for fat content and flavonoids. This indicates that the

assessed traits are highly influenced by the environmental conditions in which they are subjected to. Likewise, the effects of genotype-by-environment interaction were highly significant ( $p \leq 0.001$ ) for all mineral elements, protein, total phenolics and condensed tannins and showed significance at  $p \leq 0.05$  for fats content. These results signify the different response of genotype in the test environment.

**Table 5.3:** Combined analysis of variance showing mean square values and significant tests for nutritional and phytochemical traits among the 50 assessed genotypes across all test environments.

Source of variation	d.f	Ca	Cu	Fe	K	Mg	Na	P	Zn	Prot	Fat	Phen	Flav	Cond.tann
Block	2	52.00	6.75	3015	20205	4418	128520	3.42	0.35	0.08	54.53	0.03	24.19	0.21
Genotype (G)	49	176.26***	1.93***	6793***	541.98***	2427.93***	1039.06***	9.47***	1757.57***	22.94***	59.11 <sup>ns</sup>	14.07***	31.06 <sup>ns</sup>	23.56***
Environment(E)	3	834.81***	2.84***	11352*	968.39***	5518.01***	1825.37***	4.67***	30475.78***	578.53***	81.08 <sup>ns</sup>	311.17***	52.56 <sup>ns</sup>	305.43***
G x E	147	136.22***	2.44***	6058***	299.667***	2043.57***	9502.26***	8.99***	2988.43***	13.06***	63.84*	11.25***	27.95 <sup>ns</sup>	16.97***
Error	398	1430	5.56	2886	6258	1332	1522.45	2.07	21.27	0.08	48.25	0.07	26.52	0.08
<b>CV (%)</b>		<b>6.9</b>	<b>4.9</b>	<b>12.3</b>	<b>5.6</b>	<b>5.1</b>	<b>7.7</b>	<b>8.0</b>	<b>17.2</b>	<b>1.3</b>	<b>17.6</b>	<b>8.6</b>	<b>15.8</b>	<b>9.9</b>
<b>LSD (5%)</b>		<b>10.69</b>	<b>1.19</b>	<b>43.86</b>	<b>64.59</b>	<b>58.59</b>	<b>26.3</b>	<b>30.70</b>	<b>7.4</b>	<b>0.48</b>	<b>11.1</b>	<b>0.44</b>	<b>8.26</b>	<b>0.44</b>
<b>SE</b>		<b>30.87</b>	<b>0.74</b>	<b>23.45</b>	<b>45.67</b>	<b>29.80</b>	<b>18.6</b>	<b>27.35</b>	<b>4.61</b>	<b>0.24</b>	<b>5.61</b>	<b>0.27</b>	<b>5.15</b>	<b>0.22</b>

d.f. = degree of freedom; Ca = calcium; Cu = copper; K = potassium; Fe = iron; Mg = magnesium; Na = sodium; P = phosphorus; Zn = zinc; Prot = protein content; Phen = Phenolics; Flav = Flavonoids; Cond.tann = condensed tannins; G x E = genotype by environment interaction; CV = coefficient of variation; LSD = least significant difference; SE = standard error;\*\*\*, \*\* and \* significant at 0.001, 0.01 and 0.05 probability level.

### 5.3.2 Mean concentrations of nutritional and phytochemical traits

Mean concentrations of the studied nutritional and phytochemical traits among 50 cowpea genotypes across four environments are presented in Table 5.4. The evaluated cowpea genotypes showed variation across all test environments for all nutritional and phytochemical traits. The mean ranges of mineral elements ranged from 185.62 - 1822.51 mg.100 g<sup>-1</sup>, 10.34 – 26.76 mg.100 g<sup>-1</sup>, 6.10 - 31.5 mg.100 g<sup>-1</sup>, 527.30 - 2690.50 mg.100 g<sup>-1</sup>, 275.40 - 2212.60 mg.100 g<sup>-1</sup>, 64.00 - 940.00 mg.100 g<sup>-1</sup>, 893.70 - 3632.80 mg.100 g<sup>-1</sup>, and 12.33 - 68.46 mg.100 g<sup>-1</sup> for Ca, Cu, Fe, K, Mg, Na P and Zn, respectively.

Furthermore, the total protein content and fat content ranged from 19.84-26.94 %, fat and 1.21- 15.51 %, respectively. Moreover, the phytochemical compounds ranged from 1 .65- 6.50 mg GAE/g, 0.53- 12.17 mg CE/g and 0.60- 6.25 mg cyanidin chloride/g for total phenolic content, flavonoids and condensed tannins, respectively. Cowpea genotypes G22, G4 , G34 , G7 , G23 , G31 , G32 , G1 , G45 and G15 recorded the highest mean concentration for Fe, Ca, Cu, K, Mg, Na, P, Zn, total protein content, fat content, total phenolic content, flavonoids and condensed tannins, respectively. Further, genotypes G36, G37, G41, G32, G1, G34, and G17 recorded the lowest concentration of Ca, Cu, Fe, K, Mg, Na, P and Zn. The genotypes G25 and G20 recorded the lowest concentration of protein and fat content, respectively. Genotypes G31, G44 and G25 recorded the lowest concentrations of total phenolics, flavonoids and condensed tannins.

**Table 5.4:** The mean concentrations of nutrient elements and phytochemical traits of 50 cowpea genotypes across all locations.

Genotype code	Ca	Cu	Fe	K	Mg	Na	P	Zn	Prot.	Fat	Phenols	Flavonoids	Cond. tannins
	.....mg/100g.....								.....%.....		Mg GAE/g	Mg CE/g	Mg cyanidin chloride/g
G1	234.23	13.60	12.20	1375.50	275.40	94.00	893.70	27.53	22.52	1.59	6.50	1.32	3.08
G2	222.21	15.28	9.40	1929.80	414.00	232.00	1448.90	26.95	22.23	1.38	3.50	0.56	1.52
G3	302.23	15.14	27.20	644.00	588.40	681.00	2327.10	13.76	22.59	3.13	1.99	1.11	2.92
G4	1822.51	22.07	86.00	1245.50	2158.10	567.00	3632.80	67.97	23.19	1.75	2.41	0.66	3.71
G5	1460.1	17.89	65.40	1127.30	1591.30	323.00	2707.20	33.94	24.66	3.91	4.34	1.46	3.37
G6	252.35	26.22	12.40	2668.20	466.30	83.00	1595.30	24.21	23.97	1.62	5.05	1.23	2.34
G7	1203.9	16.75	66.40	1365.70	1773.90	675.00	3024.20	68.46	25.69	2.04	2.36	1.36	2.87
G8	449.74	10.85	33.00	577.10	532.90	143.00	1069.20	17.25	23.97	1.66	3.03	1.38	3.70
G9	734.14	14.50	37.50	739.70	899.40	134.00	1288.70	26.83	23.44	2.96	3.21	2.09	3.11
G10	710.72	15.86	41.70	830.20	868.50	656.00	2630.90	40.16	23.81	3.67	2.75	2.15	1.93
G11	566.60	11.38	29.50	624.70	657.80	153.00	1051.00	18.83	24.92	5.34	2.45	1.26	2.60
G12	587.12	17.14	38.40	1627.90	823.60	683.00	3455.00	29.97	24.23	3.98	2.80	1.87	5.42
G13	629.40	13.64	28.40	744.30	974.90	126.00	1412.40	26.67	23.22	5.41	2.07	0.55	3.07
G14	282.20	20.58	10.20	2385.80	294.30	103.00	1407.60	26.93	21.99	1.36	4.91	1.32	1.35
G15	697.33	15.75	50.90	735.10	1022.60	751.00	3412.30	16.83	23.45	2.87	2.79	1.45	6.25
G16	522.41	14.23	34.20	1599.70	735.90	676.00	2021.20	24.58	24.77	5.25	1.85	1.59	2.39
G17	326.70	10.41	33.50	529.20	532.70	98.00	944.40	12.33	25.13	3.26	2.61	1.36	1.94

G18	329.83	12.78	18.90	1573.90	327.30	657.00	2871.20	16.42	23.14	3.73	2.66	1.83	5.39
G19	409.30	15.38	11.40	2330.00	753.80	134.00	1198.80	35.81	22.52	2.02	4.49	2.09	1.92
G20	400.61	12.96	38.40	606.40	681.00	688.00	1384.20	15.37	26.75	1.21	2.52	0.54	3.53
G21	734.54	18.05	32.20	1860.70	756.50	733.00	2326.90	28.87	25.26	3.10	3.20	1.45	2.57
G22	408.20	13.29	122.60	1655.50	604.20	101.00	1302.90	21.27	22.63	1.66	4.52	1.36	1.74
G23	255.20	17.75	14.30	2690.50	444.80	96.00	1512.00	25.35	22.93	1.41	2.47	1.12	0.82
G24	975.00	14.82	39.60	1991.50	923.20	762.00	3356.30	17.81	24.63	3.28	1.87	1.98	2.90
G25	284.00	12.63	7.90	1600.20	411.10	862.00	1235.30	38.45	19.84	1.67	3.34	1.34	0.60
G26	228.80	13.21	12.30	1413.50	422.40	93.00	954.60	24.17	23.37	1.71	3.43	1.40	1.02
G27	473.30	17.62	10.70	2027.30	644.70	88.00	1538.40	26.88	23.84	1.63	4.22	2.13	3.31
G28	341.41	14.10	45.90	673.70	442.40	661.00	2172.30	24.68	25.99	3.18	2.80	0.93	2.89
G29	1346.20	18.59	80.30	1313.50	2212.60	940.00	3619.00	25.53	23.30	2.30	2.04	1.30	5.34
G30	270.30	11.69	8.30	2099.00	502.60	73.00	1046.70	43.23	22.07	1.59	4.10	1.58	2.08
G31	679.30	13.61	33.20	1803.40	501.10	703.00	2628.80	17.25	26.94	3.27	1.65	0.68	5.91
G32	308.00	10.52	28.20	527.30	535.70	82.00	895.30	12.42	22.77	6.39	2.42	1.54	1.52
G33	302.90	12.52	32.50	564.90	457.40	107.00	989.30	15.48	23.79	4.18	2.01	1.80	3.60
G34	246.80	26.76	9.40	1862.30	404.20	64.00	1201.10	25.5	23.12	1.48	4.52	1.61	0.88
G35	757.70	13.58	42.70	817.70	1042.80	145.00	1282.70	24.3	24.42	3.06	2.30	1.58	2.82
G36	185.65	14.98	10.20	1984.20	416.40	82.00	1273.20	25.41	22.65	3.48	3.79	1.85	1.89
G37	418.51	10.34	47.60	618.70	631.80	138.00	1024.60	27.44	23.58	5.51	3.19	1.38	3.19
G38	265.61	12.56	7.60	2154.40	436.20	87.00	1059.70	27.63	21.87	1.62	3.30	3.28	0.66
G39	1417.10	14.10	73.10	1122.30	1724.50	743.00	3228.00	58.65	22.22	1.65	2.57	1.54	4.22

G40	384.71	10.79	31.10	570.70	578.20	96.00	1023.60	12.77	24.28	3.77	2.08	1.05	3.39
G41	277.80	11.62	6.10	1907.20	414.00	75.00	1067.50	26.34	24.81	1.54	3.32	1.62	1.32
G42	288.60	26.00	15.10	1856.00	407.00	106.00	1352.10	25.38	22.42	1.8	4.78	1.73	2.75
G43	357.80	11.54	7.10	2174.20	420.90	77.00	1240.50	24.4	26.62	1.38	4.41	2.78	3.02
G44	473.60	11.48	30.30	555.20	579.60	195.00	1222.00	17.33	23.8	2.24	2.93	0.53	2.29
G45	326.10	14.99	10.50	2606.20	595.00	128.00	1141.10	36.36	22.02	1.77	3.76	5.17	0.66
G46	963.10	13.72	38.70	770.20	1007.80	128.00	1360.80	25.15	22.2	3.34	2.06	1.36	2.93
G47	1268.10	16.81	38.30	1639.60	855.00	753.00	3351.30	29.3	23.41	4.91	1.69	0.80	4.63
G48	318.30	12.19	10.90	1315.70	385.00	74.00	1165.70	25.95	22.95	1.95	4.79	2.08	0.71
G59	312.80	12.15	23.90	559.50	380.30	568.00	3053.20	14.29	23.53	3.94	2.82	1.17	4.12
G50	260.90	22.01	18.40	2236.20	426.40	118.00	1380.80	25.35	23.98	1.58	4.62	1.16	1.69
<b>GM</b>	<b>545.45</b>	<b>15.13</b>	<b>31.5</b>	<b>1404.63</b>	<b>718.72</b>	<b>331.00</b>	<b>1795.64</b>	<b>26.87</b>	<b>23.63</b>	<b>2.97</b>	<b>3.19</b>	<b>1.67</b>	<b>2.76</b>
<b>Min</b>	<b>185.62</b>	<b>10.34</b>	<b>6.10</b>	<b>527.30</b>	<b>275.40</b>	<b>64.00</b>	<b>893.70</b>	<b>12.33</b>	<b>19.84</b>	<b>1.21</b>	<b>1.65</b>	<b>0.53</b>	<b>0.60</b>
<b>Max</b>	<b>1822.51</b>	<b>26.76</b>	<b>122.60</b>	<b>2690.50</b>	<b>2212.60</b>	<b>940.00</b>	<b>3632.80</b>	<b>68.46</b>	<b>26.94</b>	<b>6.39</b>	<b>6.50</b>	<b>5.17</b>	<b>6.25</b>

Note: Ca= calcium; Cu= copper; K= potassium; Fe= iron; Mg=magnesium; Na= sodium; P= phosphorus; Zn= zinc; GM= grand mean; Min- minimum; Max = maximum

### 5.3.3 The correlation analysis among the nutritional and phytochemical traits

Pearson correlation coefficient values describing the level of association among the studied nutritional and phytochemical traits across the studied sites are presented in Table 5.5. Calcium had a significant and positive correlation with Fe ( $r = 0.60; p \leq 0.001$ ), Mg ( $r = 0.92; p \leq 0.001$ ), Na ( $r = 0.51; p \leq 0.001$ ) and condensed tannins ( $r = 0.48; p \leq 0.001$ ). Calcium further significantly and negatively correlated with total phenolic content ( $r = -0.40; p \leq 0.01$ ). Copper was positively and significantly correlated to K ( $r = 0.49; p \leq 0.001$ ), P ( $r = 0.27; p \leq 0.05$ ), Zn ( $r = 0.28; p \leq 0.05$ ) and total phenolic content ( $r = 0.34; p \leq 0.01$ ) while it negatively correlated with fat content ( $r = 0.31; p \leq 0.01$ ). Similarly, a significant and positive correlation was recorded for Fe with Mg ( $r = 0.71; p \leq 0.05$ ), Na ( $r = 0.40; p \leq 0.01$ ), P ( $r = 0.51; p \leq 0.001$ ), Zn ( $r = 0.28; p \leq 0.05$ ) and condensed tannins ( $r = 0.42; p \leq 0.01$ ). However, a significant and negative correlation was also recorded between Fe and total phenolic content ( $r = -0.33; p \leq 0.05$ ).

A significant and positive correlation was observed for K with total phenolic content ( $r = 0.48; p \leq 0.001$ ) and flavonoids ( $r = 0.34; p \leq 0.05$ ) and significantly and negatively correlations with condensed tannins ( $r = -0.35; p \leq 0.05$ ). Further, Mg has a significant and positive correlations with Na ( $r = 0.46; p \leq 0.001$ ), P ( $r = 0.64; p \leq 0.001$ ), Zn ( $r = 0.60; p \leq 0.001$ ) and condensed tannins ( $r = 0.48; p \leq 0.05$ ). A significant and positive correlation was also recorded for Na with P ( $r = 0.83; p \leq 0.01$ ) and condensed tannins ( $r = 0.55; p \leq 0.001$ ) while they possessed a negative and significant correlation with total phenolics ( $r = -0.52; p \leq 0.001$ ). Likewise, P had a significant and positive correlation with Zn ( $r = 0.33; p \leq 0.05$ ) and tannins ( $r = 0.65; p \leq 0.001$ ) while it negatively and significantly correlated with total phenolic content ( $r = -0.42; p \leq 0.001$ ). There was further a positive and significant correlation between total protein content and condensed tannins ( $r = 0.36; p \leq 0.05$ ) while it showed a significant but negative impact in total phenolic content ( $r = -0.28; p \leq 0.05$ ). Furthermore, a negative and significant association was observed between fat content and total phenolic content ( $r = -0.32; p \leq 0.05$ ) and also between total phenolic content and condensed tannins ( $r = -0.41; p \leq 0.05$ ).

**Table 5.5:** Pearson correlation coefficients among 50 cowpea mineral and phytochemical contents across all locations.

	Ca	Cu	Fe	K	Mg	Na	P	Zn	Protein content	Fat content	Total phenolics	Flavonoid	Condensed Tannins
	.....mg/100g.....								.....%.....	Mg GAE/g	Mg CE/g	Mg cynadin chloride/g	
Ca	-												
Cu	0.18 <sup>ns</sup>	-											
Fe	0.69 <sup>***</sup>	0.01 <sup>ns</sup>	-										
K	-0.20 <sup>ns</sup>	0.49 <sup>***</sup>	-0.38 <sup>**</sup>	-									
Mg	0.92 <sup>***</sup>	0.19 <sup>ns</sup>	0.71 <sup>***</sup>	-0.21 <sup>ns</sup>	-								
Na	0.51 <sup>***</sup>	0.05 <sup>ns</sup>	0.40 <sup>**</sup>	-0.16 <sup>ns</sup>	0.46 <sup>***</sup>	-							
P	0.71 <sup>***</sup>	0.27 <sup>*</sup>	0.51 <sup>***</sup>	-0.04 <sup>ns</sup>	0.64 <sup>***</sup>	0.83 <sup>**</sup>	-						
Zn	0.56 <sup>***</sup>	0.28 <sup>*</sup>	0.28 <sup>*</sup>	0.24 <sup>ns</sup>	0.60 <sup>***</sup>	0.21 <sup>ns</sup>	0.33 <sup>*</sup>	-					
Protein content	0.15 <sup>ns</sup>	-0.11 <sup>ns</sup>	0.17 <sup>ns</sup>	-0.20 <sup>ns</sup>	0.09 <sup>ns</sup>	0.19 <sup>ns</sup>	0.18 <sup>ns</sup>	-0.19 <sup>ns</sup>	-				
Fat Content	0.06 <sup>ns</sup>	-0.31 <sup>**</sup>	0.16 <sup>ns</sup>	-0.42 <sup>*</sup>	0.02 <sup>ns</sup>	0.04 <sup>ns</sup>	0.01 <sup>ns</sup>	-0.17 <sup>ns</sup>	0.10 <sup>ns</sup>	-			
Total Phenolic content	-0.40 <sup>**</sup>	0.34 <sup>**</sup>	-0.33 <sup>**</sup>	0.48 <sup>***</sup>	-0.37 <sup>ns</sup>	-0.52 <sup>***</sup>	-0.42 <sup>*</sup>	0.09 <sup>ns</sup>	-0.28 <sup>*</sup>	-0.32 <sup>*</sup>	-		
Flavonoids content	-0.13 <sup>ns</sup>	-0.03 <sup>ns</sup>	-0.20 <sup>ns</sup>	0.34 <sup>*</sup>	-0.08 <sup>ns</sup>	-0.17 <sup>ns</sup>	-0.15 <sup>ns</sup>	0.12 <sup>ns</sup>	-0.20 <sup>ns</sup>	-0.10 <sup>ns</sup>	0.16 <sup>ns</sup>	-	
Condensed tannins	0.48 <sup>***</sup>	-0.03 <sup>ns</sup>	0.42 <sup>*</sup>	-0.35 <sup>*</sup>	0.41 <sup>*</sup>	0.55 <sup>***</sup>	0.67 <sup>***</sup>	-0.08 <sup>ns</sup>	0.36 <sup>*</sup>	0.22 <sup>ns</sup>	-0.41 <sup>*</sup>	-0.27 <sup>ns</sup>	-

Ca= calcium; Cu= copper; K= potassium; Fe= iron; Mg=magnesium; Na= sodium; P= phosphorus; Zn= zinc; \*\*\*, \*\* and \* significant at 0.01 and 0.05 probability levels, respectively.

### 5.3.4 PCA of studied nutritional and phytochemical traits

PCA showing explained and cumulative variances of nutritional and phytochemical traits among the studied cowpea genotypes is presented in Table 5.6. Across the studied sites, PCA identified three principal components (PC's) of which PC1, PC2 and PC3 explained 36.27 %, 19.54 % and 9.85 % of total variation. The Ca, Mg, and P recorded high and positive loadings in PC1 whereas Cu, K and Zn recorded high and positive loadings in PC2. Further, the high and positive loadings were recorded for protein content, Fe, and condensed tannins in PC3.

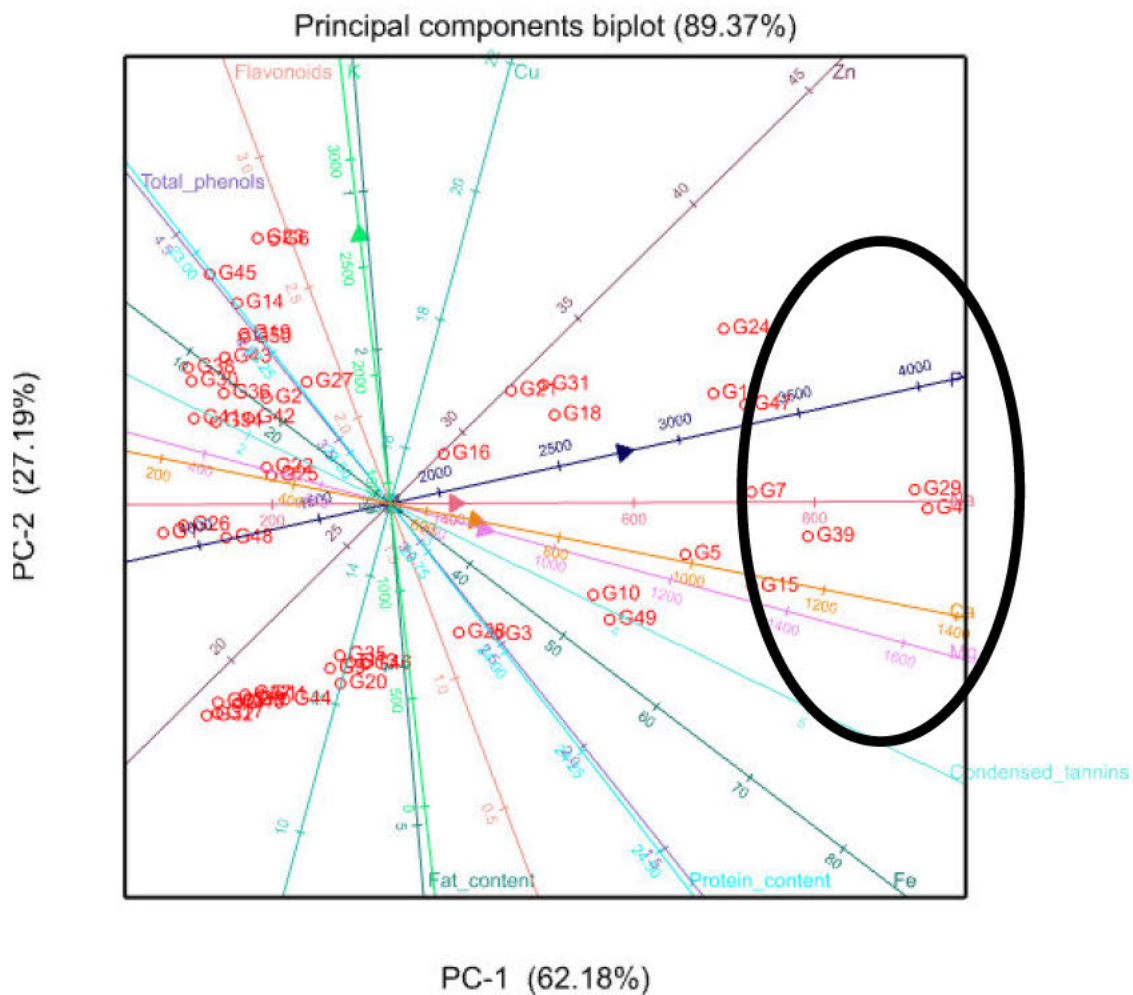
Principal component biplot showing the relationship between studied cowpea genotypes with nutritional and phytochemical traits across all test sites is shown in Figure 5.1. The genotypes with vectors that are plotted closer to a vector lines of study traits indicates the high correlation to the nutrient traits, while the genotype plotted furthest to the vector line indicates the weak association. According to Figure 1, G28 and G3 were in close proximity with protein content which indicates the high relationship amongst them. G7, G29 and G4 were specifically associated with Na, while G12 and G47 were highly associated with phosphorus. Genotypes G5, G15 and G48 were in close proximity with Ca and Mg, while G16 and G21 were specifically associated with Zn. Further, genotypes G27, G50, G14, G45 and G26 were in close proximity with total phenolics and flavonoids, while G48 and G10, and G36 were associated with condensed tannins fat content, respectively.

