Evaluating cowpea mutant genotypes for grain yield and nutritional value in South Africa

By

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DECLARATION

“I, Moshieng Ntswane, declare that the Masters research dissertation that I herewith submit for the Master of Science in Agriculture degree qualification at the University of the Free State (UFS), is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.”

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Signature Date

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LIST OF ABBREVIATIONS AND SI UNITS

a	Constant
AEC	Average environment coordination
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ARC	Agricultural Research Council
ASV	Additive main effects and multiplicative interaction stability value
AW	Atomic weight
B	Boron
b	Slope
Ca	Calcium
CEC	Cation exchange capacity
CRSP	Collaborative research support programme
Cu	Copper
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
E	Environment
Fe	Iron
G	Genotype
GE	Genotype by environment interaction
GGE	Genotype and genotype by environment interaction
Gr	Grouping
Gr.E	Grouping by environment interaction
GY	Grain yield
H^2	Broad-sense heritability
h^2	Narrow-sense heritability
HCl	Hydrochloric acid
HI	Harvest index
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component axis
ISRA	Institute Senegalais de Recherches Agricoles
K	Potassium

LSD	Least significant difference
M2	Second mutation generation
M4	Fourth mutation generation
M9	Nineth mutation generation
Masl	Meters above sea level
Max	Maximum
mc	Moisture content
Mg	Magnesium
Min	Minimum
Mn	Manganese
Mr	Molar ratio
MS_e	Mean square error
MS_g	Mean square of genotype
MS_{ge}	Mean square of genotype by environment interaction
MW	Molecular weight
Na	Sodium
NBPP	Number of branches per plant
NIR	Near-infrared spectroscopy
NPP	Number of pods per plant
NSPPx	Number of seeds per plant
NSPPy	Number of seeds per pod
OECD	Organisation for economic co-operation and development
P	Phosphorus
PA	Phytic acid
PA:Fe	Molar ratio of phytic acid with iron
PA:Zn	Molar ratio of phytic acid with zinc
PC	Principal component
PCA	Principal component analysis
PH	Plant height
PL	Pod length
PW	Pod width
PWP	Pods weight per plant
QTL	Quantitative trait locus
R	Replication

rASV	Ranking of additive main effects and multiplicative interaction stability value
Rep. (E)	Replications within environments
S	Sulphur
SA	South Africa
SSR	Simple sequence repeat
SWP	Seeds weight per plant
UCR	University of California Riverside
USA	United State of America
Zn	Zinc
100 SW	Hundred seeds weight
%	Percentage
∆A	Change in absorbance
$\sigma^2 a$	Additive varince
$\sigma^2 g$	Genotypic variance
$\sigma^2 p$	Phenotypic variance
$\sigma^2 ge$	Genotype by environment variance interaction
$\sigma^2 e$	Error/residual variance
cm	Centimeter
Da	Dalton
g	Gram
g 100 g^{-1}	Gram per 100 gram
ha	Hectare
kg ha^{-1}	Kilogram per hectare
mg 100 g^{-1}	Milligram per 100 gram
mg kg^{-1}	Milligram per kilogram
mm	Millimeter
ml	Milliliter
na	Nanometer
ppm	Parts per million

ABSTRACT

Cowpeas produce a substantial amount of grain, which is a significant source of vitamins, minerals and protein to disadvantaged people with limited access to adequate nutrients. Improved cowpea genotypes have been introduced through new breeding techniques such as mutagenesis to increase the phenotypic, genetic and nutritional diversity of the crop. The objectives of this study were: 1) to determine the phenotypic diversity and characterise cowpea mutants and normal genotypes for grain yield and yield components, to identify superior cowpea mutants and normal genotypes and to determine the correlation between all measured characteristics, 2) to evaluate the variability of cowpea mutants and normal genotypes for protein content, selected mineral elements, phytic acid and the potential bioavailability of iron (Fe) and zinc (Zn), to identify superior cowpea mutants and normal genotypes, and to determine the interrelationship between all measured characteristics, and 3) to determine genotype by environment (GE) interaction, to identify superior genotypes for grain yield and to determine the adaptability and stability of cowpea mutants and normal genotypes in South Africa. Thirty-one cowpea genotypes (16 Namibian mutants, seven International Institute of Tropical Agriculture (IITA) genotypes and eight South Africa genotypes) were planted in five different environments in South Africa during the 2021/2022 cropping season.

Significant ($P \leq 0.05$) genotype and GE interaction effects were observed for grain yield, yield components, protein content, minerals, phytic acid and potential mineral bioavailability. Broad-sense heritability (H^2) values above 50% were observed for yield components, protein, mineral elements, phytic acid and potential mineral bioavailability, while low H^2 values below 50% were observed for grain yield and Boron (B), indicating the complexity in selection and genetic improvement of these traits. Superior Namibian mutants (ShR10P12, ShR3P4, ShR4P1 and BrR11P2), IITA genotype (98K-476-8) and South African genotype (Enchore) for grain yield were identified. Superior Namibian mutants (ShL3P7-2, ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 ShR2P11, BrR11P2, ShL2P7, ShR3P4 and NKRuP5), IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White, Enchore and Glenda) for protein content, Fe and Zn concentration were also identified. The IITA genotype (IT93K-452-1) and South African genotypes (Oloyin and Orelu) had a potential of good Fe bioavailability. All cowpea mutants and normal genotypes had a potential of poor Zn bioavailability. High yielding and stable Namibian mutants (NKL9P7, ShR10P12 and ShR2P11), IITA genotype (ITOOK 1263) and South African genotype (Agrinawa) were identified. Two mega-environments, namely, 1) Taung and Mafikeng, and 2) Mafikeng, Bloemfontein, Polokwane and Potchefstroom were identified, indicating broad adaption of the genotypes. Potchefstroom and Taung were identified as ideal environments for evaluation of cowpea genotypes. Significant positive correlations between grain yield with almost all yield components were observed. Significant positive correlations of protein content with mineral elements and phytic acid were also observed, indicating the potential to simultaneously select these traits. Namibian mutants (NKR1P3, BrR11P2, ShL2P7, ShR2P11 and ShR10P12), IITA genotype (98K-476-8) and South African genotypes (Glenda, Dr Saunders, Enchore and Oloyin) were associated with high grain yield. Namibian mutants (ShR10P12, ShR3P4, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18 and IT99K-573-2-1), and South African genotypes (Agrinawa, Bechuana White and Dr Saunders) were associated with high protein content, manganese (Mn), phytic acid, Molar ratio of phytic acid with iron (PA:Fe), Molar ratio of phytic acid with zinc (PA:Zn), and ash content. These genotypes have a potential of long-term profitability to the agricultural production industry.

Keywords: cowpeas, grain yield, protein, mineral elements, mutation, phenotypic diversity, bioavailability, broad-sense heritability, stability, genotype by environment interaction

CHAPTER 1

INTRODUCTION

The *Vigna unguiculata* (L.) Walp also known as cowpea, is a self-pollinated perennial diploid of the *Fabaceae* family and subfamily *Faboideae* (Agbogidi 2010; Moussa et al. 2011). Given the world population's rapid increase, legumes (*Fabaceae*) such as cowpea (*Vigna unguiculata*), soybean (*Glycine max*) and chickpeas (*Cicer arietinum*) are regarded as one of the most crucial plant families for human nutrition (Gepts et al. 2005; Smykal et al. 2015). Africa is predominating with the largest area of cowpea production and consumption (Kareem and Taiwo 2007; Timko and Singh 2008). In 2018/2019, 15.05 million hectares of cowpea dried grain were produced worldwide, of which 8.90 million tons (96.79%) of the dried grains were produced in Africa (FAOSTATS 2020). In the past, cowpea was one of the undervalued and underutilised crops in South Africa, and as a result, agricultural improvement projects have paid little attention to it (Gerrano et al. 2020). Recently, the Agricultural Research Council (ARC) in South Africa has made significant progress in obtaining and selecting nutritious cowpea genotypes with high grain yield in an attempt to increase food security and reduce malnutrition.

Globally, it was estimated that 38.91 million people were obese or overweight due to malnutrition, with 45 million people being wasted (low height for their age), 149 million children below the age of five being stunted (looking too young for their age) and 38.91 million being overweight (WHO 2021). Due to poverty and malnutrition, almost one-third of African children experience numerous physical and mental (stunted growth, impaired immune system and pneumonia) complications (White and Broadley 2011; Mohajan 2022). Therefore, legume crops such as cowpeas can potentially contribute to the eradication of malnutrition and hunger. The whole cowpea plant is edible for both humans and animals. The crop can be consumed either as fresh leaves, immature pods or dry grains and its fodder has good value for animal feeding. Cowpeas are relatively a good source of protein with values ranging from 25.00 to 35.00% for fresh leaves, 21.00 to 29.00% for immature pods and 19.00 to 25.00% for dry grains (Okonya and Maass 2014; Gerrano et al. 2017). The crop is also rich in essential minerals [Fe, Zn, Mn and calcium (Ca)], carbohydrates (> 60%), amino acids (lysine and tryptophan), vitamins (B and C), fat (1.50%) and fibre (Hall et al. 2003; Elhardallou et al. 2015; Xiong et al. 2016). Due to its high nutritional quality, the crop has the ability to improve dietary quality by lowering cholesterol (Frosta et al. 2014).

Cowpeas can withstand the ever-changing climate better than other grain legumes such as dry beans and soybeans (Omomowo and Babalola 2021). Cowpeas are bred to withstand heat stress, drought tolerant stress and have better nitrogen fixation than drybeans and soybeans (Muhammad et al. 2010; Hall 2012). The crop is well suited to dry regions of the savannah belt where various crops may perform poorly or fail to germinate because of infertile soils or water stress caused by shortage of rainfall (Boukar et al. 2018). The crop thrives in an ideal environment with annual precipitation between 500 and 1200 mm, but recent study revealed that cowpeas can withstand annual rainfall as less as 400 mm (Ukpene and Imade 2015; DAFF 2011). In addition, most wild relatives of cowpea have been selected and used in cowpea breeding programmes due to their ability to tolerate drought stress, diseases and pest infestations (Agbicodo et al. 2009; Boukar et al. 2020; Gerrano et al. 2020). The crop also has the capacity to fix nitrogen in the soil which promotes soil health and decreases the need for inorganic nitrogenous fertilisers (Horn and Shimelis 2020).

Numerous research studies evaluated cowpea diversity using morphological, agronomic, molecular and nutritional characteristics (Ba et al. 2004; Lazaridi et al. 2017; Menssen et al. 2017). According to these studies, cowpea populations exhibit limited genetic variability, which may be related to the plant's self-pollinating reproductive mechanism, which prevents the introduction of new genetic variation. Generally, genetic variability in plant breeding can be created through cross-breeding and has proved to be a very important tool. However, cross breeding cowpea genotypes is challenging, costly and time consuming to achieve due to the self-incompatibility of the crop. Therefore, mutation breeding has been employed in cowpea breeding programmes (Horn et al. 2016). Mutation is a change that occurs in the deoxyribonucleic acid (DNA) sequence that induces genetic variation and has a chance of creating broader genetic diversity. Radiation, chemical mutagens and other appropriate biotechnology approaches have been used to induce variability in crop plants (Sikora et al. 2011; Kozgar et al. 2012; Holme et al. 2019). Cowpea mutant genotypes obtained from Namibia were gamma irradiated (Horn et al. 2016). The ARC in South Africa obtained the cowpea mutants from Namibia and normal genotypes from the IITA to compare their diversity with South African genotypes and determine their adaptability and stability with the aim of introducing new variation to the South African cowpea germplasm collection.

Cowpea mutant genotypes requires characterisation and assessment for nutritional quality and grain yield to assist with identification of potential parental genotypes which will be used to develop and improve cowpea cultivars (Gerrano et al. 2019; Mbuma et al. 2022). Therefore, breeders will be able to identify and choose the best genotypes that can be used as parents for hybridisation with the help of the knowledge on the present genetic variability among cowpea mutants and normal genotypes (Gerrano et al. 2015). Breeding populations with wide diversity should be tested in multiple environments for their adaptability and stability (Tresina and Mohan 2012; Takinami et al. 2016).

Multi-environmental trials are employed to assess the performance of genotypes and to determine their adaptability and stability in various environments (Negash et al. 2013). The change in the genotypes' performance across two or more environments is known as the GE interaction. Several statistical models have been used to quantify GE interaction, and genotype adaptability and stability before a cultivar is released and recommended for commercial production (Yan 2002; Asrat et al. 2009). These statistical methods include analysis of variance (ANOVA), genotype plus genotype by environment interaction (GGE) biplot analysis, principal component analysis (PCA) and the additive main effects and multiplicative interaction (AMMI).

The ANOVA is an important technique for determining the variability among genotypes, environments and other sources of variation in experimental trials. The PCA is used to visualise the similarity and differences between genotypes and their trait association while phenotypic correlations are used to determine the association among traits. The AMMI analysis incorporates a significant amount of GE interaction sum of squares and distinguishes between main (genotypes and environments) and interaction effects, which is essential in agricultural research (Gauch 2006). Additionally, the adaptability and stability of genotypes in multiple environments is assessed using the AMMI analysis. However, the AMMI biplot lacks the classifying capacity required to evaluate mega-environments. Mega-environments refer to the grouping of environments that have a similar influence on the performance of genotypes. The GGE biplots have been proposed and used to effectively classify superior genotypes and mega-environments (Peprah et al. 2020).

1.1 Aim of the study

The aim of this research was to quantify the variation and to characterise cowpea mutants and South African and IITA genotypes for grain yield and yield components as well as for nutritional quality traits.

1.2 Objectives

1. To determine the phenotypic diversity and characterise cowpea mutants and normal genotypes for grain yield and yield components, to identify superior cowpea mutants and normal genotypes and to determine the correlation between all measured characteristics.
2. To evaluate the variability of cowpea mutants and normal genotypes for protein content, selected mineral elements, phytic acid and the potential bioavailability of Fe and Zn, to identify superior cowpea mutants and normal genotypes and to determine the interrelationship between all measured characteristics.
3. To determine GE interaction, to identify superior genotypes for grain yield and to determine the adaptability and stability of cowpea mutants and normal genotypes in South Africa.

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CHAPTER 2

LITERATURE REVIEW

2.1 Origin, history and production of cowpea crop

Cowpea domestication was first proposed by Vavilov in 1926 with suggestions that Ethiopia was a major centre, with China and India as minor centres of domestication (Herniter et al. 2020). Other research studies revealed that wild cowpea relatives were found in the African continent, dominating other continents as the primary centre of origin (Coulibaly et al. 2002; Agbogidi 2010). The *Vigna unguiculata* ssp. *dekindtiana* var. *spontanea*, which can be found in Africa, is the wild origin of the commercial cowpea (Pasquet and Padulosi 2013). However, there are still contradicting opinions and reviews regarding the origin of wild cowpea relatives, which have high level of diversity (Ba et al. 2004; Xiong et al. 2017). The introduction of cowpea from Africa to the Indian subcontinent is speculated to be roughly 2000 to 3500 years ago (OECD 2016). During the 17th century AD, the crop was distributed to India by Spanish people via slave trade and distributed across Asia (Xiong et al. 2016). Furthermore, West African slave trade led to its cultivation reaching the southern United States of America (USA) during the early 18th century (Pakela 2006).

Currently, cowpea is produced worldwide throughout the tropical and subtropical areas (Kebede and Bekeko 2020). Approximately 8.90 million tons of dried cowpea seeds on average were produced between 2019 and 2020 worldwide with Africa accounting for about 96.79% of the world cowpea production (FAOSTATS 2020) (Figure 2.1). Europe only produces an estimated 25 732 tons of dry cowpea seeds annually. Nigeria is the largest cowpea producer globally, producing an estimated 3 million tons of annual yield, followed by Niger (Table 2.1). The top 10 cowpea producing countries (from Nigeria to Sudan) are also leading in cowpea research, hence high crop production, harvest area and grain yield. In most African countries, abiotic and biotic factors contribute to low cowpea grain yield.

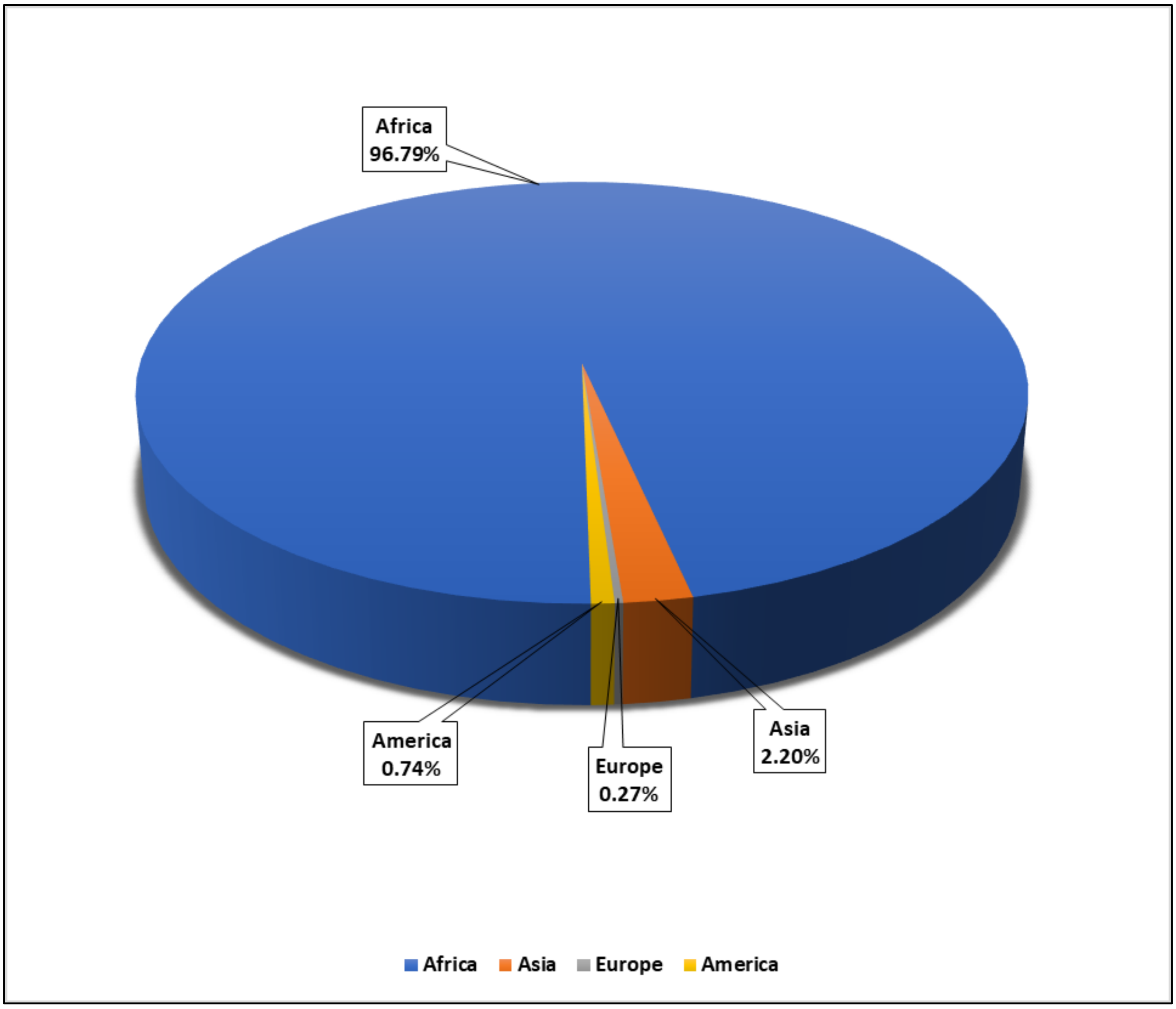

Figure 2.1 World cowpea production. Source: FAOSTATS (2020)

Table 2.1 African countries with the highest cowpea production (tons) in 2020

Rank	Country	Production (tons)	Area harvested (ha)	Yield (kg ha^{-1})
1	Nigeria	3 576 361	4 303 005	831
2	Niger	2 386 735	5 725 433	417
3	Burkina Faso	652 454	1 354 100	482
4	Ethiopia	374 332	220 037	1701
5	Kenya	246 870	298 120	828
6	Mali	215 436	454 274	474
7	Cameroon	215 016	244 058	881
8	Ghana	202 735	149 102	1360
9	Senegal	184 137	290 677	634
10	Sudan	161 000	339 780	474
11	Tanzania	127 884	112 657	1135
12	DRC	76 292	175 418	435
13	Mozambique	90 461	331 424	273
14	Malawi	41 656	97 825	426
15	Madagascar	31 069	34 122	911
16	Uganda	12 697	33 350	381
17	Mauritania	7 933	22 415	354
18	Egypt	7 180	1 853	3875
19	South Africa	4 801	109 90	437
20	Eswatini	715	2 056	348

Source: FAOSTATS (2020)

2.2 Origin, history and production of cowpea in South Africa

Studies suggest that South Africa was a centre of cowpea wild species (Fang et al. 2007; Boukar et al. 2020). An additional view was that South Africa, in the Transvaal area, was the evolutionary core of *V. unguiculata* due to the existence of the most significant original wild variations in that area (Weng et al. 2017). Southern African wild cowpea species had smaller seeds than those found in West Africa, such as *V. dekindtiana* var. *spontanea*, which have somewhat larger seeds (Boukar et al. 2015).

Literature that highlighted the history of cowpea revealed that due to the existence of the most primitive wild species (*V. rhomboidea, V. protracta, V. tenuis* and *V. stenophylla*), West and South Africa were the centre of *V. unguiculata* speciation (Pakela 2003; Boukar et al. 2020). Due to the minimal and lack of breeding programmes in the past, most wild species were eliminated in South Africa. People who were moving between countries where the *Vigna pubescens* subspecies emerged transferred the wild species across to neighbouring countries such as Mozambique, Zimbabwe, Lesotho, Botswana and Namibia (Horn et al. 2022). Later, the subspecies spread to East and West Africa. In the form of slave trade, the Spanish distributed wild cowpea species from southern African countries together with other wild species such as millet, groundnut and soybean to tropical America in the 17th century (Sedivy et al. 2017; Sousa and Raizada 2020; Panzeri et al. 2022).

Cowpeas are grown in dryland areas of South Africa, particularly in Taung and Potchefstroom in the North West. Small-scale farmers are the leading producers of cowpeas (Magloire 2005). In 2020, cowpea production in South Africa was estimated to be 4867 tons (Figure 2.2; FAOSTATS 2020). South Africa is the second lowest cowpea producing country with low yields. The area of production of the country is also very low as compared to Nigeria, which indicates that cowpea production in South Africa is given little research attention. Other factors contributing to low production and productivity are the lack of improved varieties and locally adapted cultivars, heterogeneous flowering and maturity, low yield potential and poor processing quality (Villa et al. 2005; Shiringani and Shimelis 2011). Significant progress has been made at the ARC in sourcing and selecting potential diverse cowpea population from IITA which are created for high yielding and good nutrition (Gerrano et al. 2017). However, the biggest challenge is that cowpea has narrow genetic diversity, which limits its genetic improvement (Mbuma et al. 2020, 2021, 2022).

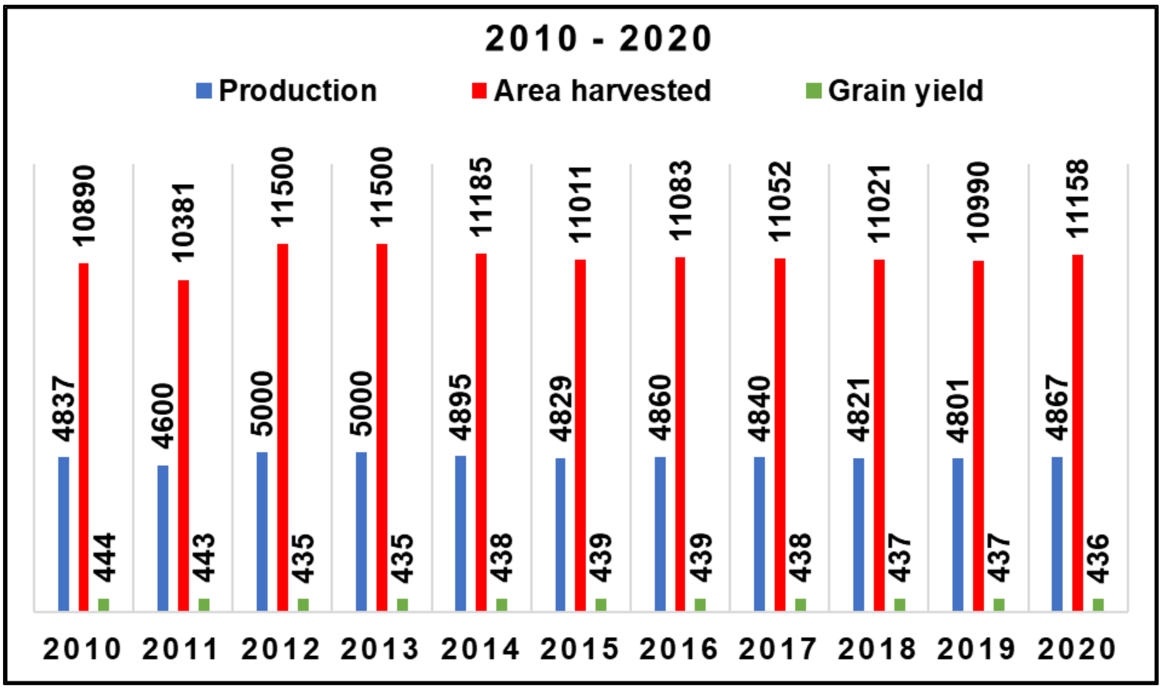

Figure 2.2 Cowpea area harvested (ha), production (ton) and grain yield (kg ha^{-1}) in South Africa from 2010 to 2020. Source: (FAOSTATS 2020)

2.3 Factors affecting productivity of cowpea

The cowpea crop productivity and improvement are known to be constrained by abiotic and biotic factors. Abiotic factors which affect plant growth and can be divided into imposed effects of stress, such as heat, cold and drought stress (Timko et al. 2007; Hatfield and Prueger 2015; Ravelombola et al. 2018). Environmental stress can cause damage to the cowpea plant which can appear as necrosis in all or a portion of the plant leaves. Cowpea plant damage can also be due to reduced rate of growth as a result of temperature, physiological malfunction, soil chemistry and water availability (Challinor et al. 2014; Iseki et al. 2021). The mentioned effects reduces the grain yield of the crop (Nunes et al. 2022). Therefore, the sustainability of crop production is threatened by environmental stress.

Insect pests and diseases are the second key production barrier for cowpea production (Afutu et al. 2017). Insect pests and diseases cause major economic crop losses. The pod borer is regarded as the most destructive and economically important cowpea insect pest predominately in sub-Saharan Africa and causing yield losses ranging from 20 to 80% (Gianessi 2013). Cowpea aphid infestation is another major constrain that causes chlorosis, leaf curling and stunted growth limiting cowpea productivity and production (Kamphuis et al. 2012; Choudhary et al. 2017; MacWilliams et al. 2020). In Africa, aphid infestation has been linked to cowpea grain yield losses which ranges from 20 to 100% (Kataria and Kumar 2013). Diseases caused by viruses, bacteria, fungi and nematodes are a major biotic constraint in cowpea productivity and production (Oliveira et al. 2012; Mbeyagala et al. 2014; Odedara and Kumar 2017). However, over the past years, natural microbial-based treatments have been effectively used in agro-ecological production to reduce crop losses and increase productivity (Omomowo and Babalola 2021). Natural microbial-based treatments etails the use microbial inoculants that can either act as biocontrol agents, biofertilizers biopesticides and

2.4 Cowpea taxonomy, botany and genetics

Cowpea [*Vigna unguiculata* (L.) Walp] is a dicotyledon which belongs to the *Fabaceae* family (Agbogidi 2010). In 1824, Savi introduced the genus *Vigna*, whose taxa had previously been referred to as *Phaseolus* (Pasquet and Padulosi 2013). The *Vigna* and *Phaseolus* are genera of the same legume tribe *Phaseoleae* with similar morphological characteristics, hence the initial confusion of referring them as the same crop. The *Vigna* taxonomy has been revised over the past years with significant breakthroughs using molecular technologies like DNA finger printing (Pasquet and Padulosi 2013; OECD 2016). It was discovered that the *unguiculata* species comprises of four groups or subspecies (spp.) namely: *unguiculata*, *biflora*, *sesquipedalis* and *textilis* (Pasquet and Gepts 2021).

The *unguiculata* is also subdivided into wild and cultivated ssp. in which the *unguiculata* is classified as a cultivated ssp., while *spontanea* and *mensensis* are classified as wild ssp. (Pasquet 2000; Ba et al. 2004). Additionally, the *unguiculata* ssp. is widely cultivated in Africa, India, the Middle East, and South America primarily for dry grain and fodder as it provides a source of protein for millions of poor people (Timko et al. 2007). Furthermore, the seeds of the wild *spontanea* ssp. are 10x smaller than cultivated *unguiculata* ssp., have small and dehiscent pods and have hard seed coats (Pasquet and Padulosi 2013; OECD 2016). Cowpea breeding programmes widely make use of the two ssp. (*unguiculata* and *spontanea*) for crop improvement. The ssp. *sesquipedialis*, commonly known as asparagus bean or “yard long bean”, is cultivated for its immature green pods in Eastern and Southern Asia (Xu et al. 2010). Cowpea is a warm-season herbaceous, annual crop that looks similar to common bean, but the leaves are less pubescent, shinier and darker green compared to the common bean (Timko et al. 2007). The growing patterns of many cowpea plants differ which include climbing, erect, bushy, trailing or indeterminate types under favourable conditions. Long bean varieties of cowpea with climbing and indeterminate growth characteristics involve regeneration of green pods throughout the growth period (Herniter et al. 2020). Their stems are slightly hairy or smooth and are rarely shaded purple (Pakela 2006). Their leaf form is either trifoliate or alternate (Pottorff et al. 2012). The inflorescence of cowpea contains two flowers. The flowers have short pedicels, are self-pollinating and a have corolla that can be in any of the following colours: purple, dirty yellow, white, light blue or violet (Ige et al. 2011). The flower opens in the morning and closes at about noon; they wilt and fall off after the flowering season. The seeds are accommodated in pods, which vary in texture, shape, size, and colour. Some plants are erect, coiled, or can be in the form of a crescent (Deshpande et al. 2018). When the pods mature, their colour could be yellow, purple or brown. Each pod contains about eight to 20 seeds, depending on the variety.

Cowpeas are diploids with 2n = 22 chromosomes. Legume crops such as chickpea (*Cicer arietinum*) and common bean (*Phaseolus vulgaris* L.) have high genomic collinearity (a pair of loci that are shared by two species but are on different chromosomes) compared with cowpea (Vasconcelos et al. 2015; Merga and Haji 2019). Cowpea has a 613 megabase genome size and maternally inherited chloroplasts (OECD 2016). The ability of the crop to develop and perform successfully is determined by genetics and a range of environmental conditions.

2.5 Nutritional value/importance and functional compounds

2.5.1 Proteins and amino acids

Cowpea is one of the most nutritious legume crops that provide populations in tropical regions with proteins and minerals (Dakora and Belane 2019). Previous research has shown that cowpea crude protein content varies with different varieties (Ravelombola et al. 2016). Protein content of cowpea genotypes was reported to be ranging from 21 to 25% (Itatat et al. 2013). Another study reported that cowpea has a comparatively higher protein content (17 to 30%) than cereals (7 to 13%) (El-Niely 2007). There is wide variation in the protein content of cowpea leaves, immature pods and the seeds. Studies have reported cowpea protein in leaves to be ranging from 25 to 32% (Okonya and Maass 2014), while for immature pods it ranges from 21 to 29% (Gerrano et al. 2017). Cowpea leaves, immature pods and seeds are all edible and mostly consumed in African countries.

Cowpea has a complex profile of proteins. The relative proportions of each protein fraction have a significant impact on the nutritional quality of the crude protein in cowpea seeds (Teka et al. 2020). The major protein fractions (classified according to their solubility in different solvents) in cowpea are glutelins (alkali) and albumins (water) followed by globulins (salt solution) and prolamins (alcohol) (Vasconcelos et al. 2010; Santos et al. 2012) (Table 2.2). Because of their protein structural features, globulin fractions are naturally resistant to digestion (Phillips et al. 2003). Albumin content in cowpea seeds range from 8.20 to 11.90% (Tchiagam et al. 2011). Globulins and albumins are graded as metabolic and enzymatic proteins including lectins, protease inhibitors and lipoxygenase (Park et al. 2010). Prolamin occurs in the lowest concentration ranging from 2.30 to 5% (Gupta et al. 2010).

Table 2.2 Types of protein fractions found in cowpea, their solubility and profile bands

Protein profile	Solubility	Protein profile bands
Glutelin	alkali	21
Albumin	water	20
Globulin	salt solution	16
Prolamin	alcohol	1

The ability of a food protein to meet the metabolic needs of humans for amino acids can also be defined as its nutritional quality (Frota et al. 2017). A protein's ability to satisfy both the physiological needs of the organism and the requirements for essential amino acids is measured by its protein quality (Kurpad 2013). Cowpea amino acid profiles vary between genotypes (Table 2.3). There are 17 basic amino acids in cowpea protein, most of which are daily required in human diet (Elhardallou et al. 2015). Isoleucine, threonine, leucine and lysine are some of the basic amino acids found in cowpea protein (Gonçalves et al. 2016). As a result, it can supplement lysine-deficient proteins in staple cereals, tubers and starchy roots (Teka et al. 2020). In comparison, mature seeds have a lower concentration of free amino acids than those in immature seeds (Jayathilake et al. 2018). High concentrations of amino acids can also indicate reduced anti-nutritional factors, and enhanced digestibility and mineral bioavailability (Sarwar et al. 2012). The total amino acid content of cowpea seeds range from 27.50 to 33.43 g 100 g^{-1} (Gupta et al. 2010).

Table 2.3 Amino acids profile of cowpea seeds

Amino acids	g 100 g^{-1}
Cysteine	0.84 – 1.08
Tryptophan	1.00 – 1.33
Methionine	1.28 – 2.06
Histidine	1.85 – 2.47
Threonine	3.89 – 5.12
Isoleucine	4.17 – 5.46
Leucine	6.45 – 8.50
Lysine	7.30 – 8.74

2.5.2 Mineral concentration

About 232 million people in Africa suffer from trace element deficiency, a problem that can be solved by legumes which have the ability of accumulating micronutrients in organs and boost brain development (Polak et al. 2015). Legumes have edible leaves and their seeds contain significant levels of dietary minerals required for human nutrition and health (Dakora and Belane 2019). Over 2 billion people are affected by insufficient Fe intake (the most widespread dietary risk factor in the world), while 49% of people worldwide are at risk from inadequate Zn diet (Brown et al. 2001). Plant breeders are focusing on the genetic improvements needed to support breeding schemes with the objective of increasing the accumulation of seed minerals in elite cowpea genotypes (Beaver and Osorno 2009).

