

BREEDING FOR GRAIN QUALITY TRAITS IN COWPEA [*VIGNA UNGUICULATA* (L)  
WALP]

**BY**

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## ABSTRACT

This study was conducted in the Northern guinea and Sudan savannas of Zaria and Kano, Nigeria to: (i) identify cowpea production constraints and assess farmer and consumer perceptions and preferences on grain quality traits in cowpea, (ii) assess the variability of grain nutritional values (protein, iron and zinc contents) of cowpea accessions from Africa and other parts of the world, (iii) determine the mode of inheritance of iron and zinc contents in cowpea grains and (iv) determine the relationship between zinc concentration and yield components. Farmers pointed out inadequate improved cowpea seed at planting time (rainy season) and lack of resistant varieties to pod sucking and pod boring insects as the major constraints to cowpea production in the areas. Farmers preferred a cowpea variety that combines high yield with rough texture, white or brown colour. Consumers' grain quality preferences were consistent with those of farmers, except for women who prioritized cooking time and oil consumption. Significant genetic variations were observed in: zinc content (1.01 to 329.15 mg/kg); iron content (10.01 to 386.3 mg/kg); protein content (1.72 to 29.93%) and other physicochemical properties of grain. Many of these variations can be generated by conventional breeding methods to address the nutritional needs in developing countries. In addition, 3 accessions: TVu-13088, TVu-13495 and TVu-9725 that combined the largest number of desirable nutritional attributes were identified which can be nominated for anti-nutritional factor testing prior to recommendation for infant diets formulation and other use. For nutrient enhancement, five accessions each were identified for protein, zinc and iron contents. Genetic diversity of 169 cowpea accessions using 119 SNP markers clustered the accessions into two main groups on genetic distances (0.00 to 0.212) with small genetic differentiation (0.26 to 0.45) between African and USA cowpea accessions. This indicates that the entire genetic diversity in the African germplasm might already have spread over cowpea-growing regions in the world as a whole, though not completely within any single region. The

Polymorphism Information Content (PIC) values ranged from 0.2366 (7344\_500 SNP) to 0.427 in two SNP markers (4749\_1972 and 14929\_258). Weak negative correlation existed between iron content and fat ( $r = -0.18$ ,  $P < 0.007$ ), iron content and carbohydrate ( $r = -0.18$ ,  $P < 0.007$ ) but iron content was positively correlated with protein ( $r = 0.26$ ,  $P < 0.001$ ) content. Fat content was negatively correlated with ash content ( $r = -0.13$ ,  $P < 0.05$ ) and protein content ( $r = -0.85$ ,  $P < 0.001$ ). Ash content correlated positively with protein ( $r = 0.14$ ,  $P < 0.05$ ) and negatively with carbohydrate ( $r = -0.23$ ,  $P < 0.001$ ). Protein was negatively correlated with carbohydrate ( $r = -0.29$ ,  $P < 0.01$ ). Zinc concentration showed weak negative significant ( $r = 0.03$ ,  $P < 0.05$ ) correlation with number of pods per plant. No significant correlation was observed between zinc concentration and grain weight. Similarly negative correlation was observed between zinc content and number of pod per plant implying an increase in zinc content may lead to decrease in pod yield. Generation mean analysis of the six basic generations was significant ( $P < 0.001$ ) among the generations. The significant variation of generation mean performance with transgressive segregants among the progenies and two fold increases in mean zinc concentration of  $F_2$  population over the high zinc content parent ( $82.54 \text{ mg/kg} > 37.70 \text{ mg/kg}$ ), implies that cowpea can be enhanced with essential micronutrients using conventional approach. Additive [a] and additive by dominance [ad] model explained the inheritance of iron and zinc content in cowpea grain. An indication of maternal effect was observed in iron content inheritance indicating non suitability to make selection in early generation of selfing. Both seed weight and plant height are predominantly under complementary gene actions in this study, suggesting the possibility of considerable amount of heterosis for seed weight and fodder yield which are the determinants for choosing cowpea varieties,. The study revealed that: Variability of grain nutritional traits (Zn, Fe and Protein) among cowpea was observed. Genetic pattern of iron and zinc content elucidated and Possibility of enhancement in cowpea using conventional approach is realized.

**DEDICATION**

To the entire family of late Alhaji Salihu Nuhu Umar Bagobiri, Tudun wada Dankadai.



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## LIST OF ABBREVIATIONS

AATF	African Agricultural Technology Foundation
AAS	Atomic absorption spectrophotometry
AOAC	Association of Analytical Chemist
ASCN	Agricultural Seed council of Nigeria
CIM	Composite interval mapping
CHO	Carbohydrates
DAP	Days after planting
DNA	Deoxyribonucleic acid
GCP	Generation Challenge Program
IAR	Institute for Agricultural Research
IBPGR	International Board of Plant Genetic Resources
IITA	International Institute of Tropical Agriculture
IM	Interval mapping
NGICA	Network for Genetic Improvement of cowpea in Africa
NACGRAB	National Center for Genetics Resources and Biotechnology
NPC	National Population Commission
SNP	Single Nucleotide Polymorphism
PIC	Polymorphism Information Content
QTL	Quantitative trait loci
TVu	Tropical <i>Vigna unguiculata</i>

## CHAPTER ONE

### 1 GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata* Walp (L.) is of vital importance to the livelihood of millions of people in West and Central Africa. It provides nutritious grain and an inexpensive source of protein for both rural and urban consumers (Joseph *et al.*, 2011). In addition, cowpea contributes to the sustainability of cropping systems and soil fertility improvements in marginal lands by providing ground cover and plant residues, fixing nitrogen, and suppressing weeds (Ajeigbe *et al.*, 2010)

Cowpea is grown in about 14.5 million hectares globally, with over 6.5 million metric tons (Fatokun *et al.*, 2012). Africa alone accounts for about 83% of the world production, with Nigeria being the world's largest producer (45.76%), followed by Niger (15%), Brazil (12%), and 5 % for Burkina Faso (Fatokun *et al.*, 2012). Cowpea is grown in all parts of Nigeria, though large production is concentrated in the northern region (Akande & Balogun, 2009). Cowpea production has been increasing at an average rate of 5% annually, with 3.5% annual growth in area and 1.5% growth in yields. Fatokun *et al.* (2012) reported that area under cowpea cultivation increased from less than 10% to nearly 20% between 1990 and 2007. The increase in production has been largely due to the expansion of areas under cultivation. In spite of this, a huge gap still remains between the potential yield and actual yield due to some production constraints such as pest and diseases, which is estimated to be account for more than 80% of yield losses in cowpea production (Fatokun, 2009)

The major driving factor for cowpea production, as for most market crops, is to supply rural surpluses to the urban areas where there is demand. Kormawa *et al.* (2000) reported that about 72% of Nigerian urban households consumed cowpea more than any other grain legume,

such as Groundnut, Soybean and Bambara groundnut. On average, a household purchased and consumed 5 kg of cowpea grain per week, spending about two hundred and forty (N240= US\$1.5) naira. Nigeria produces 2.1 million tons of cowpea on 7 million hectares and consumes around 2.7 million tons. This creates a national deficit of over 500,000 tons which is made up through imports of US \$125 million (Ishiyaku, 2013)

Consumers' preferences for grain quality traits vary from region to region. For example red to black colour cowpea with various range of grain texture is preferred in Latin America, particularly Cuba and parts of the Caribbean (IITA, 1983). In West Africa, the most preferred types of cowpeas are large sizes, white or brown grains with rough grain coat. Similarly, medium grain sizes with brown or red grains with smooth grain coat are preferred in East Africa (Langyintuo *et al.*, 2004). In Nigeria, rough grain is most preferred due to its ease of dehulling and greater swelling capacity, which is used for processed food such as "akara" and "moin moin" (Dovlo *et al.*, 1976). Similarly, white rough, large grain and brown rough, large grain were mostly preferred in the Northern and Southern part of the country. However, Hussain *et al.* (1984) reported that the choice of cowpea varieties by Nigerian women is guided predominantly by the cooking time, swelling capacity, taste and colour. Good appearance, taste, mouth feel, different flavor and nutritional qualities are also important components of grain legume quality (Negri *et al.*, 2001). Therefore, an efficient method to evaluate these attributes, in addition to physical characteristics of cowpea grain, would be useful for plant breeders to improve these aspects of grain quality.

Cowpea is a single crop species whose varietal requirements in terms of plant type, grain type, cropping system, maturity and use pattern are extremely diverse from region to region, thus making breeding programs for cowpea more complex than for most other crops (Singh *et al.*,

1997). Consequently these varying preferences show the need to develop varieties with different characteristics, as no single variety can be suitable for all regions. Therefore, there is need to combine both consumers and farmer's perceptions on cowpea grain quality traits, their needs and preferences into this research. Engaging end-users would help to understand their needs and preferences, which will enable us identify the most appropriate cowpea varieties for improvement. To achieve that, a Participatory rural appraisal (PRA) needs to be conducted to understand and include end-users' needs in the breeding objectives.

Iron and zinc deficiency are among the most common and widespread micronutrient deficiency that affects more than half of the human population (White & Broadley, 2009). Asia and Africa are the most affected countries with iron and zinc deficiency (Gómez-Galera *et al.*, 2010). A collaborative study by USAID (2006) showed the prevalence of stunted, wasted and underweight children suffering from iron and zinc deficiency in the dry and moist savannas of Nigeria. This calls for the need to identify cowpea varieties with high iron and zinc, for setting breeding program to address this problem.

Selection for nutritional quality traits such as iron and zinc using conventional breeding methods can be very difficult due to lack of discrete phenotypic classes in the progeny, subjective testing methods requiring taste panels and costly biochemical evaluation procedures. (Asante, 2012) Similarly, environmental influences in the expression of such quality traits further complicate the selection processes. Therefore, complementing the conventional breeding approaches with application of molecular markers will facilitate the selection of grain quality traits. This is because markers are less subjective, have no environmental effects on the phenotype and accelerate the identification and selection of several genomic regions involved in the

expression of complex traits to ‘assemble’ the best-performing genotype within a single, or across related, populations (Ribaut *et al.*, 2010)

Studies on variability in the level of iron and zinc have been reported on cowpea germplasm (Asante *et al.*, 2009; Pereira *et al.*, 2014). Nevertheless, there is no report on the genetic studies of iron and zinc in cowpea. There is therefore, an urgent need to characterize the cowpea germplasm available to improve grain quality traits. This will facilitate the selection of the appropriate genotypes for breeding. For example, the popular and most recent varieties: Sampea 1-14 and landraces like: Kanannado and Biyu local have appreciable physical grain quality attributes: white, brown and rough texture and large grained. Such accessions will be suitable recurrent parent for introgression.

However, the most sustainable way and readily accessible to common man is the crop that has been fortified with those essential elements. This is because fortified crops do not require recurring expenditure, robust distribution system and very careful implementation, as there is no fear of overdose, which can be harmful (Nestel *et al.*, 2006). Identifying cowpea germplasm with high micronutrient contents that will be amenable to introgression of iron and zinc without affecting their yield will pave way to fortifying this important crop. It is therefore, important to screen a number of cowpea accessions and select promising parents with good grain quality traits particularly those with higher iron, zinc and protein contents. The grain quality traits in cowpea are many, however, this study focused on grain morphological traits and nutritional quality traits, particularly, protein, iron and zinc. Each of these qualities comprises many attributes whose standards are determined by both physicochemical properties and consumers’ confirmation.

The main objective of this study was to identify and select cowpea varieties with good grain quality traits for future cowpea breeding program. The specific objectives were to:

1. Determine cowpea production constraints and grain quality traits that are mostly preferred by farmers and consumers of Sudano-Sahelian zone of Nigeria;
2. Assess the variability of grain protein content, iron concentration and zinc concentration in cowpea accessions from Africa and other parts of the world;
3. Determine the inheritance pattern of iron and zinc concentration in cowpea grains.
4. Determine the relationship between zinc concentration and yield components

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Cowpea [*Vigna unguiculata* L. (Walp)]

Cowpea is the most widely grown and most economically important indigenous African grain legume that provides an important source of protein to consumers (Langyintuo *et al.*, 2003). Xu *et al.* (2011), and Huynh *et al.* (2013) reported that cowpea is a diploid crop with 11 chromosomes ( $2n=2x=22$ ) and 630 Mb genome size. Cowpea belongs to the genus *Vigna*, section *Catiang*, species *unguiculata*. It comprises four subspecies namely: *unguiculata*, *stenophylla*, *dekindtiana* and *tenuis* (Ng & Marechal, 1985). The subspecies *unguiculata* is the only cultivated cowpea, while the other three are wild relatives. The cultivated cowpea is grouped under subspecies *unguiculata*, which is further subdivided into four cultivar groups namely; cultivar group (cv-gr) *unguiculata*, *biflora*, *sesquipedalis* and *testilis* (Ng. & Marechal, 1985). The cultivar group *unguiculata* is the most diverse of the four and is widely grown in Africa, Asia and Latin America (Ehlers *et al.*, 2002). The crop is also an important staple in many parts of the United States, South and Central America, the Caribbean, India, and Australia (Siemuri *et al.*, 2011). Several studies have shown that cowpea was probably domesticated by African farmers (Faris, 1965) and assumed to have evolved in Africa, because wild cowpeas only exist in Africa and Madagascar (Steele, 1976). Although the center of diversity of wild *Vigna* species is in southeastern Africa, West Africa is a major center of diversity of cultivated cowpea (Padulosi & Ng, 1997).

Evidence from molecular marker studies have shown that cowpea domestication occurred in northeastern Africa based on studies of amplified fragment length polymorphism (AFLP) markers by Coulibaly & Lowenberg-De Boer (2002) and could have occurred simultaneously with the domestication of sorghum and pearl millet in the third millennium B.C. (Steele, 1976).

The wild cowpea, *V. unguiculata* ssp. *unguiculata* var. *spontanea* is the likely progenitor of cultivated cowpea (Ba *et al.*, 2004). However, the most recent studies have indicated that the precise center of origin of cowpea is yet to be known (Boukar *et al.*, 2010).

## 2.2 Cowpea grain quality traits

Long-term cowpea genetic improvement through germplasm exchange have focused on developing high yielding varieties with tolerance to some biotic and abiotic constraints (Wang *et al.*, 2006; Padi, 2007). These resulted in the development of cowpea varieties that are resistant to *Striga* spp, some insects and diseases (Singh & Mligo, 2007). Crude protein content and other mineral element analysis in cowpea were reported by many authors (Ajeigbe *et al.*, 2008; Anele *et al.*, 2011), with scanty literature on the variability of cowpea grains for iron and zinc concentrations. The need to breed for cowpea consumer preference traits has become necessary because:

1. There is the need to improve children and women's diets, thereby, improving food security at large. Presently, some state Governments in Nigeria are providing dietary supplements to school children and Federal Government approves formulation of sorghum-soya in feeding school children. Almost all boarding schools are fed with cowpea diets at least three times a week.
2. The earlier developed and released cowpea varieties in Nigeria have low iron and zinc content.
3. Cowpea farmers and processors, have their preferences for grain quality traits, which are market driven.
4. Cowpea consumers in Africa are willing to pay premium price for important grain quality traits (Langyintuo *et al.*, 2004)

The morphological appearance of cowpea grains especially colour, texture and size are the common determinants of cowpea marketability in Western and Eastern Africa (USAID- Nigeria, 2008). Consumer tastes and preferences are reflected in the market through price discounts and premiums that consumers pay for visible grain characteristics. In some cases, these visible indicators are proxies for some biochemical characteristic, such as cooking time and protein (Ng *et al.*, 2011; Dugje *et al.*, 2009) as well as iron and zinc contents.

### **2.2.1 Grain size**

Determining grain size in cowpea has historically relied on measuring the weight of 100 grains, taken at random. However, this method cannot distinguish different grain shape parameters such as grain thickness or grain plumpness from grain diameter. Poersch *et al.* (2013) noted that the traditional method of grain sizing using graded sieves can be just as effective for determining grain dimensions. This, however, still means that phenotyping grain size and especially grain plumpness can be time consuming and tedious in a breeding program. Selection of these traits using marker-assisted selection (MAS) could be a valuable, time saving venture if markers linked to the genes controlling these dimensions could be identified. Grain size in cowpeas is important because it directly influences productivity and, together with colour standards, determine grain quality for commercialization (Lopes *et al.*, 2003). Variation in grain size ranges between less than 10 g per 100 grains and approximately 30 g (Ehlers & Hall, 1997)

Consumers in West Africa prefer large cowpea grain. They show preference for a grain standard from medium to large (15 to 25 g) with a minimum tolerance limit that varies from state to state in Nigeria. It is, therefore, understandable that knowledge about the genetic factors responsible for inheritance of this trait is essential for breeding programs which present great genetic variability in germplasm collections of the species.

### 2.2.2 Grain coat colour

Physical characteristics are directly related to the way cowpeas are used in food preparation. For example, cowpea grain coat and eye colours are the major factors, limiting utilization of cowpeas as food. This is important, especially when the intended use for the grains requires decortications (removal of outer layer) to remove flecks. Poor milling and winnowing may still leave some flecks for which consumers have low acceptances (Langyintuo *et al.*, 2004). In Cameroon, ‘kosai’ (cowpea fritters) without black flecks are very popular while in Ghana black flecks have little impact on the use of cowpeas for “Tubani” (steamed cowpea paste) or a mixture of cowpea with rice or “gari” (cassava chips). Preferences for different culinary roles of cowpea in the different region or countries can have impacts on the preferences for cowpea grain characteristics. Therefore, knowledge of consumer’s preferences for grain characteristics is essential to plant breeders for setting priority objectives in their breeding program.

### 2.2.3 Grain coat texture

For wide adoption of cowpea in West Africa, new cowpea varieties must have features desired by consumers as well as farmers. Four types of grain coat texture have been identified in cowpea: smooth, rough, wrinkle and loose (Fatokun *et al.*, 2012). Cowpeas with large white or brown grains with rough grain coat are preferred throughout West Africa, whereas in East Africa they prefer medium size, brown or red grains with smooth grain coat. But in some Latin American countries, particularly Cuba and part of Caribbean, black colour with various categories of grain coat texture are preferred (Obiegbuna *et al.*, 2006). A rough grain coat is preferred in West and Central Africa, since it permits easy removal of the grain coat which is essential for indigenous food preparations (Singh & Ishiyaku, 2000). The preference for cowpea grain with rough grain coat in Nigeria is due to their ease of dehulling and greater expansion capacity. Such

grains are used for cowpea diets such as “akara” and “moin moin” preparation (Dovlo *et al.*, 1976).