**Table 5.6:** Principal components, Eigen values, the proportion of variance and their percentage contributions of different mineral elements and phytochemical traits among 50 cowpea genotypes.

Traits	PC1	PC2	PC3
Ca	0.87	0.29	-0.20
Cu	0.05	0.70	0.39
Fe	0.76	0.02	-0.26
K	-0.39	0.70	0.29
Mg	0.84	0.33	-0.29
Na	0.76	0.04	0.30
P	0.84	0.24	0.30
Zn	0.35	0.69	-0.39
Protein content	0.31	-0.37	0.44
Fat content	0.19	-0.56	-0.32
Phenolic content	-0.62	0.46	0.06
Flavonoids	-0.28	0.29	-0.32
Condensed tannins	0.71	0.25	0.36
Eigen values	4.75	2.54	1.28

Proportion of variance (%)	36.27	19.54	9.85
Cumulative variance (%)	36.27	55.81	65.66

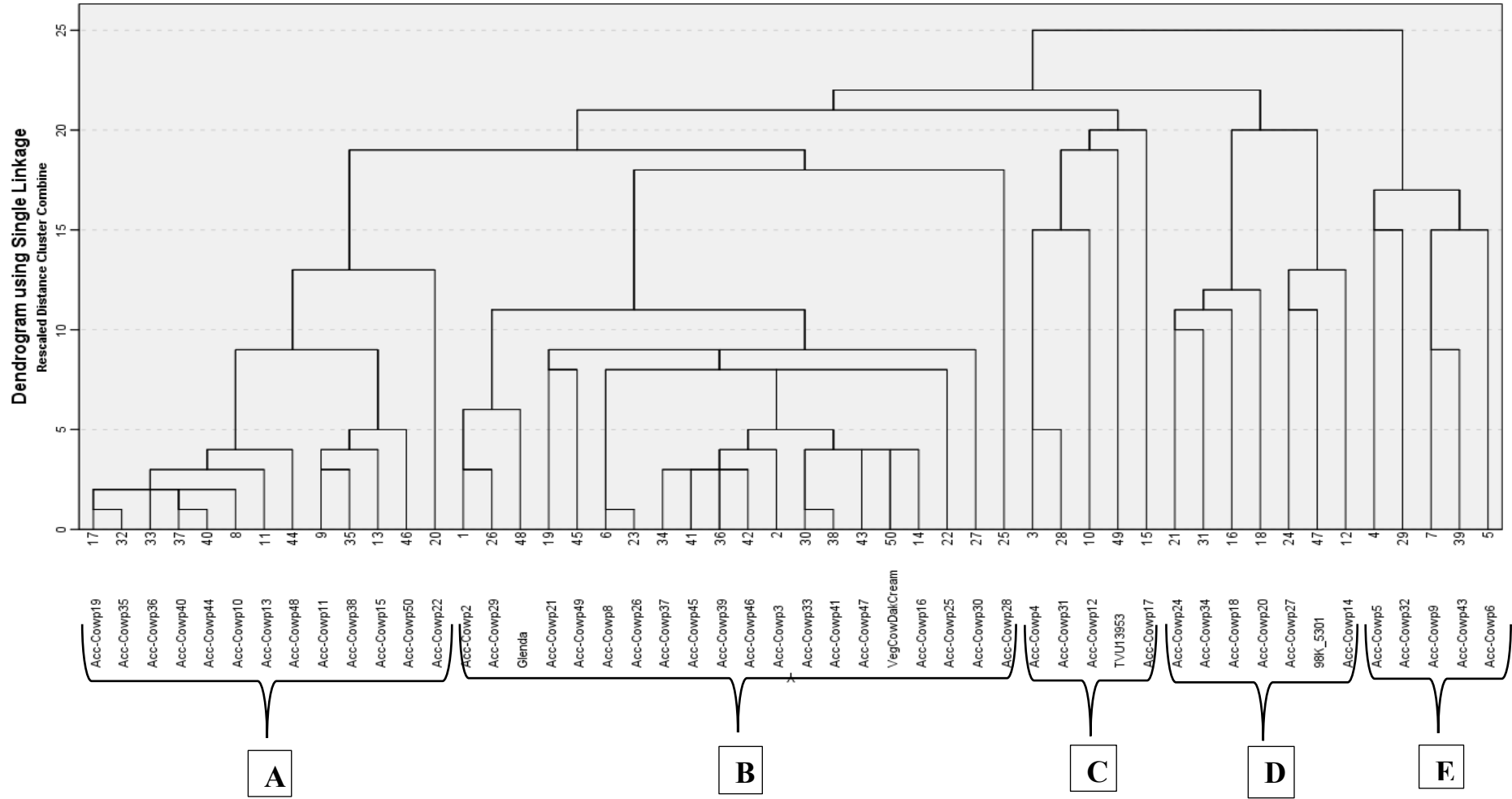
Note: Ca= calcium; Cu= copper; K= potassium; Fe= iron; Mg=magnesium; Na= sodium; P= phosphorus; Zn= zinc; GM= grand mean; Min- minimum; Max = maximum



**Figure 5.1:** Biplot analysis for principal component analysis of nutritional and phytochemical traits for the evaluated cowpea genotypes. Note: See genotype codes in Table 5.1.

### 5.3.5 Cluster analysis

The dendrogram in figure 5.2 grouped the 5 genotypes into five distinct clusters based on their similarities in the accumulation of nutritional and phytochemical properties. Cluster A comprised of 13 genotypes which are G17, G32, G33, G37, G40, G8, G11, G44, G9, G35, G13, G46 and G20 and these genotypes were mostly associated with high content of fats. The second cluster (Cluster B) comprised of high number of 20 genotypes compared to the rest of the clusters. The genotypes which were groups in Cluster B includes G1, G26, G48, G19, G45, G6, G23, G34, G41, G36, G42, G2, G30, G 38, G43, G50, G14, G50, G14, G22, G27 and G25 which were associated with the accumulation of flavonoids and total phenolics. Cluster C comprised of 5 genotypes which includes G3, G28, G10, G49 and G15 which were associated with proteins and condensed tannins. The 7 genotypes namely, G21, G31, G16, G18, G24 and G12 which were grouped in Cluster D were grouped based on their association with Zn uptake and then Cluster C comprised of 5 genotypes which were grouped based on the mineral elements including Mg, P, Na and Ca and they are genotypes G4, G29, G7, G39 and G5.



**Figure 5.2:** Dendrogram depicting the interrelatedness 50 cowpea genotypes evaluated using single linkage and Euclidean distance methods based on nutritional and phytochemical traits.

## 5.4 Discussion

The identification and cultivation of cowpea genotypes with enhanced grain nutritional and phytochemical compositions could be key to alleviate hidden hunger in South Africa. The current study investigated grain nutritional and phytochemical traits among cowpea genotypes sourced from the Agricultural Research Council genebank to identify and select suitable germplasms for breeding and cultivation. There were significant genotype, environment and genotype-by-environment effects under test sites reported for most studied nutritional and phytochemical traits (Table 5.3). The utilised environmental conditions in different locations highly contributed to the variations in nutritional composition and the level of uptake by the different cowpea genotypes. This suggests differential genotypic responses under varied test sites and further highlights the importance to recognise genetic variability in order to identify suitable genotypes.

The existence of genetic diversity among cowpea varieties for the grain nutritional and phytochemical compositions is very important for cowpea nutritional improvement in order to address global challenges such as malnutrition and food insecurity (Alidu *et al.*, 2020; Mbuma *et al.*, 2020; Sombié *et al.*, 2018). In this study, the concentrations of mineral elements were reported to be 545.45 mg 100g<sup>-1</sup>, 15.13 mg 100g<sup>-1</sup>, 31.5 mg 100g<sup>-1</sup>, 1404.63 mg 100g<sup>-1</sup>, 718.72 mg 100g<sup>-1</sup>, 331.00 mg 100g<sup>-1</sup>, 1795.64 mg 100g<sup>-1</sup> and 26.87 mg 100g<sup>-1</sup> for Ca, Cu, Fe, K, Mg, Na, P and Zn. These results were comparatively higher than the mineral element compositions reported in other recent cowpea studies in Burkina Faso (Sombié *et al.*, 2018), in Saudi Arabia (Alghamdi *et al.*, 2019) and in South Africa (Mbuma *et al.*, 2020). The observed difference among the reported results is due to existence of GEI effects and genetic variability among the assessed genotypes (Silva *et al.*, 2021; Cardona-Ayala *et al.*, 2021). Furthermore, genotypes G22, G4, G34, G7, G23, G31 and G32 recorded the highest concentration of the assessed mineral elements. Furthermore, the mean concentration for the mineral elements in cowpea genotypes in this study were higher compared to other legume crops such as Bambara groundnut (Hlanga *et al.*, 2021) and pigeon pea (Gerrano *et al.*, 2022a) which further highlights the competitiveness of cowpea with other legumes. Therefore, this result suggests that these genotypes can be recommended for selection for improved mineral element composition.

The high concentration of essential minerals such as K, Ca, Cu, Na, P, Mg, Fe and Zn makes the cowpea genotypes used in this study to be superior in the aid of addressing malnutrition in African countries especially the underdeveloped communities with limited or no access to balanced diet required for nourished bodied. For instance, the deficiencies in iron and zinc has

been identified as global health challenge especially to children and women in particular during pregnancy and reproductive age (Welch, 2002; WHO, 2004; Stein, 2010). Therefore, this suggests that the utilization of the cowpea genotypes from this study can combat the challenge. In addition, the high nutritional status in the present study could be associated to potentially enhanced genotypes for essential mineral accumulations.

Proteins are considered as an essential nutrition element in the diets of the humans (Okareh *et al.*, 2015). The deficiency in proteins is a major challenge affecting millions of children and mothers in tropical regions especially in the rural communities where there is limited intake of proteins obtained from animals due to affordability (Santos *et al.*, 2012). This implies that cowpea has a vital role as it is consumed as a cheap source of proteins to substitute the animal proteins, hence, the crop is referred to as poor man's meat. In this study, the total protein content ranged from 19.84 % to 26.94 % with a mean of 23.63%. These results concurred with the results reported by Owalabi *et al.* (2012) who assessed five varieties and reported cowpea protein content that ranges from 19.84 % to 26.61 %. Further, Asante *et al.* (2006) also studied the protein content in 32 cowpea accessions and reported ranges of 16.4% to 27.3% with an average of 22%. However, these results were comparably lower than the protein content of 46.51% reported by Adeyemi *et al.* (2012). These differences may be explained by the fact that in the current study, there was no fertilizer application and supplements that was added while Adeyemi *et al.* (2012) used the mineralised goat manure as a fertiliser during the trial establishment. It is important to note that crude protein content is significantly influenced by manure application as it is supplemented by nitrogen supplied by manure (Abebe *et al.*, 2005).

Phenolic compounds such as phenolics, flavonoids and condensed tannins are important as they act as scavengers of radicals, metal ion chelators and reducing agents (Rani *et al.* 2014; Zhao *et al.*, 2014). They form complexes with minerals and proteins making them less vulnerable to enzymatic degradation and less available for absorption (Akond *et al.* 2011; Noubissie *et al.*, 2012). In this current study, the concentration of total phenolics ranged from 1.65 to 6.50 mg GAE/g with genotype Acc-Cowp2 recording the higher and genotype Acc-Cowp34 recording the lowest phenolic contents. The reported results were higher than the ranges of 0.85 to 2.95 mg GAE/g reported by Noubissie *et al.* (2012) from seven cowpea genotypes. In addition, Yusinawan *et al.* (2021) extracted phenolic contents in cowpea seeds using different methods and reported the phenolics content ranging from 10.37- 11.93 mg GAE/g and 18.20- 20.27 mg GAE/g from two cowpea varieties which was relatively higher than the concentration reported in the current study.

The flavonoid content ranged from 0.53 to 5.17 mg CE/g which were recorded for genotypes Acc-Cowp48 and genotypes Acc-Cowp49. These results agreed with Mbuma *et al.* (2020) who reported flavonoids content ranging 0.40 to 6.11 mg CE/g. Furthermore, the condensed tannins in this study ranged from 0.60 to 6.25 mg cyanidin chloride/g which were recorded in genotype Acc-Cowp28 and Acc-Cowp17, respectively (Table 5.4). The genotypes which recorded the low concentrations of total phenolics, flavonoids and condensed tannins may be used in selecting for improved nutrition due to their nutritional importance. Although the high concentrations of phytochemical compounds in legumes may be a good antioxidant compound. However, Baloyi *et al.* (2001) disclosed that the content of polyphenols compounds in legumes have been suggested to be the possible cause of reduced nutritive value and further inhibiting the availability of nutrients.

The Pearson correlation coefficient analysis was used to measure the association amongst the assessed traits which gives an opportunity selection and improvement of nutritional status simultaneously. In this study, positive and significant correlations were observed for most of measured mineral traits (Table 5.5). For instance, Ca positively and significantly correlated with Fe, Mg, Na, P and Zn while Cu correlated with Fe and P. Further, Fe positively and significantly correlated with Na, P and Zn while Mg positively correlated with Na, P and Zn and lastly, Na with P then P with Zn. This indicate that it will be feasible to develop cowpea genotypes with increased concentrations of these essential mineral elements because selection of one would simultaneous improve the other nutrient element. The negative and significant correlation between mineral elements and phytochemical traits are expected. Ca had a negative and significant correlation phenolic content and condensed tannins, while Mg significantly and negatively correlated with condensed tannins and K correlated with flavonoids which is quite expected because phytochemical traits have compounds that lowers the availability of nutrients, Therefore, this indicates that selecting the genotypes with high concentration of phenolic, flavonoids and condensed tannins might casually reduce the contents of other essential minerals.

The negative correlations present a challenge for simultaneously selection for desirable traits which implies that the traits that associated negatively should be independently selected to allow the genetic improvement. Correspondingly, the studies by Gerrano *et al.* (2019); Mbuma *et al.* (2020); Gerrano *et al.* (2022b) also reported the significant and positive of some nutrient traits evaluated in South African agro ecologies. These results are important in cowpea

nutritional status improvement as they will contribute to selection of genotypes with potential to improve nutrition in cowpea breeding programs.

The principal component analysis (PCA) was done to further envision the performance of genotypes for nutrients and phytochemicals across all five locations. The three principal components with Eigen value  $\geq 1$  explained 65.66% of the total variation amongst 50 cowpea genotypes for the assessed nutritional and phytochemical traits. The first principal component was the most explained on the basis of genetic variation with Ca, Mg and P being the most abundant discriminatory across all environments. The high contributions of these elements indicates that they must be used as target traits for evaluation and discriminating genotype for future breeding programs. However, this does not make them superior nutritional traits based on their nutritional benefits as they are all comprised with different benefits required for human health.

The genotypes were scattered in four quadrants of the PCA biplot which indicates their genetic variation on the nutrient's traits evaluated. The cowpea genotypes portrayed association with mineral elements, proteins, fat content and phytochemical traits in the PCA biplot. For instance, genotype Acc-Cowp17, acc-Cowp12, 98K\_5301 and Glenda were in close proximity to vectors of Ca, Mg and condensed tannins which indicates that these genotypes can be selected as parental lines for recommendation for the improved nutrients accumulation. Inversely, Hlanga *et al.* (2022) states that genotypes that are not in close proximity to vectors of any particular nutrients did not accumulate significant concentration of any nutrients. Genotypes Glenda, Acc-Cowp29, Acc-Cowp22, Acc-Cowp43 and Acc-Cowp25 were not located in close proximity of any nutrients suggesting a further need for improvement in nutrient content. A very close association between P, Na, Ca, and Mg has been observed in the PCA biplot which further supports the positive and significant correlations reported for the mineral elements in table 3.

The hierarchical clustering analysis was used to differentiate the genotypes based on their associations for the accumulation of nutritional and phytochemical traits. Cluster A grouped G17, G32, G33, G37, G40, G8, G11, G44, G9, G35, G13, G46 and G20 based on their high association with high content of fats. The genotypes which were grouped in Cluster B includes G1, G26, G48, G19, G45, G6, G23, G34, G41, G36, G42, G2, G30, G38, G43, G50, G14, G50, G14, G22, G27 and G25 based on their high accumulation of flavonoids and total phenolics. Cluster C grouped G3, G28, G10, G49 and G15 which were associated with proteins

and condensed tannins. Then G21, G31, G16, G18, G24 and G12 which were grouped in Cluster D based on their association with Zn uptake and then Cluster E comprised of 5 genotypes which were grouped based on the mineral elements including Mg, P, Na and Ca and they are genotypes G4, G29, G7, G39 and G5.

## 5.5 Conclusion

The nutritional and phytochemical traits showed variations within genotypes and environmental conditions in which the genotypes were grown. The PCA biplot showed that genotypes Acc-Cowp6, Acc-Cowp17, Acc-Cowp14, 98K\_5301, Acc-Cowp9, Acc-Cowp32, Acc-Cowp9, Acc-Cowp4, Acc-Cowp16 and Acc-Cowp21 were in association with Ca, Mg, P, Na and Zn. Genotype Acc-Cowp31 and Acc-Cowp13 were highly associated with protein content while genotype Acc-Cowp39 were in close association with fat content. Further, genotypes Acc-Cowp34, Acc-Cowp18, Acc-Cowp48, Acc-Cowp22, Acc-Cowp26, Acc-Cowp49 and Acc-Cowp28 had low concentrations of total phenolic, flavonoids and condensed tannins. These genotypes can be recommended for production for superior genotypes in cowpea breeding programs targeting high nutritional value in order to assist with alleviating malnutrition among poor communities who require cheaper sources of nutrients. The nutritional contents reported in this study will provide a baseline information in the cowpea production programs for recommendation of superior genotypes for nutritional and phytochemical improvement.

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## **CHAPTER 6: ANALYSIS OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF COWPEA [*Vigna unguiculata* (L.) Walp] GENOTYPES USING SINGLE NUCLEOTIDE POLYMORPHISM MARKERS.**

### **Abstract**

Cowpea [*Vigna unguiculata* (L) Walp] is an important legume crop with immense potential for nutritional and food security, income generation and livestock feed in Sub-Saharan Africa. The crop is highly tolerant to heat and drought stresses which makes it an extremely important crop for improving resilience in crop production in the face of climate change. This study was carried out to assess the genetic diversity and population structure of 90 cowpea accessions using Single Nucleotide Polymorphism (SNP) markers. Out of 11 940 SNPs used, 5864 SNPs were highly polymorphic and maintained for genome diversity analysis. Polymorphic information content (PIC) values ranged from 0.22 to 0.32 with a mean value of 0.27. The model-based population structure analysis classified the 90 cowpea accessions into four subpopulations at  $K = 4$ , while the distance-based cluster analysis grouped the accessions into three distinct clusters. The analysis of molecular variance (AMOVA) revealed that 59% and 69% of the total molecular variation was attributed to among individual variation for model-based and distance-based populations, respectively and 18% was attributed to within individual variations. Furthermore, the low heterozygosity among cowpea accessions and the high inbreeding coefficient observed in this study suggests that the accessions reached acceptable level of homozygosity. This study would serve as a reference for future selection and breeding programs of cowpea with desirable traits and for systematic conservation of these plant genetic resources.

**Keywords:** Cowpea, genetic diversity, population structure, single nucleotide polymorphism

## 6.1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp,  $2n=2x=22$ ] is an important legume crop that belongs to the genus *Vigna*, family Fabaceae and order Fabales, with a genome size of 620 million base pairs (Badiane *et al.*, 2014). The crop is an annual herbaceous plant widely grown in tropical and subtropical regions of Sub-Saharan Africa, East Asia and other developing countries (Coulbaly *et al.*, 2002; Gbedevi *et al.*, 2021). The crop plays a major role in both human and animal nutrition and food as well as feed security and income generation for farmers and agro-traders (Gbedevi *et al.*, 2021). In addition to the crop's importance in sustaining food security, cowpea possesses high tolerance to heat and drought stresses which is extremely important in improving the resilience of the crop to the current climate change (Ehlers and Hall, 1997; Seo *et al.*, 2020).

Moreover, cowpea has significant importance to cropping systems on account of its ability to grow in low fertility soils, as a complementary crop in rotation with cereals to break the life cycle of pathogens of cereals infested in the soil and consequently improve the fertility of the soil (Carvalho *et al.*, 2017). Globally, the cowpea average yield ranges from 0.1 t ha<sup>-1</sup> to 0.59 t ha<sup>-1</sup> is lower than its expected potential yield of 1.5 t ha<sup>-1</sup> to 3 t ha<sup>-1</sup> under suitable environmental conditions (Gbaye and Holloway, 2011). This is due to the narrow genetic base of improved varieties and their susceptibility to abiotic and biotic stresses. It is of high importance to develop improved varieties of cowpea to increase productivity to help alleviate poverty in Sub-Saharan Africa and also to meet the market demand. The production of improved cowpea varieties for traits such as high yield and nutritional status will largely benefit both subsistence and commercial farmers. Therefore, characterizing genetic diversity in any crop species is important for optimal germplasm utilization, conservation, and crop improvement programs. In support, Kondwakwenda *et al.* (2020) also stated that the availability of appropriate genetic diversity is imperative for the success and sustainability of any crop breeding program.

The assessment of the genetic diversity of a particular crop is achieved using morphological, biochemical, and molecular markers (Bhandari *et al.*, 2017; Gbedevi *et al.*, 2021). Although biochemical markers are more reliable than morphological markers, they are both reported to be influenced by environmental factors. These markers provide genetic diversity information based on genotype performances using agronomic traits and may differ at different stages of growth and development as well as in the growing environment. This may hinder the real genetic variation among genotypes (Campos *et al.*, 2012; Horn and Shimelis, 2020) and reduce

the accuracy of the results obtained. Hence, there was an evolution of the development of DNA molecular markers (De Vicente *et al.*, 2004; Bucheyeki *et al.*, 2009; Sami *et al.*, 2013; Sejake *et al.*, 2021). Molecular markers are neutral to environmental effects and the genetic diversity is reviewed at the genomic DNA level; therefore, it can visualize the accurate genetic diversity among genotypes (Cui *et al.*, 2017; Gbedevi *et al.*, 2021). However, they may not be associated with any agronomic traits and needs to be supplemented with morphological markers to infer a meaningful conclusion.