Micronutrients play crucial physiological roles in processes including haemoglobin formation (Fe), protein synthesis and stabilization of plasma membranes (Zn), bone health (Ca), Fe metabolism [copper (Cu)], promotion of blood clotting and fat metabolism (Mn) and stimulation of hormonal levels (B) (Mogobe et al. 2015; Dimkpa and Bindraban 2016;

Alejandro et al. 2020). Even though cowpeas contain a significant amount of Fe and Zn, its bioavailability is low. However, if taken with meals that are high in vitamin C, Fe and Zn, absorption is improved (Messina 2016).

Large variation of mineral composition in different cowpea collections has been reported previously (Belane and Dakora 2011; Gonçalves et al. 2016; Gerrano et al. 2017; Mbuma et al. 2021). For example, a study that was conducted in South Africa reported large variation in cowpea immature pods for mineral concentrations which ranged from 60.05 to 97.78 mg kg^{-1} for Fe, 32.53 to 56.25 mg kg^{-1} for Zn, 21.05 to 47.72 mg kg^{-1} for Mn, 4.84 to 9.54 mg kg^{-1} for Cu and 21.29 to 40.31 mg kg^{-1} for B (Gerrano et al. 2017). When compared to cowpea grains, immature pods were observed to have a similar mineral concentration. For example, previous research has reported that mineral concentrations in cowpea grain ranged from 60 to 99 mg kg^{-1} for Fe, 44 to 65 mg kg^{-1} for Zn, 5 to 32 mg kg^{-1} for Mn, 8.30 to 14.70 mg kg^{-1} for Cu and 10 to 22 mg kg^{-1} for B (Belane and Dakora 2011). A recent study that was conducted in South Africa reported mineral concentration in cowpea seeds to be ranging from 1.80 to 257.30 mg kg^{-1} for Fe, 19.40 to 55.30 mg kg^{-1} for Zn, 1.70 to 21.70 mg kg^{-1} for Mn, 3.80 to 35.70 mg kg^{-1} for Cu (Mbuma et al. 2021). In Brazil, ranges of 58.50 to 69 mg kg^{-1} for Fe, 46.75 to 64.25 mg kg^{-1} for Zn, 8.25 to 16 mg kg^{-1} for Mn, 4.25 to 7 mg kg^{-1} for Cu were reported in cowpea grain (Gonçalves et al. 2020). While the immature pods and grains have similar mineral concentrations, higher values have been reported in cowpea leaves, varying from 42 to 55 mg kg^{-1} for B, 8.60 to 19.70 mg kg^{-1} for Cu, 49 to 104 mg kg^{-1} for Zn, 196 to 394 mg kg^{-1} for Mn, and 142 to 626 mg kg^{-1} for Fe (Belane and Dakora 2011). These studies have shown that there is a large variation of mineral concentration within and between collections. Furthermore, this variation may be explained by the collections' various genetic background and their growing conditions.

2.5.3 Starch, fibre and oligosaccharides

Cowpea is the most important starch-protein grain legume in the West African sub-region, with a wider range of applications than other legumes (Atuobi et al. 2011). Starches are macromolecules produced by plant tissues that are used in both food and non-food products. Cowpea seeds have a carbohydrate content that ranges from 53 to 66%, with starch accounting for most of the carbohydrate content (Huang et al. 2007; Ashogbon and Akintayo 2013). Cowpea starch is gaining popularity in research due to its resilience to shear thinning and its high amylose content (39 to 42%) (Hoover et al. 2010; Ratnaningsih et al. 2016). Due to its high amylose concentration and resistance to enzyme hydrolysis, cowpea starch could be used to make thickeners, textural modifiers and gelling agents in food formulations, and could be included in food products (Adebooye and Singh 2008; Ratnaningsih et al. 2016).

Both soluble and insoluble dietary fibre are abundant in cowpeas (Liyanage et al. 2014; Jayathilake et al. 2018). Water can dissolve soluble fibre, which stabilizes cholesterol and blood glucose level. The colon and large intestine absorb water through insoluble fibre, which does not dissolve in water, to keep waste items wet and moving smoothly (Eashwarage et al. 2017). Increased intake of fibre content is somehow associated with cardiovascular disease, diabetes, obesity, cancer and many other chronic syndromes according to numerous epidemiological studies (Lattimer and Haub 2010; Threapleton et al. 2013; Barber et al. 2020). Cowpeas contains about 12 to 14.80 g 100 g^{-1} of total dietary fibre (Kirse and Karklina 2015; Eashwarage et al. 2017).

Flatulence or gas production in the intestine is caused by the presence of oligosaccharides in legumes, which causes discomfort such as bloating and constipation (Afiukwa et al. 2012). The oligosaccharides stachyose, raffinose and verbascose are primarily responsible for this reaction to legumes, which varies depending on gender, age, intestinal microbiota composition and other factors (OECD 2016). Cooking of cowpea grains inactivate or reduce their anti-nutrients including oligosaccharides, thereby improving their nutritional quality (Wang et al. 2008; Tresina and Mohan 2012).

2.5.4 Bioactive polyphenols and antioxidants

Cowpea is high in essential bioactive compounds that can support human health in various ways, in addition to its nutritional benefits (Awika and Duodu 2017). These essential bioactive compounds are polyphenols, which include phenolic acid and flavonoids. Phenolic acid plays a role in the antioxidant properties of legumes (Xu and Chang 2009; Singh et al. 2017; Liu et al. 2020). Concentration of phenolic acids in cowpea cultivars varies greatly depending on the seed colour (Cai et al. 2003). Red cowpea phenotypes have significantly more phenolic acids than other phenotypes, while white varieties have the least (Ojwang et al. 2012).

Flavonoids play a crucial role in plant defence (Nassourou et al. 2016). Flavonoids are highly concentrated in the seed coat of cowpea and other legumes (soybeans, black beans, and kidney beans) and they have a significant influence on the choice of cowpea varieties for food consumption in most countries (Ojwang et al. 2012). Flavonoids have been studied extensively for their anti-stress and anti-inflammatory benefits (González et al. 2011; Yang et al. 2014). Previous research in South Africa reported a range of 0.36 to 0.95 mg 100 g^{-1} (Salawu et al.

2014) and 0.40 to 6.91 mg 100 g^{-1} (Mbuma et al. 2021) for total flavonoid content. In contrast to the values observed in South Africa, a higher range (0.21 to 23.95 mg 100 g^{-1}) of total flavonoids was reported in Burkina Faso (Sombié et al. 2018). Common beans showed comparative differences of total flavonoids for leaves which ranged from 2.15 to 44.59 mg 100 g^{-1}, for pods it ranged from 0.87 to 3.64 mg 100 g^{-1} and for seeds it ranged from 1.65 to 9.29 mg 100 g^{-1} (Pham et al. 2020). These studies have also indicated that flavonoid content varies among the cowpea genotypes depending on the seed colour, the darker seed coated cultivars have higher flavonoid content compared to the cream white seed coated cultivars.

Antioxidants are substances that can prevent the onset or progression of chain oxidation reactions, thereby reducing or inhibiting oxidative damage (Moreira-Araújo et al. 2017). Due to their potential function in disease prevention and health promotion, there is increasing interest in the antioxidant activity of phenolics and tannin of cowpea (Zia-Ul-Haq et al. 2013). Depending on the cultivar, the phenolic content composition and bioactive properties of cowpea can differ significantly (Sombié et al. 2018). Consuming food products that are rich in phenolic antioxidants can reduce the chances of having terminal illnesses which include diabetes, cancer and cardiovascular disease (Zhao et al. 2014).

Polymeric tannins can directly interact with proteins and carbohydrates, limiting their bioavailability, digestion and transport (King et al. 2000; Awika and Rooney 2004). Tannins can also reduce mineral availability by preventing them from being absorbed (Delimont et al. 2017). Tannis attracted interest as a potential natural supplement to reduce the calorie content of foods due to their capacity to bond carbohydrates with proteins (Dunn et al. 2015; Amoako and Awika 2016). Cowpea has a reduced tannin concentration that may be beneficial for health promotion (Awika and Duodu 2017).

2.6 Anti-nutritional compounds

Anti-nutritional factors are secondary metabolites present in plants that inhibit the absorption of nutrients in food (Soetan 2008). Trypsin inhibitors, phytic acid, and oxalic acid are the main limitations present in cowpeas that reduce nutrients' bioavailability (Preet and Punia 2000; Parmar et al. 2017; Jayathilake et al. 2018). Phytic acid and oxalic acid are the main anti-nutritional factors in legume crops (Akande et al. 2010). The quantity of anti-nutrients in cowpea depends on factors such as environmental effects, genotypical variations, and use of high-phosphate fertilizers, which is highly variable (Simion 2018).

Mineral bioavailability is reduced by the presence of phytic acid and oxalic acids as they bind with the minerals (Shukkur et al. 2006). Phytate inhibits the absorption of Mg, Ca, Zn and Fe (Akond et al. 2011). On the positive side, the ability of phytic acid to bind minerals reduces cell damage in the epithelium of the digestive tract which lowers the risk of colon cancer (Vucenik and Shamsuddin 2003). Due to lack of digestive enzymes (phytase), humans and non-ruminant animals cannot absorb phytate molecules, but ruminants can easily digest phytate because their rumen bacteria produce phytase (Klopfenstein et al. 2002). Rumen bacteria can convert dietary oxalic acid into carbon dioxide and formic acid in ruminants (Simion 2018).

2.7 History of cowpea breeding in the world

Conventional cowpea breeding began at National de Recherches Agronomiques in Senegal, and the USA in 1961. Only a few national agricultural research programmes (Nigeria, Uganda, Tanzania, Senegal, India and the USA) had a form of improvement programmes and they were maintaining various collections of cowpea germplasm (Simion 2018). Collaborative attempts to develop cowpea cultivars for Africa started before the bean/cowpea Collaborative Research Support Programme (CRSP) was established (Hall et al. 2003). The purpose of the collaboration between the University of California Riverside (UCR) and the Institute Senegalais de Recherches Agricoles (ISRA) was to produce cowpea cultivars for specific production zones in Senegal and California. Dr. L.W. Kitch of Purdue University initiated hybridizations in Cameroon in 1990, and the Purdue project later partnered with Dr. J.D. Ehlers who had joined the UCR CRSP project as a cowpea breeder in producing improved cultivars (Hall et al. 2003). The Ein El Gazal genotype was developed from a cross between the California cultivar CB5 and the Senegalese breeding line Bambey 23 for use as a dry grain cultivar in the Sahelian zone (Elawad and Hall 2002). Many consumers of cowpeas in the USA and Africa value the grain. Cowpea breeding has been going on for years throughout the world's primary cowpea-growing regions, namely, the USA (Singh 2005), Asia (Xu et al. 2017), Africa (Horn and Shimelis 2020) and Latin America (Xiong et al. 2016).

In the last three decades, international and national research organizations have achieved significant progress in breeding for pest and disease resistance, yield and nutritional value through conventional breeding (Boukar et al. 2016). Using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) marker cowpea linkage maps, quantitative trait loci for resistance to floral bud thrips were discovered (Omo-Ikerodah et al. 2008). The use of SSR markers to evaluate the genetic diversity across improved cowpea breeding lines and varieties obtained from the IITA breeding nursery demonstrated that improved cowpea varieties had a small genetic base in general (Li et al. 2001). Breeders continue to develop segregated populations in commercial breeding programmes by using superior lines as parental lines for crossings.

2.8 History of cowpea breeding in South Africa

South African wild cowpea relatives have the highest genetic diversity and most prehistoric features like bearded stigma, pod shattering, hairiness, outbreeding, and small seeds and pods (Ng and Singh 2009). The cultivated cowpea (ssp. *unguiculata*) originated from selection and domestication of perennial wild cowpea relatives, according to a review on the background of *Vigna* species (var. *dekindtiana*) (Panzeri et al. 2022). Seed dormancy and pod dehiscence were lost throughout the domestication process and when the species was domesticated through selection, it resulted in an increase in seed and pod size (Soltani et al. 2021). Various incompatibilities and linkage drag among the cultivated and wild species made it difficult to exploit genetic variability in South African wild species for crop improvement (Sharma et al. 2013). Pre-breeding presents a valuable technique for enhancing the use of genetic variability found in wild and cultivated type germplasm in such circumstances.

Cowpea is a self-pollinator, so even though some gene banks have a considerable collection of cowpea germplasm, the crop's genetic base is still limited (Boukar et al. 2020). It is vital to use alien germplasm collection, particularly from cross compatible wild relatives to broaden the genetic base of the crop (Pratap et al. 2021). Pre-breeding is used to identify desirable characteristics in susceptible genotypes/materials that cannot be utilised to breed wild species independently and introducing those qualities into genetic backgrounds that are well-adapted (Sharma et al. 2013). Currently, only pre-breeding of cowpea is practised in South Africa. At the ARC, a cowpea pre-breeding programme has been initiated with the aim of introducing novel genes or traits into the breeding programme leading to full-scale crop improvement. The ARC in South Africa has a large cowpea germplasm collection from IITA Ibadan, Nigeria and Namibia for evaluating yield potential, nutritional quality, resistance to pests and diseases, and adaptation to South African conditions.

2.9 Selection methods in cowpea breeding

Cowpea breeding programmes around the world have focused on improving qualitative and quantitative traits to improve crop productivity. Pure-line selection, pedigree, mass selection, backcross and mutation breeding are some of the methods that have been commonly and successfully used to improve cowpea (Horn and Shimelis 2020). The primary target for almost all breeding programmes is drought tolerance (Ravelombola et al. 2020), pest and disease resistance (Boukar et al. 2020), lodging tolerance and growth habit (erect) (Kim et al. 2018), light sensitivity (photo-insensitive) (Nuhu and Mukhtar 2014), higher grain yield (Gerrano et al. 2019; Mbuma et al. 2020) and nutritional value (Boukar et al. 2018; Mbuma et al. 2021, 2022).

2.9.1 Pure-line breeding

Pure-line selection is done in heterogeneous populations such as introduced germplasm collections, landraces and materials from mass selection in self-pollinated species to isolate superior genotypes. Pure-line selection is the oldest method of crop enhancement (Breseghello and Coelho 2013). This method is effective in self-fertilizing crops like sorghum (Diallo et al. 2019), pea (Yan et al. 2017), wheat (Agorastos and Goulas 2005) and cowpea (Boukar et al. 2016). It is also useful for breeding inbred lines used to create hybrids in self-pollinated crops. The main objective of the pure-line programme is to generate cowpea genotypes with improved yield, to generate genotypes that are adaptable to diverse environments and have desired characteristics such as seed type, growth habit and days to maturity (Simion 2018). Pure-line selection has been used to identify potential cowpea lines from segregating populations after application of induced mutagenesis (Horn and Shimelis 2020).

2.9.2 Pedigree selection

In pedigree selection, individual cowpea plants are selected from second filial generation and subsequent generations for progeny testing (Horn and Shimelis 2020). It is a valuable technique in a transgressive breeding programme for selecting genotypes with distinct characteristics including maturity, plant height and disease tolerance. The advantage of using pedigree breeding in cowpea breeding programmes is that it is well suited for simply inherited characters such as shape, colour, height and pod size (Nkoana 2018). Pedigree selection can provide information on the genetic components and combining ability of cowpea for morphological traits (Ayo-Vaughan et al. 2013). Such data can be utilised to create superior cowpea genotypes.

2.9.3 Backcross selection

Backcross breeding aims to increase the genetic value of a locally adapted cultivar with genetic defects such as disease susceptibility, low yield, and poor nutritional quality (Mutlu et al. 2005). It involves repeatedly backcrossing the hybrid with the recurrent parent with the desired trait, to recover the genetic background of the recurrent parent. The backcross breeding approach is frequently used to incorporate simple hereditary traits into varieties as a means of rectifying some of the deficiencies that may exist in current varieties (Collard and Mackill 2008). Additionally, the approach has been used to develop cowpea cultivars with improved productivity in hot environments (Chamarthi et al. 2019).

2.9.4 Mutation breeding

Genetic variability in plant breeding can also be obtained through mutation. Mutation breeding is a cost-effective, fast and consistent method for developing and screening crop genotypes with improved and unique agronomic traits (Raina et al. 2020). Plant propagules such as seeds can be exposed to physical and chemical mutagens, which will cause mutations (Mba et al. 2010). Finding the proper radiation dose for the target genotypes is necessary before undertaking large-scale mutagenesis (Tshilenge-Lukanda et al. 2012). Several plant traits, including disease resistance, plant height, oil quantity and quality, maturity and the size of cowpea starch granules have been successfully modified by induced mutagenesis (Goyal and Khan 2010; Singh et al. 2013).

A study was conducted in South Africa on generating drought-tolerant mutants by screening cowpea genotypes from second mutation (M2) to fourth mutation (M4) generations (De Ronde and Spreeth 2007). In Nigeria, gamma irradiation was used to create early maturing cowpea mutants with tendrils on the leaflets, thick leaves and light green pods (Adekola and Oluleye 2007). Therefore, mutation breeding has been crucial in the creation of superior cowpea genotypes.

2.10 Genetic variance, heritability and predicted selection gains

The basic concept underlying variance component approaches is to break down the overall variance of a phenotype into specific causes. The phenotypic variation is the result of genetic variation and environmental variation, while genetic variance is a measure of the variation that exists in the genetic makeup of individuals within a population (Vogt 2020). Plant breeding relies on genetic variability and new variation is critical for introducing new features into breeding programmes (Holme et al. 2019). Cowpea genetic variability must be understood to develop and improve conventional breeding programmes (Gerrano et al. 2015). Genetic variability is required to ensure long-term selective genetic gain and reduce crop vulnerability to abiotic and biotic stress (Magloire 2005). External conditions have a bigger influence on cowpea genotypes than genetic factors during the growth season (Gerrano et al. 2015). Reliable and precise assessments of the degree of phenotypic variability and heritability of yield and yield components are necessary to improve selection efficiency (Shimelis and Shiringani 2010). Researchers can utilize the total amount of genetic variation responsible for a trait in calculations of the trait's heritability once they have determined the total amount of genetic variation responsible for the trait (Govindaraj et al. 2015).

Heritability is the proportion of observable variation (the phenotype) in a population that can be attributed to genotypic variances. This could be the broad sense-heritability (H^2), which includes epistatic and dominance interaction effects, or narrow sense-heritability (h^2) which is the quantity of the additive genetic effects over the phenotypic variance (Raudonius 2017). Plant breeders commonly use heritability to evaluate the accuracy of single or multiple field trials since it affects the sensitivity of selection and is a useful statistic in quantitative breeding (Piepho and Möhring 2007). Cowpea breeders frequently prefer to evaluate the performance of all varieties on a plot mean basis since cowpea is a self-pollinated plant and thus plot mean-based broad-sense heritability is more effective (Xu et al. 2009). The high heritability for morphological and nutritional quality traits highlights the efficiency of conventional breeding for cowpea. High H^2 values of above 50% obtained from a study recommended that selection within each preliminary set for grain yield, days to 50% flowering and hundred seed weight would be desirable, given the moderate magnitude of environmental influence (Ongom et al. 2021). Estimates of heritability and genetic gain are used to assess effective genotype selection for desirable characteristics (Patel et. 2016).

Genetic gain is a valid approach for increasing crop selection efficiency (Daetwyler et al. 2014; Krchov and Bernardo 2015). Genetic gain can be defined as the measure of performance improvement obtained each year from artificial selection. The genetic gain achieved using the relevant selection procedures, including selection gain can be used to measure improvement or response to selection (Xu et al. 2020). In order to accelerate genetic gain in various legumes such as cowpeas, several modern breeding techniques have been developed, including marker-assisted breeding and genome engineering (Huynh et al. 2018; Olatoye et al. 2019). Improvements of genetic gains have been made to cowpea yield-related traits such hundred seed weight, pods per plant, harvest index, and total dry matter (Kamara et al. 2011).

2.11 Correlations and principal component analysis

The linear relationship between traits can be determined using correlation analysis. There are two types of correlations, namely genetic and phenotypic correlations. A quantitative genetic measure that indicates the genetic relationship between two characteristics is known as genetic correlation (Adetiloye et al. 2017; Sodini et al. 2018). Phenotypic correlation is the measure of the phenotypic relationship between two characters due to genetic and environmental factors. Genetic correlation is a key measurement that gives insight on the pleiotropy and linkage background as well as the selection response of complex traits (Zhang et al. 2021). Genetic correlation measures the breeding values of the the sets of parents for different traits (Hadfield et al. 2007). When two characters have a significant correlation, it is possible to select a trait of interest by focusing on the correlated trait that is easier to measure, especially when the primary trait, such as grain yield, has a complex hereditary (Ceballos 2004). Corresponding heritability, genetic advance, genotypic and phenotypic coefficients of variation will aid in the selection of superior genotypes, which will be equivalent to the amount of genetic variation available and the degree to which the characteristics are inherited (Patel et al. 2016).

The use of correlation coefficients to study the relationship between traits is essential for early plant or inbred line selection, as well as simultaneous selection when more than one trait is needed (Silva et al. 2016). This is because when compared to direct selection, indirect selection based on correlated response might result in more effective genetic development. Previous studies (Adetiloye et al. 2017; Walle et al. 2018) on genotypic and phenotypic correlation among cowpea genotypes have reported higher genotypic correlations compared with the corresponding phenotypic correlations. Their results indicated that the environmental factors had little concealing influence on the yield components. Significant positive genetic and phenotypic correlations between mineral elements (Mbuma et al. 2021), morphological traits (Mbuma et al. 2021; Owusu et al. 2021), and between morphology and nutritional quality traits combined were found (Mbuma et al. 2022), indicating a likelihood of simultaneous selection of the traits. Positive correlations are of utmost importance for traits of interest since they indicated that selection of one trait will simultaneously increase the other correlated traits. However, negative correlations are not preferred on traits of interest since they indicate that an increase in one trait will result in decreased values of the other correlated traits.

The similarity and differences between the genotypes and their association with a trait can be visualised using the principal component analysis (PCA). PCA is a statistical technique that enables you to condense the information in large data tables by using a smaller set of data that can be more easily viewed and studied (Clark and Ma'ayan 2011). Therefore, solving an eigenvalue and eigenvector problem results in the discovery of new variables known as the main principal components (PCs) and the total variation observed in the data set (Jolliffe and Cadima 2016). A study by Siwale et al. (2022) on Bambara discovered a minimum threshold eigenvalue of one with 59.36% of total variation observed. Additionaly A study on cowpea also found minimum threshold eigenvalue of one that explained 78.22% of the total variation observed in the cowpea genotypes (Mbuma et al. 2021). On a recent study by Mbuma et al. (2022), the PCA distingished four groups of cowpea genotypes with their trait association. The first group of genotypes was associated with pod width, number of seeds per pod, pod length, zinc, iron and 100 seed weight, second group was associated with calcium, manganese and copper, third group was associated with Magnesium, potassium, phosphorus and Sodium, and the fourth group was associated with flavonoids, total phenolics, number of branches and grain yield.

2.12 Genotype by environment interactions and stability analysis

The GE interaction is the change in the relative performance of genotypes when tested in multiple environments. Multi-environmental trials are helpful in evaluating the relative performance of genotypes in different environments, making it possible for breeders to give recommendations on the stability and adaptability of genotypes (Negash et al. 2013). The analysis of GE interaction provides information on specific and broad adaptation as well as the stability of genotypes (Gurmu et al. 2009; Mohammed et al. 2016).

Various statistical models have been presented to evaluate the impact of GE interaction in various legume crops (Yan 2002; Asrat et al. 2009). These methods include ANOVA, AMMI and GGE biplot analysis. The ANOVA is an important technique for determining the variability among genotypes, its limitation is that it does not determine the adaptability and stability of genotypes. Statistical analysis such as AMMI and GGE have been used mainly to quantify GE interactions and to determine the stability of tested genotypes. The advantages of utilizing the AMMI approach are that the analysis distinguishes between main and interaction effects, which is crucial for the majority of agricultural research, and integrates a significant amount of the GE interaction sum of squares (Gauch 2006). The GGE biplot was proposed to effectively classify superior genotypes under mega-environments (a collection of environments that share adapted genotypes) because the AMMI biplot lacks the classification capacity needed to evaluate mega-environments (Peprah et al. 2020).

To give suggestions for broad or targeted adaptation, the GGE analysis is utilised to assess the proportional yield potential and stability of enhanced cowpea genotypes. Significant interactions between genotypes, environments and growing season for grain yield were observed (Shiringani and Shimelis 2011), implying that genotypes had different performance across growing environments. Newly developed gamma irradiated mutants and local checks showed wide adaptability and had high grain yield in Namibian multi-environments (Horn et al. 2018). Previous studies in South Africa investigated the effects of GE interaction in cowpea genotypes and revealed significant GE interactions for grain yield and yield components, identified stable genotypes, broad and specific adaptation, and mega-environments (Gerrano et al. 2020; Mbuma et al. 2021). Significant GE interaction for cowpea grain yield has been reported in Ethiopia (Simion et al. 2018). These studies highlighted that a genotype may exhibit excellent adaptation and stability but poor performance, suggesting the need for mean yield analyses.

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CHAPTER 3

PHENOTYPIC DIVERSITY AMONG COWPEA MUTANTS AND NORMAL GENOTYPES FOR GRAIN YIELD AND YIELD COMPONENTS

3.1 Abstract

Cowpea production has the potential of contributing to solving food insecurity in African rural communities due to its high nutritional quality. Phenotypic characterization of cowpea germplasm collections is an essential step for crop improvement. The objectives of this study were to determine the phenotypic diversity and characterize cowpea mutants and normal genotypes for grain yield (GY) and yield components, to identify superior cowpea mutants and normal genotypes, and to determine the correlation between all measured characteristics. Thirty-one cowpea genotypes (16 Namibian mutants, seven IITA genotypes and eight South Africa genotypes) were planted in five different environments in South Africa during the 2021/2022 cropping season. Significant ($P \leq 0.05$) genotype, environment and GE interaction effects were observed for GY and all measured traits. High H^2 values (> 50%) were observed of the most measured traits. Low H^2 values (<50%) for GY was observed which indicated the difficulty for the genetic improvement of this trait. Superior Namibian mutants (ShR10P12, ShR3P4, ShR4P1 and BrR11P2), IITA genotype (98K-476-8) and South African genotypes (Enchore and Oloyin) were identified for GY, number of seeds per plant (NSPPx), pods weight per plant (PWP) and number of pods per plant (NPP). Significant and positive correlations between most of the measured traits were identified. The PCA showed that Namibian mutant genotypes ShL2P7, ShR4P1, NKR1P3 and BrR11P2 were associated with NSPPx, PWP and GY. Namibian mutants (ShR10P12, NKR1P3, ShR4P1 and ShL2P7), IITA genotype (98K-476-8) and South African genotype (Enchore) were associated with GY, PWP, NSPPx and NPP. The clustered heat map identified a group of Namibian mutants (NKR1P3, BrR11P2, ShL2P7, ShR2P11 and ShR10P12), IITA genotype (98K-476-8), and South African genotypes (Glenda, Dr Saunders, Enchore, and Oloyin) which were associated with GY, PWP, NSPPx, NPP, seed weight per plant (SWP) and harvest index (HI). The identified superior cowpea mutants for grain yield and yield components can be used for cowpea improvement.

Keywords: Cowpea, mutation, characterization, grain yield, diversity, broad-sense heritability

3.2 Introduction

Cowpea is a warm-season crop that provides a significant amount of protein (23 to 32%) and other essential nutrients to many people, particularly in the underdeveloped countries in Africa (Herniter et al. 2020). It is primarily grown as a vegetable, with the leaves and seeds intended for human consumption and to a lesser extent as a fodder crop, because of its nutritious content (Sapara and Javia 2014).

Like most of the legume crops, cowpea can fix nitrogen in the soil making it suitable for intercropping and resilience farming (Mndzebele et al. 2020). The crop can be cultivated under adverse environments like sandy soils, high temperatures and drought, and still produce high grain yield (Kuykendall et al. 2000; Martins et al. 2003). In comparison to other nations that produce cowpeas such as Nigeria and Niger, South African cowpea production is still quite low (FAOSTATS 2020). This could be attributed mainly to a lack of adapted and improved genotypes, as a result, the crop is mainly cultivated by subsistence farmers.

Significant progress has been made at the ARC in choosing and evaluating potential cowpea genotypes for higher yield (Gerrano et al. 2019; Mbuma et al. 2020) and enhanced nutrition (Gerrano et al. 2017, 2022; Mbuma et al. 2021, 2022). Although significant effort has been made in improving the cowpea crop, previous research also indicated the narrow genetic diversity in the existing germplasm collection, which may limit genetic improvement of the crop (Boukar et al. 2020; Mbuma et al. 2021; Gerrano et al. 2022). The crop's self-pollinating system could be a potential contributing factor for the observed limited genetic variation in cowpea germplasm collections. Therefore, genetic variation can also be created through crossing genotypes with varying characteristics. However, crossing can be very challenging, particularly in self-pollinating crops, due to poor flower structures and poor compatibility between selected genotypes in a breeding programme. For any successful breeding programme, plant breeders need to consider diversifying the germplasm collections by introducing new genetic material from different backgrounds or from other countries.

Self-pollinating legumes such as cowpea and bambara groundnut have a significantly increased genetic variation due to mutation breeding (Singh et al. 2006; Kozgar et al. 2012; Wanga et al. 2018). Considering the limited genetic variation observed in the cowpea germplasm collection, the ARC in South Africa has obtained cowpea mutants from the Namibian Radiation Regulatory Authority with the aim to test and evaluate these genotypes for their yield potential, adaptability, and stability as well as other agro-morphological characteristics in South African cowpea growing conditions. The identified superior cowpea mutants for grain yield and measured traits will be used as potential parents to diversify the existing germplasm collection.

Understanding the nature and magnitude of the phenotypic diversity present within and among cowpea germplasm collections is important for crop improvement and creation of new improved varieties (Lazaridi et al. 2017). Characterization of the available germplasm, along with the identification and selection of superior genotypes that can be utilised as suitable potential parents, is an essential step in facilitating breeding efforts (Cilliers and Swanevelder 2003; Patil et al. 2015). Characterization and diversifying the germplasm collection for grain yield, yield traits and other agro-morphological characteristics is necessary for cowpea improvement (Omirou et al. 2019; Boukar et al. 2020; Dareus et al. 2021). Hence the objectives of this study were to determine the phenotypic diversity and characterise the cowpea mutants and normal genotypes for grain yield and yield components, to identify superior cowpea mutants and normal genotypes and to determine the correlation between all measured characteristics.

3.3 Materials and methods

3.3.1 Plant material and experimental sites

Thirty-one cowpea genotypes were used in this study, which included 16 mutant genotypes obtained from Namibia, seven normal genotypes from the IITA, Ibadan, in Nigeria and eight normal genotypes were obtained from the ARC grain crops in South Africa (Table 3.1). The 16 mutant genotypes were exposed to gamma irradiation in Namibia by the Namibian Radiation Regulatory Authority (Horn et al. 2015). The gamma irradiated genotypes used in the study for evaluation were at ninth mutation (M9) stage. The IITA and South African genotypes were developed through conventional breeding.

The field trials were planted at the ARC farms in Bloemfontein (Free State province), Mafikeng (North West province), Polokwane (Limpopo province), Potchefstroom and Taung (North West province) during the 2021/2022 summer cropping season. Both Mafikeng and Polokwane had aphid infestation, which resulted in stunted growth and reduced grain yield of the infected genotypes. Namibian mutants (ShL3P7-2, NKR8P9, ShR10P10 and ShL2P7) and IITA genotype (98K-476-8) had zero grain yield in Polokwane. Namibian mutant NKR8P9 had zero grain yield in Mafikeng and were excluded from the data analysed for the the two environments. Details of the five environments and soil characteristics are given in Table 3.2 and Table 3.3, respectively.

Table 3.1 Source of collection and origin of cowpea genotypes used in the study

Namibia	IITA	South Africa
ShR10P12	ITOOK 1263	Glenda
ShR4P1	IT07K-292-10	Bechuana White
ShR9P5	IT07K-318-33	Pan 311
NKR9P9	IT99K-573-2-1	Dr Saunders
NKL9P7	98K-476-8	Agrinawa
NKR1P3	IT82E-18	Enchore
ShL3P7-2	IT93K-452-1	Orelu
ShR3P4		Oloyin
NKR8P9		
BrR11P2		
ShR10P10		
NKRuP5		
BrR11P11		
ShR2P11		
BrR4P11		
ShL2P7		

IITA = International Institute of Tropical Agriculture

Table 3.2 Characteristics of the five environments

Environments	Average minimum temperature (°C)	Average maximum temperature (°C)	Average rainfall (mm)	Average altitude (Masl)	Latitude	Longitude
Bloemfontein	4.40	30.50	545	1311	28° 55.690 S	26° 19.599 E
Mafikeng	11.20	29.10	571	1290	25° 47.410 S	25° 37.105 E
Polokwane	15.30	28.00	661	1220	23° 50.125 S	29° 41.524 E
Potchefstroom	5.40	29.20	660	1340	26° 73.407 S	27° 07.509 E
Taung	6.33	32.10	353	1087	27° 35.477 S	24° 44.776 E

Masl = Meters above sea level

Table 3.3 Soil characteristics of the five environments during the 2021/2022 cropping season

Environment	Soil type	Soil pH	Soil nutrients (mg kg^{-1})					Exchangeable cations [cmol (+) kg^{-1}]				CEC
			Fe	Mn	Zn	S	P-Brayl	Na	K	Ca	Mg	cmol (+).kg^{-1}
Bloemfontein	Fine sand	6.99	18.75	22.18	1.50	68.90	19.09	0.05	0.56	3.24	2.17	12.81
Potchefstroom	Clay	7.30	14.86	59.18	7.55	206.90	21.70	0.09	0.40	5.49	3.87	18.70
Mafikeng	Fine sand	7.34	16.13	33.98	2.49	507.00	40.38	0.02	0.90	2.05	1.58	7.09
Polokwane	Fine sand	8.55	25.78	48.60	2.41	272.50	31.22	0.24	0.55	4.35	3.61	7.83
Taung	Fine sand	7.09	12.11	45.32	2.85	355.00	10.45	0.05	0.54	2.92	1.39	7.39

Fe = Iron, Zn = Zinc, Mn = Manganese, S = Sulphur, P = Phosphorus, Na = Sodium, K = Potassium, Ca = Calcium, Mg = Magnesium, CEC = Cation exchange capacity

3.3.2 Trial design and management

The cowpea trials were laid out in a randomised complete block design with three replications across all the environments. Each genotype was grown in a two-row plot with an overall length of 5 m, a row spacing of 2 m and an intra-row spacing of 0.75 m. Three seeds were hand sown per hole in a row and pre-emergence herbicide (Dual gold) was applied to inhibit the emergence of weeds. The plants in each row were thinned during the early stage of germination to the desired plant population. To produce a healthy plant stand, irrigation was used to supplement rainfall during critical stages of seedling emergence and germination. Cultural practices were used to control pests and diseases. No fertilizers were applied, in order to simulate low-input environments.