## **2.3 Inheritance of Grain Quality Traits**

### **2.3.1 Inheritance of Grain Size**

The genetic control of the grain size is complex due to environmental influence and number of genes involved. (Drabo *et al.*, 1984) reported that seed weight was quantitatively inherited and small seed was partially dominant to large seed size. The gene action governing the inheritance of seed weight was predominantly additive but dominance and additive x additive epistatic effects were also significant. Five and eight genes were reported to control seed weight in cowpea by Lopes *et al.* (2003) and (Egbadzor *et al.*, 2013), respectively.

### **2.3.2 Inheritance of grain coat colour**

Grain coat colour is considered as one of the useful phenotypic markers in cowpea breeding due to its stable expression and convenience for observation (Xu *et al.*, 2011). Various grain coat colours such as white, cream, brown, maroon, black, red, blue and buff have been reported in cowpea (Asante, 1991; Mustapha, 2009). Similarly, Oluwatosin (2000) reported that a gene controlling red grain coat is monogenically dominant to that of cream colour. Two dominant genes which exhibit complementary epistasis were reported in bi-parental crosses consisting of Black x Cream parents and Brown x Cream parents (Oluwatosin, 2000). A general colour factor C is considered responsible for grain coat colour and its absence results in white grains (Spillman, 1912). In some genotypes, the C factor in combination with other genes conditions certain colours of grain coat. Spillman & Sando (1930) proposed that six major genes control grain coat colour in cowpea. Mustapha (2009) reported that grain coat colour is controlled by many genes, though

some of the genes may be allelic. Recently Egbadzor *et al.* (2012) reported a link between flower colour, immature pod pigmentation and grain colour in crosses between some Ghanaian cowpea germplasm. Grain coat colour is one of the major cowpea consumers' determinants in Nigeria. It is, therefore, important to consider grain colour when selecting parental lines and among the segregating population.

### **2.3.3 Inheritance of grain coat texture**

Most of the genetic studies on the inheritance of grain coat texture in cowpea focused mainly on smooth and rough textures. A smooth testa was found to be dominant over rough testa (Drabo *et al.*, 1984). Rough testa is controlled by at least two recessive genes (Franckowiak, 1973). Singh and Ishiyaku (2000) studied the segregation pattern of grain coat texture in biparental crosses involving smooth and rough parents and reported fitting ratios of smooth and rough close to 1:1 and 3:1 in the backcross involving rough parent ( $BC_1F_1$ ) and ( $BC_1F_2$ ) plants respectively. This indicated that rough grain coat is controlled by a recessive gene. Grain texture is an important morphological trait that guides consumers in selecting cowpea for processing. Therefore, incorporating consumer preferred grain texture in breeding for grain quality traits will ease adoption of new variety in Northern Nigeria.

### **2.3.4 Inheritance of protein content**

Proteins are major components of grain legumes and their nutritional and functional properties depend on the nature of soluble fractions (Vasconcelos *et al.*, 2010). Cowpea is widely adapted and nutritious grain legume constitutes one of the main sources of plant protein in Nigeria. Dry grains for human consumption are the principal product of the plant, but leaves, fresh peas, and fresh green pods are consumed by many peasants who do not have access to broadly based diet (Ehlers & Hall, 2002). Understanding the genetic mechanism for the

expression of protein content and genetic control of cowpea grain is therefore very essential for the improvement of that trait. Genetic variation for cowpea grain protein contents has been reported by Gupta *et al.* (2010) and Tchiagam *et al.* (2011a) Significant difference among cowpea protein content has also been reported by Asante *et al.* (1991); Ajeigbé *et al.* (2008) and Tchiagam *et al.* (2011b). Grain protein composition is genetically controlled but affected by environmental factors particularly nitrogen and sulphur availability (Tabe *et al.*, 2000).

### **2.3.5 Inheritance of iron and zinc contents**

Narrow variations among cowpea lines of up to 60.3 mg/kg and 40.7 mg/kg for iron and zinc were reported by Boukar *et al.* (2010). However, significant variations in grain zinc and iron concentrations were reported in different edible crops. For example 4 fold differences in grains' iron and zinc content was reported in aromatic rice compared to popular cultivars (Cakmak *et al.*, 1999; Welch & Graham, 1999). Significant genetic variation of micronutrient have been reported in maize (Bänziger & Long, 2000) with range of 9.6 to 63.2 mg/kg for grain Fe and 12.9 to 57.6 mg/kg of grain zinc. Similar variation was also reported in wheat grain with 3-4 fold increased iron and zinc content in wild type than the popular cultivars (Chhuneja *et al.*, 2006; Rawat *et al.*, 2009).

### **2.4 Association between Nutritional Quality traits**

Association studies between protein content and other physicochemical properties of cowpea was reported by many authors: For example Hussain & Basahy (1998) from Saudi Arabia, showed that ash content was positively correlated with protein content and negatively correlated with carbohydrate content. Hundred (100) seed weight was significantly negatively correlated ( $P < 0.05$ ) with protein (PC) ( $r = -0.72$ ) and iron content ( $r = -0.61$ ) (Moura *et al.*, 2012). A negative correlation between protein content and 100 seed weight was also reported by

Asante *et al.* (2004). Fat content was negatively correlated with protein. Protein content showed very high negative correlation with carbohydrate content. This indicates that selection for high protein content will result in decrease in carbohydrate content and ash content, which will make the improved line to be nutritionally superior. The total fat content in cowpea is very low, its reduction due to increase in protein will not make much difference in overall nutritional quality of the varieties (Ajeigbe *et al.*, 2008).

## **2.5 Physiological mechanisms of iron and zinc uptake**

Accumulation of minerals in grains is a complex phenomenon, which is most likely controlled by a number of genes. The movement of mineral elements from the soil to grains involves their mobilization from soils, uptake by roots, translocation to the shoot, redistribution within the plant and deposition in grains (Grusak & DellaPenna, 1999; White & Broadley, 2009). However, the genetic basis of most of these processes are unknown (Ding *et al.*, 2010). Various mechanisms for soil mineral uptake and distribution in plants have been reported to be the result of tightly controlled homeostatic mechanisms that regulate metal uptake and distribution in plants, allowing adequate but nontoxic levels of these nutrients to accumulate in plant tissues.

It has been reported that the embryo and the aleurone layer for cereal are the major depositories for grain zinc (Mazzolini *et al.*, 1985; and Choi *et al.*, 2007), while Ozturk *et al.* (2006), showed that zinc is present in the outer part of the endosperm, which contain high amounts of protein.

## 2.6 Marker Assisted Selection (MAS) for cowpea improvement

### 2.6.1 Current trends in the use of marker technologies for cowpea breeding

Molecular genetic tools and genomic resources have been developed to augment conventional cowpea breeding methods. (Muchero *et al.*, 2009a) and (Lucas *et al.*, 2011) reported a significant improvement of cowpea in the United States, India, Brazil, and many countries in Africa and Asia. These integrated genomic resources include a 1536 SNP genotyping platform, an EST-derived SNP consensus genetic map, known syntenic relationships between cowpea, *Medicago truncatula*, *Glycine max*, *Arabidopsis thaliana*, and a cowpea EST sequence collection stored in HarvEST: Cowpea database (<http://harvest.ucr.edu>). A cowpea physical map has been partially anchored to the cowpea consensus genetic map using the same SNP markers (UCR cowpea group, unpublished) and is available on <http://phymap.ucdavis.edu/cowpea>. In addition, about 500 diverse cowpea accessions have been SNP-genotyped (UCR cowpea group) and a first draft of the cowpea genome, vs.0.02, has been assembled ([www.harvest-blast.org](http://www.harvest-blast.org)). These resources enabled dissection of underlying genetic components of target agronomic traits using Quantitative Trait Locus (QTL) analysis and Association Mapping. The identified and confirmed QTLs facilitated cultivar improvement using marker-assisted breeding.

The most detailed genetic linkage map of cowpea spans a total of 2670 cM with an average of 6.43 cM between markers was reported by Ouédraogo *et al.* (2002). This cowpea linkage map revealed the importance of associating between phenotypic and genotypic data leading to understanding the biological determinants of quantitative phenotypic variation. Molecular geneticists have identified genes controlled by one or a few loci with large effects. This is particularly true in cowpea, where genetic analyses have identified the biochemical basis of many important phenotypes such as drought stress parameters (Muchero *et al.*, 2009b). However,

understanding complex trait variation is sometimes difficult, due to the nature of genetic architecture of such traits, which may involve many loci of small effect that may interact with each other or with environment (Bernardo, 2008; Collard & Mackill, 2008). Genomic Selection (GS) technologies were recently introduced and provided an economically feasible way to survey genetic variation with a resolution that is now limited more by the linkage disequilibrium (LD) in a particular mapping population than by marker density. This phenomenon has motivated the assembly of large panels of genetic diversity as well as the creation of large inter-mated populations to manipulate LD and facilitate the association of genotype with phenotype (Lie-Zhao *et al.*, 2006). Large and diverse populations increased the recombination frequency and the frequency of rare alleles in order to enhance the power to infer the effects of individual loci.

### **2.6.2 Quantitative trait loci mapping**

A quantitative trait loci (QTL) was proposed by Sax (1923), first coined by (Geldermann, 1975) and later used by many researchers to detect and locate QTL in segregating populations in crop and animal species. A QTL is defined as a region of a genome that is associated with an effect on quantitative trait. A QTL can be a single gene, or it may be a cluster of linked genes that affect the trait. QTL mapping had been reported in most crop plants for diverse traits including: yield, quality, disease and insect resistance, abiotic stresses like drought and other environmental constraints. Previous studies of grain-related traits in several crops have revealed loci controlling more than one related trait. In lentil for example, a flowering time locus was shown to be linked with the grain coat pattern locus (Slattery *et al.*, 1982; Zhi-wen *et al.*, 2005). Flowering times influence grain size in different crops (Gupta *et al.*, 2006). Similarly, pre-anthesis changes in vegetative organs can affect the amount of assimilates that are partitioned to the grain while they are developing. Also post-anthesis processes can affect the time for maturation or grain filling,

which could change the grain size (Moura *et al.*, 2012). Loci controlling flowering time or other flower morphology traits have also been associated with grain weight or grain dimension loci in model legume crops (Burgess *et al.*, 2007; Burgess *et al.*, 2007; Greenup *et al.*, 2009).

The success of modern QTL mapping depends on the availability of dense DNA marker maps for the organism involved. In recent years, comprehensive DNA marker maps have been developed for many crops (Orjuela *et al.*, 2010; Ouedraogo *et al.*, 2012). This makes the identification of QTL to be feasible and successful in crop plants, using an appropriate mapping population (MP). Such population mostly involved bi-parental progenies such as backcrosses (BC), double haploid lines (DH),  $F_2$ , or recombinant inbred lines (RILs) Crepieux *et al.* (2004); Crepieux *et al.* (2005). The QTL mapping literature has shown that a mapping population of  $N = 100$ – $150$  progenies is enough to identify QTL. Such population could be recombinant inbred lines (RIL),  $F_2$  or  $F_{2:3}$  or backcross population derived from two inbred lines coupled with reasonably good phenotypic data and genotypic data with markers spaced about 10 to 15 cM apart, are essential requirement for QTL identification (Flint-Garcia *et al.*, 2003). However, Muranty (1996) suggested the use of progenies from several parents, to achieve a high probability of obtaining more than one allele at a putative QTL and also to have a more representative estimate of the variance accounted for by a QTL.

### **2.6.3 Concept and principles of QTL**

Quantitative characters are common features of natural variation in populations of all eukaryotes, including crop plants. These traits provided a conceptual base for partitioning the total phenotypic variance in terms of additive, dominance and epistatic effects. The basic principle of determining whether a QTL is linked to a marker is to partition the mapping population into different genotypic classes based on genotypes at marker locus, and apply correlative statistics to

determine whether individuals of one genotype differ significantly with individuals of other genotype with respect to trait of interest. According to Moose & Mumm (2008), the success in using information about QTLs to increase genetic gain depends greatly on the suitable method used to determine the magnitude of QTL effects, precise estimation of QTL positions, stability of QTL effects across multiple environments, and whether QTLs are robust across relevant breeding germplasm. Prediction of QTL positions is enhanced by further fine mapping, which facilitates testing QTL effects and breeding values in additional populations. Methods for simultaneous detection and manipulation of QTL in breeding programs would thus enhance the applicability of MAS.

#### **2.6.4 Methods used in QTL detection**

The study of quantitative traits has involved statistical techniques based on means, variances and covariance of relatives. The first models described for QTL detection with various progeny types, are single marker models (Beckmann & Soller, 1990; Srivastava *et al.*, 2005). In these models, information from the phenotypic means and variances of genotypic classes at a single marker is used to test marker linked-effects and to estimate the putative QTL parameters. The most used method is based on analysis of variance (ANOVA) and considers contrasts among marker class means to test and estimate QTL effects (Tanksley *et al.*, 1992). This approach has many shortcomings; it estimates effects of the QTL which are biased by the recombination between marker and QTL and provides no information about the likely position of the QTL. Weller & Soller (2004) described a method using approximate maximum likelihood analysis and moment's methods to estimate QTL parameters and the recombination fraction between marker and QTL.

Interval mapping (IM) described by Lander and Botstein (1989) is an approach investigating the location of QTL between intervals of flanking markers by means of likelihood analysis. Furthermore, Hu & Xu (2009) compared the QTL detection powers obtained with random-effect models and fixed effects and found similar values for individual family sizes as low as 25 individuals. Statistical methods for the QTL analysis of bi-parental populations underwent successive improvements through the advent of interval mapping (Lander & Botstein, 1989) and its linearization, composite interval mapping and multiple-trait QTL mapping (Kang & Priyadarshan, 2007; Ding *et al.*, 2010).

## CHAPTER THREE

### 3.0 APPRAISAL OF FARMER AND CONSUMER PREFERENCES FOR COWPEA IN THE SUDAN SAVANNA ZONE OF NIGERIA

#### 3.1 Introduction

Adoption of existing and newly generated technologies continues to be low, despite the time and resources committed by researchers in an effort to alleviate this problem (Chambers *et al.*, 1989). Adoption of improved cowpea is still low in Nigeria (Takeshima *et al.*, 2014), even though the National and International Research Institutes developed and released various cowpea varieties. Farmers are still growing cowpea landraces such as “Kanannado” “Danmisra” and “Biyu local” (Kormawa *et al.*, 2004). This has been attributed in part to low farmer involvement in the whole process of variety or technology development. The constraints to the distribution of improved grains are on both the supply and demand sides. Takeshima *et al.* (2014) reported that one less-studied issue is the farmers and consumers’ preference for obtaining grains at planting time, when grain prices tend to be higher. While the formal grain sector is often constrained by the capacity for distributing improved grains to farmers, knowing whether farmer and consumer are willing to pay premium prices at planting time and during processing can provide potentially useful information to consider their preferences when setting breeding objectives. Knowledge of the range of plant, grain and processing traits are valuable for crop improvement programs and good market signals for the farmers (Langyintuo *et al.*, 2003)

The demand for improved cowpea varieties is likely to increase if, varieties are designed to include producers and consumers’ preferred traits, in addition to farmers’ traits, improved grains have provided 50% of the productivity gains in agriculture. The other 50% has come from

improvement in management, including timeliness, best use of fertilizer, crop protection measures and equipment (Ayinde, 2005; USAID- Nigeria, 2008).

Participatory Rural Appraisal (PRA) is one of the rapid and most important tools of identifying farmers and consumers' preferences. It reveals a number of traits that are not prioritized by breeders. Success stories of farmer and consumers' participatory breeding have been reported. These include: the consumers' preferences for sorghum-based clear beer in Tanzania (Makindara *et al.*, 2013), pearl millet breeding in Mali (Weltzien *et al.*, 1998) and nutritional quality and consumer acceptability of cowpea grain was recently reported by Ojiako & Kayode, 2014).

High-yielding cowpea varieties with desired grain nutritional characteristics could make a great impact on the Nigerian cowpea industry because farmers are likely to get good market for their product, while consumers will be willing to pay premium price for grain quality traits (Takeshima *et al.*, 2014). This would stimulate farmers to substantially increase domestic cowpea production. It is therefore, essential for Nigerian cowpea breeders to incorporate the preferences of farmers and consumers in their breeding programs. However, information on the general criteria used by farmers for selecting cowpea varieties for cultivation is lacking. Also, farmers' perceptions on grain quality in relation to consumer demand needs to be considered.

The objectives of this study were to:

1. identify farmers' production constraints and their perceptions on cowpea grain quality traits;
2. determine farmers and consumers' preferences and selection criteria for selecting cowpea varieties in Sudano-Sahelian zone of Nigeria;

## 3.2 Materials and Methods

### 3.2.1 Description of the study area

The study was conducted in the Sudan savanna and Sudano- sahelian transition zone of Kano state, Nigeria. Kano has an average annual rainfall between 500 mm to 1200 mm per annum, an average of 276 people /km<sup>2</sup> with a total of 16 million inhabitants (NPC and ICF Macro, 2009). The average household size in Kano ranges between 8 and 10 members with average income of 4000 Naira (at N140 per US dollar \$1). The major ethnic groups are Hausa and Fulani (Maiangwa & Ogungbile, 2008). Kano lies in the Sudan savanna, semiarid zone of Nigeria, on 11°34' N and 8°44' E latitude and longitude respectively. The Sudan ecology is characterized by a growing period of about 100 to 150 days. Annual rainfall, between 500 and 1000 mm, is erratic and restricted to 4 months (Inaizumi *et al.*, 1999) . Three local government areas were selected for study on the cowpea production constraints. Two sub-communities each from Bunkure (11°41'41.55"N and 8°32'17.81"E), Wudil (11°49'11.15"N and 8°54'11.41"E) and Bichi (12°11'24.63"N and 8°18'45.73"E) (Table 3.1), were selected because they are among the major cowpea growing communities in Kano State.

Cowpea consumers' preferences were assessed in Kano metropolis, which comprised Municipal, Tarauni districts and "Dawanau" market. The commercial city of Kano has the largest immigrants' population from all parts of the country and other African countries. Similarly, the largest grain (Dawanau) market in West African region is located in the city. Therefore, consumer preferences for cowpea in Kano might be representative of the whole country and all similar zones across West Africa.