Several DNA markers have been developed to determine the genetic diversity of cowpea. These include restriction fragment length polymorphism (RFLP) (Malviye *et al.*, 2012; Cui *et al.*, 2017), simple sequence repeats (SSR) (Mafakheri *et al.*, 2017), single nucleotide polymorphism (SNP) (Nkhoma *et al.*, 2020), amplified fragment length polymorphic (AFLP) (Fange *et al.*, 2007), and random amplified polymorphic DNA (RAPD) (Ba *et al.*, 2004). In recent advances in molecular genetics and molecular biology, the use of SNP markers has emerged to be the most preferred molecular marker because of its high genomic abundance, cost-effectiveness, reliability, and ease of application compared to other polymerase chain reaction (PCR)-based molecular markers (Rafalski, 2002; Kujur *et al.*, 2015). Hence, the SNP markers were used in the current study. The study by Desalegne *et al.* (2017) compared the efficiency of SNP and SSR marker-based analysis of genetic diversity in 95 cowpea accessions collected from East Africa and the International Institute of Tropical Agriculture (IITA) inbred lines. Their study revealed that SNP markers were found to be more effective than SSR markers to determine the relationship between cowpea varieties, hence, the study suggested the utilization of SNP markers in the future analysis of genetic diversity and population structure in cowpea. Similarly, the recent studies by Nkhoma *et al.* (2020) evaluated the genetic diversity in 90 cowpea genotypes using SNP markers and phenotypic traits, and the study showed that SNP markers were more efficient in discriminating the diversity among and within the cowpea genotypes evaluated.

There are sequencing technology-based tools that have emerged through SNP markers, which include genotyping-by-sequencing (GBS) and next-generation sequencing (NGS) which has been reported to be efficient, inexpensive, and fast developing in sequencing plant genomes (Elshire *et al.*, 2011; Bastien *et al.*, 2014). In addition, a new type of marker known as diversity arrays technology (DArT) has been recently developed for genotyping and genome sequencing needlessly of sequence information (Gbedevi *et al.*, 2021). In cowpea, the DArT marker has been recently used by Gbedevi *et al.* (2021) to study the genetic diversity and population

structure of 498 cowpea accessions collected from the Republic of Togo and their study revealed the presence of four major clusters among the accessions studied and the accessions were not clustered according to the regions where they were collected suggesting that the clustering did not closely resemble the geographical areas of the collections. The DArT marker has developed a GBS method called DArTseq sequencing technology for genotyping in legumes including cowpea (Ketema *et al.*, 2020; Gbedevi *et al.*, 2021), chickpeas (Farahani *et al.*, 2019), common beans (Valdisser *et al.*, 2017), and Bambara groundnut (Uba *et al.*, 2021). Hence, the objective of this study was to assess the magnitude of the genetic diversity and population structure among cowpea genotypes using single nucleotide polymorphisms (SNP) markers.

## **6.2 Materials and Methods**

### **6.2.1 Plant materials**

The study evaluated the genetic diversity of 90 cowpea accessions sourced from the Agricultural Research Council - Vegetables, Industrial and Medicinal Plants (ARC-VIMP) gene bank, Pretoria, South Africa. These accessions were collected from different parts of the world including South Africa, Kenya and Nigeria. The geographic origin and growth habits of the accessions are presented in Supplementary Table 6.1.

### **6.2.2 DNA extraction and sequencing**

The cowpea genotypes were grown in a seed germination chamber at the Biosciences eastern and central Africa -International Livestock Research Institute in Nairobi (BecA-ILRI) for genotyping. Ten-day-old leaf materials were sampled from the seedlings and the leaf samples were frozen in liquid nitrogen and stored at -80oC for genotyping. DNA extraction was done using a NucleoMag Plant DNA extraction kit (Takara Bio USA, Inc). The genomic DNA extracted was in the range of 50-100ng/ul. DNA quality and quantity were checked on 0.8% agarose gel.

Libraries were constructed according to Killian *et al.* (2012) using the DArTSeq complexity reduction method through digestion of genomic DNA using a combination of PstI and MseI enzymes and ligation of barcoded adapters and common adapters followed by PCR amplification of adapter-ligated fragments. Libraries were sequenced using Single Read sequencing runs for 77 bases. Next-generation sequencing was carried out using Hiseq2500. DArTseq markers scoring was achieved using DArTsoft14 which is an in-house marker scoring pipeline based on algorithms. Two types of DArTseq markers were scored, SilicoDArT

markers and SNP markers which were both scored as binary for the presence /absence of the restriction fragment with the marker sequence in the genomic representation of the sample. Both SilicoDArT markers and SNP markers were aligned to the cowpea reference genome, *Vunguiculata\_469\_v1.0* to identify chromosome positions.

### **6.2.3 Data analysis**

Ninety-cowpea accessions were genotyped with 11940 SNP markers. Monomorphic and SNPs with a minor allele frequency of less than 2% were filtered out and 5864 (49%) SNPs were retained for further analysis. Genotypic data were subjected to analyses of molecular variance (AMOVA) and various measures of genetic diversity within and among inferred subpopulations using GenAlex software version 6.5 (Peakall and Smouse, 2012). Genetic diversity parameters such as the number of effective alleles per locus ( $N_e$ ), Shannon's Information Index (I), gene diversity ( $H_e$ ) and the polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). The genotypic data were used to obtain a dissimilarity matrix using the Jaccard index as described by Debener *et al.* (1990). The matrix was then used to run a cluster analysis based on a neighbor-joining algorithm using the un-weighted pair group method with arithmetic average (UPGMA) in DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). Bootstrap analysis was performed for node construction using 10,000 bootstrap values.

The Bayesian genotypic clustering approach of STRUCTURE 2.3.4 (Pritchard *et al.*, 2006) was used to determine the population structure. An admixture model with independent allele frequencies, without prior population information, was used to simulate the population. The STRUCTURE program was set as follows: a burn-in period length of 100,000, and after burn-in, 100,000 Markov Chain Monte Carlo (MCMC) were used. This model assumes that the genome of each individual is a mixture of genes originating from K unknown ancestral populations. For joint inference of the population substructure, K ranging from 1 - 6 was set up, with ten independent runs for each K. The most probable value of K for each test was detected by  $\Delta K$  (Evanno *et al.*, 2005) using the STRUCTURE HARVESTER (Earl and Vonholdt, 2012). Each individual genotype was grouped into a given cluster using the 'membership coefficient' for each cluster interpreted as a probability of membership. The genotype membership was determined by the computer program CLUMPP (Jakobsson and Rosenberg, 2007).

**Table 6.1:** List of cowpea genotypes used in the study and their origin.

No. of genotypes	Genotype name	Genotype code	Origin	Growth habit	Seed shape	Seed coat color
1	Acc-Cowp2	ARC002	South Africa	Prostrate	Kidney	Red
2	Acc-Cowp3	ARC003	South Africa	Prostrate	Kidney	Brown
3	Acc-Cowp4	ARC004	South Africa	Prostrate	Kidney	Red
4	Acc-Cowp5	ARC005	South Africa	Prostrate	Rhomboid	Black
5	Acc-Cowp6	ARC006	South Africa	Prostrate	Kidney	Brown
6	Acc-Cowp9	ARC009	South Africa	Semi-erect	Rhomboid	Cream
7	Acc-Cowp10	ARC010	South Africa	Prostrate	Rhomboid	Purple
8	Acc-Cowp11	ARC011	South Africa	Climbing	Kidney	Light brown
9	Acc-Cowp12	ARC012	South Africa	Prostrate	Rhomboid	Brown
10	Acc-Cowp13	ARC013	South Africa	Prostrate	Kidney	Brown
11	Acc-Cowp14	ARC014	South Africa	Erect	Ovoid	Red
12	Acc-Cowp15	ARC015	South Africa	Prostrate	Ovoid	Red
13	Acc-Cowp16	ARC016	South Africa	Prostrate	Rhomboid	Black
14	Acc-Cowp17	ARC017	South Africa	Erect	Ovoid	Cream
15	Acc-Cowp18	ARC018	South Africa	Erect	Rhomboid	Red-speckled
16	Acc-Cowp19	ARC019	South Africa	Erect	Globose	Light brown
17	Acc-Cowp20	ARC020	South Africa	Erect	Rhomboid	Red
18	Acc-Cowp21	ARC021	South Africa	Erect	Kidney	Cream
19	Acc-Cowp22	ARC022	South Africa	Prostrate	Rhomboid	Brown
20	Acc-Cowp23	ARC023	South Africa	Unknown	Rhomboid	Brown
21	Acc-Cowp24	ARC024	South Africa	Climbing	Rhomboid	Black
22	Acc-Cowp25	ARC025	South Africa	Climbing	Rhomboid	Red
23	Acc-Cowp26	ARC026	South Africa	Semi-erect	Kidney	Grey-speckled
24	Acc-Cowp27	ARC027	South Africa	Semi-erect	Kidney	Red-speckled
25	Acc-Cowp28	ARC028	South Africa	Prostrate	Kidney	Red
26	Acc-Cowp29	ARC029	South Africa	Prostrate	Kidney	Brown
27	Acc-Cowp30	ARC030	South Africa	Semi-erect	Globose	Black
28	Acc-Cowp31	ARC031	South Africa	Prostrate	Globose	Cream
29	Acc-Cowp32	ARC032	South Africa	Prostrate	Kidney	Red
30	Acc-Cowp33	ARC033	South Africa	Prostrate	Kidney	Red
31	Acc-Cowp34	ARC034	South Africa	Semi-erect	Rhomboid	Light red
32	Acc-Cowp35	ARC035	South Africa	Climbing	Kidney	Light brown
33	Acc-Cowp36	ARC036	South Africa	Climbing	Kidney	Light brown
34	Acc-Cowp37	ARC037	South Africa	Prostrate	Kidney	Light brown
35	Acc-Cowp38	ARC038	South Africa	Climbing	Globose	Black
36	Acc-Cowp39	ARC039	South Africa	Semi-erect	Kidney	Red
37	Acc-Cowp40	ARC040	South Africa	Semi-erect	Kidney	Black
38	Acc-Cowp41	ARC041	South Africa	Erect	Ovoid	Cream
39	Acc-Cowp42	ARC042	South Africa	Unknown	Kidney	Brown
40	Acc-Cowp43	ARC043	South Africa	Semi-erect	Kidney	Brown
41	Acc-Cowp44	ARC044	South Africa	Erect	Kidney	Light brown

42	Acc-Cowp46	ARC046	South Africa	Erect	Kidney	White
43	Acc-Cowp47	ARC047	South Africa	Erect	Rhomboid	Brown
44	Acc-Cowp48	ARC048	South Africa	Prostrate	Kidney	Cream
45	Acc-Cowp49	ARC049	South Africa	Prostrate	Rhomboid	Brown
46	Acc-Cowp50	ARC050	South Africa	Prostrate	Kidney	Black
57	Acc-Cowp74	98K-5301	South Africa	Erect	Kidney	White
48	Acc-Cowp64	Glenda	South Africa	Semi-erect	Kidney	Brown
49	Acc-Cowp70	TVU13953	South Africa	Prostrate	Kidney	Cream
50	Acc-Cowp62	VegCowDakCream	South Africa	Erect	Kidney	White
51	Acc-Cowp51	CH 14	South Africa	Unknown	Kidney	Brown
52	Acc-Cowp53	BENSOGLA	South Africa	Unknown	Rhomboid	Brown
53	Acc-Cowp52	IT845-2246	Nigeria	Unknown	Rhomboid	Brown
54	Acc-Cowp54	TVU 11424	Nigeria	Unknown	Kidney	Brown
55	Acc-Cowp55	MAKATINI	South Africa	Unknown	Rhomboid	Brown
56	Acc-Cowp56	VULI	Tanzania	Unknown	Kidney	Brown
57	Acc-Cowp57	PAN 311	South Africa	Unknown	Kidney	Brown
58	Acc-Cowp58	NGOJI	South Africa	Unknown	Kidney	Brown
59	Acc-Cowp59	ENBO BUFF	South Africa	Semi-erect	Rhomboid	Brown
60	Acc-Cowp60	IT93K 1294	Nigeria	Erect	Rhomboid	Purple
61	Acc-Cowp61	OUKAWA	South Africa	Prostrate	Kidney	Cream
62	Acc-Cowp63	IT90K-76	Nigeria	Unknown	Rhomboid	Brown
63	Acc-Cowp65	IT96D-602	Nigeria	Erect	Ovoid	Cream
64	Acc-Cowp66	Bechuana White	South Africa	Unknown	Rhomboid	Brown
65	Acc-Cowp67	5431	South Africa	Erect	Rhomboid	Cream
66	Acc-Cowp68	IT90K-59	Nigeria	Unknown	Rhomboid	Brown
67	Acc-Cowp69	FAHARI	Tanzania	Semi-erect	Kidney	Brown
68	Acc-Cowp71	TVU 6345	Nigeria	Semi-erect	Crowder	Brown
69	Acc-Cowp72	ENCORE	South Africa	Unknown	Kidney	Brown
70	Acc-Cowp73	TVU 12637	Nigeria	Unknown	Kidney	Black
71	Acc-Cowp75	IT00K 1217	Nigeria	Unknown	Rhomboid	White
72	Acc-Cowp76	Veg cowpea 1	South Africa	Semi-erect	Kidney	Purple
73	Acc-Cowp77	Veg cowpea 2	South Africa	Semi-erect	Kidney	White
74	Acc-Cowp78	Veg cowpea 3	South Africa	Erect	Kidney	White
75	Acc-Cowp79	MA1	South Africa	Erect	Rhomboid	Purple
76	Acc-Cowp80	IT89U-412	Nigeria	Unknown	Kidney	Black
77	Acc-Cowp81	M346	South Africa	Erect	Rhomboid	Purple
78	Acc-Cowp82	Ukaluleni	South Africa	Prostate	Kidney	Brown
79	Acc-Cowp83	IT89D-349	Nigeria	Erect	Kidney	Brown
80	Acc-Cowp85	Mpenbeni	South Africa	Prostrate	Rhomboid	Cream
81	Acc-Cowp86	2460	South Africa	Prostrate	Kidney	Cream
82	Acc-Cowp87	Vigna Onb	South Africa	Prostrate	Kidney	Purple
83	Acc-Cowp88	MA2	South Africa	Erect	Rhomboid	White
84	Acc-Cowp89	Meter bean piet	South Africa	Prostrate	Kidney	Cream
85	Acc-Cowp91	Chappy	South Africa	Prostrate	Kidney	Cream

86	Acc-Cowp92	M217	South Africa	Erect	Rhomboid	Purple
87	Acc-Cowp93	Tatro mix	Kenya	Prostrate	Rhomboid	Brown
88	Acc-Cowp94	TV778	Nigeria	Erect	Kidney	Cream
89	Acc-Cowp7	New Line 2	South Africa	Unknown	Kidney	Cream
90	Acc-Cowp1	New Line 1	South Africa	Unknown	Kidney	Brown

Note: VegCowDakCream = Vegetable cowpea Dakama Cream.

## 6.3 Results

### 6.3.1 Allele polymorphism

SNP distribution per chromosome and the gene diversity parameters measured from 90 cowpea accessions are presented in Table 6.2. The genetic diversity parameters analysis was conducted using 5864 (49%) SNPs that remained after filtering out monomorphic and minor allele frequencies of less than 2%. The number of polymorphic SNPs per chromosome ranged from 345 on chromosome 1 to 668 on chromosome 3 with an overall mean of 488 per chromosome. The proportion of polymorphic SNPs per chromosome varied from 31.82% on SNPs with unknown chromosome origin to 57.35% SNPs on chromosome 9, with an overall mean value of 49.11% per chromosome. The mean number of effective allele ( $N_e$ ) per chromosome was the highest on chromosome 7 ( $1.48 \pm 0.013$ ), followed by chromosome 10 ( $1.47 \pm 0.013$ ) and chromosome 4 ( $1.47 \pm 0.013$ ) whilst the lowest values were observed on chromosome 9 ( $1.34 \pm 0.014$ ) and chromosome 8 ( $1.37 \pm 0.013$ ). The observed heterozygosity ranged from 7.6% to 9.6% with a mean value of 8.4%. The unbiased gene diversity (uHe) values ranged from 0.221 to 0.291 per chromosome with an overall mean of 0.267. The fixation index (FIS) values ranged from 64% on chromosome 11 to 72% on chromosome 7 with the mean value of 67%. The mean polymorphic information content (PIC) value was 0.27, in which the PIC values per chromosome ranged from 0.22 to 0.32.

**Table 6.2:** Genetic diversity within and among 90 cowpea accessions genotypes based on 5864 SNPs markers.

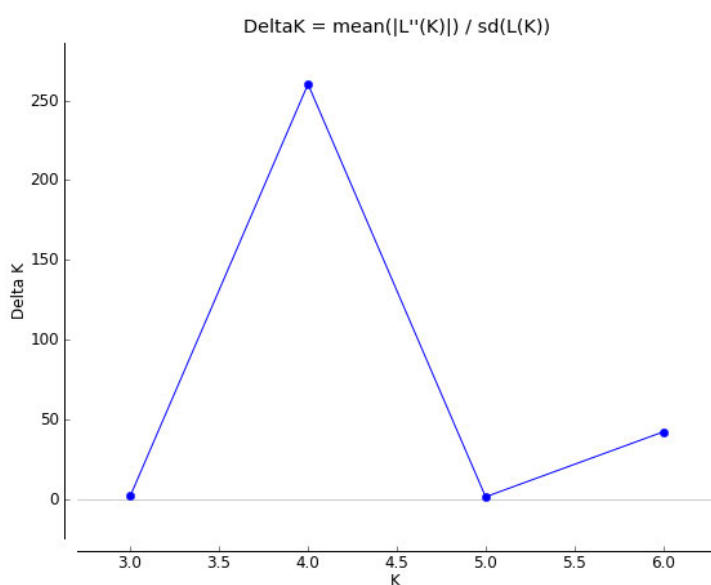
Chromosome	NSU	NPS	%P	Ne	Ho	He	FIS	PIC
1	670	345	51.49	1.43 (0.017)	0.088 (0.004)	0.269 (0.008)	0.66 (0.012)	0.27 (0.008)
2	725	377	52.00	1.41 (0.016)	0.077 (0.003)	0.258 (0.008)	0.68 (0.011)	0.26 (0.008)
3	1222	668	54.66	1.46 (0.012)	0.082 (0.003)	0.288 (0.006)	0.70 (0.008)	0.29 (0.005)
4	1123	533	47.46	1.47 (0.013)	0.094 (0.004)	0.290 (0.006)	0.67 (0.010)	0.29 (0.006)
5	965	453	46.94	1.42 (0.015)	0.081 (0.004)	0.264 (0.007)	0.68 (0.011)	0.26 (0.007)
6	915	503	54.97	1.42 (0.015)	0.075 (0.003)	0.259 (0.007)	0.68 (0.009)	0.26 (0.007)
7	1066	580	54.41	1.48 (0.013)	0.079 (0.003)	0.295 (0.006)	0.72 (0.009)	0.29 (0.006)
8	823	459	55.77	1.38 (0.014)	0.079 (0.004)	0.246 (0.007)	0.67 (0.011)	0.25 (0.007)
9	762	437	57.35	1.34 (0.014)	0.076 (0.004)	0.221 (0.007)	0.66 (0.013)	0.22 (0.007)
10	1110	538	48.47	1.47 (0.014)	0.088 (0.003)	0.289 (0.007)	0.69 (0.009)	0.29 (0.007)
11	1110	510	45.95	1.42 (0.014)	0.096 (0.004)	0.265 (0.007)	0.64 (0.011)	0.26 (0.007)
UN	1449	461	31.82	1.37 (0.015)	0.086 (0.005)	0.234 (0.008)	0.59 (0.016)	0.32 (0.008)
Overall mean	11940	5864	49.11	1.43 (0.004)	0.084 (0.001)	0.267 (0.002)	0.67 (0.003)	0.27 (0.002)

Note: %P = Polymorphism, NPS = Number of polymorphic SNPs, NSU = number of SNPs used, Ne = number of effective alleles per locus, Ho = observed heterozygosity, uHe = unbiased gene diversity, FIS = inbreeding coefficient, PIC = polymorphic information content, UN- unknown, the values within the brackets are standard error.

### 6.3.2 Population structure and clustering

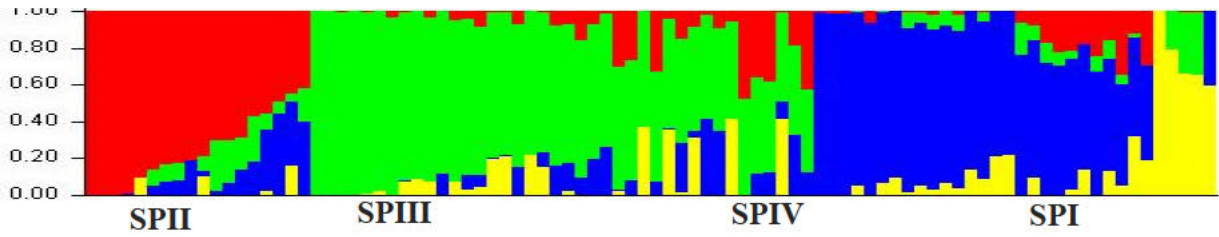
The population structure of the 90 accessions was examined using model- and distance-based structure analyses. The STRUCTURE analysis estimated the most suitable number of subpopulations was at  $K=4$  (Fig. 6.1A), indicating that the 90 accessions could be grouped into four subpopulations (SP1 – SP4) based on differences in their genetic makeup (Figure 6.1B). SP1 (yellow) contained only five accessions with admixtures from SP2 and SP3. SP2 (red) contained 18 accessions that share more admixture membership with the SP3 and SP4. SP3 (green) contained 40 accessions, which share admixture membership with the other three subpopulations. SP4 (blue) contained 27 accessions with admixture from the other subpopulations. The admixture level in the four subpopulations ranged from 50% to 63%, which indicated that these subpopulations shared more admixture memberships.

However, the distance-based cluster analysis generated using Nei's genetic distance using a neighbor-joining algorithm revealed the presence of three distinct clusters in the population represented by the 90 accessions (Figure 6.2). The clustering patterns of the two approaches were similar and constituted similar sets of accessions. For example, C1 (red) contained 41 accessions, 5 accessions from SP1, 27 from SP4, and 9 from SP3 of the STRUCTURE-generated clusters. C2 (black) consisted of 24 accessions, of which 18 accessions were from SP2 and 5 were from SP3. C3 (blue) contained 26 accessions all from SP3. The discrepancy between the two clustering approaches could result from admixtures since only 43% of the tested genotypes were considered pure.

