CHARACTERISATION OF SELECTED COWPEA ACCESSIONS USING MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR MARKERS AND DEVELOPMENT OF DUAL PURPOSE F_1 COWPEA GENOTYPES

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Plant Breeding of Chuka University

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted for the award of a degree in any other University.

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Recommendation

This thesis has been examined, passed and submitted with our approval as university supervisors

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DEDICATION

I dedicate this thesis to my lovely mum Nancy Kiarie for her unwavering support in the entire period of this study and my two sisters Cicilia Wangui and Gladys Njeri for their encouragement. I wouldn't forget to dedicate this to my niece Nancy Wangui as a form of challenge to her that she should aim higher and let the sky be the limit to her education. I also dedicate this work to my late dad Daniel Njihia who I believe is proud of me.

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ABSTRACT

Cowpea is versatile dual-purpose crop that offers many human health benefits. However, grain and leaf yield remain low, which is often associated with the use of unimproved local varieties, biotic and abiotic stresses. The dual-purpose cowpea varieties have a higher water requirement compared to grain varieties. Therefore, there is need to develop dual purpose cowpea varieties with low water requirements so as to take advantage of drought tolerance trait found in cowpea plants. The objective of this study was to characterize a selected set of germplasm using morphological, molecular and biomarkers, develop dual purpose F_1 cowpea genotypes, and to identify a set of candidate traits. This will help to identify trait variation of characters of interest and breed for diverse traits. This study used a collection of 50 cowpea accessions. In addition, a subset of six accessions was used for the production of F_1 genotypes. The six accessions were crossed using a partial diallel design to produce 15 F_1 hybrids. A Randomised Complete Block Design was used to evaluate the 50 accessions, 15 F_1 and their parents in the field under imposed water stress and well water conditions with three replications and over two cultivations. Water stress was imposed at 28 days after sowing, where watering was done at the rate of 100 ml per plant per accession after every five days until maturity. Data was collected on growth and yield variables. At 35 days after sowing, leaf samples were harvested for metabolite profiling. About 20 accessions were genotyped using 12 SSR markers. Quantitative data was subjected to analysis of variance using SAS version 9.4 and significant means separated using the Least Significant Difference at $\alpha = 0.05$. Data from SSR markers was subjected to Analysis of Molecular Variance to identify genetic variation among accessions and Euclidean distances was used for cluster analysis. The study showed that there was significant ($p < 0.05$) variation in all growth and yield variables amongst the genotypes studied. Several genotypes including NA101, NA3, NA11, MA24 and MA2 were identified to be drought tolerant and also potential genotypes for grain production. The allelic range for the genotypes that were studied was from 1 to 11 alleles which showed a wide variation among markers. The SSR markers revealed considerable genetic diversity in the cowpea population studied. Markers VM70, VM31, and VM68 were the most informative, showing high numbers of alleles, gene diversity, and PIC values. Two markers (VM61 and VM19) were monomorphic in this population. The Structure results indicate the high morphological variation observed among the accessions studied. There is significant variation in quercetin and myricetin content among the genotypes. Genotypes such as MA67 X NA11 and NA60 are particularly represented extreme cases in the concentration of these flavonoids. he results suggest potential for developing cowpea varieties with improved yield while maintaining moderate levels of beneficial flavonoids. The accumulation of the flavonoids was observed as a part of the plant's adaptive response to mitigate the adverse effects of drought stress. Grain yield was strongly influenced by traits such as number of seeds per pod, number of pods per plant, number of branches per plant, terminal leaf length and width, plant height and biomass yieldMA67 X NA60, NA20 X NA60, MA50 X MA56 and MA50 X NA60 were identified as potential genotypes for grain production. F_1 hybrids such as MA67 X NA60, NA20 X NA60 and MA50 X MA67 are potential dual-purpose genotypes due to their high yield potential for both grain and vegetable production. Genotypes MA50 X NA60 and KK06 X MA67 hybrids exhibit better drought resistance demonstrating the potential likely combination of favourable traits from parents. Several genotypes, particularly MA67 X NA60 and NA20 X NA60, demonstrated strong heterotic potential for both yield and biomass. The wide variation in heterosis across the F1 hybrids suggests that specific combining abilities play a crucial role in determining

hybrid performance. Some crosses show positive heterosis for both yield and biomass while others show contrasting responses, indicating the need for balanced selection in breeding programs. Those genotypes that show a high level of diversity as discovered in this study should be harnessed to improve accessions that are less favoured. The characterized cowpea accessions should be utilised for breeding programs aimed at developing improved varieties with desirable traits.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
BICMV	Black Eye Mosaic Virus
CABMV	Cowpea aphid borne mosaic virus
CPGMV	Cowpea Molden Mosaic Mirus
CPMMV	Cowpea Mild Mottle Virus
CPMV	Cowpea Yellow Mosaic Virus
GC-MS	Gas Chromatography Combined with Cass Spectrometry
IITA	International Institute of Tropical Agriculture
ISSR	Inter Simple Sequence Repeat
NGBK	National Gene bank of Kenya
PCR	Polymerase Chain Reaction
PVS	Participatory Varietal Selection
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SBMV	Southern Bean Mosaic Virus
SCoT	Start Condon Target
SHMV	Sun Hemp Mosaic Virus
SNPs	Single Nucleotide Polymorphism
SSRs	Simple Sequence Repeats

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Cowpea (*Vigna unguiculata* L. Walp) is an annual crop in the family Fabaceae (Ibrahim *et al*., 2017). It is a widely adapted multi-purpose legume crop that can be used in production of grain, vegetable and fodder (Dinesh, *et al*., 2022). Cowpea is drought tolerant and performs well in a wide variety of soils. It can be used as an intercrop thus help in replenishing low fertility soils (Molosiwa *et al.,* 2016). World-wide, cowpea production is estimated at approximately 15 million ha of land planted each year with the total annual production of 8.9 million metric tonnes FAOSTAT 2022). In Africa, cowpea is widely cultivated in Central and West Africa, with Nigeria being a leading producer (FAOSTAT 2022).

Cowpea has many uses. The fresh leaves, immature pods and the grains of cowpeas are nutritious and provides protein, carbohydrate, vitamins and mineral (Mekonnen *et al.*, 2022). Cowpeas are a good source of protein ranging from 23% to 25% protein on a dry-weight basis (Mamiro *et al*., 2011). Cowpea seed grain is used to supplement the protein deficiency of the predominantly carbohydrate diet of low-income families. Cowpea leaves are rich in micronutrients, nutraceuticals, beta-carotene, alpha tocopherols, flavonoids, lycopene, antioxidants and anticancer agents (Kirigia *et al.*, 2018). The rich nutritional property of cowpea leaves makes them ideal for efforts aimed at reducing food and nutrition insecurity. The nutrient composition of cowpea is superior to that of many vegetable crops (Bittenbender *et al*., 1984). Moreover, the cowpea leaves are also rich in calcium, iron, zinc, fibre and phytonutrients (Enyiukwu *et al.*, 2018). Cowpea leaves have been exploited for food, feed or fodder and medicine (Sonta *et al.*, 2019). If well managed cowpea can provide grain and vegetable to both rural and urban communities and thus offers opportunities for earning income and food security. Therefore, there is need to develop dual purpose cowpea varieties that combines such attribute to ensure profitable and sustainable cowpea production. Thereby maximizing the output from land and labour.

In Kenya cowpea yields have been low varying from 500 to 840 kg/ha (FAOSTAT 2022) compared to potential yields of 1500 to 3000 kg/ha (Avosa *et al.*, 2020). Several biotic and abiotic factors such as insect pests, diseases, poor soil fertility and drought

contribute to the reduction of cowpea yield (Gomes *et al.,* 2019). Among the abiotic factors, drought has been identified as a major limitation restricting cowpea production (Boukar *et al*., 2018). During the vegetative phase, water deficit causes leaf and plant growth reduction, alteration in the process of nutrient absorption due to low water availability in the environment, increase in stomatal resistance, and ultimately, a decrease in gaseous exchange between the environment and the plant (Olajide *et al*., 2017).

Drought conditions weaken the plants making them more vulnerable to disease infestations and insect pests attacks. Drought impairs most morphological, physiological and biochemical processes in plants, reducing plant growth, nutrient uptake, photosynthesis, and assimilate partitioning, thereby strongly reducing crop productivity (Lamaoui *et al*., 2018). Vegetable producing crops are more sensitive to drought as compared to many other crops, since most of drought adaptations tends to reduce the duration of growth (Mitri, 2001). Considering this scenario and thus cowpea germplasm has different level of drought tolerance, the cowpea productivity could be considerably improved by developing new varieties that are better adapted to water stresses, good quality and dual purpose.

Genetic diversity plays an important role in the success of any breeding programme (Govindaras *et al*., 2023). Hence, before initiating selection of traits of interest, there is need to screen a large set of genotypes to identify trait variation for characters of interest. Genetic diversity offers great opportunities for developing new and improved cultivars with desirable farmer preferred traits (Govindaras *et al*., 2023). There is need for continuous evaluation of genetic diversity in available germplasm to identify accessions with novel genes for diverse traits such as leaf quality, drought resistance, productive and leafy canopies and grain yield. To identify trait or gene of interest the available germplasm can be characterized using morphological, biochemical and molecular markers (Molosiwa *et al.,* 2016). Use of morphological characteristics is a common approach because they form the most direct measure of the phenotype, readily available and relatively cheaper requiring simple equipment. However, morphological markers are subject to environmental influences in the field that may mask the real genetic variation among genotypes.

Molecular marker techniques are powerful in determining the genetic diversity. This is because molecular markers are not influenced by the environment (Ahmed *et al*., 2017). Some of the DNA molecular markers that have been used in the analysis of genetic diversity include Restriction Fragment Length Polymorphism (Ouédraogo *et al.*, 2002), Amplified Fragment Length Polymorphism (Kolade *et al*., 2016), Simple Sequence Repeats (Chen *et al.*, 2017), Randomly Amplified Polymorphic DNA (Udensi *et al*., 2016), Single Nucleotide Polymorphisms (Carvalho *et al*., 2017), Inter Simple Sequence Repeat and Start Codon Target (Igwe *et al.*, 2017). Microsatellite or Simple Sequence Repeats (SSRs) is the most widely used marker in genetic diversity analysis due to its multiallelic nature, high reproducibility, co-dominant inheritance, abundance and extensive genome coverage that has already been reported for crops like cowpea (Kimaro *et al.,* 2020).

Generally, plant and seed morphological traits are not sufficient to characterize cowpea genotypes. Therefore, molecular and biochemical markers are also used for a better evaluation of traits variation (Carvalho *et al.,* 2017). Metabolite profiling could well-facilitate the identification of biochemical markers for plant stresses (Sumner *et al.,* 2003). Primary metabolism changes are one of the initial reactions to a variety of biotic and abiotic stimuli, but secondary metabolism is more flexible and may set up the end points for storing the knowledge gained during the adaptation process (Arbona *et al.,* 2013). Metabolite profiling of amino acids, proline and glycolate has been used to study drought response mechanisms and to predict drought tolerance (Pires *et al*., 2016; Yadav *et al.,* 2019).

Specific compounds such as sucrose, raffinose, proline, and γ-aminobutyric acid are generally accumulated in different levels during plant abiotic stresses (Bueno and lopes, 2020). Suggesting that the stress-specific plant metabolites response can be used as biomarkers for an inhibition or activation of a defined metabolic pathway. Metabolite profiling has been considered one of the most promising approaches for the detection and/or quantification of primary and secondary stress-responsive metabolites in abiotic stresses (Bueno and Lopes, 2020). Some of the metabolites related to drought include quercetin, proline and galactinols. Taste is also an important determinant of utilisation of cowpea (Biama, 2021), especially its consumption as leaf vegetable since many farmers complain about the bitter taste in cowpeas. Therefore, there is need to profile

the metabolites responsible for taste and use them a proxy in breeding for dual –purpose and/or vegetable cowpea varieties.

Dual-purpose cowpea varieties, a plant bred for leafy vegetables and grains allow farmers to exploit the nutritional benefits of both the grain and leaves (Dube and Fanadzo, 2013). Hence, it can enable the farmer with small area of land to obtain both human food and animal feed. The production potential of dual-purpose varieties is 800-1800 kg/ha but the production is as low as less than 500 kg\ha (Karanja *et al*., 2006). Some of the varieties that have been bred for dual purpose include Machakos 66, Katumani 80, and KVU. Currently, most of dual-purpose varieties are bred for medium altitude areas (1200 – 1500 m above sea level) since they have high water requirement. Therefore, there is need for breeding dual purpose varieties that are drought tolerant. Moreover, for utilisation of cowpea leaves many farmers complains about the bitter taste, for example, many consumers find Ken Kunde a bitter vegetable. Therefore, this necessitates the production of cowpea varieties with improved leaf quality attributes including taste. Moreover, attention on cowpea breeding has shifted to developing nutritionally superior dual-purpose varieties, which can be used for production of leafy vegetables and grains (Noubissié *et al.*, 2011).

Once traits of interest are identified using morphological, molecular and biomarkers, the traits need to be incorporated into one or more potential varieties and/or genotypes. After crossing it is important to estimate the genetic parameters of the hybrid genotypes in order to get information on genetic components of the trait inheritance and heritability (Fasahat *et al.*, 2016). Crop improvement depends upon the magnitude of genetic variability and the extent to which desirable characters are heritable (Sajjan *et al.*, 2016). The development of improved cultivars will depend on use of a cheap and reliable method to evaluate genetic parameters. The diallel mating system provide fairly reliable mechanism, especially in self-fertilized crops like cowpea, to assess the genetic parameters of traits on interest (Noubissie *et al.*, 2011). This can be done through estimation of general combining ability (GCA) and specific combining ability (SCA) (Jocic *et al.*, 2015).

1.2 Statement of the Problem

Cowpea is prospective crop due to its various inherent attributes, which include nutritional benefits. However, its production is constrained by increased soil moisture deficit and lack improved varieties that are in tandem with changing climatic conditions and human lifestyle. To breed for diverse traits there is need to screening a large number of genotypes to identify trait variation for characters of interest. Currently, most of the local varieties are bred either for grain or vegetable production. With increase in demand for cowpea vegetable and reduction in acreage of land suitable for production of cowpea, there is need to develop novel varieties that combine grain and leaf production. Moreover, most of consumers complain about the bitter taste of cowpea leaves. Therefore, to enhance utilisation of cowpea leafy vegetable, there is need to incorporate genes that lead to improvement of the taste attributes of the leaves on newly developed dual-purpose cowpea varieties. In addition, with increase in soil moisture stress and higher water requirement by leaf producing crops, it would be necessary to incorporate drought tolerant genes in newly developed dual purpose cowpea varieties. Estimation of genetic parameters is important since it helps in identification of superior parental combinations. Though morphological markers act as indicator of genetic variability they are highly influenced by environmental conditions, hence, the need to incorporate molecular markers for effective identification of variation of traits of interest. Moreover, metabolite profiles are good biomarkers to tag both secondary and primary metabolite that affect quality of the genotypes developed.

1.3 Objectives of the Study

1.3.1 General Objective

To characterise selected set of germplasm using morphological, molecular and biochemical markers to identify a set of candidate traits, and development of cowpea dual purpose genotypes.

1.3.2 Specific Objectives

i. To characterise selected cowpea genotypes for growth yield and quality using morphological, biochemical and molecular markers.
ii. To evaluate the performance of developed F_1 hybrid cowpea genotypes and their parents under field conditions for growth, yield and drought tolerance.

iii. To estimate the combining ability, heritability and heterosis of the F_1 cowpea hybrid genotypes

1.4 Hypotheses

$H0_1$: There is no significant variation in selected cowpea genotypes for growth, yield and leaf quality attributes using morphological, biochemical and molecular markers.

$H0_2$: There is no significant difference in the performance of the developed F1 cowpea hybrids and their parents under field conditions

$H0_3$: There is no variation in combining ability, heritability and heterosis of the developed F_1 cowpea hybrid genotypes

1.5 Justification of the Study

Breeding for improved dual-purpose variety is an alternative strategy for increasing cowpea diversification, production and utilisation. Cowpea serves as a source of nutrition for millions of people in many countries (da Silva *et al*., 2018). It is rich in protein (25%), carbohydrates, vitamins, minerals, dietary fibres, fatty acids and other nutrients (Mekonnen *et al.,* 2022). The leaves for instance, are more nutrient-dense than many other leaf vegetables. Characterisation of available genetic resources helps in identification of the traits of interest such as quality attributes, pest and disease resistance and yield potential. Consequently, this form a basic resource from which various types of varieties such as high yield, drought and pest tolerant, dual purpose and early maturing can be developed. Development of high yielding varieties (grain, vegetable or dual purpose) will help in increasing productivity and thus ensuring food security and sustainable agriculture.

Despite the advances to unveil drought response mechanisms from gene to the whole plant, drought is still one of the major yield constraints, turning crucial to select and improve tolerant cultivars with both short- and long-term acclimation mechanisms to water shortage episodes (Hasan *et al*., 2018). Drought impairs most morphological, physiological and biochemical processes in plants, reducing plant growth, nutrient uptake, photosynthesis, and assimilate partitioning, therefore strongly reducing crop productivity (Fahad *et al*.,2018). Development of dual-purpose varieties that are tolerant to drought and of good quality will help in mitigation of food security in many

areas of the country. It will also help in generation of more income to the farmers. Dual purpose varieties with will help in reduction of malnutrition due to many nutritional values that are found in cowpea grains and leaves.

CHAPTER TWO
LITERATURE REVIEW

2.1 Botany and Overview of Cowpea Production

2.1.1 Botany of Cowpea

Cowpea (*Vigna unguiculata*) is an annual herbaceous legume belonging to the family Fabaceae and is cultivated for its edible seeds or leaves (Allah *et al.,* 2017). Cowpea can either determinate or indeterminate with the vines climbing supports or trailing along the ground, to a height of 2 m (Sirisha *et al.,* 2022). Breeding and crop improvement efforts have resulted in development of erect types which are non-trailing and determinate in growth habit (Muniu, 2017). The plant is primarily self-pollinating, which leads to relatively low genetic diversity within varieties (Egbadzo *et al*., 2014). Cowpea has a well-developed rooting system. The taproot can penetrate to a depth of 2.4 m after eight weeks (Davis *et al.,* 1991). Cowpea displays apogeal type of germination with the first pair of true leaves being simple and opposite, and subsequent leaves being trifoliate with oval leaflet (6 – 15 cm long and 4 – 11 cm broad). The size and shape of the leaves vary greatly, making this an important feature for classifying and distinguishing cowpea varieties (Pottorf *et al.,* 2012). Another distinguishing feature of cowpea is the long 20 -50 cm peduncles, which hold the flowers and seed pods. One peduncle can support four or more seed pods (Davis *et al*., 1991).

Flower colour varies through different shades of purple, pink, yellow, and white and blue (National Research Council, 2012). Cowpea growth duration varies with different genotypes and is affected by environmental conditions. Green pods for use as vegetable can be harvested 45 - 90 days after sowing depending on the variety. For grains, the crop can be harvested in about 90 - 125 days after sowing when pods are fully matured. Cultivated varieties can have pods between 10 and 110 cm long (Rawal and Kanti, 1975) A pod can contain 6 to 13 seeds that are usually kidney-shaped, although the seeds become more spherical the more restricted, they are within the pod (Sheahan, 2012). Their texture and colour are very diverse. They can have a smooth or rough coat and be speckled, mottled, or blotchy. The seed colours include white, cream, green, red, brown, and black, or various combinations (Davis *et al*., 1991)

2.1.2 Cowpea Production

Cowpea is one of the most versatile and resilient food legumes among the cultivated crop plants (Carvalho *et al.*, 2017). Being a drought-tolerant and warm weather crop, it is a promising food and forage species in a typical tropical lowland climate (Alemu *et al.*, 2016). Cowpea is grown across the world on an estimated 15 million hectares of land planted each year and the total annual production is 8.9 million metric tonnes (FAOSTAT, 2022). The global estimate shows that 13.1 million hectares are utilised in the production of cowpea with Western and Eastern Africa leading in production of 7.2 million tonnes on 12 million hectares and 0.9 million tonnes on 1.1 million hectares, respectively (FAOSTAT, 2022).

According to FAOSTAT (2022), cowpea is grown on an estimated 12.3 million ha in Africa with total annual production of 5.2 million tons with the bulk of production occurring on 10.6 million ha in West Africa. The major cowpea growing countries are Nigeria, Niger, Burkina Faso, Senegal, Mali, Ghana, and Cameroon in West Africa; Kenya, Sudan, Tanzania, Somalia, Uganda, Zambia, Zimbabwe, Botswana, and Mozambique in eastern and southern Africa (FAOSTAT, 2022). Nigeria is the largest producer of cowpeas producing 3.6 million tonnes grown in approximately 4.7 million hectares which is 77170 kg/ha. Niger is the second largest producer with 5.9 million hectares and the total production is 2.6 million tonnes (FAOSTAT, 2022).

According to FAOSTAT (2022), cowpea is grown on an estimated 235,734 ha of land in Kenya with total annual production of 250,260 tonnes. The total production per hectare is 10,616 kg/ha. Cowpeas account for roughly 16% of Kenya's pulse production and 90% of the production is grown in Eastern Kenya, mainly in Kitui, Machakos, Makueni, Embu, and Tharaka-Nithi counties. The main cowpea producing areas in Kenya are Kitui, Kisii, Migori, Kakamega, Bungoma, Machakos, Makueni, Kwale, Kilifi and Tharaka Nithi.

2.1.3 Utilization of Cowpea

Cowpea has many beneficial uses such as food, feed and income generation (IITA, 2009; Simion, 2018). Cowpea is mainly consumed as dry grain or fresh vegetable. All the parts of cowpea which can be used for food and feed (fresh leaves, immature pods, and grains) are nutritious, providing protein, carbohydrate, vitamins and minerals

(Alemu *et al.,* 2016). The grain of cowpea is the most important part of the cowpea plant for human consumption containing 22 – 23 % protein (as opposed to 2 % in cassava and 10 % in maize) and a good quantity of thiamine (vitamin B1), riboflavin (vitamin B2) and niacin (vitamin B3), and richer than cereals in iron and calcium content (Ngalamu *et al*., 2015). Cowpea has low fat content, which is important in the prevention of diverse metabolic and cardiovascular diseases (Enyiukwu *et al.,* 2018). The amino acid and vitamin content of the cowpea grain supplement those of cereals. In some cases, the pods are harvested when they are full sized, just before they dry out, and then the grains are cooked and eaten as a vegetable. Cowpea residue is an important source of fodder for ruminant livestock. Cowpea forage, both the vines and leaves, either fresh, or conserved as hay or silage, is often used for fodder (Atumo, 2018).

Cowpeas play a critical role in the management of soil fertility where they are often grown in sub-Saharan Africa, in terms of nutrient improvement and resistance to certain pests (Kebede *et al.,* 2020). It is an important component of the traditional cropping systems because it fixes atmospheric nitrogen and contributes to soil fertility improvement particularly in smallholder farming systems where little or no fertilizer is used (Beshir *et al*., 2019). Cowpea can fix about 240 kg ha^{-1} of atmospheric nitrogen and make available about 60 – 70 kg ha^{-1} nitrogen available for succeeding crops grown in rotation with it (Crops Research Institute, CRI, 2006). Besides, the crop performs well even in poor soils with more than 85% sand, less than 0.2% organic matter and low levels of phosphorus (Bilatu *et al*., 2012).

2.1.4 Ecological Requirements of Cowpeas

Cowpea requires an altitude of 1500 meters above sea level (Ramirez-Jaramillo *et al.,* 2022). Above this altitude there is the problem of frost damage (Hassen *et al.,* 2010). It requires a minimum of 200 to 500 mm of rainfall during the growing season, distributed to encourage vegetative growth and around flowering (Oweis *et al.,* 2004). Moisture deficiency mainly affects vegetative growth leading to low production. Cowpeas are adapted to a wide range of soils from sandy soil to well-drained clay soils. It requires a soil pH that range from 5.6 to 6.5 (Baligar & Fageria, 2007). Below pH 4.5, plant growth is impaired through limitation of development of the Rhizobium bacteria that are responsible for the nitrogen fixation (Zahran 1999). The cowpea crop thrives in a

warm climate at optimal temperatures of 20 °C to 35 °C. The maximum temperature during flowering should not exceed 30 °C (Wang *et al.*, 2015).

2.1.5 Cowpea Production Constraints

Cowpea productivity in Kenya is generally low, ranging from 500 to 840 kg per hectare (FAOSTAT, 2022) compared to its yield potential in the range of 1500 to 3000 kg per hectare (Avosa *et al.*, 2020). The low productivity has been attributed to both biotic and abiotic factors. These factors include insect pests, diseases (fungal, viral and bacterial), poor soil fertility, metal toxicity and drought. Among the abiotic factors, drought has been identified as a major limitation restricting cowpea production (Boukar *et al*., 2018).

Drought has been reported as a major constraint in semi-arid tropics due to erratic rainfall in the beginning and towards the end of the rainy season (Olajide and Ilori 2017). The crop is usually subjected to drought stress in both seedling and terminal growth stages, and this causes substantial reduction in grain yield as well as biomass production. Drought conditions can either be intermittent when they occur at one or more intervals during the crops' growing period or terminal when there is progressive decrease in available soil moisture content resulting in severe drought (Ibitoye *et al.*, 2015). During the vegetative phase, water deficit causes leaf and plant growth reduction, alteration in the process of nutrient absorption due to low water availability in the environment, increase in stomatal resistance, and ultimately, a decrease in gaseous exchange between the environment and the plant (Boukar *et al*., 2018). Water stress leads to a decrease in plant water content and turgor reduction and results in a decrease in cellular expansion (Iwuagwu *et al.*, 2017). With climate change, it is expected soil moisture deficit will aggravate, making even the current drought tolerant varieties susceptible. Thus, there is a need for the establishment of effective breeding programs to continuously develop high-yielding and well-adapted varieties for water deficit conditions. This is because cowpea production is depending on rain-fed agricultural systems and as such severe drought will seriously affect its production.

Other cowpea production constrains are field and storage pests (aphids, leaf beetles, pod borers and bruchids) and low soil fertility. Insect pests attack cowpea both in the field and in stores (Gbaguidi *et al*., 2013). Cowpea is attacked and damaged by insect

pests in all stages of growth (Adelaide *et al* 2018), causing direct damage to the plant or act as virus vectors. Pest can cause up to 100% grain losses if not controlled (Horn *et al*., 2015). Insects causes more loses if they attack the plant at pre-flowering, post-flowering and storage (Souleymane *et al.,* 2013). Seed com, maggot, cutworm, aphids, and leafhopper occur at the pre-flowering (seedling) stage. Aphids, leaf miners, and thrips are also active insect pests at the flowering and post-flowering stage, and aphids, bean fly, bean pod borer, leaf miner, and thrips are the common insect pests at the reproductive stage (grain filling period) of cowpea (Togola *et al.,* 2020). Among the field pests of cowpea, aphid (*Aphis craccivora* (Koch) is an important vegetative stage pest of cowpea in Africa but also occurs at other growth stages. Both nymphs and adults suck plant sap and cause serious damage from the seedling to the pod bearing stage (Mofokeng *et al.,* 2021). Aphids cause damage through secretion of honeydew, which promotes the growth of sooty Moulds and other fungi on leaves, curling of leaves and delayed flowering, shrivelling of pods and, as a result, reduced photosynthetic processes and rates, finally resulting in overall yield reduction (Stoddard *et al*., 2010).

Low soil fertility is also a great constraint to cowpea production. Cowpea does not require too much nitrogen fertilizer because it fixes its nitrogen from the air using the nodules in its roots (Sudharani *et al*., 2020). However, phosphorus is critical to cowpea yield because it stimulates growth, initiates nodule formation, and influences the efficiency of rhizobium-legume symbiosis (Verbree *et al*., 2015). Therefore, cowpea requires more phosphorus than nitrogen in the form of single super phosphate (Tharanathan *et al*., 2003). In addition, it is required in large quantities in young cells such as shoot and root tips where metabolism is high and cell division is rapid (Adebooye *et al*., 2007). It also aids in flower initiation and seed and fruit development (Affrifah *et al.,* 2001).

2.1.6 Cowpea Varietal Selection

Plant breeding involves choosing strategies that increase the probability of identifying new superior genotypes and/or varieties. Participatory varietal selection (PVS) enables identification of farmers-preferred crop genotypes for targeted breeding. Farmers' knowledge, preference and acceptance of newly developed crop varieties and production technologies are important for their ultimate adoption and use (Brocke *et al*., 2010). Often PVS trials are conducted under farmers' own fields based on their own

management conditions (Tiwari *et al.,* 2009). Due to its advantage in providing detailed information about the needs of the farmers on the newly developed varieties, this technique has been widely used (Thapa *et al*., 2009). The PVS provides the breeders with valuable feedback that enables them to focus their crosses and selection criteria meet the demands of farmers.

2.2 Characterisation of Cowpea Germplasm

Characterisation of genetic diversity among cowpea germplasm is important in any hybridisation programme (Agrawal *et al.*, 2018). Additionally, characterisation helps to eliminate duplications within the germplasm and select representative samples for utilisation and conservation in gene banks. Assessing the genetic diversity of cowpea germplasm is a prerequisite for effective breeding and germplasm conservation. Characterisation of available cowpea germplasm will help to identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection as desirable genes are introgressed from diverse germplasm into available genetic base. In any crop improvement program, the source of genetic resources can be sourced from the available germplasm in gene banks and farmers ecotypes. However, these acquired germplasm need to be assessed for availability of useful traits for crop improvement (Tan *et al*., 2012).

Cowpea is primarily a self-pollinating crop and its genetic base is considered to be narrow (Asare *et al*. 2010). Presence of diversity in the germplasm of crops is essential for successful crop improvement (Varshney *et al*., 2007). Limited genetic diversity poses a threat to the survival of a species as this limits ability to respond to changes in climate, pathogen populations and agricultural practices (Manifesto *et al*., 2001). Therefore, knowledge of genetic diversity in available germplasm is very useful and promoting the efficient use of genetic variations, through supporting proper selection of cross combination among large sets of parental genotypes. Generally, genetic diversity is estimated by measuring variation in quantitative and qualitative traits. However, characterization of quantitative traits is limited by the influenced of environmental conditions (Kameswara, 2004). Germplasm can be characterised using morphological, molecular and biochemical markers.

2.2.1 Morphological Characterisation

Morphological characterization is used routinely by plant breeders for the initial description and classification of germplasm in order to select genotypes for cultivation by farmers or in breeding programmes (Krichen *et al*., 2012). The desired traits must express in the target environment and this makes morphological characterization crucial in plant breeding. Knowledge on the key traits of the germplasm helps in making decision of parent. Selection for breeding purposes and reduces the number of germplasm that a breeder would have selected for initial screening (Bonierbale *et al*., 2020). Morphological characterisation is carried out by raising germplasm in a particular experimental design, then phenotypic traits are measured under field or controlled environment. Morphological evaluations are direct, inexpensive, easy and do not require expensive technology. However, they suffer from the constraints of environmental-sensitivity and subjective characterization when compared to other methods, such as image derived phenomics (Muraya *et al*., 2017).

Morphological characterisation enables identification and quantification of genetic variation for key qualitative and quantitative traits for ideotype breeding. Knowledge of phenotypic variation and traits relationship assist plant breeders to develop the most adaptive and productive varieties (Stoilova *et al.*, 2013). In Cowpea, the genetic diversity of phenotypic traits can be assessed using standard descriptors developed by the International Board for Plant Genetic Resource (IBPGR, 1983). The key morphological traits used as markers including number of pods per plants, number of seeds per pod and seed size, which have been found to have effect on potential yield of cowpea (Siise and Massawe, 2013).

Cowpea genetic diversity studies have been carried out in several countries using morphological and physiological markers (Siise and Massawe, 2013). Morphological markers are highly dependent on the environment for expression, in fact, several limitations reduce their ability to estimate genetic diversity in plants (Mafekheri *et al.*, 2017). Some morphological traits are mainly used as markers including pod per plants, seed per pod and seed size which have effect on potential yield of cowpea (Siise & Massawe, 2013). Assessment of local and regional plant genotypes is important for identifying diversities among germplasm which can help breeder to improve some local varieties and solving production constraints. Morphological analysis is quick and

commonly used method to identify and characterize the germplasm through phenotyping. Phenotypic characteristics that are influenced by environmental factors, however, may cause elevated or deflated diversity in the desirable agronomic traits thus lowering the reliability of the method (Marinoni *et al*. 2003).

2.2.2 Molecular Characterisation

Molecular markers are nucleotide sequences that are investigated through the polymorphism present between the nucleotide sequences of different individuals (Nadeem *et al*., 2018). Different molecular markers have different characteristics making them suitable for different purposes. Currently, there are more than 30 types of molecular markers available for assessing genetic diversity (Mondini *et al*., 2009). These markers have been widely applied to measure genetic diversity in crop plants and have played an important role in the characterization of crop genetic variation.

Unlike biochemical and morphological markers, molecular markers are environmentally stable and provide a significantly high genetic polymorphism (Dar *et al.,* 2019. The limitations associated with morphological markers for analysing genetic diversity in different plant species are largely removed by the usage of DNA markers. Molecular markers are considered as an effective tool for efficient selection of desired agronomic traits, since they are based on the plant genotype and not vulnerable to environmental and development stages influences (Franco *et al.,* 2001). Cowpea genetic diversity studies have been carried out in several countries using molecular markers such as Amplified Fragment Length Polymorphism, (AFLP), (Coulibaly *et al*, 2002), Random Amplified Polymorphism DNA, (RAPD), (Prasanthi *et al*, 2012), Restriction Fragment Length Polymorphism, (RFLP), (Fatokun *et al*., 1993) and Microsatellite or Simple Sequence Repeats (SSRs) markers (Chabane *et al*., 2014). Moreover, in some studies a combination of different markers systems (morphological and DNA markers) has been used to assess cowpea genetic diversity (Siise and Massawe, 2013).

Simple Sequence Repeats markers are the most frequently used markers in analysis genetic diversity (Siise and Massawe, 2013). Simple Sequence Repeat are informative markers with desirable genetic attributes such as high viability, being multiallelic, codominant inheritance, reproducibility, relative abundance and genome coverage

(Mafakheri *et al*., 2017). Simple Sequence Repeats have been used to identify genotype, seed purity evaluation and variety protection, pedigree analysis and genetic mapping of simple and quantitative traits and marker-assisted selection breeding (Brown *et al*., 1996).

2.2.3 Metabolite Profiling

Metabolite profiling or metabolomics is the comprehensive characterisation of small molecules present in a biological sample (Hollywood *et al*., 2006). It allows for studying multiple metabolites in a cell, tissue or organism. Metabolomics utilizes analytical platforms such as gas chromatography (GC-MS), liquid chromatography–mass spectrometry (LC–MS), high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy (Johanningsmeier *et al.,* 2016). With the advent of chemometrics and advanced analytical platforms, metabolomics has greatly facilitated the understanding of the global metabolome and pathway networks (Marshall *et al*., 2017). Metabolomics approaches involve untargeted or targeted analyses, and the selection of the approach is largely dependent on the experimental question and expected outcomes (Patti *et al*., 2012). Untargeted analyses utilize an unbiased profiling or metabolic fingerprinting approach focused on uncovering the global metabolome to evaluate diverse chemical classes of metabolites associated with different pathways. On the other hand, targeted analyses rely on a priori knowledge of the class of metabolites or pathways that are of interest (Scalbert *et al*., 2009). However, the combination of these analyses is often required to obtain complete information of interest.