### **3.2.2 Site mapping and selection**

Transect walk was conducted with 2 men and 2 women in each district of the study areas. During the exercise, cowpea based foods processing and selling points were identified. Problems and opportunities associated with cowpea production, processing and consumption were also identified. The communities were selected based on the cowpea growing and processing or selling points observed during transect walk.

### **3.2.3 Sampling Procedure**

The respondents are presented in Table 3.1 below according to their village areas, gender and whether producers or consumers. Three major cowpea-growing communities: Bunkure, Wudil and Bichi, with 2 village areas each were purposively selected. Five village areas representing consumers were purposely selected from Kano metropolis, from all the villages 50 consumers representing both male and female were selected except in Dawanau where 40 respondents were selected.

Two hundred and forty (240) consumers selected from Kano metropolis were interviewed on their preferences for grain quality traits, using semi structured questionnaires (Appendix 3.2)

**Table 3.1: Number of farmers and consumers interviewed in selected cowpea growing and consuming communities**

Communities/LGA	Farmers		Consumers		Total
	Male	Female	Male	Female	
<b>Bunkure LGA:</b>					
a. Kofar gabar	34	6	-	-	<b>40</b>
b. Lautaye	38	2	-	-	<b>40</b>
<b>Wudil LGA:</b>					
a. Bakin Kasuwa	39	1	-	-	<b>40</b>
b. Kausani	33	7	-	-	<b>40</b>
<b>Bichi LGA:</b>					
a. Bichi town	37	3	-	-	<b>40</b>
b. Badume	36	4	-	-	<b>40</b>
<b>Kano Metropolis:</b>					
a. Kabara	-	-	11	39	<b>50</b>
b. Kasuwa Rimi	-	-	8	42	<b>50</b>
c. Unguwa uku	-	-	21	29	<b>50</b>
d. Hotoro	-	-	28	22	<b>50</b>
e. Dawanau	-	-	30	10	<b>40</b>
<b>Total</b>	<b>217</b>	<b>23</b>	<b>98</b>	<b>142</b>	<b>480</b>

LGA= Local Government Area

### 3.2.4 Data collection techniques

Data for varietal selection criteria and grain quality preferences for farmers and consumers were collected using two techniques. These are (1) focus group discussions (FGD) using a checklist and (2) individual interview using structured questionnaires. The questionnaires and checklist designed were edited by a team of experts: comprising of plant breeder, socioeconomics, agronomist and extension agent. The questionnaires were first pre-tested to validate the relevance of the variables and the possible responses. The questionnaire was then revised to incorporate issues that emerged from the pre-testing before it was administered to the farmers and consumers.

Focus group discussions were done with 15 farmers per community making a total of 30 per district. Agricultural extension officers with experience in working with cowpea farmers were recruited to organize the cowpea farmer groups in the communities for the FGD at each site. A standardized checklist of topics was used in the FGDs (Appendix 3.1). The topics discussed included general problems faced by cowpea farmers, grain quality preferences, marketing of cowpea and their perceptions on grain quality traits as it relates to consumer demands.

Structured questionnaires (Appendix 3.2) were administered to individual farmers that were involved in the FGD from the 5 sub-communities. This enabled individual farmers, especially those who were not confident enough to contribute during the FGD, to freely express their views. Field observations and random measures of field sizes were used to validate the data. A different set of questionnaire (Appendix 3.3) was also administered to a group of consumers from Kano metropolis. This was to measure consumers' perceptions on various cowpea grain quality attributes and determines their preferences.

Data were collated on the constraints to cowpea production, perceptions of farmer and consumers' on grain quality traits as well as their preferences for grain quality traits in cowpea.

### **3.3 Data analysis**

Both quantitative and qualitative were collected, coded and analyzed using SPSS version 17.0 (SPSS, Inc., Chicago IL). Preference ranking was used to score production constraints, most preferred variety, quality traits, and perceptions on nutrient quality traits. Average scores and ranks were used for the focus group discussion. Likert scale (Responses; 1= most important; 2= important; 3= not so important; 4= not important) was used to measure the grain quality traits. The mean perception for grain quality traits was estimated using:

$$Mean = \sum \frac{n_i - x_j}{N}$$

Where;  $n_i =$  is the number of individuals who choose the  $i^{\text{th}}$  response or attribute.

$x_j =$  the  $j^{\text{th}}$  response or attribute.

$N =$  Total number of respondents

The extent of agreement or disagreement on ranking the constraints to production was estimated using Kendal's coefficient of concordance ( $W$ ) described by Siegel & Castellan-Jr. (1988).

### 3.4 Results

#### 3.4.1 Constraints to cowpea production in the study areas

Ten production constraints were identified as the major constraints to cowpea production in the communities and ranked by farmers. Farmers scored inadequate amount of improved cowpea seeds at planting time as the most important constraint limiting cowpea production in the areas. This was followed by pest, insects, diseases, drought, parasitic weeds, high cost of inputs, lack of farm implement, poor soil fertility, high cost of labour and lack of market (Table 3.2). There was 73.05% (Kendal's value  $W=0.7305$ ) agreement among the farmers for these rankings.

**Table 3.2: Cowpea production constraints faced by farmers in Sudano-Sahelian zone of Nigeria**

Production constraint	Mean Rank	Rank
Inadequate amount of improved cowpea seeds at planting time	1.38	1
Insects (such as aphid, pod sucking bugs and thrips)	2.90	2
Lack of formal seed system that make seed available	3.09	3
Diseases (damping off, bacterial blight, <i>Fusarium wilt</i> etc)	3.66	4
Drought (seedling and terminal - mostly affecting)	4.25	5
Parasitic weeds- ( <i>Striga</i> and <i>Alectra</i> )	5.69	6
High cost of inputs (fertilizer and agrochemicals)	6.03	7
Lack of farm implements-(ploughs, thresher)	6.39	8
Poor Soil fertility (low Phosphorus level, flooding, iron toxicity)	7.08	9
High cost of labour	8.32	10

**Constraint with smallest mean rank is the most important factor limiting cowpea production in the areas.**

### **Pod boring and sucking insects**

Farmers were not familiar with most of the pod boring and sucking insects, however, when they were shown the pictures of those insects and the nature of damage caused, they identified Lepidopterans, particularly, *Maruca vitrata*, *Roptorus dentipes*, *Anoplocnemis cavipes* and parasitoids (Hymenoptera, *Braconidae*, *Aphidiinae*) of cowpea aphids. About 78% of the total farmers interviewed reported that they had observed such insects in their fields. Cowpeas cultivated under irrigation were the most affected. Field visit and farmers' opinion during FGD identified insects belonging to the members of the Hymenoptera and lepidopterans to be the major constraints in Bunkure and Wudil, respectively. Majority of the farmers (85%) applied various insecticides at different formulations and different stages of cowpea growth, to control those insects. However, 53% of farmers thought that the varieties were susceptible to Lepidopterans insects, while 47% were on the opinion that the insecticides were ineffective.

### **3.4.2 Farmer's varietal preferences**

Cowpea variety 277 popularly known as "Danbazara" by farmers was the most cultivated variety at all locations. However, farmers' preferences varied amongst the communities. Farmers in Bunkure, Wudil and Bichi districts preferred "Dankaka" (288), "Kyambas" and "Danwuri", respectively (Table 3.3). Eighty two (82%) percent of the farmers interviewed indicated their desire to have new cowpea varieties, if they would combine yield and quality attributes. During focus group discussions, Bunkure and Wudil farmers listed resistance to insects, high yield, good grain quality (good taste), and early maturity as their preferred traits. Good grain qualities were the top priorities of farmers at Bichi. They mentioned in addition to earliness, good appearance

including grain coat colour, eye colour, texture, short cooking time and swelling capacity and high yield as their traits of interest.

**Table 3.3: Farmers varietal preference ranking by community**

Variety	Bunkure	Wudil	Bichi
Danrani (288)	1	*	*
Danbazara (277)	2	2	2
Danbazara (Help me)	3	*	*
Kanannado	4	*	3
Pegeot (Fiya fiya)	*	3	*
Kyambas	*	1	*
Balami	*	4	*
Cida gero	*	*	1
Badankami	*	*	4

\*= variety is not commonly grown in a community

### **Reasons for farmers' preferences for cowpea varieties varied across communities (277, Kymbas and Danwuri)**

During focus group discussions, farmers from all the 3 districts cultivated variety Danbazara (277) mainly due to its good grain quality traits. The quality traits mentioned by the farmers included morphological attributes such as; white grain coat, rough texture, medium sized variety and cooking quality. This resulted in high demands for “277” by the consumers. Other reasons for the wide adoption of “277” included high yield performance under irrigation and rain fed conditions. Farmers in Wudil districts preferred “Kyambas” to “Danbazara” because of its adaptability to their niche and good grain quality attributes such as: medium size, white grain coat and rough texture. “Danwuri” was the most preferred variety at Bichi districts, because of its earliness and good grain quality traits that are market driven.

### 3.4.3 Farmers' preference ranking for grain quality traits

About 68% of the farmer ranked and agreed (Kendall's  $W = 0.685$ ,  $p=0.000$ ) with yield as their most important trait followed by grain quality traits and resistance to biotic and abiotic stresses (Table 3.4).

**Table 3.4: Farmers preference ranking of cowpea variety traits**

Traits	Mean Ranking	Overall Ranking
High yields	1.21	1
Good grain quality-colour, texture, size and tasty	1.68	2
Resistance to insects and pest including weeds	2.96	3
Earliness	3.88	4

Means with smallest mean rank is the most important in each column

Almost all the farmers interviewed indicated their willingness to adopt new varieties. Majority of the farmers preferred high yield (93%) and good grain quality traits (90%), especially grain coat colour and texture. Almost half of the farmers (58%) most of them from the transition zone of Bichi district, preferred for earliness in addition to grain quality traits (Table 3.5).

**Table 3.5: Farmers priorities for traits in new cowpea varieties**

Traits	Yes (%)	No (%)	Order of preference
High yields	92.54	7.46	1
Desirable grain quality-colour, texture, size and tasty	89.62	10.38	2
Resistance to insects and pest including parasitic weeds*	65.85	34.15	3
Earliness*	58.44	41.56	4

\*= very important trait in the transition zone (Bichi district) though ranked least in the overall ranking.

### 3.4.4 Farmers' perception on grain quality attributes

Farmers perceived grain colour (grain coat and eye colour), texture and size as very important grain quality traits. Cowpea grain shape and expansion ratio were regarded as important by most of the commercial farmers.

**Table 3.6: Consumers' perception on grain quality attributes**

Grain Quality Attributes	Very important (1)	Important (2)	Not so important (3)	Not important (4)	Rank score (x)	Implication
Colour	186 (77.50)*	38 (15.83)	11 (4.58)	5 (2.08)	1	Most Preferred
Grain size	149 (62.08)*	56 (23.33)	24 (10.00)	11 (4.58)	1	Most Preferred
Texture	109 (45.42)	83 (34.58)	41 (17.08)	7 (2.92)	2	Preferred
Resistance to storage insects	86 (35.83)	98 (40.83)	37 (15.42)	19 (7.92)	2	Preferred
Cooking time	54 (22.50)	96 (40.00)	64 (26.67)	36 (15.00)	2	Preferred
Grain shape	66 (27.50)	95 (39.58)	35 (14.58)	44 (18.33)	2	Preferred
Expansion capacity	61 (25.42)	99 (41.25)	55 (22.92)	25 (10.42)	2	Preferred

Numbers in bracket indicate the percentage of farmers preferred trait, the higher the percentage the more important is the trait

Consumers listed 3 most important traits in selecting for cowpea grain (Table 3.7). Both end users (75.64%) and processors (55.00%) preferred white to creamy colour grain. Majority of the end users prioritized large grains (72.49%), while processors preferred small to medium grain size (36.55% and 54.21%) respectively. Most of the processors preferred rough texture coat (86.25%), while end users showed not much preference for grain coat texture (56.19 % and 43.81%), respectively.

**Table 3.7: Consumers preferences ranking for grain quality traits**

Grain Quality Characteristics	Percent (%)	
	End user	Processor
<b>Colour</b>		
White /Cream*/**	75.60	55.00
Brown	24.40	34.70
Black	-	6.30
Those with various speckle	-	4.10
<b>Grain size</b>		
Large*	72.50	9.20
Medium**	24.50	54.20
Small	3.10	36.60
<b>Texture</b>		
Rough*/**	56.20	86.30
Smooth	43.80	13.75

\*=preferred by end-users, \*\*=preferred by processors and \*/\*\* preferred by both

Consumers' (processors and end users) preferences varied according to their need (Table 3.8). Forty five (45%) percent of the processors perceived cooking time to be the most important trait, followed by grain texture (68%), grain size (43%), oil consumption (42%) and ease of dehulling (52%) ranked as important grain attributes. They further indicated their preferences for white grains with brown eye for "akara" preparation. On the other hand, most of the end users (75%) ranked protein as the most important. Almost all the women interviewed (66%) were aware that cowpea contained micronutrients content, due to medical personnel recommendation for them to be consuming cowpea during pregnancy.

**Table 3.8: Consumer perceptions on grain quality attributes**

<b>Grain Quality Attributes</b>	<b>Most preferred (1)</b>	<b>Preferred (2)</b>	<b>Not so Preferred (3)</b>	<b>Not preferred (4)</b>	<b>Mean score (x)</b>	<b>Implication</b>
Protein*	181( <b>75.42</b> )	42(17.50)	15(6.25)	2 (0.83)	1	Most preferred
Cooking time	108 ( <b>45.00</b> )	67 (27.92)	17 (7.08)	14 (5.83)	1	Most preferred
Texture	43 (17.92)	162 ( <b>67.50</b> )	24 (10.00)	11 (4.58)	2	Preferred
Grain size	53 (22.08)	105 ( <b>43.75</b> )	55 (22.92)	27 (11.25)	2	Preferred
Oil consumption	49 (20.42)	102 (42.50)	55 (22.92)	34 (14.17)	2	Not so preferred
Dehulling	68 (28.33)	124 ( <b>51.67</b> )	33 (13.75)	15 (6.25)	2	Preferred
Micronutrients (Fe& Zn) *	66(27.5)	159 ( <b>66.25</b> )	19 (7.92)	6 (2.50)	2	Important
Colour	59 (24.58)	154 ( <b>64.17</b> )	23 (9.58)	4 (1.67)	2	Preferred
Expansion ratio	63 (26.25)	71(29.58)	81 ( <b>33.75</b> )	25 (10.42)	3	Not so preferred
Taste (Sweet/sweetness) *	51 (21.25)	64 (26.67)	94 ( <b>39.17</b> )	31 (12.92)	3	Not so preferred

- **End users' preferred trait, Number in the bracket is the percentage of consumer preference for certain trait**

Almost all (95%) the farmers interviewed grew cowpea for commercial purposes and kept about 35% of produce for home consumption. Cowpea was mainly sold in threshed grain form to traders and women processors (market women who processed cowpea into different diets). The market price for cowpea is mostly high towards planting time and during active growth of the cowpea growing season, farmers complained about storage insects during storage of excess cowpea.

Six most consumed cowpea diets are presented in Table 3.9. The high value of Kendall's coefficient of concordance ( $W= 0.708$ ,  $p= 0.000$ ) indicated the 70% agreement of the consumers' ranking for "Kosai" as the most preferred and consumed diets in Kano metropolis. This was followed by cowpea with rice cooked together, eaten with spices or stew (Wake da shinkafa); Cowpea steam paste (Moin moin); Cowpea dimples (Danwake), raw cowpea; and cowpea porridge (Table 3.9).

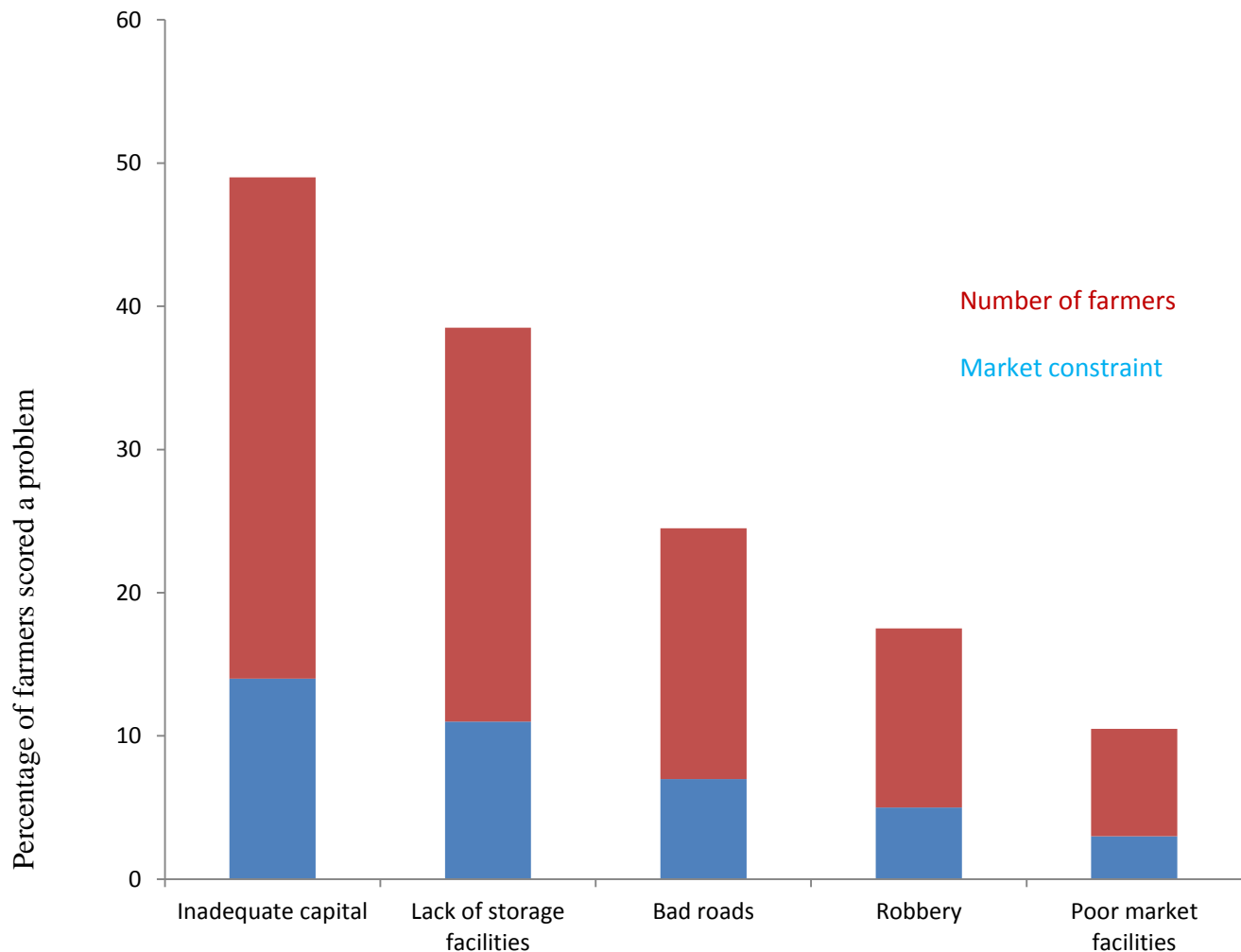
**Table 3.9: Ranking for consumer preferences for most frequently consumed diets**

<b>Cowpea dishes</b>	<b>Most Frequent Consumers</b>	<b>Mean Rank</b>	<b>Rank</b>
Akara (Kosai )*	62.70	1.38	1
Cowpea mixed with rice	59.70	2.44	2
Moin moin	48.59	3.59	3
Danwake	46.20	4.08	4
Raw cowpea	29.98	4.88	5
Cowpea porridge	42.66	6.12	7

**Diet with smallest rank is the most prepared and most consumed, \*= daily consumed**

### **3.4.5 Problems associated with Cowpea Marketing**

Problems associated with cowpea marketing as identified by marketers and farmers that do not want to sell it at harvest time due to low price are presented in Figure 3.1. Both small scale cowpea farmers and marketers identified inadequate capital as the highest problem affecting markets followed by lack of adequate storage facilities leading to high cost of transportation to the homes at harvesting time and to market.