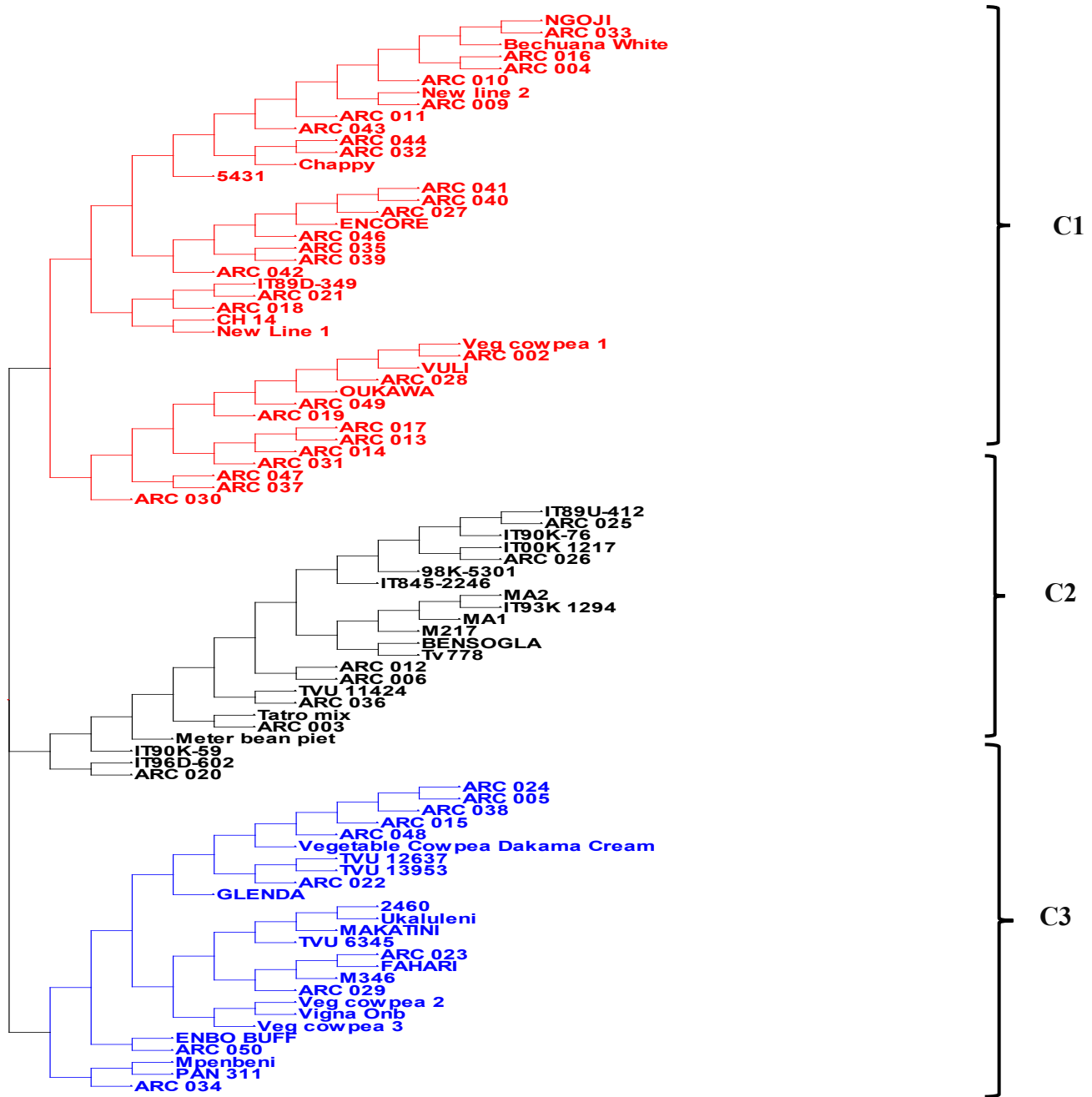


**A**

**B**



**Figure 6.1:** Population structure analysis using a Bayesian-based approach. (A). Estimation of hypothetical subpopulations using K-values showing the highest Delta k value was observed at the number of populations ( $K$ ) = 4. (B). Population structure analysis of 90 cowpea accessions.



**Figure 6.2:** Neighbor-joining analysis showing the genetic relationships among 90 cowpea accession tested using 5864 SNP markers. The different colours indicate different clusters.

### 6.3.3 Genetic diversity among subpopulations

The population genetic diversity estimates on 90 accessions were analysed based on the four subpopulations generated by STRUCTURE and three populations generated by DARwin (Table 6.3). SP3 revealed the highest values for most of the genetic parameters and displayed the highest level of genetic diversity ( $H_e = 0.247$  and  $I = 0.381$ ). SP1 displayed the lowest level of genetic diversity ( $H_e = 0.162$  and  $I = 0.189$ ) but 68.4% of the loci were fixed (Table 2). SP3 had the highest number of private alleles (508) and the highest percentage of polymorphic loci (91%). Based on the three subpopulations generated by cluster analysis, C2 revealed the highest genetic diversity for most of the genetic parameters except for the fixation index. C1, on the other hand, revealed the lowest for all the studied genetic parameters. In C1 68% of the alleles were fixed and 87% of loci were polymorphic.

**Table 6.3:** Genetic diversity within and among the 90-cowpea accession classified by growth habit.

Pop.	Na	Ne	I	Ho	He	FIS	%P	PA
Model based population structure analysis								
SPI	5	1.189 (0.006)	0.189 (0.004)	0.035 (0.002)	0.162 (0.003)	0.684 (0.007)	30.68	3
SP2	18	1.368 (0.004)	0.352 (0.003)	0.116 (0.002)	0.233 (0.002)	0.398 (0.005)	80.64	229
SP3	40	1.397 (0.004)	0.381 (0.003)	0.083 (0.001)	0.247 (0.002)	0.617 (0.004)	90.96	508
SP4	27	1.258 (0.004)	0.257 (0.003)	0.068 (0.001)	0.165 (0.002)	0.437 (0.005)	68.66	42
Overall	90	1.303 (0.002)	0.295 (0.002)	0.075 (0.001)	0.202 (0.001)	0.514 (0.003)	67.74	-
Distance-based population structure analysis								
C1	41	1.342 (0.004)	0.340 (0.003)	0.059 (0.001)	0.218 (0.002)	0.682 (0.004)	86.77	65
C2	24	1.404 (0.004)	0.387 (0.003)	0.108 (0.001)	0.254 (0.002)	0.512 (0.005)	88.93	234
C3	25	1.385 (0.004)	0.366 (0.003)	0.097 (0.001)	0.240 (0.002)	0.526 (0.005)	85.44	177
Overall	90	1.377 (0.003)	0.364 (0.002)	0.088 (0.001)	0.237 (0.001)	0.573 (0.003)	87.05	-

Note: Ne = average number of effective alleles per locus per subpopulation, I = Shannon information index, Ho = observed heterozygosity per subpopulation, He = expected heterozygosity per subpopulation, FIS = inbreeding coefficient, PA= Private alleles, %P = percentage of polymorphic loci, the values within the brackets are standard error.

### 6.3.4 Analysis of Molecular variance (AMOVA)

AMOVA was performed in both model-based and distance-based populations (Table 6.4). The results of AMOVA in model-based populations indicated that the majority of the variance occurred among individuals within populations and accounted for 59.4% of the total variation. However, 18.4% and 22.1% of the total variation was attributed to differences within individuals and among populations, respectively. Similarly, in the distance-based population, the majority of the variance was observed among individuals within the population and accounted for 68.9% of the total genetic variance. The mean fixation index within individuals was significantly high and positive in all classes of populations suggesting that outcrossing among the tested cowpea populations was low. The variation existed among populations was positive and significant suggesting that these populations were highly differentiated. Similarly, the relatively low level of variation observed within individuals was attributed to the high fixation index value.

**Table 6.4:** Analysis of molecular variance (AMOVA) among 90-cowpea accessions classified based on SNP markers.

Source	DF	SS	MS	Est. Var.	Per.Var	F-statistics
Model-based structure analysis						
Among Population	3	37575.3	12525.1	270.2	22.14	FST = 0.221 (P < 0.001)
Among Individual	86	144053.5	1675.0	725.2	59.44	FIS = 0.763 (P < 0.001)
Within Individual	90	20217.5	224.6	224.6	18.42	FIT = 0.816 (P < 0.001)
Total	179	201846.2	-	1220.0	100.00	-
Distance based structure analysis						
Among Population	2	20380.1	10190.1	143.8	12.16	FST = 0.122 (P < 0.001)
Among Individual	87	161248.8	1853.4	814.4	68.85	FIS = 0.784 (P < 0.001)
Within Individual	90	20217.5	224.64	224.6	18.99	FIT = 0.810 (P < 0.001)
Total	179	201846.4	-	1182.8	100.0	-

Note: DF =degrees of freedom, SS=sum of squares, MS= mean sum of squares, Est. Var= estimated variance, Per. Var = Percentage variation

Genetic differentiation ( $F_{ST}$ ) estimates among the subpopulations ranged from 0.103 between SP3 and SP4 to 0.239 between SP1 and SP2. The gene flow ranged from 0.8 (between SP1 and SP2) to 2.2 (between SP3 and SP4). The genetic distance among populations ranged from 0.089 between SP3 and SP4 to 0.214 between SP1 and SP2. The genetic identity (GI) ranged from 0.81 between SP1 and SP2 to 0.92 between SP3 and SP4 (Table 6.5). According to Wright (1984) standard guidelines for the interpretation of genetic differentiation, all pairs of subpopulations showed a moderate level of population differentiation. However, SP1 showed a relatively higher degree of differentiation (0.239) from the rest of the subpopulations. Gene flow among the subpopulation was relatively high between SP2, SP3, and SP4 (Table 4). The observed high genetic differentiation among the subpopulations could be explained by the low gene flow among subpopulations.

**Table 6.5:** Pair-wise estimates of gene flow (above diagonal, within the brackets), genetic differentiation ( $F_{ST}$ ) (above diagonal off brackets); genetic distance (GD) (lower diagonal off brackets) and genetic identity (GI) (lower diagonal within the brackets).

	SP1	SP2	SP3	SP4
SP1	-	0.239 (0.796)	0.142 (1.511)	0.192 (1.052)
SP2	0.214 ( 0.807)	-	0.106 (2.108)	0.149 (1.428)
SP3	0.112 (0.894)	0.103 (0.902)	-	0.103 (2.172)
SP4	0.134 (0.874)	0.135 (0.874)	0.089 (0.915)	-

## 6.4 Discussion

The analysis of genetic diversity in crops is a prerequisite for the success of any plant breeding program (Ebert and Engels, 2020). Therefore, assessing the population structure and genetic diversity of crops is fundamental to implementing efficient genetic resource management and conservation strategies. The application of high-throughput molecular markers provides a better understanding of genomic diversity and the population structure of germplasm and can speed up the identification of superior groups for further hybrid development (Serba *et al.*, 2019). The current study used 5864 SNP markers to assess the pattern and level of genetic variation and genetic structure among 90 cowpea accessions collected from various geographic origins.

The quality and the discriminatory power of a given marker system are assessed by its PIC values (Kumar *et al.*, 2020). It is important to note that SNP markers are bi-allelic in nature, hence their PIC values are restricted to 0.5, which is considered to be low or moderately informative as compared to SSR markers (Eltaher *et al.*, 2018). The mean PIC value of 0.27 reported in this study was in agreement with Gbedevi *et al.* (2021) who reported a PIC value of 0.25 but relatively higher than the one reported by Sodedji *et al.* (2021) (PIC = 0.22). The results suggest that the SNP markers that were used in this study showed a moderate level of polymorphism and revealed the existence of genetic diversity among the tested genotypes. The number of SNP markers used and the number of accessions studied might explain the observed differences among the reported PIC values and allelic polymorphism in this study and other previously reported studies. Kondwakwenda *et al.* (2020) also indicated that the observed variation in the quality and performance of SNP markers in different studies depends on the number of accessions studied, the type of markers used, and the type of germplasm studied. Nonetheless, the SNP markers used in this study were relatively informative and reliable in assessing the diversity of cowpea accessions studied.

The mean number of effective alleles per locus reported in this study was 1.43, which was comparable to the 1.41 reported by Fatokun *et al.* (2018). The gene diversity was further expressed using the probability of observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, which was the true indicator for the degree of genetic variation within and among the population assessed. The average  $H_o$  and  $H_e$  in the present study were 0.075 and 0.202, respectively for the 90 accessions which were comparable to the  $H_o$  of 0.05 and  $H_e$  of 0.31 reported by Gbedevi *et al.* (2021) for 70 cowpea accessions. Xiong *et al.* (2016), on the other hand, assessed 768 worldwide cowpea germplasm collections maintained at USDA GRIN and reported a  $H_o$  value of 0.06 and a  $H_e$  of 0.35. Nonetheless, the  $H_e$  was moderately low in this study but generally higher than the  $H_o$  for all subpopulations. Fatokun *et al.* (2018) also reported a similar trend in cowpea. Govindaraj *et al.* (2015) alluded that the low observed heterozygosity suggests a high level of inbreeding within the subpopulations.

A moderate fixation index ( $F_{IS} = 67\%$ ) was observed in this study indicating 67% of the SNP loci used were fixed in the studied accessions. However, a relatively high ( $F_{IS} = 0.83$ ) was reported by Gbedevi *et al.* (2021). The low observed heterozygosity and the relatively high rate of fixation index exhibited by the populations were explained by the fact that cowpea is a self-pollinated crop possessing a low out-crossing rate and low within-accession variability. Although the outcrossing rate in cowpea is low and ranges from less than 0.15 up to 1.58%

depending on the genotypes involved and the environment, where it is grown (Fatokun and Ng, 2007) further purification (self-pollination) of the accession is needed. The self-pollination nature and low outcrossing rate of cowpea have been reported to be the major contributor for the observed low genetic variation among cowpea accessions (Wamalwa *et al.*, 2016; Carvalho *et al.*, 2017).

Population structure analysis is the key to assessing the genetic structure of a given population and the basis for complex marker-trait association analysis (Wu *et al.*, 2015). The model-based population structure analysis grouped the 90 accessions into four subpopulations based on the peak of delta K ( $\Delta K$ ) at  $K = 4$ . The admixture level ranges from 50% in SP4 to 60% in SP1. The high proportion of admixture detected indicates either these subpopulations share the same ancestral progenitor or there was gene flow between the subpopulations. The distance-based cluster analysis using the neighbor-joining method showed the presence of three distinct clusters, which was not consistent with the results of the structure analysis. However, the pattern and the number of accessions maintained in each clustering approach were similar. The differences observed between the model based on STRUCTURE analysis and distance-based cluster analysis in the size and number of subgroups can be explained by the presence of admixtures within the subpopulations. In both model-based and distance-based clustering approaches, the grouping patterns were inconsistent with the growth habit and geographic origins of the studied accessions. Similar phenomena whereby subpopulations were grouped irrespective of the grouping criteria have also been reported by other researchers studying the genetic diversity in cowpea accessions (Sarr *et al.*, 2020; Gbedevi *et al.*, 2021; Sodedji *et al.*, 2021). In contrast, Ravelombola *et al.* (2017) reported clustering of genotypes based on growth habit resulted in two highly differentiated subpopulations.

The seed size and seed coat color preference can highly influence the genetic diversity in cowpeas (Seo *et al.*, 2020). Classification based on growth habits and other agronomic traits such as seed shape and seed coat color is significantly influenced by the breeding programs because these traits have been used to classify genotypes. Qualitative traits such as growth habits, seed size, seed shape and seed coat color are also important traits for farmers' and consumers' preferences (Nkhata *et al.*, 2010). Therefore, it is important to incorporate farmers- and consumers-preferred traits in future selections to enhance varietal adoption among farmers. In the UPGM clustering, C1 (red) was the highest group comprising 41 (46%) genotypes with brown (22%), red (12%) and cream (24%) seed coat color. Similarly, 23 accessions were grouped in C2 (black) comprises of 35% of brown seed coat color and 26

accessions grouped in C3 (blue) comprised of 35% black seed coat and 19% brown seed coat. In addition, the majority of accessions clustered in C1 and C2 were kidney-shaped while C3 was dominated with Rhomboid-shaped accessions. In terms of phenotypical variation, the erect and prostrate type were not different with respect to seed shape. The studies by Hellens *et al.*, (2010) in peas has reported the lighter seed coat color as human preference during domestication.

Similarly, C1 was dominated by erect (32%), while C2 was dominated by accessions with unknown growth habits (44%). C3 was mainly dominated by the prostrate (54%) growth habit type. Regarding the wide distribution of cowpea, accession was studied based on growth habit, the prostrate and erect types were more dominant than the semi-erect and climbing types. Growth habit is a morphologically important qualitative trait in cowpea production that highly affects crop yield and tillage method and further defines the shape of the plants and dictates how the plant should be harvested (Li *et al.*, 2022). Plant growth habit has been a major breeding target for crop improvement. Therefore, determining the genetic mechanisms that control the plant type will assist in cowpea growth development improvements. However, the results revealed that growth habits could not be used as an index for evaluating genetic diversity and for genotype classifications. The study by Khan *et al.* (2021) on bambara groundnut has also reported the same findings.

The AMOVA results revealed that the majority of the total molecular variation (59% and 69%) was due to differences among individuals within a population, 22% and 12% of the variation were attributed to the difference among the population and 18% and 19% was due to variation within individuals. In the model-based approach, the among-population variation was higher than the within individuals variation. The magnitude of variations among and within populations was further quantified by genetic differentiation observed among the populations ( $F_{ST} = 0.221$ ). The results indicate high genetic differentiation between four subpopulations based on the standard guideline of Wright (1984). The studies by Gbedevi *et al.* (2021) also reported a high genetic differentiation ( $F_{ST}$ ) value of 0.423 between two major reported subpopulations of 498 cowpea accessions assessed. However, Sarr [50] also reported a low genetic differentiation ranging from 0.018 to 0.100. The differences reported in genetic differentiations could be attributed to the diversity and number of accessions used and the number of markers involved to assess the genetic diversity.

The reported level of genetic differentiation reported in this study could be explained by the gene flow among subpopulations (Gepts and Papa, 2003). The gene flow among the studied populations ranged from 0.796 to 2.172 and according to Wright (1965), gene flow  $< 1$  indicates limited gene exchange among population. This result suggested that a moderate gene flow occurred in this study and led to high genetic differentiation between the populations. Furthermore, genetic distance was used to measure the relatedness between individuals in a population. In this study, the low pairwise genetic distance was observed ranging from 0.089 to 0.214 revealing wide genetic variations among the tested cowpea accessions. This result suggests that the accessions studied are unique and have greater potential to contribute to new varieties for breeding programs in South Africa. The understanding of genetic diversity among cowpea populations studied in this study will improve the subsequent planning in future cowpea breeding and contribute useful information in conservation and managing genetic diversity required for the vigorous and successful breeding program.

## **6.5 Conclusions**

The current study revealed the existence of genetic diversity among and within the cowpea accessions assessed and showed the effectiveness and reliability of SNP markers. The study revealed that 49% of the selected SNP markers were highly polymorphic and efficiently discriminate the tested cowpea accessions. The low heterozygosity and the high inbreeding coefficients observed among cowpea varieties indicate that the accessions reached an acceptable level of homozygosity. The model-based (structure analysis) and distance-based (UPGM) clustering approaches were used in this study. The model-based analysis revealed the presence of four subpopulations at  $K = 4$  whereas the distance-based cluster analysis classified the cowpea accessions into three distinct clusters. The subpopulations identified exhibited a high level of genetic diversity and were moderately differentiated. These subpopulations could serve as heterotic groups and a relevant source of genes for future breeding and selection of diverse cultivars with different traits. Therefore, the results obtained from this study will be highly valuable in the development of new varieties adapted to diverse environments. Consequently, this study will substantially contribute to the utilization, conservation and broadening of the use of genetic resources of cowpeas for future improvement.

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## **CHAPTER 7: COMBINING ABILITY AND GENE ACTION STUDIES FOR GRAIN YIELD AND ITS RELATED TRAITS AMONG SELECTED COWPEA [*Vigna unguiculata* (L.) Walp] GENOTYPES IN SOUTH AFRICA.**

### **Abstract**

Cowpea is a major staple crop in the Sub-Saharan Africa (SSA). It is a versatile crop broad genetic variability and can grow successfully throughout the world especially in the tropical and subtropical agro-environmental conditions. The productivity of grain yield is low in the SSA due to the use of unimproved low-yielding varieties. The development of high yielding cowpea genotypes would greatly narrow the yield gaps in cowpea. The study aimed to determine the combining ability effects and gene action controlling the yield and its related traits, determine the correlations among the yield and yield components. Ten cowpea parental lines were crossed using a half diallel mating design and 45 F<sub>1</sub> progenies were developed. The progenies and the parents were evaluated under two environments for the performance of grain yield and yield related traits. The following traits were assessed: plant height (PH), leaf length (LL), leaf width (LW), number of pods per plant (NPP), pod length (PL), pod width (PW), number of seeds per pod (NSP), hundred seed weight (HSW) and grain yield (GY). The study showed that the general and specific combining ability varied for all the assessed traits indicating the prominence of additive and non-additive genetic effects. The parental genotypes Acc-cowp17, Acc-cowp31, Acc-cowp9, Acc-cowp5, Acc-cowp38 and Acc-cowp19 recorded the desirable positive and significant general combining ability effects for grain yield and were selected for future development of high-yielding cowpea varieties' newly developed F<sub>1</sub> progenies Acc-Cowp31 x Acc-Cowp5, Acc-Cowp38 x Acc-Cowp19, Acc-Cowp9 x Acc-Cowp2, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp38, and Acc-Cowp17 x Acc-Cowp38 recorded the desired SCA effects for the yield and related traits. The selected parents and crosses are recommended for hybrid development in South Africa and similar agro ecologies of SSA after continual selection and multi-environment testing.

**Keywords:** Cowpea, diallel analysis, general combining ability gene action, specific combining ability, yield production.

## 7.1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is among the neglected, underutilized, and indigenous grain legume crops in Sub-Saharan Africa (SSA). It is a versatile crop with broad genetic variability and can grow successfully throughout the world especially in the semi-arid and arid agro-environmental conditions. The crop serves as a staple food predominantly cultivated and consumed by majority of people in the semi-arid regions of tropical Africa, Asia and South America (Carvalho, 2017). *V. unguiculata* is further one of the most preferred crops in SSA due to its attributes and also has an important part in the farming systems of the less advantaged people from these regions (Horn and Shimelis, 2020). The cowpea plant parts that are consumed as food includes the grain seed and leaves and they are both highly nutritious food which provides proteins and macro-and micro-nutrients to the human diets. In Sub-Saharan Africa, the crop accounts for approximately 85% of the cowpea global production with 14.6 million tonnes cultivated in 14.6 hectares of land (FAOSTAT, 2020).

Regardless of the vast increase of demand of cowpea in the diets of the people, there have been a noticeable gap between the yields harvested against the expected yield in the country. Cowpea yields harvested of 500 to 599 kg/ha are relatively lower than the potential yield expected for the crop which ranges between 1500 kg/ha to 3000 kg ha<sup>-1</sup> in Sub-Saharan Africa (Gbaye and Holloway, 2011; Horn *et al.*, 2015). This is highly due to the several constraints which includes the use of unimproved varieties which are susceptible to droughts, poor soil fertility and other biotic and abiotic stresses and poor management's practices during the production. Therefore, it is imperative for cowpea breeding programs to develop new improved and high yielding varieties to enhance cowpea production and productivity in SSA.

Breeding for increased grain yield in conditions susceptible to abiotic stresses, such as drought and salinity, could be best achieved by selecting varieties with potential for increased yield and resistance to stresses under optimal production conditions. Grain yield is one of the most important traits in crop improvement programs but very complex because it is genetically controlled by multiple components (Romanus *et al.*, 2008; Asíns, 2004). The expression of grain yield is based on the effects of genotype, environments and the interactions of genotype by environments and all the processes of growth and development that occurs during vegetative and reproductive stages of the crop (Quarrie *et al.*, 2006). In addition, the yield components such as population density, number of pods per plant, pod length, number of seeds per pod, hundred seed weight and mean seed weight have been associated with yield gains in cowpea and are highly influenced by both environmental and genetic factors (Wattoo *et al.*, 2009). As

a results, selection based on grain yield alone would be challenging and effective. Hence, the necessity to evaluate the yield contributing traits prior to making decision regarding the parental combinations for grain yield improvement in breeding programs (Shimelis, 2006; Lopes *et al.*, 2017).

The information of gene actions and architecture controlling economically important traits such as grain yield and yield contributing characters is vital in the breeding programmes that aims to develop new varieties. This will allow the breeders to follow appropriate breeding strategy and would greatly identify the good general and specific combiners. Romanus *et al.* (2008) stressed out that the degree of gene action involved in the expression of quantitative traits is essential for a successful development of new crop varieties through appropriate choice of parents for hybridization. There are few and insufficient studies that have been done on combining ability and gene action controlling grain yield and its yield related traits among cowpea genotypes in SSA and worldwide as a whole.