Plants synthesis a vast array of primary and secondary metabolites. Primary metabolites perform their function as molecules signalling to trigger defence response by signal transduction and pathogen recognition processes (Chen *et al.,* 2014). Primary metabolism is important for growth, development and reproduction of cells. The primary metabolites participate in the primary response by the regulation of carbohydrates, protein and lipids to infection by pathogens (Huang *et al.,* 2008). Plant growth and yield is affected by primary and secondary metabolism during pathogen infection (Liu *et al*., 2012). Secondary metabolism refers to metabolic pathways and their associated small molecular products that are non-essential for the growth and reproduction of the organism (Yang *et al*., 2018). The secondary metabolites are natural

products for protection against biotic or abiotic environmental challenges (Dixon 2001). Thus, these compounds provide increased fitness due to their antimicrobial, anti-herbivory, and/or allelopathic activities. Many natural products also have other beneficial biological functions such as flavour, fragrance, and colour attractants (Frydman *et al*., 2004). Plants protect themselves by the production of chemical compounds against pathogenic infection (Scheideler *et al.,* 2002).

In plants, the secondary metabolic pathways produce a diversity of compounds, i.e. plant secondary metabolites (PSMs). Plant secondary metabolites contain a large group of structurally diverse compounds originated from either primary metabolites or intermediates in the biosynthetic pathways of these primary metabolites (Piasecka *et al.,* 2015). Plant secondary metabolites, depending on their biosynthetic pathways, are generally classified into several large molecular families: phenolics, terpenes, steroids, alkaloids, and flavonoids (Kessler and Kalske, 2018). Plant secondary metabolites play a variety of functions such as in plant growth and developmental processes, innate immunity (Piasecka *et al*., 2015), defence response signalling (Isah, 2019), and response to environmental stresses (Yang *et al.,* 2018). In addition, PSMs also have important functions such as repelling pests and pathogens, acting as signals for symbiosis between plants and microbes, and modifying microbial communities associated with hosts (Guerrieri *et al.,* 2019).

Improvements in metabolite profiling have rendered it an important tool for addressing biological problems (Lisec *et al.,* 2006). Metabolite profiling, like any technique concerned with measuring metabolites, requires the immediate inactivation of metabolism because the turnover of metabolites, as compared with proteins and DNA or RNA, is extremely rapid. Quenching of metabolism is generally achieved by rapidly freezing samples (at a constant temperature of 60^0 C or less). In addition, the whole procedure critically requires materials of the highest purity to prevent contamination, which can easily influence the outcome of the experiment. Several metabolites have been identified which are related to drought, which include sucrose, D maltose, mannitol, betain and proline. Metabolite profiling has been gaining space to study drought response mechanisms, improving genotype selection and identifying drought tolerance proxies (Degenkolbe *et al*., 2013). Organic osmolytes found in plants include low molecular weight compounds (sugar and sugar alcohols), methylated tertiary N

compounds (glycine betaine) and amino acids (proline and glutamate) and other low molecular weight metabolites (Chen and Jiang 2010). In addition, β-alanine betaine, proline betaine, dimethylsulphoniopropionate and pinitol have also been associated with adaptation in halophytic plant species (Slama *et al.* 2015).

Genetic engineering of metabolic conduit for a number of compatible solutes such as proline, glycine betaine, sorbitol, mannitol and trehalose has led to successful demonstration that transgenic plants display increased resistance to drought stress, high salinity and cold stress (Reguera *et al*., 2012). The initial response to drought stress appears to be associated with an increase in monosaccharides, while the more delayed response seems to be associated with an increase in fructan (Kerepesi and Galiba, 2000). In many higher plants, carbohydrate metabolism is modified under dehydration stress to favour the conversion of other sugars to sucrose (Whittaker *et al.* 2001). Metabolites related to quality include quercetin, di-hydro chalcone, myricetin and catechin.

The vast numbers of plant secondary metabolites represent an extreme challenge for large-scale metabolite profiling, i.e., metabolomics, and a singular tool for profiling all primary or secondary plant metabolites currently does not exist. Natural product classes are selectively extracted through the use of optimized solvents and often analysed separately or in parallel. There exist a growing number of successful technical methods that are employed in metabolic profiling of secondary metabolites (Kopka *et al*., 2004) and the selection of any specific method is usually a compromise between sensitivity, selectivity and speed (Trethewey *et al.*,2004). GC/MS is capable of profiling many of the smaller and volatile secondary metabolites including the isoprenoids (Lange *et al.*, 2001) triterpenoids such as ὐ-amyrin (Broeckling *et al*., 2005) and phenylpropanoid aglycones such as ferulic acid (Broeckling *et el.*, 2005). However, a large number of secondary metabolites are conjugated with sugars as described above and are not amenable to GC/MS even following derivatization. Therefore, high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) and mass spectrometry (MS) detection (Achnine *et al* 2005), capillary electrophoresis-MS (Hirai *et al.*, 2005), NMR (Mesnard and Ratclitte, 2005) and/or HPLC-NMR (Exarchou *et al.*, 2005) are heavily relied upon in most approaches for metabolic profiling of secondary metabolism.

Although metabolite measurements have been carried out for decades owing to the fundamental regulatory importance of these molecules as components of metabolic pathways, the importance of some metabolites in the human diet and their use as diagnostic markers for a wide range of biological conditions, including disease and response to chemical treatment, is only now being recognized. GC-MS facilitates the identification and robust quantification of a few hundred metabolites in a single plant extract (Halket and Zaikin 2003) resulting in fairly comprehensive coverage of the central pathways of primary metabolism. The main advantages of this technology are that it has long been used for metabolite profiling and thus there are therefore stable protocols for machine setup and maintenance, and chromatogram evaluation and interpretation. Although no single analytical system can cover the whole metabolome, GC-MS has a relatively broad coverage of compound classes4, including organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates and lipophilic compounds.

2.2.4 Evaluation of Cowpea Genotypes Uunder Field Conditions

Though cowpea is reputed to be one of the most drought tolerant crops in semi-arid Africa, yield of the crop increases significantly where drought stress is minimal (Padi, 2004). Different methods have been used in the evaluation of cowpea hybrid genotypes under field conditions. The parents and the F1s are normally evaluated together. Normally, the trial is planted using randomised complete design in case hybrids are few or in randomised complete bock design in case the hybrid seeds are not limiting.

There has been considerable progress at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, on breeding for enhanced drought tolerance in cowpea adopting simple, cheap and non-destructive screening methods (Ajayi *et al*., 2018). These screening methods have been developed by cowpea scientists and their effectiveness at identifying drought tolerant cowpea varieties have been confirmed (Singh *et al.,* 2002). It is necessary to identify more efficient methods for evaluating the levels of tolerance in germplasm for crossing and selection of segregated breeding material.

Shakel *et al.* (1982) argued that when selecting genotypes with increased drought resistance, it is reasonable to propose that the evaluation be made under irrigated

conditions. Because of inconsistencies observed under water deficit conditions, they concluded that irrigated conditions might be a more reliable indicator of genotypic differences than measurements with plants under drought. Breeders typically use two strategies to improve drought tolerance. These include the exploitation of heterosis and direct selection for drought tolerance and its components (Abdelmula *et al*., 1999). The General Combining Ability (GCA) which captures only the additive portion of the genetic variance was preponderant for days to flowering, hundred seed weight, and the number of seeds per pod. Hall *et al*. (1997) and Singh *et al*. (1997) have argued that early maturing varieties depend more on drought escape mechanisms, which enable them to complete their life cycle before the occurrence of terminal drought. If, however, they are exposed to intermittent moisture stress during the vegetative or reproductive stages, they perform very poorly.

Some cowpea varieties evade terminal drought by early flowering (around 12 days), whereas other varieties respond by staying green for weeks and flower later when favourable conditions are re-established (Fatokun *et al.* 2012). An important morphological trait that may contribute to drought adaptation is the delayed leaf senescence (DLS) trait (Gwathmey *et al.* 1992). This trait enhances plant survival after a mid-season drought damages the first flush of pods, which enables a substantial second flush of pods to be produced. Cultivars with DLS also have enhanced production of forage because their leaves remain green and attached to the plant until harvest. The DLS trait allows the crop to stay alive through midseason drought and recover when rainfall resumes. Most importantly, DLS can be easily measured by visual observation using an appropriate scale. Based on the above findings determination of chlorophyll fluorescence, delayed leaf senescence (DLS), biomass, pods per plant, seeds per plant, hundred seed weight and grain yield are suitable methods for screening large number of cowpea lines.

2.3 Development of Cowpea Hybrid Genotypes

Before starting a hybrid breeding program, the breeder must have a clear objective to develop a specific hybrid. One of major feature is choice of parents to be used in making crosses. Conventionally, the development of a hybrid involves an initial step in hybridization among selected parental lines having attributes of interest such as higher yield, resistance to any of the stress causing factors. This is then followed by advancing

the progenies through repeated self-pollination until the lines are homozygous and stable before extensive testing are conducted to identify improved lines (Khanna, 2018). Cowpea, being a highly self-pollinated crop, is inherently and botanically designed to avoid outcrossing. Hybridization requires special techniques to remove the anthers just before selfing occurs (Myers, 1996). The parents of a cross should not be genetically similar in order to maximize the diversity for selection and improvement (Nkhoma *et al*., 2020).

Cowpeas are generally easier to cross than other grain legumes. Cowpea flowers are large and easy to manipulate, the keel is straight, beaked and not twisted (Boukar *et al.*, 2018). There are only a few floral nodes per raceme, which tend to have a lower rate of abortion than many other species. In all the flowers of *Vigna* species, anthesis takes place just prior or simultaneously with the opening of corolla hence, flower buds destined to open the following morning are ready for emasculation (Muhammed *et al.*, 2015). After reaching the maximum unopened size, the flower buds become pale green.

Emasculation and pollination can be done at almost any time of the day although those that are done late in the afternoon are highly successful (Watts *et al.,* 2012). Apparently cool nights provide better conditions for fertilization than the hotter day time. The bud selected for emasculation is grasped firmly but gently to avoid any stress at the fragile attachment of the bud and raceme. A cut about two thirds the width of the unopened bud is made in the centre of the bud starting from its straight edge. Small finely pointed forceps or dissecting scissors, scalpels or even long thumb-nails can be used to make a cut. The upper portion of the folded petals is then grasped by the thumb and index finger and lifted outward tearing the upper portion of the petals free. This leaves the upper portion of the style, stigma and stamens free and exposed to facilitate removal of the 10 anther sacs with a scissor or forceps (Figure 1). The scissors or forceps should be dipped in alcohol (75 - 95%) between crosses and the receptive green tipped stigma should not be touched prior to pollinating. This emasculation procedure should require no longer than 15 - 25 seconds per flower.

To expose the anther sacs, the innermost petals are removed or slipped downwards and the mass of pollen on the hairy style can be used to pollinate 4 or 5 emasculated buds.

Only the obliquely arranged disc shaped stigma at the tip of the style is receptive (Figure 2).

plates 1: Emasculation of flower bud

plates 2: Emasculated flower bud

A small tag listing the cross and date is affixed to the raceme or peduncle beneath the pollinated bud (Figure 3). The crossed flowers are left open and uncovered. To reduce thrips and other insects likely to carry pollen, an insecticide can be applied at regular intervals. A good check on the success of a cross can be made three days after anthesis. Moderate temperature and increased humidity appear to increase percentage of fruit setting in hand emasculated crosses.

plates 3: Tagging of the flower bud

2.4 Estimation of Genetic Parameters, Heritability and Heterosis

Combining ability can be used evaluate genetic parameters of morphological traits. Combining ability in crosses is the ability of the genotypes to combine among each

other during hybridization process such that desirable genes or characters are transmitted to their progenies. Two concepts of combining ability general combining ability (GCA) and specific combining ability (SCA) have had important influence on evaluation of genetic parameters in plant breeding (Sprague and Tatum, 1942). General combining ability is the mean or average performance of a line in hybrid combinations which is usually due to additive gene effects (Acquaah, 2012). Specific combining ability refers to the deviation from additivity. It also refers to the combinations that perform better or worse than they would normally be expected based on the mean performance of the lines involved. It is due to non-additive gene action (Acquaah, 2012).

Several techniques can be used for estimation of combining ability. These include top cross suggested (Jenkins and Brunaon, 1932), poly cross technique (Tysdal *et al*., 1942) diallel cross (Griffing, 1956) North Carolina design (Comstock and Robinson, 1948). The most frequently used methods in the diallel analysis are Griffing's (Griffing, 1956) diallel procedures. There are three types of diallel designs, i.e., Method I, II and III. Method I or the full diallel design is complex and uses parents, one set of F_1s and reciprocal F_1s which may not add more useful information (Nduwumuremyi *et al*., 2013). Method III consists of parents and one set reciprocals. Method II consists of parents and one set of F_1s without reciprocals (Jocic *et al.*, 2015) and is the most appropriate in estimation of combining ability.

Heritability is a measure of phenotypic variance that can be explained by genetic variation (Visscher *et al.*, 2008). Heritability is important in determining a trait response to selection. Heritability can either be broad sense (H) or narrow sense (h^2). Broad-sense heritability is the extent to which the phenotype of an individual is determined by its own genetic constitution. The narrow-sense heritability is the ratio of additive genetic variance to the total phenotypic variance (Lush, 1937). Heritability in broad sense is computed as, $H = \frac{\sigma_g^2}{\sigma_p^2}$, while heritability in narrow sense is computed as, $h^2 = \frac{\sigma_A^2}{\sigma_p^2}$, where σ_p^2 is phenotypic variance, σ_g^2 is genotypic variance and σ_A^2 is addative genetic variance.

Heterosis can be created through hybridization between carefully chosen parents in self-pollinating crops (Kadam *et al*., 2013). Heterosis is the expression of an adaptive advantage of a progeny in relation to its parents, which can be quantified by faster growth, higher final biomass, greater flower fertility, and consequently higher yields (Birchler *et al*., 2010). Heterosis is a phenomenon whereby an F_1 hybrid exhibits superior phenotypic characteristics to the mean of the two parents. Heterosis can be assessed either as mid-parent heterosis or better parent heterosis (Angui *et al*., 2020). Mid-parent (MP) heterosis is the increase in the character of the hybrid compared to the mean of the parents. Better parent (BP) heterosis (hetero-peliosis) is the increase in the character of the hybrid for example yield compared to that of the better-parent (Kant *et al*., 2011). The exploitation of heterosis in crop plants is considered to be one of the key landmark innovations in modern agriculture. Heterosis is known to affect plant growth, the photosynthetic and transpiration traits, and the root traits (Kamphorst *et al*., 2022). Heterosis can also been used to study adaptive traits like resistance to abiotic and biotic stress (Dobzhansky, 1950). Therefore, understanding the physiological mechanisms associated with heterosis can reveal ways to increase the yielding potential (grain and/leaves) and to improve plant adaptation to water stress.

CHAPTER THREE
MATERIAL AND METHODS

3.1 Study Site

The evaluation of cowpea germplasm, F_1 and their parents was carried out at Chuka University Kairini farm. The farm is located on latitude 00°23'51.4''South and longitude 037°46'24.0'' East at about 1400 m asl. The rainfall received in this region is approximately 300 -700 mm of rainfall annually and the average temperature is 28 ^{0}C. The area has volcanic foot ridge fertile soils, deep well weathered with moderate to high inherent fertility (Jaetzold *et al*., 2006).

3.2 Experimental Designs

A Randomized Complete Block Design (RCBD) was used to evaluate a set of 50 selected cowpea accessions, F_1 cowpea genotypes and their parents in the field with three replicates (Appendix I). A partial dialled mating design was used in production of F_1 cowpea hybrid genotypes.

3.3 Field Experiments

3.3.1 Evaluation of Fifty Cowpea Accessions under Field Conditions

Prior to this study, a set of 50 accessions was selected based on a preliminary evaluation of 208 accessions which include 68 from National Gene bank of Kenya 104 from International Institute of Tropical Agriculture (IITA) and 36 from farmer preferred varieties collected from western and eastern part of Kenya. The selected set of 50 cowpea accessions include 21 accessions from International Institute of Tropical Agriculture (IITA), Ibadan; Nigeria, 27 from National Gene bank of Kenya, KALRO-Muguga and 2 from farmers preferred varieties, which were used for field evaluation. The 50 cowpea accessions were selected based on variables such as earliness, drought tolerance, pest attack and leaf production potential. Furthermore, six cowpea accessions were also selected for use in production of F_1 genotypes in this study. The selection was based on the extreme performance of the 50 cowpea accessions with respect to earliness, drought tolerance, pest attack and leaf production potential.

Fifty cowpea accessions were planted under water-stress and non-stress conditions. The size of the experimental units was 4.2 m × 1.5 m. The replicates were ridged 1.0 m apart and each accession was planted in single rows 1.0 m long. Spacing within a row was

60 cm and 30 cm between the rows with three replications for each in the two water regimes.

The cowpea accessions were sown in two experiments, with one experiment irrigated from planting to plant maturity while the second experiment was irrigated from planting for 28days after which irrigation was reduced to 100ml per plant per accession every 5days using drip lines. Weed control was carried out manually using hoes. Screening of drought tolerant genotypes was carried out according to Watanabe *et al*. (1997).

3.3.2 Development of F_1 Hybrids

Six selected cowpea accessions were planted on the field for development of F_1 hybrids. The accessions were planted at a spacing of 60 cm by 30 cm. A partial dialled mating design was used in production of 15 F_1 cowpea hybrid genotypes. The parental genotypes were crossed with each other in a partial diallel mating fashion, $\frac{n(n+1)}{2}$, where n is the number of parental genotypes. Forceps with a sharp, precisely pointed tip were used for emasculation of female flowers. To prevent contamination and the delivery of undesired pollen, the forceps were sanitized using 70% alcohol after every use. In cowpea plants, a flower bud that is ready for pollination is slightly paler than its regular deep green colour (Ogunkanmi *et al.*, 2007). Mature flowers were identified during morning hours or during the afternoon hours. It was then emasculated and the pollen transferred from a selected male parent to pollinate the emasculated female parent manually by hand. After pollination, a small tag indicating the code of the male and the female flower was fixed at the peduncle beneath the pollinated bud.

3.3.3 Evaluation of F_1 Cowpea Hybrids Under Field Conditions

The developed 15 F_1 cowpea hybrids were sown in ridges at a spacing of 60 cm by 30 cm. Randomised complete block design was used to lay the experiment. The experimental field was maintained free of weeds, pest and disease. Two experiments were laid out and watered normally for the first 28days after sawing. After 28days, watering in one experiment was reduced to 100ml per plant per accession after every 5 days while in the other experiment watering continued to maturity. The screening of drought tolerance was done according to Watanabe *et al*. (1997).

3.5 Data Collection

3.5.1 Morphological Characterization

Data was collected on three random plants selected from each row using the International Board for Plant Genetic Resources (IBPGR) cowpea descriptor (Appendix II). Data was collected on both quantitative and qualitative traits. The qualitative data included: growth pattern, twining tendency, plant pigmentation, terminal leaflet shape, raceme position, pod attachment to peduncle, immature pod pigmentation, leaf colour intensity, leaf marking, mature pod curvature growth habit, plant hairiness, seed colour and flower colour. Quantitative data included: plant height, plant canopy, terminal leaf length, terminal leaf width and number of main branches, at 28 days, 42 days and 56 days after sowing (IBPGR 1983).

To evaluate for dual-purpose genotypes, data on biomass (leaf production) was collected just before the pod setting where the whole plant was uprooted in two of the replications. The plant was defoliated and the leaves were weighed and also the stem and the roots were weighed. Grain yield was determined at the end of the experiment for remaining plants. All pods from each accession were harvested and the weight was determined and recorded.

To evaluate drought tolerant genotypes, data was collected at 1 day, 14 days, 21 and 28 days after the imposition of water stress. The scoring was done using a scale of 3 - 7, where 3 = resistant plants (plants alive with green leaves); 5 = tolerant plants (plants alive with most of the leaves yellow and/or wilting); 7 = susceptible plants (plants dead and dry) according to Watanabe *at al.,* (1997).

3.5.2 Molecular Characterization

3.5.2.1 DNA Extraction

DNA was extracted from young leaf tissues of 14-day old plantlets sown on trays in the laboratory and stored at -20 ^{0}C till DNA extraction. The genomic DNA was extracted from 20 accessions using the CTAB extraction protocol with minor modification (Mace *et al*., 2003). Leaf samples were frozen in liquid Nitrogen and grounded using a pestle and mortar until a fine powder is obtained. It was put into 1.5 ml Eppendorf tube.

Pre-warmed 950 µl of C-TAB buffer and 2 µl of 2-mercaptoethanol was added into each sample before incubating at 60 ^{0}C for 30 min. The solution was mixed by inverting tubes gently at intervals of 10 min. 700 µl of 24:1 chloroform: isoamyl alcohol was added into the solution and incubated for 5 minutes at room temperature. The mixture was then centrifuged for 10 minutes at 7500 rpm, after which the supernatant will be carefully transferred into newly labelled 1.5 ml tubes. 500 µl of ice-cold isopropanol will be added to the transferred supernatant to obtain a white precipitate and then stored at 20^0 C for 30 minutes. The precipitate was then centrifuged for 20 minutes at 12,000 rpm to pelletize the DNA. To dissolve the DNA, 1 × TE buffer will be added to the pellet and stored at 4 ^{0}C. After dissolution, the extracted DNA was treated with RNase by incubating at 37 ^{0}C for 45 min. The concentrated DNA extract was stored at -20 ^{0}C until required. Quality and quantity of extracted DNA was checked using agarose gel (1%) and spectrophotometer, respectively.

3.5.2.2 Polymerase Chain Reaction and Fragment Analysis

In polymerase chain reaction assay, a set of 12 primer pairs of SSR markers was used for genotyping in this study (Table 1). The forward primers were labelled with FAM and HEX allowing multiplexing of primer products. Polymerase chain reaction (PCR) was performed using the Eppendorf master cycler gradient thermo-cycler in a total volume of 25 µl containing 2.5 µl 109 PCR buffer, 1.5 mM MgCl2-, 0.5 µl dNTP's, 0.2 µl Taq polymerase, 18 µl of distilled water, 1 µl of each primer and about 50 mg of template DNA. Multiplexing of primers were done to allow amplification of more than one target sequence by using multiple primers in the reaction mixture. Amplifications were performed at 94 ^{0}C for 10 min, followed by 40 cycles of 94 ^{0}C for 25s, annealing temperature (56 ^{0}C, 58 ^{0}C, 59 ^{0}C, 60 ^{0}C and 62 ^{0}C) for 1 min, depending on marker triplets, (see Table 1), and 72 ^{0}C for 1 min, with a final extension at 72 ^{0}C for 10 min.

Microsatellite markers that showed polymorphism after screening on a 2% agarose gel electrophoresis at 80 voltages for 45 minutes were resolved for better resolution. Primers that showed unambiguous and clear polymorphism with the PCR products were used for this study The DNA was then quantified using a Nanodrop spectrophotometer 2000C (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Fragments were analysed on an automated capillary sequencer (Saiki *et al*., 1988).

Table 1: Cowpea Simple Sequence Repeat Markers

SN	Primer Name	Oligonucleotide sequence	Size(bp)	Annealing Temp.	Dye
1	CP 171F/172R	5′CATAGTAATATGGTATGTCAGTA3′ 5′CAACCGATGTAAAAAGTGGACA3′	200	56	FAM
2	MA 61	5′GATGTTATACACAGCAGCAAC 5′GGGAATCGAAAACAGACGCTA3′	150	56	FAM
3	Vm39	5′ GAT GGT TGT AAT GGG AGA GTC 3′ 5′ AAA AGG ATG AAA TTA GGA GAG CA 3′	212	58	FAM
4	Vm70	5′ AAA ATC GGG GAA GGA AAC C 3′ 5′ GAA GGC AAA ATA CAT GGA GTC AC 3′	186	58	FAM
5	Vm68	5′ CAA GGC ATG GAA AGA AGT AAG AT 3′ 5′ TCG AAG CAA CAA ATG GTC ACA C 3′	254	59	FAM
6	Vm31	5′ CGC TCT TCG TTG ATG GTT ATG 3′ 5′ GTG TTC TAG AGG GTG TGA TGG TA 3′	200	60	FAM
7	Vm14	5′ AAT TCG TGG CAT AGT CAC AAG AGA 3′ 5′ ATA AAG GAG GGCATA GGG AGGTAT 3′	144	62	FAM
8	MA70	5′GACTAGTGCAAGTTCCCAACC3′ 5′GAAGCAGAACCCAAAGAATCT3′	180	56	HEX
9	Vm3	5′GAG CCG GGT TCA ATA GGT A 3 5′ GAG CCA GGG CAC AGG TAGT3′	171	58	HEX
10	Vm22	5′ GCG GGT AGT GTA TAC AAT TTG 3′ 5′ GTA CTG TTC CAT GGA AGA TCT 3′	217	58	HEX
11	Vm26	5′ GGC ATC AGA CAC ATA TCA CTG 3 ′ 5′ TGT GGC ATT GAG GGT AGC 3′	294	59	HEX
12	Vm19	5′ TAT TCA TGC GCC GTG ACA CTA 3′ 5′ TCG TGG CAC CCC CTA TC 3	241	60	HEX
13	Vm40	5′ TAT TAC GAG AGG CTA TTT ATT GCA 3′ 5′ CTC TAA CAC CTC AAG TTA GTG ATC 3	200	59	HEX
14	Vm13	5′ CAC CCG TGA TTG CTT GTT G 3′ 5′ GTC CCC TCC CTC CCA CTG 3′	135	63	HEX

Source: Mafakheri *et al*., 2017: Potarot, 2012, Kusi *et al*., 2018, Ouedraogo *et al*., 2021

3.5.3 Metabolite Profiling

Leaf samples of cowpea accessions was collected 35 days after sowing (Kirigia *et al.,* 2018). Samples of approximately 5 cm were cut from the middle part of the fully developed fifth trifoliate leaf of five plants per plot, bulked, and immediately frozen using dry ice. The five plants in every block were sampled within a period of 15 seconds to minimize within-block error due to metabolic changes over time. All experimental units were sampled within 60 minutes. The 18 samples from 2 randomly chosen

experimental units from each replicate were subsequently processed together as one batch with samples from each replicate. The harvested leaf tissues were stored at -80 °C for HPLC analysis.

Plant metabolites (myricetin and quercetin), were extracted following the protocol by Lisec *et al.* (2006). Ground leaf material (2 g) was extracted twice with 50 ml of acetone, 2 ml of concentrated HCl and 1 ml of 1 % solution of urotropine in water, each time. The extraction was performed in an Erlenmeyer flask with reflux on a boiling water bath for 30 min. The extract was then cooled, filtered and filled to volume with acetone (100 ml). About 25 ml of this extract was transferred to a separating funnel, 50 ml of water added and extraction with ethyl acetate repeated 3 times with 15 ml each. The ethyl acetate fractions were collected and washed three times with 50 ml of water each, then dried with anhydrous $Na2SO_4$, filtered and evaporated to dryness under low pressure. The residue was dissolved and filled to 10 ml with methanol and this solution was used for identification and quantification of flavanols by HPLC (Kulevanova *et al.*, 2002).

A Varian HPLC system equipped with a ternary pump Model 9012 and UV-Diode Array detector Model 9065 and a reverse phase column C18 (250 x 4.6 mm, 5 mm particle diameter) was used to determine the concentration of quercetin and myricetin. The mobile phase consisted of two solvents: 5 % CH3COOH (A)- Acetic acid and CH3CN (B)- acetonitrile and the elution program was as follows: 0-10 min 70% A isocratic, 10-20 min gradient to 40 % A and then 20-30 min 40 %. An isocratic again. The flow rate was set at 1.0 ml/min and the temperature at 30 ºC. The monitoring of the elution is performed in the whole UV-range and the acquisition of data for quantitative analysis at 367 nm. Calibration was made in the concentration range of 0.049 - 0.490 mg/ml myricetin and 0.086 - 0.860 mg/ml quercetin. The limit of detection (LOD) and limit of quantification (LOQ) was established by construction of a calibration curve in the low concentration region (0.005 - 0.05 mg/ml) for quercetin and myricetin.

3.6 Data Analysis

3.6.1 Morphological and Metabolite Profile Analysis

Quantitative data and metabolite concentrations was subjected to analysis of variance (ANOVA) as implemented in SAS version 9.4 (SAS 2013) to determine the variation

among tested accessions. Qualitative data was analysed using goodness of fit Chi-square test. The significant means were separated using least significant difference (LSD) at 5% significance level ($\alpha = 0.05$). The data obtained in the field evaluation and metabolite profiles were analysed using the following model.

$$Y_{ij} = \mu + \tau_i + \beta_i + \varepsilon_{ij}$$

Where $Y_{ij} = 0$verall response, μ is the population mean, $\tau_i =$ treatment effect of the i^{th} genotype, β_i is the block effect and ε_{ij} is the random error.

3.6.2 Diversity Analysis

3.6.2.1 Phenotypic Diversity

Phenotypic frequency distributions of the characters within the 50 accessions, F_1 genotypes, and their parents was calculated. The Shannon-Weaver index (H) was computed using phenotypic frequencies to assess the diversity for each character for the 50 accessions and the entire set of F_1s and their parent populations according to Hutchenson (1970) as follows:

$$H = -\sum_{i=1}^{n} P_i log_e P_i,$$

where p_1 is the proportion of accession in the i^{th} phenotype and n is the number of classes for given character.

The standardized H' ranging from 0 to 1 was obtained by dividing H by the $\log_e$ of the total number of phenotypic classes as follows:

$$H` = \frac{H}{log_e n}$$

Euclidean distances were used for cluster analysis.

3.6.2.2 Genetic Diversity

The level of polymorphism at each locus was calculated according to Botstein *et al*. (1980) and polymorphic information content (PIC) determined as:

$$PIC = 1 - \sum_{i=1}^{k} p_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2p_i^2 p_j^2$$

where p_i and p_j are the frequencies of alleles i and j, respectively.

The total number of alleles, unbiased gene diversity, allelic range, PIC (polymorphism information content), and observed heterozygosity were calculated for each locus and accessions using POPGENE and R software.

3.6.2.3 Population Structure

The software STRUCTURE (Pritchard *et al*., 2000, 2007) was used to analyse the accessions' structure based on the admixture model where each individual draws some fraction of its genome from each of the K accession. The correlated allele frequencies model (Falush *et al.*, 2003), which often improves clustering for closely related populations was used. Twenty-five runs of STRUCTURE were carried out for each set of K sub-accessions, with K values from 2 to 5. The Bayesian genotypic clustering method Instruct (Gao *et al*., 2007) was used to validate population-based approaches and to infer accession's structure using an extended Bayesian clustering approach of STRUCTURE (Pritchard *et al*., 2000) that absorbs inbreeding or selfing rate for population inference.

3.6.3 Evaluation of Combing Ability, Heritability and Heterosis of Developed F_1 Hybrids

3.6.3.1 Computation of Genetic Parameters

Griffing (1956) model II was used. The statistical model,

$$Y_{ij} = \mu + g_i + g_j + e_{ij}$$

where; Y_{ij} = mean performance of the i^{th} parental line mated to the j^{th} parental line.

μ= the population mean effect common to all observations.

g_i and g_j = the general combining ability effects of i^{th} and j^{th} parents.

s_{ij} = the interaction of the i^{th} and j^{th} parents.

e_{ij} = random error

Genetic ANOVA Model II

Source of variation	df	MS	EMS
GCA	p-1	MSgca	$\sigma_e^2 + \sigma^2_{sca} + (p+2)\sigma^2_{gca}$
SCA	$\frac{p^2-p}{2}$	MSsca	$\sigma_e^2 + \sigma^2_{sca}$
Error	$\frac{(r-1)(p^2+p-2)}{2}$	ME	σ_e^2

where, p = number of parents used and r = number of replications

3.6.3.2 Estimation of Component Variances and their Genetic Interpretations

From the EMS given in the generic ANOVA model II (3.6.3.1):

$$\sigma^2_{gca} = \frac{MS_{gca} - MS_{sca}}{p+2}$$

$$\sigma^2_{sca} = MS_{sca} - ME$$

$$\sigma^2_e = ME$$

According to Griffing's (1956) method II, these components was translated into genetic components as follows,

$$\sigma^2_{gca} = \frac{1}{2}\sigma^2_A$$

$$\sigma^2_{sca} = \frac{1}{2}\sigma^2_D$$

Accordingly,

$$\sigma^2_A = 2\sigma^2_{gca}$$

$$\sigma^2_D = 2\sigma^2_{sca}$$

3.6.3.4 Estimation of Heritability

Heritability in broad sense (H) was estimated as,

$$H = \frac{\sigma^2_g}{\sigma^2_p}$$

where: σ_g^2 is the genetic variance.

σ_p^2 is the phenotypic variance.

Heritability in narrow sense (h^2) was estimated as,

$$h^2 = \frac{\sigma_A^2}{\sigma_p^2}$$

where: σ_A^2 is the additive genetic variance.

σ_p^2 is the phenotypic variance.

3.6.3.5 Estimation of Heterosis

Heterosis was estimated according to Hallauer and Maranda (1988) as,

$$H = \frac{\overline{F_1} - \overline{M_p}}{\overline{M_p}} x100$$

where: H is heterosis

$\overline{F_1}$ is the mean performance of the F_1 hybrid.

$\overline{M_p}$ is the mean performance of mid-parent.

3.6.3.6 Estimation of Phenotypic Correlations

The phenotypic correlations (rp) between characters X and Y was calculated as follows,

$$rp = Cov(p_x, p_y)\sqrt{\sigma^2 P_x, \sigma^2 P_y}$$

3.7 Ethical Consideration

The principles regarding research ethics were observed. The study ensured approval by Chuka university Ethics committee. National Commission for Science Technology and Innovation (NACOSTI) for a research permit was obtained prior to the actual study (Appendix 3). The study ensured strict adherence to plagiarism rules with clear citations and acknowledgement of the consulted sources. Other ethical issues were strictly reviewed so that the results are credible and reported with honesty, integrity and confidentiality. The findings were then published in a transparent manner so as to help further study.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Morphological Characterisation of Selected Cowpea Accessions

4.1.1 Characterisation of Qualitative Traits

4.1.1.1 Phenotypic Variability of the Cowpea Genotypes

A wide phenotypic variability was observed among the studied accessions with respect to leaf production, seed colour, pod pigmentation, pod length, leaf shape and leaf colour (Plates 5, 6, 7 and 8). This implied that the studied accessions can be an important genetic resource to initiate a breeding program to develop varieties for different purposes. Distinctive differentiation of characters among the genotypes was observed in leaf shape, pod curvature, seed colour, leaf colour and pod colour. The results are in agreement with those of Onuminya *et al*. (2023) who found existence of wide diversity for the morphological traits in cowpea accessions.

plates 4: Phenotypic variability of cowpeas with potential leaf production

plates 5: Phenotypic variability of cowpeas with different seed colour.

plates 6: Phenotypic variability showing difference in pod pigmentation and pod length

plates 7:phenotypic variability in leaf shape and leaf colour

4.1.1.2. Phenotypic Diversity of the Cowpea Genotypes

The Shannon-Weaver index (H') of 13 cowpea plant and grain qualitative traits was compared to evaluate diversity between the different characters (Table 2). Among all the traits, growth habit showed the highest diversity index ($H' = 0.38$) followed by mature pod curvature ($H' = 0.36$). Leaf marking showed a null diversity index, indicating no variability between accessions for this trait. The polymorphism level of some morphological characteristics differed distinctly among regions, except leaf marking where no diversity was observed.

In this study, plants displayed a wide range of differences in qualitative traits studied, implying a wide genetic diversity among the accessions (Table 2). Six of the cowpea growth habits were observed, which included climbing, prostrate, semi prostrate, intermediate semi-erect, erect and acute erect. It was observed that 32% of the accessions displayed semi-erect growth habits, 22% intermediate growth habit, 4% acute erect habits while 32% had climbing habits (Table 2). About 4% of the semi-prostrate growth habit were recorded, and these could be useful as forage and good for leafy vegetables. Growth habit is a very important characteristic in the cropping system of cowpea due to its influence in harvesting. (Karikari *et al*., 2023). Moreover, cowpea with erect or semi-erect growth habit had no lodging associated problems and do not need staking to keep pods from touching the ground and thus prevent rotting of pods, which normally occurred when pods come into contact with the soil (Cobbinah *et al.*, 2011).