**Figure 3.1: Problems associated with cowpea marketing**

### 3.5 Discussion

Ranking of constraints to cowpea production by farmers in all 3 districts differed slightly among the communities; the challenges listed were similar across the districts. The highest ranked challenges were grain related issues such as; non-availability or inadequate amount of improved cowpea seed at planting time, lack of pod boring and sucking insect resistant varieties and lack of disease resistance among varieties. Agricultural Seed Council of Nigeria (ASCN) is an agency that ensures certified seeds are being sold to farmers at the right time. However, the efforts of

Nigerian government through ASCN appeared to be inadequate. There is need for the government to double its efforts through the agricultural transformation agenda towards solving farmers' problem. Majority of the low income farmers who are often associated with smaller harvests exhausted their grain stock during the lean season, and purchased grains for the coming planting season at higher price than affordable, which agrees with the findings of Takeshima *et al.* (2014).

Non availability of cowpea grains at planting time was attributed to the lack of well collaborative work between scientists, grain companies and farmers. Therefore, there is need for farmers to work with other stakeholders especially extension agents for proper guidance. For example, most of the farmers interviewed reported that some cowpea varieties popularly called "Danrani" coded: 288 and TVX-3236 became extinct despite their tolerance to drought and their high yield. The extinction was due to irregular visits by the extension officers and follow up from the researchers and lack of impact assessment from researcher's side. To my observation, the extinction from the farmers hand may be associated with natural disaster; such as insect invasion and climate change. This conformed to Bunkure Farmer's attestation when farmers lost their irrigated cowpea in 2012 (Field visit after FGD). Varaprasad & Sivaraj (2010) reported that climate change increased the risk of extinction for many species, and there may be loss of genetic variability even if the species survived. Therefore, further study is suggested to find out the causes of extinction and conservation efforts should be improved.

Lack of insect resistant (pod borer for example) varieties, resulted in high cost of production leading to either small farm sizes or abandoned cowpea cultivation. Farmers had to apply various types of insecticides at different formulations to control various insects. The farmers need insect resistant varieties, as this will help them save some money by either reducing the number of insecticide applications or not spraying at all. Farmers in the 3 locations identified

various insects that affected cowpea at both pre-flowering and post flowering stages particularly, flower thrips, pod boring and sucking insects. Pod boring insects especially *Maruca vitrata* was among the most damaging insects that causes up to 80% yield loss if not properly controlled (Fatokun, 2009). This was found in Bunkure district when they had an outbreak of such insects that resulted in 100% loss. Intensive screening of cowpea germplasm for *Maruca* resistance showed that there is no known cultivar with more than weak resistance (Murdock *et al.*, 2008). Closely related cowpea species (*Vigna vexillata*) with resistance to *Maruca* were unfortunately genetically incompatible with the cultivated species.

One of the farmers (Malam Aliyu) attested that he applied various formulations of different insecticides for more than 15 times, yet no grain was harvested from the field. Inadequate varieties with combined farmers and consumers' attributes also limited production, though Danbazara (277) was adopted by the farmers in all locations. Currently, multidisciplinary teams of scientist in collaboration with African Agricultural Technology Foundation (AATF) are working toward solving that problem in Africa, using transgenic cowpea. There is also need for more collaboration toward solving farmers' problems of such nature.

Farmers perceived colour, grain size and texture as the most important grain quality traits. Danbazara (277) was preferred by majority of the farmers in all the locations, because of its white colour, rough texture and medium size. These are the first traits considered by many farmers especially those that cultivate cowpea for commercial purpose. This result is not in agreement with the findings of Kormawa *et al* (2000) who reported brown grain cowpea as the most preferred by consumers over the white colour cowpea grain in the Nigerian markets. This would not be a surprise, because generally, in the study area the brown colour cowpea commands a

higher price and producers prefer to sell them as exports to neighboring states and countries around, while the white colour grains are sold in the area of study.

Over 80% of the farmers preferred yield and grain quality traits in all the districts visited (Bunkure, Wudil, Bichi and Kano metropolis) districts. However, farmers in Bichi area preferred grain quality traits and earliness. The latter was attributed to their proximity to cowpea consumers (Traders and processors) at Dawanau central market, while earliness was due to early cessation of rain fall. This is in conformity with the findings of Langyintuo *et al.* (2003) that even low income consumers were vigilant in identifying products that do not meet their standards, and are willing to pay a premium for products that match their preferences.

Cowpea consumers identified grain colour (scored=64%), texture (scored=68%) and size (scored=44%) as the most important grain quality traits. These are the determinants of cowpea preference and indicators for marketability of cowpea in Nigeria in particular and Africa in general. Visible characteristics are directly related to the way cowpeas are used in diets preparations. For instance, cowpea grain colour or eye colours are important considerations when the intended use for the grains required decortication to remove flecks. Poor milling and winnowing may leave some flecks for which consumers showed low tolerance to it. This agrees with the findings of Langyintuo *et al.* (2004) and Cobbinah *et al.* (2011) that consumers in Africa, preferred 'kosai' (cowpea fritters) without black flecks, while in Ghana black flecks have little impact on the use of cowpeas for 'Tubani' (steamed cowpea paste) or a mixture of cowpea with rice.

Small scale cowpea processors, who are mainly women, preferred short cooking time (45%), ease of dehulling (52%) and less oil consumption (43%). Long cooking time was identified as an undesirable characteristic of some cowpea cultivars due to relatively large amount

of energy required to process cowpea grains leading to an increasing in the cost of diets preparation and reduces income (Yeung *et al.*, 2009) Majority of the processors preferred small to medium grained cowpea due to its high expansion capacity and less oil consumption when preparing cowpea diets. They also perceived brown coloured grains are not suitable for “Akara” and “moin moin” making due to its grittiness and difficult in dehulling. This conforms to the findings of Yeung *et al.* (2009) that parenchyma cell of some cowpea cultivars clumps, creating a gritty and uncooked feeling when consumed. Cowpea varieties with smooth and wrinkled grain coat have thicker testa than those with rough grain coat, which in turn affects the rate of water absorption (Ojomo & Cheda, 1972). Similarly, cultivar with comparatively thick, smooth grain coats have a slow initial rate of water absorption, whereas thin grain coat cultivar have high initial rate and dehull better after soaking (Sefa-Dedeh *et al.*, 1979). Over 75% of the consumers mostly women rated protein and micronutrient (Fe and Zn) contents as very important and important traits, respectively.

There were significant differences between the perceptions of farmers and consumers for all grain quality attributes except for cooking quality, oil consumption and micronutrients content. This was attributed to the differences in diets and mode of cowpea processing. Processors prioritized cooking time, oil consumption during diets preparations and expansion capacity as either important or very important. While end users emphasized on the nutritional (Protein, Fe and Zinc) grain quality traits. All the farmers interviewed grew cowpea as a cash crop and recognized the importance of quality in the marketing of their produce. The specific preference for cowpea was generally similar for farmers, processors and consumers. For grain colour, white was the predominant colour (over 90%) for farmers and consumers. This is not surprising because

almost all cowpea in the Kano market are white. However, there are niche markets for brown cowpea especially in Kura and Chiromawa town along Kano Zaria road.

The study further revealed a higher preference for larger grain sizes of cowpea for end users and higher preference for small to medium sized grains for processors. These results agree with the findings of many researchers Madode *et al.* (2011); (Coulibaly *et al.* (2009) and Boys *et al.* (2007) in which they reported that consumers in West and Central Africa have their preferences for cowpea and varied from one location to another.

### **3.6 Conclusion and Recommendations**

The results of the PRA exercises in the Sudan savanna and Sudan-Sahelian transition zones of Nigeria have identified the major constraints to cowpea production and needs for cowpea smallholder farmers, processors and consumers. In all the 3 districts visited, farmers identified 10 constraints to cowpea production and emphasized on the first 5 (improved grain related issues): inadequacy of improved cowpea seeds at planting time, lack of insect resistant varieties, diseases resistant varieties and drought tolerance varieties. Cowpea varieties preferences varied across the districts, though adopted Danbazara (277) due to its high yield and good grain quality traits. This indicated the need to target for location and trait specific varieties to satisfy different farmers and consumers of different locations.

The cowpea quality traits identified, were classified according to the category of cowpea users (producers, processors or end users). Quality traits were considered by both farmers and consumers to be important criteria for making choices for cowpea varieties. Majority of the processors preferred short cooking time, an important characteristic for women who are involved in food preparation, ease of removal of the hilum and testa and less oil consumption. End users, on the other hand, considered protein, micronutrients, grain colour and size. Specific cowpea

characteristics desired by cowpea farmers, processors and consumers could increase profits for farmers and ultimately the processors as well.

It is therefore, recommended that PRA exercises should be encouraged to identify the needs of the beneficiaries along the value chain for each set of players including farmers, processors, retailers and wholesalers. This calls for the need for better targeting varieties for site specific. Processing traits such as short cooking time and less oil consumption and consumers' traits such as protein and other nutrient should also be given priority in the selection of cowpea lines, for setting breeding objectives. This will help in setting breeding objectives toward problem solving, planning, implementation, monitoring and evaluation of breeding activities as well as impact assessment. Farmers' preference may vary according to sites and blanket recommendations could mask the preference choices in locations and lead to rejection of newly developed variety.

## CHAPTER FOUR

### 4. VARIATION AMONG COWPEA ACCESSIONS FOR GRAIN QUALITY TRAITS AS REVEALED BY MORPHOLOGICAL, BIOCHEMICAL AND SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS

#### 4.1 Introduction

Cowpea is an important source of vegetable protein and is extensively grown throughout the world. Utilization of vegetable protein is gaining increasing attention due to the world's need for more low-cost dietary proteins, particularly for low income countries (Wang *et al.*, 1997). The high cost and limited availability of animal proteins in the developing countries has directed interest towards several grain and legume proteins as potential sources of vegetable protein for food use (Sathe & Salunkhe, 1981).

As a food source to over 200 million people in Africa (Fatokun *et al.*, 2012), cowpea provides the cheapest protein supplement to the urban and rural poor in Africa. An extremely important issue to note is the sheer deficiency of urban diets in relation to the nutritional needs and the impact of this on people's health (Porter *et al.*, 2003). This is particularly true in Nigeria given the general rising needs for cowpea to urban and rural poor (Ayinde, 2005). This has affected the protein and other essential element intake of most Nigerians. The eating habits of most poor people thus revolve round cowpea consumption in many forms; either through direct cooking of cowpea, processing it into "akara" (cowpeas cake), "moin-moin" or as component of other meals, as in cowpeas soup, rice and cowpeas, etc. This has made Nigeria to be the largest cowpea consumer in the world. To prepare such foods, a wide range of variation in cowpea grain quality traits is required in order to satisfy the various needs of the processors and end-users.

The physical appearance of cowpea grain such as grain coat colour, eye colour, texture and shape; short cooking time and eating qualities are very important characteristics for cowpea

consumers. Consumers in different parts of the world have specific preferences for cowpea. For example, cowpea variety (coded as 288) with medium grains size, short cooking time and white in colour is preferred by the processors in Northern Nigeria, while Ghana and Cameroon consumers prefer white, large grained and rough cowpea types (Langyintuo *et al.*, 2004). The diversity in crop varieties is essential for agricultural development for increasing food production, poverty alleviation and promoting economic growth.

Various studies have reported a wide variability in cowpea physicochemical properties, genetic variations for proteins (Kachare *et al.*, 1988; Ajeigbe *et al.*, 2008), nutritional composition, cooking time in cowpea (Onyenekwe *et al.*, 2000), mineral components of cowpea grains (Anele *et al.*, 2011) and nutritional grains quality traits in cowpea (Boukar *et al.*, 2010)

Despite much effort to assess the variability of cowpea mineral elements by many authors (Oluwatosin, 1998; Asante *et al.*, 2009; Mamiro, 2011), large gaps still remain in the assessment of cowpea diversity for grain micronutrients particularly, iron and zinc. Further screening of cowpea accessions with molecular markers and physicochemical properties will facilitate breeding for enhanced nutritional quality traits in cowpea. Cowpea germplasm collection and characterization will be initiated to explore the important nutritional grain quality traits to satisfy the growing need of such traits. Quantification of phenotypic divergence in a crop is considered a necessary pre-breeding tool (Acquaah, 2007). Genetic variation are started with the assessment of morphological variation, and followed with molecular studies to facilitate the selection process.

The specific objectives of this study were to:

1. Assess the genetic variation among cowpea accessions from Africa and other parts of the world using morphological, biochemical and molecular traits
2. Determine the association between nutrient quality traits and physicochemical properties of cowpea grain
3. Identify promising accessions for future planning for development and deployment of nutritionally (iron and zinc) enhanced cowpea varieties.

## **4.2 Materials and Methods**

### **4.2.1 Source of cowpea accessions**

A total of 200 cowpea accessions from 24 countries (Table 4.1) were collected from IITA, IAR and WACCI breeding unit, Ghana. Most of the accessions were from the primary center of diversity especially the African countries. The accessions consisted of: 4 landraces, 195 core collection genotypes including wild types and farmer's preferred variety (Table 4.1).

**Table 4.1: List, origin and source of 200 cowpea accessions selected in this study**

S/N	Accession	Origin	Source	S/N	Accession	Origin	Source	S/N	Accession	Origin	Source
1	TVu-13088	Benin	IITA	38	TVu-16566	Guinea	IITA	75	TVu-4886	Niger	IITA
2	TVu-16343	Benin	IITA	39	TVu-16574	Guinea	IITA	76	TVu-5040	Niger	IITA
3	TVu-16393	Benin	IITA	40	TVu-16575	Guinea	IITA	77	TVu-5444	Niger	IITA
4	TVu-16399	Benin	IITA	41	TVu-13457	Kenya	IITA	78	TVu-5495	Niger	IITA
5	TVu-16400	Benin	IITA	42	TVu-13468	Kenya	IITA	79	TVu-5562	Niger	IITA
6	TVu-8673	Benin	IITA	43	TVu-13495	Kenya	IITA	80	TVu-6238	Niger	IITA
7	TVu-8742	Benin	IITA	44	TVu-1801	Kenya	IITA	81	Ghana	Ghana	WACCI
8	TVu-8745	Benin	IITA	45	TVu-2651	Kenya	IITA	82	TVu-4841	Niger	IITA
9	TVu-8751	Benin	IITA	46	TVu-347	Kenya	IITA	83	TVu-1	Nigeria	IITA
10	TVu-8758	Benin	IITA	47	TVu-433	Kenya	IITA	84	TVu-10408	Nigeria	IITA
11	TVu-15164	Cam	IITA	48	TVu-8454	Kenya	IITA	85	TVu-1172	Nigeria	IITA
12	TVu-15187	Cam	IITA	49	TVu-8455	Kenya	IITA	86	TVu-1261	Nigeria	IITA
13	TVu-15197	Cam	IITA	50	TVu-11788	Malawi	IITA	87	TVu-13094	Nigeria	IITA
14	TVu-13867	C AR	IITA	51	TVu-15086	Malawi	IITA	88	TVu-13095	Nigeria	IITA
15	TVu-14090	CAR	IITA	52	TVu-15088	Malawi	IITA	89	TVu-14012	Nigeria	IITA
16	TVu-14109	CAR	IITA	53	TVu-15107	Malawi	IITA	90	TVu-14172	Nigeria	IITA
17	TVu-15251	Chad	IITA	54	TVu-15114	Malawi	IITA	91	TVu-1449	Nigeria	IITA
18	TVu-15323	Chad	IITA	55	TVu-15141	Malawi	IITA	92	TVu-1509	Nigeria	IITA
19	TVu-16461	Chad	IITA	56	TVu-15143	Malawi	IITA	93	TVu-1551	Nigeria	IITA
20	TVu-15204	Congo	IITA	57	TVu-9929	Malawi	IITA	94	TVu-15560	Nigeria	IITA
21	TVu-15223	Congo	IITA	58	TVu-14626	Mali	IITA	95	TVu-15617	Nigeria	IITA
22	TVu-15225	Congo	IITA	59	TVu-14845	Mali	IITA	96	TVu-15685	Nigeria	IITA
23	TVu-15243	Congo	IITA	60	TVu-7628	Mali	IITA	97	TVu-15686	Nigeria	IITA
24	TVu-7747	CD	IITA	61	TVu-7638	Mali	IITA	98	TVu-15694	Nigeria	IITA
25	TVu-9446	Egypt	IITA	62	TVu-7664	Mali	IITA	99	TVu-15695	Nigeria	IITA
26	TVu-9463	Egypt	IITA	63	TVu-7966	Mali	IITA	100	TVu-15719	Nigeria	IITA
27	TVu-9725	Egypt	IITA	64	TVu-8237	Mali	IITA	101	TVu-15725	Nigeria	IITA
28	TVu-1084	Ghana	IITA	65	TVu-13096	Niger	IITA	102	TVu-15762	Nigeria	IITA
29	TVu-15884	Ghana	IITA	66	TVu-14932	Niger	IITA	103	TVu-15775	Nigeria	IITA
30	TVu-15892	Ghana	IITA	67	TVu-14939	Niger	IITA	104	TVu-158	Nigeria	IITA
31	TVu-15895	Ghana	IITA	68	TVu-14967	Niger	IITA	105	TVu-15811	Nigeria	IITA
32	TVu-4489	Ghana	IITA	69	TVu-4672	Niger	IITA	106	Sampea 13	Nigeria	IAR
33	TVu-7362	Ghana	IITA	70	TVu-4701	Niger	IITA	107	TVu-1586	Nigeria	IITA
34	TVu-7999	Ghana	IITA	71	TVu-4708	Niger	IITA	108	TVu-15866	Nigeria	IITA