3.3.3 Data collection

From the five experimental environments, mature dry cowpea seeds of each genotype were collected. Data for grain yield components were recorded from five randomly selected plants per genotype in each plot. The following traits were recorded: number of branches per plant, plant height (cm), hundred seed weight (g), pod weight per plant (g), number of pods per plant, number of seeds per plant, seed weight per plant (g), pod width (cm), pod length (cm) and number of seeds per pod(Lazaridi et al. 2017). At harvest, grain yield was measured from the two rows of each genotype and converted to kilogram per hectare (kg ha^{-1}). Grain yield and harvest index were calculated according to Parker and Namuth-Covert (2017) using the following formula:

Grain yield: $$\frac{Plot\ weight\ (kg)}{Plot\ area\ (ha)}\ x\ \frac{100-14\%}{100-mc}\ x\ 10000$$

Harvest index (HI): $$\frac{Grain\ yield\ (kg\ ha^{-1})}{Biological\ yield\ (kg\ \ ha^{-1})}\ x\ 100\%$$

Where, mc = moisture content at harvest, 14% = constant of moisture content for legumes, 10000 = hectare conversion factor.

3.3.4 Data analysis

Data collected for grain yield and yield components were subjected to ANOVA using GenStat 22nd version (VSN International 2022). Least significant difference ($P \leq 0.05$) was used to separate means. Due to the distinct genetic basis of the accessible germplasm collection, Namibian cowpea mutants, IITA and South African genotypes were all regarded as fixed variables. The environments were deemed to be random variables because they are a representative sample of all potential environments that could represent the conditions for growing cowpeas in South Africa. The ANOVA was further done to determine the significant difference between the three groups of genotypes (South African genotypes, IITA genotypes and Namibian mutants) for grain yield and yield components.

The variance components and H^2 for single trials were calculated using the following equations:

$$\sigma^2 g = \frac{MS_g - MS_e}{r}$$

$$\sigma^2 e = MS_e$$

$$H^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e}$$

The variance components and H^2 across environments were calculated using the following equations:

$$\sigma^2 g = \frac{MS_g - MS_{ge}}{re}$$

$$\sigma^2 ge = \frac{MS_{ge} - MS_e}{r}$$

$$\sigma^2 e = MS_e$$

$$\sigma^2 p = \sigma^2 g + (\sigma^2 ge/e) + (\sigma^2 e/re)$$

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

Where, $\sigma^2 p$ = Phenotypic variance, $\sigma^2 g$ = Genotypic variance, $\sigma^2 ge$ = Genotype by environment variance, $\sigma^2 e$ = Error/residual variance, MS_g = Mean square of genotype, MS_{ge} = Mean square of genotype by environment, MS_e = Mean square error, g = Genotypes (fixed), r = Replications (random), e = Environments (random), H^2 = Broad-sense heritability.

PCA and clustered heat maps were done using XLSTAT software version 2022.3.2 (Addinsoft 2022). Phenotypic correlation analysis was done to determine association between grain yield and yield traits using GenStat 22nd version (VSN International 2022).

3.4 Results

3.4.1 ANOVA for single trials of cowpea mutants and normal genotypes

For Bloemfontein, the genotype (G) effect was highly significant ($P \leq 0.001$) for GY, PWP, NSPPx, NPP, pod length (PL) and number of branches per plant (NBPP), significant ($P \leq 0.01$) for SWP, HI, pod width (PW) and significant ($P \leq 0.05$) for number of seeds per pod (NSPPy) and plant height (PH) (Table 3.4). High H^2 values (> 50%) were observed for for NPP (52.50%), PL (52.88%), PWP (54.43%), GY (54.64%), NBPP (59.01%) and NSPPx (65.71%). For Potchefstroom, the G effect was highly significant ($P \leq 0.001$) for NSPPy, hundred seed weight (100 SW), HI and PW, significant ($P \leq 0.01$) for NSPPx, and significant ($P \leq 0.05$) for GY, PWP, NPP and PH. High H^2 values (> 50%) were observed for NSPPy (50.88%), HI (52.36%), 100 SW (54.62%) and PW (57.78%).

For Taung, the G effect was highly significant ($P \leq 0.001$) for NSPPx, NPP, 100 SW and PL, significant ($P \leq 0.01$) for GY, PWP and SWP and significant ($P \leq 0.05$) for NSPPy (Table 3.4). High H^2 values (> 50%) were observed for NPP (52.29%), 100 SW (55.70%), NSPPx (57.18%) and PL (65.05%). For Mafikeng, the G effect had high significant level ($P \leq 0.001$) for NSPPx, NPP and 100 SW, significant ($P \leq 0.01$) for PW and significant ($P \leq 0.05$) for PL. High H^2 values (> 50%) were observed for 100 SW (51.68%), NPP (53.83%) and NSPPx (56.00%). For Polokwane, the genotype effect was significant ($P \leq 0.01$) for 100 SW and PW. The H^2 for all the traits were below 50% in Polokwane.

3.4.2 Combined ANOVA for cowpea mutants and normal genotypes

The environmental (E) and G effects were highly significant ($P \leq 0.001$) for all traits besides for PW and SWP, respectively (Table 3.5). The GE interaction effect had high significance ($P \leq 0.001$) for GY, PWP, NSPPx and NPP, significant ($P \leq 0.01$) for HI and significant ($P \leq 0.05$) for 100 SW, PL and PH. High H^2 values were observed for PL (88.25%), NSPPy (85.21%), 100 SW (81.62%), PW (75.61%), NBPP (66.31%), PH (64.25%), HI (63.92%), NSPPx (57.83%), and NPP (51.15%). Low H^2 values were observed for GY (40.40%), PWP (40.32%) and SWP (3.37%).

3.4.3 Combined ANOVA for groupings according to origin

The origin (grouping) effect (Gr) was highly significant ($P \leq 0.001$) for NSPPy, 100 SW, PL and NBPP and significant ($P \leq 0.01$) for GY, PWP, HI and PW (Table 3.6). The environmental effect was highly significant ($P \leq 0.001$) for all yield components besides for PW. The grouping by environment (Gr.E) interaction effect was significant ($P \leq 0.01$) for GY and PWP, and significant ($P \leq 0.05$) for NPP.

Table 3.4 Analysis of variance showing mean square values and broad-sense heritability (H^2) for grain yield (GY) and yield components of cowpea at five environments

Source	Df	GY	PWP	NSPPx	NSPPy	NPP	SWP	100 SW	HI	PL	PW	NBPP	PH
Bloemfontein													
R	1	36544.00	78.00	8039.00	0.58	4.66	675.70	16.72	166.00	0.00	0.00	7.81	588.40
G	30	347975.00***	709.20***	18147.00***	6.57*	101.11***	468.90**	22.25	334.20**	6.30***	0.01**	7.14***	1880.60*
Error	30	102065.00	209.30	3755.00	3.21	31.49	200.70	12.82	123.00	1.94	0.01	1.84	889.80
σ^2_G		122955.00	249.95	7196.00	1.68	34.81	134.10	4.71	105.60	2.20	0.00	2.65	495.40
σ^2_P		225020.00	459.25	10951.00	4.89	66.30	334.80	17.54	228.60	4.12	0.01	4.49	1385.20
H^2%		54.64	54.43	65.71	34.32	52.50	40.05	26.89	46.19	52.88	42.70	59.01	35.76
Grand mean		1580.21	71.60	355.40	13.65	33.05	49.10	13.78	41.57	17.74	0.70	9.42	59.10
Potchefstroom													
R	1	1923773.00	4198.00	87713.00	4.66	428.50	28969.00	3.15	7.80	4.26	0.01	0.40	202.00
G	30	1660391.00*	3550.00*	73954.00**	5.51***	415.50*	19208.00	23.25***	444.90***	6.93	0.01***	6.92	5471.00*
Error	30	815932.00	1735.00	31961.00	1.79	187.60	19907.00	6.82	139.10	3.96	0.00	7.47	2595.00
σ^2_G		422229.50	907.50	20996.50	1.86	113.95	0.00	8.21	152.90	1.49	0.01	0.00	1438.00
σ^2_P		1238161.50	2642.50	52957.50	3.65	301.55	19557.50	15.04	292.00	5.44	0.01	7.20	4033.00
H^2%		34.101	34.34	39.65	50.88	37.79	0.00	54.62	52.36	27.31	57.78	0.00	35.66
Grand mean		2451.85	113.18	471.00	14.34	41.60	97.00	17.30	57.02	17.76	0.72	11.89	122.00
Taung													
R	1	2511742.00	5621.00	68512.00	0.47	471.60	3790.00	22.97	1983.40	1.36	0.04	18.65	0.10
G	30	1122663.00**	2378.70**	51528.00***	4.26*	460.30***	1356.00**	17.06***	267.70	8.40***	0.09	6.42	476.80
Error	30	403921.00	846.80	14038.00	1.95	144.20	511.70	4.85	195.90	1.78	0.07	7.08	368.00
σ^2_G		359371.00	765.95	18745.00	1.16	158.05	422.15	6.10	35.90	3.31	0.01	0.00	54.40
σ^2_P		763292.00	1612.75	32783.00	3.11	302.25	933.85	10.95	231.80	5.09	0.08	6.75	422.40
H^2 %		47.08	47.49	57.18	37.23	52.29	45.21	55.70	15.49	65.05	14.54	0.00	12.88
Grand mean		1886.29	86.70	411.00	13.99	38.10	61.60	14.66	35.28	17.62	0.71	10.68	60.00

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, R = Replication, G = Genotype, σ^2_G = Genotypic variance, σ^2_P = Phenotypic variance, Df = degrees of freedom, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, NSPPy = Number of seeds per pod, NPP = Number of pods per plant, SWP = Seed weight per plant, 100 SW = Hundred seed weight, HI = Harvest index, PL = Pod length, PW = Pod width, NBPP = Number of branches per plant, PH = Plant height

Table 3.4 Continued

Source	Df	GY	PWP	NSPPx	NSPPy	NPP	SWP	100 SW	HI	PW	PL	NBPP	PH
Mafikeng													
R	1.00	8725.00	18.10	984.00	3.95	0.02	2.40	2.27	249.49	2.76	0.01	24.07	1441.00
G	29.00	430916.00	901.60	28418.00***	6.23	226.47***	476.80	17.52***	152.97	5.31*	0.02**	5.94	4105.00
Error	29.00	255179.00	532.90	8015.00	3.51	67.98	284.50	5.58	98.20	2.46	0.01	4.31	2291.00
σ^2_G		87868.50	184.35	10201.50	1.36	79.25	96.15	5.97	27.39	1.43	0.01	0.82	907.00
σ^2_P		343047.50	717.25	18216.50	4.87	147.23	380.65	11.55	125.59	3.88	0.01	5.13	3198.00
H^2%		25.61	25.70	56.00	27.93	53.83	25.26	51.68	21.81	36.88	49.76	15.95	28.36
Grand mean		1162.92	53.20	271.00	12.71	28.20	34.70	13.07	30.57	15.79	0.69	13.33	118.00
Polokwane													
R	1.00	750238.00	1551.70	19929.00	0.31	204.02	925.40	9.63	187.90	0.88	0.000	3.77	536.00
G	25.00	290456.00	600.20	19608.00	7.11	139.53	283.20	12.69**	165.00	3.96	0.02**	3.36	1256.00
Error	24.00	278476.00	576.40	12379.00	5.07	89.54	280.30	4.83	108.90	3.02	0.01	2.77	1050.00
σ^2_G		5990.00	11.90	3614.50	1.02	24.99	1.45	3.93	28.05	0.47	0.01	0.30	103.00
σ^2_P		284466.00	588.30	15993.50	6.09	114.54	281.75	8.76	136.95	3.49	0.01	3.07	1153.00
H^2%		2.11	2.02	22.60	16.76	21.82	0.52	44.87	20.48	13.54	48.76	9.64	8.93
Grand mean		1233.24	56.20	288.00	12.85	27.40	39.80	14.81	50.89	15.58	0.68	11.50	70.70

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, R = Replication, G = Genotype, σ^2_G = Genotypic variance, σ^2_P = Phenotypic variance, Df = degrees of freedom, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, NSPPy = Number of seeds per pod, NPP = Number of pods per plant, SWP = Seed weight per plant, 100 SW = Hundred seed weight, HI = Harvest index, PL = Pod length, PW = Pod width, NBPP = Number of branches per plant, PH = Plant height

Table 3.5 Analysis of variance showing mean square values and broad-sense heritability (H^2) for grain yield (GY) and yield components of cowpea across five environments

Source	Df	GY	PWP	NSPPx	NSPPy	NPP	SWP	100 SW	HI	PL	PW	NBPP	PH
R	1	251005.39	547.10	4928.00	0.30	58.00	651.00	2.08	839.60	0.55	0.04	44.87	1993.09
E	4	16973416.64***	37050.91***	451861.02***	35.215***	2352.45***	38278.07***	153.02***	7192.20***	69.48***	0.02	130.82***	62433.12***
G	30	1186317.23***	2505.03***	74339.12***	18.517***	479.67***	4665.00	56.06***	591.50***	22.30***	0.08***	12.842***	5691.23***
GE	114	707089.07***	1495.09***	31349.34***	2.74	234.34***	4508.00	10.31*	213.41**	2.62*	0.02	4.33	2034.87*
Error	147	398677.87	841.42	14965.00	3.02	109.20	4510.00	7.25	142.50	2.61	0.02	4.71	1413.32
σ^2_G		47922.82	100.99	4299.00	1.58	24.53	15.70	4.58	37.81	1.97	0.01	0.85	365.64
σ^2_{GE}		154205.60	326.84	8192.00	-0.14	62.57	-1.00	1.53	35.45	0.01	0.00	-0.19	310.78
σ^2_P		118631.72	250.50	7433.90	1.85	47.97	466.50	5.61	59.15	2.23	0.01	1.28	569.12
H^2%		40.40	40.32	57.83	85.21	51.15	3.37	81.62	63.92	88.25	75.61	66.31	64.25

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, R = Replication, E = Environment, G = Genotype, GE = Genotype by environment interaction, σ^2_G = Genotypic variance, σ^2_{GE} = Genotype by environment interaction variance, σ^2_P = Phenotypic variance, Df = degrees of freedom, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, NSPPy = Number of seeds per pod, NPP = Number of pods per plant, SWP = Seed weight per plant, 100 SW = Hundred seed weight, HI = Harvest index, PL = Pod length, PW = Pod width, NBPP = Number of branches per plant, PH = Plant height

Table 3.6 Analysis of variance showing mean square values for grain yield (GY) and yield components of cowpea three groups based on their origins

Source	Df	GY	PWP	NSPPx	NSPPy	NPP	SWP	100 SW	HI	PL	PW	NBPP	PH
R	1	257314.00	561.00	5111.00	0.28	56.30	687.00	2.39	846.20	0.53	0.04	44.57	2027.00
Gr	2	3320023.00**	7055.00**	13889.00	39.32***	175.50	7454.00	175.58***	1146.20**	41.62***	0.10**	80.17***	637.00
E	4	17312153.00***	37780.00***	440890.00***	29.53***	2442.90***	38457.00***	159.83***	7405.20***	73.58***	0.02	128.94***	61453.00***
Gr.E	8	1440523.00**	3080.00**	44549.00	6.31	368.40*	5172.00	17.76	182.80	2.29	0.02	3.78	1897.00
Error	281	548066.00	1156.00	26446.00	4.17	185.50	4479.00	11.66	203.60	4.21	0.02	4.90	2050.00
Total	296	1685.10	77.19	363.00	13.55	33.91	57.40	14.76	42.91	16.96	0.70	11.33	86.30

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, R = Replication, Gr = Grouping, E = Environment, Gr.E = Grouping by environment interaction, Df = degrees of freedom, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, NSPPy = Number of seeds per pod, NPP = Number of pods per plant, SWP = Seed weight per plant, 100 SW = Hundred seed weight, HI = Harvest index, PL = Pod length, PW = Pod width, NBPP = Number of branches per plant, PH = Plant height

3.4.4 Mean values of cowpea mutants and normal genotypes across environments

Grain yield ranged from 827.11 to 2505.21 kg ha^{-1} (Table 3.7). Namibian mutants (ShR10P10, ShR10P12, ShR3P4, ShR4P1, ShL2P7, ShR9P5, NKR1P3, NKR8P9, NKRuP5 and BrR11P2), IITA genotypes (IT99K-573-2-1, ITOOK 1263 and 98K-476-8) and South African genotype (Enchore) had high mean values for GY which were above the average mean (1667.23 kg ha^{-1}) (Figure 3.1A). Number of seeds per plant ranged from 134.10 to 550.50. Namibian mutants (ShR10P10, ShR10P12, ShR3P4, ShR4P1, NKR1P3, BrR11P11, BrR11P2 and BrR4P11), IITA genotypes (IT82E-18 and 98K-476-8), and South African genotypes (Pan 311, Enchore, Glenda and Dr Saunders) had high mean values for NSPPx, which were above the average mean (357.70) (Figure 3.1B). Number of pods per plant ranged from 18.37 to 52.37. Namibian mutants (ShR10P10, ShR10P12, ShR3P4, ShR4P1, ShL2P7, NKR1P3, BrR11P2), IITA genotype (98K-476-8), and South African genotypes (Oloyin, Dr Saunders, Enchore and Glenda) had high mean values for NPP, which were above the average mean (33.67) (Figure 3.1C). Number of seeds per pod ranged from 10.83 to 15.88. Namibian mutants (ShR10P12, ShR3P4, ShR4P1, NKL9P7, NKR1P3, NKR9P9, NKRuP5, BrR11P11, BrR11P2 and BrR4P11), IITA genotypes (IT07K-292-10 and IT82E-18), and South African genotypes (Pan 311 and Bechuana White) had high mean values for NSPPy, which were above the average mean (13.47) (Figure 3.1D).

Pod weight per plant ranged from 37.82 to 115.09 g. Namibian mutants (ShR10P10, ShR10P12, ShL2P7, ShR3P4, ShR4P1, ShR9P5, NKR1P3, NKRuP5, NKR8P9 and BrR11P2), IITA genotypes (IT99K-573-2-1, ITOOK 1263 and 98K-476-8), and one South African genotype (Enchore) had high mean values for PWP, which were above the average mean (76.36 g) (Figure 3.2A). Seed weight per plant ranged from 31.60 to 158.80 g. Namibian mutants (ShR10P10, ShR10P12, ShR2P11, ShR3P4, ShR9P5, ShL2P7, ShR4P1, NKR1P3, NKRuP5, NKR8P9 and BrR11P2), IITA genotypes (IT99K-573-2-1, IT07K-318-33, Oloyin and 98K-476-8), South African genotype (Oloyin) had high mean values for SWP, which were above the average mean (54.13 g) (Figure 3.2B). Hundred seed weight ranged from 8.89 to 20.05 g. Namibian mutants (ShR10P10, ShL2P7, ShL3P7-2, ShR2P11, NKL9P7, ShR9P5, NKR1P3, NKR8P9, ShR10P12 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT93K-452-1, IT99K-573-2-1 and ITOOK 1263) and South African genotypes (Oloyin and Orelu) had high mean values for 100 SW which were above the average mean (14.84 g) (Figure 3.2C). Harvest index ranged from 20.42 to 55.65%. Namibian mutants (ShR10P10, ShR10P12, ShR4P1, ShL2P7, ShR9P5, NKR1P3, NKR8P9, NKR9P9, NKRuP5, BrR11P11 and BrR11P2), IITA genotypes (IT82E-18 and IT93K-452-1) and South African genotypes (Oloyin, Pan 311, Enchore and Glenda) had high mean values for HI, which were above the average mean (43.13%) (Figure 3.2D).

Pod length ranged from 12.15 to 20.72 cm. Namibian mutants (ShR10P10, ShR3P4, ShR9P5, NKL9P7, NKR8P9, NKR9P9, NKRuP5, BrR11P2, NKR1P3 and BrR4P11), IITA genotypes (IT07K-292-10, IT82E-18, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Bechuana White and Enchore) had high mean values for PL, which were above the average mean (16.94 cm) (Figure 3.3A). Pod width ranged from 0.49 to 0.95 cm. Namibian mutants (ShR10P10, ShR3P4, ShR4P1, ShR9P5, NKL9P7, NKR8P9, NKR9P9, NKRuP5, BrR11P11, BrR11P2 and BrR4P11), IITA genotypes (IT07K-318-33, IT82E-18 and ITOOK 1263), and South African genotypes (Pan 311 and Enchore) had high mean values for PW, which were above the average mean (0.70 cm) (Figure 3.3B). Number of branches per plant ranged from 9.30 to 13.60. Namibian mutants (ShR10P10, NKR8P9 and BrR11P2), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18, IT93K-452-1, IT99K-573-2-1, ITOOK 1263 and 98K-476-8), and South African genotypes (Agrinawa, Dr Saunders, Bechuana White and Enchore) had high mean values for NBPP, which were above the average mean (11.37) (Figure 3.3C). Plant height ranged from 45.40 to 146.10 cm. Namibian mutants (ShR10P10, ShR10P12, NKL9P7, NKR8P9, NKR9P9, BrR11P11 and BrR4P11), IITA genotypes (IT07K-318-33, IT93K-452-1 and ITOOK 1263), and South African genotypes (Orelu, Agrinawa, Bechuana White and Dr Saunders) had high mean values for PH, which were above the average mean (86.09 cm) (Figure 3.3D).

3.4.5 Mean values of cowpea groupings across environments

Significant differences were observed between the three groupings for grain yield and yield components except for NBPP. Namibian mutants had high mean values for GY (1820.17 kg ha^{-1}), PWP (83.46 g), NSPPx (374.00), NSPPy (14.03), SWP (64.30 g), 100 SW (15.41 g), HI (44.66%) and PW (0.73 cm) compared to the IITA and South African genotypes (Table 3.8). The IITA genotypes had high mean values for NBPP (12.46) compared to the Namibian mutants and South African genotypes. South African genotypes had high mean values for NPP (35.11) compared to the Namibian mutants and IITA genotypes. The IITA genotypes had low mean values for NSPPx (346.00), HI (37.93%), and PH (82.90 cm) compared to the Namibian mutants and South African genotypes. South African genotypes had low mean values for GY (1427.44 kg ha^{-1}), PWP (65.35 g), NSPPy (12.93), SWP (46.20 g), 100 SW (12.93 g), PL (16.04 cm) and PW (0.67 cm) compared to the Namibian mutants and IITA genotypes.

Table 3.7 Mean values of grain yield (GY) and yield components of each of the 31 genotypes across five environments

Genotypes	GY (kg ha^{-1})	NSPPx	NPP	NSPPy	PWP (g)	SWP (g)	100 SW (g)	HI (%)	PL (cm)	PW (cm)	NBPP	PH (cm)
ShL3P7-2	827.11	134.10	18.37	10.83	37.82	31.60	16.62	20.42	15.21	0.69	11.29	54.30
ShR10P10	2137.67	387.80	39.00	12.22	98.16	70.80	17.62	47.38	17.54	0.78	11.67	146.10
ShR10P12	2020.43	405.20	35.80	14.10	92.78	66.60	16.32	51.83	16.60	0.66	10.90	102.50
ShR2P11	1623.83	297.90	31.30	12.34	74.26	54.50	15.55	40.56	15.13	0.63	10.10	51.10
ShR3P4	1832.62	397.20	34.60	14.30	83.82	54.80	13.56	40.96	18.02	0.77	10.10	85.00
ShR4P1	1821.71	442.90	35.80	15.32	83.35	59.20	12.55	49.42	16.95	0.71	11.20	75.30
ShR9P5	1778.31	352.80	31.30	13.34	81.50	158.80	16.25	48.64	18.05	0.75	10.10	78.10
98K-476-8	2045.84	510.60	52.37	12.41	93.46	68.50	13.01	35.06	16.75	0.55	13.17	59.40
Agrinawa	1083.22	291.20	33.60	11.92	49.77	34.10	11.09	35.99	16.32	0.65	13.10	100.20
Bechuana White	1541.96	332.90	32.10	13.90	70.95	46.50	13.38	42.37	18.04	0.70	11.60	101.90
BrR11P11	1586.49	370.50	31.90	14.08	72.65	48.40	12.78	43.57	15.91	0.71	11.00	97.70
BrR11P2	2505.21	550.50	45.90	15.66	115.09	76.70	13.81	49.60	17.71	0.75	11.50	81.00
BrR4P11	1489.06	363.40	28.50	15.88	68.07	45.40	12.97	42.64	17.67	0.73	10.60	93.10
Dr Saunders	1238.94	486.80	42.80	13.10	56.69	43.30	8.89	38.91	12.15	0.49	13.20	101.90
Enchore	1732.54	373.90	38.90	12.26	79.29	52.00	13.08	43.61	17.57	0.74	13.10	58.20
Glenda	1481.47	406.40	40.90	12.84	67.78	52.10	12.00	51.69	15.77	0.59	11.20	59.50
IT07K-292-10	1526.34	279.10	28.20	15.03	69.70	49.20	16.86	28.70	17.44	0.69	11.50	81.00
IT07K-318-33	1502.33	316.70	32.10	12.70	68.67	55.70	15.33	34.72	16.78	0.74	13.50	87.60
IT82E-18	1631.98	377.40	32.10	15.02	74.69	46.40	12.74	46.10	17.30	0.73	11.60	64.30
IT93K-452-1	1395.87	312.60	32.00	12.30	63.79	45.80	14.96	45.32	15.23	0.63	12.20	107.30
IT99K-573-2-1	1872.75	351.50	32.50	12.14	85.48	62.80	17.70	40.53	17.83	0.70	11.80	79.70
ITOOK 1263	1712.82	290.80	32.90	12.10	78.55	51.10	16.47	36.07	19.48	0.77	13.60	92.80
NKL9P7	1549.43	309.90	27.80	14.64	70.91	48.30	15.12	38.09	17.88	0.78	10.60	126.00
NKR1P3	2230.24	523.70	42.40	14.34	102.15	68.80	16.21	46.84	17.04	0.70	10.90	67.60
NKR8P9	1748.91	270.20	23.92	13.45	80.34	56.30	20.05	55.26	20.72	0.79	11.38	114.90
NKR9P9	1605.38	301.10	27.70	13.78	73.74	48.50	14.63	44.81	17.59	0.73	9.30	97.10
NKRuP5	1751.85	349.70	25.90	15.40	80.72	55.50	18.66	44.76	17.73	0.79	10.40	83.30
Oloyin	1648.07	333.20	36.30	12.23	75.24	55.70	15.55	48.79	16.08	0.63	10.80	65.20
Orelu	1126.25	233.30	26.60	11.86	51.50	38.50	16.08	37.30	16.12	0.59	10.50	126.90
Pan 311	1567.06	383.70	29.70	15.34	71.55	47.40	13.37	51.48	16.23	0.95	10.00	84.40
ShL2P7	2068.53	351.60	40.50	12.75	94.73	57.80	16.70	55.65	16.22	0.63	10.67	45.40
LSD (P = 0.05)	558.01	108.11	9.23	1.54	25.64	59.35	2.38	10.55	1.43	0.12	1.92	33.22
Min mean	827.11	134.10	18.37	10.83	37.82	31.60	8.89	20.42	12.15	0.49	9.30	45.40
Max mean	2505.21	550.50	52.37	15.88	115.09	158.80	20.05	55.65	20.72	0.95	13.60	146.10
Grand mean	1667.23	357.70	33.67	13.47	76.36	54.13	14.84	43.13	16.94	0.70	11.37	86.09
CV%	37.90	34.20	31.00	12.90	38.00	118.90	18.10	27.70	9.50	18.60	19.10	43.70

LSD = Least significant difference, CV = Coefficient of variation, NSPPx = Number of seeds per plant, NPP = Number of pods per plant, NSPPy = Number of seeds per pod, PWP = Pod weight per plant, SWP = Seed weight per plant, 100 SW = Hundred seed weight, HI = Harvest index, PL = Pod length, PW = Pod width, NBPP = Number of branches per plant, PH = Plant height

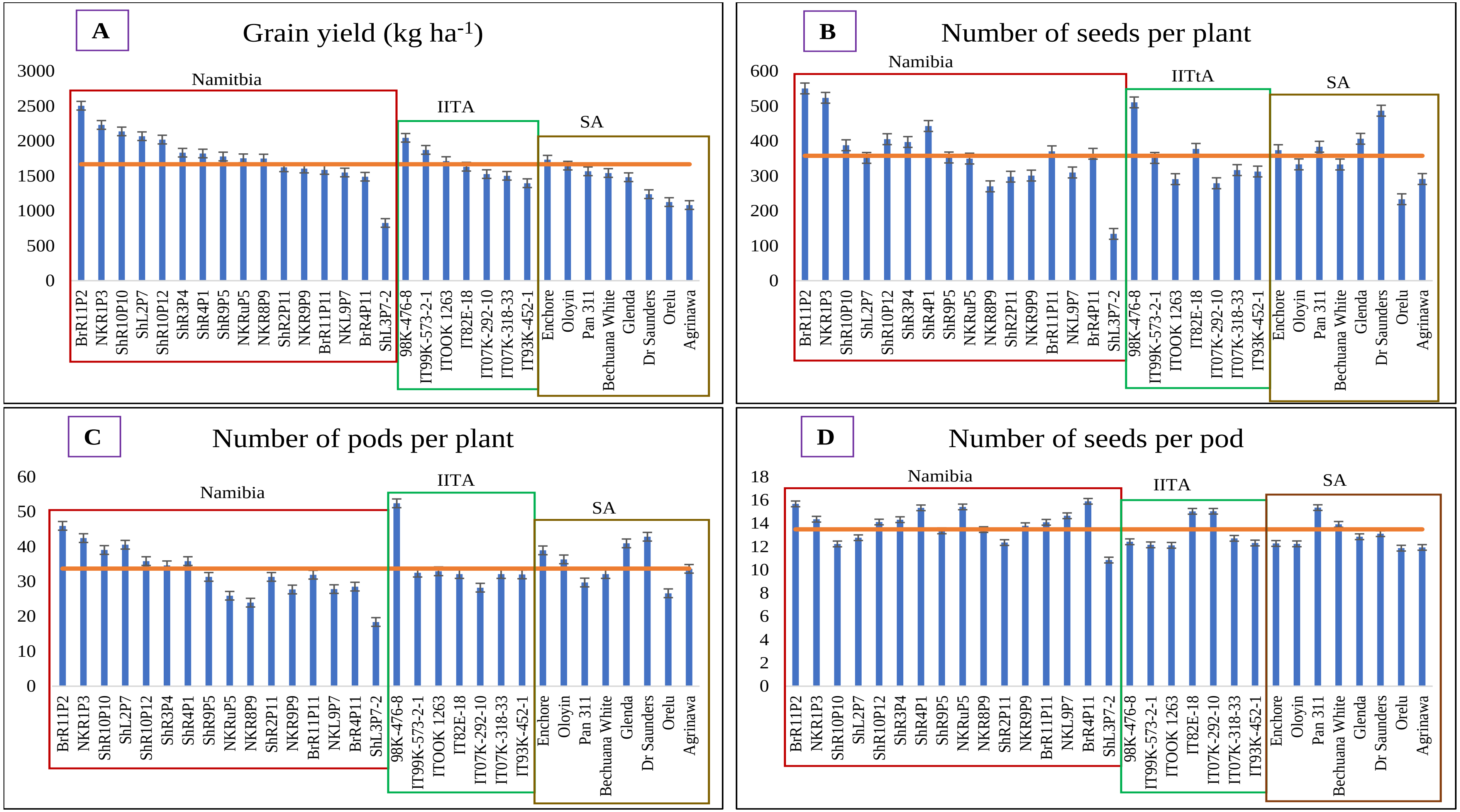

Figure 3.1 Mean value performance of Namibian mutants, IITA (International Institute of Tropical Agriculture) and SA (South Africa) genotypes for grain yield (A), number of pods per plant (B), number of seeds per plant (C) and number of seeds per pod (D). Blue bars = Traits, Orange line = Average mean

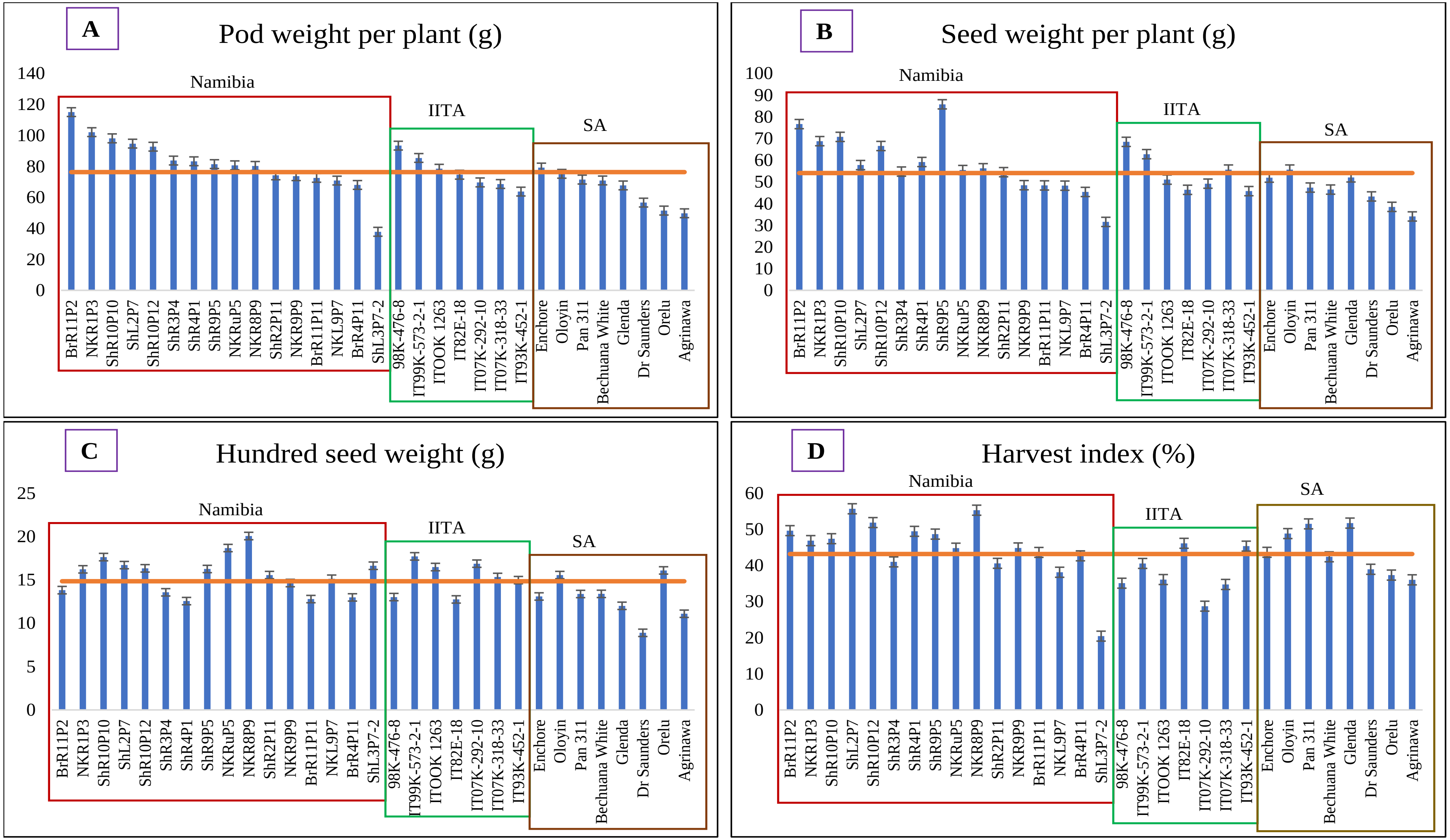

Figure 3.2 Mean value performance of Namibian mutants, IITA (International Institute of Tropical Agriculture) and SA (South Africa) genotypes for pod weight per plant (A), seed weight per plant (B), hundred seed weight (C) and harvest index (D). Blue bars = Traits, Orange line = Average mean