On twinning tendency, 54% of the accessions exhibited pronounced twinning tendency, 32% exhibited intermediate tendency and 12% exhibited slight twinning character (Table 2). The distribution of twining tendency suggests that multiple genetic factors are at play. The distribution of twining tendency showed a range of phenotypic expression rather than distinct. The alleles controlling twinning tendency may occupy different loci and their effect on each other, thus producing different phenotypes. Consequently, the different levels of expression could be the result of various alleles with potentially epistatic effects contributing to the overall phenotype in differing degrees. The findings of this study are consistent with those of Barik *et al*. (2023) who identified three groups of twining tendency in cowpea, that is none, slight and intermediate.

About 26% of the accessions had determinate growth pattern while 74% had indeterminate growth pattern (Table 2). The distribution of growth patterns in the studied cowpea accessions suggests that growth habit in cowpea is likely a qualitative trait. The clear distinction between determinate and indeterminate growth patterns suggests that this trait is likely controlled by one or a few major genes. The predominance of the indeterminate growth pattern (74%) suggests that the allele(s) responsible for this pattern might be more common or possibly dominant in the studied accessions. Growth pattern is an important trait in a breeding program. The determinate pattern is associated with uniform grain maturity. Hence, allows for adoption of mechanized harvesting especially large-scale production (Kumar *et al*., 2015). Indeterminate growth pattern is associated with continuous production and non-uniform grain maturity within the planting season.

Genotypes of the present study revealed great variation for the traits plant pigmentation, leaf colour, pod colour, flower colour and seed colour (Table 2). The results of this study were agreement with those of Egbadzor *et al*. (2014) and Supriya (2022) who reported significant variation regarding these traits in cowpea and French bean, respectively. In plant pigmentation, 8% of the accessions displayed no pigment, 28% very slight plant pigmentation, 54% displayed moderate pigmentation, 8% displayed intermediate pigmentation and 2% displayed extreme pigmentation. The fact that only a small percentage of accessions show extreme pigmentation further suggests that strong pigmentation might be due to the cumulative effect of multiple alleles, each contributing incrementally to the trait. Ishiyaku and Singh (2004) suggested two genes with dominant and recessive epistasis condition pigmentation on vegetative regions of the cowpea plant. In case of seed colour, the types observed were: white (4%), cream (36%), brown (20%), grey (20%), Marouane (4%) and black (16%). The distribution of seed colours in cowpea accessions studied suggests that seed colour is likely a qualitative trait with multiple categories. The distinct colour categories observed indicate that the trait is likely controlled by a few genes with different alleles, leading to a variety of phenotypes. The presence of specific colour types, such as cream, brown, grey, and black, suggests that different alleles may produce distinct colours rather than a continuous range, which is typical of qualitative traits. Genome-wide association studies have identified four specific genes that play a direct role in determining cowpea

seed colour, revealing qualitative inheritance for this trait in cowpea (Xiong *et al*, 2024).

The flower colour of the cowpea accessions was either pink (2%), white (16%) or violet (82%) among the accessions studied, suggesting that flower colour in cowpea might be a qualitative trait. Qualitative traits are controlled by one or a few genes, resulting in distinct and easily observable phenotypes. The relatively clear separation into distinct colour categories (pink, white, violet) without intermediate forms further supports the idea that flower colour is controlled by a few genes with dominant or recessive alleles rather than by multiple genes as in quantitative traits. These results are in agreement with those of Rawal *et al*., (1976) who showed that flower colour show monogenic inheritance.

About 94% of the cowpea accessions had the raceme distributed throughout the plant canopy whereas 6% of the plants had the raceme above the canopy, suggests that this trait might be a qualitative trait. The distinct separation into two categories implies that this trait could be controlled by one or a few genes with different alleles that determine the positioning of the raceme. Raceme position is a good attribute to cowpea architecture since it facilitates easy visibility of pods for harvesting. It was observed that when the racemes are held at the same level or within the canopy the pods become hidden within the canopy making harvesting more difficult and strenuous. The findings of this study are in agreement with those of Pandey and Ngarm (1985) and Bennett-Lartey and Ofori (1999) who showed that varieties with their racemes above the canopy are easier and cheaper to harvest than those with racemes below the canopy. Such accessions will also enhance the use of mechanical harvesters since they will not require the pulling up of the whole plant during harvesting.

The results of this study revealed that 54% of the accessions had straight pods, 44% had slightly curved pods while 2% were curved (Table 2), suggesting that pod shape may be influenced by a multiple allele. The majority of accessions falling into the straight or slightly curved categories, with only a small percentage having curved pods points to the possibility of multiple alleles contributing to the shape of the pod. Genetics and association studies have implicated three to four genes controlling pod shape in cowpea (Nwofia, 2014). Pod straightness increases the market value of fresh

bean where consumer preferences differ with regard to colour, shape and even taste between regions (Salk *et al*., 2008; Yanmaz, 2010). Consequently, directional selection pressure may partly explain why straight pods had a highest frequency as farmers may prefer straight pods.

On the immature pod pigmentation, 60% of the accessions had no pigmentation, 14% had pigmented tip, 4% had pigmented sutures, 4% had pigmented valves, 6% had pigmented splashes and 12% were uniformly pigmented. The distribution of immature pod pigmentation in cowpea accessions suggests that pod pigmentation has complex inheritance. The variety of pigmentation patterns indicates that multiple genes may be involved, each contributing to different aspects of pod pigmentation, resulting in a range of phenotypes. The results of this study were consistent with those of Porter *et al*., (1974) who reported six different patterns of pod pigmentation. Mustapha and Singh (2008) revealed that pod pigmentation followed two patterns of inheritance, that is, monogenicity and biogenicity inheritance. According to Bennet-Lartey and Ofori (1999) the pods of many cowpea cultivars contain anthocyanin, which is either partially or wholly purple.

On terminal leaf shape, 34% of the accessions had globose shape, 64% had sub globose shape while only 2% had sub hesitate shape. The distribution of terminal leaf shapes in cowpea accessions suggests that leaf shape could be influenced by multiple genetic factors. The relatively small percentage of accessions showing the sub-hastate shape further supports the idea of a quantitative inheritance pattern, where different alleles at multiple loci might produce a spectrum of leaf shapes. However, without clear intermediate forms or a broader distribution of leaf shapes, it could also be indicative of a qualitative trait with different alleles influencing distinct categories of leaf shape. Several other studies have studied the inheritance of the leaf shape in cowpea and reported that it is a qualitative trait (Saunders, 1960; Ojomo, 1977; Oluwatosin, 2002). However, further genetic analysis would be needed to determine the precise mode of inheritance for terminal leaf shape. Pottorff *et al*. (2012) identified one major QTL, which is associated with the hastate and sub-globose leaf shape in the cowpea recombinant inbred line population. Hutchinson *et al*. (2017) showed that globose leaf shape are preferred attributes for vegetable cowpea production and thus preferred by

farmers, implying that this leaf shape may appear in higher proportion than expected in cowpea varieties bred for vegetable production as a result of directional selection.

Table 2: Phenotypic frequency and diversity of qualitative straits for fifty cowpea accessions

Variable	Description	Frequency	Percentage	Shannon waver Index(H)	Standardised Shannon-Weaver index(H')
Immature pod pigmentation	None	30	60.0	1.27	0.32
	Pigmented tip	7	14.0		
	pigmented sutures	2	4.0		
	Pigmented valves	2	4.0		
	Splashes of pigment	3	6.0		
	Uniformly pigmented	6	12.0		
	total	50	100.0		
Plant hairiness	Glabrescent	13	26.0	0.94	0.24
	short appressed	30	60.0		
	Pubescent	7	14.0		
	Total	50	100.0		
Terminal leaflet shape	Globose	17	34.0	0.74	0.19
	Sub globose	32	64.0		
	Sub hastate	1	2.0		
Growth pattern	Determinate	13	26.0	0.58	0.15
	Indeterminate	37	74.0		
Leaf marking	present	50	100	0	0
	Absent	0	0		
Leaf color intensity	Pale green	9	18.0	1.00	0.26
	Intermediate green	27	54.0		
	Dark green	14	28.0		
	Total	50	100.0		
Growth Habit	Acute erect	2	4.0	1.50	0.38
	Semi-erect	16	32.0		
	Intermediate	11	22.0		
	Semi prostrate	2	4.0		
	Prostrate	3	6.0		
	Climbing	16	32.0		
	Total	50	100.0		

Table 2 (continued

Variable	Description	Frequency	Percentage	Shannon waver Index(H)	Standardised Shannon-Weaver index(H')
Twinning tendency	None	2	4.0	1.10	0.28
	slight	6	12.0		
	Intermediate	16	32.0		
	Pronounced	26	52.0		
	Total	50	100.0		
Flower colour	violet	41	82	0.54	0.14
	white	8	16		
	Pink	1	2		
Raceme position	Mostly above canopy	0	0	0.77	0.20
	in upper canopy	3	6		
	throughout	47	94		
Mature pod curvature	straight	27	54	1.41	0.357
	Slightly curved	22	44		
	curved	1	2		
	coiled	0	0		
Seed colour	Brown	10	20	0.71	0.18
	cream	18	36		
	Black	8	16		
	Gray	10	20		
	White	2	4		
	Maroon	2	4		

4.1.1.3 Principal Component Analysis

The principal component analysis was carried out using 13 morphological traits. The first three principal components explained 47.97% of total phenotypic variation (Table 3). The Eigen values for PC1, PC2 and PC3 were 2.46, 1.88 and 1.41, respectively. This imply that the explained variance for PC1, PC2 and PC3 are 42.70%, 32.73% and 24.57%, respectively. Other studies have reported similar results (Rekha *et al*., 2013; Arora *et al*., 2018). The finding of this study was also in agreement with the principle of Syafii *et al*. (2015) that the first principal component accounts for maximum variability in the data with respect to succeeding components. The PC1 gave the highest loading for twinning tendency, PC2 had the highest loading for plant hairiness while

PC3 had the highest loading for plant pigmentation. Those accessions that had high loading could be highly diverse. The variable leaf marking had a factor loading of zero in all the three PC, indicating that the variable has little to no influence on the principal component.

In case of PC1, twining tendency, growth habit and growth pattern had high and positive loading indicating that the variables and the principal component increase together. This suggests that these variables are strongly associated with PC1, contributing significantly to the variance captured by PC1. On the other hand, terminal leaf shape, plant hairiness and seed colour had low and negative loading, implying these variables are not highly correlated with the PC1. Moreover, the negative loading indicates an inverse relationship, where the variable decreases as the principal component increases.

For PC2, it was observed that growth pattern, twinning tendency plant hairiness and seed colour had high and positive factor loading. This means that, the listed descriptors are the most effective characters for distinguishing cowpea accessions variation with PC2. For terminal leaf shape, leaf colour intensity, mature pod curvature and plant pigmentation there was low and negative factor loading. In the case of PC3, seed colour, terminal leaf shape, growth habit, plant pigmentation and twinning tendency had a positive and high loading which shows they have a positive correlation with PC3. Immature pod pigmentation, raceme position, mature pod curvature and plant hairiness had a negative and high loading which shows a negative correlation with PC3.

Twinning tendency had a higher loading in PC1 than in PC2 and PC3. This suggests that twining tendency is strongly associated with PC1, contributing significantly to the variance captured by PC1. Similarly, plant pigmentation had a higher loading in PC3 than in PC1 or PC2. Moreover, plant hairiness had a higher loading in PC2 than in PC1 or PC3. These results demonstrate that different variables contributed differently to different components. Generally, the loadings reveal, which variables are most influential in the data's variance and how the components relate to the underlying structure of the data. As per Hair *et al*. (2009), the loading effect of any traits greater than ±0.3 was regarded meaningful and significant.

Table 3: Principal component analysis of 50 cowpea accessions for 13 morphological traits

Principle component analysis	Principle component Axis		
	PC1	PC2	PC3
Eigen Value	2.458	1.884	1.414
Variation explained	2.458	1.884	1.414
Cumulative % of variation	20.49	36.19	47.97
Variables		Factor loading	
Terminal leaf shape	-0.40725	-0.43175	0.24520
Growth habit	0.56664	-0.22428	0.43494
Growth pattern	0.56086	0.57159	0.08687
Twinning tendency	0.74006	0.40932	0.17918
Plant pigmentation	0.31238	-0.20176	0.55269
Plant hairiness	-0.23093	0.61255	-0.29724
Leaf colour intensity	0.59290	-0.50281	-0.17927
Leaf marking	0.00000	0.00000	0.00000
Immature pod pigmentation	0.34909	0.12903	-0.58039
Flower colour	0.13757	-0.15022	0.06092
Raceme position	0.54896	-0.05416	-0.27390
Seed colour	-0.03129	0.55730	0.46656
Mature pod curvature	0.40465	-0.36291	-0.25640

Cluster analysis revealed the existence of diversity among the fifty cowpea accessions for the 13 morphological characters studied. Thus, the genotypes belonging to the distant clusters could be used for cowpea breeding program to get a wider range of variability. However, most of accessions clustered together, suggesting high level of relationship. The most divergent accessions were NA31, NA82, NA11, NA60, NA64, ES5, NA29 and MA30, which were highly discriminated from other accessions. The findings of this study are in agreement with those of Padulosi (1993) who reported that differences among cowpea varieties may also be due to it being a self-pollinated crop. Moreover, there is likelihood that most of the cowpea accessions are still segregating morphologically based on some common agromorphological traits. Generally, this study has shown that there were variations or polymorphism in some morphological characteristics expressed by the cowpea accessions, which were segregating in one trait or the other. The cluster analysis confirmed segregation among the cowpea accessions as it sorted the cowpea accessions into three groups with respect to the segregation patterns of their morphological traits. Furthermore, the high level of relationship

reported among cowpea accessions may also be due to it being a self-pollinated crop (Kouam *et al*., 2012). Overall, the variation observed among the 50 cowpea genotypes suggests that phenotypic variation can reveal diversity existing among cowpea genotypes. These results are consistent with those of Molosiwa *et al*. (2016), Moolendra *et al*. (2018) and Walle *et al*. (2019).

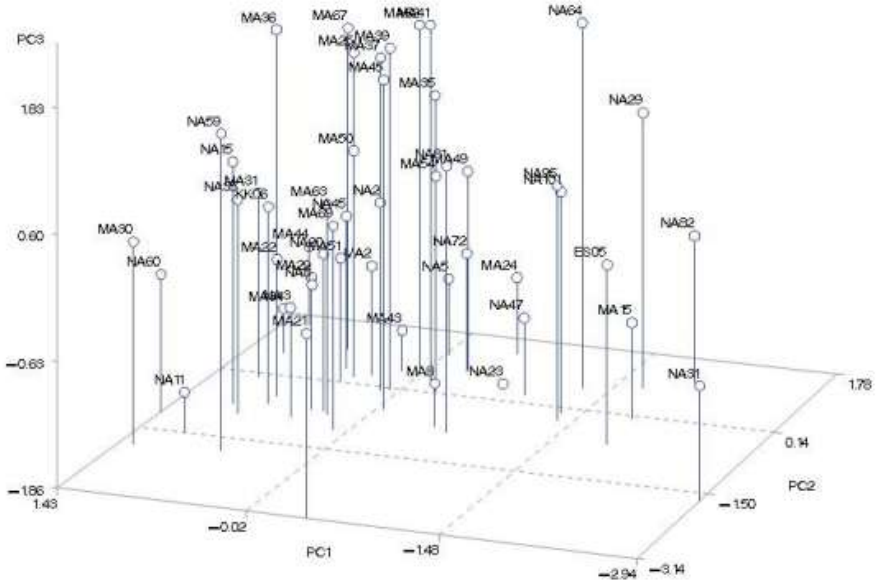

Figure 1: Cluster analysis of accessions based on thirteen Qualitative traits

4.1.2 Evaluation of Selected Cowpea Accessions under Normal Conditions

The test of the fitted model showed that it was adequate ($p < 0.05$) in explaining the linear relationship between cowpea accessions and all trait measured (Appendix 4). There was a significant interaction ($p < 0.05$) between the genotypes (cowpea accessions) and trial for all variables measured (Appendix 5). On testing the effects genotypes, the results indicated that there was significant ($p < 0.05$) effect of genotypes on all traits measured (Appendix 6). However, the blocking of the genotypes was found to be effective ($p > 0.05$).

At 28 DAS, the plant height ranged from 8.99 cm (NA8) to 21.50 cm (NA59; Table 4). The plant width varied from 24.22 cm to 36.11 cm with MA49 having the narrowest width and MA36 showing the widest width. The terminal leaf length ranged from 7.55 cm (MA41) and to 13.37 cm (MA50) While terminal leaf width ranged from 3.94 cm (MA50) to 8.08 cm (MA41) (Table 4). At 56 DAS the plant height ranged from 12.6

cm to 35.22 cm with NA8 being the shortest plants and MA21 the tallest plants. The number of branches ranged between 4.28 and 7.89 with NA8 showing lowest number of branches and MA24 showing the highest number of branches.

Table 4: Means of plant height (PH), plant width (PW), terminal leaf length (TLL), terminal leaf width (TLW) and number of branches (NOB) at different days after sowing under well water condition in Trial 1

Genotypes	PH28DAS	PW28DAS	TLL42DAS	TLW42DAS	PH56DAS	NOB56DAS
NA59	25.33a	31.22l	10.49ef	7.66b	31.89a	6.00jk
MA67	21.56b	36.44b	9.08qrs	6.46ij	26.44c	6.44fg
MA15	20.44c	35.67c	10.26fg	7.40cd	27.78b	6.67de
MA62	19.67d	31.22l	8.63tu	5.34st	25.44de	6.89c
MA63	19.50d	33.33gh	9.67lm	6.14l	24.00g	5.56no
MA69	19.44d	26.78s	9.71klm	7.44bc	26.56c	5.67mn
MA49	19.11d	33.89ef	9.06qrs	5.84nop	25.67d	7.67a
NA95	18.22e	36.19bc	9.77jkl	6.16kl	20.89o	5.22q
NA47	18.22e	28.56q	9.31opq	5.29tu	23.44ghi	5.44op
MA41	18.11e	27.33r	10.23fgh	8.01a	22.89ijk	6.33gh
MA54	18.11e	34.89d	10.83bcd	7.34cd	25.33def	6.78cd
MA29	18.11e	36.00bc	9.98ijkl	6.73fgh	23.00ijk	6.79cd
MA30	17.33f	29.44p	11.10bc	7.21cde	27.78b	6.67de
MA51	17.33f	30.44mn	9.33nopq	6.04lmn	24.72f	5.44op
NA101	17.33f	36.00bc	9.93hijkl	6.40ijk	21.61mn	5.00r
NA5	17.33f	31.33l	7.28y	4.09x	18.11qr	5.89kl
MA37	16.78fg	33.89ef	9.16pqr	6.97ef	21.39no	6.22hi
NA35	16.50gh	28.83q	10.90ghi	5.28tu	16.67s	4.00v
MA43	16.44ghi	32.67ij	9.08qrs	6.62ghi	22.17lm	6.33gh
MA39	16.44ghi	31.99k	10.58de	7.43bc	22.56kl	6.67de
NA2	16.3ghij	27.33r	8.06wx	5.88no	27.00c	6.00jk
MA35	16.3ghij	33.89ef	10.62de	5.74opqr	24.89ef	6.56ef
MA36	16.1ghijk	31.11l	10.06ghij	6.28jkl	22.89ijk	5.89kl
NA64	16.0hijkl	30.89lm	8.21w	5.36st	18.33qr	5.67mn
NA45	15.8ijklm	34.22e	9.04qrs	5.78opq	23.22hij	5.44op
NA11	15.8ijklm	38.50a	9.82ijkl	6.13lm	22.56kl	5.67mn
NA15	15.7jklm	29.67op	7.76x	5.34st	22.44kl	5.33pq
NA23	15.6klmn	20.56x	8.90rst	5.69opqr	23.89g	4.56st
MA8	15.4klmn	32.44jk	8.56u	5.30tu	21.78mn	7.11b
MA31	15.4klmn	34.33e	9.63lmn	5.62pqr	25.67d	5.78lm
MA25	15.3lmno	24.56v	9.12qrs	6.50hij	23.78gh	5.22q
NA60	15.2mnop	25.89t	11.14b	7.16de	21.67mn	4.56st
MA2	14.89nopq	33.33gh	10.74de	7.23cd	21.78mn	6.78cd
NA3	14.67opqr	36.44b	9.81ijkl	6.49hij	19.39p	6.11ij
NA72	14.56pqr	30.44mn	10.02ghijk	6.04lmn	16.00t	4.33u
ES5	14.50qrs	32.83hij	9.62lmno	5.30tu	14.00u	5.78lm
KK06	14.44qrs	33.67fg	8.54uv	5.57qrs	19.78p	5.44op
MA21	14.44qrs	32.78ij	8.81stu	5.79opq	22.67jkl	6.22hi

Table 4(continued)

Genotypes	PH28DAS	PW28DAS	TLL42DAS	TLW42DAS	PH56DAS	NOB56DAS
MA34	14.11rst	30.33no	9.91ijkl	6.76fg	26.94c	6.33gh
MA24	13.83st	27.22rs	10.81cde	5.09uv	23.43ghi	6.56ef
MA44	13.83st	33.17ghi	9.93hijkl	6.40ijk	17.00s	4.50stu
NA81	13.44tu	32.89hij	9.44mnop	5.50rst	18.22qr	5.33pq
NA82	12.82uv	18.44y	7.93wx	4.69w	17.78r	5.00r
MA45	12.33vw	31.17l	8.64tu	5.89mno	18.11qr	4.67s
MA22	12.22vw	25.67tu	9.77jkl	6.82fg	18.55q	5.44op
MA50	12.22vw	36.33b	13.44a	2.05z	15.56t	5.44op
NA31	12.00w	22.33w	8.68tu	4.21x	15.56t	6.29ghi
NA20	9.78x	22.33w	8.23vw	5.00v	11.11w	4.56st

[a]Means followed by the same letters are not significantly different at 5% probability level. Where, NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accessions, KK = Kakamega accessions, DAS = days after sowing CV = Coefficient of variation, LSD = Least Significant Difference

In trial 2, at 28 DAS the plant height ranged from 8.89 cm and 24.00 cm with NA8 being the shortest and MA67 being the tallest plants (Table 5). Plant width range was between 23.83 cm (NA35) and 39.17 cm (MA31). Terminal leaf length range was between 7.55 cm and 13.37 cm with NA8 showing the shortest length and MA50 showing the longest leaves. The terminal leaf width range was from 3.94 cm (MA50) to 8.01 cm (MA67). At 56 DAS the plant height ranged from 12.61 cm to 35.22 cm with NA8 being the shortest plant and MA21 being the tallest plants. The number of branches ranged from 4.28 to7.89 with NA8 having the least number of branches and MA24 having the highest number of branches.

Table 5: Means of plant height (PH), plant width (PW), terminal leaf length (TLL), terminal leaf width (TLW) and number of branches (NOB) at different days after sowing under well water condition in Trial 2

Genotypes	PH28DAS	PW28DAS	TLL42DAS	TLW42DAS	PH56DAS	NOB56DAS
NA59	21.50b	31.6ghijklmnopq	9.54lmno	6.62hijk	29.89c	5.22op
MA67	24.00a	30.78jklmnopqrs	10.27defg	8.01a	26.1efgh	
MA15	21.00bc	31.89fghijklmnop	10.21defghi	7.03efg	31.78b	7.00c
MA62	19.33ef	31.33ijklmnopq	8.92qr	6.09nop	26.4defg	6.67d
MA63	17.78jkl	31.11ijklmnopqr	9.58klmno	6.2mnop	26.22efg	5.89ij
MA69	14.89stu	31.89efghijklmno	9.56lmno	6.57ijk	25.89fghi	6.11gh
MA49	18.56gh	24.22xy	9.87ghijkl	5.17t	24.2klmn	5.89ij
NA95	12.44y	32.22efghijklmno	8.72rs	5.00tu	16.44w	5.4mno
NA47	16.89mno	33.44defghij	10.21defghi	5.86pqr	23.44no	6.00hi
MA41	18.67fgh	30.50klmnopqrs	10.81b	8.08a	30.06c	7.50b
MA54	17.22lmn	34.67bcdefg	10.39cdef	6.91fg	25.00hijk	6.33ef

Table 5(continued)

Genotypes	PH28DAS	PW28DAS	TLL42DAS	TLW42DAS	PH56DAS	NOB56DAS
MA29	18.00hij	30.78jklmnopqr	9.44mno	6.56ijk	26.8defg	6.11gh
MA30	18.11hi	32.33efghijklmn	10.49bcde	7.64b	27.56d	6.22fg
MA51	21.00bc	32.56efghijklm	9.33nop	6.73ghij	29.56c	6.22fg
NA101	14.33uv	31.11ijklmnopqr	10.36cdef	6.63hijk	25.50ghij	5.22op
NA5	17.56jklm	33.00defghijkl	8.87tu	5.01tu	20.56rs	6.44e
MA37	17.44jklm	34.44bcdefgh	9.54lmno	6.81fghi	23.22no	6.00hi
NA35	14.83stu	23.83y	8.68rs	5.76qrs	23.8lmno	5.5lmn
MA43	16.47nop	25.67uvwxy	10.22defgh	7.04ef	24.78ijkl	6.22fg
MA39	17.22lmn	34.00cdefghi	10.38cdef	6.48jkl	27.33de	6.22fg
NA2	20.67cd	28.44qrstuv	8.77r	6.88fgh	25.00hijk	5.78jk
MA35	15.89pqr	27.78rstuvw	9.31opq	4.14wx	21.44qr	5.44mn
MA36	16.67nop	36.11abcd	9.66jklmno	5.87pqr	26.89def	6.00hi
NA64	14.56tu	24.78wxy	8.28uv	4.72uv	18.67uv	5.44mn
NA45	15.22rst	29.78mnopqrst	9.39nop	6.84fghi	26.78def	5.11p
NA11	17.78jkl	35.00bcde	9.82hijklm	6.84fghi	26.4defg	5.67kl
NA15	17.33klmn	34.78bcdef	10.01fghijk	7.22de	23.00op	5.89ij
NA23	20.56cd	31.8fghijklmnopq	10.84b	6.86fghi	23.22no	5.78jk
MA8	20.00de	37.67ab	9.8ghijklm	5.67rs	18.44v	6.67d
MA31	14.89stu	39.17a	7.89vw	5.09t	24.2klmn	5.78jk
MA25	17.33klmn	34.56bcdefg	9.60lmno	6.77fghij	26.89def	6.78d
NA60	15.90pqr	33.33defghijk	10.86b	7.50bcd	23.6mno	4.78q
MA2	15.78pqr	31.4ghijklmnopq	10.30defg	7.07ef	24.9ijkl	6.00hi
NA3	14.67tu	34.11cdefghi	10.71bc	7.58bc	24.7jklm	6.78d
NA72	17.56jklm	32.78defghijklm	9.96ghijkl	6.42klm	22.89op	5.33no
ES5	12.78xy	27.44stuvwx	10.08fghij	4.32w	16.63w	5.44mn
KK06	14.67tu	29.00opqrstu	9.41no	5.51s	22.00pq	5.89ij
MA21	20.67cd	30.00lmnopqrs	8.78r	6.00nopq	35.22a	6.11gh
MA34	16.11opq	29.56nopqrst	10.12efghi	6.2lmno	23.klmno	5.56lm
MA24	16.33nop	31.4ghijklmnopq	9.66jklmno	5.87pqr	26.7defg	7.89a
MA44	15.50qrs	33.50defghij			26.0fghi	5.5lmn
NA81	17.22lmn	33.89cdefghi	9.01pqr	6.24lmn	25.56ghij	4.67qr
NA82	13.22wx	26.44tuvwxy	9.59klmno	5.93opqr	21.56qr	4.78q
MA45	19.00fg	37.00abc	9.71ijklmn	5.73qrs	22.78op	5.11p
MA22	17.00mno	33.94cdefghi	10.36cdef	7.32cde	23.5mno	6.83cd
MA50	17.44jklm	33.00defghijkl	13.37a	3.94x	19.22tuv	5.11p
NA31	15.83pqr	30.44klmnopqrs	10.87b	7.38bcd	20.11st	6.22fg
NA20	13.67vw	28.67pqrstu	10.53bcd	6.68hijk	15.56w	4.56rs
NA29	13.50wx	25.11vwxy	7.67w	4.73uv	19.78stu	4.44st
NA8	8.89z	28.58pqrstu	7.55w	4.44vw	12.61x	4.28t
LSD	0.3	0.96	0.28	0.22	1.17	0.3
R	0.997	0.98	0.99	0.99	0.98	0.96

[a] Means followed by the same letters are not significantly different at 5% probability level. Where, NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accessions, KK = Kakamega accessions, DAS = Days after sowing CV = Coefficient of variation, LSD = Least Significant Difference.

In trial 1, the grain yield ranged from 230.56 kg/ha to 3345.37 kg/ha with NA35 being the lowest yielding and MA24 being the highest yielding (Table 6). In trial 2, the yield ranged from 460.28 kg/ha to 3856.23 kg/ha with NA29 being the lowest yielding and NA3 being the highest yielding (Table 6). In trial 1, the flowering dates ranged from 44.33 to 52.00 with NA8 being the latest flowering and NA3 being the earliest flowering. In trial 2, the range of flowering ranged from 60.33 to 44.33 with NA35 being the latest flowering and MA29 being the earliest flowering.

In trial 1, the terminal leaf length had a highly significant and positive correlation ($p < 0.05$) with plant height at 28 DAS, plant width, terminal leaf width, plant height at 56 DAS, number of branches, grain yield and days to 50% flowering (Table 7). Terminal leaf width had highly significant and positive correlation ($p < 0.05$) with plant width, number of primary branches per plant, plant height at 56 DAS, grain yield and days to 50% flowering. Alam and Hossain (2008) got the same results. It was observed that plant width had highly significant positive correlation with number of primary branches per plant ($r = 0.775^{**}$) suggesting that increase in plant width would lead to simultaneous increase in primary branches per plant. Plant width also had significant and positive correlation ($p < 0.05$) with grain yield and plant height at 56 DAS. These results are in agreement with those of Paiva and Costa (1988) reported similar result in okra. Plant height at 28 DAS had no significant correlation.

In trial 2, there was a negative correlation between terminal leaf length and number of days to 50% flowering ($r = -0.358^{*}$). It was also observed that there was a highly significant positive correlation ($p < 0.05$) between terminal leaf width and plant height at 56 DAS and number of branches. The correlation between plant height at 28 DAS and terminal leaf width plant height at 56ADS and number of branches was positive significant ($p < 0.05$). Plant height was significantly positively correlated ($p < 0.05$) with yield. Number of branches per plant showed high significant correlation ($p < 0.01$) with yield. Similarly, it exhibited significant correlation ($p < 0.05$) with plant height at 28 DAS.

Table 6: Means of grain yield (GY) and days to 50% flowering (DTF) under well water conditions in two trials

Genotypes	DTF Trial 1	DTF Trial 2	GY Trial 1	GY Trial 2
NA59	48.00ef	51.00jk	627.78w	1073.70vw
MA67	48.00ef	48.33o	750.00v	1579.31ijk
MA15	49.33cd	55.33c	363.89x	1259.48st
MA62	50.67b	54.67d	766.67uv	2193.67d
MA63	49.33cd	48.67no	933.33opq	1421.90no
MA69	49.33cd	49.00mn	2233.33e	1415.37op
MA49	47.33fg	52.33g	922.22opqr	2262.69c
NA95	48.67de	53.00f	580.56w	1197.31u
NA47	49.33cd	53.00f	1472.22fg	1194.49u
MA41	50.67b	47.67p	1477.78fg	1202.70tu
MA54	45.00i	52.33g	1369.44hi	1368.43opq
MA29	46.67gh	44.33u	1298.61i	1551.20jk
MA30	49.33cd	51.67hi	1376.39hi	1697.82g
MA51	48.00ef	53.67e	1472.22fg	1309.44rs
NA101	48.00ef	47.33pq	3219.44b	2200.68d
NA5	49.33cd	48.67no	997.22no	1242.18tu
MA37	48.00ef	53.00f	1333.33i	1529.88klm
NA35	50.67b	60.33a	230.56z	1258.58st
MA43	49.33cd	48.67no	974.54nop	1527.76klm
MA39	48.00ef	55.67c	1527.78f	2082.95e
NA2	48.00ef	52.00gh	861.11qrst	1246.54tu
MA35	49.33cd	51.00jk	2190.28e	1362.27pqr
MA36	47.56f	50.67kl	1331.02i	1479.49mn
NA64	49.33cd	48.67no	830.56stuv	935.97x
NA45	50.67b	51.67hi	1296.30ij	1131.76qrs
NA11	48.00ef	45.67t	3149.07b	1290.63d
NA15	49.33cd	54.33d	787.04tuv	843.89y
NA23	49.33cd	51.33ij	1191.67k	1207.30tu
MA8	49.33cd	51.00jk	1191.67jk	1353.24qr
MA31	48.00ef	51.67hi	2356.94d	1351.11qr
MA25	50.67b	51.67hi	903.70pqrs	1476.23mn
NA60	50.00bc	48.33o	930.86opq	1600.43hij
MA2	50.67b	47.67p	2639.82c	1862.56f
NA3	44.33i	48.67no	3193.52b	3856.23a
NA72	48.00ef	47.00qr	819.70utv	1853.24f
ES5	48.00ef	51.33ij	768.52uv	1016.33w
KK06	46.00h	47.67p	788.89tuv	1026.44w
MA21	49.33cd	49.00mn	1159.88kl	1627.50hi
MA34	50.67b	49.33m	919.44opqr	1541.57kl
MA24	48.67de	47.33pq	3345.37a	2422.50b
MA44	46.00h	47.67p	1434.26gh	1651.53gh
NA81	48.00ef	50.33l	1158.33kl	1489.68lm
MA36	47.56f	50.67kl	1331.02i	1479.49mn
NA64	49.33cd	48.67no	830.56stuv	935.97x
NA45	50.67b	51.67hi	1296.30ij	1131.76qrs
NA11	48.00ef	45.67t	3149.07b	1290.63d
NA15	49.33cd	54.33d	787.04tuv	843.89y

Table 6(continued)

Genotypes	DTF Trial 1	DTF Trial 2	GY Trial 1	GY Trial 2
NA23	49.33cd	51.33ij	1191.67k	1207.30tu
MA8	49.33cd	51.00jk	1191.67jk	1353.24qr
MA31	48.00ef	51.67hi	2356.94d	1351.11qr
MA25	50.67b	51.67hi	903.70pqrs	1476.23mn
NA60	50.00bc	48.33o	930.86opq	1600.43hij
MA2	50.67b	47.67p	2639.82c	1862.56f
NA3	44.33i	48.67no	3193.52b	3856.23a
NA72	48.00ef	47.00qr	819.70utv	1853.24f
ES5	48.00ef	51.33ij	768.52uv	1016.33w
KK06	46.00h	47.67p	788.89tuv	1026.44w
MA21	49.33cd	49.00mn	1159.88kl	1627.50hi
MA34	50.67b	49.33m	919.44opqr	1541.57kl
MA24	48.67de	47.33pq	3345.37a	2422.50b
MA44	46.00h	47.67p	1434.26gh	1651.53gh
NA81	48.00ef	50.33l	1158.33kl	1489.68lm
NA82	52.00a	56.33b	270.83yz	1349.06qr
MA45	46.00h	46.33s	1084.26lm	1368.95opq
MA22	46.00h	49.00mn	2251.30e	2062.92e
MA50	46.00h	45.67t	1044.44mn	1123.13v
NA31	49.33cd	50.33l	1216.67jk	1854.26f
NA20	48.00ef	46.67rs	323.61xy	801.54y
NA29	48.00ef	52.33g	844.44rstu	460.28z
NA8	52.00a	55.33c	1543.06f	956.10x
LSD	0.69	0.46	57.80	81.337
R	0.96	0.996	0.997	0.998
CV	0.88	0.56	2.37	3.29

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accession, KK = Kakamega accessions, DAS = Days after sowing CV = Coefficient of variation, LSD = Least Significant Difference.