Combining ability studies has been proven to be of great importance in providing baseline knowledge for the parent's selection based on the performance of hybrid (Owusu *et al.*, 2018; Pandey and Singh, 2010; Raut *et al.*, 2017). Combining ability analysis infer the gene action implicated during the expression of a certain trait using the ratios of general and specific combining ability effects. The general combining ability (GCA) is effective in predicting additive gene action contributing to heterosis while the specific combining ability (SCA) predicts the dominant gene action. It is further alluded that both these genetic effects are important as they will assist in the interpretation of genetic basis of quantitative traits (Rajani *et al.*, 2001; Ayo-Vaughan *et al.*, 2013). Combining ability analysis has been successfully used in various studies in cowpea to identify superior combinations for improved yield production (Owusu *et al.*, 2020; Ayo-Vaughan *et al.*, 2013; Romanus *et al.*, 2008) and other legume crops including pigeon pea (Bharate *et al.*, 2011), Chickpea (Jha *et al.*, 2018) and groundnuts (Shaibu *et al.*, 2018).

There are several mating designs that are used in the plant breeding programmes for progeny development and identifying the type of gene actions involved in inheritance of a specific character in question. Frankel and Galun (2012) declared that the mating designs selection are determined by the targeted size of population, type of crossing used for the crop, pollen dispersal and the presence of male sterility mechanism. This therefore necessitates the use of appropriate mating design during the development of superior progenies for the success of

improvement (Mahalingam *et al.*, 2011). Thus, several studies described and contrasted different mating designs which includes biparental, polycross, top cross, North Carolina, Line x tester and diallel mating (Griffing, 1956; Kempthorne, 1957; Comstock and Robinson, 1948). In this study, the diallel mating design will be used. The understanding of the nature of gene actions controlling inheritance of traits related to yield production will be of paramount importance in formulation of efficient breeding programmes that aims to improve cowpea yields as well as identifying good general and specific combiners for yield and yield related traits. Therefore, the objective of the present study was to determine the combining ability effects and gene action controlling the yield and its related traits, determine the correlations among the yield and yield components and also to determine the heritability of the yield and related traits among cowpea selected parental lines and their progenies.

## **7.2 Materials and Methods**

### **7.2.1 Plant Materials and generation of crosses**

Ten cowpea genotypes selected from a preliminary evaluation trial based on high grain yield (Table 7.1). The 10 selected lines were crossed between November and March using the 10 x 10 half diallel mating design to develop 45 F1 progenies in a glasshouse (25°7345 S, 28°2453 E) at the ARC-VIMP, Pretoria. The day and night temperatures in the glasshouse were set at 28 °C and 16 °C, respectively, and relative humidity ranged between 50% and 55%. The environment resembled field growing conditions, while excluding incoming rainfall, but irrigation was supplied every two days in a week. The stagger planting of 10 parents was done in 2-week interval to synchronize flowering and pollen supply. The crosses were done manually following the standard procedure by Myers (1996) and all 45 F1 progenies were generated successfully.

**Table 7.1:** The geographical region, seed coat colour and growth habit for the parental genotypes used for the study.

Parental genotypes	Geographical origin	Growth habit	Seed shape	Seed coat colour
Acc-Cowp2	South Africa	Prostrate	Kidney	Red
Acc-Cowp5	South Africa	Prostrate	Rhomboid	Black
Acc-Cowp9	South Africa	Semi-erect	Rhomboid	Cream
Acc-Cowp10	South Africa	Prostrate	Rhomboid	Purple
Acc-Cowp19	South Africa	Erect	Globose	Light brown
Acc-Cowp31	South Africa	Prostrate	Globose	Cream
Acc-Cowp32	South Africa	Prostrate	Kidney	Red
Acc-Cowp38	South Africa	Climbing	Globose	Black
Acc-Cowp47	South Africa	Erect	Rhomboid	Brown
Acc-Cowp17	South Africa	Erect	Ovoid	Cream

### 7.2.2 Description of the study site for progeny evaluation

The field evaluation of the progenies was conducted at the two selected sites in the Agricultural Experimental Farm in South Africa. The first site was Brits located in the Northwest province and the second site is in Loskop located at Groblersdal, Limpopo province. The first experimental site was Brits (25.6276° S, 27.7816° E, 1083 m above sea level), characterized by clay loam soil with a soil pH of 4.5 – 5.5 with an annual rainfall of 629 mm and mean daily minimum and maximum temperatures of 18.3 °C and 28.9 °C. The second experimental site was Loskop (25.1773° S, 29.3936°E, 920 m above sea level) characterised by loam soil with a soil pH of 5.0-6.2, an annual rainfall of 772 mm and a daily minimum and maximum temperature of 10.0 °C and 21.0 °C, respectively.

### 7.2.3 Trial design and management

Ten parents and 45 F1 progenies were evaluated at the field in an 11 x 5 alpha-lattice experimental design with three replicates during the September to February planting season. Each F1 progeny and parent was sown 6 m<sup>2</sup> plot with two rows which are 3 m long. The intra- and inter-row spacing was 0.3 and 1 m, respectively. Two seeds were sown per hole and were

later thinned after two weeks of emergence. There was no fertilizer input added. Further, the trials were rainfed, however sprinkler irrigation system was used to supply supplemental moisture where necessary. The chemical and cultural practices as per recommendation for cowpea were used during the field management practices.

#### **7.2.4 Data Collection**

The quantitative data were collected on the following agronomic traits: plant height (PH) in cm, leaf length (LL) in cm, leaf width (LW) in cm, number of pods per plant (NPP) which were counted pre plant, pod length (PL) in cm, pod width (PW) in mm, number of seeds per pod (NSP) which were counted per pod, hundred seed weight (HSW) in grams which measured from randomly selected 100 seeds after harvest per plot. Thereafter, the grain yield in was measured in grams plant<sup>-1</sup> and later converted to tons per hectare (t ha<sup>-1</sup>).

#### **7.2.5 Statistical analysis**

##### **7.2.5.1 Analysis of variance**

The data collected were subjected was subjected Genstat 20<sup>th</sup> edition (VSN International, Hempstead, UK). The mean values were separated at 5% probability level using Fischer's unprotected least significant difference (LSD). Subsequently, the Pearson correlation analysis was conducted to deduce trait and genotype association using Genstat 20<sup>th</sup> (VSN International, Hempstead, UK) software.

##### **7.2.5.2 Combining ability analysis.**

The general combining ability (GCA) and specific combining ability (SCA) were determined using the mean performance across all test environment based on Griffing's methods II and I (Griffing, 1956) using the analysis of genetic designs using the Plant Breeding Tools Version 1.4 (Belko et al., 2014). The fixed model for the analysis was as follows:

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + E_k + E_{kg_i}$$

$$X_{ij} = \mu + g_i + g_j + S_{ij} + E_k + E_{kg_i} + E_{kg_j} + E_{Sij} + e_{ijk}$$

Where,  $X_{ij}$  is the mean phenotypic observation of the character measured on the  $i^{\text{th}}$  cross with  $j^{\text{th}}$  in the  $k^{\text{th}}$  replicates,  $\mu$  is the overall meal effect,  $g_i$  and  $g_j$  are the GCA effects of the  $i^{\text{th}}$  and  $j^{\text{th}}$  parental genotypes,  $S_{ij}$  is the SCA effects of the cross between the  $i^{\text{th}}$  and  $j^{\text{th}}$  in the  $k^{\text{th}}$  replication and  $e_{ijk}$  is the environmental effects associated with the  $ijk^{\text{th}}$  individual observation,  $E_k + E_{kg_i} + E_{kg_j} + E_{Sij} = \text{GCA} \times \text{Environment}$  and  $\text{SCA} \times \text{Environment}$  interaction effects, respectively .

The significance effects of GCA and SCA source of variation were determined using the interactions corresponding with the environment as error terms. Further, the mean squares of the GCA x Environment and SCA x Environment interactions were tested using the pooled error variance according to Griffing (1956). The significance of GCA and SCA effects were determined by the t-test using their respective standard errors at 5% level of significance (Singh and Chaudhary, 1985).

### 7.2.5.3 Gene Action and Heritability Estimates

The additive genetic variance ( $\sigma^2A$ ), dominance genetic variance ( $\sigma^2D$ ), broad sense heritability ( $h^2B$ ) and narrow-sense heritability ( $h^2n$ ) were calculated from the GCA and SCA component analysis using the equations (Griffing *et al.*, 1956; Hallauer *et al.*, 2010) which are as follows:

$$\sigma^2A = 4\sigma^2gca$$

$$\sigma^2D = 4\sigma^2sca$$

$$h^2B = \frac{2\sigma^2GCA}{2\sigma^2GCA + \sigma^2AE + \delta^2e}$$

$$h^2n = \frac{2\sigma^2GCA + \sigma^2SCA}{2\sigma^2GCA + \sigma^2SCA + \delta^2e}$$

Where,  $4\sigma^2gca$  and  $4\sigma^2sca$  are an additive and dominance variance, respectively, which represents the genetic effects, while  $\sigma^2gca + \sigma^2sca + \delta^2e$  is an additive, dominance and an environmental variance which represents the phenotypic effects.

### 7.2.5.4 Correlation analysis

The Pearson correlation coefficients was computed to determine the relationship between the assessed traits under two using the SPSS version 25 (SPSS Inc., Chicago, IL, USA, 2018).

## 7.3 Results

### 7.3.1 Genotype, environmental and their interaction effects

A combined analysis of variance including the data collected from the parents and their progenies showing the mean squares and the level of significance of the yield and its yield components across two locations is presented in Table 7.2. The genotypic effects were significant at  $P \leq 0.01$  for all trial except the LL and NSP. The significant environmental effects were highly significant for all traits except for the LL and NSP. Furthermore, the significant differences due to genotype and environmental interaction effects were observed for all traits except the LL and NSP.

**Table 7.2:** Mean squares and significance tests for the assessed yield and yield related traits among the 10 parental cowpea genotypes and their 45 progenies evaluated in two South African locations.

Source of variation	d.f	NB	PH	LL	LW	NPP	PL	PW	NSP	HSW	GY
Replication	2	5.95**	2.16**	3245 <sup>ns</sup>	0.27**	9.60	0.07 <sup>ns</sup>	0.10 <sup>ns</sup>	0.29***	137.79**	0.01**
Block (Rep)	4	3.18**	5.84 <sup>ns</sup>	3297 <sup>ns</sup>	0.29 <sup>ns</sup>	24.62 <sup>ns</sup>	0.08 <sup>ns</sup>	0.07 <sup>ns</sup>	0.49 <sup>ns</sup>	0.74 <sup>ns</sup>	0.01 <sup>ns</sup>
Genotype (G)	54	9.62**	144.79**	3341 <sup>ns</sup>	8.49**	184.35**	17.79**	1.16**	21.45**	137.46 <sup>ns</sup>	0.60**
Environment(E)	1	80.51**	283.42**	2186 <sup>ns</sup>	66.31**	801.84**	0.14**	0.26*	45.10**	140.33 <sup>ns</sup>	3.72**
G x E	54	12.43**	140.72**	3299 <sup>ns</sup>	7.38**	209.47**	13.35**	1.45**	20.77**	137.43 <sup>ns</sup>	0.63**
Residual	218	0.49	13.50	3290	0.11	52.80	0.18	0.08	0.66	137.56	0.01

Notes: NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*, \*\*\* denotes the significance at 0.05, 0.01 and 0.001.

### 7.3.2 The mean performance of cowpea parents and their hybrids for assessed traits.

The combined mean performances of parents and their crosses for assessed yield related traits across two South African agro ecologies are presented in Table 7.3. The NB of parents ranged from 10.50 to 8.33 for the parents Acc-Cowp47 and Acc-Cowp38, respectively. The NB of progenies ranged from 6.82 to 12.83 with a combined grand mean of 9.22. The crosses Acc-Cowp32 x Acc-Cowp31 recorded the highest NB while the crosses Acc-Cowp38 x Acc-Cowp19 recorded the lowest NB. The PH of newly developed cowpea hybrids ranged from 92.10 cm to 317.17 cm. The crosses Acc-Cowp47 x Acc-Cowp9 and Acc-Cowp17 x Acc-Cowp47 recorded the highest PH of 317.17 cm and 275.61 cm, respectively while the crosses Acc-Cowp47 x Acc-Cowp5 and Acc-Cowp32 x Acc-Cowp2 recorded the lowest PH of 92.10 cm and 92.20 cm, respectively. The parental genotypes ranged between 260.58 and 110.75 cm. The parental genotype Acc-Cowp31 and Acc-Cowp32 recorded the highest PH of 260.58 cm and 251.98 cm, respectively, while parental lines Acc-Cowp17 and Acc-Cowp10 recorded the lowest PH of 110.75 cm and 133.02 cm, respectively. The crosses had the tallest PH compared to the parental lines.

The highest LL of 10.00 cm was expressed by the parent Acc-Cowp5 compared to the lowest LL of 7.90 cm recorded by the parent Acc-Cowp47. Among the crosses, the highest LL was recorded by Acc-Cowp10 x Acc-Cowp5 (12.20 cm, Acc-Cowp32 x Acc-Cowp5 (11.90 cm) and Acc-Cowp47 x Acc-Cowp9 (11.40 cm). The lowest LL was recorded for hybrids Acc-Cowp9 x Acc-Cowp2 (7.30 cm), Acc-Cowp47 x Acc-Cowp5 (7.60 cm) and Acc-Cowp32 x Acc-Cowp2 (7.60 cm). Among all the crosses, 53% of the crosses had the LL < 10 cm while the 47% of crosses possessed the LL >10 cm. The LW of parental lines ranged from 8.23 cm to 2.68 cm. Genotypes Acc-Cowp9 had the highest LW while the parental genotype Acc-Cowp5 had the lowest LW. The LW of progenies ranged from 8.82 cm to 3.40 cm. The highest LW was expressed by crosses Acc-Cowp31 x Acc-Cowp9, Acc-Cowp10 x Acc-Cowp2 and Acc-Cowp17 x Acc-Cowp47 with an LW of 8.82cm, 7.45 cm and 7.41 cm, respectively. The lowest LW of 3.40 cm, 4.08 cm and 4.31 cm was recorded for crosses Acc-Cowp32 x Acc-Cowp5, Acc-Cowp5 x Acc-Cowp2 and Acc-Cowp32 x Acc-Cowp10, respectively.

NPP varied from 236.17 to 57.50 across all parental genotypes. The parental genotype that recorded high NPP of 236.17 and 171.67 were Acc-Cowp2 and Acc-Cowp10, respectively whereas the parental genotypes Acc-Cowp5 and Acc-Cowp9 recorded the low NPP of 57.50 and 94.83, respectively. The crosses recorded a considerably high NPP as compared to parental genotypes which varied from 84.00 to 266.17. The crosses Acc-Cowp32 x Acc-Cowp31, Acc-Cowp17 x Acc-Cowp31 and Acc-Cowp17 x Acc-Cowp5 recorded the highest NPP of 266.17, 264.17 and 244.50, respectively. Comparably, the crosses Acc-Cowp38 x Acc-Cowp5, Acc-Cowp9 x Acc-Cowp5 and Acc-Cowp38 x Acc-Cowp32 recorded the lowest NPP of 79.67, 84.00 and 89.67, respectively.

PL of parental lines varied from 14.77 cm to 19.28 which were recorded for parental genotypes Acc-Cowp32 and Acc-Cowp19. The newly developed hybrids recorded a high PL which varied from 13.76 cm to 21.85 cm. The crosses that expressed the high PL were Acc-Cowp47 x Acc-Cowp38 (21.85 cm) and Acc-Cowp38 x Acc-Cowp9 (20.94 cm) and the lowest PL were recorded by crosses Acc-Cowp10 x Acc-Cowp5 (13.76 cm) and Acc-Cowp47 x Acc-Cowp5 (13.97 cm). Furthermore, the PW ranged from 5.00 mm and 6.94 mm for parental genotypes with genotype Acc-Cowp38 possessing the highest PW and Acc-Cowp5 recording the lowest PW. Furthermore, the PW of progenies ranged between 4.78 mm to 6.98 mm. The highest PW were recorded by crosses Acc-Cowp47 x Acc-Cowp38 and Acc-Cowp47 x Acc-Cowp2 with a PW of 6.98 mm and 6.94 mm, respectively whereas the lowest PW of 4.78 mm and 5.26 mm

were recorded by crosses Acc-Cowp17 x Acc-Cowp5 and Acc-Cowp17 x Acc-Cowp19, respectively.

The NSP of parental genotypes varied from 11.83 to 27.67 with a mean of 14.55. The parents Acc-Cowp38 and Acc-Cowp19 recorded the highest NSP of 17.67 and 16.86, respectively and the lowest NSP was recorded by parental genotypes Acc-Cowp38 (11.83) and Acc-Cowp17 (12.67). The NSP of newly developed crosses ranged from 11.17 to 20.67 with a mean of 15.13. The highest NSP among the progenies was recorded by Acc-Cowp47 x Acc-Cowp38 (20.67) and Acc-Cowp38 x Acc-Cowp10 whereas the lowest NSP among the progenies were recorded by Acc-Cowp10 x Acc-Cowp5 (11.17) and Acc-Cowp47 x Acc-Cowp5 (12.17). HSW for the parental genotypes ranged from 20.00g to 26.00 g with genotype Acc-Cowp38 and Acc-Cowp32 recording the highest HSW of 26.00g while the parental genotypes Acc-Cowp9, Acc-Cowp19 and Acc-Cowp31 recorded the lowest HSW of 20.00g, 22.00g and 22.00g, respectively. Furthermore, the crosses recorded the highest HSW compared to the parents which ranged from 19.00g to 35.75g with a mean of 24.33g. The crosses Acc-Cowp17 x Acc-Cowp31 recorded the highest NSP of 19.00 while Acc-Cowp38 x Acc-Cowp19 recorded the lowest HSW of 19.00 g.

Moreover, the low GY for the parents was recorded by Acc-Cowp9 ( $0.25 \text{ t ha}^{-1}$ ) and the high GY was recorded by parental genotype Acc-Cowp2 ( $0.85 \text{ t ha}^{-1}$ ) with a mean of  $0.61 \text{ t ha}^{-1}$  across all parental genotypes. GY of newly developed progenies varied from  $0.38 \text{ t ha}^{-1}$  for Acc-Cowp5 x Acc-Cowp2 and  $1.89 \text{ t ha}^{-1}$  for Acc-Cowp31 x Acc-Cowp5 with a mean of  $0.94 \text{ t ha}^{-1}$ . The highest GY yielders were Acc-Cowp5 x Acc-Cowp2 ( $1.89 \text{ t ha}^{-1}$ ), Acc-Cowp32 x Acc-Cowp31 ( $1.50 \text{ t ha}^{-1}$ ) and Acc-Cowp17 x Acc-Cowp5 ( $1.42 \text{ t ha}^{-1}$ ) and the lowest GY yield among crosses were Acc-Cowp5 x Acc-Cowp2 ( $0.38 \text{ t ha}^{-1}$ ), Acc-Cowp17 x Acc-Cowp2 ( $0.48 \text{ t ha}^{-1}$ ) and Acc-Cowp32 x Acc-Cowp2 ( $0.51 \text{ t ha}^{-1}$ ). Among the 45 progenies evaluated, 25 % had an outstanding performance greater than  $1 \text{ t ha}^{-1}$  ( $\text{GY} > 1 \text{ t ha}^{-1}$ ) and then 75 % yielded below  $1 \text{ t ha}^{-1}$  ( $\text{GY} < 1 \text{ t ha}^{-1}$ ).

**Table 7.3:** Combined means for grain yield and yield related traits evaluated among 10 parents and their 45 progenies of cowpea tested under two South African agro-ecologies.

<b>Genotypes</b>	<b>NB</b>	<b>PH</b>	<b>LL</b>	<b>LW</b>	<b>NPP</b>	<b>PL</b>	<b>PW</b>	<b>NSP</b>	<b>HSW</b>	<b>GY</b>
Parents										
Acc-Cowp2	8.50	137.70	9.80	4.33	236.17	17.01	5.93	14.00	25.00	0.85
Acc-Cowp5	9.50	168.21	10.00	2.68	94.83	16.09	5.00	15.67	24.00	0.75
Acc-Cowp9	9.33	185.96	184.40	8.23	57.50	15.90	5.85	12.33	20.00	0.25
Acc-Cowp10	9.67	133.02	10.70	6.38	171.67	16.48	5.65	14.00	24.00	0.92
Acc-Cowp19	8.50	197.06	9.80	6.48	104.33	19.28	6.32	16.83	22.00	0.46
Acc-Cowp31	9.00	260.58	9.20	4.31	114.50	15.88	6.23	15.17	22.00	0.31
Acc-Cowp32	9.50	251.98	9.00	4.29	85.67	14.77	5.69	11.83	26.00	0.79
Acc-Cowp38	8.33	211.08	8.20	6.86	115.00	17.02	6.94	17.67	26.00	0.52
Acc-Cowp47	10.50	189.36	7.90	6.58	116.50	17.40	5.89	15.33	22.00	0.44
Acc-Cowp17	9.00	110.75	9.40	6.52	124.67	14.42	5.25	12.67	23.00	0.79
Crosses										
Acc-Cowp5 x Acc-Cowp2	9.17	159.95	11.10	4.08	116.67	16.97	5.90	16.67	21.00	0.38
Acc-Cowp9 x Acc-Cowp2	8.00	146.18	7.30	5.02	106.00	16.44	6.17	15.00	22.00	0.76
Acc-Cowp10 x Acc-Cowp2	9.17	225.28	11.00	7.45	225.17	17.54	6.04	15.17	26.00	1.20
Acc-Cowp19 x Acc-Cowp2	9.17	185.71	9.00	6.85	107.67	16.45	5.95	13.67	20.00	0.75

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Acc-Cowp31 x Acc-Cowp2	8.33	204.60	10.10	7.07	261.00	17.00	5.95	13.67	20.00	1.36
Acc-Cowp32 x Acc-Cowp2	9.67	92.20	7.60	5.53	136.00	15.41	5.45	18.00	26.00	0.51
Acc-Cowp38 x Acc-Cowp2	9.17	187.90	10.20	7.01	234.33	16.29	6.18	13.33	24.00	0.90
Acc-Cowp47 x Acc-Cowp2	9.17	262.41	11.00	6.41	136.17	16.99	6.94	15.00	25.00	0.80
Acc-Cowp17 x Acc-Cowp2	8.50	172.90	11.00	4.87	147.17	18.50	5.93	16.50	21.00	0.48
Acc-Cowp9 x Acc-Cowp5	9.67	161.71	8.50	5.93	84.00	16.77	5.91	15.00	32.00	0.68
Acc-Cowp10 x Acc-Cowp5	9.17	239.62	12.10	6.77	202.83	13.76	5.45	11.17	27.00	1.04
Acc-Cowp19 x Acc-Cowp5	10.67	171.75	7.90	5.56	109.00	17.84	6.23	17.00	28.00	0.99
Acc-Cowp31 x Acc-Cowp5	8.67	234.81	10.00	6.85	115.50	17.40	6.76	15.17	21.00	1.89
Acc-Cowp32 x Acc-Cowp5	8.50	188.69	11.90	3.40	100.83	14.89	6.45	13.33	25.00	0.61
Acc-Cowp38 x Acc-Cowp5	7.83	204.08	10.10	5.49	79.67	16.37	6.57	14.17	28.00	0.83
Acc-Cowp47 x Acc-Cowp5	8.33	92.10	7.40	4.59	123.50	13.97	6.00	12.17	23.00	1.13
Acc-Cowp17 x Acc-Cowp5	9.67	138.04	9.40	5.55	244.50	16.82	4.78	15.50	23.00	1.42
Acc-Cowp10 x Acc-Cowp9	7.50	253.84	10.00	5.17	216.83	17.90	5.95	14.00	23.00	1.34
Acc-Cowp19 x Acc-Cowp9	11.67	170.28	9.50	5.00	93.00	14.77	6.03	16.50	23.00	0.55
Acc-Cowp31 x Acc-Cowp9	10.17	252.69	10.70	8.82	133.00	19.56	6.16	18.00	23.00	0.85
Acc-Cowp32 x Acc-Cowp9	11.17	197.18	8.60	7.00	216.33	14.87	5.90	13.00	21.00	1.05
Acc-Cowp38 x Acc-Cowp9	8.33	238.75	11.20	6.75	97.00	20.94	5.90	14.67	21.00	0.71