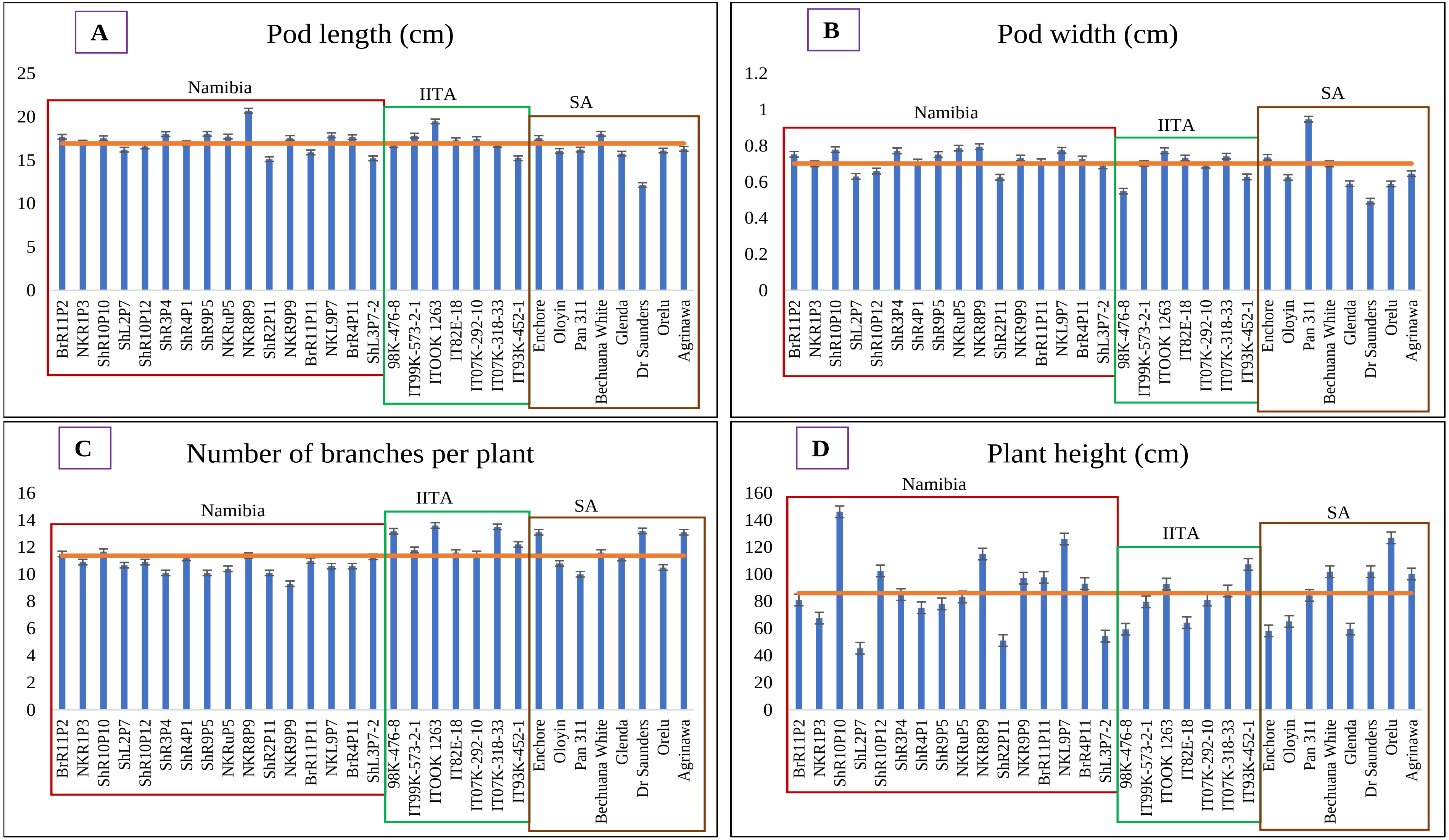

Figure 3.3 Mean value performance of Namibian mutants, IITA (International Institute of Tropical Agriculture) and SA (South Africa) genotypes for pod width (A), pod length (B), number of branches per plant (C) and plant height (D). Blue bars = Traits, Orange line = Average mean

Table 3.8 Mean values for grain yield (GY) and yield components of cowpea three groupes based on their origin

Origin	GY (kg ha^{-1})	PWP (g)	NSPPx	NSPPy	NPP	SWP (g)	100 SW (g)	HI (%)	PL (cm)	PW (cm)	NBPP	PH (cm)
IITA	1670.85^{b}	76.40^{b}	346.00^{c}	13.14^{b}	34.25^{b}	54.30^{b}	15.35^{b}	37.93^{c}	17.31^{a}	0.69^{b}	12.46^{a}	82.90^{b}
Namibia	1820.17^{a}	83.46^{a}	374.00^{a}	14.03^{a}	33.17^{c}	64.30^{a}	15.41^{a}	44.66^{a}	17.27^{b}	0.73^{a}	10.66^{c}	87.30^{a}
SA	1427.44^{c}	65.35^{c}	355.00^{b}	12.93^{c}	35.11^{a}	46.20^{c}	12.93^{c}	43.77^{b}	16.04^{c}	0.67^{c}	11.69^{b}	87.30^{a}
LSD	250.13	11.55	50.60	0.60	4.17	19.82	1.05	4.86	0.63	11.55	0.72	15.02

At $P = 0.05$, means that share the same letter of the alphabet are not significantly different, IITA = International Institute of Tropical Agriculture, SA = South Africa, LSD = Least significant difference, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, NSPPy = Number of seeds per pod, NPP = Number of pods per plant, SWP = Seed weight per plant, 100 SW = Hundred seed weight, HI = Harvest index, PL = Pod length, PW = Pod width, NBPP = Number of branches per plant, PH = Plant height

3.4.6 *Phenotypic correlation*

Highly significant ($P \leq 0.001$) and positive correlations were found for GY with PWP (r = 1.00), NSPPx (r = 0.69), NPP (r = 0.60), HI (r = 0.57), for PWP with NSPPx (r = 0.69), NPP (r = 0.60), HI (r = 0.57), for NSPPx with NPP (r = 0.84), and for PW with PL (r = 0.63) (Table 3.9). Significant ($P \leq 0.01$) and positive correlation was found for NSPPx with HI (r = 0.45), for SWP with GY (r = 0.50) and PWP (r = 0.05), for PL with 100 SW, for NSPPy with NSPPx (r = 0.44) and PL (r = 0.46).

Significant ($P \leq 0.05$) and positive correlations were found for PL with GY (r = 0.41) and PWP (r = 0.41), for NSPPy with GY (r = 0.36) and PWP (r = 0.36), and for NPP with HI (r = 0.37). Significant ($P \leq 0.05$) and negative correlations were observed for 100 SW with NSPPx (r = -0.41) and NPP (r = -0.42), for PW with NPP (r = -0.40), and for NBPP with HI (r = -0.35) and NSPPy (r = -0.40). The heat map showed strong positive correlation between GY, PWP, NSPPx and NPP (Figure 3.4). Weak correlations were observed between NSPPx with NPP, PL, PW, PH and was only significant to 100 SW.

Table 3.9 Pearson correlations for grain yield (GY) and yield components

Traits	GY	PWP	NSPPx	NPP	HI	SWP	100 SW	PL	PW	NSPPy	NBPP
PWP	1.00***										
NSPPx	0.69***	0.69***									
NPP	0.60***	0.60***	0.84***								
HI	0.57***	0.57***	0.45**	0.30							
SWP	0.50**	0.50**	0.32	0.24	0.34						
100 SW	0.25	0.26	-0.41*	-0.42*	0.07	0.23					
PL	0.41*	0.41*	-0.11	-0.22	0.19	0.26	0.53**				
PW	0.23	0.23	-0.11	-0.40*	0.20	0.15	0.34	0.63***			
NSPPy	0.36*	0.36*	0.44**	-0.01	0.35	0.10	-0.14	0.26	0.46**		
NBPP	-0.10	-0.10	0.12	0.37*	-0.35*	-0.19	-0.26	-0.09	-0.29	-0.40*	
PH	-0.11	-0.11	-0.15	-0.25	0.02	-0.09	0.13	0.19	0.19	0.06	0.02

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, NPP = Number of pods per plant, HI = Harvest index, SWP = Seed weight per plant, 100 SW = Hundred seed weight, PL = Pod length, PW = Pod width, NSPPy = Number of seeds per pod, NBPP = Number of branches per plant, PH = Plant height

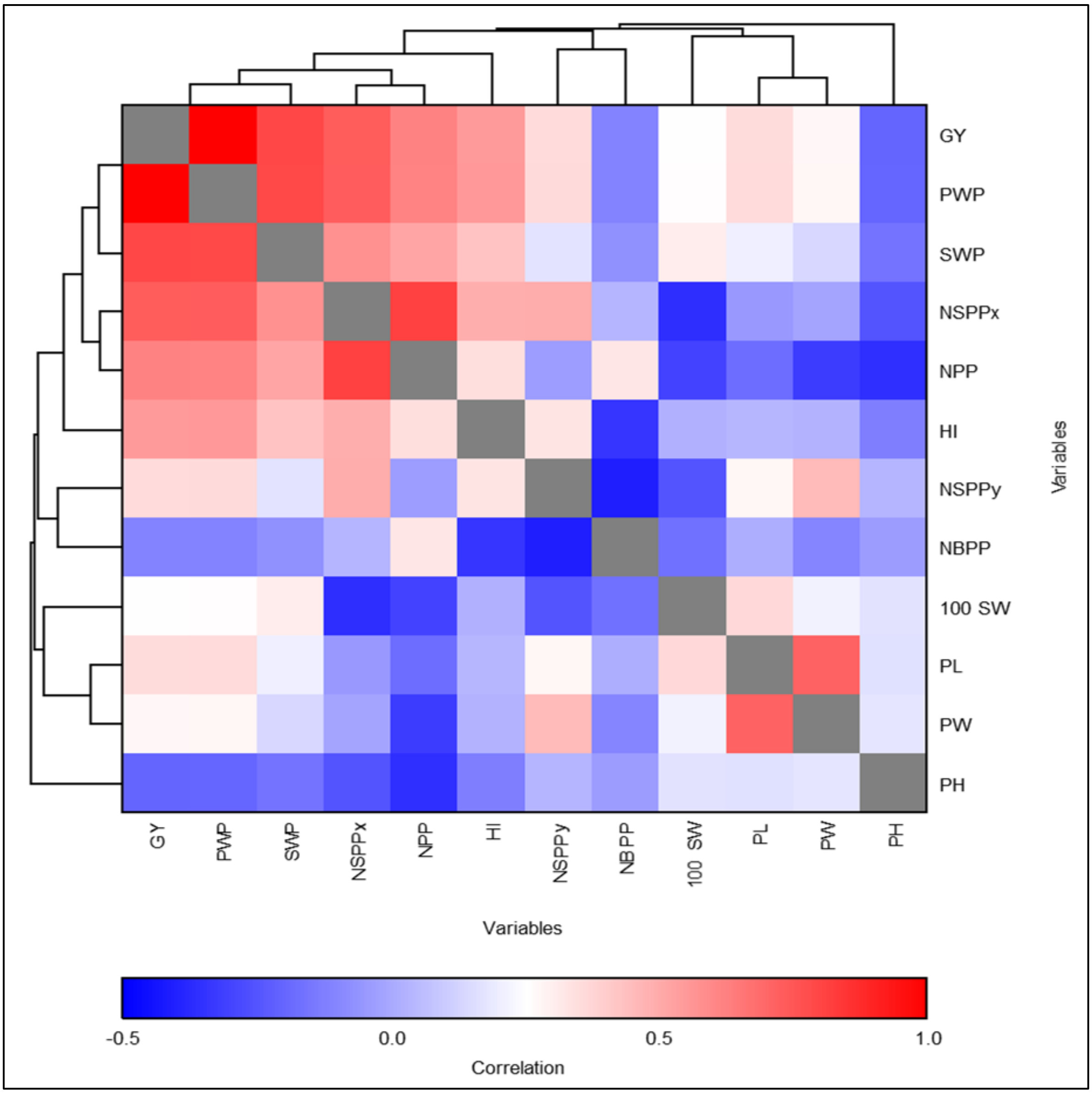

Figure 3.4 Heat map showing phenotypic correlations between cowpea grain yield (GY) and yield components. PWP = Pod weight per plant, SWP = Seed weight per plant, NSPPx = Number of seeds per plant, NPP = Number of pods per plant, HI = Harvest index, NSPPy = Number of seeds per pod, NBPP = Number of branches per plant, 100 SW = Hundred seed weight, PL = Pod length, PW = Pod width, PH = Plant height

3.4.7 Principal component analysis (PCA)

The PCA (Table 3.10) showed that the first four principal components (PCs) had eigenvalues of greater than one and accounted 79.55% of the total variation. PC1, PC2, PC3 and PC4 explained 34.55%, 23.99%, 11.67% and 9.34% of the total variation, respectively. Only PC1 and PC2 were interpreted because they contributed most of the variation in the dataset. The PC1 was strong and positively affected by GY, HI, NSPPx, and PWP. NBPP, NPP and NSPPx had a positive effect on PC2, whereas 100 SW, PH, PL and PW had a substantial negative affect.

The PCA biplot was used to visualise genotype performance and their trait associations (Figure 3.5). The PC1 and PC2 explained 58.54% of the total variation. South African genotype Enchore and IITA genotype 98K-476-8 were associated with NPP. Namibian mutants (ShL2P7, ShR4P1, NKR1P3 and BrR11P2) were associated with NSPPx, PWP and GY. South African genotypes (Dr Saunders and Glenda) and IITA genotypes (IT07K-318-33 and IT82E-18) were associated with NBPP. Namibian mutants (ShR3P4, NKRuP5 and NKR8P9), IITA genotype (IT99K-573-2-1) and South African genotype (Pan 311) were associated with 100 SW, PW and PL. Namibian mutants (ShR10P12, ShR10P10 and ShR9P5) were associated with NSPPy, HI and SWP. Namibian mutants (NKR9P9, NKL9P7 and BrR4P11) and IITA genotypes (IT07K-292-10 and ITOOK 1263) were associated with PH. The PCA showed positive correlations between NPP, NSPPx, PWP and GY, however, these traits were negatively correlated with PH. Positive correlations were observed between SWP, HI, NSPPy, 100 SW, PW and PL and negative correlation of these traits with NBPP.

Table 3.10 Principal component analysis (PCA) of grain yield and yield components in cowpea genotypes

Traits	PC1	PC2	PC3	PC4
100 SW	0.123	-0.713	-0.537	-0.193
NBPP	-0.194	0.507	-0.474	0.585
GY	0.955	0.008	-0.195	0.029
HI	0.698	-0.083	0.217	-0.168
NPP	0.581	0.771	-0.157	0.102
NSPPx	0.766	0.553	0.203	0.152
NSPPy	0.511	-0.239	0.707	0.155
PH	-0.107	-0.337	0.071	0.636
PL	0.393	-0.688	-0.272	0.306
PWP	0.955	0.004	-0.195	0.031
PW	0.292	-0.743	0.188	0.247
SWP	0.590	-0.110	-0.289	-0.308
Eigenvalue	4.15	2.88	1.40	1.12
Variation%	34.55	23.99	11.67	9.34
Cumulative%	34.55	58.54	70.21	79.55

PC = Principal component, 100 SW = Hundred seed weight, NBPP = Number of branches per plant, GY = Grain yield, HI = Harvest index, NPP = Number of pods per plant, NSPPx = Number of seeds per plant, NSPPy = Number of seeds per pod, PH = Plant height, PL = Pod length, PWP = Pod weight per plant, PW = Pod width, SWP = Seed weight per plant

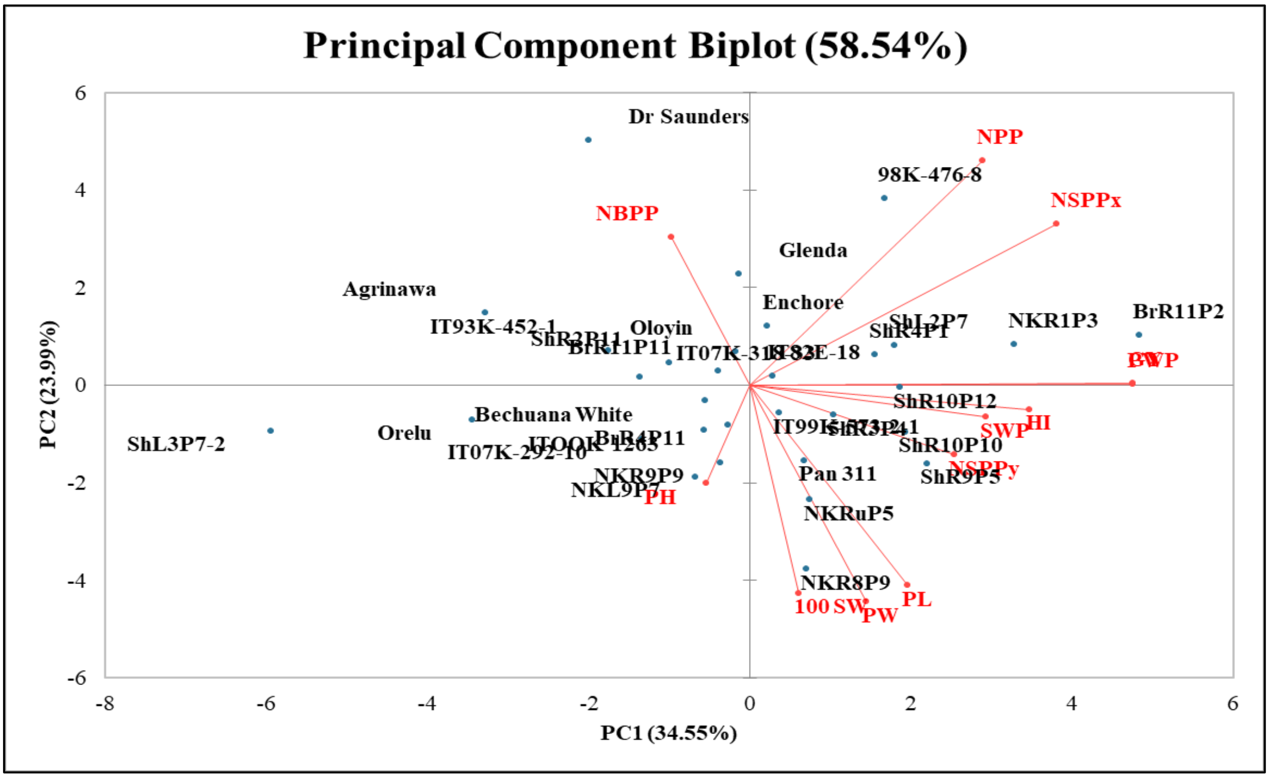

Figure 3.5 Principal component analysis (PCA) showing the associations among the genotypes with their traits. PC = Principal component, 100 SW = Hundred seed weight, NBPP = Number of branches per plant, PH = Plant height, NPP = Number of pods per plant, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, PL = Pod length, PW = Pod width, NSPPy = Number of seeds per pod, SWP = Seed weight per plant, GY = Grain yield, HI = Harvest index

3.4.8 Clustered heat map of measured traits in cowpea mutant and normal genotypes

One distinct grouping of characteristics (GY, PWP, NSPPx, SWP, HI, NSPPy, PL, PW and 100 SW) was identified using the clustering heat map (Figure 3.6). The PH and NBPP were not associated with any cluster or sub-clusters. The clustering heat map also revealed three distinctive groupings of genotypes, namely; 1) Namibian mutants (ShR10P10 and ShL3P7-2), IITA genotypes (IT93K-452-1, IT99K-573-2-1, IT07K-292-10, ITOOK 1263 and IT07K-318-33) and South African genotypes (Orelu and Agrinawa) which were associated with PL, PW, 100 SW, PH and NBPP, 2) Namibian mutants (NKR8P9, NKRuP5, NKR9P9, NKL9P7, BrR4P11, BrR11P11, ShR4P1 and ShR3P4), IITA genotype (IT82E-18) and South African genotypes (Bechuana white and Pan 311) which were associated with HI, NSPPy, PL and PW, and 3) Namibian mutants (NKR1P3, BrR11P2, ShL2P7, ShR2P11 and ShR10P12), IITA genotype (98K-476-8) and South African genotypes (Glenda, Dr saunders, Enchore and Oloyin) which were associated with GY, PWP, NSPPx, NPP, SWP and HI.

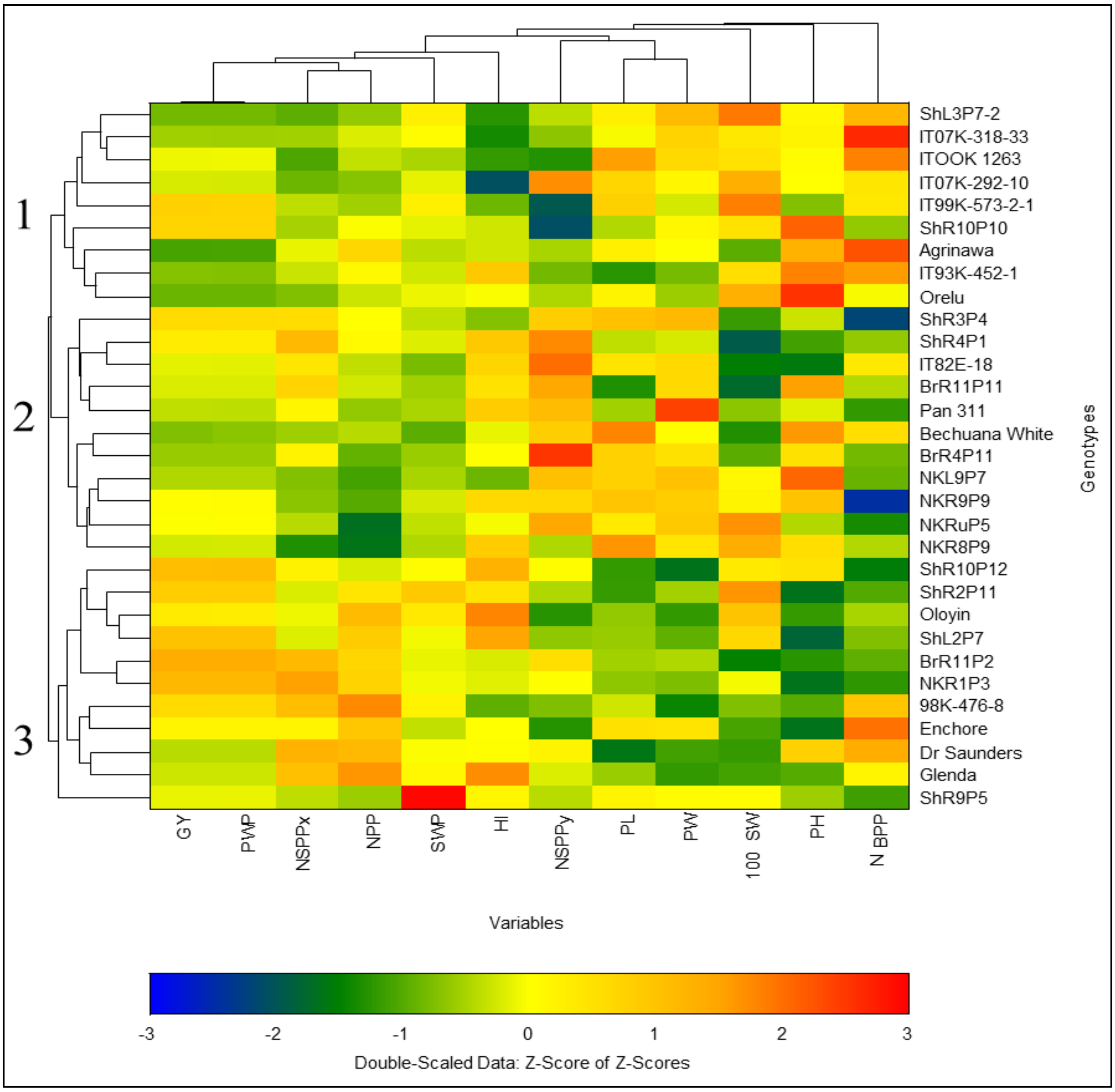

Figure 3.6 Clustered heat map showing the groupings of genotypes based on the measured traits. 100 SW = Hundred seed weight, NBPP = Number of branches per plant, PH = Plant height, NPP = Number of pods per plant, PWP = Pod weight per plant, NSPPx = Number of seeds per plant, PL = Pod length, PW = Pod width, NSPPy = Number of seeds per pod, SWP = Seed weight per plant, GY = Grain yeld, HI = Harvest index

3.5 Discussion

3.5.1 Analysis of variance for cowpea mutants and normal genotypes

Highly significant genotype effects indicated the presence of genetic variation among the tested genotypes for grain yield and measured traits. Due to the high genetic diversity among the tested mutants and South African genotypes, it is possible to identify and choose superior mutants for grain yield and yield components, allowing for the future use of this germplasm collection diversity for crop improvement. Previous studies have also reported significant variation among cowpea genotypes for grain yield and yield related traits (Gerrano et al. 2015; Viswanatha and Yogeesh 2017).

Significant GE interaction for grain yield and other measured traits indicated that cowpea mutants and the normal genotypes performed differently across diverse environments. A polygenic trait called grain yield is primarily controlled by minor additive genetic effects (Mbuma et al. 2021; Siwale et al. 2022). The phenotype of polygenic traits such as grain yield is mostly influenced by the environment, resulting in significant and large GE interaction. The results also reveal that cowpea genotypes should be tested for adaptation and stability over a diverse range of conditions and seasons before cultivar commercialisation. It is noteworthy that these cowpea mutants and normal genotypes were only evaluated in five environments but only in one season, thus, future studies should investigate the adaptability and stability of these mutants in multiple season trials.

The H^2 values were above 50% for GY, PWP, NSPPx, NPP, NBPP and PL in Bloemfontein, indicating that the genotypic variance contributed more to the variation than the phenotypic variance. High H^2 value for GY is very important because then plant breeders can directly select for the trait. The H^2 values for NSPPy, HI, 100 SW and PW were above 50% in Potchefstroom, indicating that these traits could be selected with high precision in this environment. The H^2 values for NPP, 100 SW, NSPPx and PL were above 50% in Taung. The H^2 values for 100 SW, NPP and NSPPx were also above 50% in Mafikeng; selection of these traits would also be done with good accuracy in this environment. The H^2 values were below 50% for all the measured traits in Polokwane, indicating that selection of these traits in this environment would be done with low precision. Additionally, it was noted that Polokwane and Mafikeng had aphid infestation, which affected the growth and the resulting grain yield. Therefore, the H^2 values of all the traits do not represent the nature of the genotypes in Polokwane, but rather the environmental influence.

On average, H^2 values were above 50% for NSPPx, NSPPy, NPP, 100SW, HI, PL, PW, NBPP and PH across environments, indicating that the variation observed in these traits were largely influenced by genotypic differences, which also suggested that selection for these traits could be done with high precision. When compared to South African genotypes, previous studies on cowpea mutant genotypes found significant differences for yield traits with H^2 values above 50%, indicating that mutagenesis can be utilised to improve the crop (Horn et al. 2016; Diouf et al. 2020). On the other hand, the H^2 values for GY, PWP and SWP were below 50%, implying that phenotypic variation observed for these traits was mainly due to the environmental effects. Similarly, low H^2 value for grain yield was reported by Mofokeng et al. (2020) on cowpea genotypes tested under different South African conditions.

3.5.2 Combined ANOVA for groupings according to origin

Highly significant effects of the genotype groupings according to origin for almost all the traits indicated the existence of large genetic variability among the Namibian mutants, IITA and South African genotypes. This genetic variation could be used to diversify the cowpea germplasm collection in South Africa and further exploited for crop improvement. The Namibian Radiation Regulatory Authority successfully developed cowpea mutant genotypes with desired traits such as high grain yield, drought tolerance, and pests and diseases resistance that contributed to the existing genetic diversity of the crop (Omoigui et al. 2012; Horn et al. 2016; Horn and Shimelis 2020). The grouping by environment interaction was significant for grain yield and other yield components, indicating that genotype groupings performed differently across the testing environments. The wide diversity observed in this study for grain yield and yield components indicate that cowpea mutants introduce new variation into the existing germplasm collection. Horn et al. (2018) evaluated Namibian mutants in multiple environments and reported GE interactions to be significant for grain yield, indicating genotype performance varied among test environments.

3.5.3 Mean values of cowpea mutants and normal genotypes across environments

Namibian mutants (ShR10P12, ShR3P4, ShR4P1 and BrR11P2), IITA genotype (98K-476-8) and South African genotype (Enchore) had mean values above the average mean for GY (1667.23 kg ha^{-1}), NSPPx (357.70), PWP (76.36 g) and NPP (33.67) indicating the possibility of broad or wide adaptation of these genotypes. The identified genotypes with high grain yield across the test environments should also be evaluated for nutritional composition, and disease and pest resistance. Interesting was that the Namibian mutant genotypes showed better performance for grain yield and yield components compared to the IITA genotypes and South

African genotypes, which suggested that mutants were better performing than the normal genotypes and could be used to widen the genetic bases of cowpea breeding populations. African countries with a high rate of hunger and malnutrition could benefit greatly from the improved cowpea genotypes with high grain production and good nutrition.

Namibian mutants (NKL9P7, NKR1P3, NKR9P9, NKRuP5, ShR10P10 and ShL2P7), IITA genotypes (IT07K-292-10, IT82E-18 and IT93K-452-1) and South African genotypes (Pan 311 and Bechuana White) had mean values above the average mean for NSPPy (13.47), 100 SW (14.84 g), PL (16.94 cm) and PW (0.70 cm). These traits are closely related with grain yield, so plant breeders can select these secondary traits for grain yield improvement. Namibian mutants (ShL3P7-2 and NKR8P8), IITA genotypes (IT07K-318-33, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Orelu, Agrinawa, Dr Saunders, Bechuana White and Enchore) had high mean values for NBPP (11.37) and PH (86.09 cm) which were above the average mean, indicating that these genotypes could be exploited as fodder and as a vegetative crop. These genotypes can be used as a start toward addressing the protein-calorie undernourishment and Fe deficiency in African regions through consumption of their leaves as a vegetable. The results of nutrient profiling of cowpea leaves demonstrated its wide range of nutrients, including protein, carbohydrates, Fe, Zn, magnesium, calcium and potassium (Enyiukwu et al. 2018).

3.5.4 Mean values of cowpea groupings across environments

Significant differences were observed between the groupings for almost all the traits except for NBPP, indicating that the source of origin of the genotypes contributed to the variation observed. The Namibian mutant group was superior to IITA genotypes and South African genotypes for GY, PWP, NSPPx, NSPPy, SWP, 100 SW, HI, PW and PH. This indicates that Namibian mutants outperformed the South African normal genotypes in terms of GY and related traits, and it also indicates the possibility of adaptation in South African conditions. Breeding efforts to boost grain yield, which will help the world to increase food production must consider the variation available in cowpea mutants (Goyal and Khan 2010). The IITA genotypes also had high GY, PWP, NSPPy, SWP, 100 SW, PL and NBPP compared to the South African genotypes, indicating that the diversity of the mutants can be exploited to improve the South African cowpea genotypes.

3.5.5 Phenotypic correlation

Significant and positive correlations observed for GY with HI, NPP, NSPPx, NSPPy, PL, and PWP suggested that an enhancement of one of these yield components will increase GY. Previous research has also revealed a positive correlation between grain yield, the number of branches per plant and the number of pods per plant (Mbuma et al. 2021). Significant and positive correlation between most of the yield traits indicated the likelihood of simultaneously selecting and improving grain yield and traits related to yield. Grain yield is a complex trait, so direct selection is complicated, however, selection of secondary traits can lead to grain yield improvement (Oladejo et al. 2011). Significantly positive correlations were also found between PW with PL and 100 SW, implying that selecting for any of these traits will improve the other traits. These traits also contributed to grain yield; therefore, their selection is important for high grain yield breeding. Furthermore, a recent study revealed that the number of pods per plant and pod weight showed a significant positive phenotypic correlation with pod yield per plant, indicating the potential for simultaneous selection of these traits (Kalambe et al. 2019). Given that NBPP and PH were negatively correlated with GY in this study, an improvement in one of these characteristics would decrease the GY.

3.5.6 Principal component analysis (PCA)

The PCA showed that Namibian mutants (ShR10P12, NKR1P3, ShR4P1 and ShL2P7), IITA genotype (98K-476-8) and South African genotype (Enchore) were associated with GY, PWP, NSPPx and NPP suggesting that such genotypes could be used in the breeding programme as parental lines for the development of high yielding cultivars. However, these traits (GY, PWP, NSPPx and NPP) were negatively correlated with PH, which was associated with the Namibian mutants (NKL9P7, NKR9P9 and BrR4P11), IITA genotypes (ITOOK 1263 and IT07K-292-10), and South African genotypes (Orelu and Bechuana White), indicating that the genotypes associated with high plant height will have a reduced grain yield. The PCA biplot also showed that Namibian mutants (ShR10P12, ShR3P4, ShR10P10, ShR9P5, NKR8P9 and NKRuP5), IITA genotype (IT99K-573-2-1) and South African genotype (Pan 311) were associated with SWP, HI, NSSPy, PL, PW and 100 SW, indicating that selecting for these traits will promote high GY in these genotypes since these traits showed positive correlations with GY. Namibian mutants (BrR11P11 and ShR2P11), IITA genotypes (IT93K-452-1 and IT07K-318-33 and IT82E-18), and South African genotypes (Oloyin, Glenda and Dr Saunders) were associated with SWP, HI, NSSPy, PL, PW and 100 SW and all these traits were negatively associated with NBPP. These findings imply that genotypes associated with NBPP and PH will have low grain yield performance compared to those associated with the other traits. However, a recent study conducted in South African genotypes reported positive correlations between PH, NBPP and GY and these traits were closely associated with the South African normal genotypes, indicating that South African genotypes are bred for both high grain yield and as leafy vegetable (Gerrano et al. 2022). These results could also indicate that the Namibian mutants and IITA genotypes had an influence on the negative association observed for PH and NBPP, suggesting different stature of the plants, hence the large variation for the three groups (Namibian mutants, IITA genotypes and South African genotypes).