Table 7:Correlation analysis for plant height, plant width, terminal leaf length, terminal leaf width, number of branches, grain yield and days to 50% flowering at different growth stages in well water conditions in Tria1 and 2.

	PH28	PW28	TLL42	TLW42	PH56	NOB56	GY	DTF
PH28		0.78**	0.58**	0.784**	0.91**	0.766**	0.228	0.74**
PW28	0.269		0.63**	0.655**	0.71**	0.775**	0.440**	0.78**
TLL42	0.258	0.216		0.557**	0.58**	0.568**	0.376**	0.61**
TLW42	0.433**	0.244	0.366*		0.80**	0.687**	0.368**	0.67**
PH56	0.642**	0.198	0.096	0.547**		0.801**	0.346*	0.72**
NOB56	0.455**	0.271	0.209	0.451**	0.49**		0.394**	0.78**
GY	0.089	0.190	0.268	0.362*	0.23*	0.456**		0.341*
DTF	0.101	0.196	0.358*	0.125	0.04	0.045	0.150	

**Correlation is significant at the 0.01 level (2-tailed) DTF -days to 50% flowering; *Correlation is significant at the 0.05 level (2-tailed), Upper diagonal = Trial 1, Lower diagonal = Trial 2. Where, PH28 = Plant height at 28 days after sowing, PW28 = plant width at 28 days after sowing, TLL42 = terminal leaf length at 42 days after sowing,

TLW42 = terminal leaf width at 42 days after sowing, PH56 = plant height at 42 days after sowing, NOB = number of branches at 56 days after sowing.

The findings of this study revealed that there was great variation with respect to all traits studied. Great trait variation in a plant breeding program is crucial for several reasons, all of which contribute to the development of improved plant varieties that can meet specific agricultural, environmental and market needs. Selection of agronomic traits is key to speed up genetic improvement to increase cowpea yield output (Mofokeng *et al*., 2020). The results of these study revealed a wide range of number branches per plant for the studied accessions. These was consistent with findings of Odeseye *et al* 2020 who reported the lowest value of 3 branches per plant. Apte *et al.* 2020 and Nkouannessi (2005) found similar results in cowpea accessions they studied.

Generally, the result of this current study revealed that cowpea accessions with higher number of branches were also high yielding. The results of this study showed that the height of plant varied significantly among the cowpea accessions studied. Generally, it was observed that the tallest accessions also had higher grain yield. This could be attributed to the increased number of nodes, which leads to high number of branches. Adetiloye *et al*. (2017) also reported similar results.

Selection of plant leaf traits such as terminal leaf length and terminal leaf width is important in cowpea improvement programs. In the present study, terminal leaf length and terminal leaf width displayed a great phenotypic variation. However, the variation observed in these was lower than that reported by Gerrano *et al*. (2019), probably due to difference in number of accessions assessed. The leaf characteristics are important selection index since they affect the rate of photosynthesis, which directly affects plant growth, productivity and yield. Evans (2013) demonstrated that leaf characteristics, including chlorophyll content affect plant productivity. The chlorophyll pigment is responsible for capturing sunlight to drive photosynthesis (Taiz and Zeiger, (2010). Therefore, increased leaf length and width may lead to increase in yield.

The findings of this study revealed that there was a positive correlation between number of branches, plant height and grain yield. Generally, taller plant had higher number of branches and grain yield. Plant width was also found to have a positive correlation with

plant height, terminal leaf length and width, number of branches and grain yield. This shows that an increase in plant width (plant canopy spread) would lead to an increase in grain yield. The positive correlation among most of the traits studied may suggest the presence of strong association among them due to preponderance of genetic variance and genetic factors in the expression of these traits. Sheidu, and Igyuve (2023) found similar results when they correlate several cowpea traits. Meena *et al*. (2015) showed that the positive significant correlation observe among the traits will make selection and improvement of desirable traits easy. Several high yielding cowpea accessions were identified in the current study. These included NA3, MA24, NA101, NA11 and MA22. These accessions could be selected for adoption and for incorporation in breeding activities to develop breeding populations with superior yield potential. Selecting high yield cowpea accessions is pivotal in cowpea breeding.

The results in this study revealed that the great variation in number of days to 50% flowering in the accession studied. In general, shorter accessions flowered earlier relative taller plants. The results are in agreement to those of Madakbas and Ergin (2011) who found out that the dwarf varieties were early flowering. Adeigbe *et al*. 2015 also reported that there were variations in days to flowering among the genotypes and the results of this study followed the same trend. In this current study, generally, it was observed that highest yielding accessions were also early flowering. Singh *et al*. (1997) emphasized that early flowering varieties are often associated with higher grain yield, especially in environments where the growing season is limited by factors like drought or temperature extremes. The current studies identified two very early flowering accessions, i.e., MA29 and NA3(44-48) and one very late flowering accession, i.e., NA35(60DAS). According to Togola *et al*. (2019) those genotypes that had fewer days between 37 and 45, 46 and 55 and more than 55 days after sowing are classified as early, medium and late flowering, respectively.

4.1.3 Performance of Cowpea Genotypes under Water Stressed Conditions

4.1.3.1 Screening of Selected Cowpea Accessions for Drought Tolerance

The scoring of the severity of drought revealed that the accessions were classified as tolerant, resistant and susceptible to drought (Table 8; Appendix 7). The expression of the accessions varied with duration of drought imposition. Some were tolerant at 7 and 14 days after drought imposition and became susceptible at 21 or 28 DAS after

imposition of drought (Appendix 7). Some of the accessions remained resistant to drought even at 28 days after the imposition of drought, suggesting that they were resistant to drought. Some accessions were resistant at 7 14 and 21 days after imposition of drought and later became susceptible and some became tolerant (Table 8)

Table 8:Tolerance and susceptibility levels of cowpea genotypes at 21days after imposition of drought in trial 1 and 2

Treatment	Severity level 28DA1T2	Conclusion	Severity level 28DA1T2	conclusion
NA59	3	Resistant	3	Resistant
MA67	5	Resistant	5	Tolerant
MA15	5	Tolerant	5	Tolerant
MA62	5	Tolerant	5	Tolerant
MA63	3	Resistant	3	Resistant
MA69	3	Resistant	3	Resistant
MA49	3	Resistant	5	Tolerant
NA95	5	Resistant	3	Resistant
NA47	3	Resistant	3	Resistant
MA41	5	Tolerant	5	Tolerant
MA54	5	Tolerant	5	Tolerant
MA29	5	Tolerant	3	Resistant
MA30	5	Tolerant	5	Tolerant
MA51	3	Resistant	5	Tolerant
NA101	3	Resistant	3	Resistant
NA5	5	Tolerant	5	Tolerant
MA37	5	Tolerant	5	Tolerant
NA35	7	susceptible	7	susceptible
MA43	3	Resistant	5	Resistant
MA39	5	Resistant	3	Resistant
NA2	3	Resistant	3	Resistant
MA35	3	Resistant	3	Resistant
MA36	3	Resistant	5	Tolerant
NA64	5	Tolerant	5	Tolerant
NA45	5	Tolerant	5	Tolerant
NA11	3	Resistant	3	Tolerant
NA15	5	Tolerant	5	Tolerant
NA23	5	Tolerant	5	Tolerant
MA8	5	Tolerant	5	Tolerant
MA31	3	Resistant	5	Tolerant
MA25	3	Resistant	3	Resistant
NA60	5	Resistant	3	Resistant
MA2	3	Resistant	3	Resistant
NA3	3	Resistant	3	Resistant
NA72	3	Resistant	5	Tolerant
ES5	5	Tolerant	5	Tolerant
KK06	5	Tolerant	5	Tolerant
MA21	5	Resistant	3	Resistant

Table 8 (continued)

Treatment	Severity level 28DA1T2	Conclusion	Severity level 28DA1T2	conclusion
MA34	3	Resistant	5	Tolerant
MA24	3	Resistant	3	Resistant
MA44	3	Resistant	5	Tolerant
NA81	3	Resistant	5	Tolerant
NA82	7	Susceptible	7	Susceptible
MA45	3	Resistant	5	Tolerant
MA22	3	Resistant	3	Resistant
MA50	5	Tolerant	5	Tolerant
NA31	3	Resistant	5	Tolerant
NA20	5	Tolerant	5	Tolerant
NA29	5	Tolerant	5	Tolerant
NA8	3	Resistant	5	Tolerant

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accession, KK = Kakamega accessions, DAI = Days after imposition. Drought response level;3 = Resistant plants,5 =Tolerant plants and 7=susceptible plants.

The fifty cowpea genotypes displayed a range of expression with respect to drought tolerance, ranging from susceptible to tolerant to drought. Suggesting that it is possible to select the studied material for drought tolerance. Twenty-four accessions kept greener than the drought sensitive NA82 and NA35. In these accessions (NA82 and NA35), the plants were dry or were completely dead. Accessions such as MA24, MA22, MA2, NA3, NA101 and NA11 remained green in both trials, suggesting these accessions are good candidate for selection for drought tolerance. The drought tolerant accessions were observed to display a characteristic stay green. Accessions with delayed leaf senescence enhance plant survival under drought conditions and thus improved plant productivity. Di Fozon *et al.* (2000) reported that genetic variation exists for foliar senescence and genotypes and plants with leaves which remain green for longer periods than normal are defined as "stay-green". The results of this current study were in agreement with the findings of Belko *et al.* (2012) who reported that leaf senescence caused by drought stress varied across cowpea genotypes.

4.1.3.2 Evaluation of Selected Cowpea Accessions under Water Stressed Conditions

The test of the fitted model showed that it was adequate ($p < 0.05$) to explain the linear relationship between genotypes and all traits measured (Appendix 8). There was a significant interaction ($p < 0.05$) between the genotypes and trial for all variables

measured (Appendix 9). On testing the effects genotyped, the results indicated that there was significant ($p < 0.05$) effect of genotypes on all traits measured (Appendix 10). However, the blocking of the genotypes was found to be effective ($p > 0.05$).

Plant height at 28 DAS ranged from 7.89 cm to 25.33 cm with NA59 having the tallest plant and NA8 having the shortest plant. The plant width range was between 18.44 cm and 37.88 cm with NA3 having the widest plant canopy and NA82 having the lowest plant canopy (Table 9). Terminal leaf length ranged from 6.46 cm to 13.44 cm with MA50 having the longest terminal leaf and NA8 having the shortest terminal leaf. Terminal leaf width range from 2.05 cm (MA50) to 8.01cm (MA41). Plant height at 56 DAS ranged from 9.22 cm to 31.89 cm with NA59 having the tallest plants and NA8 having the shortest plants. Number of branches, ranged between 1.00 (MA15) to 5.94 (MA8).

In trial 2, plant height at 28 DAS ranged from 4.67 cm to 15.56 cm with MA39 having the shortest plants and KK06 having the tallest plant (Table 10). Plant width ranged from 8.00 cm (MA39) to 24.00 cm (KK06). Terminal leaf length ranged from 4.67 cm to 10.22 cm with NA20 having the shortest terminal leaf length and MA50 having the longest terminal leaf. Terminal leaf width range from 1.64 cm (MA50) to 6.94 cm (MA22). Plant height at 56 DAS ranged from 10.94 cm to 30.75 cm with NA82 having the lowest plant height and MA36 having the highest plant. The number of branches ranged from 2.89 (MA39) to 5.78 (MA24).

Table 9: Means of plant height (PH), plant width (PW), terminal leaf length (TLL), terminal leaf width (TLW) and number of branches (NOB) at different days after sowing under water stressed condition in Trial 1

Treatment	PH28DAS	PC28DAS	TLL42 DAS	TLW42 DAS	PH56DAS	N0B56 DAS
NA59	11.17jk	10.00v	5.06st	3.11tu	16.67jk	2.00t
MA67	15.78a	19.33ijk	7.00hi	4.83ghi	17.89fghij	4.78d
MA15	8.00qr	11.00uv	5.50r	4.00nop	16.00klm	1.00v
MA62	11.33ij	18.56klm	6.39lm	4.11no	14.33no	3.67klm
MA63	13.78c	24.67a	5.72q	3.83pq	20.00bcd	3.33o
MA69	15.00b	24.44a	8.56b	6.06a	20.33bc	4.89cd
MA49	13.33de	18.61klm	6.50l	3.44r	19.89bcde	4.50e
NA95	11.33ij	17.00no	6.00op	3.28rst	12.56p	3.56mn
NA47	12.11fg	16.18opq	7.33g	3.94opq	17.56ghijk	4.56e
MA41	16.17a	21.50def	6.08o	4.42lm	14.83	5.83a

Table 9 (Continued)

Treatment	PH28DAS	PC28DAS	TLL42DAS	TLW42DAS	PH56DAS	N0B56DAS
MA54	12.56ef	21.94cde	8.28c	5.22de	17.61ghijk	5.28b
MA29	12.83de	22.67bc	7.42g	5.50c	23.33a	4.50e
MA30	11.5ghij	17.67lmn	7.61ef	4.83ghi	21.17b	4.83cd
MA51	12.56ef	20.72fgh	7.14h	4.92fg	16.39jkl	5.17b
NA101	8.56pq	19.78hij	7.78e	5.06ef	18.7defgh	3.78ijk
NA5	11.8ghij	18.78jkl	5.83pq	3.33rs	12.11pq	3.44no
MA37	13.78c	19.33ijk	7.33g	5.06ef	18.7defgh	3.78ijk
NA35	10.67kl	16.67nop	7.72e	4.92fg	10.83rs	3.67klm
MA43	8.33q	14.50s	6.03o	3.81q	17.06hijk	3.44no
MA39	10.39l	19.83hij	8.75a	5.33cd	16.56jk	4.50e
NA2	13.67cd	19.78hij	6.78jk	5.06ef	18.33efghi	3.89hi
MA35	12.61ef	21.11defg	7.47fg	5.17de	19.78bcde	5.17b
MA36	13.67c	20.99efg	8.07d	5.27d	16.00klm	4.11g
NA64	7.44r	16.22opq	4.72u	2.17x	7.56t	2.56r
NA45	9.00op	12.50t	6.75k	3.17stu	9.83rs	2.83q
NA11	11.44hij	17.00no	7.06hi	5.06ef	16.89ijk	4.33f
NA15	9.67mn	10.50uv	5.75q	3.17stu	14.33no	2.50r
NA23	9.44no	11.22u	5.39r	2.89v	16.78ijk	1.22u
MA8	15.06b	22.22bcd	7.33g	4.67ijk	19.83bcde	5.94a
MA31	13.44cd	21.89cde	7.78e	4.72hijk	14.89lmn	3.89hi
MA25	11.22ijk	18.33klm	6.94ij	4.89fgh	17.11hijk	4.11g
NA60	11.7ghij	14.61s	6.14no	4.44lm	16.83ijk	2.83q
MA2	12.50ef	23.17b	7.67e	4.83ghi	17.72ghij	4.61e
NA3	8.17q	18.61klm	6.78jk	4.64jk	16.33jklm	4.50e
NA72	6.11s	14.28s	5.17s	3.28rst	9.17st	3.58lmn
ES5	11.8ghij	15.78pqr	6.78jk	4.72hijk	13.11op	3.00p
KK06	11.5ghij	21.00efg	8.00d	4.75ghij	17.00ijk	3.67klm
MA21	11.6ghij	17.56mn	6.28mn	4.17n	16.39jkl	4.28f
MA34	12.67ef	20.17ghi	7.00hi	4.58jkl	20.17bcd	4.83cd
MA24	12.06fgh	19.11ijk	6.69k	4.39m	19.44cdef	4.00gh
MA44	11.83ghi	21.56cdef	7.72e	5.17de	14.72mno	4.94c
NA81	9.33no	14.78rs	8.06d	4.56klm	17.33ghijk	4.50e
NA82	8.33q	13.00t	4.83u	2.33wx	8.00t	2.33s
MA45	13.00de	17.67lmn	7.44fg	5.06ef	17.22ghijk	4.56e
MA22	10.67kl	25.00a	7.77e	5.72b	17.00ijk	4.78d
MA50	9.17nop	17.17no	8.02	1.31y	7.67t	3.67klm
NA31	10.17lm	20.67fgh	8.72ab	5.33cd	18.8cdefg	3.83ij
NA20	5.75s	10.80uv	3.58v	2.47w	10.83qr	2.00t
NA29	9.39no	15.83pqr	4.89tu	2.33wx	13.39nop	3.06p
NA8	8.56pq	15.39qrs	5.47r	3.06uv	9.72rs	3.06p
LSD	0.7	0.55	0.31	0.25	0.63	0.2
R	0.99	0.997	0.98	0.99	0.996	0.99
CV	2.7	1.07	2.06	2.59	1.77	2.1

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accessions KK = Kakamega accessions, DAS = Days after sowing CV = Coefficient of variation, LSD = Least Significant Difference

Table 10: Means of plant height (PH), plant width (PW), terminal leaf length (TLL), terminal leaf width (TLW) and number of branches (NOB) at different days after sowing under water stressed condition in Trial 2

Treatment	PH28DAS	PW28DAS	TLL42DAS	TLW42DAS	PH56DAS	NOB56DAS
NA59	7.00t	13.00u	5.67wx	4.00rst	15.00uv	5.50ab
MA67	11.56ij	14.56t	6.56qrs	4.17opqrs	21.58fg	3.92lmn
MA15	12.44fgh	16.4opqrs	8.67cd	5.94b	16.50rst	3.9mnop
MA62	13.67c	17.44lmn	6.33rst	4.06qrst	18.9jklmno	4.33ghi
MA63	9.33o	18.33jkl	8.00fgh	5.22efghi	17.33pqrs	3.50qrs
MA69	13.78c	20.33defg	8.44cde	5.50cd	17.83nopq	3.6opqrs
MA49	12.50efg	20.0defgh	7.22mn	4.39no	20.06hij	5.02cd
NA95	7.11t	14.33t	6.28stu	4.11pqrst	15.17uv	3.56pqrs
NA47	11.67i	18.00klm	7.78ghij	4.50mn	19.06ijklm	4.83de
MA41	13.72c	19.44ghi	7.28klm	5.44cde	22.78e	5.22bc
MA54	12.61efg	20.72cde	7.56jk	5.08ghij	17.9mnopq	5.22bc
MA29	9.78n	19.89efgh	7.56jk	5.22efghi	22.46ef	4.33ghi
MA30	10.83l	17.94klm	7.22mn	4.50mn	21.56fg	5.56a
MA51	13.78c	20.89cd	7.11mno	4.67lm	20.44gh	3.9lmno
NA101	10.33m	20.56def	8.72c	5.94b	30.11a	5.11cd
NA5	12.78de	18.56ijk	6.00uv	3.67uv	15.89tu	5.00cd
MA37	12.67ef	23.78a	7.11mno	5.36cdef	19.0ijklmn	4.06jklm
NA35	12.56efg	15.56s	7.67ij	4.83kl	17.56pqr	3.1nopqr
MA43	10.11m	19.50ghi	7.25lmn	5.31defg	18.5lmnop	4.22ijk
MA39	4.67w	8.00x	6.67pq	3.47st	25.50c	2.89v
NA2	11.49ij	17.78klm	6.67pq	4.94jk	20.00hij	3.1nopqr
MA36	14.78b	22.17b	7.00no	4.36kl	30.75a	5.08cd
NA64	8.61q	14..11t	4.94y	2.83xy	11.50z	3.06uv
NA45	9.00p	12.00v	7.67ij	3.00wx	14.17vwx	4.50fgh
NA11	12.78de	19.67fgh	7.22mn	5.2defgh	19.11ijklm	4.33ghi
NA15	9.78n	11.33vw	5..56x	3.61v	12.11yz	4.22ijk
NA23	14.50b	16.7nopqr	6.33rst	4.33nop	17.78opq	3.33stu
MA8	7.67rs	10.61w	5.89vw	3.47v	17.78opq	3.9klmn
MA31	9.56no	17.2mnop	6.56qrs	4.06qrst	18.8klmno	4.56efg
MA25	13.00d	20.56def	6.61pqr	5.06hijk	20.00hij	3.7nopqr
NA60	11.78i	15.89rs	8.06fg	6.11b	22.17ef	4.67ef
MA2	12.17h	17.39lmno	7.75hij	5.56c	24.33cd	4.61efg
NA3	8.50q	20.0defgh	8.39de	5.89b	28.11b	4.33ghi
NA72	7.94r	17.1mnop	7.33klm	4.11pqrst	16.89qrst	4.00klm
ES5	11.33jk	16.11qrs	6.89op	4.39no	13.61wx	3.17tuv
KK06	15.56a	24.00a	6.39qrst	4.28nopq	19.89hijk	5.22bc
MA21	11.78i	17.89klm	6.44qrst	4.22opqr	20.17hi	4.83de
MA34	7.67rs	14.22t	6.61pqr	4.20opqr	13.44wx	3.89lmno
MA24	12.44fgh	19.11hij	7.51jkl	5.23efgh	24.00d	5.78a
MA44	9.78n	17.94klm	7.66ij	5.00ijk	16.08tu	3.8lmnop
ES5	11.33jk	16.11qrs	6.89op	4.39no	13.61wx	3.17tuv
KK06	15.56a	24.00a	6.39qrst	4.28nopq	19.89hijk	5.22bc
MA21	11.78i	17.89klm	6.44qrst	4.22opqr	20.17hi	4.83de
MA34	7.67rs	14.22t	6.61pqr	4.20opqr	13.44wx	3.89lmno

Table 10(Continued)

Treatment	PH28DAS	PW28DAS	TLL42DAS	TLW42DAS	PH56DAS	NOB56DAS
MA24	12.44fgh	19.11hij	7.51jkl	5.23efgh	24.00d	5.78a
MA44	9.78n	17.94klm	7.66ij	5.00ijk	16.08tu	3.82lmnop
NA81	11.17k	17.83klm	8.17ef	5.58c	17.33pqrs	5.08cd
NA82	7.50s	11.17vw	4.83y	2.86xy	10.94z	3.17tuv
MA45	11.67i	16.33pqrs	6.17tuv	4.83kl	18.5lmnop	4.68ef
MA22	12.33gh	21.67bc	9.78d	6.94a	21.83ef	4.50fgh
MA50	9.44o	17.56lmn	10.22a	1.64z	15.78tu	4.00klm
NA31	5.50v	14.25t	7.67ij	5.1fghij	13.22xy	4.11jkl
NA20	6.22u	11.94v	4.67y	2.72y	16.17stu	3.44rst
NA29	9.33o	19.44ghi	7.06mno	3.89tu	10.94z	3.94klmn
NA8	8.78pq	16.33pqrs	5.94vw	3.22w	14.50vw	4.11jkl
LSD	0.64	1.14	0.18	0.17	1.66	0.16
R	0.98	0.98	0.99	0.99	0.96	0.99
CV	3.22	3.52	1.5	2.33	5.95	2.26

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accessions, KK = Kakamega accessions, DAS = Days after sowing CV = Coefficient of variation, LSD = Least Significant Difference.

In trial1, grain yield ranges from 218.50 Kg/ha to 1836.18 Kg/ha while in trial 2, it ranged from 80.56 kg/ha to 2462.96 kg/ha (Table 11). The number of days to 50% flowering (DTF) ranged from 39.33 to 46.33 days with MA45, MA51, NA20 and MA50 being the earliest flowering and NA82, NA23, MA43, NA29 and MA63 being the latest flowering accessions. In trial 1, accessions NA35, NA72, NA20, MA51 and NA82 were in the lowest grain yielding category while NA3, NA11, MA22, MA24, MA29 and MA49 were in the highest grain yielding category. In trial 2, accessions MA24, NA11, NA3, MA22 and NA101 were the highest grain yielding category while NA35, NA82, NA64, MA15 and NA20 were the lowest grain yielding category. The accessions MA24, NA3, NA11, and MA22 performed well in both trial 1 and trial 2. These accessions were also found to be resistant to drought based on their high yielding potential under drought conditions.

Table 11: Means of grain yield and days to 50% flowering in the water stressed experiment

Genotypes	DTFT1	DTFT2	YieldT1	YieldT2
NA59	41.33efg	46.33j	407.82u	344.44uv
MA67	42.33cde	44.00o	1118.95fg	351.85uv
MA15	43.33bc	51.33b	646.19r	188.89w
MA62	43.33bc	50.00c	1075.33hi	421.30qrstu
MA63	44.00b	44.67n	1047.94ij	645.37mn
MA69	41.67defg	45.67kl	1011.00jkl	858.33hij
MA49	41.00efgh	47.33hi	1836.18a	672.22lm
NA95	41.33efg	49.00d	959.70lm	462.04pqr
NA47	43.33bc	49.00d	1032.96ijk	341.98uv
MA41	41.67defg	43.00qr	316.52vw	472.22pq
MA54	41.33efg	47.33hi	1140.26fg	587.04no
MA29	42.00cdef	42.00tu	1329.47d	437.04pqrst
MA30	41.33efg	48.33ef	850.08o	474.07pq
MA51	39.67hi	48.67de	266.47wxy	692.59lm
NA101	41.33efg	42.67rs	1187.42ef	1900.93d
NA5	42.33cde	46.00jk	844.09o	450.93pqrs
MA37	42.00cdef	48.33ef	1204.96e	914.81ghi
NA35	43.00bcd	56.33a	218.50y	174.07w
MA43	44.00b	45.00mn	746.70p	464.81pq
MA39	40.33ghi	51.33b	695.90pqr	830.56jk
NA2	43.00bcd	48.00fg	977.05l	515.74op
MA35	43.00bcd	46.00jk	962.94lm	974.07g
MA36	41.33efg	47.00i	970.58l	1224.07f
NA64	43.00bcd	45.00mn	745.77p	313.89v
NA45	40.33ghi	47.67gh	865.14no	411.11qrstu
NA11	40.33ghi	42.33st	1511.56c	2462.96a
NA15	43.33bc	49.67c	552.00s	480.56pq
NA23	44.00b	48.33ef	480.16t	944.44g
MA8	43.00bcd	46.33j	917.30mn	716.67lm
MA31	43.33bc	47.00i	658.31qr	568.21no
NA64	43.00bcd	45.00mn	745.77p	313.89v
NA60	40.67ghi	44.67n	1136.28fg	316.67v
MA2	41.44efg	43.33pq	1300.74d	1958.33d
NA3	41.33efg	43.33pq	1743.86b	2303.79a
NA72	40.67fghi	42.67rs	247.64xy	380.56rstuv
ES5	40.67fghi	45.33lm	654.90r	479.63pq
KK06	40.67fghi	43.67op	847.79o	315.28v
MA21	41.33efg	45.33lm	1196.05e	369.44stuv
MA34	42.33cde	46.00jk	1296.36d	375.00stuv
MA24	42.33cde	42.33st	1535.31c	2144.44c
MA44	40.33ghi	44.00o	1169.52efg	505.56op
NA81	43.00bcd	45.67kl	870.85no	925.00gh
NA82	46.33a	47.67gh	220.12y	80.56x
MA45	39.33j	42.00tu	431.03tu	753.70kl
MA22	41.00efgh	43.33pq	991.70kl	1421.30e
MA50	39.67hi	41.67u	416.03u	838.89ij
NA31	40.67fghi	47.33hi	1077.26hi	905.56ghij

Table 11 (Continued)

Treatment	DTFT1	DTFT2	YieldT1	YieldT2
NA20	39.67hi	42.67rs	292.64wx	172.22w
NA29	44.00b	48.33ef	302.76vw	350.00uv
NA8	41.33efg	49..00d	352.10v	464.81pq
LSD	1.50	0.495	53.08	83.114
R	0.79	0.992	0.996	0.997
CV	2.21	0.66	3.78	5.42

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, ES = Eastern accessions, KK = Kakamega accessions, DAS = Days after sowing CV = Coefficient of variation, LSD=Least Significant Difference.

In Trial 1, there was positive significant correlation ($p < 0.05$) between the plant height at 28 DAS, plant width, terminal leaf length, terminal leaf width, number of branches and grain yield (Table 12). In trial 2, there was positive significant correlation ($p < 0.05$) between plant height at 28 DAS, plant width at 28 DAS, terminal leaf width, plant height at 56 DAS and number of branches (Table 12). There was negative significant correlation (-0.40**) between days to 50% flowering and grain yield.

Table 12: Correlation analysis for plant height, plant width, terminal leaf length and width, number of branches, grain yield and days to 50% flowering at different growth stages in water stressed conditions in Tria1 and 2.

	PH 28	PW28	TLL42	TLW42	PH56	NOB56	Yield	DTF
PH 28		0.66**	0.46**	0.558**	0.587**	0.63**	0.34*	-0.01
PW28	0.70**		0.65**	0.658**	0.501**	0.77**	0.44**	-0.13
TLL 42	0.24	0.45**		0.738**	0.473**	0.66**	0.37**	-0.31*
TLW 42	0.42**	0.54**	0.54**		0.695**	0.63**	0.44**	-0.23
PH56	0.349*	0.42**	0.37**	0.493**		0.47**	0.60**	0.008
NOB	0.351*	0.43**	0.21	0.283*	0.399**		0.39**	-0.26
Yield(N)	0.156	0.32*	0.41**	0.435**	0.571**	0.26		-0.096
DTF	0.001	-0.27	-0.19	-0.140	-0.241	-0.26	-0.40**	

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed). Upper diagonal=T1, Lower diagonal T2

Where, PH28 = Plant height at 28 days after sowing, PW28=plant width at 28days after sawing, TLL42=terminal leaf length at 42days after sawing, TLW42=terminal leaf width at 42days after sawing, PH56=plant height at 42days after sawing, NOB=number of branches at 56days after sawing.

Selecting cowpea accessions for drought tolerance involves identifying genotypes that exhibit specific traits associated with better performance under water limited conditions. In this study delayed leaf senescence (stay green trait) was shown to be trait that can be incorporated with other traits to provide a good selection index for drought

tolerant genotypes. Other traits that can be used to generate a good selection index for drought tolerance may include root architecture (deep and extensive root systems), stomatal conductance and transpiration efficiency, phenological adaptation (early flowering and maturity), biomass partitioning, water use efficiency and secondary metabolite profiles. Gomes *et al*. (2020) highlighted the advantages of an integrated morphological, physiological and biochemical analysis, to identify response mechanisms and potential probes for drought tolerance in cowpea, aiming to support selection and breeding strategies to obtain high yield and drought tolerant varieties. Bhatnagar-Mathur *et al*. (2010) showed that different mechanisms such as drought escape may make a plant tolerant to drought. Drought escape may involve rapid phenological development like early flowering (Gaur *et al.,* 2008).

In this study early flowering and stay green traits were used to characterise drought tolerant accessions. Six accessions, which were MA45, MA51, NA20, MA50, MA24 and NA11, were early flowering, with days to 50% being less than 42 days. On the other hand, four accessions (MA62, MA15, MA39 and NA35) registered the longest days to 50% flowering, more than 55days. The findings of this are consistent with those of Seleiman *et al*. (2021) which stated that delayed senescence is one the strategies that are adopted by plants under water deficit. There was negative significant correlation between days to 50% flowering and grain yield, perhaps indicating that there is yield penalty for any reduction of crop maturity duration below the optimum. These findings are similar to those of Caliskan *et al*. (2008). However, selection for earliness is still a common practice in breeding crops for drought tolerance. However, it had been observed that delayed flowering and maturation can prevent the formation of proper quality seeds in cowpea (Aliyu *et al*., 2022).

The high yielding of NA101, NA3, NA11, MA2 and MA24 reflects the ability of these accessions to tolerate and respond to a wide range of temperature and moisture stress conditions. Plant growth and development change because of physiological alterations in response to water deficiency. Studies have found out that different molecular, biochemical, physiological, morphological and ecological traits and processes of the plants are impaired under drought stress conditions (Ortiz *et al*., 2015). The identification of genotype(s) adapted to drought stress is expected to facilitate the breeding efforts toward development of drought tolerant varieties. In this current study,

the evaluation of cowpea accessions under water limited conditions showed wide range of phenotypic variation for grain yield and all other agronomic traits measured, indicating that selection of drought tolerant accessions can be effective. These results were in agreement with those of Bowman *et al.* (2017).

Understanding the correlations between variables is essential in plant breeding, as it provides valuable insights into how different traits are related. These correlations can significantly influence the decisions made during the selection of genotypes of interest, particularly when the goal is to improve multiple traits simultaneously. There was positive significant correlation between PH28 DAS and gain yield, indicating that selecting for taller plants at this early stage could have a favourable impact on grain yield. Plant width (canopy spread) showed positive significant correlations with traits like terminal leaf width, plant height, number of branches and grain yield. This suggests that plants with wider canopies might develop larger leaves, be taller, and produce more branches, retain more soil moisture, thus potentially contributing to overall plant robustness, ultimately leading to higher grain yield. The results of this study are supported by those of Walle *et al.,* (2018), Neyhart*et al.* (2019) and Hassani *Hassani*. (2024).

4.2 Molecular Characterisation of Selected Cowpea Accessions

4.2.1 Genetic Diversity of Selected Cowpea Accessions

In total, 69 alleles were observed for the 12 SSR markers across all 20 cowpea accessions analysed (Table 13). The number of alleles per locus ranged from 1 to 11 with a mean value of 10.6. The majority of SSR loci scored were highly polymorphic displaying PIC values ranging from 0 to 0.92 with a mean of 0.49. In most of the cases the PCR amplification product size range was higher than five, suggesting greater genetic diversity for cowpea accessions. Genetic diversity ranged from 0.00 to 0.85 with mean of 0.53. The heterozygosity ranged from 0.00 to 0.85 with a mean of 0.49. The F_{IS} values ranged from -0.22 to 0.76. Positive F_{IS} values indicate a deficiency of heterozygotes, while negative values suggest an excess of heterozygotes.

Table 13: Molecular characterisation of accessions

SSR Marker	Number of alleles	Gene diversity	Observed heterozygosity	F_{IS}	PIC	Allelic range
VM68	10	0.79	0.50	0.37	0.77	234 - 254
VM40	8	0.63	0.15	0.76	0.61	170 - 195
VM39	6	0.77	0.20	0.74	0.73	196 - 210
VM3	2	0.50	0.40	0.2	0.37	402 - 404
VM61	1	0.00	0.00	*m	0.00	181
VM22	2	0.35	0.45	-0.22	0.29	221 - 223
VM70	11	0.85	0.40	0.53	0.84	267 - 295
VM26	9	0.72	0.85	-0.18	0.67	72 - 287
VM31	10	0.83	0.77	0.07	0.81	188 - 227
VM19	1	0.00	0.00	*m	0.00	119
CP171	6	0.59	0.35	0.41	0.55	85 - 122
MA70	3	0.30	0.35	-0.17	0.28	269 - 274

F_{IS} =Inbreeding coefficient, *m = monomorphic PIC = polymorphism information content

Generally, the PIC values were high, meaning most of the SSR markers can distinguish between many different alleles at a given locus. This is particularly useful in the differentiation of cowpea accessions, as it indicates a high level of genetic diversity at that specific marker. In this study, the observed PIC were within the range of those observed by Kuruma *et al.* (2010), but higher than those observed by Gumede *et al*. (2022). High PIC values may also reflect the presence of rare alleles (Agrama and Tuinstra, 2003). Markers with a high number of variants, or high levels of polymorphism, can indeed indicate introgression among different genotypes (Padulosi and Bordoni, 2007).