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Acc-Cowp47 x Acc-Cowp9	12.00	317.17	11.40	5.57	98.50	16.85	6.31	14.00	24.00	0.66
Acc-Cowp17 x Acc-Cowp9	7.67	171.37	7.90	4.87	101.50	15.29	6.35	12.67	27.00	0.76
Acc-Cowp19 x Acc-Cowp10	10.17	168.82	9.90	6.27	118.50	14.63	6.16	13.33	25.00	0.74
Acc-Cowp31 x Acc-Cowp10	9.17	196.13	9.10	4.97	131.67	13.53	6.04	13.00	23.00	0.86
Acc-Cowp32 x Acc-Cowp10	7.83	147.48	9.40	4.31	116.50	18.26	5.42	15.50	22.00	0.67
Acc-Cowp38 x Acc-Cowp10	10.17	160.21	10.50	7.30	176.50	19.37	6.22	18.33	25.00	1.00
Acc-Cowp47 x Acc-Cowp10	11.50	147.43	10.90	5.99	136.67	16.66	6.18	14.67	23.00	0.55
Acc-Cowp17 x Acc-Cowp10	8.33	243.61	10.20	7.11	181.83	16.36	5.98	15.00	20.00	0.62
Acc-Cowp31 x Acc-Cowp19	9.17	152.91	9.80	6.01	201.17	18.48	5.92	17.00	27.00	1.03
Acc-Cowp32 x Acc-Cowp19	9.33	155.45	10.40	6.39	102.83	19.35	6.50	18.00	26.00	0.81
Acc-Cowp38 x Acc-Cowp19	12.83	234.44	9.20	6.56	148.67	17.95	6.41	16.67	35.74	0.91
Acc-Cowp47 x Acc-Cowp19	10.50	116.27	10.50	5.98	219.33	19.57	6.42	16.67	25.00	0.99
Acc-Cowp17 x Acc-Cowp19	8.67	254.01	9.90	5.91	180.67	16.18	5.26	14.17	26.00	1.03
Acc-Cowp32 x Acc-Cowp31	6.67	193.07	8.50	6.13	266.17	16.91	6.04	14.83	21.00	1.50
Acc-Cowp38 x Acc-Cowp31	10.67	173.70	8.30	6.42	150.67	16.94	6.22	15.33	23.00	0.77
Acc-Cowp47 x Acc-Cowp31	11.00	133.89	11.10	6.62	126.17	16.99	5.75	14.50	24.00	0.94
Acc-Cowp17 x Acc-Cowp31	9.00	245.78	10.90	7.05	264.17	19.08	6.13	16.17	19.00	0.93
Acc-Cowp38 x Acc-Cowp32	9.17	156.81	8.10	5.00	89.67	17.04	5.96	14.83	25.00	0.54

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Acc-Cowp47 x Acc-Cowp32	9.50	221.22	9.00	4.49	139.17	15.88	6.28	12.50	29.00	0.59
Acc-Cowp17 x Acc-Cowp32	7.50	164.02	9.10	6.42	242.83	15.69	6.32	13.83	22.00	1.32
Acc-Cowp47 x Acc-Cowp38	7.67	258.65	8.90	6.40	188.00	21.85	6.98	20.67	24.00	1.32
Acc-Cowp17 x Acc-Cowp38	8.50	204.57	8.80	4.96	191.67	16.77	6.51	15.33	30.00	1.03
Acc-Cowp17 x Acc-Cowp47	6.67	275.67	8.00	7.41	178.50	18.01	6.40	17.17	22.00	0.91
<b>GM</b>	9.22	191.26	12.80	5.93	150.15	16.90	6.06	15.02	24.09	0.85
<b>Min</b>	6.67	92.10	7.30	2.68	57.50	13.53	4.78	11.17	19.00	0.25
<b>Max</b>	12.83	317.17	12.80	8.82	266.17	21.85	6.78	20.67	35.74	1.89
<b>CV (%)</b>	2.50	1.90	8.32	5.40	4.80	2.50	4.50	5.40	13.27	8.50
<b>LSD (5%)</b>	1.12	5.91	12.30	0.52	5.90	0.68	0.44	1.31	1.88	0.12

NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*, \*\*\* denotes the significance at 0.05, 0.01 and 0.001. See genotypes (G) in Table 2.

### **7.3.3 The analysis of variance of GCA and SCA effects of parents and F1 progenies**

The summary of analysis of variance in Table 7.4 shows the mean squares and the significant tests for the GCA and SCA effects for the assessed traits across two locations. The genotypic effects were highly significant for NB only at  $P \leq 0.001$  and the rest of the traits did not have a significant effect. The environmental effects were significant for NB, PH, LW, NPP, NSP, HSW and GY and showed no significant effects on LL, PL and PW. The genotypic x environmental interaction effects were significant for all traits except for LL and PL. The GCA effects were significant for LW, PL, NSP and HSW whereas the SCA effects were significant for PW only. The GCA x environmental interactions effects were highly significant for all traits while the SCA x environmental interaction effects were significant for all the traits except PH and LL.

**Table 7.4:** Mean squares and significant tests of general and specific combining ability effects for yield and yield relates traits across two sites in South Africa.

Source of variation	d.f.	NB	PH	LL	LW	NPP	PL	PW	NSP	HSW	GY
Rep	4	3.18***	5.84 <sup>ns</sup>	3297.55 <sup>ns</sup>	0.30*	24.62 <sup>ns</sup>	0.07 <sup>ns</sup>	0.07 <sup>ns</sup>	0.49 <sup>ns</sup>	0.74*	0.01*
Genotype (G)	54	9.62	14479.67 <sup>ns</sup>	3341.49 <sup>ns</sup>	8.49 <sup>ns</sup>	18435.38 <sup>ns</sup>	17.78 <sup>ns</sup>	1.16 <sup>ns</sup>	21.45 <sup>ns</sup>	41.76 <sup>ns</sup>	0.60 <sup>ns</sup>
Environment (E)	1	80.51**	28342.24**	2185.94 <sup>ns</sup>	66.30***	80184.05***	0.14 <sup>ns</sup>	0.26 <sup>ns</sup>	45.10***	137.95**	3.72***
GCA	9	11.90 <sup>ns</sup>	10572.42 <sup>ns</sup>	6060.57 <sup>ns</sup>	15.72*	31072.60 <sup>ns</sup>	23.46*	2.35 <sup>ns</sup>	29.04*	43.82**	0.411 <sup>ns</sup>
SCA	45	9.16 <sup>ns</sup>	15261.12 <sup>ns</sup>	2797.68 <sup>ns</sup>	7.04 <sup>ns</sup>	18907.93 <sup>ns</sup>	16.65 <sup>ns</sup>	0.93***	19.94 <sup>ns</sup>	41.35 <sup>ns</sup>	0.64 <sup>ns</sup>
G x E	54	12.43***	14072.21**	3298.83 <sup>ns</sup>	7.38***	20947.68***	13.34 <sup>ns</sup>	1.45***	20.77***	34.07***	0.63***
GCA x Env	9	14.37***	19307.51**	6299.99*	5.22***	31986.59***	7.34**	1.03***	11.23***	8.58***	0.77***
SCA x Env	45	12.04***	13025.14 <sup>ns</sup>	2698.59 <sup>ns</sup>	7.81***	18739.90***	15.55**	1.54***	22.67***	39.16***	0.61***
Residual	216	0.49	13.53	3289.17	0.10	52.92	0.18	0.08	0.66	0.33	0.01

Note: G = GCA= general combining ability, SCA= specific combining ability; d.f. = degrees of freedom; Rep= replication; Env= environment; GCA x Env = general combining ability by environment interaction ; SCA x Env = specific combining ability by environment interaction; NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*,\*\*\* denotes the significance at 0.05, 0.01 and 0.001.

#### 7.3.4 General combining effects of parental genotypes.

The general combining effects of the parental genotypes for yield and yield related traits under contrasting locations are presented in Table 7.5. The parental line exhibited the varied GCA effects for the different evaluated traits. The significant and positive GCA effects were the most desirable in this study. For NB, the GCA effects varied from 0.04 to 0.74. The positive and significant GCA effects for NB was recorded for parental genotypes Acc-Cowp19 ( $P \leq 0.01$ ; 0.64) and Acc-Cowp47 ( $P \leq 0.01$ ; 0.49) whereas a negative but significant GCA effects were recorded for parental genotypes Acc-Cowp2 ( $P \leq 0.05$ ; -0.350, Acc-Cowp17 ( $P \leq 0.001$ ; -0.74). The PH GCA effects ranged from 1.03 to 17.08. The positive and significant GCA effects were recorded Acc-Cowp9 ( $P \leq 0.05$ ; 14.77), Acc-Cowp31 ( $P \leq 0.001$ ; 17.08) and Acc-Cowp38 ( $P \leq 0.01$ ; 11.46) and then the significant but negative GCA effects were recorded for parental genotypes Acc-Cowp2 ( $P \leq 0.05$ ; -15.94) and Acc-Cowp5 ( $P \leq 0.05$ ; -14.72).

The GCA effects for LL varied from 2.18 for Acc-Cowp10 to 26.09 for Acc-Cowp9. The positive and significant GCA effects for LL were recorded for genotype Acc-Cowp9 ( $P \leq 0.001$ ; 26.09). The GCA effects for LW varied 0.12 for Acc-Cowp47 to 0.97 for Acc-Cowp5. The significant and positive GCA effects for LW were recorded for Acc-Cowp38 ( $P \leq 0.05$ ; 0.37) and Acc-Cowp9 ( $P \leq 0.05$ ; 0.45) whereas the significant but negative GCA effects were recorded for parental genotypes Acc-Cowp5 ( $P \leq 0.01$ ; -0.970 and Acc-Cowp32 ( $P \leq 0.05$ ; -0.66).

For NPP, GCA effects varied from 5.46 under Acc-Cowp38 to 32.54 under Acc-Cowp9. Positive and significant GCA effects for NPP were recorded for Acc-Cowp2 ( $P \leq 0.05$ ; 24.24), Acc-Cowp31 ( $P \leq 0.05$ ; 18.90) and Acc-Cowp17 ( $P \leq 0.01$ ; 27.54). On the contrast, negative GCA effects were recorded for Acc-Cowp5 ( $P \leq 0.01$ ; -23.79) and Acc-Cowp9 ( $P \leq 0.01$ ; -32.79). Furthermore, the GCA effects varied from 0.02 under Acc-Cowp2 and 0.97 under Acc-Cowp38 for PL. The significant and positive GCA effects for PL were recorded for Acc-Cowp19 ( $P \leq 0.01$ ; 0.66) and Acc-Cowp47 ( $P \leq 0.05$ ; 0.48). Comparably, the negative and significant GCA effects for PL were recorded for Acc-Cowp5 ( $P \leq 0.01$ ; -0.74), Acc-Cowp10 ( $P \leq 0.05$ ; -0.41), Acc-Cowp32 ( $P \leq 0.01$ ; -0.67) and Acc-Cowp17 ( $P \leq 0.05$ ; -0.20). For PW, the GCA effects ranged from 0.02 to 0.35. The significant and positive GCA effects were recorded for Acc-Cowp38 and Acc-Cowp47 while the significant but negative GCA effects for PW were recorded for Acc-Cowp5 ( $P \leq 0.01$ ; -0.22), Acc-Cowp10 ( $P \leq 0.05$ ; -0.16) and Acc-Cowp17 ( $P \leq 0.01$ ; -0.20).

The GCA effects for NSP ranged from 0.20 under Acc-Cowp2 to 1.12 under Acc-Cowp38. The significant and positive GCA effects for NSP were recorded for Acc-Cowp38 ( $P \leq 0.01$ ; 1.12) and Acc-Cowp19 ( $P \leq 0.01$ ; 0.95). NSP further recorded the significant but negative GCA effects for Acc-Cowp9 ( $P \leq 0.05$ ; -0.65), Acc-Cowp10 ( $P \leq 0.05$ ; -0.59) and Acc-Cowp32 ( $P \leq 0.05$ ; -0.64). For HSW, the GCA effects varied from 0.05 for Acc-Cowp10 to 1.37 for Acc-Cowp31. The significant and positive GCA effects were recorded for Acc-Cowp5 ( $P \leq 0.01$ ; 1.17), Acc-Cowp32 ( $P \leq 0.05$ ; 0.54) and Acc-Cowp38 ( $P \leq 0.01$ ; 0.940). Moreover, the GCA effects of GY varied from 0.02 for Acc-Cowp38 and Acc-Cowp32 to 0.12 Acc-Cowp10 and Acc-Cowp31. GY recorded the significant and positive GCA effects for Acc-Cowp5 ( $P \leq 0.01$ ; 0.09), Acc-Cowp31 ( $P \leq 0.01$ ; 0.12) and Acc-Cowp17 ( $P \leq 0.05$ ; 0.06) and further recorded the significant but negative GCA effects for parental genotypes Acc-Cowp9 ( $P \leq 0.01$ ; -0.12) and Acc-Cowp47 ( $P \leq 0.05$ ; -0.05).

**Table 7.5:** General combining ability effects for yield and yield related traits among 10 parental cowpea genotypes evaluated across two sites.

<b>Genotypes</b>	<b>NB</b>	<b>PH</b>	<b>LL</b>	<b>LW</b>	<b>NPP</b>	<b>PL</b>	<b>PW</b>	<b>NSP</b>	<b>HSW</b>	<b>GY</b>
Acc-Cowp2	-0.34*	-15.94*	-2.74 <sup>ns</sup>	-0.19 <sup>ns</sup>	24.24*	-0.02 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.20 <sup>ns</sup>	-0.68*	-0.04 <sup>ns</sup>
Acc-Cowp5	-0.06 <sup>ns</sup>	-14.72*	-2.69 <sup>ns</sup>	-0.97**	-23.79**	-0.74**	-0.22**	-0.31 <sup>ns</sup>	1.17**	0.09**
Acc-Cowp9	0.28 <sup>ns</sup>	14.77*	26.09***	0.45*	-32.54***	-0.06 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.65*	-0.55*	-0.12**
Acc-Cowp10	0.08 <sup>ns</sup>	-4.61 <sup>ns</sup>	-2.18 <sup>ns</sup>	0.24 <sup>ns</sup>	16.51 <sup>ns</sup>	-0.41*	-0.16*	-0.59*	-0.08 <sup>ns</sup>	0.04 <sup>ns</sup>
Acc-Cowp19	0.64**	-8.34 <sup>ns</sup>	-2.93 <sup>ns</sup>	0.19 <sup>ns</sup>	-13.51 <sup>ns</sup>	0.66**	0.07 <sup>ns</sup>	0.95**	0.33 <sup>ns</sup>	-0.05 <sup>ns</sup>
Acc-Cowp31	-0.05 <sup>ns</sup>	17.08***	-2.83 <sup>ns</sup>	0.28 <sup>ns</sup>	18.90*	0.14 <sup>ns</sup>	0.07 <sup>ns</sup>	0.23 <sup>ns</sup>	-1.37**	0.12**
Acc-Cowp32	-0.26 <sup>ns</sup>	-6.98 <sup>ns</sup>	-3.35 <sup>ns</sup>	-0.66*	-5.83 <sup>ns</sup>	-0.67**	-0.08 <sup>ns</sup>	-0.64*	0.54*	-0.02 <sup>ns</sup>
Acc-Cowp38	-0.04 <sup>ns</sup>	11.46*	-3.25 <sup>ns</sup>	0.37*	-5.46 <sup>ns</sup>	0.97**	0.35**	1.12**	0.94**	-0.02 <sup>ns</sup>
Acc-Cowp47	0.49**	8.31 <sup>ns</sup>	-3.06 <sup>ns</sup>	0.12 <sup>ns</sup>	-6.06 <sup>ns</sup>	0.48*	0.20**	0.23 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.05*
Acc-Cowp17	-0.74***	-1.03 <sup>ns</sup>	-3.07 <sup>ns</sup>	0.17 <sup>ns</sup>	27.54**	-0.36*	-0.20**	-0.30 <sup>ns</sup>	-0.39 <sup>ns</sup>	0.06*

Note: NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*, \*\*\* denotes the significance at 0.05, 0.01 and 0.001.

### 7.3.5 Specific combining ability effects for the F<sub>1</sub> progenies

The specific combining ability effects for the progenies for the evaluated yield and yield related traits under two different locations are presented in Table 7.6. The SCA effects had a wide variations among the 45 progenies for the assessed ten characters. NB recorded the significant and positive SCA effects of 1.05, 1.52, 0.71, 1.92, 2.00, 1.71, 3.00, 1.53, 1.34 and 1.53 for crosses Acc-Cowp32 x Acc-Cowp2, Acc-Cowp19 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp10, Acc-Cowp38 x Acc-Cowp19, Acc-Cowp38 x Acc-Cowp31, Acc-Cowp47 x Acc-Cowp31 and Acc-Cowp38 x Acc-Cowp32, respectively. The crosses Acc-Cowp9 x Acc-Cowp2, Acc-Cowp38 x Acc-Cowp5, Acc-Cowp47 x Acc-Cowp5, Acc-Cowp10 x Acc-Cowp9, Acc-Cowp38 x Acc-Cowp9, Acc-Cowp17 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp10, Acc-Cowp32 x Acc-Cowp31, Acc-Cowp47 x Acc-Cowp38 and Acc-Cowp17 x Acc-Cowp47 recorded significant but negative SCA effects.

The significant and positive SCA effects for PH were recorded for 9 crosses including Acc-Cowp10 x Acc-Cowp2 ( $P \leq 0.05$ ; 54.58), Acc-Cowp47 x Acc-Cowp2 ( $P \leq 0.001$ ; 78.78), Acc-Cowp10 x Acc-Cowp5 ( $P \leq 0.01$ ; 67.69), Acc-Cowp10 x Acc-Cowp9 ( $P \leq 0.05$ ; 52.42), Acc-Cowp47 and Acc-Cowp17 x Acc-Cowp47 ( $P \leq 0.01$ ; 77.14). The negative SCA effects for PH were recorded for crosses Acc-Cowp9 x Acc-Cowp2 ( $P \leq 0.05$ ; -43.91), Acc-Cowp32 x Acc-Cowp2 ( $P \leq 0.01$ ; -70.14), Acc-Cowp47 x Acc-Cowp5 ( $P \leq 0.01$ ; -92.75), Acc-Cowp47 x Acc-Cowp19 ( $P \leq 0.05$ ; -47.53), Acc-Cowp47 x Acc-Cowp19 ( $P \leq 0.01$ ; -74.96), Acc-Cowp38 x Acc-Cowp31 ( $P \leq 0.05$ ; -46.09) and Acc-Cowp47 x Acc-Cowp31 ( $P \leq 0.01$ ; -82.75).

For LL, the SCA effects ranged from 0.38 for Acc-Cowp47 x Acc-Cowp5 to 28.88 under Acc-Cowp9 x Acc-Cowp2 and there were no positive SCA effects recorded for these traits. However, LL recorded a significant negative SCA effects on some crosses including Acc-Cowp9 x Acc-Cowp2, Acc-Cowp9 x Acc-Cowp5, Acc-Cowp10 x Acc-Cowp9, Acc-Cowp19 x Acc-Cowp9, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp38 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp9 and Acc-Cowp17 x Acc-Cowp10. The LW expresses the significant and positive GCA effects on 12 crosses out 45 whereas the negative SCA effects were expressed on 11 crosses out 44. The crosses Acc-Cowp10 x Acc-Cowp5 (1.57), Acc-Cowp10 x Acc-Cowp2 (1.47), Acc-Cowp32 x Acc-Cowp9 (1.28) recorded the highest positive and significant SCA effects in LW and then the crosses Acc-Cowp17 x Acc-Cowp9 (-1.67), Acc-Cowp19 x Acc-Cowp9 (-1.57) and Acc-Cowp17 x Acc-Cowp38 (-1.50).

The SCA effects in NPP ranged from 0.90 under Acc-Cowp47 x Acc-Cowp32 to 104.56 under Acc-Cowp32 x Acc-Cowp9. Out of the 44 crosses, 10 crosses expressed the significant and positive SCA effects while 3 of them possessed the significant but negative SCA effects. The highest progenies that had the positive effects includes Acc-Cowp32 x Acc-Cowp9 (104.56), Acc-Cowp32 x Acc-Cowp31 (102.95) and Acc-Cowp17 x Acc-Cowp5 (90.59) and the progenies that express the negative SCA effects included Acc-Cowp19 x Acc-Cowp2 (-53.21), Acc-Cowp17 x Acc-Cowp2 (-54.76) and Acc-Cowp31 x Acc-Cowp10 (-53.90). Furthermore, the PL recorded the SCA effects that varied between 0.02 for Acc-Cowp31 x Acc-Cowp2 to 3.50 for Acc-Cowp47 x Acc-Cowp38. Out of 45 crosses, 22% of crosses expressed the significant and positive SCA effects whereas 13% of crosses expressed the negative SCA effects for PL. For PW, there was a considerable variation on SCA ranging from 0.03 for Acc-Cowp38 x Acc-Cowp10 to 0.86 for Acc-Cowp17 x Acc-Cowp5. The crosses Acc-Cowp31 x Acc-Cowp5 (0.85), Acc-Cowp47 x Acc-Cowp2 (0.70) and Acc-Cowp32 x Acc-Cowp5 (0.68) expressed the highest significant and positive SCA effects on PW.

SCA effects for NSP ranged from 0.01 for Acc-Cowp47 x Acc-Cowp10 to 4.29 for Acc-Cowp47 x Acc-Cowp38. Out of 45 crosses, 9 recorded the significant but negative SCA effects including Acc-Cowp47 x Acc-Cowp32 (-2.10), Acc-Cowp10 x Acc-Cowp5 (-2.95) and Acc-Cowp47 x Acc-Cowp2 (-2.79). Similarly, 9 crosses recorded the positive SCA effects including Acc-Cowp47 x Acc-Cowp38 (4.29), Acc-Cowp32 x Acc-Cowp2 (3.65) and Acc-Cowp31 x Acc-Cowp9 (3.39). For HSW, the SCA effects ranged from 0.17 for Acc-Cowp38 x Acc-Cowp10 to 7.13 for Acc-Cowp9 x Acc-Cowp5. 8 crosses out of 45 crosses recorded significant and positive SCA effects which includes Acc-Cowp9 x Acc-Cowp5 (7.13), Acc-Cowp17 x Acc-Cowp38 (5.48) and Acc-Cowp47 x Acc-Cowp32 (4.93). Similarly, 8 crosses out of 45 recorded the negative SCA effects including Acc-Cowp19 x Acc-Cowp2 (-3.87), Acc-Cowp38 x Acc-Cowp9 (-3.46) and Acc-Cowp17 x Acc-Cowp10 (-3.31) crosses.

Estimates of the SCA effects for GY varied from 0.01 for Acc-Cowp38 x Acc-Cowp9 to 0.83 for Acc-Cowp31 x Acc-Cowp5. The positive and significant SCA effects for GY were recorded for 12 crosses including Acc-Cowp31 x Acc-Cowp5 (0.83), Acc-Cowp17 x Acc-Cowp19 (0.55) and Acc-Cowp10 x Acc-Cowp9 (0.58). Then 3 crosses out of 45 crosses recorded the negative SCA effects including Acc-Cowp17 x Acc-Cowp2 (-0.39), Acc-Cowp17 x Acc-Cowp10 (-0.33) and Acc-Cowp17 x Acc-Cowp31 (-0.28).

**Table 7.6:** Specific combining ability effects for yield and yield related traits among 45 progenies of cowpea assessed under two sites.