3.5.7 Clustered heat map of measured traits in cowpea mutant and normal genotypes

Based on grain yield and yield components, the clustered heat map identified three separate groups of cowpea genotypes. In cluster 1, Namibian mutants (ShR10P10 and ShL3P7-2), IITA genotypes (IT93K-452-1, IT99K-573-2-1, IT07K-292-10, ITOOK 1263 and IT07K-318-33) and South African genotypes (Orelu and Agrinawa) were associated with PL, PW, 100 SW, PH and NBPP, indicating that these genotypes could be used as cowpea vegetable crop. It is also highly recommended that these genotypes should be evaluated for nutritional value even though they were not associated with high grain yield, this is because previous research has reported a negative correlation between the grain yield and nutritional quality traits of cowpea (Mbuma et al. 2022). It can also be noted that most of the genotypes in cluster 1 are IITA genotypes and the mean values of the grouping indicated that these genotypes had high NBPP compared to the other groups, further indicating their potential to be exploited as a vegetable and fodder. In cluster 2, Namibian mutants (ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR9P9, NKRuP5 and NKR8P9), IITA genotype (IT82E-18) and South African genotypes (Pan 311 and Bechuana White) were associated with HI, NSPPy, PL and PW, which suggested that most of the Namibian mutants are highly associated with traits that have a positive influence on cowpea grain yield.

In cluster 3, Namibian mutants (ShR10P12, ShR2P11, ShL2P7, BrR11P2, ShR9P5 and NKR1P3), IITA genotype (98K-476-8) and South African genotypes (Enchore, Dr Saunders and Glenda) were associated with GY, PWP, NSPPx, NPP and SWP, which indicated that such genotypes could be further exploited for nutritional value, abiotic stresses, disease and pest resistance. Successfully breeding for cowpea genotypes that possess high grain yield, good nutritional value, resistance to diseases and pests as well as tolerance to abiotic stresses would ensure sustainability of cowpea crop production and productivity, which will ultimately combat the issues associated with food security and malnutrition. The genotypes were not clustered according to their source of origin but rather their trait association, demonstrating relatedness among genotypes from various genetic bases.

3.6 Conclusions

Significant differences between cowpea genotypes and groupings, and GE interaction for grain yield and grain yield components were observed, indicating that there is a sufficient genetic variation in cowpea germplasm colletions for these traits which can be exploited for further crop improvement. Significant GE interaction suggested that cowpea genotypes should be tested for adaptability and stability for grain yield and other important traits before their recommendation for release and commercial production. The high H^2 values for all yield traits indicated the potential for genetic improvement and high selection efficiency for these traits. Low H^2 for GY indicated the complexity in genetic improvement of grain yield. Namibian mutants (ShR10P12, ShR2P11, ShL2P7, BrR11P2 and NKR1P3), IITA genotype (98K-476-8) and South African genotype (Enchore) had high grain yield, indicating that these genotypes can be further studied for adaptability and stability and be considered as potential parents for future breeding and crop improvement. Positive correlations observed between grain yield and measured traits indicated a possibility for simultaneous selection and improvement of yield traits. Namibian mutants (ShR10P12, NKR1P3, ShR4P1 and ShL2P7), IITA genotype (98K-476-8) and South African genotype (Enchore) were associated with GY, PWP, NSPPx and NPP. The clustered heat map also identified a group of Namibian, IITA and South African genotypes (Glenda, Dr Saunders, Enchore, 98K-476-8, NKR1P3, BrR11P2, ShL2P7, Oloyin, ShR2P11 and ShR10P12) which were associated with GY, PWP, NSPPx, NPP, SWP and HI. These identified superior cowpea mutants could be used as potential parents to diversify the current narrow germplasm collection and for the development of cowpea genotypes with high grain yield.

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CHAPTER 4

VARIATION IN SEED PROTEIN, SELECTED MINERALS, PHYTIC ACID AND POTENTIAL MINERAL BIOAVAILABILITY OF COWPEA MUTANTS AND NORMAL GENOTYPES

4.1 Abstract

Cowpea is an important legume crop in African countries and has the potential of eradicating hunger and malnutrition due to its high protein content and other essential mineral elements. The objectives of this study were to evaluate the variability of cowpea mutants and normal genotypes for protein content, selected mineral elements, phytic acid, and the potential bioavailability of Fe and Zn, to identify superior cowpea mutants and normal genotypes, and to determine the interrelationship between all measured characteristics. Thirty-one cowpea genotypes (16 Namibian mutants, seven IITA genotypes and eight South Africa genotypes) were planted at five different environments in South Africa during the 2021/2022 summer cropping season. Significant ($P \leq 0.05$) genotype and GE interaction effects were observed for protein, minerals and phytic acid concentration. High H^2 values (> 50%) were observed for almost all the traits except for boron (B) indicating high potential selection efficiency for these traits. Superior Namibian mutants (ShL3P7-2, ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 ShR2P11, BrR11P2, ShL2P7, ShR3P4 and NKRuP5), IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White, Enchore and Glenda) that combined high protein, Fe and Zn were identified. The IITA genotype, IT93K-452-1 and South African genotypes Oloyin and Orelu had a potential of good Fe bioavailability while Zn had a potential of poor bioavailability in all mutants and genotypes. Significant and positive correlations were observed between most of the traits. Namibian mutants (ShR10P12, ShR3P4, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18 and IT99K-573-2-1), and South African genotypes (Agrinawa, Bechuana White and Dr Saunders) were associated with high values for protein, Mn, phytic acid, molar ratios of phytic acid with Fe and Zn, and ash. Namibian mutants (ShL3P7-2, ShR2P11, ShR10P10, NKL9P7 and ShL2P7), IITA genotypes (IT93K-452-1 and 98K-476-8), and South African genotypes (Orelu, Oloyin, Pan 311 and Glenda) were associated with high values for phytic acid, Fe, Zn, Cu, B and moisture. These genotypes could be a good source of the essential minerals and should be further studied for adaptability in South African growing conditions.

Keywords: cowpea mutants, phytic acid, protein content, mineral concentration, variability, bioavailability

4.2 Introduction

Protein and micronutrient deficiencies cause food insecurity and malnutrition among the poor populations in developing countries. About 232 million people in Africa suffer from protein and mineral element deficiency such as Fe and Zn, a problem that can be solved by the capability of legumes to accumulate micronutrients for enhancement of human diets (Dakora and Belane 2019). Therefore, the development of improved cowpea cultivars that contain improved protein content with high mineral content, potential of good mineral bioavailability and in combination with good grain yield is crucial for assuring food, nutritional, health and income security for various countries.

Cowpea is a warm-season legume crop that many people, particularly in developing countries, rely on for calories and protein (Herniter et al. 2020). Cowpea grain comprises of a high protein content (23 to 32%), moderate quantity of lysine, minerals (Zn, Fe, Mn, B and Cu), vitamins A and B, amino acids (isoleucine and leucine), carbohydrates (< 60%) and anti-nutritional factors (tannin and phytic acid) (Elhardallou et al. 2015; Baptista et al. 2017). The crop contains anti-nutritional agents that not only make the grain less vulnerable to insect attack but also make it harder for both animals and humans to digest and absorb nutrients (Maia et al. 2000; Parmar et al. 2017; Simion 2018).

Two common examples of significant dietary elements in legumes that are inhibited by phytic acid are Fe and Zn (Sandberg 2002). Phytic acid, also known as phytate, is regarded an anti-nutritional factor, as it reduces cowpea mineral bioavailability and it also has antioxidant properties with significant health benefits such as prevention of cancer cell formation (Carbas et al. 2020). The potential bioavailability of some minerals in legume crops such as cowpea, soybean and lentils can be determined by the molar ratio of phytic acid to the minerals contained in the diet; for example, a phytic acid to Zn molar ratio greater than 15:1 implies limited mineral bioavailability (Akande et al. 2010). To minimize phytic acid concentration in cowpea seeds and provide nutrient-dense food products, techniques such as seed soaking and heating can be employed (Afiukwa et al. 2011). Additionally, the organic acids produced during fermentation may enhance the Fe uptake (Sandberg 2002). Genetic engineering and mutation breeding offer the possibility to completely eradicate anti-nutrients, but because these substances also have benefits, genetic modifications can be applied to make food crops more nutrient-dense (Welch and Graham 2004).

The availability and accessibility of intra-specific genetic diversity of species is crucial for breeding for sustainable crop development (Olasupo et al. 2018). The development of improved genotypes with superior properties such as increased stress tolerance and good nutrient content, is possible through mutation breeding and other biotechnological techniques (Chaudhary et al. 2019). Mutagenesis is one of the popular techniques for increasing genetic diversity for crop development. Gamma-ray mutation has been employed to improve the genetic diversity of cowpea in Burkina Faso (Gnankambary et al. 2020) and Namibia (Horn et al. 2016).

Levels of diversity among cowpea genotypes can be determined through phenotypic characterisation and assessment of mineral elements and protein content (Stoilova and Pereira 2013). Several studies using morphological, agronomic and nutritional characteristics have evaluated diversity in cowpea populations in South Africa and IITA (Gerrano et al. 2015, 2017, 2019, 2020, 2022; Molosiwa et al. 2016; Lazaridi et al. 2017; Mbuma et al. 2020, 2021, 2022; Mofokeng et al. 2020). For years, the ARC in South Africa has been maintaining a large cowpea germplasm collection, with genotypes that are either locally bred, or derived from other breeding programmes. However, the available cowpea germplasm collection has a narrow genetic diversity. Therefore, the ARC in South Africa sourced cowpea mutants from Namibia with the aim of introducing new diversity to the germplasm collection. Hence, the objectives of this study were to evaluate the variability of cowpea mutants and normal genotypes for protein content, selected mineral elements, phytic acid and the potential bioavailability of Fe and Zn, to identify superior cowpea mutants and normal genotypes and to determine the interrelationship between all measured characteristics.

4.3 Materials and methods

4.3.1 Plant material and experimental sites

Plant material and experimental sites are in accordance with Chapter 3 in section 3.3.1.

4.3.2 Trial design and management

Trial design and management are in accordance with Chapter 3 in section 3.3.2.

4.3.3 Data collection and analysis

4.3.3.1 Protein, ash and moisture contents

Dry seeds were harvested from each genotype from the five environments. The seeds were used to determine the crude protein (%), total ash (%) and moisture (%) contents using near-infrared spectroscopy (NIR) (Model DA 7250, Perten, Instruments AB, Sweden) explained by Weng et al. (2017). The NIR plate was filled to level with seeds and then scanned on NIR per genotype. The analyses of every genotype was repeated three times and the averages were recorded.

4.3.3.2 Mineral composition

A 1KA, A10 Yellowline (Merck Chemicals Pty Ltd) mini flour industrial grinder was used to finely grind the dry seeds and the flour was sieved through a 0.5 mm sieve. A sample of 0.5 g of flour was dry ashed in a muffle furnace for five hours at 550°C before the flour of each sample could be used to extract Fe, Zn, Mn, Cu, and B using 1N HCl. The Optical Emission Spectroscopy (IRIS/AP HR DUO Themo Electron Corporation) was used to measure the mineral concentrations in the aqueous extracts explained by Ataro et al. (2008). The mineral elements were expressed as ppm and reported as mg kg^{-1} which is the same as ppm.

4.3.3.3 Phytic acid analysis and molar ratios of phytic acid to iron and zinc

Finely ground cowpea flour sample (0.25 g) was transferred into 14 ml plastic tubes. The phytic acid was extracted by adding 5% trichloroacetic acid and mechanically shaking the samples on a belly dancer. After centrifuging at 12 000 g for 10 minutes, the absorbance of the sample extracts was read at 500 nm (JENWAY, Spectrophotometer 7315) described by Dragiĉević et al. (2011). Phytic acid standard solutions were made using phytic acid dodecasodium salt from rice. A standard curve was then drawn by measuring different concentrations of phytic acid. The phytic acid was calculated as follows:

$$Phytic\ acid\ (\mathrm{mg}\ kg^{-1}) = (\Delta A - a)/b$$

Where, a = Constant from slope, ΔA = Change in absorbance, b = Slope of the standard curve.

The potential mineral bioavailability was determined using molar ratios for phytate concentration/mineral concentration (Fe/Zn) as described by Queiroz et al. (2011). Only the Fe and Zn potential bioavailability were studied because of their deficiency in the diets of many people and the detrimental effects of these deficiencies. The following molar ratios (MR) were determined to estimate to what extent phytic acid inhibited the potential bioavailability of Fe and Zn:

$$\mathrm{MR} = \frac{\{Phytic\ acid\ conc\ (mg\ kg^{-1})/MW\ phytic\ acid\ (Da)\}}{\{Mineral\ conc\ (mg\ kg^{-1})/AW\ mineral\ (Da)\}}$$

Where, conc = Concentration, MW = Molecular weight (660 Da), AW mineral = Atomic weight of Zn (65 Da) or Fe (56 Da), mineral conc = Zinc or Fe concentration (mg kg^{-1}) in the sample, PA:Fe = molar ratio of phytic acid with iron, PA:Zn = molar ratio of phytic acid with zinc.

4.3.4 Statistical analysis

Data collected for protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash content were subjected to ANOVA using GenStat 22nd version (VSN International 2022). Least significant difference ($P \leq 0.05$) was used to separate means. Because their genetic backgrounds are distinct from those of any other germplasm collection, the Namibian mutants, IITA, and South African genotypes were considered to be a fixed variable. The five environments were demeed to be a random variable because they are a representative sample of all potential environments that could represent the conditions for growing cowpeas in South Africa. The variance components and H^2 were calculated as mentioned in Chapter 3 in section 3.3.4.

PCA was done to determine association between protein, mineral elements, phytic acid, potential mineral bioavailability, moisture and ash content using GenStat 22nd version (VSN International 2022). PCA and clustered heat maps were done using XLSTAT software version 2022.3.2 (Addinsoft 2022).

4.4 Results

4.4.1 ANOVA for protein, selected minerals, phytic acid, potential mineral bioavailability, moisture and ash of cowpea mutants and normal genotypes

In Bloemfontein, the genotype (G) effect was highly significant ($P \leq 0.001$) for Zn, Mn and ash, significant ($P \leq 0.01$) for Cu, and significant ($P \leq 0.05$) for phytic acid and PA:Zn (Table 4.1). High H^2 values (> 50%) were observed for Mn (51.80%), ash (57.53%) and Zn (59.83%). Low H^2 values (< 50%) were observed for PA:Fe (7.27%), moisture (10.67%), Fe (15.68%), protein (23.03%), B (25.06%), PA:Zn (36.92%), phytic acid (38.11%) and Cu (40.78%). In

Potchefstroom, the G effect was highly significant ($P \leq 0.001$) for Mn, phytic acid, and ash, significant ($P \leq 0.01$) for Zn, and significant ($P \leq 0.05$) for Fe and PA:Zn. High H^2 values (> 50%) for Mn (51.23%), phytic acid (54.44%) and ash (68.35%). Low H^2 values (< 50%) were observed for Cu (0.00%), B (0.00%), moisture (17.10%), PA:Fe (19.14%), protein (22.64%), PA:Zn (36.88%), Fe (37.60%) and Zn (44.01%).

In Taung, the G effect was highly significant ($P \leq 0.001$) for Zn, Cu, phytic acid and ash, significant ($P \leq 0.01$) for protein, Mn and PA:Zn, and significant ($P \leq 0.05$) for moisture. High H^2 values (> 50%) were observed for phytic acid (56.27%), Zn (60.04%), Cu (70.74%) and ash (85.47%). Low H^2 values (< 50%) were observed for B (4.89%), Fe (8.99%), PA:Fe (18.93%), moisture (28.96%), PA:Zn (46.22%), protein (48.63%) and Mn (49.51%). In Mafikeng, the G effect was highly significant ($P \leq 0.001$) for Zn, Cu and ash, significant ($P \leq 0.01$) for protein and Mn, and significant ($P \leq 0.05$) for Fe and PA:Zn. High H^2 values (> 50%) were observed for Zn (52.83%), Cu (59.15%) and ash (78.54%). Low H^2 values (< 50%) were observed for PA:Fe (19.14%), moisture (23.62%), phytic acid (25.00%), B (25.80%), Fe (29.46%), PA:Zn (36.88%), Mn (41.21%) and protein (44.17%).

In Polokwane, the G effect was highly significant ($P \leq 0.001$) for protein and phytic acid, and significant ($P \leq 0.01$) for ash, moisture, Mn and PA:Zn. High H^2 values (> 50%) were observed for ash (52.86%), moisture (53.85%), phytic acid (59.37%) and protein (67.23%). Low H^2 values (< 50%) were observed for Fe (0.00%), B (0.00%), Cu (8.83%), Zn (16.21%), PA:Fe (18.93%), PA:Zn (46.22%) and Mn (49.83%).

4.4.2 Combined ANOVA for protein, selected minerals, phytic acid, potential mineral bioavailability, moisture and ash of cowpea mutants and normal genotypes

The G effect was highly significant ($P \leq 0.001$) for protein, Zn, Cu, Mn, phytic acid, PA:Fe, PA:Zn, Ash, and moisture, and significant ($P \leq 0.05$) for Fe (Table 4.2). The GE interaction was highly significant ($P \leq 0.001$) for phytic acid, significant ($P \leq 0.01$) for ash and significant ($P \leq 0.05$) for protein and Cu. The H^2 values were high (> 50%) for almost all the traits except for B concentration which had a H^2 value of less than 50%.

4.4.3 Combined ANOVA for cowpea groupings of genotypes for all traits

The effect of groupings (Gr) were highly significant ($P \leq 0.001$) for protein, Mn and ash, significant ($P \leq 0.01$) for Zn and moisture, and significant ($P \leq 0.05$) for phytic acid and PA:Zn (Table 4.3). The E effect was highly significant ($P \leq 0.001$) for almost all the traits besides for ash which was significant at $P \leq 0.01$. The grouping by environment (Gr.E) interaction was non-significant ($P > 0.05$) for all the traits.

Table 4.1 Analysis of variance showing mean square values and broad-sense heritability (H^2) for protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash of cowpeas at five environments

Source	Df	Protein	Fe	Zn	Cu	Mn	B	Phytic acid	PA:Fe	PA:Zn	Moisture	Ash
Bloemfontein												
R	1	6.23	5.52	4.94	1.78	4.13	281.03	1914692.00	7.25	6.46	0.02	0.03
G	30	3.45	25.16	9.94***	1.47**	3.42***	4.91	1018106.00*	2.88	8.38*	0.20	0.08***
Error	30	2.16	18.34	2.50	0.62	1.09	2.94	456192.00	2.49	3.86	0.16	0.02
σ^2_G		0.65	3.41	3.72	0.43	1.17	0.98	280957.00	0.20	2.26	0.02	0.03
σ^2_P		2.81	21.75	6.22	1.05	2.26	3.92	737149.00	2.69	6.12	0.18	0.05
H^2 (%)		23.03	15.68	59.83	40.78	51.80	25.06	38.11	7.27	36.92	10.67	57.53
Mean		22.72	53.60	34.56	7.14	11.53	15.98	7363.24	11.71	21.17	13.40	4.00
Potchefstroom												
R	1	14.43	209.61	9.29	6.45	0.10	119.29	268730.00	4.66	0.07	0.29	0.00
G	30	2.03	35.28*	10.84**	1.60	1.99***	3.44	1131635.00***	2.49	7.77*	0.06	0.09***
Error	30	1.28	16.00	4.22	1.61	0.64	3.60	333829.00	1.69	3.58	0.04	0.02
σ^2_G		0.38	9.64	3.31	0.00	0.68	0.00	398903.00	0.40	2.09	0.01	0.04
σ^2_P		1.66	25.64	7.53	1.61	1.32	3.52	732732.00	2.09	5.68	0.05	0.06
H^2 (%)		22.64	37.60	44.01	0.00	51.23	0.00	54.44	19.14	36.88	17.10	68.35
Mean		21.56	48.68	35.53	6.79	11.54	17.55	6118.89	10.71	17.12	11.80	4.18
Taung												
R	1	1.54	1408.39	204.13	25.17	39.52	69.20	2771746.00	11.75	4.32	4.73	0.63
G	30	1.59**	76.72	21.56***	3.44***	6.35**	17.60	639312.00***	2.96	6.70**	0.23*	0.14***
Error	30	0.55	64.06	5.38	0.59	2.15	15.96	178925.00	2.02	2.46	0.13	0.01
σ^2_G		0.52	6.33	8.09	1.43	2.10	0.82	230193.50	0.47	2.12	0.05	0.06
σ^2_P		1.07	70.39	13.47	2.02	4.25	16.78	409118.50	2.49	4.58	0.18	0.07
H^2 (%)		48.63	8.99	60.04	70.74	49.51	4.89	56.27	18.93	46.22	28.96	85.47
Mean		22.66	61.73	39.78	7.98	14.43	20.60	6655.10	2.02	16.71	12.21	4.35
Mafikeng												
R	1	2.65	143.08	175.45	0.28	2.68	16.60	137577.00	4.66	0.07	0.01	0.00
G	29	2.86**	35.46*	15.08***	1.21***	2.47**	3.83	1138350.00	2.49	7.77*	0.12	0.14***
Error	29	1.11	19.32	4.66	0.31	1.03	2.26	683002.00	1.69	3.58	0.07	0.02
σ^2_G		0.88	8.07	5.21	0.45	0.72	0.79	227674.00	0.40	2.09	0.02	0.06
σ^2_P		1.98	27.39	9.87	0.76	1.75	3.04	910676.00	2.09	5.68	0.10	0.08
H^2 (%)		44.17	29.46	52.83	59.15	41.21	25.80	25.00	19.14	36.88	23.62	78.54
Mean		21.95	42.70	28.53	5.25	10.93	15.00	6194.85	10.71	17.12	12.62	4.02
Polokwane												
R	1	1.86	394.65	203.20	6.65	0.96	10.14	1201498.00	11.75	4.32	0.12	0.04
G	25	4.82***	37.96	26.13	1.61	1.77**	1.15	676997.00***	2.96	6.70**	0.17**	0.06**
Error	25	0.94	46.60	18.84	1.35	0.59	2.03	172576.00	2.02	2.46	0.05	0.02
σ^2_G		1.94	0.00	3.65	0.13	0.59	0.00	252210.50	0.47	2.12	0.06	0.02
σ^2_P		2.88	42.28	22.49	1.48	1.18	1.59	424786.50	2.49	4.58	0.11	0.04
H^2 (%)		67.23	0.00	16.21	8.83	49.83	0.00	59.37	18.93	46.22	53.85	52.86
Mean		19.66	36.90	24.42	5.98	9.84	13.50	5611.87	9.33	16.71	12.95	3.88

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, R = Replication, G = Genotype, σ^2_G = Genotypic variance, σ^2_P = Phenotypic variance, Df = Degrees of freedom, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

Table 4.2 Analysis of variance showing mean square values and broad-sense heritability (H^2) for protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash of cowpeas across five environments

Source	Df	Protein	Fe	Zn	Cu	Mn	B	Phytic acid	PA:Fe	PA:Zn	Moisture	Ash
R	1	6.07	90.61	21.54	28.80	4.72	12.15	456527.00	3.49	10.81	0.43	0.13
G	30	7.12***	73.43*	41.80***	4.35***	10.08***	8.23	5865911.00***	12.36***	31.15***	0.33***	0.40***
E	4	96.92***	5570.33***	2245.86***	68.70***	185.78***	456.43***	59419469.00***	140.59***	564.17***	24.15***	2.16***
GE	114	1.80*	34.95	9.89	1.24*	1.52	5.96	2467028.00***	2.47	6.96	0.11	0.03**
Error	148	1.32	45.15	10.18	0.94	1.37	8.61	381971.00	3.59	10.58	0.12	0.02
σ^2_G		0.53	3.85	3.19	0.31	0.86	0.23	339888.30	0.99	2.42	0.02	0.04
σ^2_{GE}		0.24	0.00	0.00	0.15	0.07	0.00	1042528.50	0.00	0.00	0.00	0.01
σ^2_P		0.71	7.34	4.18	0.44	1.01	0.82	586591.10	1.24	3.12	0.03	0.04
H^2 (%)		74.71	52.40	76.34	71.40	84.97	27.60	57.94	80.00	77.66	66.47	92.15

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, R = Replication, G = Genotype, E = Environment, GE = Genotype by environment interaction, σ^2_G = Genotypic variance, σ^2_P = Phenotypic variance, Df = degrees of freedom, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

Table 4.3 Analysis of variance showing mean square values for protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash of three cowpea groups based on their origins

Origin	Df	Protein	Fe	Zn	Cu	Mn	B	Phytic acid	PA:Fe	PA:Zn	Moisture	Ash
R	1	6.01	89.37	21.24	28.91	4.78	12.36	456527.00	3.42	10.59	0.43	0.13
Gr	2	14.49***	105.19	82.43**	3.55	22.36***	0.67	6774882.00*	8.09	37.18*	0.69**	0.63***
E	4	94.57***	5814.61***	2339.45***	68.81***	180.93***	456.10***	59419469.00***	148.29***	590.46***	24.14***	2.00**
Gr.E	8	0.88	28.97	13.23	1.78	2.28	1.92	2644219.00	2.16	9.15	0.16	0.03
Error	282	2.04	43.83	12.83	1.38	2.16	7.71	1687553.00	4.05	11.14	0.13	0.06
Mean		21.72	48.67	32.52	6.62	11.65	16.53	6167.79	11.50	20.11	12.59	4.09

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, R = Replication, Gr = Grouping, E = Environment, Gr.E = Grouping by environment interaction, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

4.4.4 Cowpea mutants and normal genotype performance for all characteristics across environments

Protein for seeds ranged from 19.57 to 23.56% (Table 4.4). Namibian mutants (ShL3P7-2, ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 and NKRuP5), IITA genotypes (IT82E-18, IT99K-573-2-1 and ITOOK 1263), South African genotype (Oloyin) had mean values above the average mean for protein (21.71%) (Figure 4.1A). Fe ranged from 43.70 to 55.25 mg kg^{-1}. Namibian mutants (ShL3P7-2, ShR2P11, BrR11P2, NKR8P9, NKR9P9 and ShL2P7), IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1 and ITOOK 1263), and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White and Enchore) had mean values above the average mean for Fe (48.81 mg kg^{-1}) (Figure 4.1B). Zn ranged 28.85 to 37.90 mg kg^{-1}. Namibian mutants (ShL3P7-2, ShR2P11, ShR3P4, BrR11P2, NKR8P9, NKR9P9 and ShL2P7), IITA genotypes (IT82E-18, IT93K-452-1 and ITOOK 1263), and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White, Enchore and Glenda) had mean values above the average mean for Zn (32.60 mg kg^{-1}) (Figure 4.1C). Cu ranged from 5.45 to 8.35 mg kg^{-1}. Namibian mutants (ShL3P7-2, ShR10P10, ShR2P11, ShR3P4, ShR4P1, BrR11P2, BrR4P11, NKL9P7, NKR9P9 and ShL2P7), IITA genotype (IT93K-452-1) and South African genotypes (Oloyin, Orelu, Pan 311 and Bechuana White) had mean values above the average mean for Cu (6.63 mg kg^{-1}) (Figure 4.1D).

Mn ranged from 8.55 to 13.75 mg kg^{-1} (Table 4.4). Namibian mutants (ShR2P11, ShR3P4, ShR4P1, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and ShL2P7), IITA genotypes (IT07K-292-10, IT82E-18 and ITOOK 1263), and South African genotypes (Oloyin, Agrinawa, Bechuana White, Dr Saunders and Enchore) had mean values above the average mean for Mn (11.63 mg kg^{-1}) (Figure 4.2A). B ranged from 14.85 to 19.00 mg kg^{-1}. Namibian mutants (ShL3P7-2, ShR2P11, ShR3P4, BrR11P11, BrR11P2, BrR4P11, NKR8P9, NKR9P9 and NKRuP5), IITA genotypes (98K-476-8, IT82E-18, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Orelu, Pan 311, Agrinawa, Bechuana White and Enchore) had mean values above the average mean for B (16.53 mg kg^{-1}) (Figure 4.2B). Phytic acid ranged from 4417.64 to 7877.27 mg kg^{-1}. Namibian mutants (BrR4P11, NKL9P7, ShR3P4, ShR9P5, BrR11P2, NKR8P9, NKR9P9, NKRuP5 and ShL2P7), IITA genotypes (IT82E-18, IT07K-292-10, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Pan 311, Glenda, Bechuana White, Dr Saunders and Enchore) had mean values above the average mean for phytic acid (6167.79 mg kg^{-1}) (Figure 4.2C). Namibian mutants (ShL3P7-2, ShR10P10, ShR10P12, ShR2P11, ShR4P1, BrR11P11, NKR1P3, NKR8P9 and ShL2P7), IITA genotypes (98K-476-8, IT07K-318-33 and IT93K-452-1), and South African genotypes (Oloyin, Orelu and

Agrinawa) had mean values below the average mean for phytic acid (6167.79 mg kg^{-1}). Molar ratio of phytic acid to Fe ranged from 9.05 to 14.03. The PA:Fe was below 10 for IITA genotype (IT93K-452-1) and South African genotypes (Oloyin and Orelu) (Figure 4.2D). Molar ratio of phytic acid to Zn ranged from 16.33 to 24.05. The PA:Zn was above 15 for all the genotypes (Figure 4.3A).

Moisture ranged from 12.12 to 12.94% (Table 4.4). Namibian mutants (ShR2P11, ShR3P4, ShR4P1, BrR4P11, NKL9P7 and ShL2P7), IITA genotypes (98K-476-8, IT07K-292-10, IT07K-318-33, IT93K-452-1 and IT99K-573-2-1), and South African genotypes (Oloyin, Orelu, Pan 311, Dr Saunders and Glenda) had mean values above the average mean for moisture (12.59%) (Figure 4.3B). Ash ranged from 3.54 to 4.37%. Namibian mutants (ShR10P10, ShR3P4, ShR4P1, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKL9P7, NKR8P9, NKR9P9 and NKRuP5), IITA genotypes (IT07K-318-33, IT82E-18 and ITOOK 1263), and South African genotypes (Pan 311, Bechuana White and Enchore) had mean values above the average mean for ash (4.09%) (Figure 4.3C).

4.4.5 Cowpea group performance for all characteristics across environments

All the groupings were significantly different ($P \leq 0.01$) for protein, Fe, Zn, Mn, ash and moisture (Table 4.5). The groupings were not significantly different for B and PA:Zn. IITA and South African groups were not significantly different for Cu, PA:Fe and phytic acid. Namibian mutants had high mean values for protein (21.93%), ash (4.15%), Cu (6.72 mg kg^{-1}) and PA:Zn (20.42) compared to the IITA and South African genotypes. The IITA genotypes had high mean values for moisture (12.72%), phytic acid (6398.60 mg kg^{-1}) and PA:Fe ratio (11.77) compared to the Namibian mutants and South African genotypes. South African genotypes had high mean values for Fe (49.95 mg kg^{-1}), Zn (33.54 mg kg^{-1}) and Mn (12.18 mg kg^{-1}) compared to the Namibian mutants and IITA genotypes.

South African genotypes had low mean values for protein content (21.20%), ash content (4.00%), PA:Fe molar ratio (11.14) and PA:Zn molar ratio (19.28) compared to the Namibian mutants and IITA genotypes. Namibian mutants had low mean values for moisture (12.55%), Fe (47.97 mg kg^{-1}), Zn (31.85 mg kg^{-1}) and phytic acid (5965.59 mg kg^{-1}) compared the IITA and South African genotypes. The IITA genotypes had lower mean values for Cu (6.35 mg kg^{-1}), Mn (11.09 mg kg^{-1}) and B (16.41 mg kg^{-1}) compared to the Namibian mutants and South African genotypes.