A high total number of alleles was identified across 12 SSR markers. The presence of such a high number of alleles per locus indicates substantial allelic diversity among the cowpea accessions. These findings are in agreement with those of Cholastova and Knotova (2012) and Adjebeng-Danquah *et al*. (2020) who found high allelic frequency was detected across the accessions. High allelic diversity is a desirable feature in breeding programs as it suggests the presence of multiple genetic variations that can be utilized for improving traits of interest. The relatively high mean gene diversity observed suggests that the cowpea accessions possess a wide range of genetic differences, which is beneficial for breeding efforts aimed at improving multiple traits simultaneously. The presence of genetic diversity in crop populations cannot be fully captured by morphological characteristics alone. While morphological traits provide

visible differences, they often do not reflect the underlying genetic variability accurately. This is particularly true for traits that are influenced by environmental conditions, making it challenging to distinguish between genetic and environmental effects. Molecular markers can detect polymorphisms at the DNA level, revealing genetic variation that is not visible through morphological assessment. Several studies have use different types of molecular markers to assess genetic diversity (Nkhoma *et al*., 2020; Gumede *et al*., 2022; Potts *et al*., 2024). These studies revealed that the accessions studied exhibited considerable variation across geographical regions, sub-species, and improvement status. Such detailed information is crucial for understanding the genetic structure of cowpea accessions and for identifying unique genotypes or alleles that might be valuable in breeding programs.

The moderate to high observed heterozygosity observed indicates that there is considerable genetic variation within the cowpea accessions. High heterozygosity is often associated with greater adaptability and survival, especially under variable environmental conditions. Generally, gene diversity was higher in observed heterozygosity, suggesting presence of inbreeding. Inbreeding reduces the number of heterozygous individuals compared to what is expected based on allele frequencies (Schmidt *et al*., 2021).

The VM40 locus showed the highest F_{IS} (0.76), indicating a significant deficiency of heterozygotes while the VM22 and VM26 loci showed negative F_{IS} values, suggesting an excess of heterozygotes. The variation in F_{IS} values could also reflect the diverse origins of the cowpea accessions in this study, possibly representing adaptation to different environments. According to Gomes *et al*., (2020), two landraces exhibited negative F_{IS} values, indicating that these landraces are less related than expected which could imply fewer homozygotes and consequent crossbreeding.

4.2.2 Population Structure of the Selected Cowpea Accessions

Structure analysis predicted K = 5 as the optimum subpopulations, suggesting that at least five distinct group exist in the studied cowpea accessions (Figure 5). Sarr *et al*. 2020 reported three populations in their studied accessions. The accessions assessed in this study were expected to be highly differentiated as they were obtained from different regions Sarr *et al.* (2020) suggested that high intra-regional diversity could be linked to

the presence of many different accessions in each region. While the low genetic diversity between regions could be partly explained by the distribution of the same cowpea varieties in different regions consequently, having high gene flow and thus high rate of inbreeding that would be expected among subpopulations. These Structure results support the high morphological variation observed on the studied accessions. However, low observed heterozygosity was observed for some loci, probably suggesting substantial exchange of cowpea accessions between adjacent counties of villages, which could lead to long distance seed-mediate gene flow as seeds are moved from one place to another. Gbedevi *et al*. (2021) results revealed a moderate level of diversity among cowpea germplasm which was attributed to farmer-to-farmer seed exchange.

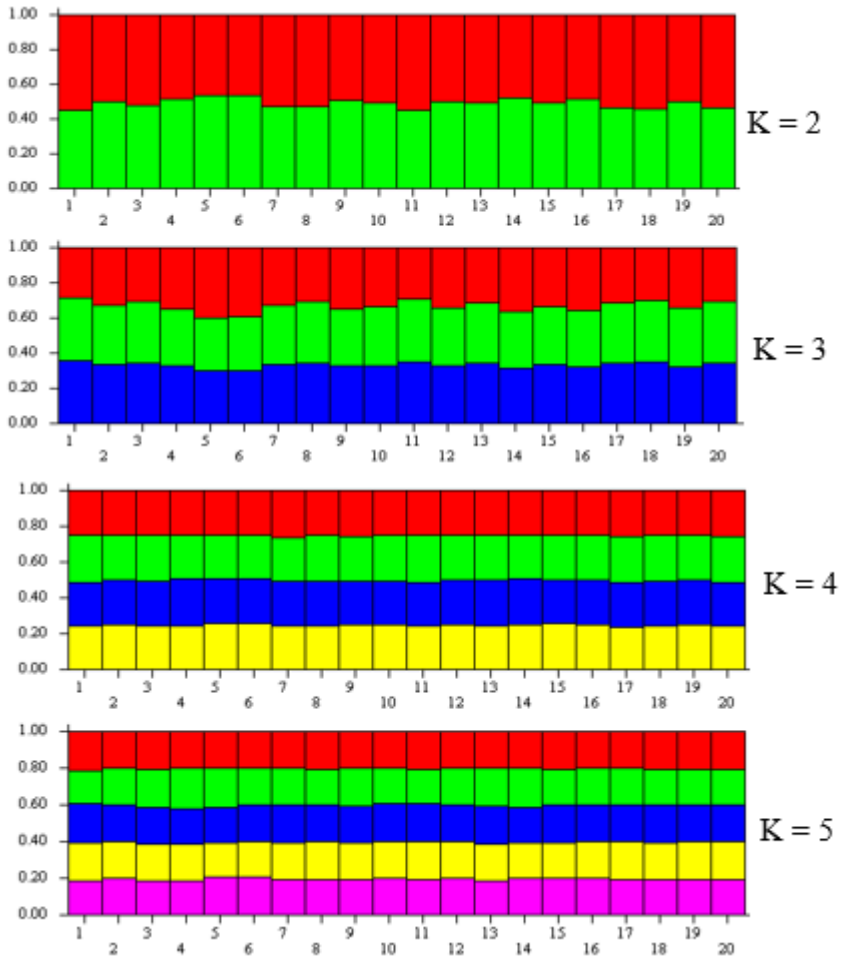

Figure 2: Bar plots of the STRUCTURE analysis. Each of the 20 cowpea accessions is represented by a vertical bar being partitioned in K = 2 up to K = 5 coloured segments that designate the population's estimated membership fraction in the inferred subgroups. Accessions numbering is according to serial number given in Appendix 11.

4.3 Biochemical Characterization of Cowpea Genotypes using Quercetin and Myricetin as Biomarker for Drought Tolerance and Leaf Quality

The test of the fitted model showed that it was adequate ($p < 0.05$) in explaining the linear relationship between genotypes, quercetin and myricetin (Appendix 12). There was a significant effect ($p < 0.05$) of genotypes on concentration of quercetin and myricetin (Appendix 13). Quercetin concentration ranged from 1.61 mg/kg to 516.77 mg/kg with F_1 cowpea genotype MA67 X NA11 yielding the lowest amount and cowpea accession NA60 yielding the highest amount (Table 14). Myricetin concentration ranged from 0 to 19.26 mg/kg with NA11 X NA60 and NA60 yielding no detectable amount and NA20 yielding the highest amount.

Table 14: Concentration of myricetin and quercetin flavonoid, grain yield and drought susceptibility index of 10 cowpea accessions and 8 F_1 cowpea hybrids grown under water-stress conditions

Genotypes	Quercetin (mg/kg)	Myricetin (mg/kg)	Grain yield (kg/ha)	Drought sensitivity index at 28 DAI
NA60	516.77a	0i	1080.74e	3
NA20	239.00b	19.26a	311.30i	5
NA64	143.84c	19.16a	745.77p	5
NA5	133.75d	7.29e	844.09o	5
MA67	133.04d	9.34d	1563.33bc	3
NA11	122.73e	12.22c	1344.81d	5
MA2	90.95f	3.61g	1300.74d	3
KK06	69.25g	15.38b	1014.4ef	5
MA22	66.04g	7.80e	991.70kl	3
MA50	45.39h	5.83f	730.74h	3
MA67 X NA60	36.44i	5.78f	1375.93d	3
MA50 X NA11	23.47j	4.13g	1435.19cd	3
MA50 X MA67	21.12jk	11.74c	3220.37a	3
KK06 X NA11	15.04k	3.56g	938.89efg	3
NA20 X NA60	5.61l	1.81h	1668.52b	5
NA11 X NA60	4.76l	0i	794.44gh	5
MA50 X NA60	2.23l	2.15h	781.48gh	3
MA67 X NA11	1.61l	2.18h	881.48fgh	5
LSD	2.07	1.41	182.85	
R	0.9999	0.99	0.982	
CV	1.20	10.25	9.51	

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAI = Days after imposition of drought, CV = Coefficient of variation, LSD = Least Significant Difference.

The finding of this study showed that there was positive significant correlation ($p < 0.05$) between quercetin and DSI at 21 DAI, suggesting that quercetin production might be triggered by drought stress at this stage (Table 15). There was also positive significant correlation ($p < 0.05$) between myricetin and DSI at 21 DAI, reinforcing the link between drought stress and increased production of these flavonoids. There was weak negative non-significant correlation ($p > 0.05$) between grain yield and the two flavonoids (quercetin and myricetin). The weak negative correlations with quercetin and myricetin, indicating that higher concentrations of these compounds might be associated with stress conditions leading to reduced yield. There was weak negative non-significant correlation ($p > 0.05$) between grain yield and DSI at 14, 21 and 28 DAI.

Table 15: Correlation analysis for concentration quercetin and myricetin, drought severity index at 7, 14, 21 and 28 days after imposition of drought.

	DSI7DAI	DSI14DA1	DSI21DAI	DSI28DAI	Grain yield	Quercetin
DSI7DAI						
DSI14DA1	0.343					
DSI21DAI	0.271	0.553*				
DSI28DAI	0.239	0.582*	0.564*			
Grain yield	0.164	-0.341	-0.431	-0.359		
Quercetin	0.164	0.341	0.474*	0.15	-0.11	
Myricetin	0.257	0.33	0.550*	0.29	-0.088	0.545*

Where DAI = Days after imposition of drought; DSI = Drought severity index

The finding of this study showed that there was significant effect of genotypes on quercetin and myricetin concentrations, highlighting that there is genetic variability in cowpea with respect to these important flavonoids. Studies has shown that concentration of phenolic compounds, including flavonoid in crops varies with environmental conditions (Gitonga *et al*., 2022). Plants generally secrete secondary metabolites such as flavonoids, alkaloids, terpenoids and steroids in response to different plant stress (Roy *et al*., 2022). The wide range in quercetin and myricetin concentration, underscores the potential for selecting genotypes with specific traits, such as high flavonoid content. The high concentration of flavonoid can be used as biochemical markers in a plant breeding program. Similarly, the variation in myricetin concentration, with some genotypes showing no detectable levels, indicates the possibility of breeding for or against this compound depending on the desired outcome.

The findings from genotypes such as MA67 X NA11 and NA60 are particularly notable, as they represent extremes cases in the concentration of these flavonoids. The finding of this current study is in agreement with those of Molee and Waraasathaporn (2016) who found out that drought stress led to a significant increase in the concentration of phenolic compounds. The accumulation of these phenolic compounds was observed as a part of the plant's adaptive response to mitigate the adverse effects of drought stress.

The analysis indicates that drought severity at 21 days after the imposition of drought is particularly influential in enhancing the production of quercetin and myricetin. However, this increase in flavonoid concentration is associated with a decrease in grain yield, suggesting a trade-off between stress tolerance and productivity. Studies has shown that drought stress affects vegetative and reproductive growth processes and synthesis of secondary metabolites in plants (Yang *et al*., 2020). Generally, genotypes with high concentration of myricetin and quercetin had higher grain yield and lower DSI, probably indicating that secondary metabolites are a more conservative strategy by plants to cope with drought in the aerial parts. This observation is in agreement with the findings of Goufo *et al*. (2017) who found out that secondary metabolites accumulated differently in roots, but similarly in leaves, supporting that they are a more conservative strategy to cope with drought by plant biomass. This uniform response in the aerial parts likely serves as a protective mechanism to maintain vital physiological functions and mitigate oxidative damage during periods of water scarcity. The conservative nature of this response underscores the importance of these metabolites in the plant's overall drought tolerance strategy.

The finding of the study showed that the studied genotypes displayed wide phenotypic and genetic variation. The accumulation of quercetin and myricetin varied significantly among different cowpea genotypes under drought stress. This may suggest that roots may employ different strategies or metabolic pathways to handle drought conditions, potentially focusing on maintaining root function and structure. Under drought conditions, certain root architectures may be more effective in accessing deeper water sources or in minimizing water loss, which can reduce stress and thereby influence the plant's metabolic pathways. Plants with more extensive or deeper root systems might experience less severe drought stress, potentially leading to different profiles of

secondary metabolite production compared to plants with less efficient root systems. Ryu and Cho (2015) suggested that genetic factors influencing root architecture may also be linked to the pathways responsible for secondary metabolite production.

The genotypes with high amount of quercetin and myricetin can be used in breeding programs to significantly improve the quality of legume vegetable leaves. The presence of high amount quercetin and myricetin in legume vegetable leaves may enhance their nutritional quality, supports health, aids in stress resistance, and increases marketability, making them vital components of both human diets and agricultural systems. Studies has shown that flavonoids contain indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche *et al*., 2016). Flavonoids such as quercetin, myricetin and kaempferol possess antimutagenic, anticancer, and antihypertensive activities. Kruger and Taylor (2016) and Galani *et al*. (2018) revealed that nutritional composition of legumes and pulses influences consumer preferences and thus their marketability. Given the growing consumer interest in functional foods with added health benefits, cowpea genotypes with high in quercetin and myricetin can be used in development of vegetable or dual-purpose cowpea varieties and be marketed as premium products. This can create niche markets and add economic value for producers.

4.4 Development of F_1 Genotypes for Dual purpose and Drought Tolerance

4.4.1 Development of F1 Genotypes

The study successfully developed 10 F_1 hybrid cowpea, out of the 15 F_1 hybrids that were supposed to be developed. The hybrids developed included MA50 X MA67, KK06 X MA67, MA50 X NA60, NA60 X NA20, MA50 X NA11, MA67 X NA60, KK06 X NA11, MA67 X NA11, NA11 X NA60 and MA50 X NA20. Some parents such as NA60 and MA50 were identified as poor donors while others like KK06 and MA67 were poor receptors, leading to production of 10 instead of 15 F_1 hybrids. When KK06 and MA67 were used as female, the success rate for the cross was 0%. However, they were also identified as good donors and when used as male the success rate of the cross was more than 90%. Genotypes acting as good pollen donors and poor pollen receptors could be as a result of abortion of the embryo or other forms of reproductive failure.

In this study, it was observed that cowpea pollen is typically available in the morning, and it is most efficient to emasculate the tiny flower and pollinate them immediately while the flower is readily identified. The findings of this study are in agreement with those of Alghamdi and El- (2021) and Reddy and Reddy (2022) who found out that timing of pollen availability and flower emasculation are critical factors in hybridisation success rate. The findings of this current study are also consistent with those of Abolhasani and Khosravi (2023) and Sharma and Reddy (2023) who showed that immediate pollination and efficiency handling tiny flowers with precision and speed increase the probability of successful crosses. The implication is that by performing both emasculation and pollination promptly in the morning, the likelihood of successful cross was increased and the risk of missing the optimal window for pollination was reduced.

4.4.2 Phenotypic Frequency and Diversity of Qualitative Traits for Six Cowpea Parents and their 10 F_1 Genotypes

4.4.2.1 Phenotypic Frequency and Diversity of Qualitative Traits

The findings of this showed that the majority (62.5%) of genotypes had their produced racemes throughout the canopy, with 25% and 12.5% had their raceme positions at the same level as the canopy and above the canopy, respectively (Table 16). Standardised Shannon-Weaver index of 0.36 indicates that raceme has moderate diversity among the studied genotypes. About 75% of the genotypes showed no twinning, 6.25% showed slight twinning tendency, 6.25% had intermediate twinning tendency while 12.5% showed pronounced twinning tendency. Standardised Shannon-Weaver index of 0.33 indicates that twinning tendency has moderate diversity among the studied genotypes. About 18.75% of the genotypes had acute erect growth habit, 37.5% had intermediate, 25% had semi prostrate while 18.75% had prostrate growth habit. Standardised Shannon-Weaver index of 0.46 indicates that growth habit has high diversity among the studied genotypes. About 87.5% of the genotypes had violet flowers while 12.5% had white flowers. Standardised Shannon-Weaver index of 0.14 indicates that flower colour has low diversity among the studied genotypes.

The findings of this study revealed that 25% of the genotypes had brown seeds, 37.5% had cream colour, 18.75 had black seeds, 6.25% had grey seeds and 12.5% had white flowers (Table 16). Standardised Shannon-Weaver index of 0.53 indicates that seed

colour has high diversity among the studied genotypes. The majority (62.5%) of the genotypes had straight pods, while 31.25% were slightly curved and 6.25% were curved pods. Standardised Shannon-Weaver index of 0.11 indicates that mature pod curvature has low diversity among the studied genotypes. A majority (43.75%) of the genotypes had no plant pigmentation, while 12.5% had very slight pigmentation, 37.5% had moderate pigmentation and 6.25% had intermediate plant pigmentation. Standardised Shannon-Weaver index of 0.36 indicates that plant pigmentation has moderate diversity among the studied genotypes. On immature pod pigmentation the observed frequencies were 25.0% had no pigmentation, 6.25% had a pigmented tip, 6.25% had pigmented valves, 18.75% had splashes of pigment and 43.75% were uniformly pigmented. Standardised Shannon-Weaver index of 0.38 indicates that plant pigmentation has moderate diversity among the studied genotypes.

The results of this study revealed that the majority (62.5%) of the genotypes were glabrescent, while 25.0% were short appressed and 12.5% were pubescent (Table 16). Standardised Shannon-Weaver index of 0.32 indicates that plant hairiness has moderate diversity among the studied genotypes. On terminal leaflet shape, 37.5% were globose and 62.5% were sub globose. Standardised Shannon-Weaver index of 0.24 indicates that terminal leaflet shape has moderate diversity among the studied genotypes. The leaves of all genotypes studied had some leaf markings. Consequently, this trait had no diversity. The majority (81.25%) of genotypes had intermediate pattern and 18.75% had determinate growth pattern. Standardised Shannon-Weaver index of 0.24 indicates that growth habit has high diversity among the studied genotypes.

Table 16:Frequency table of the F1 genotypes and their parents

Variable	Description	Frequency	Percentage (%)	Shannon waver Index(H)	Standardised Shannon-Weaver index(H')
Immature pod pigmentation	None	7	43.8	1.06	0.38
	Pigmented tip	1	6.3		
	pigmented sutures	0	0		
	Pigmented valves	1	6.3		
	Splashes of pigment	3	18.8		
	Uniformly pigmented	4	25.0		
	total				
Plant hairiness	Glabrescent	10	62.5	0.89	0.32
	short appressed	4	25.0		
	Pubescent	2	12.5		
	Total				
Terminal leaflet shape	Globose	6	37.5	0.67	0.24
	Sub globose	10	62.5		
	Sub hastate	0	0		
Growth pattern	Determinate	3	18.8	0.49	0.17
	Indeterminate	13	81.3		
Leaf marking	present	16	100.0	0	0
	Absent	0	0		
Leaf color intensity	Pale green	9	56.3	0.67	0.24
	Intermediate green	3	18.8		
	Dark green	4	25.0		
	Total				
Raceme position	Mostly above canopy	2	12.5	0.93	0.33
	in upper canopy	4	25		
	throughout	10	62.25		

Table 16 (Continued)

Variable	Description	Frequency	Percentage (%)	Shannon waver Index(H)	Standardised Shannon-Weaver index(H')
Growth Habit	Acute erect	3	18.75	1.27	0.46
	Semi-erect	0	0		
	Intermediate	6	37.5		
	Semi prostrate	4	25.0		
	Prostrate	3	18.75		
	Climbing	0	0		
	Total				
Plant pigmentation	None	7	43.8	1.04	0.36
	very slight	2	12.5		
	Moderate	6	37.5		
	Intermediate	1	6.3		
	Extensive				
	Total				
Flower colour	violet	14	87.5	0.38	0.14
	white	2	12.5		
	Pink	0	0		
Twinning tendency	None	12	75.0	1.02	0.36
	slight	1	6.25		
	Intermediate	1	6.25		
	Pronounced	2	12.5		
Mature pod curvature	straight	10	62.5	0.32	0.11
	Slightly curved	5	31.25		
	curved	1	6.25		
	coiled	0	0		

Table 16 (Continued)

Variable	Description	Frequency	Percentage (%)	Shannon waver Index(H)	Standardised Shannon-Weaver index(H')
Seed colour	Brown	4	25	1.47	0.53
	cream	6	37.5		
	Black	3	18.75		
	Gray	1	6.25		
	White	2	12.5		

The Shannon-Weaver index revealed that majority of the traits had medium to high diversity, with leaf marking showing no diversity. The diversity of traits is crucial for breeding programs as they reflect the genetic variability within the population. Raceme position is a crucial trait in breeding programs, particularly for improving harvesting efficiency, whether done manually or mechanically. Yuan *et al*. (2020) demonstrated that structural traits such raceme position and growth habit that influence crop productivity are important trait in plant breeding.

The results of this study suggest that most of the genotypes studied may need no staking since majority of them did not show trailing tendency. Such genotypes may have an advantage since the associated cost and labour involved in providing stakes to prevent rotting would not be necessary and this can reduce the cost of production. It was observed that erect genotypes may have high reproductive efficiency while the semi-erect ones seem to have higher total pod yield than the spreading types. These observations are consistent with results of Miklas and Porch (2020) who highlighted the importance of plant architecture on productivity of legume crops.

Leaf colour, shape, and texture are indeed crucial traits in vegetable cowpea production, especially when considering aspects such as chlorophyll concentration, plant health, and the marketability of the produce. Timko and Singh (2021) highlighted various traits important for cowpea production, including leaf colour, shape and texture, and their impact on the plant's overall health and productivity. Barker and Lee (2020) provide insights into the genetic basis of leaf colour and its relation to chlorophyll concentration in cowpea. Chlorophyll concentration is a key factor influencing the plant's photosynthetic efficiency, growth, and overall health. Higher chlorophyll content is

typically associated with more vigorous plants, which can result in better yield and quality of both leaves and pods.

4.4.2.2 Principal Component Analysis

The PC1 explained the highest variation (26.7%) in the data, with strong loadings on traits such as growth pattern, twinning tendency, and raceme position (Table 17). The PC2 explained an additional 21.3% of the variation, highlighting traits like plant pigmentation and leaf colour intensity. The PC3 adds another 13.8% to the variation explained, with notable loadings on traits like growth habit and flower colour. In PC1, growth pattern, twining tendency and raceme position, with factor loading of 0.82662, 0.92509 and 0.82069, respectively, had strong positive contribution, meaning variations in these factors were a major factor in differentiating the cowpea genotypes studied. In PC 2, plant pigmentation and leaf colour intensity, with factor loading of 0.84588 and 0.71596, respectively, had strong positive contribution, implying that variations in these factors plays an important role in PC2. In PC 3, growth habit, flower colour and mature pod curvature, with factor loading of 0.79849, -0.60085 and 0.45174, respectively, had strong contribution to the differentiation along PC3.

Table 17: Principal component (PC) analysis of 13 qualitative traits for six parents and their F1 hybrids accessions

	PC1	PC2	PC3
Eigen Value	3.206	2.556	1.659
Variation explained	3.189	2.447	1.78
Cumulative % of variation	26.7	48.0	61.8
	Variables		
Terminal leaf shape	-0.43926	0.40847	-0.1171
Growth habit	0.18645	0.26030	0.79849
Growth pattern	0.82662	-0.12314	-0.08247
Twinning tendency	0.92509	-0.06439	-0.09622
Plant pigmentation	-0.03815	0.84588	0.13018
Plant hairiness	0.15930	-0.36620	-0.41968
Leaf colour intensity	0.42240	0.71596	-0.33386
Leaf marking	0.00	0.00	0.00
Immature pod pigmentation	0.51869	0.48220	0.01450
Flower colour	0.29749	0.14466	-0.60085
Raceme position	0.82069	-0.06486	0.11428
Seed colour	0.33091	-0.7662	0.33025
Mature pod curvature	0.30514	0.30831	0.45174

The first three principal components explained 61.8% of the total variability among 16 genotypes evaluated for 13 traits. This was higher than that reported by Fatokun *et al*. (2017). Twinning tendency showed the highest loading in PC1, plant pigmentation showed the highest loading in PC2 while growth habit showed the highest loading in PC3. This implies that these factors are key traits in the differentiation of genotype in their respective PC. Rani and Dubey (2020) demonstrated that a high factor loading, whether positive or negative, indicate that it is a major factor in differentiating the genotypes. According to Hair *et al*. (2009), the factor loading effect of any traits greater than ±0.3 is regarded meaningful and significant in differentiating genotypes. Chahal and Gosal (2002) showed that the largest absolute value influencing the cluster more than those with lower absolute values closer to zero.

Cluster analysis of genotypes was performed on the basis of 13 characters and is presented in Figure 2. The analysis grouped 16 cowpea genotypes into 4 major clusters. The most diverse genotypes were NA11 X NA60, MA50 X MA67, MA67, NA20 X NA60 and NA11.This indicated the existence of diversity among the accessions. However, most of the genotypes were clustered together showing close relationship for these genotypes.

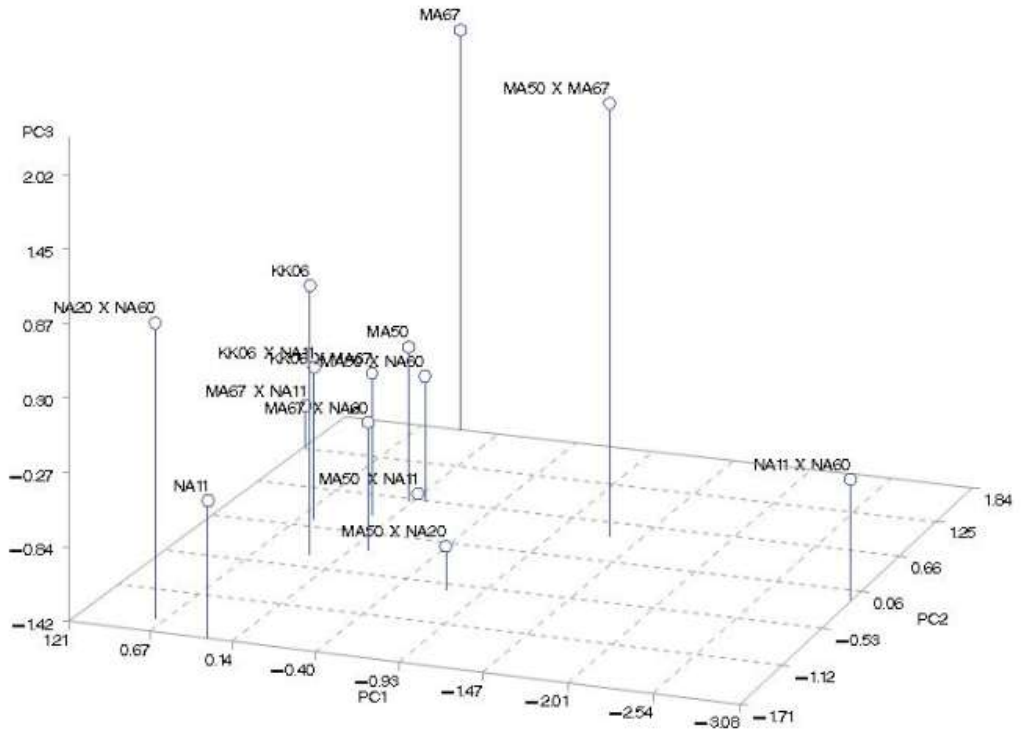

Figure 3: Cluster analysis of 16 cowpea genotypes based on thirteen qualitative traits

4.4.3 Evaluation of Developed F1 Hybrid Cowpea Genotypes and their Parents under Normal Conditions

The test of the fitted model showed that it was adequate ($p < 0.05$; Appendix 14). There was a significant interaction ($p < 0.05$) between the genotypes and trial for all variables measured (Appendix 15). On testing the effects treatments, the results indicated that there was significant ($p < 0.05$) effect of genotypes on all traits measured (Appendix 16). However, the blocking of the genotypes was effective ($p > 0.05$; Appendix 16).

Plant height range at 28 days ranged from 5.67 cm to 16.00 cm with NA11 having the shortest plant and MA50 X NA11 having the tallest plant (Table 18). Plant width ranged from 10.67 cm (MA50) to 34.55 cm (MA50 X MA67). On terminal leaf length, MA67 X NA11 had the smallest length of 4.25 cm while MA67 X NA60 had the largest length of 11.00 cm. The terminal leaf width ranges from 1.75 cm (MA67 X NA11) to 8.50 cm (MA67 X NA60). Plant height at 56 days, ranged from 7.50 cm to 23.67 cm with NA11 having the shortest plants and NA60 having the tallest plants. Number of branches ranged from 2.67 to 8.00 with MA50 X MA67 having the tallest plants and NA11 having the shortest plants.

In trial 2, plant height at 28 DAS ranged from 4.00 cm to 15.00 cm with MA50 having the tallest plants and MA50 X NA20 having the shortest plants. (Table 19). The plant width ranged from7.27 cm to 30.0 cm with MA50 having the largest plant width and KK06 showing the smallest plant width. Terminal leaf length ranged from 4.00 cm (MA50 X NA20) to 11.00 cm (MA67 X NA60) while terminal leaf width ranged from 2.50 cm (MA50 X MA67) to 7.33 cm (MA67 X NA60). Plant height at 56DAS ranged from 6.00 cm to 25.00 cm with MA50 having the tallest plants and MA50 X NA20 having the shortest plants. The number of branches range from 1.00 to 5.00 with MA50 having the highest number of branches and MA50 X NA20 having the least number of branches.

Table 18:Means of plant height (PH), plant width (PW), terminal leaf length (TLL), terminal leaf width (TLW), number of main branches (NOB) for cowpea genotypes under well-watered conditions in Trial 1

Genotypes	PH(28DAS)	PW(28DAS)	TLL(42DAS)	TLW(42DAS)	PH56DAS	NOB56DAS
MA50 X NA11	16.00a	21.00f	6.50hi	4.00g	20.00c	7.00b
MA67 X NA60	15.00ab	30.00b	11.00a	8.50a	19.00d	6.00c
KK06	14.00abc	23.00e	5.57k	3.83gh	16.00e	4.00f
NA60	13.00bc	21.33f	7.33ef	6.00d	23.67a	6.67b
KK06 X NA11	13.00bc	29.50b	7.75de	4.50f	16.00e	5.50cd
MA50 X MA67	12.50bcd	34.55a	8.00d	4.75f	13.00g	8.00a
MA50 X NA20	12.00cd	30.00b	7.00fg	3.50hi	9.00i	3.00gh
NA20	11.67cd	16.33g	6.17ij	4.83f	22.50b	4.67e
MA67	11.67cd	15.67g	6.67gh	5.33e	14.33f	5.00de
NA11 X NA60	10.00de	24.50d	5.75jk	3.25i	11.50h	4.50ef
MA50 X NA60	10.00de	28.00c	10.50b	6.50c	19.00d	5.00de
MA50	8.00ef	10.67i	6.33hi	3.83gh	20.33c	3.33g
NA20 X NA60	8.00ef	30.00b	10.00c	7.50b	19.00d	7.00b
MA67 X NA11	7.00f	20.50f	4.25l	1.75j	9.00i	5.00de
NA11	5.67f	13.33h	5.67k	4.00g	7.50j	2.67h
KK06 X MA67			8.00d	4.50f		
LSD	2.63	1.16	0.448	0.439	0.881	0.604
R	0.935	0.998	0.993	0.992	0.998	0.987
CV	10.27	2.33	2.748	3.999	2.33	5.2

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing CV = Coefficient of variation, LSD = Least Significant Difference.

In trial 1, plant biomass yield ranges from 1566.11 kg/ha (NA11 X NA60) to 12014 kg/ha (KK06 X MA67) and in trial 2 the range was from 1351.67 kg/ha (MA50 X NA20) to 12467kg/ha (MA50 X MA67) (Table 20). In trial 1, grain yield ranged from 555.56kg/ha to 9644.45kg/ha with NA11 X NA60 having the lowest grain yield and MA67 X NA60 having the highest grain yield. In trial 2, the range was between 30.56 kg/ha and 960.56 kg/ha with NA20 having the lowest grain yield and MA67 X NA60 having the highest gain yield. The days to 50% flowering ranged from 38.00 to 46.00 with NA11 X NA60 flowering the earliest and MA50 X MA67 flowering latest in both trials.

Table 19:Means of plant height (PH), plant width (PW), terminal leaf length (TLL), terminal leaf width (TLW), number of main branches (NOB) for cowpea genotypes under well-watered conditions Trial 2

Genotypes	PH(28DAS)	PW(28DAS)	TLL(42DAS)	TLW(42DAS)	PH56DAS	NOB56DAS
MA50 X NA11	9.83d	20.73cd	7.67fg	5.00e	17.67f	4.00c
MA67 X NA60	10.67c	23.50b	11.00a	7.33a	21.00d	5.33a
KK06	8.00f	7.27j	5.00l	3.00i	11.67i	1.67g
NA60	11.00c	19.33ef	7.00hi	5.33d	19.00e	3.00e
KK06 X NA11	13.00b	23.25b	8.00ef	5.17de	20.33d	3.67cd
MA50 X MA67	11.00c	30.00a	9.00c	2.50j	15.50h	4.50b
MA50 X NA20	4.00h	11.50i	4.00m	3.00i	6.00j	1.00h
NA20	13.33b	17.67g	8.67cd	6.67b	23.33c	3.33de
MA67	12.67b	14.67h	7.67fg	5.67c	26.00a	3.33de
NA11 X NA60	7.50fg	21.00c	6.50ij	3.50g	11.50i	3.00e
MA50 X NA60	9.00e	18.33fg	7.33gh	4.17f	12.00i	3.33de
MA50	15.00a	30.00a	10.33b	6.83b	25.00b	5.00a
NA20 X NA60	8.00f	21.00c	8.33de	5.67c	15.67h	5.00a
MA67 X NA11	12.67b	19.75de	6.67i	3.33gh	16.67g	2.33f
NA11	10.67c	12.67i	5.67k	3.17hi	6.00j	2.00fg
KK06 X MA67	7.00g	15.00h	6.00jk	3.00i	12.00i	2.00fg
LSD	0.7	1.2	0.599	0.20	0.77	0.44
R	0.988	0.99	0.98	0.997	0.997	0.98
CV	3.39	3.27	4.06	2.15	2.34	6.624

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

In trial 1, the pod length ranged from 6.80 cm to 12.00 cm with NA20 having the shortest pods and MA50 X MA67 having the longest pods (Table 21). In trial 2, the pod length ranged from 6.90 cm to 16.56 cm with NA60 having the shortest pods and MA67 X NA60 having the longest pods. Pods per plant ranged from 8.00 to 127.00 with NA11 X NA60 having he lowest number of pods per plant and MA50 X MA67 having the highest number of pods per plant while in trial 2, NA11 X NA60 still had the lowest number (8.00) and MA50 X MA67 of pods per plant also had the highest number (143.00) of pods per plant. Genotype NA11 X NA60 had the least number of seeds per pod (4.00) in both trials while MA67 X NA60 had the highest number (14.00 and 15.67), in trial 1 and 2 respectively; (Table21). Pod weight ranged from 67.78 (NA11 X NA60) to 1105.56g (MA50 X MA67) in trial 1 while in trial 2 the range from 54.72 (NA20) to 397.22g (MA50). In trial 1, 100 seed weight ranged from 2.40 g to 17.52 g with KK06 X MA67 having the lowest 100 seed weight and MA50 X MA67 showing the highest 100 seed weight. In trial 2, 100 seed weight ranged from 3.22g to 13.38 g

with MA50 X NA60 having the lowest 100 seed weight and NA60 having the highest 100 seed weight (Table 22).