<b>Crosses</b>	<b>NB</b>	<b>PH</b>	<b>LL</b>	<b>LW</b>	<b>NPP</b>	<b>PL</b>	<b>PW</b>	<b>NSP</b>	<b>HSW</b>	<b>GY</b>
Acc-Cowp5 x Acc-Cowp2	0.39 <sup>ns</sup>	-0.65 <sup>ns</sup>	3.74 <sup>ns</sup>	-0.69 <sup>ns</sup>	-33.93 <sup>ns</sup>	0.83 <sup>ns</sup>	0.08 <sup>ns</sup>	1.98*	-3.29*	0.52***
Acc-Cowp9 x Acc-Cowp2	-1.16*	-43.91*	-28.88**	-1.17*	-35.85 <sup>ns</sup>	-0.37 <sup>ns</sup>	0.16 <sup>ns</sup>	0.65 <sup>ns</sup>	-0.81 <sup>ns</sup>	0.08 <sup>ns</sup>
Acc-Cowp10 x Acc-Cowp2	0.21 <sup>ns</sup>	54.58*	3.14 <sup>ns</sup>	1.47**	34.27 <sup>ns</sup>	1.07 <sup>ns</sup>	0.16 <sup>ns</sup>	0.76 <sup>ns</sup>	2.49*	0.35**
Acc-Cowp19 x Acc-Cowp2	-0.34 <sup>ns</sup>	18.73 <sup>ns</sup>	1.82 <sup>ns</sup>	0.92*	-53.21*	-1.09 <sup>ns</sup>	-0.16 <sup>ns</sup>	-2.29**	-3.87**	-0.01 <sup>ns</sup>
Acc-Cowp31 x Acc-Cowp2	-0.50 <sup>ns</sup>	-12.21 <sup>ns</sup>	2.91 <sup>ns</sup>	1.05 <sup>ns</sup>	67.71*	0.02 <sup>ns</sup>	-0.15 <sup>ns</sup>	-1.56*	-1.48 <sup>ns</sup>	0.43**
Acc-Cowp32 x Acc-Cowp2	1.05*	-70.14**	0.88 <sup>ns</sup>	0.45 <sup>ns</sup>	-32.55 <sup>ns</sup>	-0.79 <sup>ns</sup>	-0.51*	3.65***	1.99 <sup>ns</sup>	0.28**
Acc-Cowp38 x Acc-Cowp2	0.32 <sup>ns</sup>	1.13 <sup>ns</sup>	3.34 <sup>ns</sup>	0.90*	65.40*	-1.56*	-0.21 <sup>ns</sup>	-2.79**	-0.18 <sup>ns</sup>	0.12 <sup>ns</sup>
Acc-Cowp47 x Acc-Cowp2	-0.20 <sup>ns</sup>	78.78***	3.99 <sup>ns</sup>	0.55 <sup>ns</sup>	-32.17 <sup>ns</sup>	-0.36 <sup>ns</sup>	0.70***	-0.23 <sup>ns</sup>	1.66 <sup>ns</sup>	0.04 <sup>ns</sup>
Acc-Cowp17 x Acc-Cowp2	0.36 <sup>ns</sup>	-1.38 <sup>ns</sup>	4.05 <sup>ns</sup>	-1.03*	-54.76*	1.99*	0.09 <sup>ns</sup>	1.80*	-1.38 <sup>ns</sup>	-0.39**
Acc-Cowp9 x Acc-Cowp5	-0.29 <sup>ns</sup>	-29.59 <sup>ns</sup>	-27.68**	0.52*	-9.82 <sup>ns</sup>	0.67 <sup>ns</sup>	0.09 <sup>ns</sup>	0.94 <sup>ns</sup>	7.13***	-0.14 <sup>ns</sup>
Acc-Cowp10 x Acc-Cowp5	-0.07 <sup>ns</sup>	67.69**	4.19 <sup>ns</sup>	1.57**	59.96*	-1.98*	-0.23 <sup>ns</sup>	-2.95**	1.67 <sup>ns</sup>	0.05 <sup>ns</sup>
Acc-Cowp19 x Acc-Cowp5	0.86 <sup>ns</sup>	3.55 <sup>ns</sup>	0.68 <sup>ns</sup>	0.41 <sup>ns</sup>	-3.85 <sup>ns</sup>	1.02 <sup>ns</sup>	0.31 <sup>ns</sup>	1.34 <sup>ns</sup>	2.50*	0.09 <sup>ns</sup>
Acc-Cowp31 x Acc-Cowp5	-0.44 <sup>ns</sup>	41.20 <sup>ns</sup>	2.71 <sup>ns</sup>	1.61 <sup>ns</sup>	-29.76 <sup>ns</sup>	1.09 <sup>ns</sup>	0.85***	0.23 <sup>ns</sup>	-2.68*	0.83***
Acc-Cowp32 x Acc-Cowp5	-0.40 <sup>ns</sup>	19.13 <sup>ns</sup>	5.18 <sup>ns</sup>	-0.89*	-19.69 <sup>ns</sup>	-0.59 <sup>ns</sup>	0.68**	-0.73 <sup>ns</sup>	-0.24 <sup>ns</sup>	-0.31 <sup>ns</sup>
Acc-Cowp38 x Acc-Cowp5	-1.29*	16.09 <sup>ns</sup>	3.27 <sup>ns</sup>	0.16 <sup>ns</sup>	-41.23 <sup>ns</sup>	-0.76 <sup>ns</sup>	0.38 <sup>ns</sup>	-1.66 <sup>ns</sup>	1.72 <sup>ns</sup>	-0.09 <sup>ns</sup>
Acc-Cowp47 x Acc-Cowp5	-1.32*	-92.75***	0.38 <sup>ns</sup>	-0.49 <sup>ns</sup>	3.19 <sup>ns</sup>	-2.66**	-0.04 <sup>ns</sup>	-2.77**	-1.73 <sup>ns</sup>	0.23 <sup>ns</sup>

Acc-Cowp17 x Acc-Cowp5	1.25 <sup>ns</sup>	-37.47 <sup>ns</sup>	2.37 <sup>ns</sup>	0.42 <sup>ns</sup>	90.59 <sup>***</sup>	1.03 <sup>ns</sup>	-0.86 <sup>***</sup>	1.09 <sup>ns</sup>	-1.23 <sup>ns</sup>	0.42 <sup>**</sup>
Acc-Cowp10 x Acc-Cowp9	-2.08 <sup>**</sup>	52.42 <sup>*</sup>	-26.71 <sup>**</sup>	-1.45 <sup>ns</sup>	82.71 <sup>**</sup>	1.46 <sup>*</sup>	0.07 <sup>ns</sup>	0.21 <sup>ns</sup>	-0.23 <sup>ns</sup>	0.58 <sup>**</sup>
Acc-Cowp19 x Acc-Cowp9	1.52 <sup>*</sup>	-27.41 <sup>ns</sup>	-26.47 <sup>**</sup>	-1.57 <sup>**</sup>	-11.09 <sup>ns</sup>	-2.73 <sup>**</sup>	-0.08 <sup>ns</sup>	1.17 <sup>ns</sup>	-0.35 <sup>ns</sup>	-0.13 <sup>ns</sup>
Acc-Cowp31 x Acc-Cowp9	0.71 <sup>*</sup>	29.59 <sup>ns</sup>	-25.39 <sup>*</sup>	2.16 <sup>**</sup>	-3.51 <sup>ns</sup>	2.57 <sup>**</sup>	0.06 <sup>ns</sup>	3.39 <sup>***</sup>	1.08 <sup>ns</sup>	0.01 <sup>ns</sup>
Acc-Cowp32 x Acc-Cowp9	1.92 <sup>**</sup>	-1.87 <sup>ns</sup>	-26.95 <sup>**</sup>	1.28 <sup>*</sup>	104.56 <sup>***</sup>	-1.29 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.73 <sup>ns</sup>	-2.78 <sup>**</sup>	0.34 <sup>**</sup>
Acc-Cowp38 x Acc-Cowp9	-1.14 <sup>*</sup>	21.27 <sup>ns</sup>	-24.46 <sup>*</sup>	0.01 <sup>ns</sup>	-15.15 <sup>ns</sup>	3.12 <sup>***</sup>	-0.49 <sup>*</sup>	-0.83 <sup>ns</sup>	-3.46 <sup>**</sup>	0.01 <sup>ns</sup>
Acc-Cowp47 x Acc-Cowp9	2.00 <sup>**</sup>	102.83 <sup>***</sup>	-24.46 <sup>*</sup>	-0.92 <sup>*</sup>	-13.05 <sup>ns</sup>	-0.47 <sup>ns</sup>	0.07 <sup>ns</sup>	-0.60 <sup>ns</sup>	0.79 <sup>ns</sup>	-0.02 <sup>ns</sup>
Acc-Cowp17 x Acc-Cowp9	-1.09 <sup>*</sup>	-33.64 <sup>ns</sup>	-27.90 <sup>**</sup>	-1.67 <sup>**</sup>	-43.65 <sup>ns</sup>	-1.19 <sup>ns</sup>	0.52 <sup>**</sup>	-1.41 <sup>*</sup>	3.82 <sup>**</sup>	-0.03 <sup>ns</sup>
Acc-Cowp19 x Acc-Cowp10	0.22 <sup>ns</sup>	-9.48 <sup>ns</sup>	2.25 <sup>ns</sup>	-0.09 <sup>ns</sup>	34.65 <sup>ns</sup>	-2.51 <sup>**</sup>	0.18 <sup>ns</sup>	-2.05 <sup>*</sup>	0.72 <sup>ns</sup>	-0.09 <sup>ns</sup>
Acc-Cowp31 x Acc-Cowp10	-0.08 <sup>ns</sup>	-7.59 <sup>ns</sup>	1.27 <sup>ns</sup>	-1.48 <sup>**</sup>	-53.90 <sup>*</sup>	-3.11 <sup>***</sup>	0.08 <sup>ns</sup>	-1.66 <sup>*</sup>	0.70 <sup>ns</sup>	-0.15 <sup>ns</sup>
Acc-Cowp32 x Acc-Cowp10	-1.21 <sup>*</sup>	-32.19 <sup>ns</sup>	2.12 <sup>ns</sup>	-1.19 <sup>*</sup>	-44.33 <sup>ns</sup>	2.44 <sup>**</sup>	-0.40 <sup>*</sup>	1.71 <sup>*</sup>	-2.04 <sup>*</sup>	-0.21 <sup>ns</sup>
Acc-Cowp38 x Acc-Cowp10	0.91 <sup>ns</sup>	-37.89 <sup>ns</sup>	3.15 <sup>ns</sup>	0.77 <sup>*</sup>	15.29 <sup>ns</sup>	1.91 <sup>*</sup>	-0.03 <sup>ns</sup>	2.78 <sup>**</sup>	0.17 <sup>ns</sup>	0.14 <sup>ns</sup>
Acc-Cowp47 x Acc-Cowp10	1.71 <sup>*</sup>	-47.53 <sup>*</sup>	3.37 <sup>ns</sup>	-0.30 <sup>ns</sup>	-23.94 <sup>ns</sup>	-0.31 <sup>ns</sup>	0.08 <sup>ns</sup>	0.01 <sup>ns</sup>	-1.13 <sup>ns</sup>	-0.29 <sup>ns</sup>
Acc-Cowp17 x Acc-Cowp10	-0.22 <sup>ns</sup>	57.99 <sup>*</sup>	2.60 <sup>ns</sup>	0.77 <sup>*</sup>	-12.37 <sup>ns</sup>	0.23 <sup>ns</sup>	0.29 <sup>ns</sup>	0.87 <sup>ns</sup>	-3.31 <sup>*</sup>	-0.33 <sup>**</sup>
Acc-Cowp31 x Acc-Cowp19	-0.65 <sup>ns</sup>	-47.09 <sup>*</sup>	2.71 <sup>ns</sup>	-0.39 <sup>ns</sup>	45.63 <sup>ns</sup>	0.78 <sup>ns</sup>	-0.28 <sup>ns</sup>	0.79 <sup>ns</sup>	4.22 <sup>**</sup>	0.12 <sup>ns</sup>
Acc-Cowp32 x Acc-Cowp19	-0.27 <sup>ns</sup>	-20.49 <sup>ns</sup>	3.88 <sup>ns</sup>	0.93 <sup>*</sup>	-27.97 <sup>ns</sup>	2.46 <sup>**</sup>	0.45 <sup>*</sup>	2.67 <sup>**</sup>	1.10 <sup>ns</sup>	0.14 <sup>ns</sup>
Acc-Cowp38 x Acc-Cowp19	3.00 <sup>***</sup>	40.06 <sup>ns</sup>	2.61 <sup>ns</sup>	0.08 <sup>ns</sup>	17.49 <sup>ns</sup>	-0.58 <sup>ns</sup>	-0.07 <sup>ns</sup>	-0.42 <sup>ns</sup>	-2.65 <sup>ns</sup>	0.24 <sup>*</sup>
Acc-Cowp47 x Acc-Cowp19	0.14 <sup>ns</sup>	-74.96 <sup>**</sup>	3.67 <sup>ns</sup>	-0.26 <sup>ns</sup>	88.75 <sup>**</sup>	1.54 <sup>*</sup>	0.09 <sup>ns</sup>	0.46 <sup>ns</sup>	0.93 <sup>ns</sup>	0.17 <sup>ns</sup>

Acc-Cowp17 x Acc-Cowp19	-0.45 <sup>ns</sup>	72.12 <sup>**</sup>	3.08 <sup>ns</sup>	-0.38 <sup>ns</sup>	16.48 <sup>ns</sup>	-1.02 <sup>ns</sup>	-0.67 <sup>**</sup>	-1.51 <sup>ns</sup>	2.18 <sup>*</sup>	0.55 <sup>**</sup>
Acc-Cowp32 x Acc-Cowp31	-2.25 <sup>**</sup>	-8.29 <sup>ns</sup>	1.91 <sup>ns</sup>	0.58 <sup>*</sup>	102.95 <sup>***</sup>	0.53 <sup>ns</sup>	-0.01 <sup>ns</sup>	0.23 <sup>ns</sup>	-2.18 <sup>*</sup>	-0.17 <sup>ns</sup>
Acc-Cowp38 x Acc-Cowp31	1.53 <sup>*</sup>	-46.09 <sup>*</sup>	1.58 <sup>ns</sup>	-0.15 <sup>ns</sup>	-12.93 <sup>ns</sup>	-1.08 <sup>ns</sup>	-0.26 <sup>ns</sup>	-1.04 <sup>ns</sup>	-0.33 <sup>ns</sup>	0.02 <sup>ns</sup>
Acc-Cowp47 x Acc-Cowp31	1.34 <sup>*</sup>	-82.75 <sup>***</sup>	4.15 <sup>ns</sup>	0.29 <sup>ns</sup>	-36.83 <sup>ns</sup>	-0.53 <sup>ns</sup>	-0.57 <sup>*</sup>	-0.98 <sup>ns</sup>	1.14 <sup>ns</sup>	-0.09 <sup>ns</sup>
Acc-Cowp17 x Acc-Cowp31	0.57 <sup>ns</sup>	38.47 <sup>ns</sup>	3.99 <sup>ns</sup>	0.67 <sup>*</sup>	67.53 <sup>*</sup>	2.39 <sup>*</sup>	0.21 <sup>ns</sup>	1.21 <sup>ns</sup>	-2.93 <sup>ns</sup>	-0.28 <sup>**</sup>
Acc-Cowp38 x Acc-Cowp32	1.53 <sup>*</sup>	-38.92 <sup>ns</sup>	1.86 <sup>ns</sup>	-0.64 <sup>*</sup>	-49.19 <sup>*</sup>	-0.16 <sup>ns</sup>	-0.37 <sup>ns</sup>	-0.66 <sup>ns</sup>	-0.51 <sup>ns</sup>	-0.20 <sup>ns</sup>
Acc-Cowp47 x Acc-Cowp32	0.05 <sup>ns</sup>	28.64 <sup>ns</sup>	2.66 <sup>ns</sup>	-0.89 <sup>*</sup>	0.90 <sup>ns</sup>	-0.82 <sup>ns</sup>	0.09 <sup>ns</sup>	-2.10 <sup>*</sup>	4.93 <sup>*</sup>	0.03 <sup>ns</sup>
Acc-Cowp17 x Acc-Cowp32	-0.72 <sup>ns</sup>	-19.23 <sup>ns</sup>	2.74 <sup>ns</sup>	0.99 <sup>*</sup>	70.97 <sup>**</sup>	-0.18 <sup>ns</sup>	0.54 <sup>**</sup>	-0.24 <sup>ns</sup>	-1.84 <sup>ns</sup>	0.42 <sup>**</sup>
Acc-Cowp47 x Acc-Cowp38	-2.01 <sup>**</sup>	47.63 <sup>*</sup>	2.45 <sup>ns</sup>	-0.01 <sup>ns</sup>	49.36 <sup>*</sup>	3.50 <sup>***</sup>	0.37 <sup>ns</sup>	4.29 <sup>***</sup>	-0.37 <sup>ns</sup>	0.54 <sup>***</sup>
Acc-Cowp17 x Acc-Cowp38	0.06 <sup>ns</sup>	2.89 <sup>ns</sup>	2.32 <sup>ns</sup>	-1.50 <sup>***</sup>	19.43 <sup>ns</sup>	-0.74 <sup>ns</sup>	0.31 <sup>ns</sup>	-0.51 <sup>ns</sup>	5.45 <sup>**</sup>	0.14 <sup>ns</sup>
Acc-Cowp17 x Acc-Cowp47	-2.30 <sup>**</sup>	77.14 <sup>**</sup>	1.29 <sup>ns</sup>	1.20 <sup>ns</sup>	6.86 <sup>ns</sup>	0.99 <sup>ns</sup>	0.35 <sup>ns</sup>	2.21 <sup>*</sup>	-1.66 <sup>ns</sup>	0.05 <sup>ns</sup>

Notes: NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*, \*\*\* denotes the significance at 0.05, 0.01 and 0.001.

### 7.3.6 Gene action and heritability estimates

The estimates for gene action and heritability from the crosses among the assessed yield and yield-related traits are presented in Table 7.7. There were variations in gene action and heritability among the assessed traits. The additive variance ( $\sigma^2A$ ) ranged from 0.01 for LL to 106.55 for NPP. An additive variance of zero was recorded for PH and G. The dominance variance ( $\sigma^2D$ ) varied from 0.03 for GY to 1490.65 for PH and the zero was recorded for NB, LW, PW and NSP. In overall, the  $\sigma^2A$  was higher than  $\sigma^2D$  for many traits including NB, LW, NPP, PW, NSP and HSW except for PH, LL and GY which recorded high  $\sigma^2D$  than  $\sigma^2A$ . The broad-sense heritability ( $h^2B$ ) ranged from 0.01 for NB and NPP to 0.10 for PL. Further, the  $h^2n$  ranged from 0.03 for HSW to 0.06 for LW, however, an  $h^2n$  of zero was recorded for PH, LL, NPP and GY.

**Table 7.7:** Gene action and heritability estimates for yield and yield related traits among 10 cowpea parents and 45 F1 progenies evaluated across two sites in South Africa.

Traits	$\sigma^2A$	$\sigma^2D$	$h^2B$	$h^2n$	Residual
NB	0.04	0.00	0.01	0.05	0.49
PH	0.04	1490.65	0.08	0.04	13.53
LL	0.01	66.05	0.02	0.07	3289.17
LW	0.63	0.00	0.06	0.06	0.10
NPP	106.55	0.00	0.01	0.07	52.92
PL	0.78	1.40	0.10	0.04	0.18
PW	0.11	0.00	0.05	0.05	0.08
NSP	1.14	0.00	0.04	0.04	0.66
HSW	1.84	1.46	0.06	0.03	0.33
GY	0.14	0.03	0.03	0.06	0.02

Notes:  $\sigma^2A$  : additive variance,  $\sigma^2D$  : dominance variance,  $h^2B$  : broad-sense heritability,  $h^2n$  : narrow-sense heritability, NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*, \*\*\* denotes the significance at 0.05, 0.01 and 0.001.

### 7.3.7 The associations of the assessed yield and yield-related traits

The Pearson's correlation coefficients showing the considerable associations between the assessed traits across all two locations are presented in Table 7.8. The high significant and positive effects were recorded between the several traits. A significant and positive correlation between NB and PH ( $r = 0.11$ ;  $P \leq 0.05$ ) and NB with GY ( $r = 0.17$ ;  $P \leq 0.05$ ). NB also had a negative and significant relations with NPP ( $r = 0.18$ ;  $P \leq 0.01$ ). PH has a significant and positive correlations with PW ( $r = 0.12$ ;  $P \leq 0.05$ ) and GY ( $r = 0.10$ ;  $P \leq 0.05$ ). Moreover, a significant and negative correlations were recorded between NB and NPP ( $r = 0.18$ ;  $P \leq 0.01$ ). PH had a positive and significant correlations with PW ( $r = 0.12$ ;  $P \leq 0.05$ ) and GY ( $r = 0.10$ ;  $P \leq 0.05$ ). Furthermore, the significant and positive associations were observed between LL and NPP ( $r = 0.05$ ;  $P \leq 0.01$ ), NSP ( $r = 0.06$ ;  $P \leq 0.01$ ) and GY ( $r = 0.05$ ;  $P \leq 0.05$ ). A considerably high significant correlations were also recorded between NPP and GY ( $r = 0.71$ ;  $P \leq 0.05$ ) and PL ( $r = 0.11$ ;  $P \leq 0.05$ ). Additionally, PL had a significant and positive correlations with PW ( $r = 0.39$ ;  $P \leq 0.01$ ), NSP ( $r = 0.70$ ;  $P \leq 0.01$ ) and GY ( $r = 0.06$ ;  $P \leq 0.01$ ). Moreover, a significantly high and positive associations were recorded between PW and NSP ( $r = 0.87$ ;  $P \leq 0.01$ ), HSW ( $r = 0.91$ ;  $P \leq 0.01$ ) and GY ( $r = 0.19$ ;  $P \leq 0.01$ ). There were also positive and significant correlations between NSP and GY ( $r = 0.06$ ;  $P \leq 0.01$ ).

**Table 7.8:** Pearson correlation coefficients of grain yield and yield components among 10 cowpea parental lines and their 45 progenies evaluated across two locations in South Africa.

Traits	NB	PH	LL	LW	NPP	PL	PW	NSP	HSW	GY
NB	-									
PH	0.11*	-								
LL	-0.03 <sup>ns</sup>	0.07 <sup>ns</sup>	-							
LW	0.04 <sup>ns</sup>	0.02 <sup>ns</sup>	0.08 <sup>ns</sup>	-						
NPP	0.18**	0.03 <sup>ns</sup>	0.05**	0.07 <sup>ns</sup>	-					
PL	0.04 <sup>ns</sup>	0.07 <sup>ns</sup>	0.05 <sup>ns</sup>	0.21 <sup>ns</sup>	0.11*	-				
PW	0.02 <sup>ns</sup>	0.12*	0.01 <sup>ns</sup>	0.05 <sup>ns</sup>	0.09 <sup>ns</sup>	0.39**	-			
NSP	0.01 <sup>ns</sup>	0.09 <sup>ns</sup>	0.06**	0.19 <sup>ns</sup>	0.07 <sup>ns</sup>	0.70**	0.81**	-		
HSW	0.10 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	0.08 <sup>ns</sup>	0.01 <sup>ns</sup>	0.71 <sup>ns</sup>	0.91**	0.04 <sup>ns</sup>	-	
GY	0.17**	0.10*	0.07*	0.11 <sup>ns</sup>	0.71**	0.06**	0.19**	0.06**	0.71 <sup>ns</sup>	-

NB: number of branches, PH: plant height, LL: leaf length, LW: leaf width, NPP: number of pods per plant, PL: pod length, PW: pod width, NSP: number of seeds per pod, HSW: hundred seed weight, GY: grain yield, ns: non-significant differences, \*, \*\*, \*\*\* denotes the significance at 0.05, 0.01 and 0.001.