**Table 4.1: (Cont'd) List, origin and source of 200 cowpea accessions selected in this study**

S/N	Accession	Origin	Source	S/N	Accession	Origin	Source	S/N	Accession	Origin	Source
35	TVu-997	Ghana	IITA	72	TVu-4747	Niger	IITA	109	TVu-16414	Nigeria	IITA
36	TVu-999	Ghana	IITA	73	TVu-4776	Niger	IITA	110	TVu-16483	Nigeria	IITA
37	TVu-16521	Guinea	IITA	74	TVu-4808	Niger	IITA	111	TVu-16504	Nigeria	IITA
112	TVu-18	Nigeria	IITA	142	TVu-990	S. Africa	IITA	172	Biyu local	Nigeria	IAR
113	TVu-301	Nigeria	IITA	143	TVu-10454	Tanzania	IITA	173	TVu-1985	USA	IITA
114	TVu-320	Nigeria	IITA	144	TVu-15355	Tanzania	IITA	174	TVu-1986	USA	IITA
115	TVu-3629	Nigeria	IITA	145	TVu-15360	Tanzania	IITA	175	TVu-202	USA	IITA
116	TVu-3710	Nigeria	IITA	146	TVu-15381	Tanzania	IITA	176	TVu-2155	USA	IITA
117	TVu-409	Nigeria	IITA	147	TVu-2672	Tanzania	IITA	177	TVu-232	USA	IITA
118	SB_sata	Nigeria	IAR	148	TVu-7188	Tanzania	IITA	178	TVu-233	USA	IITA
119	TVu-43	Nigeria	IITA	149	TVu-7293	Tanzania	IITA	179	TVu-297	USA	IITA
120	TVu-4536	Nigeria	IITA	150	TVu-8330	Tanzania	IITA	180	TVu-30	USA	IITA
121	TVu-4545	Nigeria	IITA	151	TVu-8612	Togo	IITA	181	TVu-315	USA	IITA
122	TVu-4558	Nigeria	IITA	152	TVu-8619	Togo	IITA	182	TVu-332	USA	IITA
123	TVu-486	Nigeria	IITA	153	TVu-117	Uganda	IITA	183	TVu-337	USA	IITA
124	TVu-50	Nigeria	IITA	154	TVu-1184	Uganda	IITA	184	TVu-374	USA	IITA
125	TVu-526	Nigeria	IITA	155	TVu-1185	Uganda	IITA	185	TVu-384	USA	IITA
126	TVu-59	Nigeria	IITA	156	TVu-1272	Uganda	IITA	186	TVu-387	USA	IITA
127	TVu-746	Nigeria	IITA	157	TVu-1283	Uganda	IITA	187	TVu-393	USA	IITA
128	TVu-857	Nigeria	IITA	158	TVu-1330	Uganda	IITA	188	TVu-408	USA	IITA
129	TVu-875	Nigeria	IITA	159	TVu-401	USA	IITA	189	TVu-415	USA	IITA
130	TVu-971	Nigeria	IITA	160	TVu-1004	USA	IITA	190	TVu-430	USA	IITA
131	TVu-1706	Pakistan	IITA	161	TVu-1016	USA	IITA	191	TVu-456	USA	IITA
132	TVu-11462	Philippines	IITA	162	TVu-1030	USA	IITA	192	Baban wake	Nigeria	IAR
133	TVu-11503	Philippines	IITA	163	TVu-1036	USA	IITA	193	TVu-497	USA	IITA
134	TVu-14396	Senegal	IITA	164	TVu-1037	USA	IITA	194	TVu-566	USA	IITA
135	TVu-10745	S. Leone	IITA	165	TVu-1045	USA	IITA	195	TVu-697	USA	IITA
136	TVu-1171	S. Africa	IITA	166	TVu-1251	USA	IITA	196	TVu-13305	Zambia	IITA
137	TVu-1236	S. Africa	IITA	167	TVu-1494	USA	IITA	197	TVu-15058	Zambia	IITA
138	TVu-2397	S. Africa	IITA	168	TVu-1560	USA	IITA	198	TVu-16637	Zambia	IITA
139	TVu-274	S. Africa	IITA	169	TVu-1562	USA	IITA	199	TVu-8407	Zambia	IITA
140	TVu-36	S. Africa	IITA	170	TVu-1616	USA	IITA	200	Maikube	Nigeria	IAR
141	TVu-84	S. Africa	IITA	171	TVu-1715	USA	IITA				

**IITA= International Institute of Tropical Agriculture, IAR= Institute for Agricultural Research, Zaria-Nigeria, WACCI= West Africa center for crop Improvement, breeding unit, SB\_sata= Saka babba sata (landrace), Cam= Cameroon, CD= Cote'd voire,**

#### 4.2.2 Soil sampling and analyses

Soil physicochemical properties of the trial site and soil used for pot experiment were analyzed in order to know the approximate soil mineral content. This is because plant uptake soil mineral elements and translocate to other tissues including grains (White and Broadley, 2009). The availability of such elements in the soil may confound the end results of the iron and zinc concentration in the grain.

Soil samples were collected from the Institute for Agricultural Research farm in Samaru. The samples were taken from 2 layers (0-15 cm and 15-30 cm) at 6 different locations within the experimental area as described by Anderson & Ingram, (1998). Un-decomposed plant materials were sorted out then the soil was air dried and the samples ground and passed through a 2 mm sieve to obtain the fine earth. The sieved soil samples were stored in thick polythene bags for laboratory analyses and subsequent screen house studies.

Soil auger was driven gently into the soil until 0-15cm was completely buried. The auger was then removed and pushed out into a moisture can. This was repeated for 15-30 cm for different portions. The samples were then oven-dried at 105°C for 24 hours. The density of the soil was calculated as:  $Bulk\ density = \frac{Oven\ dry\ mass\ of\ clod}{Volume\ of\ soil}$

#### Particle size distribution

Particle size distribution was determined following Walkley & Black (1934). Forty grams (40 g) of the fine earth fraction of the soil was taken into a plastic bottle and mixed with 100 ml of 5% calgon (sodium hexametaphosphate) solution was added. The content of the bottle was then shaken on a mechanical shaker for 2 hours after which it was transferred into a 1.0 litre measuring cylinder and topped up to the mark with distilled water. The suspension was then agitated with a plunger and five minutes thereafter, the density of the suspension (silt and clay) was taken using a

hydrometer. The hydrometer reading of the suspension was taken again after eight hours (clay). The suspension temperatures  $T_1$  and  $T_2$  were recorded during the 5 minute and 8 hour hydrometer readings. The contents of the cylinder after the eight hour reading were emptied onto a 47  $\mu\text{m}$  sieve. The sand retained on the sieve was then washed off into a moisture can and dried at 105°C for 24 hours, after which the dry weight of the sand was recorded. Blank sample hydrometer readings at five minutes and eight hours respectively were also taken for the 5% calgon topped up to 1.0 liter. The particle size distribution was then determined using the formulae below.

$$\text{Percentage clay} = \frac{(\text{8 hour reading} - \text{Correction for Temperature})}{\text{Oven dry mass of the sample}} \times 100$$

$$\text{Percentage sand} = \frac{\text{oven dry weight of particles retained on the 47 sieve}}{\text{Oven dry mass sample}} \times 100$$

$$\text{Percentage silt} = \% (\text{Clay and Silt}) - \text{Percentage of Clay}$$

The temperature for density of the soil particle suspensions was corrected by subtracting increase in weight of the particles from the blank hydrometer reading (Day, 1965). The correction was made because, for every 1°C increase in temperature, above 19.5°C, there is an increase of 0.3 in the density of the particles in suspension.

The moisture content of the soil at field capacity was determined by saturating 500 g of soil sample with water and covered with a plastic and allowed to drain for 3 days. Sub samples were then taken and oven dried at 105°C. The gravimetric water content was determined as the difference in mass between moist soil and oven-dried soil per oven-dried soil.

### **Soil chemical analysis**

The pH of the fine earth of the two soils was determined in a 1:1 soil to distilled water ratio. Ten gram (10 g) soil was weighed and 10 mL of distilled water added, stirred vigorously and allowed to stand for 30 minutes. A microprocessor pH 213 meter was calibrated, and then inserted into the supernatant of the soil solution and the pH read. Available iron and zinc contents

in the soil were measured by atomic absorption spectrometry (AAS-240FS from Varian Company) after digestion with acid. The organic carbon content of the soil was determined using the wet combustion method of Walkley and Black (1934). Total N of the soil and available phosphorus were determined by using the Kjeldahl digestion procedure as outlined by Anderson & Ingram (1998).

#### **4.2.3 Phenotypic characterization of the accessions**

Field trial was established in 2012, at the experimental station of the Institute for Agricultural Research, Samaru, Zaria-Nigeria (11° 09' 31.38" N and 7° 38' 15.19"E). Two hundred cowpea (200) accessions were planted out in an augmented block design as described by Khairwal *et al* (2007) with 2 checks; Sampea-13 (early maturing) and Biyu local (late maturing), in a 9 m row per accession per plot. Data were taken on the days to 50% flowering, growth habit, flower colour, pod placement, 100 grain weight, grain coat colour, grain coat pattern and grain coat texture following Mahalakshmi *et al.* (2007) cowpea descriptors.

##### **4.2.3.1 Physicochemical analyses of cowpea grain**

The proximate analysis was conducted on 200 cowpea accessions based on the procedure of the Association of Analytical Chemist (AOAC, 2000). During the analysis, moisture, fiber, ash, crude fats, proteins and carbohydrates contents were determined and expressed in percentages (%).

##### **Digestion of cowpea grain**

Two grams of the dried cowpea grain were taken at random from each accession, grinded with pestle and mortar. Half gram (0.5g) of the cowpea powder was taken and transferred to a digestion flask, containing 0.5 g digestion mixture, and 20 ml of concentrated sulfuric acid added to the mixture. The solution was heated until it became clear and frothing ceased. Then it was

boiled briskly for another 2 hours by adding 20 ml mixture of perchloric acid and nitric acid; 10:10 ml (v/v), cooled and to the digest about 50 ml water was added in 5 ml portions with mixing. The digest was then transferred to a 100 ml volumetric flask and the volume made up to the mark. This was done 3 times for each sample. All the proximate values were reported in percentage (%) in accordance with the AOAC (2000) procedures.

### **Moisture Content Determination**

Plastic dishes were washed and dried to a constant weight in an oven at 100°C. They were later removed and cooled in a desiccator and weighed ( $W_1$ ). Two grams of the grounded powder of each accession were placed in the weighed moisture dish ( $W_2$ ). The dish containing the sample was kept in an oven for about 3 hours, the sample was removed and cooled in the desecrator and weighed  $W_3$ .

The percentage (%) of moisture was calculated as  $\frac{W_2 - W_3}{W_2 - W_1} \times 100$

### **Determination of Ash content**

For ash content determination, crucibles were rinsed, dried in the oven, cooled in the desiccators and weighed ( $W_1$ ). Two grams of the grounded sample were placed in the crucibles and weighed ( $W_2$ ). They were transferred into the Muffle Furnace and incinerated at 55° C, then removed and cooled in the desiccator and then weighed ( $W_3$ ).

The percentage (%) of Ash was calculated as  $\frac{C_2 - C_3}{W} \times 100$

### **Determination of Fibre**

Two grams of the sample was placed in a beaker containing 1.2 ml of  $H_2SO_4$  per 100 ml of solution and boiled for about 30 minutes, the residue was filtered and washed with hot water. The residue was then transferred to a beaker containing 1.2 gram of NaOH per 100ml of solution

and boiled for about 30 minutes, the residue was washed with hot water and dried in an oven and weighed ( $C_2$ ), the weighed sample was incinerated in a Furnace at  $550^\circ\text{C}$ , removed and allowed to cool, and weighed ( $C_3$ ). Fiber content was estimated from the loss in weight of the crucible and its content on ignition as shown below:

The percentage (%) of Fibre was then calculated as:  $\frac{C_2 - C_3}{W} \times 100$

### **Determination of Lipids (Fat)**

Lipid was determined using Soxhlet apparatus after 8 hours of refluxing. Oven dried digests were transferred to a desiccator and allowed to cool before the weight was taken.

The percentage (%) Fat was calculated as: =  $\frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$

### **Determination of Protein**

The nitrogen value, which is the precursor for protein of a substance, was determined by microkjeldahl method described by Hussain *et al.* (2010), involving digestions, distillation and finally titration of the sample. The nitrogen value was converted to protein by multiplying the factor of 6.25. One tablet of copper catalyst and 25 ml concentrated Sulfuric acid  $\text{H}_2\text{SO}_4$  were added to the digest in the fume cupboard and heated till solution assumed a green colour. The digest was cooled and wash any black particle that appeared at the mouth and neck of the flask was washed down with distilled water. The digest was then transferred into 250 ml flask with several washings with distilled water. This was followed with distillation using the Markham distillation apparatus. After 15 minutes of steaming, 100 ml conical flask containing 10ml of boric indicator was placed under the condenser. Ten Mill (10 ml) of the digests was pipetted into the body of the apparatus via the small funnel aperture; washed down with distilled water followed by 10ml of 40% NaOH solution. Ammonium sulphate was then collected after 7 minutes of steaming. The solution in the receiving flask was then titrated using N/100 (0.01N)

hydrochloric acid and calculated the Nitrogen content and hence the protein content of each sample. A blank solution was run along with each sample, until all the 200 samples were exhausted.

#### **Determination of Carbohydrate (CHO) content**

Carbohydrate content was determined by following the method described by Pearson (1976). In this method, carbohydrate was obtained by subtracting the sum of the percentages of moisture, ash, crude protein and crude fiber from 100:

$$\text{Carbohydrate content} = 100 - (\% \text{ of moisture} + \% \text{ Ash} + \% \text{ Protein} + \% \text{ Fibre})$$

#### **4.2.3.2 Iron and zinc content determination**

The Harvest plus crop sampling protocol for micronutrients determination (Fatokun & Stangoulis, 2008) was used for this study. At 95% pod maturity, 100 g of matured pods were collected from 10 plants from each accession and the samples placed in clean, well labeled paper bags. The collected samples were dried at 60°C for 3 days in a contaminant-free oven. The pods were threshed by gentle twisting of the pods with hand over a dust-free plastic tray. Grains of 5 g from each of the accession were collected based on quartering procedure of Harvestplus (Fatokun & Stangoulis, 2008). The Quartering procedure is done by spreading the grains in a circular form on a clean acid-washed tray. The circle was divided into four roughly equal parts and discarded the two diametrically opposite quarters, and remixed the remaining two parts. This was repeated until 5-10 g grains were obtained.

The grain was ground using non-contaminating zirconium pestle and mortar. The milled samples were packaged in cleaned, paper envelopes and then digested with H<sub>2</sub>SO<sub>4</sub> acid. Atomic Absorption Spectrophotometry (AAS) analysis was employed for both iron and zinc concentration following Benton-Jones (1989) technique, based on nitric perchloric acid digestion.

A total of 0.25 mg of each sample was acid digested with 5 ml of a 2:1 mixture of 65% nitric acid (HNO<sub>3</sub>) and 70% perchloric acid (HClO<sub>4</sub>) in 50 ml Taylor digestion tubes for 2 hours. This was followed by a heat treatment of 200°C for another 2 hours and resuspension in 25 ml of deionized water. The concentration of iron and zinc grains of individual plant samples of each generation were then read using Atomic Absorption Spectrophotometer (AAS240SF, from Varian Company) machine. Readings were then evaluated against standard curves prepared from iron and zinc diluted to a concentration of 100 mg/kg and zinc diluted to 50 mg/kg. Measurement conditions were as recommended by the manufacturer for analysis of Zn and Fe in a cellulose based matrix and acquisition time of 60 seconds. Grain samples were scanned by pouring 0.2 ml of each sample into AAS cups sealed at one end by 4 µm Poly-4 film.

The actual iron and zinc concentration were estimated using the formular:

$$\text{Actual concentration} \left( \frac{\text{mg}}{\text{kg}} \right) = \frac{\text{Concentration of a sample} \times \text{Volume of the digests}}{\text{weight of a sample}} - \text{concentration of the blank solution}$$

### **Selection of parental lines**

Contrasting parental lines were selected, based on the field evaluation data and laboratory assessment of iron, zinc, protein and other physicochemical properties of the cowpea grain. Parental materials were selected using Mulamba & Mock (1978) rank summation index (Appendix 4.1) adopted from cowpea descriptors of IBPGR (1983) and Mahalakshmi *et al.* (2007) scoring system (Table 4.2). Conversely, these descriptors did not specify the clear range for low, medium and high protein, iron and zinc content.

**Table 4.2 Scoring system used to characterize the 200 cowpea accessions (Mahalakshmi *et al.*, 2007)**

Traits	Score		
	1	2	3
Maturity period	Early (70-75days)	Medium (80-95 days)	late maturing (> 100 days)
Growth habit	Erect	Semi-erect	prostrating type
Shattering	Normal	Low shattering	High shattering
100 grain weight( g)	20g and above	Between 15 & 19g	less than 10g
Protein content (mg/kg)	21-29%,	10-20%	less than 10%
Zinc content (mg/kg)	300 and above	Between 100-290	below 100
Iron content (mg/kg)	100 and above	50-99	less than 50
Fibre content (%)	3.1- 4.9%,	2.1-3.0%	1.0-2.0%
Carbohydrate (%)	71-78%,	60- 70%	less than 59%
Fat (%)	11-22%,	6-10%	1-5%
Grain coat colour	White	Brown	Others
Grain coat texture	Rough	Smooth	Wrinkle

**1= scale for early maturing or high content; 2= medium maturing/content and 3= late maturing or low content**

#### 4.2.4 Molecular analysis

##### 4.2.4.1 Characterization of cowpea accessions using SNP markers

Thirty of the 200 accessions were discarded due to undesirable traits (susceptibility to insects and diseases, shattering etc) recorded against them. As a result, 170 cowpea accessions were selected based on the adopted Mahalakshmi *et al.* (2007) criteria outlined in Table (4.1) and rank summation index (Appendix 4.1), and genotyped using 119 SNP. Five seeds of each accession were planted in a pot (21cm height and 23 cm width) in the screen house of the Institute for Agricultural Research, Zaria, Nigeria in 2013.