Table 4.4 Mean values of protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash of each of 31 genotypes across five environments

Genotypes	Protein (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Phytic acid (mg kg^{-1})	PA:Fe	PA:Zn	Moisture (%)	Ash (%)
ShL3P7-2	21.74	54.31	33.92	6.78	10.96	17.68	5121.09	10.23	19.08	12.53	3.73
ShR10P10	21.48	48.56	32.42	6.90	10.59	15.62	4999.27	11.05	19.37	12.42	4.17
ShR10P12	21.27	47.50	30.10	6.55	10.70	16.40	5883.64	10.78	19.67	12.33	4.01
ShR2P11	20.53	49.65	33.35	6.85	11.85	16.80	6051.82	10.89	18.13	12.72	4.04
ShR3P4	22.60	47.85	32.70	6.70	13.00	16.55	6659.09	12.16	20.74	12.84	4.24
ShR4P1	22.35	47.20	32.60	6.90	12.15	14.85	5905.36	10.90	18.44	12.74	4.18
ShR9P5	20.79	45.50	29.25	5.70	11.35	15.15	6604.55	12.68	23.18	12.56	4.17
BrR11P11	23.56	46.75	30.55	6.00	11.00	17.60	6001.82	10.98	19.96	12.58	4.29
BrR11P2	21.59	49.15	33.65	7.50	12.60	17.50	6636.36	12.16	20.09	12.51	4.21
BrR4P11	22.04	46.00	31.25	7.35	11.80	19.00	6375.91	12.11	21.47	12.65	4.29
NKL9P7	22.87	47.90	31.80	8.00	11.40	15.90	6287.27	11.49	20.58	12.61	4.23
NKR1P3	21.28	46.75	29.25	6.00	11.90	16.05	5805.45	10.85	20.16	12.59	4.06
NKR8P9	22.51	50.56	34.48	6.33	11.46	16.98	4417.64	11.98	20.90	12.50	4.26
NKR9P9	22.66	49.05	33.15	6.75	12.20	16.65	6564.55	11.61	20.94	12.43	4.22
NKRuP5	22.15	43.70	30.45	6.15	10.95	16.65	6760.00	13.39	22.90	12.16	4.18
ShL2P7	21.54	51.43	33.42	7.22	11.65	15.43	5375.64	11.32	20.16	12.62	4.00
98K-476-8	21.22	49.24	31.99	6.22	10.52	16.80	4760.18	10.09	18.33	12.78	3.83
IT07K-292-10	21.47	48.45	31.80	5.75	11.90	15.90	6387.27	11.32	20.38	12.79	3.83
IT07K-318-33	21.23	44.55	28.85	6.05	8.55	14.85	5998.18	11.61	21.67	12.70	4.10
IT82E-18	22.67	49.50	33.30	6.45	12.35	16.80	7835.45	14.03	24.05	12.59	4.29
IT93K-452-1	20.98	52.45	34.40	7.20	11.00	16.45	5715.45	9.68	17.16	12.70	4.02
IT99K-573-2-1	22.01	45.45	31.75	6.45	10.95	17.30	6216.36	11.64	19.74	12.94	3.96
ITOOK 1263	22.98	52.10	37.90	6.35	12.20	16.85	7877.27	13.57	20.86	12.51	4.37
Oloyin	22.50	55.25	36.45	7.25	12.75	15.65	5872.73	9.05	16.33	12.76	3.54
Orelu	21.47	51.10	34.50	8.35	10.85	16.60	5664.73	9.57	16.66	12.66	3.80
Pan 311	21.69	48.90	33.75	7.05	11.55	16.95	6527.27	11.57	19.93	12.70	4.27
Agrinawa	19.57	46.50	30.55	5.45	13.75	17.35	6140.91	11.45	20.42	12.38	4.05
Bechuana White	20.55	50.15	32.65	6.70	13.70	17.15	6937.27	11.94	21.65	12.12	4.13
Dr Saunders	21.39	47.50	31.95	6.20	11.80	15.70	6640.91	12.36	20.93	12.67	3.79
Enchore	21.20	51.90	34.25	6.05	11.70	17.20	6974.55	11.83	20.31	12.59	4.29
Glenda	21.26	48.30	34.25	6.25	11.35	16.05	6203.64	11.33	18.09	12.65	4.09
LSD (P = 0.05)	1.02	5.94	2.82	0.86	1.04	2.59	546.02	1.67	2.87	0.31	0.13
Min mean	19.57	43.70	28.85	5.45	8.55	14.85	4417.64	9.05	16.33	12.12	3.54
Max mean	23.56	55.25	37.90	8.35	13.75	19.00	7877.27	14.03	24.05	12.94	4.37
Grand mean	21.71	48.81	32.60	6.63	11.63	16.53	6167.79	10.00	15.00	12.59	4.09
CV%	5.30	13.80	9.80	14.60	10.10	17.70	10.00	16.50	16.20	2.80	3.50

LSD = Least significant difference, CV = Coefficient of variation, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

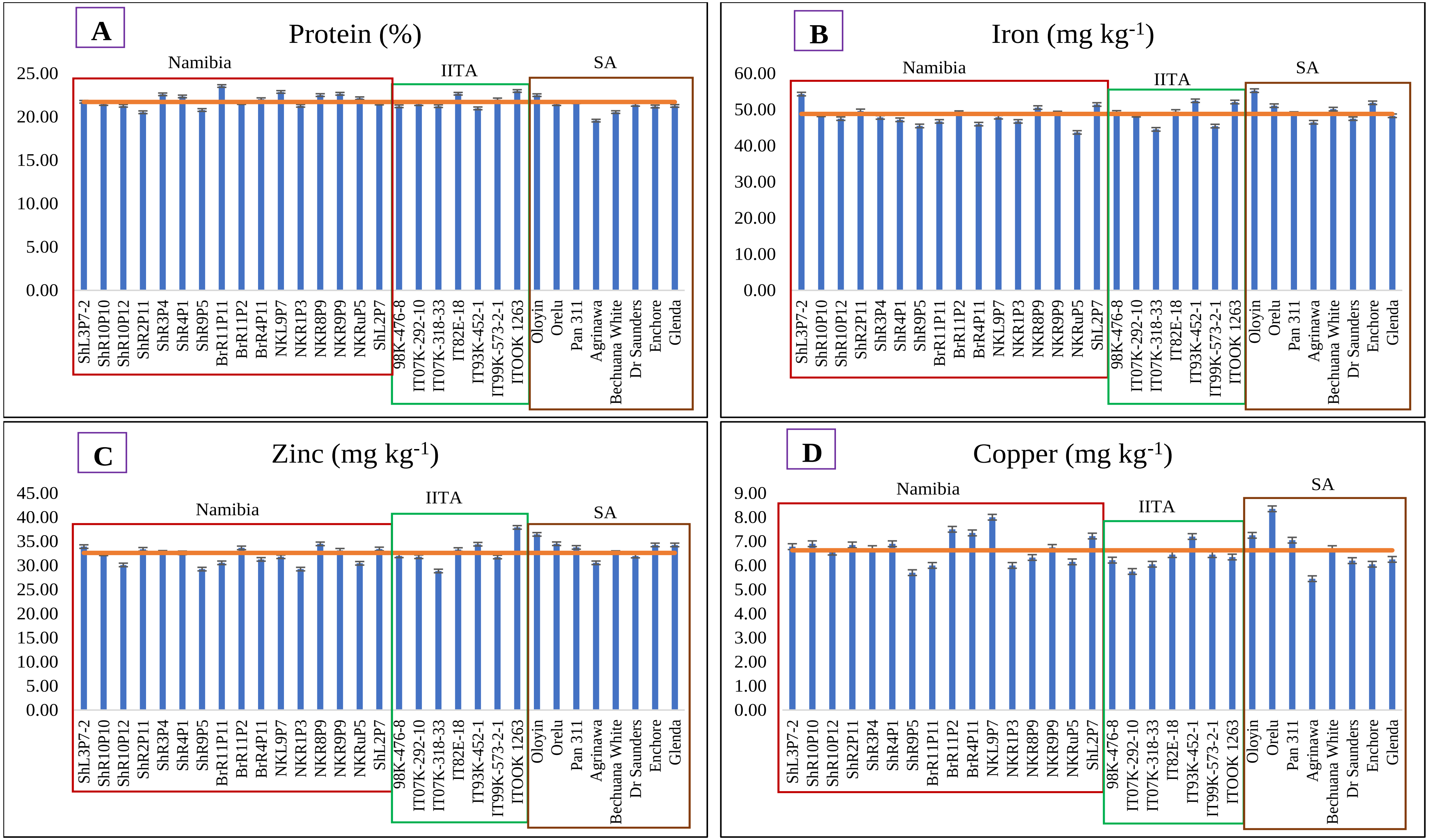

Figure 4.1 Mean value performance of Namibian mutants, IITA (International Institute of Tropical Agriculture) and SA (South Africa) genotypes for protein (A), iron (B), zinc (C) and copper (D). Blue bars = Traits, Orange line = Average mean

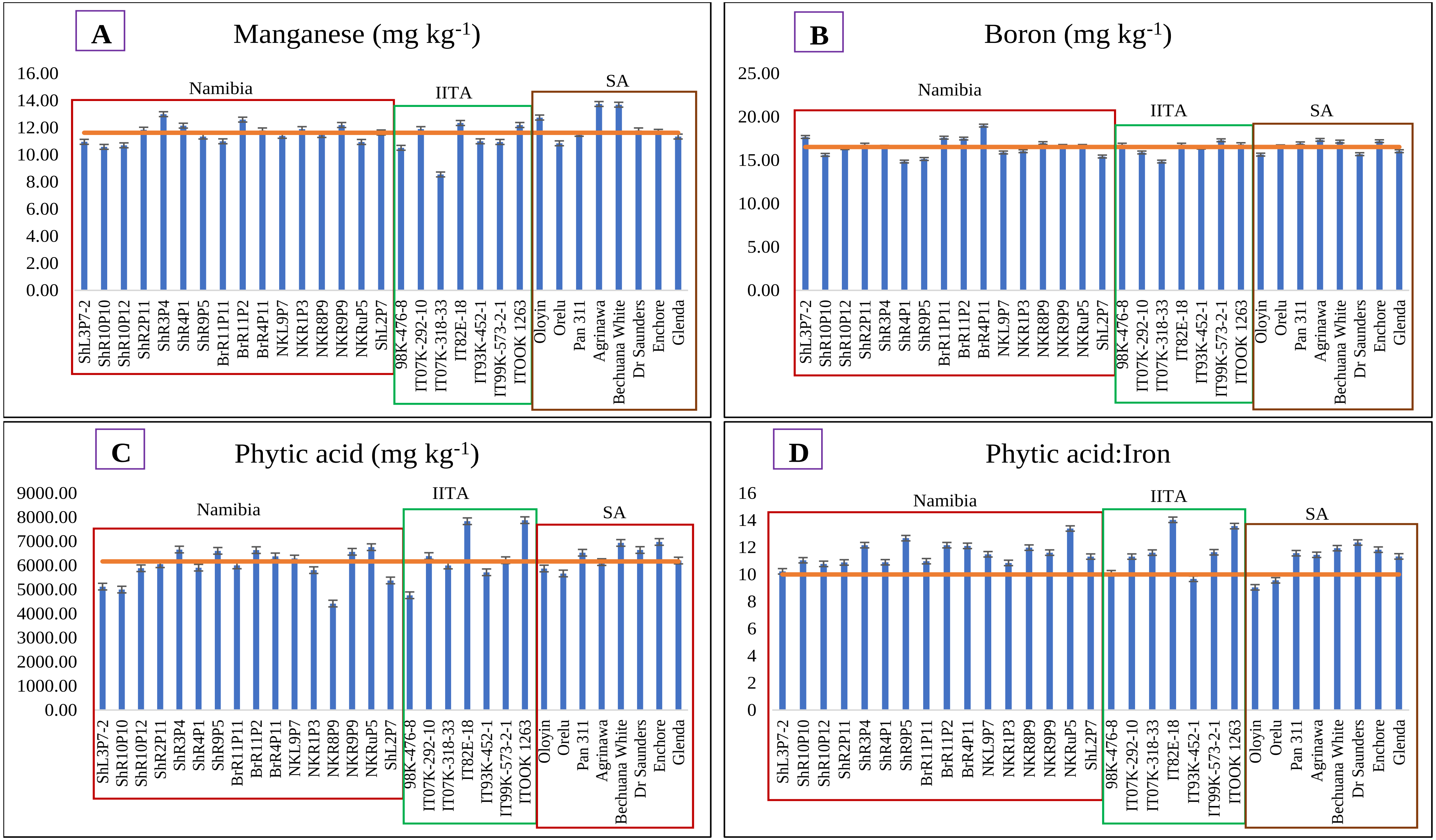

Figure 4.2 Mean value performance of Namibian mutants, IITA (International Institute of Tropical Agriculture) and SA (South Africa) genotypes for manganese (A), boron (B), phytic acid (C) and phytic acid:iron (D). Blue bars = Traits, Orange line = Average mean

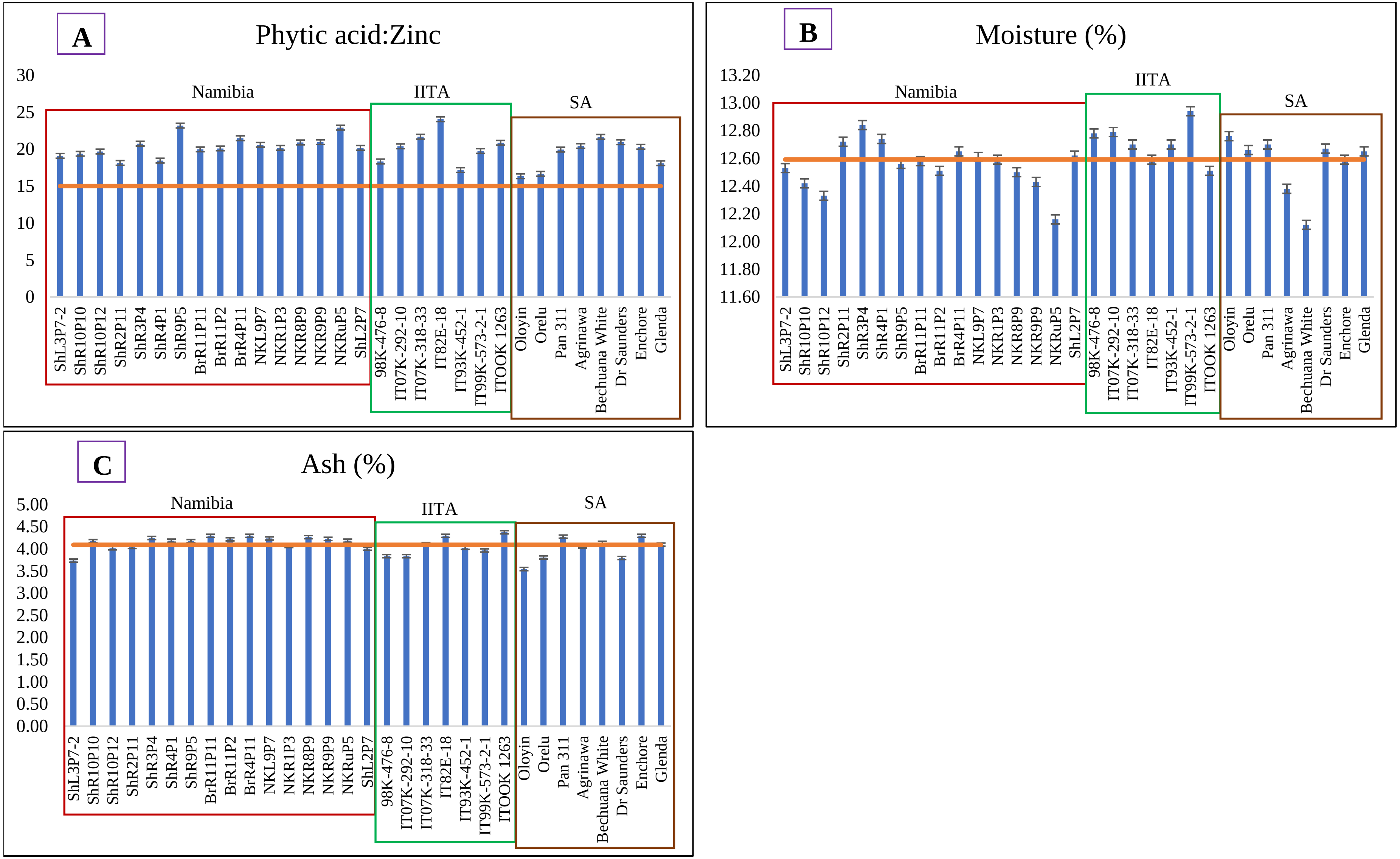

Figure 4.3 Mean value performance of Namibian mutants, IITA (International Institute of Tropical Agriculture) and SA (South Africa) genotypes for phytic acid:zinc (A), moisture (B) and ash (C). Blue bars = Traits, Orange line = Average mean

Table 4.5 Mean values of cowpea genotype groupings based on their origin

Origin	Protein	Fe	Zn	Cu	Mn	B	Phytic acid	PA:Fe	PA:Zn	Moisture	Ash
Namibia	21.93^{a}	47.97^{c}	31.85^{c}	6.72^{a}	11.62^{a}	16.56^{b}	5965.59^{c}	11.57^{b}	20.42^{a}	12.55^{c}	4.15^{a}
IITA	21.82^{b}	48.80^{b}	32.88^{b}	6.35^{c}	11.09^{c}	16.41^{c}	6398.60^{a}	11.77^{a}	20.36^{b}	12.72^{a}	4.07^{b}
SA	21.20^{c}	49.95^{a}	33.54^{a}	6.66^{b}	12.18^{b}	16.58^{a}	6370.25^{b}	11.14^{c}	19.28^{c}	12.57^{b}	4.00^{c}
LSD	0.40	1.87	1.01	0.33	0.42	0.78	366.37	0.57	0.94	0.10	0.07

At $P = 0.05$, means that are followed by the same letter of the alphabet are not significantly different, IITA = International Institute of Tropical Agriculture, LSD = Least significant difference, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

4.4.6 Phenotypic correlation

Highly significant ($P \leq 0.001$) and positive correlations were found for Fe with Zn ($r = 0.83$), phytic acid with PA:Fe ($r = 0.69$), PA:Fe with PA:Zn ($r = 0.88$) and ash ($r = 0.66$), PA:Zn with ash ($r = 0.57$). (Table 4.6, Figure 4.4). Significant ($P \leq 0.01$) and positive correlations were found for phytic acid with PA:Zn ($r = 0.52$). Significant ($P \leq 0.05$) and positive correlations were found for Fe with Cu ($r = 0.39$), Zn with Cu ($r = 0.42$), Mn with phytic acid ($r = 0.41$) and phytic acid with ash ($r = 0.42$). Significant ($P \leq 0.01$) and negative correlation was found for Fe with PA:Zn ($r = -0.51$). Significant ($P \leq 0.05$) and negative correlation was found for Fe with PA:Fe ($r = -0.41$), Zn with PA:Zn ($r = -0.43$), Cu with PA:Zn ($r = -0.43$), and PA:Zn with moisture ($r = -0.40$).

Table 4.6 Pearson correlations for protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash

Traits	Protein	Fe	Zn	Cu	Mn	B	Phytic acid	PA:Fe	PA:Zn	Ash
Fe	0.07									
Zn	0.27	0.83***								
Cu	0.24	0.39*	0.42*							
Mn	-0.06	0.24	0.30	0.00						
B	0.08	0.09	0.15	0.08	0.26					
Phytic acid	0.16	-0.15	0.10	-0.14	0.41*	0.12				
PA:Fe	0.21	-0.41*	-0.11	-0.35	0.20	0.13	0.69***			
PA:Zn	0.12	-0.51**	-0.43*	-0.43*	0.09	0.08	0.52**	0.88***		
Ash	0.30	-0.35	-0.08	-0.10	0.10	0.23	0.42*	0.66***	0.57***	
Moisture	0.16	0.08	0.14	0.13	-0.20	-0.17	-0.14	-0.31	-0.40*	-0.28

$^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, Zn = Zinc, Fe = Iron, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

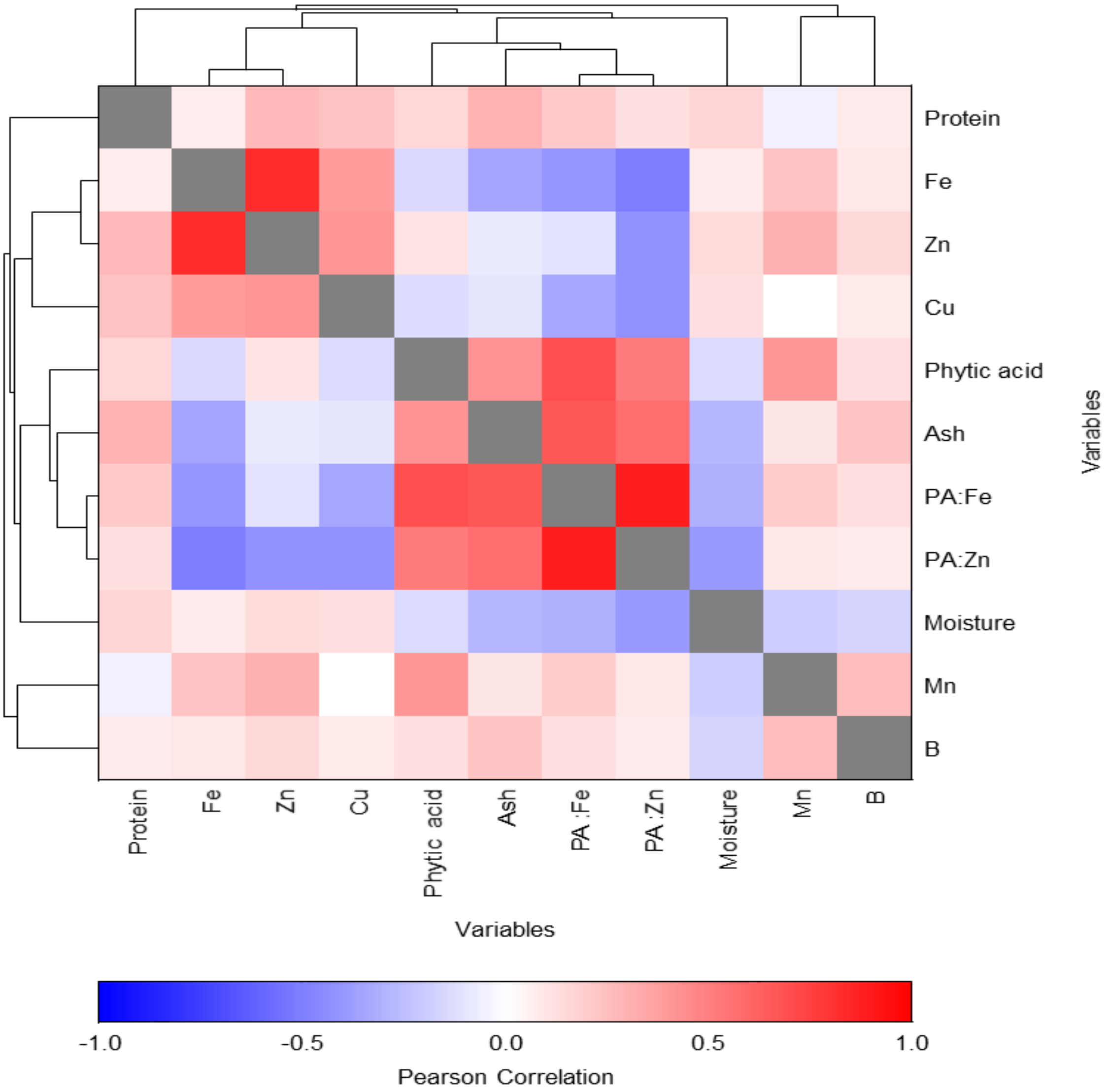

Figure 4.4 Heat map representing phenotypic correlation between measured traits for cowpea genotypes. Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

4.4.7 Principal component analysis (PCA)

The PCA revealed that the first four PCs contributed for 75.41% of the total variation and had eigenvalues of equal to or greater than one (Table 4.7). The PC1, PC2, PC3 and PC4 explained 33.39%, 20.71%, 12.27% and 9.05% of the total variation, respectively. Only PC1 and PC2 were interpreted because they contributed for most of the variation in the dataset. Ash, PA:Fe, PA:Zn and phytic acid all contributed the most to PC1, while Fe had large and negative impact on the PC1. Fe, Zn, Mn, B, and phytic acid contributed most to the variation in PC2 on the PC2.

The performance of genotypes and their association with the traits were visualised using the PCA biplot. The PC1 and PC2 explained 54.10% of the total variation (Figure 4.5). Namibian mutant (BrR11P2), IITA genotype (ITOOK 1263) and South African genotypes (Pan 311 and Enchore) were associated with protein, Mn and B. Namibian mutants (BrR4P11, NKR9P9 and ShR3P4), IITA genotype (IT82E-18) and South African genotype (Bechuana White) were associated with phytic acid, PA:Fe ratio and ash. Namibian mutant (NKRuP5) was associated with PA:Zn. Namibian mutants (ShR4P1, ShR2P11 and ShL2P7), IITA genotypes (IT07K-292-10, IT99K-573-2-1 and 98K-476-8) and South African genotype (Glenda) were associated with moisture. Namibian mutant (NKR8P9) was associated with high Cu, Fe and Zn. The PCA showed positive correlations between the Fe, Zn and Cu, and these characteristics were inversely associated with the PA:Zn ratio. Protein, Mn, B, phytic acid, PA:Fe, and ash content all had positive associations, but these characteristics had negative association with moisture content.

Table 4.7 Principal component analysis (PCA) of 11 traits in cowpea genotypes

Traits	PC1	PC2	PC3	PC4
Protein	0.05	0.29	0.65	0.07
Fe	-0.34	0.40	-0.15	-0.09
Zn	-0.23	0.53	0.03	-0.16
Cu	-0.26	0.27	0.25	0.32
Mn	0.08	0.40	-0.46	-0.24
B	0.07	0.30	-0.18	0.61
Phytic acid	0.33	0.31	-0.01	-0.40
PA:Fe	0.47	0.16	0.09	-0.14
PA:Zn	0.48	-0.01	0.03	-0.02
Ash	0.37	0.19	0.23	0.26
Moisture	-0.23	-0.07	0.43	-0.43
Eigenvalue	3.67	2.28	1.35	1.00
Variation (%)	33.39	20.71	12.27	9.05
Cumulative (%)	33.39	54.10	66.36	75.41

PC = Principal component, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

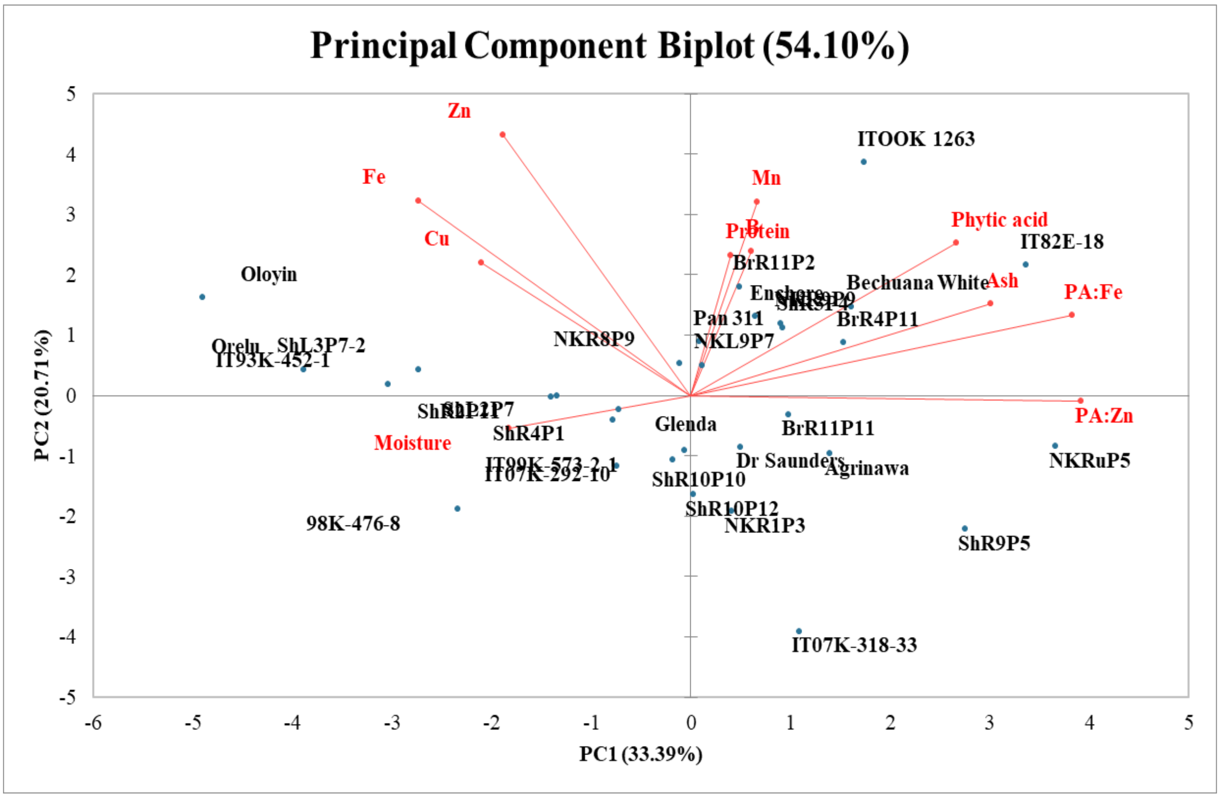

Figure 4.5 Principal component analysis (PCA) displaying genotypes with their associated traits. PC = Principal component, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

4.4.8 Clustered heat map for protein, mineral elements, phytic acid, potential mineral bioavailability, moisture and ash in cowpea mutant and normal genotypes

The clustering heat map distinguished two distinctive groups of traits (Figure 4.6). The first group is made up of protein, Mn, B, phytic acid, PA:Fe, PA:Zn and ash. The second group is made up of Fe, Zn and Cu. The heat map of clustering also differentiated between two distinct genotype groups, namely, 1) Namibian mutants (ShL3P7-2, ShR10P10, ShR2P11, ShR4P1, NKL9P7, NKR8P9 and ShL2P7), IITA genotypes (ITOOK 1263, IT93K-452-1 and 98K-476-8) and South African genotypes (Orelu, Oloyin, Pan 311, Enchore and Glenda), which were associated with high Fe, Zn, Cu, B, phytic acid and moisture, and 2) Namibian mutants (ShR10P12, ShR3P4, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18 and IT99K-573-2-1) and South African genotypes (Agrinawa, Bechuana White and Dr Saunders), which were associated with high protein, Mn, phytic acid, PA:Fe, PA:Zn and ash.

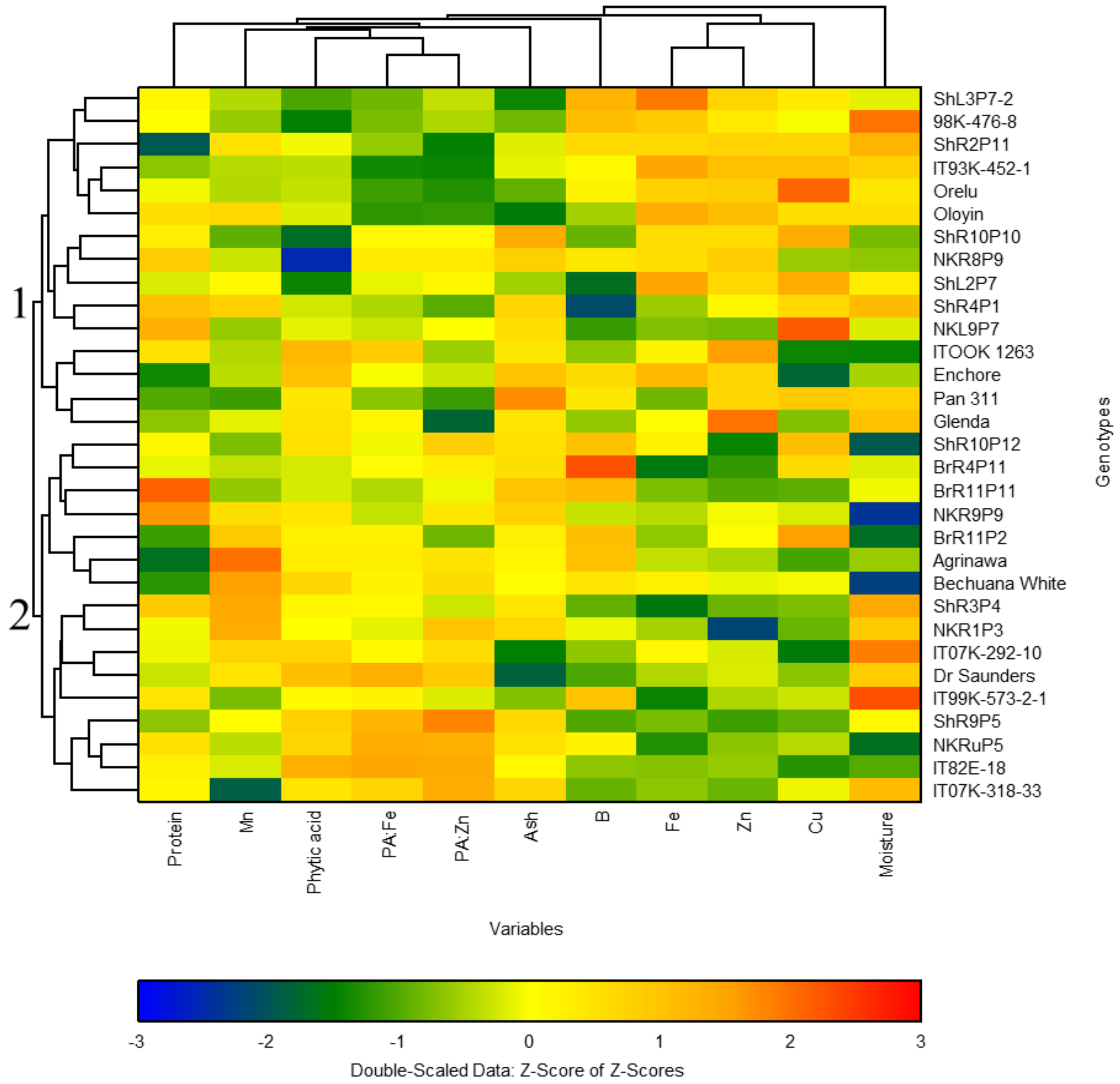

Figure 4.6 Clustered heat map showing the associations between the genotypes with the measured traits. Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, B = Boron, PA:Fe = Molar ratio of phytic acid with iron, PA:Zn = Molar ratio of phytic acid with zinc

4.5 Discussion

4.5.1 ANOVA for for protein, selected minerals, phytic acid, potential mineral bioavailability, moisture and ash of cowpea mutants and normal genotypes

Significant genotype effects for protein content, selected mineral elements (Fe, Zn, Mn and Cu) except for B, phytic acid concentration, PA:Zn molar ratio and ash content indicated the existence of genetic variability among the tested genotypes. The significant variation observed between the cowpea mutants and normal genotypes indicate that superior mutants for protein, selected mineral elements (except for B), (low) phytic acid concentration, PA:Zn molar ratio and ash content can be identified and selected for further crop improvement. To tackle nutritional deficiency in poor African countries, it is crucial to develop cowpea genotypes with high nutritional content. Therefore, before starting a breeding programme, cowpea germplasm collections must be evaluated for genetic diversity. Highly significant genotype effects for seed protein content and mineral elements were observed in previous studies conducted in South Africa (Mbuma et al. 2021; 2022). Similar findings were also observed in cowpea leaves (Gerrano et al. 2022). The genotype effect for B concentration, PA:Fe molar ratio and moisture content were non-significant in almost all the environments, which indicated cowpea mutants and normal genotypes performed similarly in most of the environments, indicating that identifying and selecting superior genotypes for these characteristics would be difficult.

The GE interaction was only significant for protein content, Cu, phytic acid concentration and ash content, indicating inconsistent performance of the genotypes in tested environments for these characteristics. Significant GE interaction also suggests the need for evaluation of cowpea genotypes in multiple environments and seasons to quantify their stability and adaptability before commercial release and recommendation. Due to the increased potential of crop temporal and spatial instability, GE interactions could be a barrier to crop improvement. The GE interaction was found to be significant for cowpea protein, Fe and Zn content, suggesting that different cowpea genotypes exhibit different responses to different environments (Araújo et al. 2021). A study on GE interactions reported non-significant variations for protein and Cu in cowpea breeding populations, indicating that the environments had limited influence on the performance of the genotypes (Gerrano et al. 2019).

The H^2 values were above 50% for protein content (Polokwane), Zn concentration (Bloemfontein, Taung and Mafikeng), Cu concentration (Taung and Mafikeng), Mn concentration (Bloemfontein and Potchefstroom), and phytic acid concentration (Potchefstroom, Taung and Polokwane). These results indicated that the genotypic variance of these traits contributed more to the variation than the phenotypic variance. Across the environments, H^2 values were above 50% for almost all the measured traits except for B concentration, indicating that selection of these characteristics can be done with high precision. Previous studies (Ravelombola et al. 2016; Weng et al. 2019) have reported high H^2 values (> 50%) for protein content in cowpea breeding populations. Protein is of high importance in legume crops and the ability of the trait to be inherited to the progenies is of interest for crop improvement.

4.5.2 Combined ANOVA for cowpea groupings of genotypes for all characteristics

The significant grouping effect of the three sources of germplasm for protein content, Zn, Mn, phytic acid, PA:Zn molar ratio, ash and moisture content, indicated the presence of wide variation between the three groups of genotypes for these traits. The significant grouping effect also suggested that superior groups for each trait could be identified and selected. The variation between cowpea mutants and IITA genotypes also indicate the possibility of introducing new variation and broadening the South African cowpea germplasm genetic diversity. High genetic diversity is very important in plant breeding populations for crop improvement. The IITA and Namibian Radiation Authority has managed to obtain different cowpea groups in Africa and alter their genetic diversity through techniques such as mutation breeding to develop improved lines with good nutrition to contribute to overcoming malnutrition in undeveloped countries (Boukar et al. 2018; Horn and Shimelis 2020). Non-significant Gr.E interaction for all the measured traits indicated that the genotype groups performed similarly in all the environments. This could also suggest that environments had little to no influence on the performance of each group for nutritional quality traits.