#### 7.4 Discussion

The yield gaps in cowpea grain yield productivity of the obtained (0.1 t/ha to 0.5 t/ha) compared to the expected (1.5 t/ha to 3 t/ha) in the Sub-Saharan African regions are considerably high. The low yields are mainly associated with the use of unimproved varieties of cowpea with low-yielding potentials. This can be improved by developing high-yielding varieties with good adaptability that are characterized by superior agronomic traits. This therefore necessitates the improvement of cowpea varieties which are superior yielders which possess good agronomic performance for sustainable production of cowpea in the SSA regions. Hence, the current study intends to determine the combining ability and gene action of cowpea yield and its related trait among the cowpea genotypes and further identify traits that are useful for future selection of parental genotypes for development of new hybrids for advancements and variety release.

The analysis of variance revealed the significant genotypic, environmental and their interaction effects for almost all traits except LL and NSP which suggest and confirms the considerable genetic diversity among the parental genotypes and their progenies for the assessed traits. The reported variations are useful as the possibility of selection of parental genotypes immensely depends on the existence of allelic heterogeneity on the genotypes which signifies the dominance of additive variance (Ramalho *et al.*, 1993). Further, the significant genotype x environment interaction effects reported for the assessed traits indicates the need for further testing of newly developed progenies under numerous environments for precise recommendations with narrow or broad adaptability to a certain environments. Similarly, significant variations among cowpea parents and their progenies were also reported in the previous studies (Mukati *et al.*, 2014; Wankhande and Manjare, 2016; Owusu *et al.*, 2018; Joshi *et al.*, 2022).

The grain yield and its components are a good criterion with high contributions to explore the genetic variations which further determines high-yielding varieties. The mean performance of cowpea varieties showed greater variations, however, the mean performance of progenies showed a noticeable variation compared to their parents. Diaz *et al.* (2017) explained that this is due to good recombination related to hybrid vigor. In the current study, the best performing families were Acc-Cowp32 x Acc-Cowp31, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp10 x Acc-Cowp5, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp31, Acc-Cowp47 x Acc-Cowp38, Acc-Cowp47 x Acc-Cowp38, Acc-Cowp47 x Acc-Cowp38, Acc-Cowp17 x Acc-Cowp31 and Acc-Cowp5 x Acc-Cowp2 for NB, PH, LL, LW, NPP, PL, PW, NSP, HSW and GY respectively. The GY yield levels of the cowpea progenies were much higher ( $\geq 1$  t/ha) than

the respective parents ( $\leq 1$  t/ha) and comparatively higher than the yield levels reported from the recent study by Ezin *et al.* (2023). The good response of the progenies in GY in this study suggest the good recombination of additive and non-additive allele attained from different parents (Daud *et al.*, 2021). The identified cowpea families will be useful for further improvement of GY in South Africa or identical environments. The recent studies by (Ezin *et al.*, 2023; Jahun *et al.*, 2023) also reported that the progenies showed greater variation in the mean performance and they performed better than the parental genotypes.

The development of new varieties highly depends on the hybrids derived from parental genotypes which are characterized by best and desirable yield and yield related traits. Combining ability studies has been reported to be of great importance in providing information for the selection of parents based on the performance of hybrid (Owusu *et al.*, 2018; Raut *et al.*, 2017). It infers the gene action implicated during the expression of a certain trait using the ratios of general and specific combining ability effects (Sprague and Tatum, 1942). The high GCA inherent reflects the predominance of additive gene action suggesting that the parental GCA possessing high GCA for the certain trait could serve as a good source for selection to develop superior generations with good trait of interest (Mangena *et al.*, 2022). Furthermore, Avo-Vaughan *et al.* (2013) alluded that the parents that are good combiners are ideal to progeny development as they result to high probability of good SCA effects which then allows the development of superior and desired varieties. In the current study, the analysis of variance for GCA and SCA effects showed significant genotypic, environment and their interaction effects which indicates the additive and non-additive gene action contributed to the expression of trait studied as stated by Griffing (1956) which then signifies the possibility of developing new hybrids (Silva *et al.*, 2004; Owusu *et al.*, 2018).

The significant effects reported on the interaction of SCA x environment and GCA with the environment for the measured traits highlights the influence of environmental variance on the ability of parents passing the desirable traits to their progenies. The two different good combination of different alleles from different parents in a cross resulted in high SCA effects (Yohane *et al.*, 2022). The parental genotypes in this study recorded the high and significant GCA effects and high trait performance for a character of interest. The parents such as Acc-Cowp17, Acc-Cowp31, Acc-Cowp9, Acc-Cowp5, Acc-Cowp38, and Acc-Cowp19 were good combiners which exhibited the desirable GCA effects and traits performance. These parents possessed the significant and positive GCA effects making them the important parental genotypes for improving these traits. For instance, Acc-Cowp31 and Acc-Cowp5 were good

combiners for the production of GY and HSW. This indicates that these parents can be recommended for breeding for increased productivity of grain yield. However, these parents also had significant but negative GCA effects for NPP which suggests the need to use the large number of parents to maximize the probability of identification of good combiners when dealing with a large number of parameters (Shumbusha *et al.*, 2018).

The high SCA effects and its performance for increased grain yield were expressed by crosses between Acc-Cowp31 x Acc-Cowp5, Acc-Cowp38 x Acc-Cowp19, Acc-Cowp9 x Acc-Cowp2, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp38, and Acc-Cowp17 x Acc-Cowp38. The traits such as NB, NPP, PL and NSP are among the most important yield-determining agronomic traits that influence grain yield in cowpea. The crosses Acc-Cowp38 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9 and Acc-Cowp47 x Acc-Cowp38 recorded high SCA effects for the expression of traits including NB, NPP, PL and NSP. Furthermore, the progenies in this study exhibited high performance in all traits compared to parental genotypes. This indicates that the parental genotypes that combined and resulted in these crosses are identified as valuable germplasm resources for breeding for novel cowpea cultivars with increased GY. The number of pods per plant has been reported to have direct effects on grain yield production implying that they are the main contributors to grain yield and should be selected for improvement (Nwofia *et al.*, 2012; Almeida *et al.*, 2014).

The heritability and gene action analysis were used to provide further information on the inheritance of studied traits from parents to progeny. The additive variance in this study was larger than the dominance variance for most traits including NB, LW, NPP, NSP and HSW except for PH, PL, PW and GY suggesting that the inheritance of these traits was mainly controlled by additive gene action. The broad-sense heritability found in these traits was higher than narrow-sense heritability which further confirms that the expression of traits is mainly controlled by additive gene action. Similar results were also reported (Romanus *et al.*, 2008; Jahun *et al.*, 2023) whereby the additive gene action mainly controlled traits including NSP, PL and HSW. The low narrow-sense heritability as compared to broad-sense heritability reported in this study may be due to the influence of additive gene effects which indicates that the phenotypic expression of genotypes for the assessed traits could be reliable for selection of promising genotypes (Owusu *et al.*, 2018). In addition, the significant variation between narrow- and broad-sense heritability may be due to the high genotype by environment influence

on genotypes, which then suggest further multi-environment testing of genotypes for cultivar release in South Africa.

The significant and positive correlations were recorded between GY and PH, NB, LL, NPP, PL.PW, NSP and HSW was of great importance in the direct selection for multi-trait improvement and for the simultaneous improvement of traits. Similarly, the previous studies (Nwofia et al., 2012; Gupta *et al.*, 2023) recorded the significant and positive correlations between GY and NPP, PL, HSW and NSP. The reported association patterns of NPP and PL is explained because when the longer pod led length is attained, more space available for seed development resulting to high number of seeds per pod and high accumulation of grain yield. The presence of the significant and positive relationships between yield and its related traits confirms the linkages of the best genes that controls the traits expression.

### **7.5 Conclusion**

The current study aimed to determine the combining effects and gene action for yield and yield related traits for to select desirable parental lines and progenies for future advancement programs. The study revealed that trait expression was controlled both additive and non-additive effect with the additive gene action shown to be the important in controlling traits including NB, LW, NPP, NSP and HSW. The parents Acc-cowp17, Acc-cowp31, Acc-cowp9, Acc-cowp5, Acc-cowp38 and Acc-cowp19 were identified as good combiners for grain yield and its associated traits productivity. These parents could be exploited as donors since they possess good alleles for yield traits related to the production of grain yield. The newly developed progenies Acc-Cowp31 x Acc-Cowp5, Acc-Cowp38 x Acc-Cowp19, Acc-Cowp9 x Acc-Cowp2, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp38, and Acc-Cowp17 x Acc-Cowp38 were found to be the best performing due to their desirable SCA effects for enhanced grain yield. Significant and positive correlation were identified between grain yield and plant height, number of branches, number of pods per plant, pod length and number of seeds per pods and are recommended as selection criteria to improve grain yield in cowpea. The selected parents and progenies and the progenies could be useful genetic resources for future development of improved cowpea varieties incorporating these traits.

## 7.6 References

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## **CHAPTER 8: OVERVIEW OF RESEARCH FINDINGS**

### **8.1 Introduction**

The human populations in the Sub-Saharan African regions have been on the rise thus posing a great threat to food and nutritional security to the millions of people. The major climate variability experienced in the world has greatly affects the food production in food insecurity and malnutrition prone regions of Sub-Saharan Africa. Cowpea (*Vigna unguiculata* (L) Walp) plays an important role in food and nutritional security. The crop has a potential to contribute to resilience and enhancement of agricultural sustainability in the face of adverse environmental conditions serving a genetic resource for future crop improvements. All these attributes make cowpea a novel and viable crop to sustainable food systems. The crop has been statistically reported as the second most cultivated among the important legume crops after groundnuts, common beans, soybeans and peas.

There has been a considerable gap between the actual and potential yield of cowpea in the Sub-Saharan Africa. The grain yield has been estimated to range between 0.1 to 0.5 t/ha which is lower than the expected potential yield of 1.5 to 3 t/ha. This is associated with lack of improved high yielding and nutrient rich varieties and the biotic and abiotic stresses. Hence the need for the production of superior cowpea genotypes with enhance yield and nutritional quality for variety development in the Sub-Saharan Africa. The current study sought to (1) to quantify the genotype by environment interaction effect and select cowpea genotypes with high grain yield and average adaptation across selected cowpea growing environments in South Africa, (2) to assess the phenotypic variability and correlation relationship analysis in cowpea based on yield and yield related traits, (3) to evaluation the variations of nutritional content and phytochemical compositions among cowpea genotypes under diverse environments, (4) to assess the genetic diversity among cowpea genotypes using single nucleotide polymorphism (SNP) markers, and to select distinct and complementary genotypes for developing improved cultivars and to (5) to determine the combining ability effects and gene action controlling the yield and yield-related traits among selected cowpea parental genotypes and their progenies.

### **8.2 Summary and Implications of research findings**

#### **8.2.1 Influence of genotype and environment on grain yield among cowpea (*Vigna unguiculata* (L) walp) genotypes under dry land farming system.**

Fifty cowpea genotypes sourced from the Agricultural Research Council, originated from different areas of South Africa, Kenya and Nigeria were used for this study. The field trials

were conducted first season at Brits, Loskop and Roodeplaat during the 2019/2020 planting season and for the second season, the trials were conducted at Brits, Loskop, Mafikeng and Polokwane during the 2020/ 2021 planting season. The experiments were laid out at 10 x 5 alpha lattice design replicated three times at all sites. At physiological maturity, when the plants and pods turned brown in colour, the pods were harvested and grain yield data were recorded in grams/plant for each genotype, and later converted to t/ha. The main findings of the study were as follows:

- The grain yield revealed significant genotype, environment and genotype x environment interaction effects ( $P \leq 0.01$ ).
- The AMMI analysis revealed that the GEI effects accounted more of total variation of 57% than genotype (29%) and environment (13%).
- Cowpea genotypes Acc-Cowp38, Acc-Cowp2 and 98K\_5301 recorded high grain yield of 0.47 t/ha, 0.45 t/ha and 0.43 t/ha, respectively, across all test environments.
- The GGE biplot revealed two mega-environments of which the first mega- environment involved E4 (Brits 2020/2021), E5 (Loskop 2020/2021), E6 (Mafikeng, 2020/2021) and E7 (Polokwane 2020/2021) and the second mega environment involved E2 (Loskop 2019/2020), E3 (Roodeplaat 2019/2020) and E1(Brits 2019/2020).
- Genotypes Acc-cowp38, Acc-cowp2 and Acc-cowp9 were identified as the ideal genotypes across all environments.
- Environments E1 (Brits 2019/2020) and E3 (Roodeplaat 2019/2020) were revealed as the ideal environments possessing high discriminatory power for grain yield among the studied cowpea genotypes.

The significant and high effects of genotype x environment in this study highlights the importance of evaluating the stability of cowpea genotypes across different environmental conditions for the effective selection of stable and high-yielding genotypes. The environmental effects also played a major role in the performances, growth and development among cowpea genotypes allowing the identification superior lines with specific and narrow adaptations. The cowpea genotypes Acc-Cowp38, Acc-Cowp2, Acc-Cowp9, Acc-Cowp5 and Acc-Cowp39 were selected as high yielding and stable genotypes and could be recommended for production in South Africa and similar agro-ecological areas for incorporation in the future programs targeting genetic gains from grain yield improvements.

### **8.2.2 Phenotypic variability and correlation relationship analysis in cowpea [*Vigna unguiculata* (L.) Walp] based on yield and yield related traits.**

The study assessed the relationships between the yield and yield related traits for direct and indirect selection of superior genotypes. The field trials were conducted using fifty cowpea genotypes. first season at Brits, Loskop and Roodeplaat during the 2019/2020 planting season and for the second season, the trials were conducted at Brits, Loskop, Mafikeng and Polokwane during the 2020/ 2021 planting season. The experiments were laid out at 10 x 5 alpha lattice design replicated three times at all sites. The data recorded included the plant height (PH), number of branches (NB), leaf length (LL), leaf width (LW), number of pods per plant (NPPa0, pod length (PL), pod width (PW), number of seeds per pod (NSP), hundred seed weight (HSW) and grain yield (GY). The findings were as follows:

- The ANOVA revealed significant differences ( $P \leq 0.01$ ) on the effects of genotype, environment, and genotype x environment interactions on all studied traits.
- Genotypes such as Acc-Cowp47 were selected for superior for traits such as NB, LL, and LW, then Acc-Cowp10 for plant height, then Acc-Cowp38 for NPP, PW, NSP, HSW and GY and then Acc-Cowp2 for PL.
- The study further indicates that NPP, PL, NSP and HSW had significant and positive correlations with grain yield.
- the PCA biplot identified NB, NPP, PL, PW, NSP, and HSW as the important traits in the production of grain yield.
- Genotypes Acc-Cowp2, 98K\_5301, Acc-Cowp4, Acc-Cowp17 and Acc-Cowp9 were grouped together based on their high exhibition of NPP, PW, NSP, PL, HSW and GY.

The phenotypic characterization and understanding the relationship between yield and yield related traits is the pre-requisite research for the improvement of any neglected crop such as cowpea for maintaining sustainable genetic advancement. The analysis of phenotypic variations among genotypes for different traits and their associations is of great importance for any successful breeding programs. The significant and positive correlations reported in this study is very essential as allows the simultaneous selections of the traits as they have direct proportional influence against each other. However, the negative correlations between the traits would pose a challenge for simultaneous selections. The selected genotypes are recommended for future programs targeting increased yield and yield related traits.

### 8.2.3 Variation in grain nutritional and phytochemical compositions among cowpea [*Vigna unguiculata* (L) Walp] genotypes grown under dryland farming system in South Africa.

The study assessed the variability of cowpea grain nutritional and phytochemical compositions among the 50 cowpea genotypes to select the superior lines possessing high nutritional content and low phytochemical traits for cultivar development with improved nutritional quality. The cowpea samples were analysed for essential minerals including calcium (Ca), iron (Fe), potassium (K), sodium (Na), phosphorus (P), and zinc (Zn) and for protein, fats contents. Further analysed for phytochemical traits including flavonoids, condensed tannins and phenolic contents. The findings of the study were:

- The combined ANOVA across all test environments highlighted the significant effects ( $P \leq 0.001$ ) for all nutritional and phytochemical traits for genotype, environment, and genotype by environment interaction evaluated except for flavonoids and fat content.
- Genotypes Acc-Cowp6, Acc-Cowp17, Acc-Cowp14, 98K\_5301, Acc-Cowp9, Acc-Cowp32, Acc-Cowp9, Acc-Cowp4, Acc-Cowp16 and Acc-Cowp21 recorded the highest concentration of the assessed mineral elements.
- Genotype Acc-Cowp31 and Acc-Cowp13 were highly associated with protein content while genotype Acc-Cowp39 were in close association with fat content.
- Genotypes Acc-Cowp34, Acc-Cowp18, Acc-Cowp48, Acc-Cowp22, Acc-Cowp26, Acc-Cowp49 and Acc-Cowp28 had low concentrations of total phenolic, flavonoids and condensed tannins.
- The PCA depicted genotype Acc-Cowp17, acc-Cowp12, 98K\_5301 and Glenda were near vectors of Ca, Mg and condensed tannins.
- Ca positively and significantly correlated with Fe, Mg, Na, P and Zn then Cu correlated with Fe and P., then Fe with Na, P and Zn while Mg positively correlated with Na, P and Zn and lastly, Na with P then P with Zn.
- Further, Ca had a negative and significant correlation phenolic content and condensed tannins, while Mg with condensed tannins and K correlated with flavonoids.

The high concentration of nutrients and low phytochemical traits is important in his study as it allows recommendation for consumption in malnutrition prone regions, however, the lines that exhibited low nutritional contents are of cause of concern in preventing nutrient deficiencies diseases and provision of essential minerals. The negative correlations between essentials

minerals is undesirable but greatly expected since it was reported that phytochemical traits have compounds that lowers the availability of nutrients suggesting that selecting the genotypes with high concentration of phenolic, flavonoids and condensed tannins might casually reduce the contents of other essential minerals. Hence the genotypes possessing the high concentration of essential minerals and proteins, but low phytochemical traits were selected in this study to improve the nutritional quality in the future programs for cultivar development.

#### **8.2.4 Analysis of genetic diversity and population structure of cowpea [*Vigna unguiculata* (L.) Walp] genotypes using single nucleotide polymorphism markers.**

90 cowpea accessions sourced from the Agricultural Research Council - Vegetables, Industrial and Medicinal Plants (ARC-VIMP) gene bank, Pretoria, South Africa. These accessions were collected from different parts of the world including South Africa, Kenya and Nigeria. The accessions were genotyped with 11940 SNP markers. Monomorphic and SNPs with a minor allele frequency of less than 2% were filtered out and 5864 (49%) SNPs were retained for further analysis. The findings of the study were as follows:

- The mean PIC value of 0.27 reported in this study showed a moderate level of polymorphism and a moderate fixation index ( $FIS = 67\%$ ).
- The average observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity in the present study were 0.075 and 0.202, respectively for the 90 accessions.
- The AMOVA results in model-based populations indicated that the majority of the variance occurred among individuals within populations and accounted for 59.4% of the total variation and 18.4% and 22.1% of the total variation was attributed to differences within individuals and among populations, respectively.
- The model-based population structure analysis grouped the 90 accessions into four subpopulations based on the peak of delta K ( $\Delta K$ ) at  $K = 4$ .
- On the contrary, the distance-based cluster analysis using the neighbour-joining method showed the presence of three distinct clusters, which was not consistent with the results of the structure analysis.
- In both model-based and distance-based clustering approaches, the accessions were not grouped based on the growth habit and geographic origins of the studied accessions.

The population structure and genetic diversity analysis is fundamental for efficient genetic resource management and conservation strategies. The application of high-throughput molecular markers such as SNP provides a better understanding of genomic diversity and the

population structure of germplasm and can speed up the identification of superior groups for further hybrid development. The moderate level of polymorphism and revealed the existence of genetic diversity among the tested genotypes. The magnitude of variations among and within populations was further quantified by genetic differentiation observed among the populations ( $F_{ST} = 0.221$ ) indicated high genetic differentiation between four subpopulations. The SNP markers used in this study were highly informative and allowed the design of new varieties adapted to diverse environments that will substantially contribute to the utilization, conservation and broadening of the use of genetic resources of cowpeas for future improvement.

### **8.2.5 Combining ability and gene action studies for grain yield and its related traits among selected cowpea [*Vigna unguiculata* (L.) Walp] genotypes.**

Ten selected parental genotypes and 45 progenies crosses which were derived using the half diallel mating design were used to determine the combining ability effects and gene action controlling the yield and its related traits. These parents and progenies were evaluated at the field in an 11 x 5 alpha-lattice experimental design with three replicates at two locations namely Brits and Loskop. The quantitative data were collected on the following agronomic traits: plant height (PH) in cm, leaf length (LL) in cm, leaf width (LW) in cm, number of pods per plant (NPP) which were counted pre plant, pod length (PL) in cm, pod width (PW) in mm, number of seeds per pod (NSP) which were counted per pod, hundred seed weight (HSW) in grams which measured from randomly selected 100 seeds after harvest per plot. Thereafter, the grain yield in was measured in grams plant<sup>-1</sup> and later converted to tons per hectare (t ha<sup>-1</sup>). The findings were as follows:

- The parents and their parents expressed the significant ( $P \leq 0.01$ ) genotypic, environmental and genotype x environmental effects were significant at  $P \leq 0.01$  for all trial except the LL and NSP.
- The GCA effects showed the significant ( $P \leq 0.05$ ) effects of parents only on NSP, HSW, LW and PL whereas the SCA effects showed significant ( $P \leq 0.05$ ) effects on progenies only on PW.
- The parents such as Acc-Cowp17, Acc-Cowp31, Acc-Cowp9, Acc-Cowp5, Acc-Cowp38, and Acc-Cowp19 were good combiners and exhibited the desirable GCA effects.

- The newly developed progenies Acc-Cowp31 x Acc-Cowp5, Acc-Cowp38 x Acc-Cowp19, Acc-Cowp9 x Acc-Cowp2, Acc-Cowp47 x Acc-Cowp9, Acc-Cowp31 x Acc-Cowp9, Acc-Cowp32 x Acc-Cowp9, Acc-Cowp47 x Acc-Cowp38, and Acc-Cowp17 x Acc-Cowp38 were found to be the best performing due to their desirable SCA effects.
- The additive and non-additive effect with the additive gene action shown to be the important in controlling traits including NB, LW, NPP, NSP and HSW.
- The significant and positive correlations were recorded between GY and PH, NB, LL, NPP, PL.PW, NSP and HSW

The significant general and specific combining ability effects of parent and their progenies observed in this study were important in revealing the gene actions that controls the traits expression in the parents and their genotypes. Both additive and non-additive gene actions were important in the expression of yield and yield related traits, however additive gene effects were more important in the expression of traits such as NB, LW, NPP, NSP and HSW which was further confirmed by low narrow-sense heritability as compared to broad-sense heritability reported for these traits due to the influence of additive gene effects. The best combiners which constantly showed high performances in the traits across all environments should be evaluated further for advancement in multi-environments. The selected parents and progenies and the progenies could be useful genetic resources for future development of improved cowpea varieties incorporating these traits.

### **8.3 Conclusion and recommendations**

The research findings reported in the study were complex and useful in addressing the objectives of the study. However, based on the findings, it is recommended that cowpea improvement should be expanded and incorporates the genomic and mapping approaches to identify the genes and quantitative trait loci (QTL'S)A that are associated with the agronomic traits assessed in this study. Further, to improve the nutritional content of the varieties reported in this study, the approaches such as biofortification could be used improving nutritional quality of food by elimination anti-nutritional traits with the aid of increasing the nutrient content availability and accessibility. The suggested approaches will sustain cowpea yield productivity and nutritional quality.