At 21 days after planting (DAP), the youngest and most fresh leaf sample was detached from the parent plant, placed on the Harris Cutting Mat, and gently pushed the Harris Uni-Core vertically through the leaf to take 4 discs from each genotype. The plunger was used to eject the sample disc from the Harris Uni-Core into the corresponding well of 96 well plate. The plates were well labeled according to the number of germplasm contained and blank well as suggested by the Kbioscience sampling protocol. Possible cross-contaminants were eliminated (Chum and Andre, 2009) by rinsing plunger and cutting end of the tool in a sodium hypochlorite (2% NaClO) solution and distilled water. The Harris cutting mat was periodically cleaned with the same decontaminants as the cutting tool, to further reduce the chance of cross-contamination.

A perforated heat seal was placed on top of the storage plate containing the final number of samples disc. A conventional heat-sealer was used at 155°C for 2 seconds to seal up the plates. Similarly, a brief visual inspection was carried out to ensure that the wells were sealed. The plates were packaged with small desiccant bag to remove air. The second bag was then sealed up for additional protection. The plates were then shipped to Kbioscience in UK for genotyping.

### **SNP Genotyping**

KASPar technology was used for genotyping at Kbioscience also known as LGC genomic laboratory in the United Kingdom. One hundred and nineteen (119) SNP markers (Appendix 4.2) were selected from the 1152 optimized SNP markers for cowpea, by the Generation challenge Program (GCP). Genotyping was conducted using KASP primer mix, KASP master mix and the extracted DNA samples, by following Kbioscience protocol as described by Thomson (2013).

### **4.3 Data analysis**

The 9 grain quality traits were recorded in 200 accessions. Descriptive statistic was performed on quality traits using MS excel (2010). The descriptive statistics generated were used

to construct frequency graphs for each of the accession quality traits recorded. Principal component analysis (PCA) was performed using GenStat 15th edition, to determine the most significant variables associated with nutritional grain quality traits. PROC CORR for correlation analysis using Pearson's method was run to determine the association between nutritional quality traits (protein, iron and zinc) and physicochemical parameters. Power marker was used to determine, the number of allele per locus, allele frequency, gene diversity also known as expected heterozygosity ( $H_e$ ). The allele frequency data was converted to bi-allelic and then estimated gene diversity at each locus according to Nei *et al.* (1983) and polymorphism information content (PIC) were also estimated using Power marker version 3.25 (Liu & Muse, 2005). Following the equation of Botstein *et al.* (1980)

$H_e = 1 - \sum P_j^2$  : Where;  $P_{ij}$  = is the frequency of  $j$  allele for  $I$  locus across summed of locus

$$PIC = 1 - \sum_{i=1}^n P_i^2$$

Where;  $P_i$  is the frequency of the  $j^{\text{th}}$  allele for the  $i^{\text{th}}$  marker, and summed over  $n$  alleles, it was used to represent the information value of a marker for detecting  $P^{\text{th}}$   $j^{\text{th}}$   $i^{\text{th}}$  respectively. It depends on the number of detectable alleles and their frequency. Genetic distance (GD) for each pair of population was calculated using Nei *et al.* (1983) and a dendrogram using unweighted pair group method with arithmetic average (UPGMA) was generated using Power marker 3.25 (Liu & Muse, 2005) and viewed on Mega 5.0 software.

## 4.4 Results

### 4.4.1 Soil physicochemical properties of the trial site

The soil physicochemical properties are presented in Table 4.3. The level of iron and zinc content in the soil were generally low with less than 3 and 1 Cmol/kg for iron and zinc, respectively. Particle size distribution revealed that the soil was generally sandy-loam. Particle sizes were evenly distributed with approximately 1:1:4 ratios of clay silt and sand respectively. The surface soils were sandy loam, while the second layer (15-30 cm) was sandy-clay with small amount of loamy soil. The organic carbon content was low (ranged from 0.16 to 0.37), with adequate range (6.10-8.60 mol/kg) of cation exchange capacity (CEC) within the plots. The soil was generally acidic with pH ranging from 5.7 to 6.10.

**Table 4.3: Soil physicochemical properties of the evaluation site in 2013 and 2014 rainy seasons**

Source/ Location	Particle size Distribution Corrected to 20 C (%)				pH ratio 1:2.5 (%)				[ Cmol 1kg]				
	Clay	silt	sand	Textural Class	Water	Cacl <sub>2</sub>	OC	TN	Ca	Zn	K	Fe	CEC
R1 (1-33)	16	20	64	S L	5.90	4.80	0.37	0.350	3.20	0.42	0.23	2.65	6.80
R1(34-67)	24	20	56	SCL	6.20	4.60	0.27	0.104	4.00	0.16	0.22	1.48	8.60
R2 (68-102)	14	20	66	SL	6.10	4.80	0.31	0.350	4.40	0.39	0.18	2.57	8.20
R2(103-136)	22	18	60	SCL	5.90	4.50	0.21	0.082	4.00	0.20	0.16	1.30	7.80
R3 (137-169)	16	16	68	SL	5.70	4.60	0.31	0.105	2.40	0.38	0.23	2.65	6.10
Plant (168-200)	24	14	62	SCL	5.80	4.80	0.16	0.082	3.40	0.22	0.17	1.39	6.50

SL= sandy loam, SCL= sandy clay loam in the textural classification column, Numbers within the brackets in the first column are the accessions grown within a range, R1, R2 and R3= different portions where soil was collected and analyzed.

## 4.4.2 Variations among cowpea accessions at morphological and molecular level

### 4.4.2.1 Descriptive analysis of growth habit and grain quality traits for 200 cowpea

#### Accessions

Frequency distribution of the six qualitative traits: grain coat texture, grain coat and eye colour pattern, 100 grain weight, shattering, growth habit and maturity period of the 200 cowpea accessions are presented in Fig. 4.1a to 4.1f, respectively.

One hundred and thirty four of the accessions (67%) had smooth grain coat texture, while only 66 (33%) showed rough grain coat texture (Fig. 4.1a).

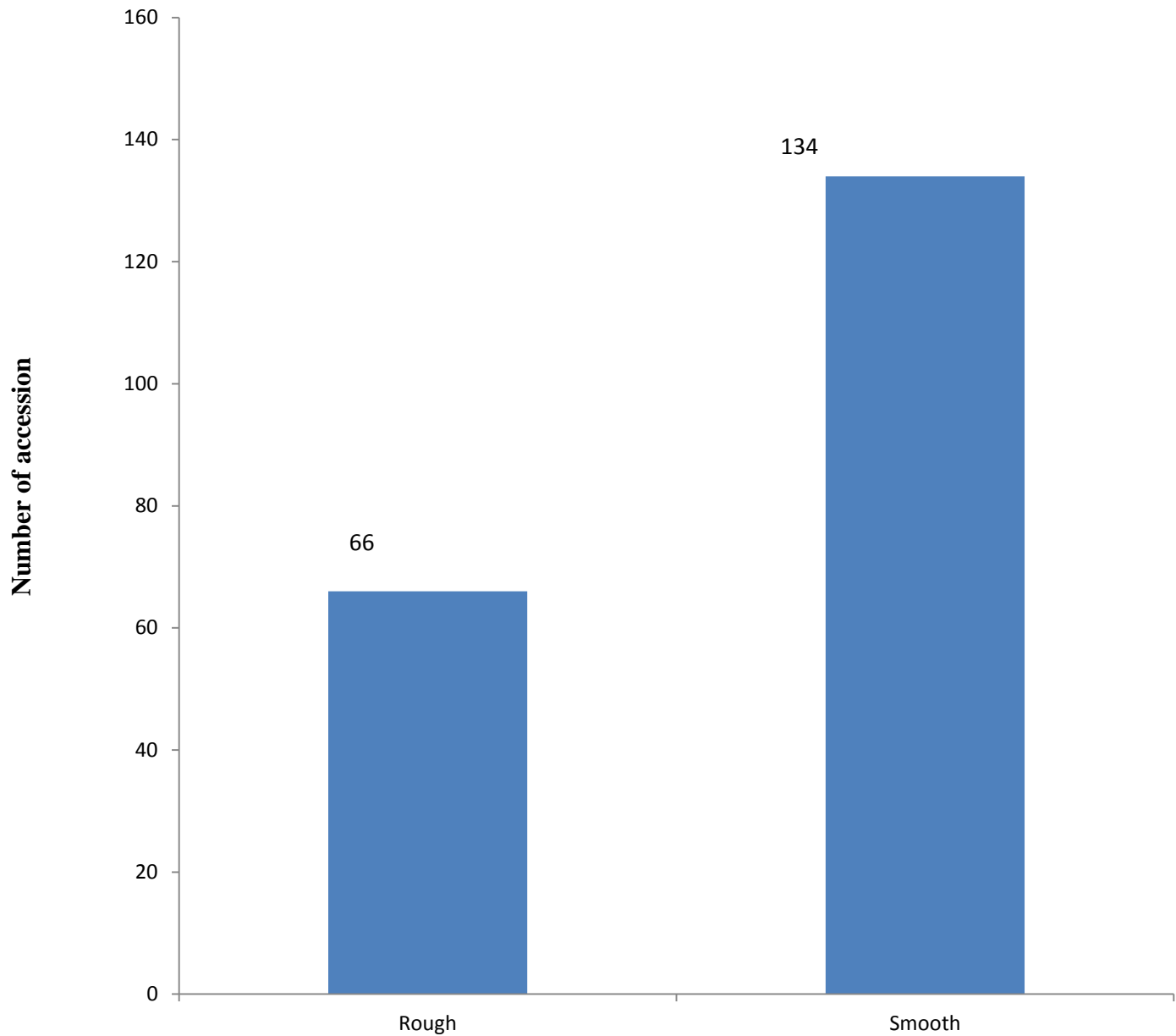
Five descriptive grain coat colour pattern: Holstein, small eye, solid, Watson and Whippoorwill were observed (Fig. 4.1b). Sixty five (32.5%) of the accessions were Holstein, with definite eye margin extended over large portion of their grains, followed by 46 (23%) accessions with solid colour. Other colours observed included: 38 (19%) accessions having small eye, 29 (14.5%) Watson and 22 (11%) Whippoorwill.

More than half (54%) had medium grain size followed by the large grained (35%) accessions and small grained cowpea accessions had the least (11 %) number (Fig. 4.1c).

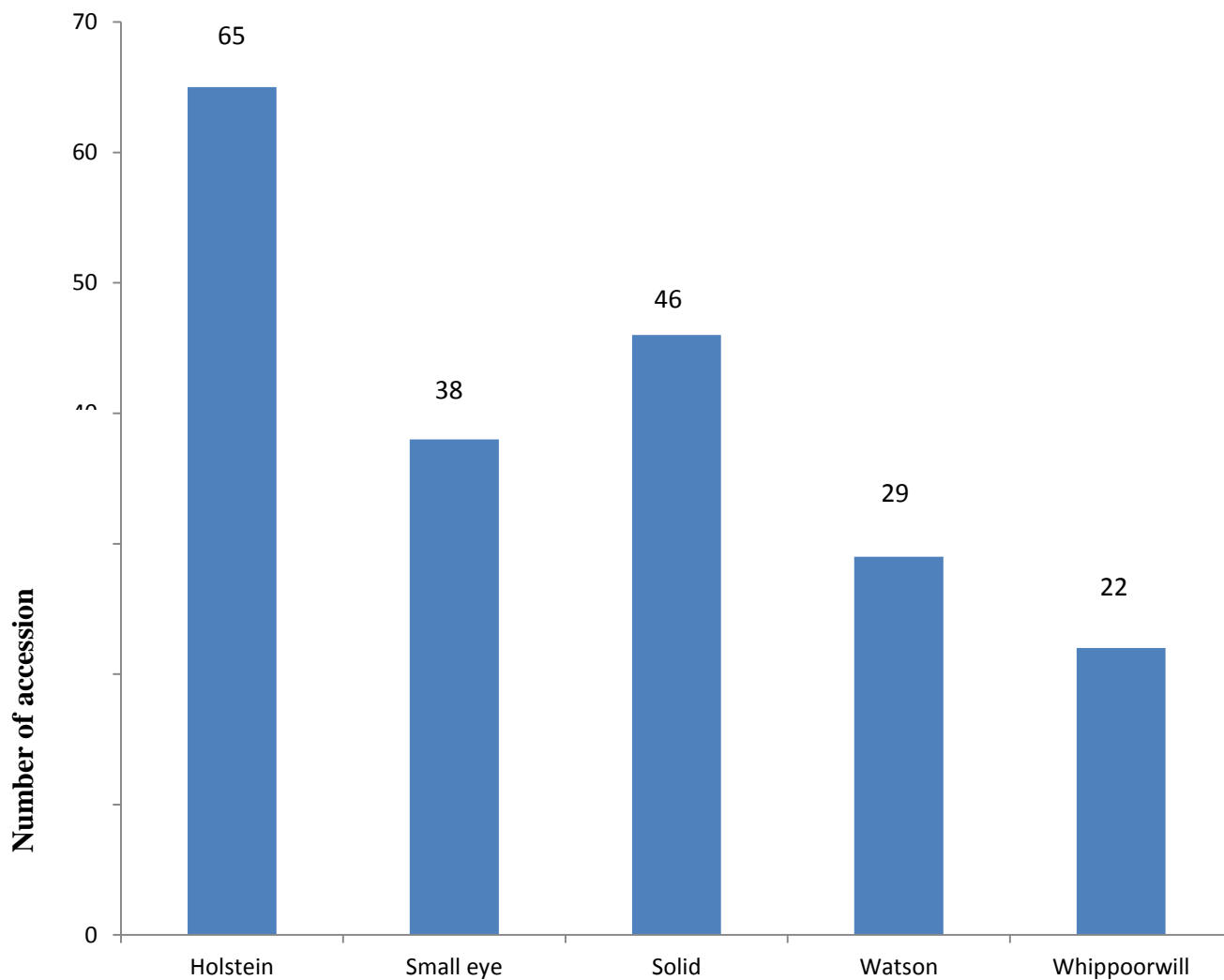
One hundred and seventy nine (89.5%) accession were normal (did not shatter), while 21 (10.5%) of the accessions showed a level of pod shattering before 10 % pod maturity and runs throughout the remaining period of maturity. Most of these accessions were small grained type (Fig. 4.1 d).

Eighty six (43.0%) of the accessions showed semi erect growth habit, followed by 69 (34.5%) prostrating type and 45 (22.5%) erect type (Fig. 4.1 e)

Ninety seven (48.5%) of the 200 accessions matured late, followed by 68 (34.0%) and 35 (17.5%) for the medium and early maturing respectively (Fig. 4.1 f).



**Figure 4.1 a: Cowpea descriptors (IBPGR, 1983; Mahalakshmi *et al*, 2007) showing the distribution of seed coat texture.**



**Figure 4.1 b: Distribution of seed coat colour pattern in cowpea accessions (Saunders, 1959)**

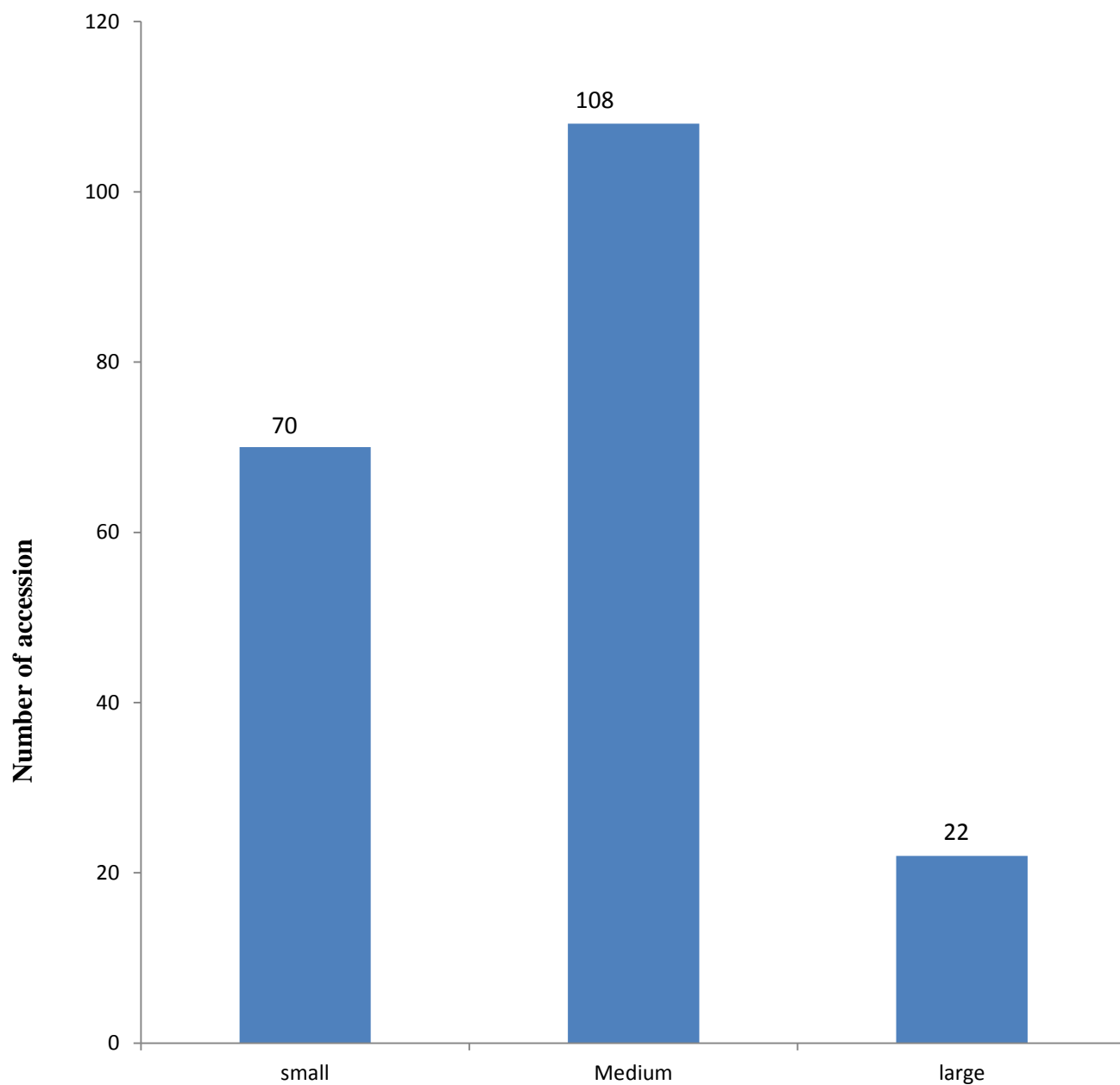
**Holstein**= seed colour has definite eye margin which extends over a large portion of the seed coat

**Small eye**= Colour around the hilum is discontinuous, consisting of two separate elongated colour on either side of, or parallel to, the hilum.