4.5.3 Cowpea mutants and normal genotype performance for all characteristics across environments

Superior Namibian mutants (ShL3P7-2, ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 ShR2P11, BrR11P2, ShL2P7, ShR3P4 and NKRuP5), IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1, IT99K-573-2-1 and ITOOK 1263) and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White, Enchore and Glenda) for protein content, Fe and Zn concentration were identified. Cowpea genotypes that combine high protein content, Fe and Zn concentration are of high importance as they have the potential to address malnutrition. Superior genotypes with high protein, Fe and Zn content should be evaluated for yield stability and adaptability, and subsequently these genotypes could be released and recommended for commercial production.

Superior Namibian mutants (ShR10P10, ShR3P4, ShR4P1, ShR9P5, BrR11P11, BrR11P2, ShR2P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 and NKRuP5), IITA genotypes (98K-476-

8, IT82E-18, IT99K-573-2-1, IT07K-292-10 and ITOOK 1263), and South African genotypes (Pan 311, Bechuana White, Enchore, Orelu, Agrinawa, Dr Saunders and Glenda) for ash, Cu, Mn and B were identified. Genotypes with high mean values for Cu, Mn and B concentrations are very important for the human diet as these traits could improve malnutrition and other dreaded diseases associated with lack of essential minerals. Ash content represents the total mineral content in the seed after combustion and is usually the first step in the process of determining concentrations of specific minerals. A recent study found that the ash content of cowpeas ranges from 2.97 to 3.47% (Gondwe et al. 2019), which is within the range of the ash content (3.54 to 4.37%) identified in the present study; indicating that the cowpea genotypes are an excellent source of minerals.

Namibian mutants (ShL3P7-2, ShR10P10, ShR10P12, ShR2P11, ShR4P1, BrR11P11, NKR1P3, NKR8P9 and ShL2P7), IITA genotypes (98K-476-8, IT07K-318-33 and IT93K-452-1), and South African genotypes (Oloyin, Orelu and Agrinawa) had mean values below the average mean (6167.79 mg kg^{-1}) for phytic acid concentration. Genotypes with low phytic acid concentration are essential as they would be associated with a good potential mineral bioavailability and can be a good source of nutrition for people with mineral deficiency. However, genotypes with high phytic acid concentration are not recommended even if they are associated with high mineral content due their mineral binding capacity. Phytic acid hinders mineral bioavailability, reducing their absorption in the gut when legumes are consumed (Gupta et al. 2015).

The PA:Fe molar ratio was below 10 for IITA genotype (IT93K-452-1) and South African genotypes (Oloyin and Orelu) indicating a potential of good Fe bioavailability. The PA:Zn molar ratio was above 15 for all the genotypes, indicating potential of poor Zn bioavailability. A study on cowpea mineral bioavailability found that only one genotype exhibited a moderate Zn bioavailability (Diouf et al. 2020). These findings imply that increased phytic acid levels in cowpea seeds will result in decreased Zn bioavailability. A previous study revealed that soaking cowpea seeds in vinegar for 24 hours allowed reduction of phytic acid which resulted in increased Fe bioavailability and low Zn bioavailability (Diouf et al. 2020). Furthermore, the study revealed that soaking cowpea seeds in tap water for 24 hours provided a good Fe bioavailability and a moderate Zn bioavailability.

4.5.4 Cowpea group performance for all characteristics across environments

The significant differences between the genotype groupings observed for protein, Fe, Zn and Mn, ash and moisture, indicated that the variation observed was influenced by the source of origin. The Namibian mutant group was superior to IITA genotypes and South African genotypes for protein content and Cu indicating that when breeding for high protein and Cu, breeders can choose the Namibian mutants and use them as parents. The IITA group had high moisture and phytic acid compared to Namibian mutants and South African groups. Thus, the IITA genotypes' seeds should be well dried and possibly be stored separately since high moisture in the seeds causes post-harvest diseases. The high phytic acid concentration also indicated that the potential bioavailability of minerals would be reduced in these genotypes. The South African genotype group was superior to Namibian mutants and IITA genotypes for Fe, Zn and Mn indicating that these genotypes could be used as parents when breeding for improved nutritional value, particularly the mineral elements. A study on cowpea nutritional diversity in leaves reported that South African genotypes were superior to IITA genotypes for Fe, Zn and Mn concentration (Gerrano et al. 2022).

4.5.5 Phenotypic correlation

The Fe, Zn and Cu concentration were significant and positively inter-correlated, suggesting that these mineral elements could be selected simultaneously. Mineral elements are required to support human body development, growth and health. To improve the mineral elements, it is crucial to consider the possibility of indirect or simultaneous selection of these characteristics. Significant correlation between Fe and Zn concentration has also been reported in previous studies (Dakora and Belane 2019; Mbuma et al. 2021; Gerrano et al. 2022). A recent study on cowpea genotypes found positive correlations of Cu with Fe and Zn (Mbuma et al. 2022). A high protein content will have a favourable impact on the mineral concentrations in cowpea genotypes, for which there was a positive correlation observed in the study for protein content with most of the mineral elements. There were positive correlations between the protein content and most of the mineral elements, which indicated that these traits can be simultenously selected and improved. Protein is required to fuel energy and carry oxygen throughout the blood of the human body while micronutrients are necessary for maintaining healthy biological functioning. Successfully breeding of superior cowpea cultivars with high protein content coupled with essential mineral elements would address malnutrition and undernourishment in most poor populations that depends on this crop as part of their daily diet.

Significant and positive correlations were observed for phytic acid with Mn, PA:Fe and ash, PA:Zn with ash, and PA:Fe with PA:Zn and ash. However, the molar ratios of the minerals (PA:Zn and PA:Fe) indicate to what extent the minerals are bioavailable. Therefore, implementations to reduce the phytic acid concentration is crucial. The phytic acid concentration of cowpea can be reduced by soaking the seeds in vinegar with water for 24 hours before cooking (Diouf et al. 2020).

4.5.6 Principal component analysis (PCA)

Namibian mutants (BrR11P2), IITA genotype (ITOOK 1263) and South African genotypes (Pan 311 and Enchore) were associated with protein, Mn and B, suggesting that these genotypes could be used to improve these traits. The protein content, Mn and B concentration were negatively correlated with moisture content, indicating a dilution effect due to high moisture content.

Namibian mutants (NKR9P9, ShR3P4, BrR4P11 and NKRuP5), IITA genotype (IT82E-18), and South African genotype (Bechuana White) were associated with ash, PA:Zn molar ratio, PA:Fe molar ratio and phytic acid which could indicate that the potential mineral bioavailability of these genotypes could be reduced. However, phytic acid is also an antioxidant, which could have health benefits such as reducing the chance of having cancer, therefore these genotypes have potential of improving human health when consumed.

High Cu, Fe, and Zn concentrations were associated to the Namibian mutant (NKR8P9), and these characteristics were negatively associated with PA: Zn molar ratio. This suggests that this mutant has an improved levels of micronutrients and that it could be utilised as parental line in genetic improvement breeding programme. Previous studies have reported a positive association of IITA genotypes with high Fe, Zn and Cu (Gerrano et al. 2019).

4.5.7 Clustered heat map for protein, mineral elements, phytic acid, potential mineral bioavailability, moisture and ash in cowpea mutant and normal genotypes

Based on the protein, selected mineral elements, phytic acid, moisture and ash content, the clustered heat map differentiated between two distinct clusters of cowpea genotypes. The cowpea genotypes did not cluster according to their origin and genetic background. In cluster 1, Namibian mutants (ShL3P7-2, ShR10P10, ShR2P11, ShR4P1, NKL9P7, NKR8P9 and

ShL2P7), IITA genotypes (ITOOK 1263, IT93K-452-1 and 98K-476-8), and South African genotypes (Orelu, Oloyin, Pan 311, Enchore and Glenda) were associated with high Fe, Zn, Cu, B, moisture and low phytic acid, indicating that these genotypes can be used as parental genotypes in breeding programmes. Furthermore, since the genotypes are associated with low phytic acid concentration, they can also be released as new cultivars due to their association with the mineral elements which are responsible healthy body functioning. In cluster 2, Namibian mutants (ShR10P12, ShR3P4, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18 and IT99K-573-2-1), and South African genotypes (Agrinawa, Bechuana White and Dr Saunders) were associated with high protein, Mn, phytic acid, PA:Fe, PA:Zn, and ash, indicating that genotypes associated with high protein content and Mn concentration might have a reduced potential mineral bioavailability due to the presence of high phytic acid concentration.

4.6 Conclusions

The significant effects of genotype and groupings indicated the presence of wide variation between three populations (Namibian mutants, IITA and South African genotypes) which can be exploited for crop improvement. The significant GE interaction emphasised the significance of evaluating cowpea genotypes in various environments and seasons. Almost all of the measured characteristics had high H^2 values, which suggested that they may respond well to selection. Superior Namibian mutants (ShL3P7-2, ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 ShR2P11, BrR11P2, ShL2P7, ShR3P4 and NKRuP5), IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1, IT99K-573-2-1 and ITOOK 1263), and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White, Enchore and Glenda) that combine protein, Fe and Zn were identified. The IITA genotype (IT93K-452-1) and South African genotypes (Oloyin and Orelu) were found to have a potential of good Fe bioavailability. Significant and positive correlations were observed between the nutritional quality traits indicating possibility of simultaneous improvement of the nutritional quality traits. Namibian mutants (ShR10P12, ShR3P4, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18 and IT99K-573-2-1), and South African genotypes (Agrinawa, Bechuana White and Dr Saunders) were associated with high protein, Mn, phytic acid, PA:Fe, PA:Zn, and ash. Namibian mutants (ShL3P7-2, ShR2P11, ShR10P10, NKL9P7 and ShL2P7), IITA genotypes

(IT93K-452-1 and 98K-476-8), and South African genotypes (Orelu, Oloyin, Pan 311 and Glenda) were associated with phytic acid, Fe, Zn, Cu, B concentration and moisture content.

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CHAPTER 5

EVALUATION OF GENOTYPE BY ENVIRONMENT INTERACTION AND STABILITY OF COWPEA MUTANTS AND NORMAL GENOTYPES FOR GRAIN YIELD

5.1 Abstract

The underutilised, highly nutritive legume crop known as cowpea has the potential to address global concerns of hunger and undernourishment. Nonetheless, the crop has a relatively limited productivity and production, which is mainly attributed to the lack of genotypes with high grain yield that are adapted and stable under diverse growing conditions. Cowpea breeding trials are conducted in multi-environments to evaluate the genotypes for stability and adaptability of commercial characteristics. The objectives of this study were to determine GE interaction, to identify the superior genotypes for grain yield and to determine the adaptability and stability of cowpea mutants and normal genotypes in South Africa. Thirty-one cowpea genotypes (16 Namibian mutants, seven IITA genotypes and eight South Africa genotypes) were planted in five different environments in South Africa during the 2021/2022 cropping season. High significant ($P \leq 0.001$) genotype effect for grain yield indicated the presence of large variation among the cowpea genotypes. Highly significant ($P \leq 0.001$) GE interaction effects for grain yield indicated that genotypes performed differently across environments. Ten Namibian mutants (BrR11P2, NKR1P3, ShR10P10, ShR10P12, ShR3P4, ShR4P1, ShR9P5, NKR8P9, NKRuP5 and ShL2P7), three IITA genotypes (98K-476-8, IT99K-573-2-1 and ITOOK 1263) and one South African genotype (Enchore) with high grain yield were identified. Superior mutants for grain yield can be used as parents to broaden the genetic base of cowpea germplasm collections in South Africa. Namibian mutants (NKL9P7, ShR10P12 and ShR2P11), IITA genotype (ITOOK 1263) and South African genotype (Agrinawa) were the most stable genotypes. Two mega-environments, namely, 1) Taung and Mafikeng, and 2) Mafikeng, Bloemfontein, Polokwane and Potchefstroom were identified. Namibian mutants (ShR10P12 and NKR8P9) and IITA genotype (IT99K-573-2-1) were located in the two mega-environments, which indicated the potential of broad adaptation of these genotypes for grain yield within these mega-environments. Genotypes with high grain yield that showed broad adaptation and good stability can be recommended for release and commercial production.

Keyword: cowpea, grain yield, AMMI biplot, GGE biplot, stability, adaptability

5.2 Introduction

The cowpea is a valuable orphan grain legume crop that is native to Africa and is farmed extensively in dry and hot regions around the world using low-input agricultural production methods (Horn and Shimelis 2020). The crop is of utmost importance because of its nutritional quality, which mainly benefits poor populations in underdeveloped regions, specifically in Asia, Africa and Central America (Bjornlund et al. 2020). Cowpea is consumed either as a leafy vegetable or grain crop. The crop has a dual purpose, it can be used for animal and human feeding.

Despite the well-known health benefits of cowpeas, South Africa has very low rates of crop production, resulting in the country ranking the second last of the top 20 cowpea producing countries in Africa (FAOSTATS 2020). The yield produced is very low compared to the area available for production of the crop. This could be attributed to the lack of improved cowpea varieties in terms of yield potential, adaptability and stability in South African growing conditions.

The Mpumalanga, Limpopo, North West and KwaZulu-Natal provinces of South Africa are where subsistence farmers primarily produce cowpeas (Asiwe 2009). The diverse environmental conditions characterise the primary cowpea growing regions in South Africa. The differences across environments generally influence the performance of cowpea genotypes, resulting in a change in ranking from one environment to another, a phenomenon called GE interactions (Mbuma et al. 2020). Significant GE interactions in breeding programmes could result in low heritability values and thus reduces the selection efficiency. Significant and large GE interaction is also an indication that the environment plays a significant role in the observed phenotypic variations of the crop, suggesting the necessity to evaluate cowpea genotypes in multiple environments and seasons (Simion et al. 2018).

Significant GE interactions for grain yield and morphological characteristics have been reported in South Africa (Shiringani and Shimelis 2011; Gerrano et al. 2019, Mbuma et al. 2021). These studies have also identified genotypes that were stable or unstable, adapted or not adapted to the environmental conditions. In addition, mega-environments were also identified. A mega-environment is defined as a collection of environments that constantly provide similar superior genotypes. In Nigeria, significant GE interaction for grain yield has been reported (Ishiyaku et al. 2017). In Ethiopia, significant GE interaction for grain yield and morphological characteristics has been reported (Simion et al. 2018). These studies evaluated different breeding populations under dirvese environments, highlighting how the performance of cowpea genotypes is influenced by the genetic make-up of the materials and the characteristics of the environment. Thus, the efficiency of selection of cowpea genotypes could be improved if the nature and extent of GE interaction is understood (Yousaf and Sarwar 2008; Simion et al. 2018; Mbuma et al. 2021). Furthermore, knowledge on the nature of GE interaction could provide suitable strategies for breeding genotypes for broad or specific adaptation (Asio et al. 2005; Cooper and Messina 2021).

In cowpea breeding populations, lack of genetic diversity has been reported for grain yield and morphological traits (Mbuma et al. 2021). This is mostly linked to the reproductive system and the incompatibility of parental genotypes. Plant breeders must consider broadening the cowpea germplasm collection by incorporating new genetic materials from various origins or countries for successful breeding. Self-pollinating crops like cowpea and bambara groundnut could benefit greatly from increased genetic variability of desirable traits through mutation breeding and mutagenesis (Kozgar et al. 2012; Singh et al. 2013; Wanga et al. 2018). Considering the narrow genetic diversity observed in the cowpea germplasm collection, the ARC in South Africa has obtained cowpea mutants from the Namibian Radiation Regulatory Authority with the aim to test and evaluate these genotypes for their yield potential, adaptability, and stability as well as other agro-morphological characteristics in South African growing conditions. The identified superior cowpea mutants that show good adaptation and stability for grain yield could therefore be used as potential parents to diversify the existing germplasm collection. Therefore, the objectives of this study were to determine GE interaction, to identify superior genotypes for grain yield and to determine the adaptability and stability of cowpea mutants and normal genotypes in South Africa.

5.3 Materials and methods

5.3.1 Plant material and experimental sites

Plant material and experimental sites are in accordance with Chapter 3 in section 3.3.1).

5.3.2 Trial design and management

Trial design and management are in accordance with Chapter 3 in section 3.3.2.

5.3.3 Data collection

Data collection and the formula to determine the grain yield was mentioned in Chapter 3 in section 3.3.3.

5.3.4 Statistical analysis

GenStat 22nd edition statistical software (VSN International 2022) was used for a mixed model and combined ANOVA across the five environments to compare the 31 genotypes performance across environments and to analyse their interactions. Genotypes were considered fixed while environment was considered random. The AMMI and GGE analyses were done using the same software. The AMMI was used to determine the stability of genotype performance across different environments while GGE was used to determine the adaptability of the genotypes across environments. Environment-centred PCA serves as the foundation for GGE biplot analysis, whereas double-centred PCA is used for AMMI analysis. Plant breeders can concentrate on the genotype effect (G) and GE interaction by using the GGE model, which offers further visualisation than the AMMI and eliminates the influence of the environment (E) (Ding et al. 2008). Therefore, GGE was conducted to support the AMMI results with identification of superior genotypes and mega-environments.

5.4 Results

5.4.1 The ANOVA for AMMI analysis of cowpea grain yield

Genotype, environment and GE interaction effect were highly significant ($P \leq 0.001$) for grain yield (Table 5.1) and accounted for 14.62%, 27.89% and 33.11% of the total variation, respectively. Significant ($P \leq 0.001$) interaction principal component axes (IPCA 1) and significant ($P \leq 0.01$) IPCA 2 were identified and accounted for 48.15% and 28.96% of the GE interaction, respectively.

Table 5.1 Additive main effects and multiplicative interaction (AMMI) analysis of variance (ANOVA) for grain yield of 31 genotypes

Source	Df	Sum squares	Mean squares	Total variation (%)	GEI (%)
Genotypes	30	35589505.00	1186317.00***	14.62	
Environments (E)	4	67893664.00	16973416.00***	27.89	
Rep. (E)	5	5362816.00	1072563.00*	2.20	
Interactions	114	80608160.00	707089.00***	33.11	
IPCA 1	33	38811996.00	1176121.00***		48.15
IPCA 2	31	23342932.00	752998.00**		28.96
GEI error	50	18453233.00	369065.00		22.89
Pooled error	144	54019877.00	375138.00		
Total	309	243474023.00	787942.00		

$^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, Rep. (E) = Replications within environments, IPCA = Interaction principal component axis, GEI = Genotype by environment interaction, Df = Degrees of fredom

5.4.2 Mean value performance and stability ranking of genotypes across environments

In Bloemfontein, eight Namibian mutants (BrR4P11, NKL9P7, ShR10P12, ShR10P10, ShR9P5, BrR11P2, NKR1P3 and ShL2P7), five IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1, IT99K-573-2-1 and IT07K-318-33), and three South African genotypes (Oloyin, Pan 311 and Enchore) had higher grain yield than the trial mean (1580.21 kg ha^{-1}) (Table 5.2). In Mafikeng, five Namibian mutants (ShR10P12, NKR1P3, ShR3P4, NKR8P9 and ShR2P11), four IITA genotypes (98K-476-8, IT07K-292-10, IT07K-318-33 and ITOOK 1263), and four South African genotypes (Oloyin, Pan 311, Bechuana White and Glenda) had higher grain yield than the trial mean (1165.65 kg ha^{-1}).

In Polokwane, eight Namibian mutants (BrR11P11, BrR11P2, ShR3P4, ShR4P1, NKR8P9, NKRuP5, ShL2P7 and ShR10P10), four IITA genotypes (98K-476-8, IT07K-292-10, IT82E-18 and ITOOK 1263) and four South African genotypes (Pan 311, Bechuana White, Enchore and Glenda) had higher grain yield than the trial mean (1252.16 kg ha^{-1}). In Potchefstroom, 10 Namibian mutants (BrR11P2, BrR11P11, NKR1P3, NKRuP5, ShR4P1, ShR10P12, ShR9P5, NKR9P9, ShR3P4 and ShL2P7), two IITA genotypes (IT82E-18 and IT99K-573-2-1), and one

South African genotype (Enchore) had higher grain yield than the trial mean (2451.85 kg ha^{-1}). In Taung, nine Namibian mutants (ShR10P10, ShR10P12, ShR9P5, BrR11P2, BrR4P11, NKR8P9, NKRuP5, NKR1P3 and ShL2P7), three IITA genotypes (98K-476-8, IT99K-573-2-1 and ITOOK 1263) and one South African genotype (Oloyin) had higher grain yield than the trial mean (1886.29 kg ha^{-1}).

On average, 10 Namibian mutants (BrR11P2, NKR1P3, ShR10P10, ShR10P12, ShR3P4, ShR4P1, ShR9P5, NKR8P9, NKRuP5 and ShL2P7), three IITA genotypes (98K-476-8, IT99K-573-2-1 and ITOOK 1263), and one South African genotype (Enchore) had higher grain yield across the environments than the trial mean (1667.23 kg ha^{-1}).

The AMMI stability value (ASV) was used to identify stable genotypes across the five environments. Namibian mutants NKL9P7 (ASV = 0.81), ShR10P12 (ASV = 2.94) and ShR2P11 (ASV = 6.02), South African genotype Agrinawa (ASV = 4.29) and IITA genotype ITOOK 1263 (ASV = 7.57) were identified as stable genotypes compared to Namibian mutants ShR10P10 (ASV = 30.04), ShR4P1 (ASV = 31.11) and BrR11P2 (ASV = 66.39), and South African genotypes Pan 311 (ASV = 30.09) and Oloyin (ASV = 40.23) which were the least stable genotypes.

Table 5.2 Mean values of all genotypes' performance across environments for cowpea grain yield (kg ha^{-1})

Genotypes	Bloemfontein	Mafikeng	Polokwane	Potchefstroom	Taung	Mean	ASV	rASV
ShL3P7-2	748.62	449.29	412.05	865.17	1660.41	827.11	23.63	23
ShR10P10	2131.37	947.46	1722.61	2258.22	3628.66	2137.66	30.04	27
ShR10P12	1732.15	2147.71	862.08	2958.01	2402.20	2020.43	2.94	2
ShR2P11	1576.95	1815.59	561.39	2359.56	1805.66	1623.83	6.02	4
ShR3P4	1083.31	1934.22	1544.89	3309.00	1291.66	1832.62	23.67	24
ShR4P1	1571.21	847.77	1562.82	3621.98	1504.79	1821.71	31.11	29
ShR9P5	1760.40	736.69	1171.58	3137.91	2084.95	1778.31	17.68	18
BrR11P11	1314.83	870.06	1769.26	2552.35	1425.94	1586.49	11.25	10
BrR11P2	1748.64	780.55	1753.69	5661.09	2582.05	2505.20	66.39	31
BrR4P11	2161.58	877.67	840.85	1400.05	2165.14	1489.06	25.05	25
NKL9P7	1844.78	934.85	950.82	2308.30	1708.42	1549.43	0.81	1
NKR1P3	2052.92	1951.59	1155.46	2767.42	3223.82	2230.24	18.30	19
NKR8P9	1201.22	1247.44	1333.89	2408.99	2553.02	1748.91	10.63	8
NKR9P9	1311.02	745.13	761.71	3379.62	1829.44	1605.38	26.42	26
NKRuP5	1429.28	1132.44	1345.67	2943.06	1908.83	1751.86	11.04	9
ShL2P7	1842.15	989.12	1653.47	3216.56	2641.34	2068.53	14.03	12
98K-476-8	1690.23	1918.27	1630.79	2246.77	2743.16	2045.84	19.43	21
IT07K-292-10	1539.80	1432.32	1556.64	2120.72	982.24	1526.34	16.93	14
IT07K-318-33	2104.87	1278.87	925.90	2383.40	818.60	1502.33	15.41	13
IT82E-18	1918.05	1008.34	1546.03	2653.62	1033.85	1631.98	17.23	15
IT93K-452-1	1759.92	734.12	1073.75	2313.69	1097.88	1395.87	9.97	7
IT99K-573-2-1	2438.97	577.82	860.80	2874.02	2612.16	1872.75	17.61	17
ITOOK 1263	957.17	1262.43	1905.10	2225.85	2213.54	1712.82	7.57	5
Oloyin	1587.49	1185.03	944.21	1330.50	3193.12	1648.07	40.23	30
Orelu	1275.34	1087.47	690.99	1148.88	1428.55	1126.25	21.99	22
Pan 311	1652.79	1525.66	1863.04	1237.81	1555.99	1567.06	30.09	28
Agrinawa	998.10	447.73	1058.04	1805.32	1106.91	1083.22	4.29	3
Bechuana White	1266.44	1239.58	1455.52	2025.38	1722.86	1541.96	8.80	6
Dr Saunders	1182.00	1150.36	1191.20	2047.14	623.98	1238.94	17.54	16
Enchore	2112.47	1121.43	1367.15	2745.26	1316.39	1732.54	13.09	11
Glenda	992.43	1758.11	1345.55	1701.70	1609.56	1481.47	19.35	20
Mean	1580.21	1165.65	1252.16	2451.85	1886.29	1667.23		

ASV = Additive main effects and multiplicative interaction stability value, rASV = Ranking of additive main effects and multiplicative interaction stability value

5.4.3 Adaptability, additive main effects and multiplicative interaction (AMMI) biplot analysis

The AMMI biplot was used to visualise the genotype mean performance for grain yield for the five environments according to their stability (Figure 5.1). The Namibian mutant BrR11P2 had the high grain yield and it was located close to the Potchefstroom environment. The Namibian mutants (ShR10P10, ShR10P12 and NKR1P3), and IITA genotypes (ITOOK 1263 and 98K-476-8) had high grain yield and were located close to the Taung environment.

The stability of the cowpea genotypes across the environments was demonstrated using the AMMI biplot (Figure 5.2). Principal components (PC 1 and PC 2) contributed for 48.18% and 28.96% of the overall variation. Genotypes close to the horizontal cartesian plane are regarded as the most stable genotypes while those that are far away are the least stable genotypes. The Namibian mutants (NKL9P7, ShR10P12 and NKRuP5,), IITA genotype (ITOOK 1263) and South African genotypes (Agrinawa and Bechuana White) were the most stable. The length of the vectors in the biplot indicates the interaction of the environments with each other. Polokwane, Mafikeng and Bloemfontein environments indicated little GE interaction while Potchefstroom and Taung indicated high GE interactions.

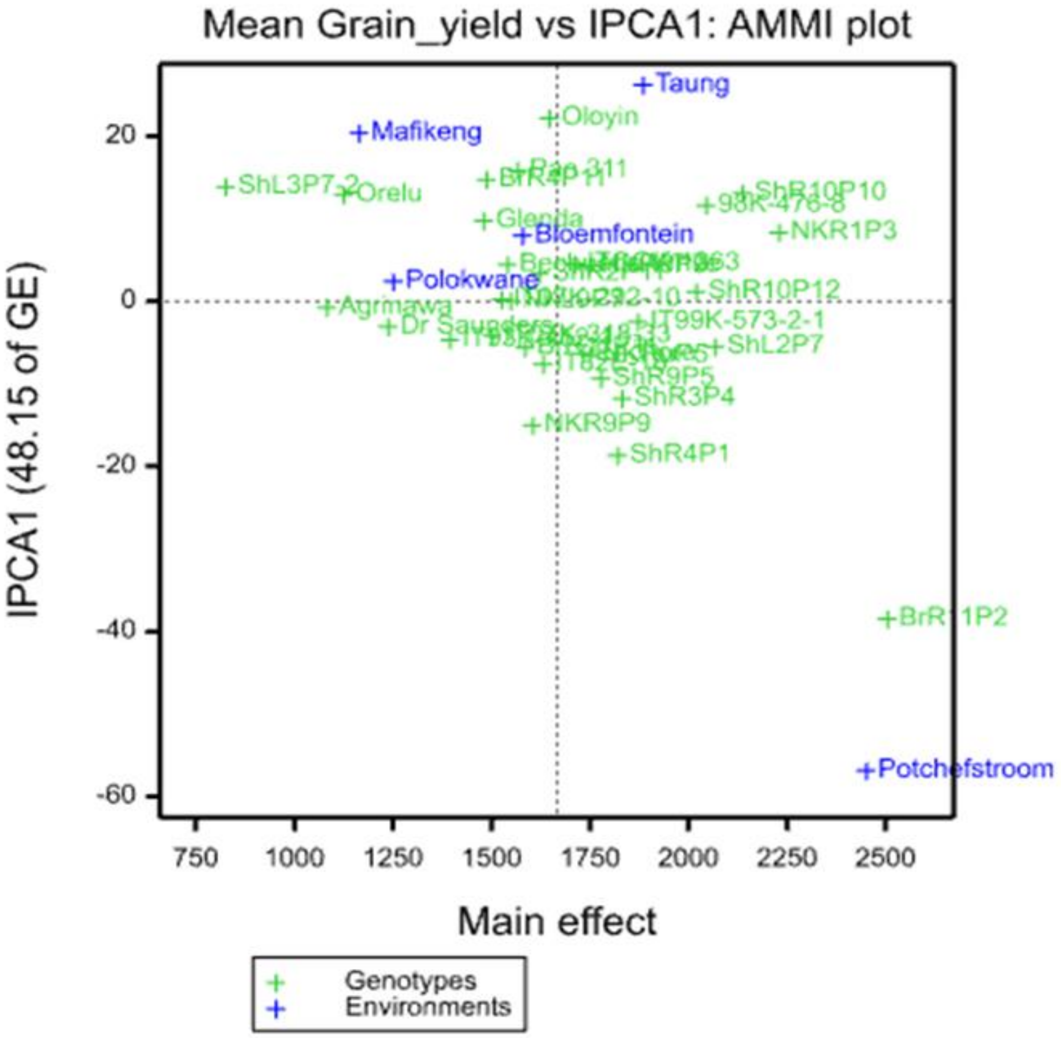

Figure 5.1 Adaptability, additive main effects and multiplicative interaction (AMMI) biplot for grain yield of 31 genotypes across five environments. IPCA = Interaction principal component axis, GE = Genotype by environment interaction

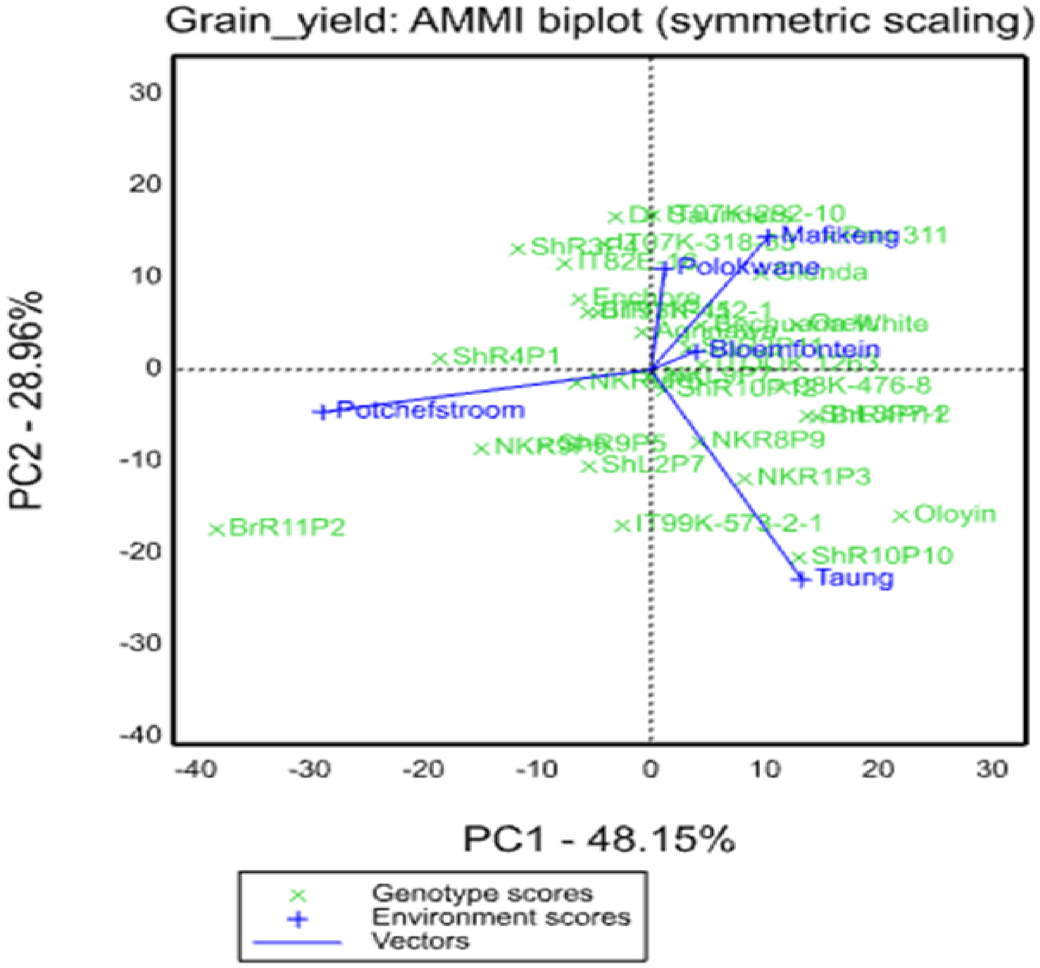

Figure 5.2 Adaptability, additive main effects and multiplicative interaction (AMMI) biplot for grain yield stability of 31 genotypes across five environments. PC = Principal component

5.4.4 Genotype plus genotype by environment interactions (GGE) biplot analysis

Figure 5.3 shows a 'which won where' biplot view of the relationship between genotypes and environments. The biplots for grain yield explained 76.31% of the total variation observed, of which 47.15% and 29.16% were explained by the PC1 and PC2, respectively. Two mega-environments were identified. The first mega-environment (Mafikeng, Bloemfontein, Polokwane and Potchefstroom) was associated with five Namibian mutants (BrR11P2, ShR10P12, NKR8P9, ShR4P1 and NKR9P9), two IITA genotypes (IT99K-573-2-1 and IT07K-318-33), and three South African genotypes (Oloyin, Bechuana White and Enchore). The second mega-environment (Taung and Mafikeng) was associated with four Namibian mutants (ShR10P10, ShR10P12, NKR8P9 and NKR1P3), and three IITA genotypes (IT99K-573-2-1, 98K-476-8 and ITOOK 1263).

The Namibian mutants (ShR10P10 and BrR11P2) were situated at the vertexes of the "which won where" polygon of the two mega-environments which indicated that these genotypes were best performing and most adapted in those environments. The Namibian mutants (BrR11P2, NKR1P3, ShR10P10, ShR4P1 and NKR9P9) had the highest grain yield and were adapted to Potchefstroom, Taung and Bloemfontein, while Namibian mutants (ShL3P7-2 and BrR4P11), and South African genotypes (PAN 311, Agrinawa, Dr Saunders and Oloyin) had the lowest grain yield and were not adapted to any specific environment.