Table 20: Means of grain yield, biomass and days to 50% flowering for cowpea genotypes in the well-watered experiment in trial 1 and 2

Genotypes	DT50 %F T1	DT50 %F T2	Biomass yield T1	Biomass yield T2	Grain Yield T1	Grain Yield T2
MA50 X NA11	40.0cd	41.0c	4395.56c	2698.33cd	1022.41i	428.33g
MA67 X NA60	45.0ab	45.0ab	5016.67b	3356.30b	9644.45a	960.56a
KK06	44.0b	44.0b	2352.22e	1276.67fg	933.89i	118.33j
NA60	39.0de	39.0de	1350.00gh	2148.33e	1582.04g	383.33g
KK06 X NA11	45.0ab	45.0ab	1785.56f	3596.67b	3105.56e	626.67e
MA50 X MA67	46.0a	46.0a	5468.44a	1505.56f	4861.11c	786.7bc
MA50 X NA20	39.0de	39.0de	512.22i	1320.00fg	1139.1hi	255.56h
NA20	41.0c	40.0cd	4128.89c	5362.78a	857.04	30.56k
MA67	39.0de	40.0cd	956.67h	2497.78de	1560.74g	128.89ij
NA11 X NA60	38.0e	38.0ef	1078.33h	872.78i	555.56j	188.89hi
MA50 X NA60	45.0ab	44.0b	1181.11gh	2372.78de	4105.56d	756.39d
MA50	39.0de	37.0f	1580.00fg	2872.78c	1123.2hi	526.67f
NA20 X NA60	46.0a	46.0a	5544.44a	2422.78de	7338.89b	847.22b
MA67 X NA11	44.0b	45.0ab	2790.00d	1545.93f	853.70i	387.78g
NA11	44.0b	44.0b	2678.33de	1110.00gh	2190.74	150.00ij
KK06 X MA67	40.0cd	40.0cd	5234.44ab	3252.78b	1344.4gh	715.00d
LSD	1.67	1.25	399.98	351.4	289.21	69.48
R	0.93	0.96	0.988	0.979	0.997	0.987
CV	2.37	1.78	8.33	8.82	6.57	9.14

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

In trial 1, the results of this study shows that plant width had a significant positive correlation ($p < 005$) with terminal leaf length, number of branches, grain yield, biomass, pod length and seeds per pod (Table 23) Terminal leaf length had positive significant correlation ($p < 0.05$) with terminal leaf width, grain yield, biomass yield, seeds per pod and pods per plant. Terminal leaf width had a positive significant correlation ($p < 0.05$) with plant height grain and biomass yield. Grain yield had a positive significant correlation ($p < 0.05$) with biomass yield, seeds per pod, pods per plant and days to 50% flowering, and seeds per pod had a positive significant correlation ($p < 0.05$) with pods per plant.

Table 21: Means of cowpea pod length, pod weight and seeds per pod in the well-watered experiment in trial 1 and 2

Genotypes	PL T1	PLT2	PW NT1	PWNT2	Seeds/pod T1	Seeds/pod T2
MA50 X NA11	11.83a	15.13b	275.19gh	117.50gh	7.33de	8.3def
MA67 X NA60	10.67b	16.56a	225.00i	204.72e	14.00b	15.67a
KK06	8.57d	7.97ij	335.56ef	60.56ij	7.67d	5.00i
NA60	8.07def	6.90k	359.26e	57.78ij	6.67ef	7.67f
KK06 X NA11	10.00c	12.67cd	966.67b	103.6ghi	8.00d	6.67g
MA50 X MA67	12.00a	12.33de	1105.56a	275.00bc	15.67a	12.33c
MA50 X NA20	8.70d	8.67i	470.00cd	253.33cd	6.00fg	9.00d
NA20	6.80g	11.83ef	483.70cd	54.72j	8.00d	5.67hi
MA67	8.53d	13.40c	504.07c	140.83fg	4.67h	6.00gh
NA11 X NA60	7.73ef	9.60h	67.78j	109.44gh	4.00h	4.00j
MA50 X NA60	8.67d	10.80g	198.33i	172.78ef	12.67c	1433b
MA50	8.33de	9.77h	360.00e	397.22a	5.67g	8.67de
NA20 X NA60	11.83a	15.67b	242.96hi	213.06de	12.33c	13.00c
MA67 X NA11	11.17b	11.43fg	309.72fg	146.39fg	4.67h	8.00ef
NA11	7.60f	7.57jk	447.04d	311.11b	5.67g	5.67hi
KK06 X MA67	10.97b	7.97ij	227.78i	78.61hij	12.67c	12.33c
LSD	0.65	0.7423	46.32	46.32	0.791	0.75
R	0.966	0.986	0.993	0.951	0.989	0.989
CV	4.15	3.995	6.76	16.48	5.60	5.079

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

In trial 2, plant width was found to have a positive significant correlation ($p < 0.05$) with terminal leaf length, plant height at 56 DAS, number of branches, grain and biomass yield (Table 23). Terminal leaf length had a positive significant correlation ($p < 0.05$) with terminal leaf width, plant height, number of branches, biomass yield, pod length and pods per plant. The grain yield had a positive significant correlation ($p < 0.05$) with seeds per pod, pod per plant and days to 50% flowering. Terminal leaf width was positively significantly correlated ($p < 0.05$) to plant height, number of branches, biomass yield and pod length, while number of branches were positively significantly correlated ($p < 0.05$) to grain yield, pod length and pods per plant.

Table 22: Means of cowpea pods per plant and 100 seed weight in the normal watered experiment in trial 1 ad 2

Treatment	Pods/plant T1	Pods/plant T2	100 SW T1	100SWT2
MA50 X NA11	30.67i	65.00f	6.78ef	11.99b
MA67 X NA60	119.00b	134.00b	5.10fg	10.98bc
KK06	48.67fg	64.67f	16.78a	7.68gh
NA60	52.00f	64.67f	16.40ab	13.38a
KK06 X NA11	73.67d	93.00d	4.20gh	8.33fg
MA50 X MA67	127.00a	143.00a	17.52a	11.85b
MA50 X NA20	14.00j	14.00j	14.70bc	5.55i
NA20	63.33e	62.00g	13.00cd	9.44ef
MA67	52.67f	52.67h	17.51a	4.00j
NA11 X NA60	8.00j	8.00j	8.33e	9.61e
MA50 X NA60	60.67e	72.67e	8.65e	3.22j
MA50	39.00h	39.00j	13.10cd	9.75cde
NA20 X NA60	100.00c	112.00c	11.67d	9.67de
MA67 X NA11	25.00i	43.67i	13.45cd	8.58efg
NA11	42.67gh	44.00i	17.41a	6.87h
KK06 X MA67	38.00h	44.00i	2.40h	10.91cbd
LSD	7.0179	1.184	1.88	1.27
R	0.990	0.9997	0.969	0.95
CV	7.529	1.07	9.645	8.61

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

Table 23: Correlation analysis for plant height, plant width, terminal leaf length, terminal leaf width, number of branches, grain yield days to 50% flowering, grain yield. Biomass yield,100 seed weight, pod length, seeds per pod, pods per plant and days to 50% flowering at different growth stages in well water conditions in Trial 1 and 2

	PH 28	PW28	TLL42	TLW42	PH56	NOB56	Grain Yield	Biomass yield	100SW	PL	Seeds/pod	Pods/plant	DTF
PH28		0.379	0.25	0.271	0.428	0.444	0.123	0.158	-0.315	0.208	0.277	0.237	-0.103
PW28	0.52*		0.606*	0.36	-0.08	0.528*	0.583*	0.303**	-0.336	0.559*	0.685**	0.508	0.508
TLL42	0.66**	0.8**		0.9**	0.415	0.446	0.842**	0.343**	-0.425	0.339	0.796**	0.676**	0.431
TLW42	0.555*	0.382	0.76**		0.56*	0.447	0.828**	0.347**	-0.21	0.152	0.646**	0.722**	0.352
PH56	0.79**	0.50*	0.76**	0.8**		0.425	0.199*	0.228	-0.276	-0.033	0.303	0.32	-0.09
NOB56	0.492	0.8**	0.94**	0.69**	0.7**		0.474	0.625*	-0.214	0.71**	0.611*	0.626*	0.327
Grain Yield	-0.031	0.54*	0.493	0.112	0.04*	0.580*		0.515*	-0.242	0.465	0.740**	0.840**	0.7**
Biomass yield	0.451	0.218**	0.547*	0.70**	0.7**	0.392	0.085		-0.279	0.7**	0.700**	0.607*	0.461
100SW	0.134	0.484	0.361	0.168	0.221	0.378	0.245	0.174		-0.349	-0.319	-0.016	-0.03
PL	0.234	0.437	0.66**	0.533*	0.492	0.73**	0.423	0.384	0.098		0.558*	0.429	0.457
Seeds/pod	-0.187	0.358	0.421	0.147	-0.012	0.464	0.894**	0.168	0.114	0.424		0.796**	0.62*
Pods/plant	0.237	0.441	0.610*	0.246	0.259	0.66**	0.682**	0.236	0.3	0.61*	0.597*		0.7**
DTF	0.033	0.067	0.14	-0.172	-0.16	0.21	0.552*	-0.09	-0.062	0.43	0.409	0.771**	

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed). Upper diagonal=T1, Lower diagonal T2

Where, PH28 = Plant height at 28 days after sowing, PW28 = plant width at 28 days after sowing, TLL42 = terminal leaf length at 42 days after sowing, TLW42 = terminal leaf width at 42 days after sowing, PH56 = plant height at 42days after sowing, NOB = number of branches at 56 days after sowing.

The findings of the study showed a significant variation in all traits studied. The variation observed indicates a wide range of genetic diversity among the cowpea genotypes studied. This genetic diversity is crucial to enhance breeding programs for increasing grain yield, improving resilience to environmental stresses, or enhancing other agronomic characteristics. Several genotypes such as MA67 X NA60, NA20 X NA60, MA50 X MA56 and MA50 X NA60 were identified as potential genotypes for grain production. The results of this study showed that grain yield was strongly influenced by traits such as number of seeds per pod, number of pods per plant, number of branches per plant, terminal leaf length and width, plant height and biomass yield. The finding of this study is in agreement with those of Aremu *et al.*, (2007) who showed that number of branches, length of the pod, number of pods per plants and number of seed per pod are the highest contributor to grain yield. Thap *et al*., 2021) reported a positive correlation between plant height and grain yield in cowpea. Yields of genotypes largely depend on plant traits such as yield components (Atakora *et al*., 2023) The finding of this current study demonstrate that integration of various plant traits can enhance overall grain yield in cowpea. Consequently, guiding future breeding efforts towards more productive and resilient varieties.

This study identified KK06 X MA67, MA67 X NA60 and MA50 X MA67 as potential genotypes for vegetable production as they consistently produced high biomass yield in both trials. The finding of this study was that F_1 hybrids such as MA67 X NA60, NA20 X NA60 and MA50 X MA67 were potential dual-purpose genotypes due to their high yield potential for both grain and vegetable production. The biomass yield was strongly influenced by plant width, terminal leaf length and width, and number of branches per plant. This finding implies that by focusing on these traits a breeding program can develop varieties tailored toward biomass production. The leaf area index, which is a function of plant height, number of leaves per plant, number branches per plant and leaf dimensions constitute the plant architecture, which determines the quality of solar radiation intercepted (Liu *et al*, 2010). Bhagwati *et al*., (2017) showed that strong correlation between plant height and plant biomass yield. Therefore, variation of these traits offers a plant breeder opportunity enhancing photosynthesis and thus boost biomass yield. Keller *et al*. (2024) linked photosynthesis and yield, revealing that a strategy to improve photosynthesis and light use efficiency accelerates selection for high yield potential genotypes.

4.4.4 Screening of F_1 Hybrids and Their Parents for Drought Tolerance

Drought severity was classified into resistant genotypes (3), tolerant genotypes (5) and susceptible genotypes (7) (Appendix7). Some genotypes showed resistance up to day 28 after imposition of drought while some were only resistant in the first 7 or 14 days after imposition and then they became susceptible. Accession NA20 was resistant at 7 days after imposition of drought, it was tolerant at 14 and 21 days after imposition and was susceptible as the drought imposition increased (Table 24 and 25).

Table 24: Scores of hybrids and their parents for drought severity index at 7, 14, 21 and 28 days after imposition of drought in two trials

Treatment	7DAT1	14DA1T1	21DAIT1	28DAIT1	7DAIT2	14DA1T2	21DAIT2	28DAIT2
MA50 X NA11	3	3	3	3	3	3	3	5
MA67 X NA60	3	5	3	3	3	3	5	5
KK06	3	3	5	5	3	3	3	3
NA60	3	3	3	3	3	3	3	5
KK06 X NA11	3	3	3	3	3	3	5	5
MA50 X MA67	3	3	3	3	3	3	3	3
MA50 X NA20	3	3	5	5	3	3	3	5
NA20	3	5	5	7	3	5	5	7
MA67	3	3	5	5	3	5	5	3
NA11 X NA60	3	4	3	5	3	5	5	5
MA50 X NA60	3	3	3	3	3	3	3	5
MA50	3	3	5	5	3	5	3	5
NA20 X NA60	3	3	3	3	3	3	3	3
MA67 X NA11	3	3	3	5	3	3	3	5
NA11	5	5	5	5	5	5	5	5
KK06 X MA67	3	3	3	3	3	3	3	3

NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, 3 =Resistant plants, 5 =Tolerant genotypes, 7 = Susceptible genotypes.

The findings of this study showed that different cowpea genotypes exhibit varying levels of resistance, tolerance, and susceptibility to drought stress. This variation is crucial as it indicates genetic diversity in the response to drought, which can be leveraged in breeding programs. Some genotypes, such as MA50 X MA67 and KK06 X MA67, consistently show resistance across all growth and development points. These genotypes have a stable response to drought, maintaining a low level of stress expression, which is a desirable trait for cultivation in water-limited environment. The study also revealed that some genotypes exhibit changes in drought stress expression over time. For instance, NA20 and MA67 initially show resistance but become more

susceptible as the drought period extends. The hybrids, in many cases, show improved or stable resistance compared to their parental lines. For example, MA50 X NA60 and KK06 X MA67 hybrids exhibit better drought resistance, demonstrating the potential likely combination of favourable traits (genes) from both parents, which might result in a more robust physiological response to drought. The findings of this study were in agreement with those of Carvalho *et al*. (2019)

Table 25:Tolerance and susceptibility levels of cowpea genotypes at 21 days after imposition of drought in trial 1 and 2

Treatment	DSI28DA1 Trial 1	Conclusion	DSI28DA1 Trial 2	conclusion
MA50 X NA11	3	Resistant	5	Tolerant
MA67 X NA60	3	Resistant	3	Tolerant
KK06	3	Resistant	3	Resistant
NA60	3	Resistant	5	Tolerant
KK06 X NA11	3	Resistant	5	Tolerant
MA50 X MA67	3	Resistant	3	Resistant
MA50 X NA20	3	Tolerant	5	Tolerant
NA20	5	Resistant	5	Resistant
MA67	5	Tolerant	5	Resistant
NA11 X NA60	5	Tolerant	5	Tolerant
MA50 X NA60	3	Resistant	3	Tolerant
MA50	5	Tolerant	5	Tolerant
NA20 X NA60	3	Resistant	3	Resistant
MA67 X NA11	3	Resistant	5	Tolerant
NA11	5	Tolerant	5	Tolerant
KK06 X MA67	3	Resistant	3	Resistant

NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DSI = Drought sensitivity index, DA1 =Days after imposition

In this study it was observed that several genotypes retained their green leaves under drought stress for up to four weeks. Some of these genotypes includes MA50 X NA11, MA67 X NA60, NA60 and KK06 X NA11. Tengey *et al*. (2023) showed that delayed senescence is one of the mechanisms that cowpea uses to cope with water-stresses. By postponing the aging process of the leaves, the plant can continue with photosynthesis and maintain its energy reserves longer, which helps sustain growth and yield during periods of drought. This mechanism allows the plant to better withstand prolonged water deficit and maintain productivity under challenging conditions. Several genes are associated with the stay-green trait, which helps plants retain green leaves under stress. These includes SST2 (Borrill *et al*., 2019), SGR (Koo *et al*., 2011), OsSGR (Yang *et al*., 2011) and CRFs (Ashikari *et al*., 2005). Gene SST2 impact on chlorophyll retention

makes it a valuable target for breeding drought-resistant crops. Gene SGR role in controlling chlorophyll degradation suggests incorporating this gene could improve stress tolerance and extend the productive period of crops. Gene OsSG has a regulatory effect on the leaf senescence process, thereby influencing the plant's response to drought. Gene CRFs can impact the stay-green phenotype by modulating leaf aging and stress responses. Thus, the stay-green trait involves a complex network of genetic and physiological mechanisms. Key genes like SST2, SGR, and OsSGR directly influence chlorophyll retention and leaf senescence, while CRFs modulate broader hormonal responses that affect stress tolerance (Yang *et al*., 2011; Borrill *et al*., 2019). Therefore, understanding these genes and their interactions provides valuable insights for developing crops that can withstand water stress more effectively.

4.4.5 Evaluation of Developed F1 Hybrids under Water Stressed Conditions.

The test of the fitted model showed that it was adequate ($p < 0.05$) to explain the linear relationship between genotypes and all traits measured (Appendix 17). There was a significant interaction ($p < 0.05$) between the genotypes and trial for all variables measured (Appendix 18). On testing the effects genotyped, the results indicated that there was significant ($p < 0.05$) effect of genotypes on all traits measured (Appendix 19). However, the blocking of the genotypes was found to be effective ($p > 0.05$).

Plant height at 28DAS range from 5.33cm to 13.00cm with NA20 being the shortest plant. And MA50 X NA11 being the tallest plant. Plant width ranged between 9.00cm and 28.50cm with NA20 showing lowest width and MA50 X MA67 showing the highest width (Table 26). Terminal leaf length ranged from 3.67cm (NA20) to 8.33cm (MA67 X NA11) while terminal leaf width was from 2.00cm (NA20) to 5.67cm (MA67 X NA60). Plant height at 56DAS ranged from 4.67cm to 24.00cm with NA20 being the shortest mean and MA50 X NA11 being the tallest plant. Number of branches ranged from 1.00 to 4.50 with NA20 showing the least number and MA50 X MA67 showing the highest number.

Table 26: Means of cowpea accessions; Plant height (PH) at 28DAS and 56DAS, Plant width (PW) Terminal leaf length (TLL), Terminal leaf width (TLW), Number of main branches (NOB): Stressed experiment Trial 1

Genotypes	PH (28DAS) T1	PC (28DAS) T1	TLL (42DAS) T1	TLW (42DAS) T1	PH (56DAS) T1	NOB 56DAS T1
MA50 X NA11	13.00a	22.70c	8.00c	5.17b	24.00a	3.33b
MA67 X NA60	9.83de	21.90d	7.33e	5.67a	17.67c	2.67d
KK06	9.83e	19.00f	6.67gh	4.00ef	15.33e	2.33e
NA60	8.67f	15.33i	5.33k	4.17de	12.33g	2.33e
KK06 X NA11	8.07g	17.90g	6.33ij	3.83efg	12.33g	3.00c
MA50 X MA67	10.00d	28.50a	10.50a	2.75j	17.50c	4.50a
MA50 X NA20	7.83gh	17.67g	5.00l	3.50gh	15.33e	2.33e
NA20	5.33i	9.00l	3.67m	2.00k	4.67h	1.00g
MA67	11.43b	18.83f	6.50hi	3.33hi	16.33d	3.00c
NA11 X NA60	7.45h	22.00d	6.25j	3.75fg	13.50f	2.50de
MA50 X NA60	7.50h	16.50h	7.00f	4.00ef	13.00f	1.00g
MA50	10.33cd	21.23e	7.67d	4.50cd	15.33e	2.67d
NA20 X NA60	10.57c	13.67j	7.00f	4.83bc	12.00g	2.67d
MA67 X NA11	11.67b	12.00k	8.33b	4.67c	18.67b	3.33b
NA11	9.83e	15.00i	7.67d	5.17b	15.33e	2.00f
KK06 X MA67	10.75c	25.00b	6.75g	3.00ij	13.00f	3.50b
LSD	1.05	1.208	0.466	0.32	0.57	0.423
R	0.992	0.999	0.98	0.993	0.998	0.989
CV	3.68	2.158	2.87	3.24	1.69	3.765

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

In trial 2, plant height at 28DAS ranged from 4.00cm to17.33cm with MA50 X NA11 being the shortest plant and KK06 X MA67 being the tallest plant (Table 27). Plant width was between 11.67cm and 60.33cm with NA20 showing the lowest width and MA67 X NA11 showing the highest width. Terminal leaf length ranged from 5.00cm (NA20) to 9.00cm (MA50 X MA67) while terminal leaf width was from 2.33cm (MA67 X NA11) to 6.17cm (NA20 X NA60) (Table 27). Plant height at 56DAS ranged from 7.50cm to 19.00cm with NA20 being the shortest plant and MA50 X MA67 being the tallest plant. Number of branches ranged from 2.67 and 7.67 with and NA20 having the lowest number and MA50 X NA11 having the highest number of branches.

Table 27: Means of cowpea accessions; Plant height (PH) at 28DAS and 56DAS, Plant width (PW) Terminal leaf length (TLL), Terminal leaf width (TLW), Number of main branches (NOB): Stressed experiment Trial 2

Genotypes	PH (28DAS T2	PC (28DAS) T2	TLL (42DAS) T2	TLW (42DAS) T2	PH 56DAS) T2	NOB (56DAS) T2
MA50 X NA11	17.33a	28.67d	7.50e	4.83cd	17.67d	7.67a
MA67 X NA60	12.00cd	23.00g	7.75de	6.00a	18.00cd	5.00de
KK06	10.33ef	20.67h	8.33bc	5.00bc	18.67ab	4.67ef
NA60	15.00b	20.00h	7.00f	4.83cd	13.67g	4.00g
KK06 X NA11	11.00de	30.00c	9.00a	6.00a	15.00f	6.00c
MA50 X MA67	13.00c	32.00b	9.00a	4.00e	19.00a	7.00b
MA50 X NA20	10.00ef	17.00i	6.00g	3.00f	9.00i	4.00g
NA20	6.00g	11.67j	5.00h	2.67g	7.50j	2.67h
MA67	14.67b	21.00h	8.17cd	6.17a	17.67d	5.00de
NA11 X NA60	12.00cd	24.00g	7.00f	3.00f	9.00i	4.00g
MA50 X NA60	16.67a	28.00de	7.33ef	4.17e	12.00h	5.00de
MA50	17.00a	25.33f	8.00cd	5.17a	18.33bc	5.33d
NA20 X NA60	12.00cd	27.00e	8.61ab	6.17a	15.33ef	5.33d
MA67 X NA11	17.00a	60.33a	5.83g	2.33h	12.33h	5.00de
NA11	9.67f	17.67i	7.00f	4.67d	15.67e	4.33fg
KK06 X MA67	4.00h	12.00j	8.00cd	3.00f	8.00j	3.00h
LSD	0.501	0.55	2.62	1.67	0.54	0.314
R	0.998	0.998	0.68	0.69	0.997	0.97
CV	2.8	1.64	20.77	22.20	1.94	5.86

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

The result indicates significant difference among the trait studied. In trial 1, biomass yield ranges from 197.78kg/ha to 5022.22kg/ha with NA20 showing the least and MA50 XMA67 showing the highest biomass. In trial 2, the yield ranged from 257.78kg/ha to 1827.78kg/ha with MA67 showing the lowest yield and MA67 X NA60 showing the highest yield (Table 28). In trial1, grain yield was from 311.30kg/ha to 3220.37kg/ha with NA20 showing the lowest yield and MA50 X MA67 showing the highest yield. In trial 2, the range was from 56.61kg/ha to 562.50kg/ha with KK06 showing the lowest yield and MA50 X MA67 showing the highest yield The range of days to 50% flowering in trial 1 was from 36.00 to 44.00 days with KK06 being the earliest flowering and MA67 X NA60 being the latest flowering while in trial 2, MA67 was the earliest flowering while NA20 X NA60 was the latest flowering.

Table 28:Means of Developed hybrids and parents on grain yield, biomass and days to 50% flowering in the water stressed experiment on trial 1 and 2

Genotypes	DT50 %F TI	DT50% F T2	Biomass T1	Biomass T2	Yield T1	Yield T2
MA50 X NA11	39.0ef	37.0f	2623.9d	1370.56c	1435.19cd	203.89e
MA67 X NA60	44.0a	43.0ab	4123.3c	1827.78a	1375.93d	506.7bc
KK06	35.0h	40.0de	1738.3e	658.89gh	1014.44ef	56.61i
NA60	38.0fg	37.0f	790.0ij	620.56hi	1080.74e	199.44e
KK06 X NA11	42.0bc	41.0cd	1678.9ef	295.56l	938.89efg	544.44a
MA50 X MA67	41.0cd	42.0bc	5022.2a	765.00fg	3220.37a	562.50a
MA50 X NA20	37.0gh	40.0de	197.8	659.4fgh	490.74i	100.0gh
NA20	40.0de	39.0e	2687.78	788.89f	311.30i	147.78f
MA67	37.0gh	36.0f	567.2jk	257.78l	1563.33bc	110.00g
NA11 X NA60	39.0ef	36.0f	956.67hi	487.22j	794.44gh	106.1gh
MA50 X NA60	43.0ab	42.0bc	1018.ghi	1690.56b	781.48gh	538.9ab
MA50	36.0h	40.0de	1216.1gh	962.78e	730.74h	354.17d
NA20 X NA60	43.0ab	44.0a	4481.1bc	515.56ij	1668.52b	497.50c
MA67 X NA11	42.0bc	40.0de	2457.2d	537.22hij	881.48fgh	215.00e
NA11	36.0h	39.0e	1364.4fg	1491.67ij	1344.81d	72.22hi
KK06 X MA67	41.0cd	39.0e	4846.1ab	1192.22d	811.11gh	385.56d
LSD	1.67	1.727	371.75	129.7	182.85	358.75
R	0.92	0.9	0.988	0.982	0.982	0.988
CV	3.2	7.4	2.8	3.7	2.77	6.11

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

In trial 1, Pod length range was from 10.17cm (NA11 X NA60) to 14.80cm (NA11) while in trial 2, the range was from 9.07cm (KK06 X MA67) to 16.20cm (NA20 X NA60) (Table 29). In trial 1, number of seeds per pod, ranged from 3.00 to 12.00 seeds with MA67 showing the lowest number and MA50 X MA67 showing the highest number and in trial 2, the range was from 3.00 to 12.33 with KK06 showing the lowest number and MA67 X NA60 showing the highest number. Pod weight in trial 1 ranged from 109.17cm (NA11 x NA60) to 2233.33cm (NA20 X NA60) while in trial 2 the range was from 54.44(MA50) to 260.56(MA67 X NA60).

Table 29:Means of Developed hybrids and the parents on pod length (PL), pod weight (PW), and seeds per pod, in the water stressed experiment on trial 1 and 2

Genotypes	PL T1	PLT2	PW DT1	PWDT2	S/pod T1	S/pod T2
MA50 X NA11	12.67fgh	13.10fg	968.33d	203.89cd	4.33f	6.00e
MA67 X NA60	14.70a	11.93h	622.22e	260.56b	11.33a	12.33a
KK06	13.87bcd	12.60g	520.56ef	182.22d	4.00fg	3.00i
NA60	13.43cde	10.97i	226.85hij	191.67cd	5.67de	5.33ef
KK06 X NA11	14.67a	9.33k	1158.33c	127.78e	6.33d	5.33ef
MA50 X MA67	14.87a	14.60bc	201.67hij	96.11efg	12.00a	8.67c
MA50 X NA20	11.33i	10.97i	586.11e	130.00e	4.00fg	5.33ef
NA20	13.37def	13.40ef	578.15e	91.67efg	5.67de	4.33gh
MA67	13.80bcd	13.87de	331.11ghi	78.89fg	3.00h	5.00fg
NA11 X NA60	10.17j	11.70h	109.17j	118.33ef	3.00h	3.33i
MA50 X NA60	12.33gh	9.97j	1353.89b	78.61fg	8.67c	11.00b
MA50	14.40ab	13.27ef	134.81j	54.44g	3.67fgh	3.67hi
NA20 X NA60	14.17abc	16.20a	2233.33a	105.28ef	10.33b	7.67d
MA67 X NA11	13.00efg	14.93b	170.00ij	230.00bc	3.33gh	5.67ef
NA11	14.80a	14.07cd	355.93gh	322.22a	5.33e	5.67ef
KK06 X MA67	12.00hi	9.07k	394.44fg	237.78bc	9.67b	8.33cd
LSD	0.7	0.6	162.12	46.32	0.84	0.84
R	0.934	0.98	0.980	0.92	0.98	0.978
CV	3.31	2.86	15.64	17.71	7.83	7.87

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

The range of pods per plant was from 7.00 to 11.00 with MA50 X NA20 showing the lowest number and MA50 X MA67 showing the highest number. In trial 2, the range was from 1.00 to 99.67 with NA11 X NA60 showing the lowest number and MA50X MA67 showing the highest number (Table 30). The range for 100 seed weight in trial 1 was from 2.40g to 17.52g with KK06X MA67 showing the lowest weight and MA50 X MA67 showing the highest weight. In the trial 2, the range was from 3.22g to 13.38g with MA50 X NA60 showing the lowest weight and NA60 showing the highest weight.

In trial 1, it was observed that number of pods per plant had a positive significant correlation ($p < 0.05$) with terminal leaf length, grain and biomass yield, pod length and number of seeds per pod (Table 31). It was also observed that biomass yield was positively significantly correlated ($p < 0.05$) to terminal leaf length and width, plant height and number of branches while grain yield was positively significantly correlated ($p < 0.05$) with plant width, terminal leaf length and number of branches. The number of branches had a positive significant correlation ($p < 0.05$) with plant height, terminal

leaf length and leaf width, while number of seeds per pod were positively significantly correlated ($p < 0.05$) with grain yield and biomass.

In trial 2, terminal leaf length had a positive significant correlation ($p < 0.05$) with terminal leaf width, plant height, number of branches and grain yield (Table 31). Plant height had a positive significant correlation ($p < 0.05$) with number of branches, biomass yield and pods per plant while grain yield had a positive significant correlation ($p < 0.05$) with number of seeds per pod, number pod per plant and days to 50% flowering. The number of seeds per pod was also positively significantly correlated ($p < 0.05$) with pods per plant and days to 50% flowering.

Table 30: Means of Developed hybrids and their parents on 100 seed weight and pods per plant in the water stressed experiment in both trial 1 and 2

Genotypes	Pods/plant T1	Pods/plant T2	100 SW T1	100SWT2
MA50 X NA11	21.00g	32.67g	14.60d	10.78cd
MA67 X NA60	56.67b	97.00b	9.44fg	8.75ef
KK06	28.67d	27.00h	6.48ij	7.63f
NA60	29.00d	50.00d	17.25b	18.65a
KK06 X NA11	23.00f	51.00d	15.02cd	5.30g
MA50 X MA67	111.00a	99.67a	7.26hi	9.96cde
MA50 X NA20	7.00j	10.67j	17.10bc	7.34f
NA20	25.67e	35.00f	8.22ghi	3.42h
MA67	37.67c	36.67f	5.10jk	8.43ef
NA11 X NA60		1.00k	10.83ef	11.56c
MA50 X NA60	17.00h	42.00e	9.29fgh	5.42g
MA50	20.67g	21.00i	7.28hi	2.67h
NA20 X NA60	57.67b	72.00c	3.40l	8.44ef
MA67 X NA11	12.00i	26.00h	12.15e	13.48b
NA11	29.00d	34.67fg	19.70a	8.70ef
KK06 X MA67	22.00fg	19.00i	4.80jk	9.56de
LSD	1.799	2.05	2.08	1.67
R	0.999	0.999	0.96	0.958
CV	3.24	2.9998	11.91	11.42

[a]Means followed by the same letters are not significantly different at 5% probability level. NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions, KK = Kakamega accessions, DAS = Days after sowing, CV = Coefficient of variation, LSD = Least Significant Difference

Table 31: Correlation analysis for plant height, plant width, terminal leaf length, terminal leaf width, number of branches, grain yield and days to 50% flowering, grain yield. Biomass yield,100 seed weight, pod length, seeds per pod, pods per plant and days to 50% flowering at different growth stages in water stressed conditions in Tria1 and 2

	PH28T1	PC28T1	TLL42T1	TLW42T1	PH56T1	NOB56T1	Yield T1	Biomass T1	100 SW T1	PL T1	Seeds/pod T1	Pods/plant T1	DTF TI
PH 28T2		0.366	0.650**	0.510*	0.80**	0.672**	0.377	0.415	-0.153	0.242	-0.011	0.132	-0.061
PC28T2	0.631**		0.572*	-0.022	0.512*	0.674**	0.580*	0.438	-0.218	-0.039	0.323	0.48	-0.018
TLL42T2	0.114	-0.002		0.342	0.69**	0.691**	0.732**	0.595*	-0.141	0.37	0.351	0.568*	0.182
TLW42T2	0.275	-0.119	0.701**		0.577*	0.042	0.007	0.019**	0.307	0.242	-0.003	-0.11	0.087
PH56T2	0.506*	0.218	0.657**	0.748**		0.598*	0.444	0.145**	0.183	0.048	-0.098	0.104	-0.085
NOB56T2	0.643**	0.492	0.559*	0.456	0.73**		0.666**	0.616*	-0.164	0.183	0.219	0.501	0.106
Yield T2	0.065	0.18	0.548*	0.348	0.255	0.385		0.501	-0.114	0.458	0.478*	0.898**	0.095*
Biomass T2	-0.003	-0.205	-0.125	0.674**	0.08*	0.037	0.391		-0.495	0.307	0.797**	0.646*	0.591
100SWT2	0.23	0.275	-0.086	-0.133	-0.021	0.029	-0.221	-0.173		-0.089	-0.345	-0.371	-0.241
PLT2	0.273	0.358	-0.033	0.12	0.408	0.291	-0.097	-0.182	0.044		0.353	0.595*	0.047
Seeds/pod T2	0.036	0.071	0.223	0.168	0.095	0.184	0.737**	0.66**	0.015	-0.151		0.694**	0.689**
Pods/plant T2	0.098	0.123	0.46	0.496	0.529*	0.437	0.693**	0.217	0.067	0.268	0.665**		0.281
DTF T2	-0.117	0.196	0.32	0.225	0.202	0.195	0.763**	0.286	-0.358	0.121	0.642**	0.630*	

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed). Upper diagonal = T1, Lower diagonal T2

Where, PH28 = Plant height at 28 days after sowing, PW28 = plant width at 28days after sawing, TLL42=terminal leaf length at 42days after sowing, TLW42=terminal leaf width at 42 days after sowing, PH56 = plant height at 42days after sowing, NOB=number of branches at 56days after sowing.

The finding of the study revealed a wide range of performance of the studied genotypes under water stressed conditions. The study identified several genotypes such as MA50 X MA67, KK06 X MA67, MA67 X NA60 and MA50 X NA11, which exceled in biomass production under water-limited conditions. These genotypes can serve as valuable resources in developing drought-tolerant crops and optimizing biomass production in challenging environments. Further exploration of their traits and performance can provide insights into improving agricultural resilience and productivity. The genotype MA50 X MA67 did not show any significant difference on the biomass yield both under well-watered and water-stressed condition. This may indicate that MA50 X MA67 is inherently resilient to water stress, possibly due to water use efficiency water use or adaptive physiological traits. It had been shown that various physiological traits can be used in breeding approaches related to drought tolerance in plants (Blum, 2017). Studies has also shown that plants responses to combined stress conditions can provide insights into why some genotypes might show stable performance (Zhao and Huang, 2015).