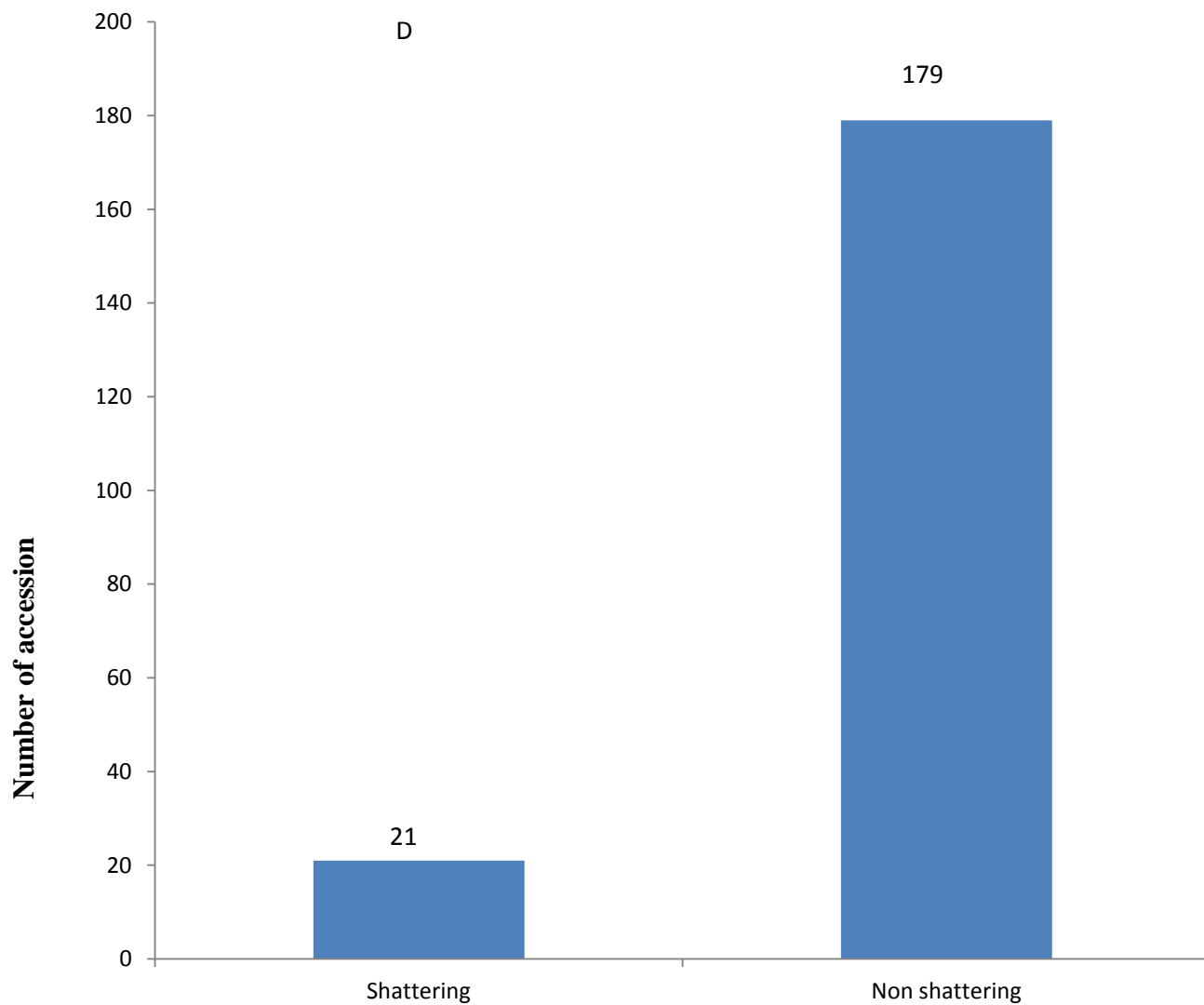
**Solid** = Seed colour extends more or less evenly over the entire seed coat.

**Watson** = Edges of coloured area around hilum are not sharply demarcated but are broken up into separate fine spots especially at the micropylar end of seed.

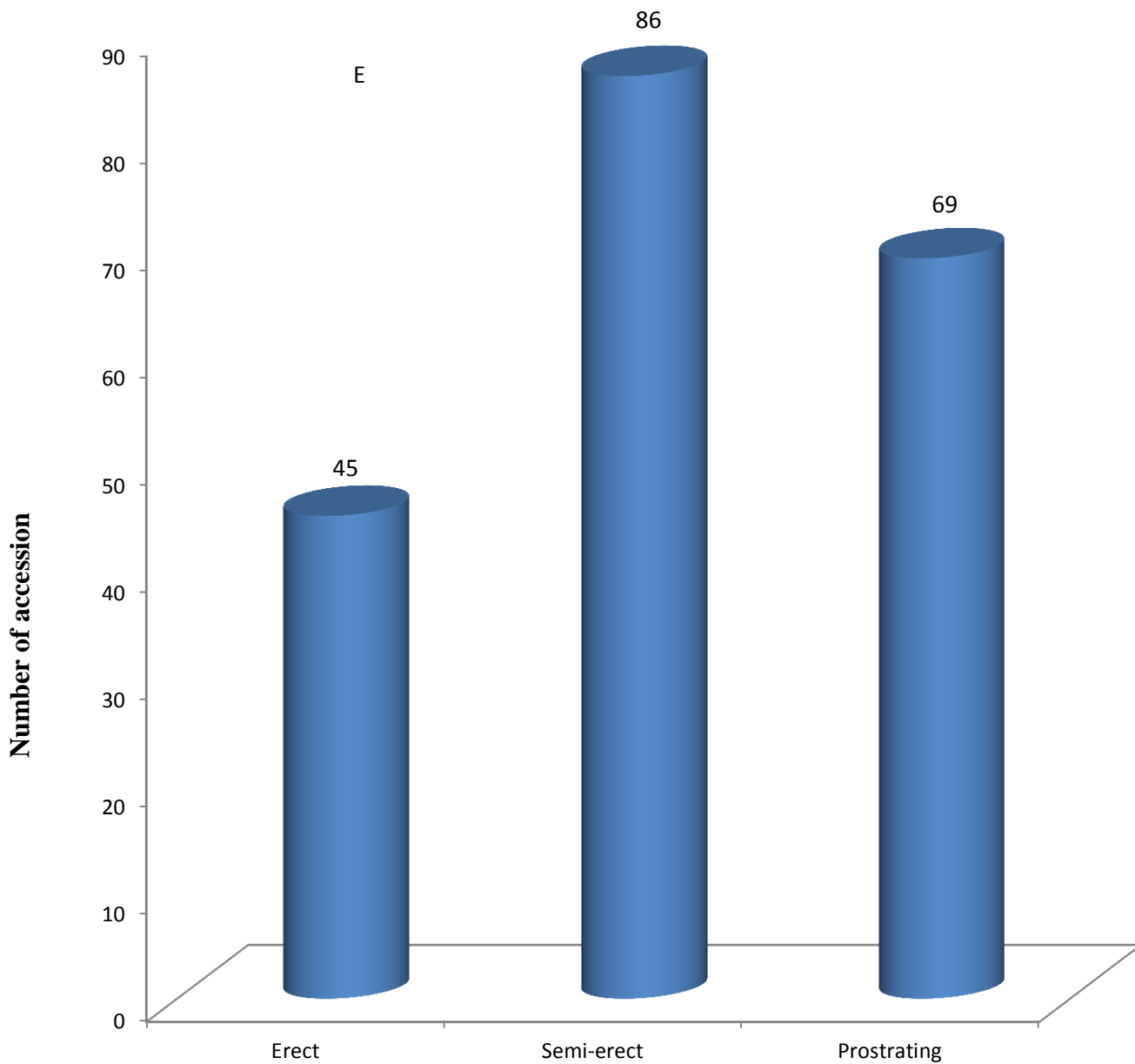
**Whippoorwill** = Seed coat has irregular areas of dark shade separated by lighter area.



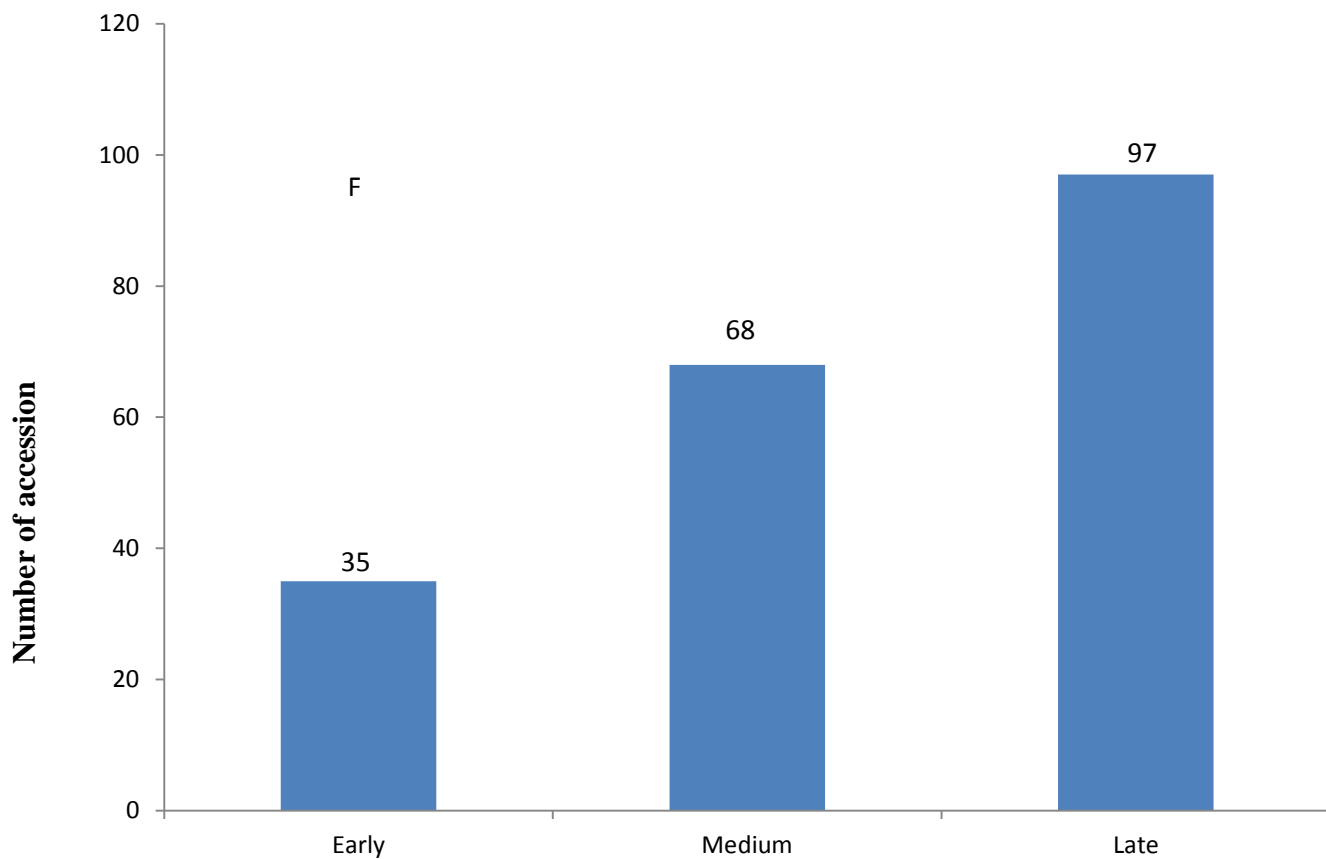
**Figure 4.1 c: Distribution of seed size based on 100 seed weight (g) Cowpea descriptors (Mahalakshmi *et al*, 2007)**



**Figure 4.1 d: Distribution of cowpea accessions based on shattering or non-shattering**  
Mahalakshmi *et al*, 2007



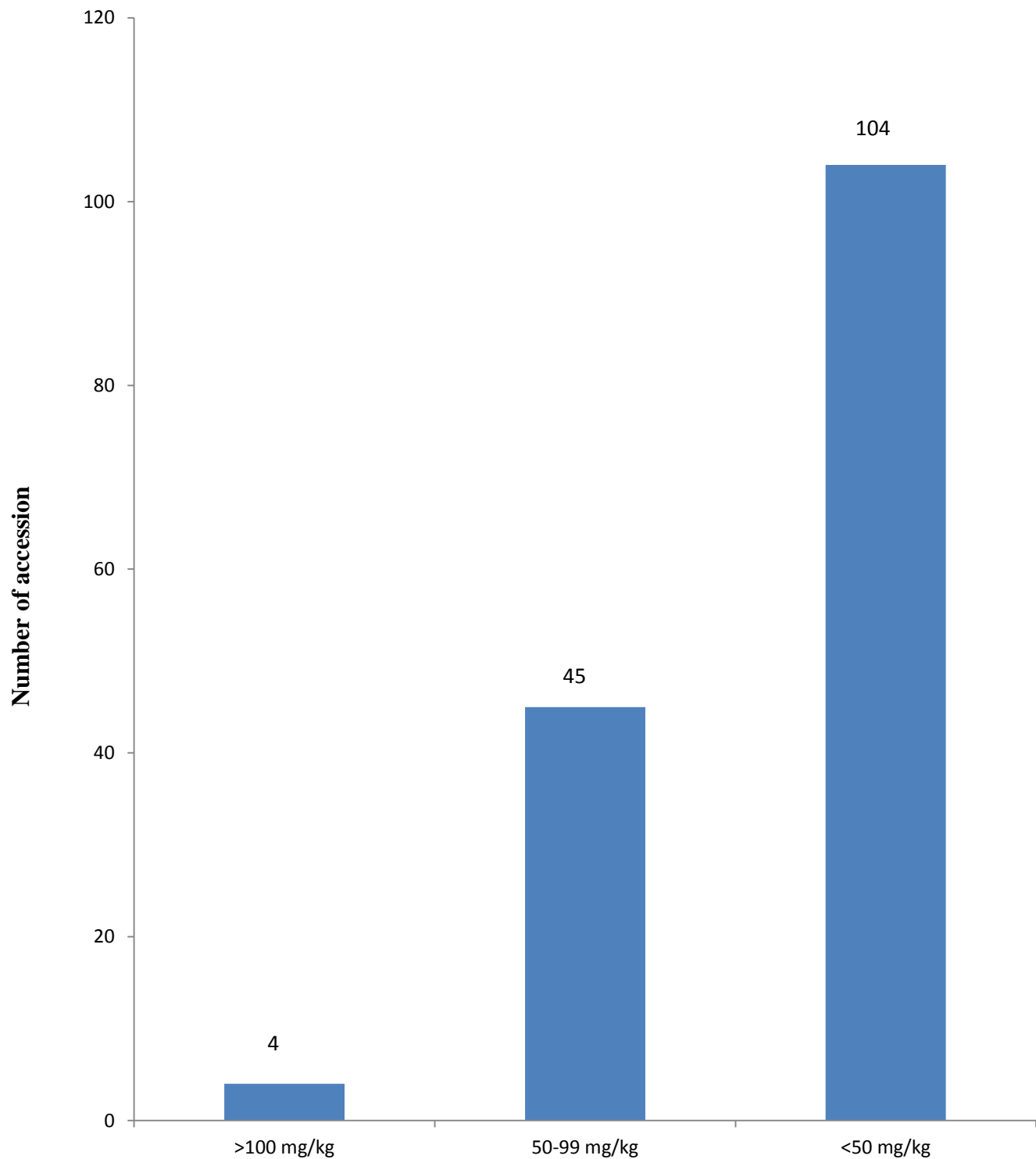
**Figure 4.1 e: Distribution of growth habit among 200 cowpea accessions (Mahalakshmi *et al*, 2007)**