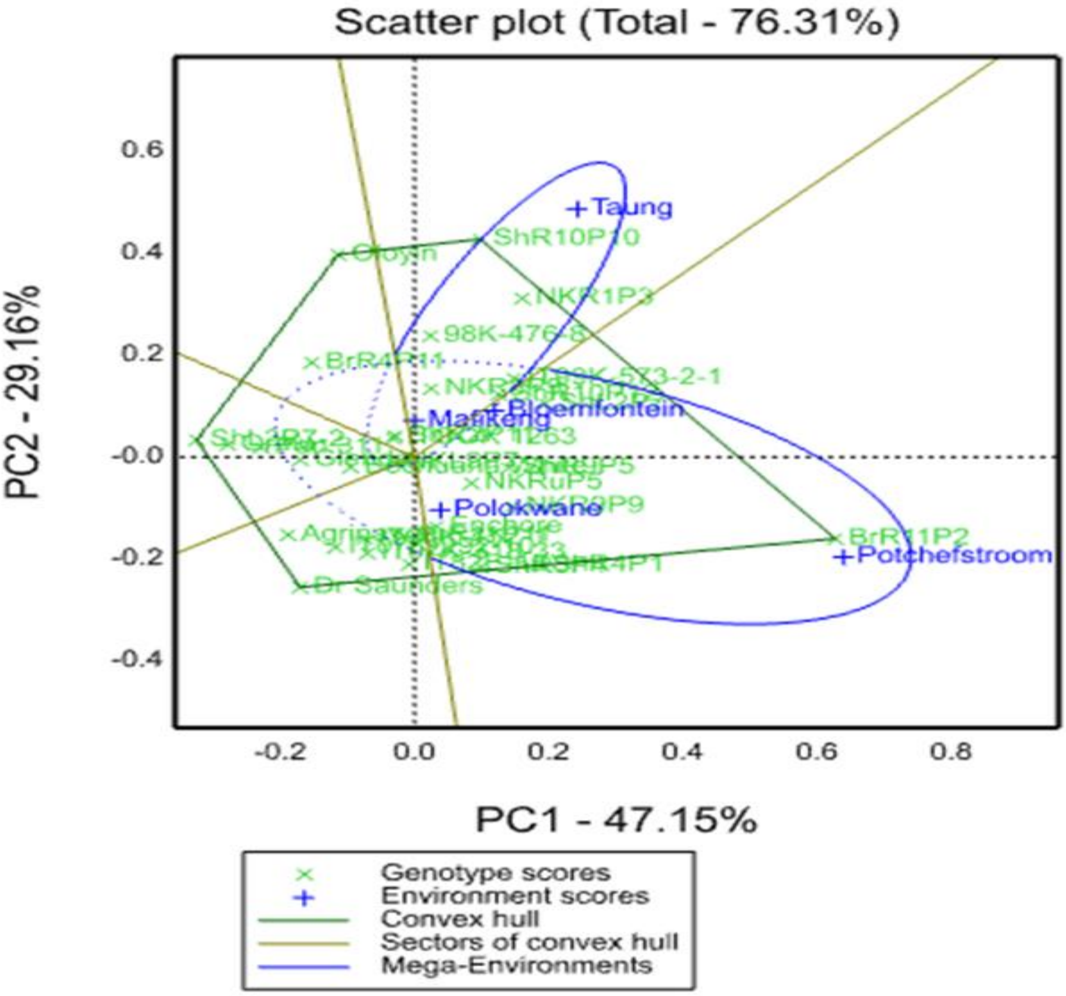

Figure 5.3 Genotype plus genotype by environment interactions biplot highlighting the mega-environments for cowpea grain yield. PC = Principal component

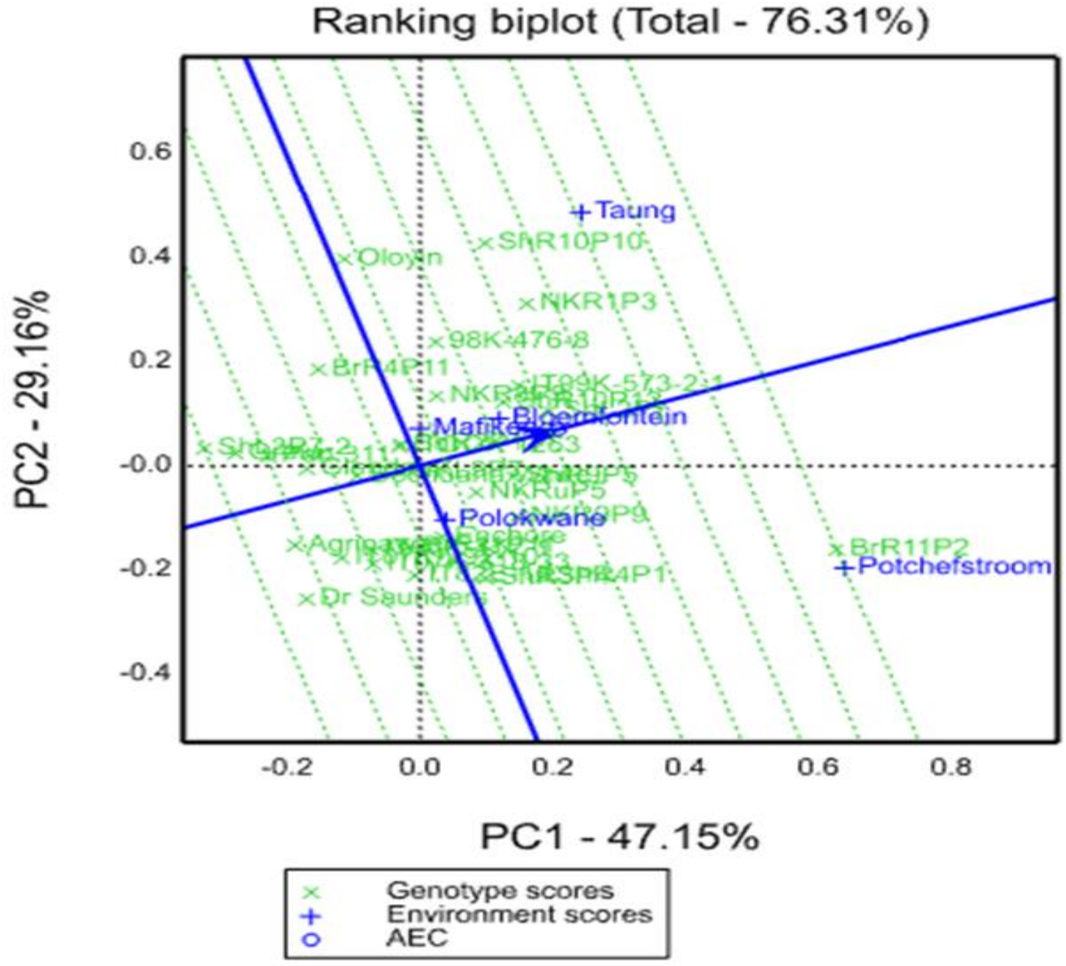

Figure 5.4 Genotype plus genotype by environment interactions biplot ranking indicating the mean grain yield performance of 31 cowpea genotypes. PC = Principal component, AEC = Average environment coordination

5.4.5 Ideal genotypes and environments for cowpea grain yield

The ideal genotype on the GGE biplot, which compares all other genotypes to it, is the one that is situated in the middle of the circular rings (Figure 5.5). Genotypes that are located near the epicentre are regarded as being close to the ideal genotype. The ideal genotypes were Namibian mutants (BrR11P2, ShR10P12, NKR8P9, NKRuP5, NKR1P3 and ShR10P10), and IITA genotypes (98K-476-8 and IT99K-573-2-1). Potchefstroom was the most ideal planting site because the environment was closest to the epicentre (Figure 5.6). Taung and Bloemfontein were also close to epicentre making them the second most ideal environments. Even though Mafikeng and Polokwane were part of the mega-environments, they are far away from the epicentre.

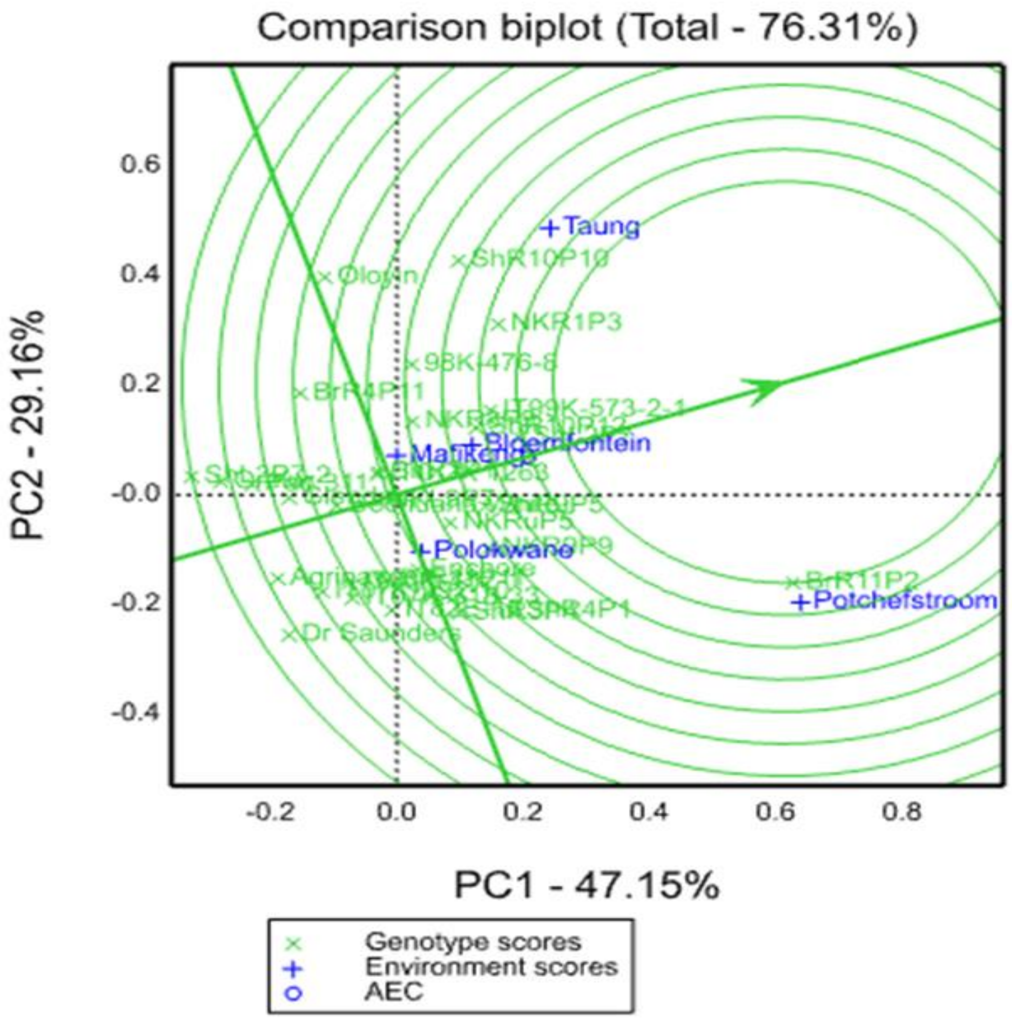

Figure 5.5 Genotype plus genotype by environment interactions biplot showing the ideal genotypes according to the average grain yield of cowpea across the five environments. PC = Principal component, AEC = Average environment coordination

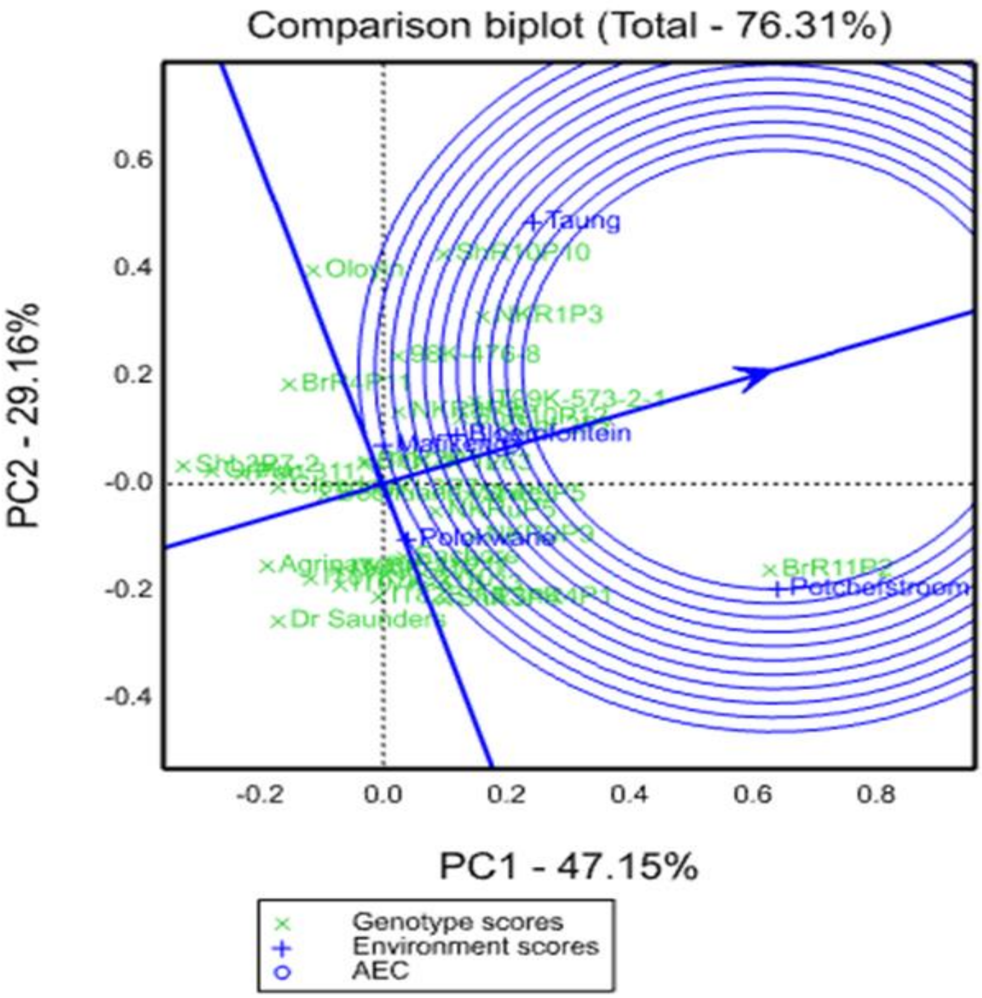

Figure 5.6 Genotype plus genotype by environment interactions biplot showing ideal environments for cowpea grain yield. PC = Principal component, AEC = Average environment coordination

5.5 Discussion

5.5.1 The ANOVA for AMMI analysis of cowpea grain yield

Significantly high ($P \leq 0.001$) genotype main effects for grain yield indicated wide variations between the cowpea mutants, IITA and South African genotypes. The significant variation indicated that superior Namibian mutants, IITA and South African genotypes could be identified and selected for grain yield. The significant genotypic variation for grain yield could be attributable to the variations in the genetic backgrounds of the materials since the cowpea mutants were obtained from Namibia, the IITA genotypes from Nigeria, and the normal genotypes from the ARC in South Africa. Previous research investigated the diversity in Namibian cowpea mutations for grain yield and morphological characteristics and reported significant variation among genotypes (Horn et al. 2018). Significant variation of South African cowpea breeding populations has been reported for grain yield and yield traits (Gerrano et al. 2015, 2019, 2022; Mbuma et al. 2021). These studies investigated different breeding populations in diverse environments, further highlighting that the variation present in cowpea populations could be attributed to different genetic backgrounds and environmental factors.

The environment effect was highly significant which indicated the differences of the environmental impact on the performance of the genotypes. These could be explained by the diverse environmental conditions in which these cowpea genotypes were planted and evaluated. Significant GE interaction effects for grain yield were observed when cowpea genotypes and growing environments interacted, indicating that genotypes varied in performance in accordance with the environment. The differences in genotype performance across environments could be attributed to the variation in soil pH which ranged from acidic to alkaline, which affects plant growth, seed germination and ultimately grain yield (Uguru et al. 2011; Gentili et al. 2018). The fluctuation observed in the genotype performance across environments for grain yield could also be attributed to the severe pest and disease infestation in some of the test environments (Mafikeng and Polokwane), mainly aphids, which were caused by high temperature and humidity of these environments. Most of the genotypes survived the aphid infestation besides for Namibian mutants (ShL3P7-2, NKR8P9, ShR10P10 and ShL2P7) and IITA genotype (98K-476-8) in Polokwane, and Namibian mutant (NKR8P9) in Mafikeng which resulted in reduced grain yield. The significant GE interactions for cowpea grain yield indicate that genotypes with good adaptability and stability should be identified in test environments through further testing with techniques such as AMMI and GGE analysis. In South Africa, significant GE interactions for grain yield and morphological characteristics have previously been reported (Shiringani and Shimelis 2011; Gerrano et al. 2019; Mbuma et al. 2021). Significant GE interactions for superior cowpea genotypes have also been reported for grain yield, maturity and grain size in Nigeria (Ishiyaku et al. 2017; Nassir et al. 2021). These studies highlighted the importance of quantifying the nature and magnitude of GE interaction in diverse environments. It is noteworthy that the current study was conducted in multi-environments for one season, therefore, further research should consider evaluating cowpea mutant genotypes in multi-seasons. Previous studies (Gerrano et al. 2020; Mbuma et al. 2020) on cowpea have reported significant genotype by season interaction effects for grain yield and morphological characteristics.

5.5.2 Mean value performance of genotypes across the environments

Namibian mutants ShR10P12 and NKR1P3 (Bloemfontein, Mafikeng, Potchefstroom and Taung), BrR11P2 and ShL2P7 (Bloemfontein, Polokwane, Potchefstroom and Taung), and IITA mutant 98K-476-8 (Bloemfontein, Mafikeng, Polokwane and Taung), Namibian mutants ShR10P10 (Bloemfontein, Polokwane and Taung), ShR3P4 (Mafikeng, Polokwane and Potchefstroom), ShR9P5 (Bloemfontein, Potchefstroom and Taung), NKRuP5 (Polokwane, Potchefstroom and Taung), NKR8P9 and IITA genotype ITOOK 1263 (Mafikeng, Polokwane and Taung), IT82E-18 (Bloemfontein, Polokwane and Potchefstroom), IT99K-573-2-1 (Bloemfontein, Potchefstroom and Taung), and South African genotypes Oloyin (Bloemfontein, Mafikeng and Taung) and Pan 311 (Bloemfontein, Mafikeng and Polokwane) had high grain yield in at least three environments, indicating the broad adaptation of these cowpea genotypes. This means genotypes showing high grain yield in multiple environments have broad adaptation to those environments. Overall, the Namibian mutants and IITA genotypes had higher grain yield than the South African genotypes in all the tested environments. These results indicate that these improved genotypes will contribute to the ARC cowpea germplasm collection for future crop improvement of South African genotypes. Additionally, the diverse adaptation of these cowpea mutants and genotypes suggested the possibility to use them as parental lines for future cross breeding for development of broadly adapted cultivars, to meet future demands and to mitigate climate change.

Namibian mutants ShR4P1 and BrR11P11 (Polokwane and Potchefstroom), BrR4P11 (Bloemfontein and Taung), IITA genotype ITOOK 1263 and South African genotype Bechuana White (Mafikeng and Polokwane), IITA genotype IT07K-318-33 (Bloemfontein and Mafikeng), South African genotypes Glenda (Mafikeng and Polokwane) and Enchore (Bloemfontein and Potchefstroom), Namibian mutant NKL9P7, IITA genotype IT93K-452-1 and South African genotype Orelu (Bloemfontein), and Namibian mutants ShR2P11 and NKR9P9 (Mafikeng and Potchefstroom) had high grain yield in at least one environment, which indicated specific adaptation of these cowpea genotypes. These genotypes are therefore not stable across environments. To understand the extent of their stability, these genotypes should be replanted for more seasons and additional environments. Furthermore, cowpea mutants and genotypes with specific adaptation to environments should be exploited for niche breeding in South Africa.

On average, the Namibian mutants BrR11P2, NKR1P3, ShR10P10, ShR10P12, ShR3P4, ShR4P1, ShR9P5, NKR8P9, NKRuP5 and ShL2P7, IITA genotypes 98K-476-8, IT99K-573-

2-1 and ITOOK 1263, and South African genotype Enchore had high grain yield compared to the grand mean across the environments which indicated the possibility of broad adaptation. Most of the superior genotypes across the environments were mutants, suggesting that high grain yield across the environments could be expected from the mutants. The identified superior mutants for grain yield can be used as parents for future combinations to broaden the existing narrow genetic diversity in the cowpea germplasm collection.

5.5.3 Adaptability, additive main effects and multiplicative interaction (AMMI) biplot analysis and stability ranking of genotypes

Namibian mutant (BrR11P2) had the highest grain yield and was located close to Potchefstroom on the biplot indicating specific adaptation. Namibian mutants (ShR10P10, ShR10P12 and NKR1P3), and IITA genotypes (ITOOK 1263 and 98K-476-8) had high grain yield and were located close to Taung which indicated adaptation to the environment. Additionally, both Taung and Potchefstroom were high yielding, but unstable environments. These two environments are found in the same province (North West); therefore, the GE interaction is due to the different soil characteristic of the environments (Table 5.3). The most stable cowpea genotypes were Namibian mutants (NKL9P7, ShR10P12 and ShR2P11), IITA genotype (ITOOK 1263) and South African genotype (Agrinawa), indicating the potential of these genotypes to be planted in diverse environments. Since AMMI biplots do not provide a simple measure to identify superior genotypes, AMMI stability ranking is used to help identify stable genotypes (Mbeyagala et al. 2021; Gumede et al. 2022). Through the ASV value, stable genotypes such as Namibian mutants (NKL9P7, ShR10P12 and ShR2P11), IITA genotype (ITOOK 1263) and South African genotype (Agrinawa) were identified. However, most of the stable genotypes did not have high grain yield, thus, during the selection process, breeders should consider stable genotypes with high grain yield and those stable genotypes with low grain yield should be discarded since low grain yield plus stability is an indication of consistently poor grain yield (Yan and Tinker 2006).

5.5.4 Genotype plus genotype by environment interactions (GGE) biplot analysis, ideal genotypes and environments for cowpea grain yield

Namibian mutants (BrR11P2 and ShR10P10) were situated at the corner of the polygon, which suggested that these genotypes had the highest grain yield at all the five environments. The

GGE biplot identified two mega-environments indicating the possibility of reducing the number of testing environments, particularly the ones that have a similar influence on the performance of genotype for grain yield. Namibian mutants (BrR11P2, ShR10P12, NKR8P9, ShR4P1 and NKR9P9), IITA genotypes (IT99K-573-2-1 and IT07K-318-33), and South African (Oloyin, Bechuana White and Enchore) were located in the major mega-environment (Potchefstroom, Bloemfontein, Polokwane and Mafikeng), which indicated the potential broad-adaptation of these genotypes. The advantage of a mega-environment is that the number of testing sites can be reduced, thus saving operational costs for resource limited breeding programmes. Namibian mutants (ShR10P10, ShR10P12, NKR8P9 and NKR1P3), and IITA genotypes (IT99K-573-2-1, 98K-476-8 and ITOOK 1263) were located in the second mega-environment (Taung and Mafikeng), which indicated potential specific adaptation. It is noteworthy that both Taung and Mafikeng are located in the same province and some of their weather conditions, soil types and nutrition are similar as mentioned in table 3.2 and table 3.3, hence, they appear to influence these genotypes similarly. In the case of this study, Mafikeng was adversely affected by aphid infestation, which may have influenced this data. In a resource limited breeding programme, multiple environments within one mega-environment indicate redundancy, meaning that some environments can be dropped to save costs (Gerrano et al. 2020; Mbuma et al. 2020).

Namibian mutants (BrR11P2, ShR10P12, NKR8P9, NKRuP5, NKR9P9, NKR1P3 and ShR10P10), and IITA genotypes (IT99K-573-2-1 and 98K-476-8) had high grain yield and showed broad adaptation and stability making them ideal genotypes. Thus, these cowpea mutants could be selected for release and commercial production. These ideal cowpea mutants can also be used as parents to broaden the existing germplasm collection and they can be further exploited for genetic improvement of the crop.

An environment that can effectively identify superior genotypes and be able to accurately represent the overall environments is known as the ideal environment. Potchefstroom and Taung were identified as ideal environments indicating that these environments should be considered for the evaluation and selection of cowpea genotypes particularly for grain yield. Previous research also identified Potchefstroom as an ideal environment for cowpea production (Gerrano et al. 2020).

5.6 Conclusions

Grain yield was greatly influenced by the environments, genotypes and the GE interactions. Namibian mutants (BrR11P2, NKR1P3, ShR10P10 and ShL2P7), IITA genotypes (98K-476-8, IT99K-573-2-1 and ITOOK 1263), and South African genotype (Enchore) had high grain yield across environments and these genotypes can be considered for release and commercial production. Generally, cowpea mutants from Namibia and IITA genotypes had higher grain yield compared to South African genotypes. The genetic base of the cowpea germplasm collection in South Africa could be expanded by using superior mutants for grain yield as parents. The AMMI ANOVA showed that most of the phenotypic variation was attributed GE interaction. Stable genotypes (Namibian mutants NKL9P7, ShR10P12 and ShR2P11, IITA genotype ITOOK 1263 and South African genotype Agrinawa) were identified. Two mega-environments, namely, 1) Taung and Mafikeng, and 2) Mafikeng, Bloemfontein, Polokwane and Potchefstroom were identified. Namibian mutants (BrR11P2, ShR10P12, NKR8P9, NKRuP5, NKR9P9, NKR1P3 and ShR10P10), and IITA genotypes (IT99K-573-2-1 and 98K-476-8) had high grain yield and showed broad adaptation and stability, thus, they should be considered as suitable parents for crop breeding and genetic development in the future.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1 General discussion

Characterisation of cowpea germplasm collections is important and offer opportunities for plant breeders to select genotypes with wide diversity for crop improvement. Therefore, it is necessary to understand phenotypic and genetic diversity, heritability, genetic variance and correlations between yield and yield components of the advanced breeding lines in order to select and develop adapted cowpea genotypes (Addisu and Shumet 2015). However, cowpea natural genetic variability is too limited for breeding due to the crop being self-pollinating, therefore induced mutation offers the creation of a significant amount of genetic variation in a relatively short time period. The objectives of this study were: 1) to determine the phenotypic diversity and characterise cowpea mutants and normal genotypes for grain yield and yield components, to identify superior cowpea mutants and normal genotypes and to determine the correlation between all measured characteristics, 2) to evaluate the variability of cowpea mutants and normal genotypes for protein content, selected mineral elements, phytic acid and the potential bioavailability of Fe and Zn, to identify superior cowpea mutants and normal genotypes and to determine the interrelationship between all measured characteristics, and 3) to determine GE interaction, to identify the superior genotypes for grain yield and to determine the adaptability and stability of cowpea mutants and normal genotypes in South Africa.

Significant genotype effects showed the existence of wide genetic diversity between the tested genotypes for grain yield, grain yield components, protein, selected mineral elements, phytic acid, potential mineral bioavailability, moisture and ash. The wide genetic diversity observed indicates that large phenotypic diversity exists among the tested Namibian mutants and normal genotypes which can be used in the cowpea improvement programmes. Previous study on cowpea has also reported significant variations among the cowpea genotypes for grain yield, protein and selected minerals (Mbuma et al. 2021).

Significant GE interaction for grain yield, yield components, protein content, selected minerals, phytic acid and potential mineral bioavailability indicated that the cowpea mutants and normal genotypes showed different performances across the test environments. Significant variation was observed for genotype groups of all the yield components indicating that there are wide differences between the Namibian mutants, IITA and South African genotypes. The high H^2 values (above 50%) between the different environments and across environments for grain yield, yield traits, protein, selected mineral elements, phytic acid and potential mineral bioavailability indicate that the traits can be selected with high efficiency. High H^2 between different cowpea populations is very important for plant breeders to be able to select traits of interest without difficulties.

Superior Namibian mutants (ShR10P12, ShR3P4, ShR4P1, BrR11P2, NKL9P7, NKR1P3, NKR9P9, NKRuP5, ShR10P10 and ShL2P7), IITA genotypes (98K-476-8, IT07K-292-10, IT82E-18 and IT93K-452-1) and South African genotype (Pan 311, Enchore and Bechuana White) were identified for good grain yield and yield components indicating that the genotypes can be released to the market and to small-scale farmers to contribute to overcoming hunger in hunger in African communities. The genotypes with high grain yield and yield components can be selected as parental lines to improve cowpea genotypes with low grain yield. Superior Namibian mutants (ShR10P10, ShR2P11, ShR9P5, ShL3P7-2, ShR3P4, ShR4P1, BrR11P11, BrR4P11, NKL9P7, NKR8P9, NKR9P9 ShR2P11, BrR11P2, ShL2P7, ShR3P4 and NKRuP5), IITA genotypes (98K-476-8, IT82E-18, IT93K-452-1, IT99K-573-2-1, IT07K-292-10 and ITOOK 1263), and South African genotypes (Oloyin, Orelu, Pan 311, Bechuana White, Enchore, Agrinawa, Dr Saunders and Glenda) for protein, selected mineral elements, phytic acid, ash and moisture were identified. The identified superior genotypes showed good nutrition and can potentially contribute to resolve hunger, mineral deficiency and malnutrition in rural areas where the people do not have access to meat products. A recent study identified superior IITA and South African genotypes for selected mineral elements which suggested that the genotypes could be used to advance the nutritional quality of the cowpea sold on South African markets (Mbuma et al. 2021). Furthermore, the genotypes mentioned in the study were identified to have low phytic acid concentration, which indicates better mineral bioavailability. However, PA:Fe indicated that only IITA genotype (IT93K-452-1) and South African genotypes (Oloyin and Orelu) were Fe bioavailable, while the PA:Zn showed a potential of poor Zn bioavailability in all the tested genotypes.

Grain yield was found to have significant and positive relationships with the yield related traits, indicating the possibility of simultaneously selecting yield related traits and indirectly improving grain production. The complexity of grain yield causes complications with directly selecting for grain yield, therefore, selecting for secondary grain yield traits is crucial to improve the production of the crop. Significant and positive correlations were discovered between protein content, selected mineral elements, phytic acid, ash, and moisture content, suggesting the possibility of selecting nutritional quality attributes simultaneously.

The PCA showed that Namibian mutants (ShR10P12, NKR1P3, ShR4P1 and ShL2P7), IITA genotype (98K-476-8) and South African genotype (Enchore) were associated with high grain yield, number of seeds per plant, pod weight per plant and number of pods per plant, but negatively associated with PH. The results suggest that selecting genotypes with high grain yield will affect the PH of the crop. Additionally, high PH can be linked to stalk lodging and breaking causing the cowpea plants to lose the pods or seeds. The cluster identified Namibian mutants (ShR10P12, ShR3P4, ShR9P5, BrR11P11, BrR11P2, BrR4P11, NKR1P3, NKR9P9 and NKRuP5), IITA genotypes (IT07K-292-10, IT07K-318-33, IT82E-18 and IT99K-573-2-1), and South African genotypes (Agrinawa, Bechuana White and Dr Saunders) which were associated with high protein content, Mn, phytic acid, PA:Fe, PA:Zn and ash, indicating that the genotypes can be selected to supplement the nutritious legumes already available like soybeans and chickpeas.

Using AMMI analysis, supported by the ranking stability value, Namibian mutants (NKL9P7, ShR10P12 and ShR2P11), IITA genotype (ITOOK 1263) and South African genotype (Agrinawa) were identified to be more stable than the rest of the tested genotypes indicating consistent grain yield production across the environments. The GGE biplot identified two mega-environments. Namibian mutants (BrR11P2 and ShR10P10) were situated at the corner of the polygon, which indicated that the Namibian mutants were superior to the IITA and South African genotypes. Namibian mutants (BrR11P2, ShR10P12, NKR8P9, ShR4P1 and NKR9P9), IITA genotypes (IT99K-573-2-1 and IT07K-318-33), and South African (Oloyin, Bechuana White and Enchore) were located in the main mega-environment (Potchefstroom, Bloemfontein, Polokwane and Mafikeng). These results indicate broad adaptation of the genotypes to different testing environments. A similar study conducted in Nigeria found the IITA genotypes to be broadly adapted compared to commercial genotypes (Oladejo et al. 2011). Namibian mutants (BrR11P2, ShR10P12, NKR8P9, NKRuP5, NKR9P9, NKR1P3 and ShR10P10), and IITA genotypes (IT99K-573-2-1 and 98K-476-8) were found to be ideal genotypes for high grain yield, stability and adaptability. These genotypes are of importance in South African breeding programmes as they can be included in breeding activities to improve cowpea productivity and broaden the genetic diversity. Potchefstroom and Taung were identified as ideal environments to test for grain yield, stability and adaptation.

6.2 Conclusions

The significant effects of genotype, groupings, GE interaction and grouping by environment interactions showed that there are huge phenotypic variations present between the Namibian mutants, IITA and South African genotypes. The high H^2 values across the environments for grain yield, grain yield traits, protein, selected mineral elements, phytic acid and potential mineral bioavailability indicated the potential for good response to selection. On average, superior Namibian mutants (ShR10P12, ShR3P4, ShR4P1, BrR11P2, NKL9P7, NKR1P3, NKR9P9, NKRuP5, ShR10P10 and ShL2P7), IITA genotypes (98K-476-8, IT07K-292-10, IT82E-18 and IT93K-452-1) and South African genotype (Pan 311, Enchore and Bechuana White) for grain yield, yield components, protein, selected mineral elements, phytic acid, ash and moisture content were identified. The genotypes could be used as potential parents to genetically improve the South African germplasm collection. The potential mineral bioavailability of the genotypes needs to be improved. The study identified simultaneous correlation of the traits for grain yield and secondary yield traits. The study also identified simultaneous correlation of the traits for nutritional quality traits. Stable Namibian mutants (NKL9P7, ShR10P12 and ShR2P11), IITA genotype (ITOOK 1263) and South African genotype (Agrinawa) were identified. Namibian mutants (BrR11P2 and ShR10P10) were found to be broadly adapted to the South African growing conditions. The identified ideal environments can be used for future evaluation and testing of genotypes before releasing new varieties.

6.3 Recommendations for future research

1. Research is required to determine seasonal variation and stability of cowpea mutants and normal genotypes for nutritional quality traits, grain yield and yield related traits.

2. There is a need for research to evaluate cowpea mutants and normal genotypes for heat tolerance, drought tolerance, disease and pest resistance.

3. There is a need to study the interrelationships between nutritional quality traits, grain yield and yield related traits in cowpea breeding populations.

4. A study on combining ability among the cowpea mutants and normal genotypes is required to determine the potential of these genotypes as parents for nutritional quality traits, grain yield and yield related traits.

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