Most of the F_1 hybrid genotypes had higher grain yield than their parents. This reflects the beneficial effects of hybrid vigour, genetic complementation and probably increased plant vigour. These findings are in agreement with those of Owusu *et al*. (2018) who showed that many of hybrids had higher grain than most of the parents. The study also identifies three F_1 hybrid genotypes that yielded high than any of its parent, which included MA50 X MA67, NA20 X NA60 and MA67 X NA60 under water-stressed conditions. In this current study, it was observed that there was a strong influence of number of seeds per pod and number of pods per plant on grain yield of the genotypes under water-limited conditions. According Hall (2004) and Kumar (2005) drought and heat tolerant genotypes produced a greater number of pods than the sensitive types under extreme environmental conditions.

The genotype studied displayed a significant variation in days to 50% flowering. The results are similar to those of Manggoel and Uguru (2011) who established that days to 50% flowering were significantly different in the varieties. The results of the study showed that 50% flowering was positively significantly correlated to grain yield and number of seeds per pod but was non-significantly correlated to biomass. This implies that late flowering genotypes gave higher grain yield and numbers of seeds per pod than

early flowering genotypes. This suggests that plants that take longer time to reach 50% flowering may have a longer grain-filling period, which can be beneficial for grain development and accumulation. This extended period allows the plant to accumulate more resources and support higher grain production. Studies has shown that plants grown in water-limited environments generate substantial genotype × environment interactions (GEI) for grain yield, with much of the yield and GEI variation resulting from variations in flowering time and their effects on canopy development (Liu and Zhang 2009; Wang *et al*., 2020). The genotypes that flower later might develop a larger canopy, which can enhance light interception and photosynthesis, potentially increasing grain yield. Dawo *et al.* 2007 showed that late flowering allows increased vegetative growth, subsequently producing sufficient photosynthates enabling development of increased number of pods per plant. However, the development of optimum canopy is necessary for high grain yield (Ishiyaku and Singh, 2003).

In breeding for enhanced drought tolerance, it is necessary to identify efficient methods to evaluate levels of tolerance in germplasm for crossing and selection of segregated breeding materials. Phenotypic variation was observed on different growth variables, with most important variable including plant height, number of branches, number of seeds per pod and number of pods per plant. These variables can be used for drought tolerance selection criteria. However, most of traits studied were quantitative traits that are influenced by environment conditions (Stoilova & Pereira, 2013).

4.5 Determination of Combining Ability, Heritability and Heterosis of Developed F_1 Cowpea Genotypes

4.5.1 Combining Ability and Heritability

The test of the fitted model showed that it was adequate ($p < 0.05$) to explain the variation for all traits measured according to Griffing's method 2 (Appendix 20). There was a significant effect ($p < 0.05$) of both general combining ability (GCA) and specific combining ability (SCA) on all the variables measured (Appendix 21). The GCA ranged from 3.64 in drought tolerance index to 28956.18 in grain yield, while SCA ranged from 6.59 in drought tolerance index to 70121.18 in grain yield (Table 32). The GCA:SCA ratio ranged from 0.41 in grain yield to 4.63 in pod width. The heritability in broad sense (H) ranged from 0.90 in 100 seed weight to 0.99 in pod width, while heritability in narrow sense (h2) ranged from 0.28 in grain yield to 0.79 in pod width.

Table 32: General combining ability, specific combining ability and heritability of various traits of cowpea genotypes

Variable	GCA	SCA	GCA:SCA ratio	H	h^2
Grain yield	28956.18	70121.18	0.41	0.95	0.28
100 Seed weight	7.66	4.36	1.76	0.90	0.58
Pod width	44657.65	9674.58	4.63	0.95	0.79
Days to 50% flowering	8.19	16.62	0.49	0.96	0.32
Pod length	5.44	9.04	0.60	0.99	0.37
Number of pods per plant	2743.50	3284.42	0.84	0.99	0.46
Drought tolerance index	3.64	6.59	0.55	0.96	0.34
Number of seeds per pod	23.63	33.97	0.70	0.99	0.41

Where H = broad sense heritability and h^2 = narrow sense heritability

The finding of this study revealed that the SCA for grain yield, days to 50% flowering, pod length, number of pods per plant, drought tolerance index and number of seeds per pod was higher than GCA, indicating that non-additive gene effects are more important for these traits in the studied cowpea genotypes. The GCA:SCA ratio for these traits was less than one implying that non-additive effects dominate over additive effects for these traits. The results of this study showed that the GCA for 100 seed weight and pod width was higher than SCA, implying that additive gene effects are more influential for these traits. The GCA:SCA ratio for these traits was greater than one supporting the dominance of additive genetic effects for these traits. The findings of this study were in agreement with those of Pessoa *et al*. (2024) who found out that both additive and non-additive genetic effects influence the behaviour of inherited traits cowpea. The result indicated that the GCA and SCA varied for all characters measured signifying the prominence of both additive and non-additive genetic components in the present study. These results are in agreement with those of Owusu *et al*. (2018) who demonstrated that GCA and SCA varied in 12 traits they studied in cowpea.

These results highlight the complex genetic nature of these traits in cowpea. Combining ability illustrates the breeding value of parental lines to generate hybrids, and thus aids in the recognition of parents with high GCA and parental combinations with high SCA (Sprague and Tatum 1942; Griffings 1956). Traits such as 100 seed weight and pod width, which are predominantly controlled by additive genes, are more amenable to

improvement through simple selection methods. However, traits like grain yield and drought tolerance, influenced by non-additive effects, might require more sophisticated breeding strategies such as hybridization or marker-assisted selection to achieve significant genetic gains. The SCA that are supposed to be manifestation of non-additive gene action, are very important for discrimination of crosses for their genetic worth as breeding materials, especially for hybrid production. The high SCA value of these hybrids indicates that the expression of these traits is determined by dominance, epistatic and various other gene interactions (Griffings 1956; Baker 1978). Generally, the crosses showing highly significant and desirable SCA effects were associated with high performance for respective traits in specific hybrids. This finding was consistent with those of Abd El-Azeem *et al*. (2021) who demonstrated the importance of SCA effects on production of superior hybrids varieties.

The broad sense heritability for most of traits was high indicating that most of the phenotypic variation observed was more likely due to genetic factors. However, most traits registered low to moderate narrow sense heritability, suggesting that large proportion of the genetic variance is due to the dominant genetic effects. Response to selection is higher for traits with higher narrow sense heritability (Schmidt *et al*. 2019). Narrow sense heritability reflects the proportion of phenotypic variance due to additive genetic variance alone (Falconer and Mackay, 1996). Therefore, it is a more direct measure of the potential for response to selection. Traits with high broad sense heritability but low narrow sense heritability, such as grain yield, days to flowering and pod length, suggest that while these traits are genetically controlled, their improvement through selection may be limited by non-additive genetic effects or environmental variability. Traits with both high broad sense heritability and narrow sense heritability, such as pod width and number of seeds per pod, are good candidates for selection as they have a substantial additive genetic component. Traits with high narrow sense heritability have been found to have accurate estimations of genetic gain, thus substantial reducing the selection cycles in a breeding program (Seck *et al*., 2023)

4.5.2 Heterosis

The findings of this study showed that the heterosis for grain yield under well-watered conditions ranged from -70.55% (NA11 X NA60) to 513.75 (MA67 X NA60) in trial 1 and from -29.17(NA11 X NA60) to 478.43 (KK06 X MA67) in trial 2 (Table 33). In

case of biomass yield, the heterosis ranged from -82.06 (MA50 X NA20) to 331.15 (MA50 X MA67) in trial 1 and form -67.94 (MA50 X NA20) to 201.40 (KK06 X NA11) in trial 2. Under water-stressed conditions, the heterosis for grain yield ranged from -68.86 (KK06 X MA67) to 180.75 (MA50 X MA67) and biomass yield the ranged -89.87 (MA50 X NA20) to 507.61 (MA67 X NA60) in trial 1 (Table 32). The heterosis for grain yield ranged from -60.15 (MA50 X NA20) to 227.48 (MA67 X NA60) and biomass yield ranged from -72.51 (KK06 X NA11) to 316.19 (MA67 X NA60) in trial 2.

In well-watered conditions, the hybrid MA67 X NA60 performed exceptionally well in both grain and biomass yield in Trial 1 and Trial 2, indicating strong heterosis. The hybrid KK06 X NA11 also had positive grain yield heterosis in both trials. The hybrid MA50 X NA20 had negative heterosis for both grain and biomass yield in both trials, indicating it underperforms relative to its parents. The hybrid NA11 X NA60 showed negative heterosis for both traits in both trials, suggesting poor hybrid performance. The hybrid MA67 X NA60 appeared to be the most consistently high-performing hybrid, with strong positive heterosis for both traits. Generally, the hybrids MA50 X NA11, MA50 X NA20, and NA11 X NA60 showed negative heterosis, indicating they may not be as favourable for high yield. Biomass yield seems to vary more compared to grain yield, suggesting that it might be highly influenced by small fluctuation in the environmental factors compared grain yield.

Under water-stress conditions, the hybrid MA50 X NA11 had positive heterosis for biomass yield in both trials. The hybrid MA67 X NA60, KK06 X MA67 and MA50 X MA67 also had extremely high heterosis for both biomass and grain yield in both trials. The hybrid MA50 X MA67 had positive heterosis for grain yield and biomass yield in both trials. The hybrid MA67 X NA60 stands out for its consistently high positive heterosis in both grain and biomass yield across all conditions, indication good genotype consistence performance. The hybrids MA50 X NA11 and KK06 X NA11 exhibit variable performance, with positive heterosis under certain conditions but negative under others, suggesting variable genotype performance. Generally, the hybrids MA67 X NA60, KK06 X NA11, MA50 X MA67, MA50 X NA60 and NA20 X NA60 identified as best specific combiner for grain yield and its major attributes

while in biomass KK06 X MA67, MA67 X NA60 and MA50 X NA11were the best combiner for biomass yield.

Table 33: Heterosis of grain and biomass yield for 10 F1 cowpea hybrid genotypes under well-watered and water-stressed conditions in two trials

Genotypes	Yield Trial 1	Yield Trial 2	Biomass Trial 1	Biomass Trail 2
Well-watered conditions				
MA50 X NA11	-38.30	26.60	106.44	35.50
MA67 X NA60	513.75	275.06	334.97	44.48
KK06 X NA11	98.78	367.07	-29.01	201.40
MA50 X MA67	262.24	117.94	331.15	-43.93
MA50 X NA20	15.05	-8.28	-82.06	-67.94
NA11 X NA60	-70.55	-29.17	-46.46	-46.43
MA50 X NA60	203.53	66.24	-19.38	-5.49
NA20 X NA60	501.78	309.38	102.39	-35.49
MA67 X NA11	-54.49	178.08	53.51	-14.3
KK06 X MA67	7.79	478.43	216.39	72.36
Water-stressed conditions				
MA50 X NA11	38.29	-4.36	103.36	11.68
MA67 X NA60	4.07	227.48	507.61	316.19
KK06 X NA11	-20.41	745.14	8.22	-72.51
MA50 X MA67	180.75	142.37	463.24	25.35
MA50 X NA20	-5.81	-60.16	-89.87	-24.71
NA11 X NA60	-34.49	-21.88	-11.19	-53.87
MA50 X NA60	-13.72	94.68	1.52	113.54
NA20 X NA60	139.72	186.56	157.69	-26.84
MA67 X NA11	-39.38	135.98	27.21	-38.58
KK06 X MA67	-68.86	362.80	320.39	160.12

Where KK = Kakamega accessions, NA = IITA accessions, MA = NGBK-KALRO-Muguga accessions

The studied F1 hybrid genotypes showed a great range of estimates of heterosis for both grain and biomass yield under well-watered and water-stressed conditions. This variability reflects the genetic diversity and the different responses of each genotype to the environmental conditions. The F1 hybrid genotypes with high positive heterosis were indicative of strong SCA (Falconer and Mackay, 1996; Schmidt *et al*. 2019). Heterosis is positively correlated with SCA than GCA, indicating that SCA can be used in heterosis prediction to develop potential hybrids (Yu *et al*., 2020). The significant variation in heterosis among the different hybrids suggests that certain combinations of parental lines are more suited for specific conditions. For instance, the F_1 genotype NA20 X NA60 exhibited strong heterosis for grain yield under well-watered conditions but not for biomass yield under water stress, indicating a genotype-environment

interaction. The high heterosis observed in certain hybrids, especially under adverse conditions, underscores the potential for using these genotypes in breeding programs aimed at improving drought tolerance and yield stability in cowpea. The genotype MA67 X NA60 appears particularly promising due to its consistently high heterosis across all trials. The differential expression of heterosis in morphological traits is largely due to the complex interactions between a subset of loci that control these traits (Farinati *et al.,* 2023). The importance of genetic diversity for selecting parents in a breeding program has also been repeatedly emphasized (Sawant *et al*., 2009).

The range of heterosis estimates provides valuable insights into the genetic potential of these hybrids and their adaptability to varying environmental conditions, which is crucial for selecting superior genotypes in breeding programs. Burgess *et al*. (2023) demonstrated that through selecting superior parents, the growth and yield attributes of the crop can be improved. However, some crosses gave very high negative heterosis for grain yield and biomass. The negative heterosis could result from genetic incompatibility between the parental lines. When the parental genotypes are too genetically diverse or incompatible, the resulting hybrids may suffer from issues such as poor vigour, reduced growth, or impaired physiological functions, leading to lower yields (Fujimoto *et al*., 2018; Calvo-Baltanás *et al*., 2021). Negative epistasis, where gene interactions between the parental lines have an adverse effect on the hybrid, can lead to reduced performance in terms of yield and biomass.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Cowpea has high economic potential for the Sub-Saharan African (SSA) region due to its low production cost and higher economic return. The fresh leaves, immature pods and the grains of cowpeas are nutritious and provides protein, carbohydrate, vitamins and mineral. Among the abiotic factors, drought has been identified as a major limitation restricting cowpea production. Genetic diversity offers great opportunities for developing new and improved cultivars with desirable farmer preferred traits.

Due to the increase in demand for cowpea vegetable and reduction in acreage of land suitable for production of cowpea, there is need to develop novel varieties that combine grain and leaf production. With increase in soil moisture stress and higher water requirement by leaf producing crops, it would be necessary to incorporate drought tolerant genes in newly developed dual purpose cowpea varieties. This study was to help in morphological molecular and biochemical characterisation of selected cowpea accessions, to evaluate the performance of developed F_1 cowpea hybrids and their parents and to estimate the combining ability, heritability and heterosis of the F_1 hybrid genotypes.

Fifty cowpea germplasm accessions were grown for evaluation and were planted in two cultivations for morphological evaluation. F_1 cowpea genotypes were also planted and evaluated and data was collected. Scoring for the experiment that was under water stress was done to screen the susceptibility and resistance level on accessions and data was collected on a scale of 3(Resistant) 5(Tolerant) and 7(susceptible). Since morphological characterisation is not sufficient to characterize cowpea molecular and biochemical characterisation was done for further analysis. This study, therefore, aimed to assess the degree of hybrid vigour and extent of heritability in F_1 hybrids in developed genotypes. Data on plant height, plant width, terminal leaf length, terminal leaf width, number of branches, grain yield and days to 50% flowering. In the hybrids part, data on pod length, pod weight, seeds per pod, 100 seed weight and pods per plant was collected.

Cowpea genotypes MA24, NA11, MA2, NA3 and MA22 are recommended for use as source for developing drought tolerance breeding programmes based on their

impressive performance under water-stressed condition. Genotypes MA45, MA44, MA49 and NA59 with its moderate tolerance to drought is recommended for use as source for developing delayed leaf senescence in cowpea breeding programme which is an important mechanism because it can enhance drought adaptation of early cowpea cultivars by enabling them to produce a greater second pod flush if the first flush is damaged by drought. Also, their genetic studies demonstrated that combining the delayed leaf senescence and heat tolerance traits could breed cowpea cultivars with enhanced yield stability.MA24, NA3, NA101, NA11 and MA22 were also identified with high grain yield.

The number of alleles per locus ranged from 1 to 11 and VM70 was the most diverse with 11 alleles. Gene diversity: Ranged from 0 to 0.85, with VM70 showing the highest diversity (0.85). Polymorphism Information Content (PIC): Ranged from 0 to 0.84, with VM70 being the most informative marker. Inbreeding coefficient (FIS): Values ranged from -0.22 to 0.76, indicating varying levels of inbreeding across loci. The predominantly positive FIS values align with cowpea's self-pollinating nature, but some negative values suggest selection effects. This molecular data provides valuable insights into the genetic diversity and structure of the cowpea population, which can be useful for breeding programs and conservation efforts. The results suggest potential for developing cowpea varieties with improved yield while maintaining moderate levels of beneficial flavonoids. Quercetin: Ranged from 1.61 to 516.77 mg/kg. NA60 had the highest content (516.77 mg/kg) while Myricetin: Ranged from 0 to 19.26 mg/kg. NA20 had the highest content (19.26 mg/kg).

MA50X MA67, MA67 X NA60 and KK06 X MA67 were associated with dual purpose production and also tolerance to drought. They had the highest production for grain yield and biomass production, and were also identified with tolerance to drought since they had delayed leaf senescence and also performed well in the water stressed experiment. Genotypes NA20 X NA60 and MA50 X NA60 could be incorporated in cowpea improvement for grain yield improvement and MA50 X NA11 and MA50 X MA67 can be incorporated for grain production.

Heterosis was high and positive in MA67 X NA60, KK06 X MA67, NA20 X NA60 and MA50 X MA67. Thus, heterosis levels observed in the crosses can enable cowpeas

yield improvement. This information will be helpful in the development of new higher-yielding cowpea varieties. Genotypes MA50 X MA67, NA20 X NA60 MA50 X NA11 and MA67 X NA60 performed better than their parents in the water stressed experiment. Genotype MA67 X NA60 maintained positive heterosis across trials. The heterosis observed in the F1 hybrids and the genetic component analysis offer insights into the genetic improvement programme.

The GCA for 100 seed weight and pod width was found to be higher than SCA. This is an implication that additive gene effects are more influential for these traits. Heterosis is positively correlated with SCA than GCA, indicating that SCA can be used in heterosis prediction to develop potential hybrids. Most traits registered low to moderate narrow sense heritability, suggesting that large proportion of the genetic variance is due to the dominance genetic effects.

5.2 Conclusion

Based on the findings of the current study, there is potential in improving the productivity of cowpea. The cowpea germplasm studied exhibits significant genetic diversity, providing a valuable resource for breeding programs. Variation among cowpea lines in leaf shape, growth pattern, habits, pigmentation, maturity and seed characteristics are indicators of potential value of a germplasm collection for use in breeding and crop improvement. The set of cowpea varieties evaluated in this study differed in a wide range of plant and seed characteristics. The leaf characteristics are important selection index since they affect the rate of photosynthesis, which directly affects plant growth, productivity and yield.In this study, accessions NA3, NA101, NA11, MA24 and MA22 were found to be potential genotypes for grain production.

Overall, the SSR markers revealed considerable genetic diversity in the cowpea population studied. Two markers (VM61 and VM19) were monomorphic in this population. Markers VM70, VM31, and VM68 were the most informative, showing high numbers of alleles, gene diversity, and PIC values. This molecular data provides valuable insights into the genetic diversity and structure of the cowpea population, which can be useful for breeding programs and conservation efforts. Some genotypes (MA67) show a good balance of flavonoid content, yield, and drought tolerance. There's

potential to develop cowpea varieties with improved yield while maintaining moderate levels of flavonoids, although careful selection is needed to balance these traits.

Accessions MA50 X MA67, MA67 X NA60 and KK06 X MA67 were identified to be potential genotypes for dual purpose. The strong correlation between characters like plant height, terminal leaf width, terminal leaflet length, number of branches seeds per pod and pods per plant had very high level of contribution to the total variability observed among the accessions in relation to grain and biomass yield, making these characters important for discriminating among accessions of cowpea. Moreover, for any breeding work to be successful in cowpea, these characters must be considered. Based on the findings of the current study, there is potential in improving the productivity of cowpea in the country.

The highest heterosis were observed in MA67 X NA60 and MA50 X MA67 hence it has greater potential for higher selection response in the trait's improvement. This study provides valuable information on the genetic diversity, heritability, and combining ability of cowpea germplasm. The heterosis observed in the F_1 hybrids and the genetic component analysis offer insights into the genetic control of key traits. The significance of GCA and SCA for all traits suggested that additive and non-additive genetic variances are important in the inheritance of the studied traits. The GCA to SCA ratios pointed out the higher influence of additive gene effect The novel dual-purpose genotypes developed have the potential to contribute significantly to enhanced cowpea production systems, addressing both food and feed security challenges. These findings lay a foundation for future cowpea breeding programs aimed at developing improved varieties with optimal grain and fodder characteristics. Traits with high broad sense heritability but low narrow sense heritability, such as grain yield, days to flowering and pod length, suggest that while these traits are genetically controlled, their improvement through selection may be limited by non-additive genetic effects. This integrated approach, combining genetic, biochemical, and agronomic data, offers a robust foundation for future cowpea improvement efforts, addressing both productivity and quality attributes.

5.3: Recommendations of the Study

i. Utilize the characterized cowpea accessions for breeding programs aimed at developing improved varieties with desirable traits.
ii. Utilize the developed dual-purpose F_1 cowpea genotypes in breeding program to develop dual-purpose varieties.
iii. Utilize flavonoids (myricetin and quercetin) as biomarkers in breeding for drought and leaf quality in cowpea.
iv. Utilize the findings of molecular markers in Kenyan cowpea breeding programs to serve as guide for selecting trait of interest.

5.4 Recommendations for Further Studies

i. Conduct replicated yield trials for promising genotypes from evaluation experiments across different agro-ecological zones to assess their adaptability and stability and release of superior genotypes to the farming community as varieties.
ii. Selection and advancement of promising developed dual-purpose F1 cowpea genotypes.
iii. Expand the genetic diversity analysis to include more cowpea accessions from core collection.
iv. Conduct further study to identify quantitative trait loci (QTLs) or genes associated drought tolerance, grain and biomass yield, and leaf quality.
v. Explore the potential of advanced breeding techniques such as marker-assisted selection (MAS) and genomic selection to accelerate the development of improved cowpea varieties.
vi. Evaluate the nutritional factors in both the grains and vegetables of the developed dual-purpose genotypes.

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APPENDICES

Appendix 1: Field layout

BLOCK 1	BLOCK 2	BLOCK 3
MA24	NA3	NA45
MA29	NA5	MA35
NA8	NA11	MA44
NA101	NA15	NA81
NA3	NA29	MA31
NA82	NA31	MA54
NA29	NA60	NA3
MA36	NA20	MA69
MA39	MA2	MA34
NA11	MA22	NA15
NA60	MA25	NA67
MA30	NA101	MA51
MA49	MA31	NA59
MA25	MA21	MA21
MA2	MA37	MA47
MA51	MA39	MA36
MA21	MA62	NA20
MA37	KK6	KK6
NA45	MA54	MA22
MA34	NA95	MA39
MA31	MA50	MA8
MA69	MA30	MA25
NA64	NA59	MA2
NA95	MA49	MA67
MA35	MA29	NA60
MA8	MA34	NA20
MA63	MA8	NA35
MA67	MA63	MA49
NA20	NA47	MA24
NA47	MA44	NA5
MA54	NA2	NA95
MA43	NA81	NA11
NA81	NA8	MA37
NA5	NA23	MA50
MA41	NA64	ES5
MA15	NA82	NA82
ES5	ES5	NA29
NA31	MA24	NA64
NA72	MA35	MA29
KK6	MA36	MA62
MA62	MA15	MA15
NA2	MA41	MA30
NA23	MA43	MA63
NA35	NA45	NA23
NA15	MA51	MA43
MA50	MA69	NA72
MA22	MA67	NA101
NA59	NA72	NA31
NA80	MA45	NA8
MA44	NA80	MA41

Appendix 2: Scoring of Morphological Variables (Qualitative and Quantitative Traits)

a) The qualitative traits will be evaluated using different scoring scales.

1- Growth pattern 1= Determinate (apical bud of main stem) 2= Indeterminate

2- Twining tendency 0 = None 3 = Slight 5 = Intermediate 7 = Pronounced
3- Plant pigmentation (recorded for stem, branches, petioles and peduncles in the 6th week after planting) 0 = None 1 = Very slight 3 = Moderate at the base and tips of petioles 5 = Intermediate 7 = Extensive 9 = Solid
4- Terminal leaflet shape (recorded for the terminal leaflet of a young, mature leaf in the 6th week after planting) 1 = Globose 2 = Sub-globose 3 = Sub-hastate 4 = Hastate
5- Plant hairiness (of stems, leaves and pods) 3 = Glabrescent 5 = Short appressed hairs 7 = Pubescent to hirsute
6- 6-Raceme position (recorded when peduncles have reached full length) 1 = Mostly above canopy 2 = In upper canopy 3 = Throughout canopy
7- Pod attachment to peduncle (recorded when pods were fully-grown) 3 = Pendant 5 = 30 – 90° down from erect 7 = Erect
8- Mature pod curvature: 0 = Straight 3 = Slightly curved 5 = Curved 7 = Coiled
9- Immature pod pigmentation (pattern of pigment distribution on full grown immature pod) 0 = None 1 = Pigmented tip 2 = Pigmented sutures 3 = Pigmented valves, green sutures 4 = Splashes of pigment 5 = Uniformly pigmented
10- Seed shape 1 = Kidney 2 = Ovoid 3 = Crowder 4 = Globose 5 = Rhomboid
11- Testa texture 1 = Smooth 3 = Smooth to rough 5 = Rough (fine reticulation) 7 = Rough to wrinkled 9 = Wrinkled (coarse folds on the testa)
12- Leaf colour intensity: 3 = Pale green 5 = Intermediate green 7 = Dark green
13- Leaf marking (presence/absence of V mark on leaflets) 0 = Absent 1 = Present
14- Splitting of testa 0 = Absent 1 = Present (testa split exposing cotyledons)
15- Flower colour 1=White 2=Violet 3=Mauve-pink 4=Other

(b) The quantitative traits will be evaluated as follows:

1- Seed weight (g) Weight of 100 seeds with moisture content of 12%.
2- Terminal leaflet length (mm) Mean length of 10 terminal leaflets from 4 randomly selected plants.
3- Terminal leaflet width (mm) Mean width of 10 terminal leaflets measured on the broadest part of 3 randomly selected plants.
4- Number of main branches the branches whose origin is in the leaf axils on the main stem; recorded in the 8th week after planting. Mean of 3 randomly selected plants.
5- Number of nodes on main stem Recorded between three to four weeks after planting. Mean of 4 randomly selected plants.
6- Number of pods per peduncle Mean of 3 randomly selected peduncles.
7- Peduncle length (mm) Recorded when peduncles have reached full length. Mean length of 10 peduncles, one from each of 3randomly selected plants will be measured.
8- Number of pods per plant Mean number of mature pods from 4 randomly selected plants
9- Pod length (cm) Mean of the 10 longest mature pods from 4 randomly selected plants.
10- Plant height (cm) Mean of 3 randomly selected plants at the 8th week after sowing.
11- Pod weight (g) Mean weight of 4 longest mature pods from 3 randomly selected plants.
12- Number of seeds per pod Mean number of seeds of the 4 longest mature pods from 3 randomly selected plants.

Appendix 3: National Commission for Science, Technology and Innovation Permit

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Appendix 4: Test of model adequacy for plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to flowering and grain yield: Unstressed Trials 1 and 2

Trial	Variable	Source of variation	DF	SS	MS	F-Value	P-Value
1	PH28DAS	model	51	966.78	18.96	39.82	<.0001
		error	97	46.18	0.48		
		corrected total	148	1012.96			
	PW28DAS	model	51	1536.97	30.14	6.96	<.0001
		error	91	393.76	4.33		
		corrected total	142	1930.73			
	TLL42DAS	model	50	138.46	2.77	46.75	<.0001
		error	96	5.69	0.06		
		corrected total	146	144.16			
	TLW42DAS	model	50	146.99	2.94	85.06	<.0001
		error	95	3.28	0.03		
		corrected total	145	150.27			
	PH56DAS	model	51	2553.79	50.07	99.80	<.0001
		error	94	47.16	0.50		
		corrected total	145	2600.96			
	NOB56DAS	model	51	82.76	1.62	88.11	<.0001
		error	96	1.77	0.02		
		corrected total	147	84.53			
	GYKGHA	model	51	41337604.37	810541.26	637.03	<.0001
		error	98	124692.99	1272.38		
		corrected total	149	41462297.36			
	DT50%F	model	51	415.1	8.14	44.86	<.0001
		error	97	17.6	0.18		
		corrected total	148	432.69			
T2	PH28DAS	model	51	854.78	16.76	570.51	<.0001
		error	89	2.61	0.03		
		corrected total	140	857.40			
	PW28DAS	model	51	1515.01	29.71	98.13	<.0001
		error	88	26.64	0.30		
		corrected total	139	1541.64			
	TLL42DAS	model	51	175.02	3.43	129.12	<.0001

	error	89	2.37	0.03		
	corrected total	140	177.38			
TLW42DAS	model	51	142.54	2.79	168.50	<.0001
	error	89	1.48	0.02		
	corrected total	140	144.02			
PH56DAS	model	51	2553.79	50.07	99.80	<.0001
	error	94	47.16	0.50		
	corrected total	145	2600.96			
NOB56DAS	model	51	82.76	1.62	88.11	<.0001
	error	96	1.77	0.02		
	corrected total	147	84.53			
GYKGHA	model	51	23659889.14	463919.39	432.20	<.0001
	error	98	105167.73	1073.14		
	corrected total	149	23765056.87			
DT50%F	model	51	1502.07	29.45	369.74	<.0001
	error	98	7.8	0.08		
	corrected total	149	1509.87			

Appendix 5: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to flowering and grain yield for combined trials unstressed growth trials

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P Value
1 & 2	PH28DAS	Blocks	2	0.137	0.068	0.20	0.8177
		Genotypes	49	1204.86	24.59	72.35	<.0001
		Trial	1	2528.19	2528.187	7438.43	<.0001
		Genotypes*Trial	49	624.65	12.75	37.51	<.0001
	PC28DAS	Blocks	2	5.69	2.84	1.28	0.2806
		Genotypes	49	1721.61	35.13	15.81	<.0001
		Trial	1	13521.52	13521.52	6082.98	<.0001
		Genotypes*Trial	49	1387.36	28.31	12.74	<.0001
	TLL42DAS	Blocks	2	0.086	0.043	1.00	0.3713
		Genotypes	49	230.54	4.71	108.88	<.0001

	Trial	1	476.14	476.14	11019.1	<.0001
	Genotypes*Trial	49	83.25	1.734	40.14	<.0001
TLW42DAS	Blocks	2	0.130	0.065	2.50	0.0845
	Genotypes	49	219.52	4.480	172.75	<.0001
	Trial	1	192.49	192.493	7422.52	<.0001
	Genotypes*Trial	49	68.806	1.433	55.27	<.0001
PH56DAS	Blocks	2	0.99	0.495	0.82	0.4403
	Genotypes	49	3620.83	73.89	122.89	<.0001
	Trial	1	2017.61	2017.61	3355.25	<.0001
	Genotypes*Trial	49	1360.77	27.77	46.18	<.0001
NOB56DAS	Blocks	2	0.037	0.019	0.76	0.4702
	Genotypes	49	95.594	1.95	79.01	<.0001
	Trial	1	179.106	179.106	7253.23	<.0001
	Genotypes*Trial	49	54.53	1.113	45.06	<.0001
DT50%F	Blocks	2	0.67	0.335	2.58	0.0780
	Genotypes	49	1323.47	25.95	200.00	<.0001
	Trial	1	260.04	260.04	2004.14	<.0001
	Genotypes*Trial	49	611.353	13.008	100.25	<.0001
Yield kg/ha	Blocks	2	1981.83	990.91	0.68	0.5068
	Genotypes	49	79645054.74	1561667.74	1075.47	<.0001
	Trial	1	2024584.80	2024584.80	1394.26	<.0001
	Genotypes*Trial	49	21648812.54	460613.03	317.21	<.0001

Appendix 6: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to flowering and grain yield unstressed growth trials.

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P Value
1	PH28DAS	Blocks	2	2.91	1.457	3.06	0.05
		Genotypes	49	963.94	19.67	41.32	<.0001
	PW28DAS	Blocks	2	8.36	4.177	0.97	0.38
		Genotypes	49	1532.277	31.27	7.23	<.0001
	TLL42DAS	Blocks	2	0.01	0.005	0.08	0.92
		Genotypes	49	138.47	2.88	48.69	<.0001
	TLW42DAS	Blocks	2	0.07	0.04	1.02	0.36
		Genotypes	49	146.90	3.06	88.55	<.0001
	PH56DAS	Blocks	2	0.89	0.44	0.88	0.42
		Genotypes	49	2553.32	52.11	103.85	<.0001
	NOB56DAS	Blocks	2	0.09	0.04	2.33	0.10
		Genotypes	49	82.70	1.69	91.63	<.0001
	GYKGHA	Blocks	2	1433.52	716.76	0.56	0.57
		Genotypes	49	41336170.85	843595.32	663.01	<.0001
	DT50%F	Blocks	2	0.25	0.127	0.70	0.50
		Genotypes	49	414.67	8.46	46.65	<.0001
2	PH28DAS	Blocks	2	0.02	0.01	0.31	0.74
		Genotypes	49	854.68	17.44	593.73	<.0001
	PW28DAS	Blocks	2	0.62	0.31	1.03	0.36
		Genotypes	49	1514.22	30.90	102.08	<.0001
	TLL42DAS	Blocks	2	0.10	0.05	1.96	0.15
		Genotypes	49	174.94	3.57	134.33	<.0001
	TLW42DAS	Blocks	2	0.12	0.06	3.72	0.03
		Genotypes	49	142.39	2.90	175.19	<.0001
	PH56DAS	Blocks	2	0.72	0.36	0.77	0.47
		Genotypes	49	2602.52	53.11	113.26	<.0001
	NOB56DAS	Blocks	2	0.008	0.004	0.13	0.87

	Genotypes	49	68.34	1.39	44.35	<.0001
GYKGHA	Blocks	2	3066.29	1533.14	0.88	0.42
	Genotypes	49	56358344.50	1150170.30	661.87	<.0001
DT50%F	Blocks	2	0.57	0.28	3.60	0.03
	Genotypes	49	1501.49	30.64	384.69	<.0001

Appendix 7: Mean of Drought severity of cowpea genotypes at 7,14 21 and 28 days after imposition of drought.

Treatment	7DAIT1	14DA1T1	21DAIT1	28DAIT1	7DAIT2	14DA1T2	21DAIT2	28DAIT2
NA59	3	3	3	3	3	3	3	3
MA67	3	3	3	3	3	3	3	5
MA15	3	3	5	5	3	3	5	5
MA62	3	3	5	5	3	3	5	5
MA63	3	3	3	3	3	3	3	3
MA69	3	3	3	3	3	3	3	3
MA49	3	3	5	3	3	3	5	5
NA95	3	3	3	5	3	3	3	3
NA47	3	3	3	3	3	3	5	3
MA41	3	3	5	5	3	3	5	5
MA54	3	3	5	5	3	3	5	5
MA29	3	3	3	5	3	3	5	3
MA30	3	3	5	5	3	3	5	5
MA51	3	3	4	3	3	3	3	5
NA101	3	3	3	3	3	3	3	3
NA5	3	3	5	5	3	3	5	5
MA37	3	3	5	5	3	3	5	5
NA35	3	5	5	5	3	5	5	7
MA43	3	3	3	3	3	3	3	3
MA39	3	3	3	5	3	3	3	3
NA2	3	3	3	3	3	3	3	3
MA35	3	3	3	3	3	3	3	3
MA36	3	3	5	3	3	3	3	5
NA64	3	3	3	5	3	5	5	5
NA45	3	3	5	5	3	3	5	5
NA11	3	3	3	3	3	3	3	3
NA15	3	5	5	5	3	5	5	5
NA23	3	5	5	5	3	5	5	5
MA8	3	5	5	5	3	5	5	5
MA31	3	3	5	3	3	3	3	5
MA25	3	3	3	3	3	3	3	3
NA60	3	3	3	5	3	3	3	3
MA2	3	3	3	3	3	3	3	3
NA3	3	3	3	3	3	3	3	3

NA72	3	3	3	3	3	3	3	5
ES5	3	5	5	5	3	3	5	5
KK06	3	5	5	5	3	5	5	5
MA21	3	3	3	5	3	3	3	3
MA34	3	3	5	3	3	3	3	5
MA24	3	3	3	3	3	3	3	3
MA44	3	3	5	3	3	3	3	5
NA81	3	3	5	3	3	3	3	5
NA82	3	5	5	5	3	5	5	7
MA45	3	3	3	3	3	3	3	5
MA22	3	3	3	3	3	3	3	3
MA50	3	5	5	5	3	3	5	5
NA31	3	3	3	3	3	3	3	5
NA20	5	5	5	5	5	5	5	5
NA29	3	5	5	5	3	5	5	5
NA8	3	3	3	3	3	3	5	5

Appendix 8: Test of model adequacy for plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to flowering and grain yield.: water stressed Trials 1 and 2

Trial	Variable	Source of variation	DF	SS	MS	F-Value	P-Value
1	PH28DAS	model	17	515.15	30.30	132.51	<.0001
		error	19	4.35	0.23		
		corrected total	36	519.50			
	PW28DAS	model	17	5007.46	294.56	976.15	<.0001
		error	19	5.73	0.30		
		corrected total	36	5013.19			
	TLL42DAS	model	17	43.81	2.58	57.35	<.0001
		error	19	0.85	0.04		
		corrected total	36	44.66			
	TLW42DAS	model	17	57.61	3.39	154.76	
		error	19	0.42	0.02		<.0001
		corrected total	36	58.02			
	PH56DAS	model	17	473.19	27.83	456.19	<.0001
		error	17	1.04	0.06		
		corrected total	34	474.23			
	NOB56DAS	model	17	53.75	3.16	93.42	<.0001
		error	17	0.58	0.03		
		corrected total	34	54.32			
	GYKGHA	model	51	23659889.1	463919.4	432.20	<.0001
		error	98	105167.73	1073.14		
		corrected total	149	23765056.9			

	DT50%F	model	51	308.81	6.06	7.07	<.0001
		error	98	83.96	0.86		
		corrected total	149	392.77			
2	PH28DAS	model	17	152.72	8.98	123.28	<.0001
		error	25	1.82	0.07		
		corrected total	42	154.54			
	PW28DAS	model	17	956.47	56.26	634.68	<.0001
		error	25	2.22	0.009		
		corrected total	42	958.69			
	TLL42DAS	model	17	91.61	5.39	379.42	<.0001
		error	25	0.36	0.01		
		corrected total	42	91.97			
	TLW42DAS	model	17	39.96	2.35	61.88	<.0001
		error	25	0.95	0.04		
		corrected total	42	40.91			
	PH56DAS	model	17	729.64	42.92	514.99	<.0001
		error	25	2.08	0.08		
		corrected total	42	731.72			
	NOB56DAS	model	17	19.26	1.13	44.17	<.0001
		error	23	0.59	0.03		
		corrected total	40	19.85			
	GYKGHA	model	51	41378678.9	811346.65	458.09	<.0001
		error	69	122210.51	1771.17		
		corrected total	120	41500889.4			
	DT50%F	model	51	1285.78	25.21	296.87	<.0001
		error	98	9.16	0.09		
		corrected total	149	1294.93			

Appendix 9: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to flowering and grain yield for combined trials stressed growth trials

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P value
1 & 2	PH28DAS	Blocks	2	0.71	0.357	2.25	0.1080
		Genotypes	49	1408.49	28.74	181.66	<.0001
		Trial	1	1432.77	1432.77	9054.54	<.0001

	Genotypes*Trial	49	559.33	11.41	72.14	<.0001
PW28DAS	Blocks	2	0.78	0.39	1.55	0.2149
	Genotypes	49	2834.99	57.85	229.45	<.0001
	Trial	1	10607.37	10607.37	42067.5	<.0001
	Genotypes*Trial	49	1749.91	35.71	141.63	<.0001
TLL42DAS	Blocks	2	0.053	0.026	1.03	0.3591
	Genotypes	49	265.80	5.42	212.54	<.0001
	Trial	1	495.70	495.70	19422.7	<.0001
	Genotypes*Trial	49	108.60	2.216	86.84	<.0001
TLW42DAS	Blocks	2	0.019	0.01	0.56	0.5694
	Genotypes	49	259.29	5.29	307.49	<.0001
	Trial	1	199.24	199.244	11577.7	<.0001
	Genotypes*Trial	49	68.66	1.40	81.42	<.0001
PH56DAS	Blocks	2	0.09	0.045	0.09	0.9149
	Genotypes	49	3774.13	77.023	152.81	<.0001
	Trial	1	2149.51	2149.51	4264.44	<.0001
	Genotypes*Trial	49	973.54	19.868	39.42	<.0001
NOB56DAS	Blocks	2	0.008	0.004	0.36	0.6967
	Genotypes	49	163.98	3.35	285.33	<.0001
	Trial	1	249.48	249.48	21270.3	<.0001
	Genotypes*Trial	49	72.80	1.49	126.67	<.0001
DT50%F	Blocks	2	0.69	0.35	2.28	0.1050
	Genotypes	49	983.58	19.29	126.53	<.0001
	Trial	1	1422.22	1422.22	9331.16	<.0001
	Genotypes*Trial	49	548.78	11.68	76.61	<.0001

	Yield kg/ha	Blocks	2	2492.21	1246.10	0.91	0.4040
		Genotypes	49	46748099.47	916629.40	670.32	<.0001
		Trial	1	1214638.29	1214638.29	888.25	<.0001
		Genotypes*Trial	49	14561424.43	309817.54	226.57	<.0001

Appendix 10: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to flowering and grain yield Stressed growth trials.

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P value
1	PH28DAS	Blocks	2	0.67	0.33	1.86	0.16
		Genotypes	50	1368.32	27.37	152.15	<.0001
	PW28DAS	Blocks	2	0.29	0.14	1.35	0.26
		Genotypes	50	3083.21	61.66	581.63	<.0001
	TLL42DAS	Blocks	2	0.16	0.08	2.09	0.13
		Genotypes	49	196.93	4.02	105.82	<.0001
	TLW42DAS	Blocks	2	0.06	0.03	1.20	0.30
		Genotypes	49	186.16	3.8	160.91	<.0001
	PH56DAS	Blocks	2	0.36	0.18	1.23	0.30
		Genotypes	50	3099.97	62.00	428.09	<.0001
	NOB56DAS	Blocks	2	0.02	0.01	0.60	0.54
		Genotypes	50	95.12	1.90	128.57	<.0001
	GYKGHA	Blocks	2	2858.63	1429.31	1.33	0.26
		Genotypes	49	23657030.51	482796.54	449.89	<.0001
	DT50%F	Blocks	2	5.37	2.69	3.14	0.05
		Genotypes	49	303.44	6.19	7.23	<.0001
2	PH28DAS	Blocks	2	0.14	0.07	0.51	0.60
		Genotypes	49	698.07	14.25	107.05	<.0001
	PW28DAS	Blocks	2	0.66	0.33	0.80	0.45
		Genotypes	49	1709.30	34.88	84.38	<.0001
	TLL42DAS	Blocks	2	0.01	0.004	0.40	0.67
		Genotypes	49	179.60	3.67	341.57	<.0001

TLW42DAS	Blocks	2	0.008	0.004	0.42	0.66
	Genotypes	49	149.04	3.04	308.57	<.0001
PH56DAS	Blocks	2	0.32	0.16	1.85	0.16
	Genotypes	49	1752.18	35.76	417.13	<.0001
NOB56DAS	Blocks	2	0.036	0.02	2.29	0.11
	Genotypes	49	138.45	2.83	362.46	<.0001
GYKGHA	Blocks	2	1248.19	624.09	2.06	0.14
	Genotypes	49	41328419.20	843437.13	2781.72	<.0001
DT50%F	Blocks	2	0.38	0.19	2.06	0.13
	Genotypes	49	1285.39	26.23	280.80	<.0001

Appendix 11: Genotypes subjected to population structure

Serial number	Genotype	Origin
1	NA23	IITA
2	MA67	GBK
3	MA69	GBK
4	NA72	IITA
5	KK06	Western
6	MA30	GBK
7	MA23	GBK
8	MA50	GBK
9	MA45	GBK
10	MA54	GBK
11	TR3	Tharaka
12	ES5	Eastern
13	MA36	GBK
14	MA24	GBK
15	NA20	IITA
16	MA46	GBK
17	NA81	IITA
18	NA11	IITA
19	MA44	GBK
20	NA60	IITA

Appendix 12: Test of model adequacy for the metabolite profiling

Variable	Source of variation	df	SS	MS	F-Value	P-Value
Quercetin	model	19	770124.49	40532.87	29776.4	<.0001
	error	31	42.20	1.3612		
	corrected total	50	770166.69			
Myricetin	model	19	1332.56	70.13	149.98	<.0001
	error	24	1.22	0.47		

	corrected total	43	1343.79	

Appendix 13: Analysis of variance for the biochemical characterisation

Variable	Source of variation	df	SS	MS	F value	p-value
Quercetin	Genotypes	17	769839.33	45284.67	33267.2	<.0001
Myricetin	Genotypes	17	1313.06	77.23	165.18	<.0001

Appendix 14: Test of model adequacy for plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to 50% flowering grain yield 100seed weight, pod weight, pod length, seeds per pod and pods per plant.: Un stressed Trials 1 and 2

Trial	Variable	Source of variation	df	SS	MS	F-Value	P-Value
T1	PH28DAS	model	16	253.66	15.85	12.65	<.0001
		error	14	17.55	1.25		
		corrected total	30	271.21			
	PW28DAS	model	16	1430.68	89.42	368.18	<.0001
		error	14	3.4	0.24		
		corrected total	30	1434.08			
	TLL42DAS	model	17	74.26	4.37	124.96	<.0001
		error	14	0.49	0.03		
		corrected total	31	74.75			
	TLW42DAS	model	17	61.02	3.59	107.10	<.0001
		error	14	0.47	0.03		
		corrected total	31	61.49			
	PH56DAS	model	16	833.28	52.08	370.38	<.0001
		error	14	1.97	0.14		
		corrected total	30	835.25			
	NOB56DAS	model	16	68.23	4.26	64.59	<.0001
		error	14	0.92	0.07		
		corrected total	30	69.15			
	GYKGHA	model	17	310261.2	182505.9	606.72	<.0001
		error	30	902430.2	30081.0		
		corrected total	47	311161.5			
	Biomass	model	17	146623.6	86236.7	149.89	<.0001

		error	30	172049.7	57535.0		
		corrected total	47	148384.2			
	DT50%F	model	17	397.25	23.37	23.37	<.0001
		error	30	30.00	1.00		
		corrected total	47	427.25			
	100SW	model	17	1115.24	65.60	41.99	<.0001
		error	30	46.88	1.56		
		corrected total	47	1162.119			
	PL	model	17	83.67	4.92	25.09	<.0001
		error	30	5.89	0.20		
		corrected total	47	89.56			
	PW	model	17	4669.61	274.68	89.69	<.0001
		error	30	91.88	3.06		
		corrected total	47	4761.48			
	Seeds/pod	model	17	626.72	36.87	163.60	<.0001
		error	30	6.76	0.23		
		corrected total	47	633.48			
	Pods/plant	model	17	53697.10	3158.65	178.33	<.0001
		error	30	531.38	17.71		
		corrected total	47	54228.48			
2	PH28DAS	model	17	244.13	14.36	110.57	<.0001
		error	23	2.99	0.13		
		corrected total	40	247.12			
	PW28DAS	model	17	1051.46	61.85	166.67	<.0001
		error	22	8.16	0.37		
		corrected total	39	1059.62			
	TLL42DAS	model	17	120.35	7.08	73.56	<.0001
		error	24	2.31	0.10		
		corrected total	41	122.66			
	TLW42DAS	model	17	93.90	5.52	516.64	<.0001
		error	24	0.26	0.01		
		corrected total	41	94.16			
	PH56DAS	model	17	1390.09	81.77	513.56	<.0001
		error	24	3.82	0.16		
		corrected total	41	1393.92			
	NOB56DAS	model	17	57.17	3.36	65.20	<.0001

		error	24	1.24	0.05		
		corrected total	41	58.41			
	GYKGHA	model	17	3982346.45	234255.67	134.93	<.0001
		error	30	52083.34	1736.11		
		corrected total	47	4034429.79			
	Biomass	model	17	60666855.21	3568638.54	80.36	<.0001
		error	30	1332252.01	44408.40		
		corrected total	47	61999107.22			
	DT50%F	model	17	420.38	24.73	43.96	<.0001
		error	30	16.88	0.56		
		corrected total	47	437.25			
	100SW	model	17	680.99	40.06	40.06	<.0001
		error	30	30.00	1.00		
		corrected total	47	710.99			
	PL	model	17	191.72	11.28	87.85	<.0001
		error	29	3.72	0.13		
		corrected total	46	195.44			
	PW	model	17	88.37	5.20	20.79	<.0001
		error	30	7.50	0.25		
		corrected total	47	95.87			
	Seeds/pod	model	17	576.35	33.90	166.06	<.0001
		error	30	6.1	0.20		
		corrected total	47	582.48			
	Pods/plant	model	17	65032.13	3825.42	7587.61	<.0001
		error	30	15.13	0.50		
		corrected total	47	65047.25			

Appendix 15: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to 50% flowering grain yield 100seed weight, pod weight, pod length, seeds per pod and pods per plant.: Un stressed combined trial

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P value

PH28DAS	Block	2	0.55	0.276	0.50	0.6094
	trial	1	0.97	0.97	1.77	<.0001
	Genotypes	19	224.82	11.83	21.52	<.0001
	Trial*Genotypes	10	273.56	27.35	49.74	<.0001
PW28DAS	Block	2	0.215	0.108	0.00	0.9963
	trial	1	1641.41	86.39	3.01	<.0001
	Genotypes	19	70.62	70.62	2.46	0.0053
	Trial*Genotypes	10	4.89	0.212	0.01	<.0001
TLL42DAS	Block	2	0.505	0.25	3.51	0.0395
	trial	1	0.485	0.485	6.73	<.0001
	Genotypes	15	139.50	9.30	129.04	<.0001
	Trial*Genotypes	15	54.74	3.65	50.64	<.0001
TLW42DAS	Block	2	0.04	0.02	1.12	0.3365
	trial	1	0.537	0.537	27.56	<.0001
	Genotypes	15	117.85	7.857	403.07	<.0001
	Trial*Genotypes	15	39.357	2.62	134.61	<.0001
PH56DAS	Block	2	0.25	0.127	0.84	0.4408
	trial	1	3.42	3.42	22.55	<.0001
	Genotypes	15	1723.54	114.90	757.64	<.0001
	Trial*Genotypes	15	439.20	31.37	206.86	<.0001
NOB56DAS	Block	2	0.0114	0.0057	0.10	0.9026
	trial	1	46.197	46.197	831.67	<.0001
	Genotypes	15	86.885	5.79	104.28	<.0001
	Trial*Genotypes	15	31.82	2.27	40.92	<.0001
DT50%F	Block	2	0.063	0.031	0.04	0.9620

	trial	1	802.50	53.50	66.42	<.0001
	Genotypes	15	0.00	0.00	0.00	<.0001
	Trial*Genotypes	15	12.00	0.80	0.99	<.0001
Yield kg/ha	Block	2	17784.8	8892.4	0.55	0.5792
	trial	1	114368479.8	114368479.8	7085.14	<.0001
	Genotypes	15	184419591.3	12294639.4	761.65	<.0001
	Trial*Genotypes	15	129758859.9	8650590.7	535.91	<.0001
100SW	Block	2	5.06	2.53	2.04	0.1387
	trial	1	1178.07	78.54	63.29	<.0001
	Genotypes	15	72.61	72.61	58.51	<.0001
	Trial*Genotypes	15	613.03	40.87	32.93	<.0001
PL	Block	2	0.173	0.0865	0.53	0.5895
	trial	1	17.009	17.009	104.80	<.0001
	Genotypes	15	182.11	12.14		<.0001
	Trial*Genotypes	15	86.67			<.0001
PW	Block	2	15625.003	7812.501	1.55	0.2194
	trial	1	5183073.592	5183073.59	1031.51	<.0001
	Genotypes	15	6943005.58	462867.039	92.12	<.0001
	Trial*Genotypes	15	7721679.80	514778.65	102.45	<.0001
Pod/plant	Block	2	37.02	18.51	1.98	0.1467
	trial	1	2511.26	2511.26	268.61	<.0001
	Genotypes	15	116222.16	7748.14	828.76	<.0001
	Trial*Genotypes	15	2436.906	162.46	17.38	<.0001
Seeds/pod	Block	2	0.297	0.148	0.70	0.5019
	trial	1	4.17	4.17	19.57	<.0001

	Genotypes	15	1109.958	73.997	347.48	<.0001
	Trial*Genotypes	15	92.50	6.17	28.96	<.0001

Appendix 16: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to 50% flowering grain yield 100seed weight, pod weight, pod length, seeds per pod and pods per plant.: Un stressed Trials 1 and 2

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	
1	PH28DAS	Blocks	2	1.45	0.76	0.58	0.5737
		Genotypes	14	248.02	17.72	14.13	<.0001
	PW28DAS	Blocks	2	0.28	0.139	0.57	0.5766
		Genotypes	14	1239.27	88.52	364.49	<.0001
	TLL42DAS	Blocks	2	0.05	0.02	0.56	0.5359
		Genotypes	15	65.94	4.40	125.76	<.0001
	TLW42DAS	Blocks	2	0.007	0.004	0.11	0.90
		Genotypes	15	58.82	3.92	117.01	<.0001
	PH56DAS	Blocks	2	0.41	0.21	1.46	0.26
		Genotypes	14	8.221	58.73	417.67	<.0001
	NOB56DAS	Blocks	2	0.022	0.111	0.17	0.85
		Genotypes	14	65.38	4.67	70.74	<.0001
	GYKGHA	Blocks	2	60604.3	30302.1	1.01	0.377
		Genotypes	15	310199576.9	20679971.8	687.48	<.0001
	Biomass	Blocks	2	320000.1	160000.0	2.78	0.0780
		Genotypes	15	146282384.5	9752159.0	169.50	<.0001
	DT50%F	Blocks	2	2.00	1.00	1.00	0.38
		Genotypes	15	395.25	26.35	26.35	<.0001
	100SW	Blocks	2	3.13	1.56	1.00	0.38
		Genotypes	15	1112.12	74.14	47.45	<.0001
	PL	Blocks	2	0.12	0.60	0.31	0.74
		Genotypes	15	83.56	5.57	28.40	<.0001

	PW	Blocks	2	6.13	3.06	1.00	0.38
		Genotypes	15	4663.48	310.90	101.52	<.0001
	Seeds/pod	Blocks	2	0.07	0.04	0.16	0.85
		Genotypes	15	626.65	41.78	185.39	<.0001
	Pods/plant	Blocks	2	69.29	34.65	1.96	0.159
		Genotypes	15	53627.81	3575.19	201.85	<.0001
2	PH28DAS	Blocks	2	0.77	0.39	1.04	0.37
		Genotypes	15	1038.84	69.26	186.62	<.0001
	PW28DAS	Blocks	2	0.03	0.01	1.68	0.21
		Genotypes	15	13.51	0.90	108.70	<.0001
	TLL42DAS	Blocks	2	0.54	0.27	2.82	0.08
		Genotypes	15	120.17	8.01	83.25	<.0001
	TLW42DAS	Blocks	2	0.09	0.05	4.21	0.03
		Genotypes	15	92.70	6.18	578.03	<.0001
	PH56DAS	Blocks	2	0.12	0.06	0.37	0.69
		Genotypes	15	1367.58	91.17	572.61	<.0001
	NOB56DAS	Blocks	2	0.05	0.02	0.47	0.63
		Genotypes	15	56.28	3.75	72.75	<.0001
	GYKGHA	Blocks	2	3472.22	1736.11	1.0	0.38
		Genotypes	15	3978874.23	265258.28	152.79	<.0001
	Biomass	Blocks	2	95258.50	47629.25	1.07	0.35
		Genotypes	15	60571596.70	4038106.45	90.93	<.0001
	DT50%F	Blocks	2	1.13	0.56	1.00	0.38
		Genotypes	15	419.25	27.95	49.69	<.0001
	100SW	Blocks	2	2.00	1.00	1.00	0.38
		Genotypes	15	678.989	45.27	45.27	<.0001
	PL	Blocks	2	0.34	0.17	1.34	0.28
		Genotypes	15	191.16	12.74	99.27	<.0001
	PW	Blocks	2	0.50	0.25	1.00	0.38
		Genotypes	15	87.87	5.86	23.43	<.0001
	Seeds/pod	Blocks	2	0.54	0.27	1.33	0.28
		Genotypes	15	575.81	38.39	188.02	<.0001

	Pods/plant	Blocks	2	0.88	0.44	0.87	0.43
		Genotypes	15	65031.25	4335.42	8599.17	<.0001

Appendix 17: Test of model adequacy for plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to 50% flowering grain yield 100seed weight, pod weight, pod length, seeds per pod and pods per plant.: Stressed Trials 1 and 2

Trial	Variable	Source of variation	DF	SS	MS	F-Value	P-Value
T1	PH28DAS	model	17	515.15	30.30	132.51	<.0001
		error	19	4.35	0.23		
		corrected total	36	519.5			
	PW28DAS	model	17	5007.46	294.56	976.15	<.0001
		error	19	5.73	0.30		
		corrected total	36	5013.19			
	TLL42DAS	model	17	43.81	2.58	57.35	<.0001
		error	19	0.85	0.04		
		corrected total	36	44.66			
	TLW42DAS	model	17	57.61	3.39	154.76	<.0001
		error	19	0.42	0.02		
		corrected total	36	58.02			
	PH56DAS	model	17	473.19	27.83	456.19	<.0001
		error	17	1.04	0.06		
		corrected total	34	474.23			
	NOB56DAS	model	17	53.73	3.16	93.42	<.0001
		error	17	0.58	0.03		
		corrected total	34	54.32			
	GYKGHA	model	17	20218884.62	1189346.15	98.92	<.0001
		error	30	360712.03	12023.73		
		corrected total	47	20579596.65			
	Biomass	model	17	114621463.2	6742439.0	145.67	<.0001
		error	30	1388576.6	46285.9		
		corrected total	47	116010039.8			
	DT50%F	model	17	343.25	20.19	20.19	<.0001
		error	30	30.00	1.00		
		corrected total	47	373.25			
	100SW	model	17	1187.49	69.85	54.97	<.0001
		error	30	38.13	1.27		
		corrected	47	1225.62			

		corrected total					
	PL	model	17	133.02	7.82	50.69	<.0001
		error	30	4.633	0.15		
		corrected total	47	137.65			
	PW	model	17	3328558.66	195797.57	253.75	<.0001
		error	30	23148.15	771.61		
		corrected total	47	3351706.81			
	Seeds/pod	model	17	423.40	24.91	100.94	<.0001
		error	29	7.16	0.25		
		corrected total	46	43.55			
	Pods/plant	model	16	27808.80	1738.05	1502.02	<.0001
		error	28	32.40	1.16		
		corrected total	44	27841.20			
2	PH28DAS	model	17	152.72	0.02	123.28	<.0001
		error	25	1.82	10.17		
		corrected total	42	154.54			
	PW28DAS	model	17	956.47	56.26	634.68	<.0001
		error	25	2.21	0.09		
		corrected total	42	958.69			
	TLL42DAS	model	17	91.61	5.39	379.42	<.0001
		error	25	0.36	0.01		
		corrected total	42	91.97			
	TLW42DAS	model	17	39.96	2.35	61.88	<.0001
		error	25	0.95	0.04		
		corrected total	42	40.91			
	PH56DAS	model	17	729.64	42.92	514.99	<.0001
		error	25	2.08	0.08		
		corrected total	42	731.72			
	NOB56DAS	model	17	19.26	1.13	44.17	<.0001
		error	23	0.59	0.03		
		corrected total	40	19.85			
	GYKGHA	model	17	1647558.69	96915.22	196.25	<.0001
		error	30	14814.82	493.83		
		corrected total	47	1662373.51			
	Biomass	model	17	9960399.38	585905.85	96.85	<.0001
		error	30	181485.37	6049.51		
		corrected total	47	10141884.74			
	DT50%F	model	16	256.93	16.06	15.05	<.0001

		error	28	29.87	1.07		
		corrected total	44	286.80			
	100SW	model	17	368.08	21.65	37.12	<.0001
		error	30	17.50	0.58		
		corrected total	47	385.58			
	PL	model	17	410.67	24.16	121.91	<.0001
		error	30	5.94	0.198		
		corrected total	47	416.62			
	PW	model	17	454532.87	26737.23	34.65	<.0001
		error	30	23148.15	771.61		
		corrected total	47	477681.02			
	Seeds/pod	model	17	317.04	18.65	75.38	<.0001
		error	29	7.17	0.25		
		corrected total	46	324.21			
	Pods/plant	model	17	35138.63	2066.98	1369.11	<.0001
		error	30	45.29	1.51		
		corrected total	47	35183.92			

Appendix 18: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to 50% flowering grain yield 100seed weight, pod weight, pod length, seeds per pod and pods per plant.: Stressed combined trials

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P-Value
1$2	PH28DAS	Block	2	0.147	0.074	0.52	0.6000
		trial	1	138.67	138.66	973.92	<.0001
		Genotypes	15	492.84	32.856	230.76	<.0001
		Trial*Genotypes	15	168.47	11.23	78.88	<.0001
	PW28DAS	Block					
		trial					
		Genotypes					
		Trial*Genotypes					
	TLL42DAS	Block	2	0.19	0.095	3.61	0.0351
		trial	1	6.085	6.085	231.01	<.0001
		Genotypes	15	91.03	6.068	230.38	<.0001
		Trial*Genotypes	15	33.158	2.21	83.92	<.0001
	TLW42DAS	Block	2	0.093	0.046	1.56	0.2209
		trial	1	2.87	2.87	96.50	<.0001
		Genotypes	15	67.966	4.53	152.10	<.0001
		Trial*Genotypes	15	28.758	1.917	64.36	<.0001
	PH56DAS	Block	2	0.194	0.097	1.29	0.2849
		trial	1	5.11	5.11	68.01	<.0001
		Genotypes	15	966.156	64.41	857.29	<.0001
		Trial*Genotypes	15	250.10	16.67	221.92	<.0001
	NOB56DAS	Block	2	0.046	0.02	0.71	0.4995
		trial	1	75.117	75.117	2307.28	<.0001

	Genotypes	15	51.85	3.457	106.18	<.0001
	Trial*Genotypes	15	16.36	1.09	33.51	<.0001
DT50%F	Block	2	4.13	2.06	2.07	0.1352
	trial	1	484.45	32.30	32.57	<.0001
	Genotypes	15	0.90	0.90	0.90	<.0001
	Trial*Genotypes	15	111.60	7.97	7.99	<.0001
Yield kg/ha	Block	2	13216.75	6608.38	1.02	0.3659
	trial	1	17959325.2	1795935.2	2777.29	<.0001
	Genotypes	15	13141163.3	876077.6	135.48	<.0001
	Trial*Genotypes	15	8686666.9	579111.1	89.56	<.0001
100SW	Block	2	3.56	1.78	1.98	0.1467
	trial	1	191.51	191.51	212.90	<.0001
	Genotypes	15	640.37	42.69	47.46	<.0001
	Trial*Genotypes	15	911.41	60.77	67.55	<.0001
PL	Block	2	0.69	0.345	1.98	0.1461
	trial	1	67.335	67.335	387.16	<.0001
	Genotypes	15	403.845	26.92	154.80	<.0001
	Trial*Genotypes	15	138.945	9.263	53.26	<.0001
PW	Block	2	3086.42	1543.21	2.07	0.1352
	trial	1	1412765.164	1412765.4	1891.97	<.0001
	Genotypes	15	2100570.506	140038.0	187.54	<.0001
	Trial*Genotypes	15	1679434.598	111962.3	149.9	<.0001
Pods/plant	Block	2	1.05	0.527	0.36	0.7005
	trial	1	2444.01	2444.011	1661.09	<.0001
	Genotypes	15	58947.29	3929.81	2670.94	<.0001
	Trial*Genotypes	15	3988.49	284.89	193.63	<.0001
Seeds/pod	Block	2	0.19	0.095	0.38	0.6871
	trial	1	0.010	0.010	0.04	<.0001
	Genotypes	15	673.83	44.92	178.00	<.0001
	Trial*Genotypes	15	64.82	4.32	17.12	<.0001

Appendix 19: Analysis of variance for effect of treatments on plant height, Plant width, Terminal leaf length, Terminal leaf width and number of branches days to 50% flowering grain yield 100seed weight, pod weight, pod length, seeds per pod and pods per plant.: Stressed Trials 1 and 2

Trial	Variable	Source of variation	DF	Type III SS	Mean Square	F Value	P-Value
T1	PH28DAS	Blocks	2	0.13	0.06	4.51	0.02
		Genotypes	15	91.42	6.09	429.11	<.0001
	PW28DAS	Blocks	2	0.07	0.04	0.97	0.39
		Genotypes	15	39.16	2.61	68.74	<.0001
	TLL42DAS	Blocks	2	0.006	0.03	0.72	0.5
		Genotypes	15	43.71	2.91	64.85	<.0001
	TLW42DAS	Blocks	2	0.024	0.012	0.55	0.59
		Genotypes	15	57.007	3.80	173.58	<.0001
	PH56DAS	Blocks	2	0.04	0.02	0.29	0.75
		Genotypes	15	152.61	10.17	139.62	<.0001
	NOB56DAS	Blocks	2	0.24	0.12	1.34	0.28
		Genotypes	15	937.82	62.52	705.28	<.0001
	GYKGHA	Blocks	2	37624.86	1882.43	1.56	0.2258
		Genotypes	15	20181259.8	134547.3	111.90	<.0001
	Biomass	Blocks	2	354201.4	177100.7	3.83	0.03

		Genotypes	15	11426726.7	761787.4	164.58	<.0001
	DT50%F	Blocks	2	2.00	1.00	1.00	0.38
		Genotypes	15	341.25	22.75	22.75	<.0001
	100SW	Blocks	2	2.54	1.27	1.00	0.38
		Genotypes	15	1184.95	79.00	62.16	<.0001
	PL	Blocks	2	0.30	0.15	0.98	0.39
		Genotypes	15	132.71	8.84	57.32	<.0001
	PW	Blocks	2	1543.21	771.61	1.00	0.38
		Genotypes	15	3327015.5	22180.0	287.45	<.0001
	Seeds/pod	Blocks	2	0.18	0.089	0.36	0.7
		Genotypes	15	423.32	28.22	114.38	<.0001
	Pods/plant	Blocks	2	3.60	1.80	1.56	0.23
		Genotypes	14	27805.20	1986.1	171.37	<.0001
2	PH28DAS	Blocks	2	0.04	0.02	0.29	0.75
		Genotypes	15	152.61	10.17	139.62	<.0001
	PW28DAS	Blocks	2	0.24	0.12	1.34	0.28
		Genotypes	15	937.82	62.52	705.28	<.0001
	TLL42DAS	Blocks	2	0.13	0.06	4.51	0.02
		Genotypes	15	91.42	6.09	429.11	<.0001
	TLW42DAS	Blocks	2	0.07	0.04	0.97	0.39
		Genotypes	15	39.16	2.61	68.74	<.0001
	PH56DAS	Blocks	2	0.14	0.07	0.86	0.44
		Genotypes	15	729.31	48.62	583.40	<.0001
	NOB56DAS	Blocks	2	0.04	0.02	0.85	0.44
		Genotypes	15	18.54	1.24	48.18	<.0001
	GYKGHA	Blocks	2	987.65	493.83	1.00	0.38
		Genotypes	15	1646571.0	109771.4 0	222.29	<.0001
	Biomass	Blocks	2	20304.79	10152.39	1.68	0.20
		Genotypes	15	9940094.6	662672.9 7	109.54 4	<.0001
	DT50%F	Blocks	2	2.13	1.07	1.00	0.38
		Genotypes	14	254.80	18.20	17.06	<.0001
	100SW	Blocks	2	1.17	0.58	1.00	0.38
		Genotypes	15	366.91	24.46	41.93	<.0001
	PL	Blocks	2	0.60	0.30	1.50	0.24
		Genotypes	15	410.08	27.34	137.97	<.0001
	PW	Blocks	2	1543.21	771.61	1.00	0.38
		Genotypes	15	452989.66	30199.32	39.14	<.0001
	Seeds/pod	Blocks	2	0.83	0.41	1.67	0.21
		Genotypes	15	315.75	21.05	85.09	<.0001
	Pods/plant	Blocks	2	8.04	4.02	2.66	0.09
		Genotypes	15	35130.58	2342.04	155.30	<.0001

Appendix 20: Model for General Combining Ability and Specific Combining Ability using Griffing's Method 2

Variable	Source	df	SS	MS	F-value	p-value
GY	Model	15	92138330.65	6142555.38	1398.88	<0.0001
	Error	32	140513.81	4391.06		

	Corrected total	47	92278844.46			
100 SW	Model	15	589.0374703	39.2691647	31.03	<0.0001
	Error	32	40.5000000	1.2656250		
	Corrected total	47	629.5374703			
PW	Model	15	3471502.789	231433.519	90.55	<0.0001
	Error	32	81790.137	2555.942		
	Corrected total	47	3553292.926			
DFT	Model	15	289.3125000	19.2875000	19.29	<0.0001
	Error	32	32.0000000	1.0000000		
	Corrected total	47	321.3125000			
PLN	Model	15	152.0382813	10.1358854	95.63	<0.0001
	Error	32	3.3916667	0.1059896		
	Corrected total	47	155.4299479			
NPP	Model	15	30872.11979	2058.14132	4390.70	<0.0001
	Error	32	15.00000	0.46875		
	Corrected total	47	30887.11979			
DTI	Model	15	23.25000000	1.55000000	2.07	0.0416
	Error	32	24.00000000	0.75000000		
	Corrected total	47	47.25000000			
NSP	Model	15	558.0781250	37.2052083	336.16	<0.0001
	Error	32	3.5416667	0.1106771		
	Corrected total	47	561.6197917			

Where, GY = grain yield, SW = Seed weight, PW = Pod width, DFT = Days to 50% flowering, PLN = Pod length, NPP = number of pods per plant, DTI = Drought tolerance index and NSP = number of seeds per pod

Appendix 21: Analysis of Variance for General Combining Ability and Specific Combining Ability using Griffing's Method 2

Variable	Source	MS	p-value
Grain yield	Parent 1	286264.54	<0.0001
	Parent 2	326058.88	<0.0001
	Parent1*parent2	74512.23	<0.0001
100 Seed weight	Parent 1	66.06	<0.0001
	Parent 2	67.7	<0.0001
	Parent1*parent2	5.632	0.0034
Pod width	Parent 1	401191.378	<0.0001
	Parent 2	337792.02	<0.0001
	Parent1*parent2	12230.5148	<0.0001
Days to 50% flowering	Parent 1	81.37	<0.0001
	Parent 2	84.98	<0.0001
	Parent1*parent2	17.62	<0.0001
Pod length	Parent 1	48.4	<0.0001

	Parent 2	56.95	<0.0001
	Parent1*parent2	9.15	<0.0001
Number of pods per plant	Parent 1	24522.37	<0.0001
	Parent 2	25943.43	<0.0001
	Parent1*parent2	3284.89	<0.0001
Drought tolerance index	Parent 1	35.79	0.00601
	Parent 2	36.38	0.00196
	Parent1*parent2	6.98	0.0476
Number of seeds per pod	Parent 1	225.23	<0.0001
	Parent 2	220.98	<0.0001
	Parent1*parent2	34.08	<0.0001