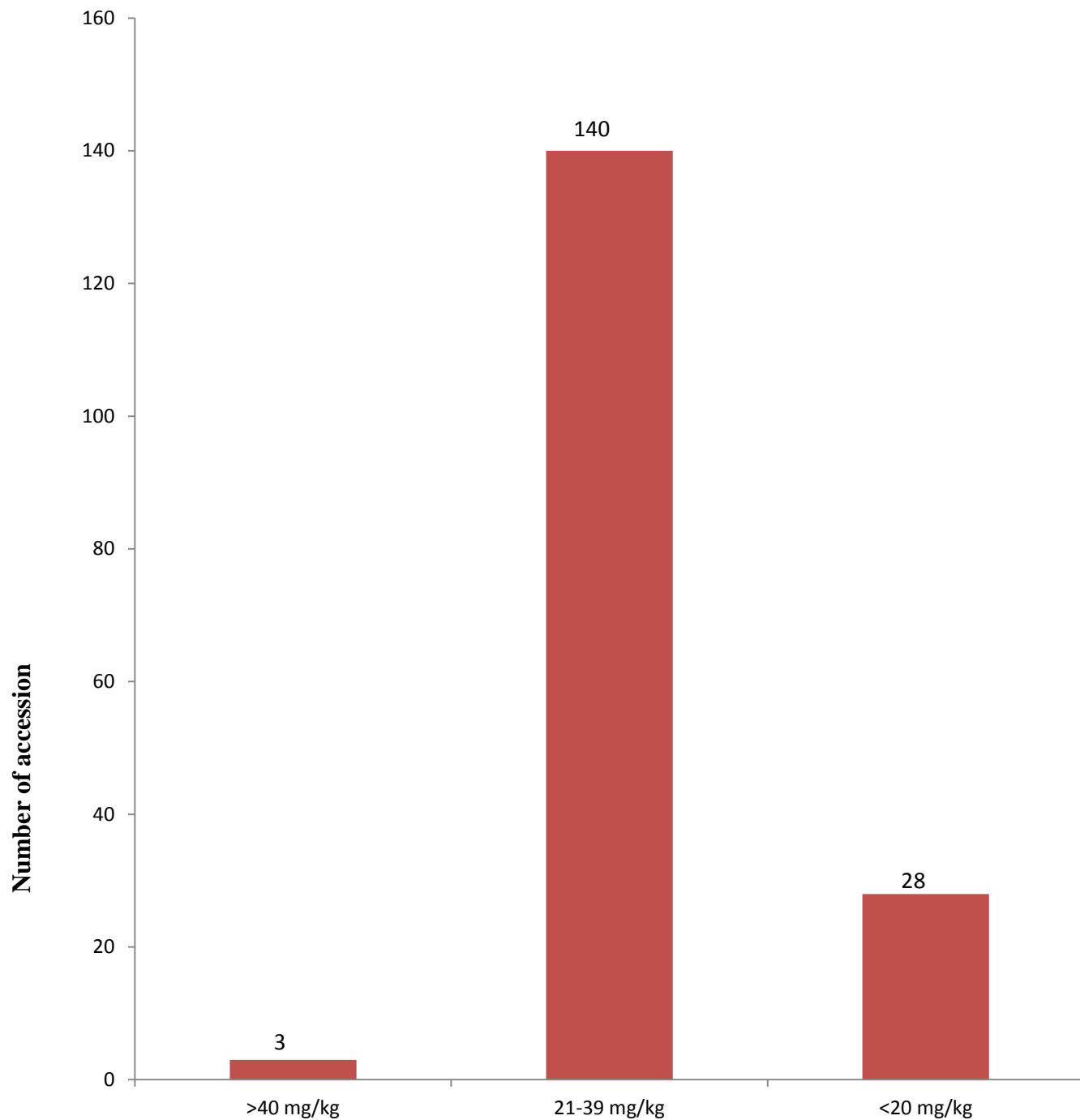
**Figure 4.1 f: Distribution of maturity periods of the cowpea accessions used Mahalakshmi *et al*, 2007**

#### **4.4.2.2 Variation in iron, zinc and protein contents within 153 African cowpea accessions**

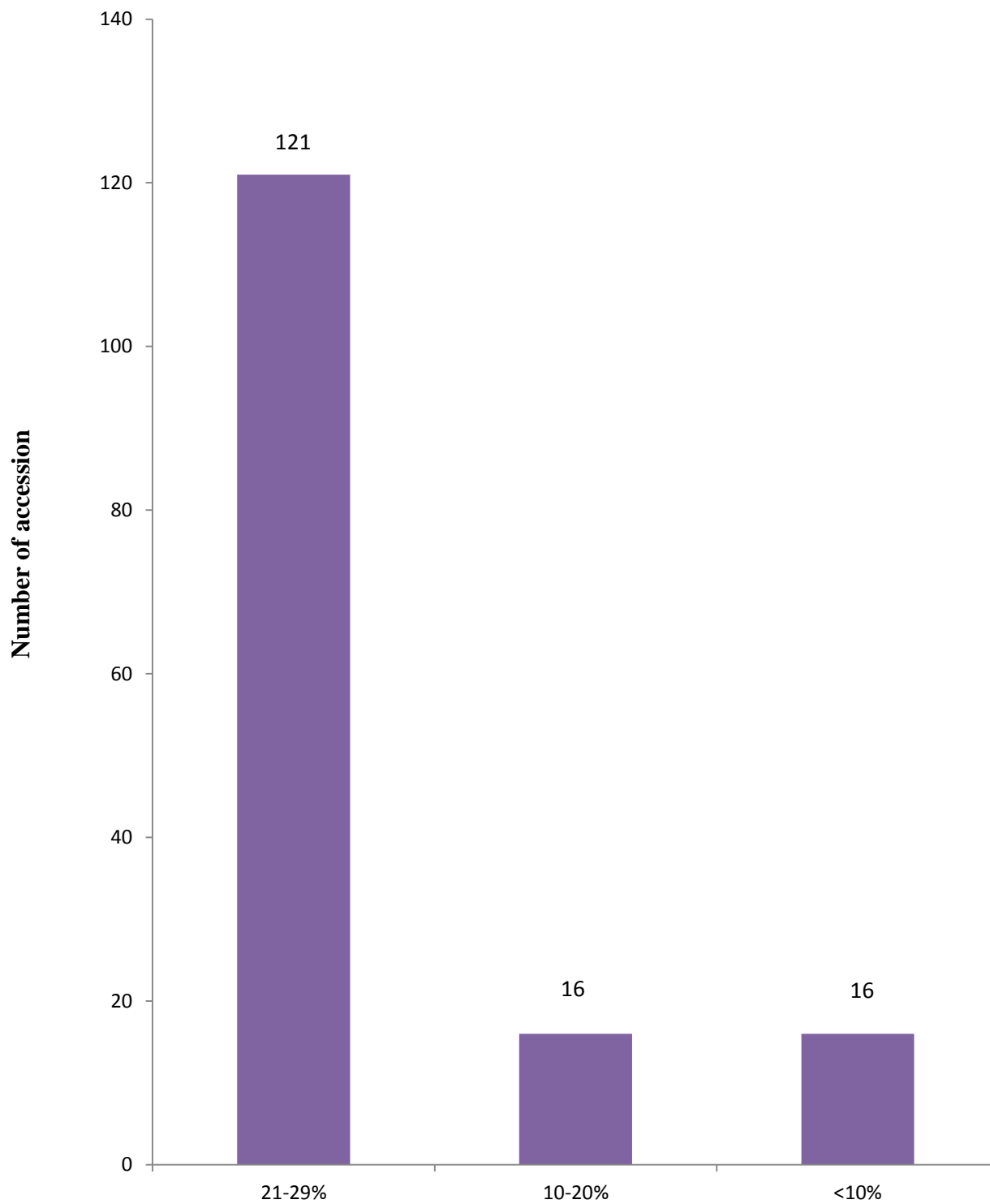
The accessions were grouped into 3 groups namely; high, medium and low iron, zinc and protein contents, respectively (Fig. 4.2 a, b and c). Four of the accessions had high iron content (mean iron concentration >100 mg/kg), 45 accessions had intermediate iron content (50-99 mg/kg) and 104 showed low iron content of less than 50 mg/kg (Fig 4.2a). The four accessions with high iron content were TVu-1330, TVu-13495, TVu-8751 and 16400 with a mean grain iron concentration >100 mg/kg. Almost all the accessions (140) showed medium grain zinc concentration of 21-39 mg/kg and more than two-third (121) of the accessions had high protein content ranged between 21 and 29% (Fig 4.2b and C).



**Figure 4.2 a: Frequency distribution of African cowpea accessions based on grain iron concentration as determined by Atomic absorption spectrophotometry**



**Fig 4.2 b: Frequency distribution of African cowpea accessions based on zinc concentration as determined by Atomic absorption spectrophotometry (AAS)**



**Figure 4.2 c: Frequency distribution of African cowpea accessions based on percentage grain protein content**

Variation in iron concentration, zinc concentration, protein contents and other physicochemical parameters are presented in appendix 4.2. Due to non-positive correlation of 3 important grain nutritional values (iron, zinc and protein), top 10 and least 10 of the accessions are selected each from iron, zinc and protein set and presented in Table 4.4. Mean grain iron concentration ranged from 1.01 to 325.15 mg/kg with a mean of 45.83 mg/kg. The highest iron concentration was recorded in TVu-1330 (329.15 mg/kg) followed by TVu-13495 (131.65 mg/kg) and TVu-16400 (123.53 mg/kg) and they originated from Zambia, Malawi and Benin. The accession from Ghana had the lowest iron concentration of 10.9 mg/kg (Table 4.4).

Mean zinc content range from 10.01 mg/kg to 52.03 mg/kg with a mean of 26.66 mg/kg among 153 accessions (Table 4.5). The highest zinc concentration was recorded in TVu-15251 (52.03 mg/kg), followed by TVu-301 (51.8 mg/kg) and TVu-9725 (40.12 mg/kg) and they originated from Congo, Nigeria and Ghana respectively. TVu-15058 and TVu-1801 from Zambia and Malawi had the lowest zinc concentration of 10 and 13.46 mg/kg respectively (Table 4.4). The mean percentage of protein content was 20.9 %. It ranged from 1.7 % in TVu-544 from Nigeria to 29.9% for TVu-1185 from Uganda (Table 4.4).

**Table 4.4 Different set of accessions representing top 10, least 10, overall mean, standard deviation and ranges of iron, zinc and protein contents of 153 African cowpea accessions**

	Accession	Iron content	Accession	Zinc content	Accession	Protein content
Top ten	TVu-1330	329.15	TVu-15251	52.03	<b>TVu-13495</b>	<b>29.93</b>
	<b>TVu-13495</b>	<b>131.65</b>	TVu-301	51.80	<b>TVu-13088</b>	<b>29.89</b>
	TVu-16400	123.53	<b>TVu-9725</b>	<b>40.12</b>	TVu-15187	29.59
	TVu-8751	108.65	TVu-7999	38.75	TVu-347	29.41
	<b>TVu-9725</b>	<b>99.42</b>	<b>TVu-13088</b>	<b>38.30</b>	TVu-13468	29.37
	TVu-999	98.05	TVu-15686	37.45	TVu-13867	29.30
	TVu-4489	93.97	TVu-15107	36.62	TVu-13457	28.71
	<b>TVu-13088</b>	<b>92.01</b>	TVu-15141	36.24	TVu-7362	28.59
	TVu-15892	89.55	TVu-15719	36.15	TVu-8745	27.80
	TVu-16521	87.68	TVu-15685	35.88	TVu-997	27.80
least ten	TVu-15694	3.60	TVu-158	17.50	TVu-2397	2.69
	Ghana	3.28	TVu-15164	17.21	TVu11462	2.60
	TVu-1	1.50	TVu-8407	16.37	TVu-746	2.56
	TVu-16483	1.50	TVu-14845	15.15	TVu-59	2.44
	TVu-15694	1.13	TVu-13495	15.07	TVu-1706	2.38
	Ghana	1.12	TVu-433	15.00	TVu-875	2.17
	TVu-1	1.12	TVu-8742	14.87	TVu-971	2.17
	TVu-16483	1.11	TVu-8454	14.54	TVu-1171	2.16
	TVu-1184	1.10	TVu-15058	13.46	TVu-526	2.09
	TVu-1185	1.01	TVu-1801	10.00	TVu-14845	1.72
	<b>Mean</b>	<b>45.83</b>		<b>26.66</b>		<b>20.98</b>
	<b>STD</b>	<b>34.20</b>		<b>6.35</b>		<b>6.76</b>
	<b>Range</b>	<b>1.01-329.15</b>		<b>10.0 - 52.03</b>		<b>1.72 - 29.93</b>

STD= Standard deviation of the population; mean= population mean

#### 4.4.2.3 Variation in physicochemical properties of the United States and three Asian cowpea accessions

The first three accessions (TVu-320, TVu-329 and TVu-3710) on the table 4.5 are from Philippines, they showed no significant variation among them in all the physicochemical properties analyzed. The U.S. accessions had mean iron content ranging from 1.04 mg/kg for TVu-30 to 58.36 mg/kg for TVu-1616 with a population mean of 17.73 mg/kg (Table 4.5). Zinc mean content varied significantly (16.25 to 386.53 mg/kg) among the accessions. TVu-332 had the least mean zinc content of 16.25 mg/kg, while TVu-1616 had the highest mean zinc content of 386.53 mg/kg (Table 4.5). Protein content among American accessions ranged from 1.21 % to 23.10 % shown by TVu-84 and TVu-8330, respectively.

**Table 4.5: Mean physicochemical properties of the United State and three Asian cowpea accessions**

<b>Accessions</b>	<b>Iron (mg/kg)</b>	<b>Zinc (mg/kg)</b>	<b>% Moisture</b>	<b>% Fat</b>	<b>% Ash</b>	<b>% Protein</b>	<b>% Fibre</b>	<b>% CHO</b>
TVu-320	14.84	25.38	4.72	2.54	2.41	20.18	2.90	70.15
TVu-3629	15.84	23.19	6.74	2.58	3.65	18.16	2.55	68.87
TVu-3710	34.52	27.81	6.64	2.19	2.91	22.05	2.00	66.24
TVu-274	1.12	25.35	6.58	22.12	2.45	2.23	2.85	66.62
TVu-36	1.41	25.29	6.48	22.14	2.98	2.17	2.25	66.62
TVu-84	9.92	27.87	7.05	22.08	2.06	1.21	2.65	67.60
TVu-990	1.14	36.99	6.58	22.00	2.65	1.33	1.70	67.44
TVu-10454	17.80	23.43	6.00	2.48	2.32	22.16	1.85	67.04
TVu-1236	24.44	35.86	8.44	20.96	2.69	2.07	2.15	65.84
TVu-15360	4.80	38.59	7.00	2.69	2.22	22.70	1.05	65.39
TVu-15381	2.16	21.87	5.02	2.51	2.36	22.14	2.04	67.97
TVu-2672	1.13	27.77	7.04	2.02	3.99	22.28	2.70	64.67
TVu-7188	1.12	27.81	5.96	2.27	3.86	22.44	1.74	65.47
TVu-7293	1.13	23.92	6.30	2.82	2.54	22.74	2.05	65.60
TVu-8330	1.11	36.21	6.27	2.03	2.75	23.10	1.82	65.85
TVu-8612	14.31	31.44	4.38	2.21	2.01	22.84	1.64	68.56
TVu-8619	14.16	25.85	6.32	2.62	3.98	22.66	1.66	64.42

**Table 4.5: (Cont'd) Mean physicochemical properties of the United State and three Asian cowpea accessions**

<b>Accessions</b>	<b>Iron (mg/kg)</b>	<b>Zinc (mg/kg)</b>	<b>% Moisture</b>	<b>% Fat</b>	<b>% Ash</b>	<b>% Protein</b>	<b>% Fibre</b>	<b>% CHO</b>
TVu-117	1.12	24.46	6.57	2.07	2.17	22.46	1.72	66.73
TVu-1272	13.51	19.58	6.39	2.05	3.80	22.74	2.80	65.02
TVu-1251	14.22	22.18	3.15	2.84	2.96	22.18	2.95	68.87
TVu-1494	10.96	26.11	5.17	2.16	2.93	22.74	2.75	67.08
TVu-1560	17.26	22.73	5.35	2.12	2.67	22.42	1.30	67.44
TVu-1562	32.51	22.38	3.56	2.98	2.37	22.28	2.75	68.84
TVu-1616	58.36	386.53	4.13	2.65	2.03	22.48	2.75	68.71
TVu-1715	26.68	21.49	4.50	2.26	2.15	22.18	1.75	68.91
TVu-1985	33.37	26.66	4.34	2.56	2.56	22.54	1.10	68.00
TVu-1986	33.75	25.70	4.20	2.33	1.55	22.80	1.40	69.12
TVu-202	1.72	19.84	4.45	2.14	2.29	22.62	1.05	68.50
TVu-2155	10.73	164.80	4.04	2.51	2.37	23.10	1.24	67.98
TVu-232	20.95	45.02	4.39	2.49	2.00	22.64	2.90	68.48
TVu-233	35.17	36.19	5.92	2.12	2.55	22.18	4.82	67.23
TVu-297	13.60	18.24	4.50	2.27	2.18	22.42	2.60	68.63
TVu-30	1.04	21.47	5.78	2.82	2.75	22.84	1.46	65.81
TVu-315	14.31	18.38	4.41	2.65	2.28	22.16	3.80	68.50
TVu-332	24.09	16.57	4.18	2.57	2.88	22.80	2.75	67.57
TVu-337	27.79	19.55	4.43	2.64	2.56	22.18	1.15	68.19
TVu-374	8.98	24.93	0.81	6.90	2.20	22.18	2.10	67.91
TVu-384	8.87	23.97	0.25	6.73	2.50	19.42	2.90	71.10
TVu-387	32.40	19.70	0.89	6.59	2.43	21.16	2.45	68.93
TVu-393	25.27	21.13	0.16	7.17	2.69	22.10	1.14	67.88
TVu-408	1.19	20.00	1.69	7.52	2.99	20.92	1.98	66.88
TVu-415	43.93	20.97	0.70	7.13	2.42	22.12	2.05	67.63
TVu-430	42.88	19.41	1.01	6.28	3.99	21.84	2.15	66.88
TVu-456	41.34	26.14	0.92	6.30	5.23	21.80	2.95	65.25
TVu-566	27.93	26.62	0.64	6.18	2.80	22.64	2.80	67.74
TVu-697	26.96	25.75	1.24	6.06	2.84	22.12	1.86	67.74
TVu-13305	21.40	20.42	0.92	6.12	2.67	22.74	2.54	67.55
<b>Mean</b>	<b>17.73</b>	<b>35.99</b>	<b>4.39</b>	<b>5.48</b>	<b>2.72</b>	<b>19.98</b>	<b>2.20</b>	<b>67.44</b>
<b>STD</b>	<b>14.3</b>	<b>56.38</b>	<b>2.26</b>	<b>5.99</b>	<b>0.67</b>	<b>6.41</b>	<b>0.76</b>	<b>1.43</b>
<b>Range</b>	<b>1.04-58.36</b>	<b>16.57-386.53</b>	<b>0.16-8.44</b>	<b>2.02-22.14</b>	<b>1.55-5.23</b>	<b>1.21-23.10</b>	<b>1.05-4.82</b>	<b>64.42-71.10</b>

All accessions are from USA except the first 3 that are from Philippines; CHO= carbohydrate; STD= standard deviation.

#### 4.4.3 Principal Component Analysis

Principal component analysis was performed to identify the most contributing traits to the total variability observed. The results of the Principal component analysis for the 9 nutritional grain quality traits are presented in Table 4.6. The traits analyzed were: iron, zinc, protein, moisture content, fats content, ash content, fibre content, carbohydrate content and 100 grain weight. The first 4 principal components axis had eigenvalues greater than one (eigenvalues  $>1$ ) and contributed to more than half (63.00%) of the total variation, hence, they were retained. The first principal component contributed 23.36% of the total variation, which were contributed by fat (0.5836), protein (0.6482), and grain iron concentration (0.3258). Fifteen point twenty four percent (15.24%) of the total variation was contributed by 100 grain weight (0.4057), carbohydrates (0.5313) and fibre (0.3562) clustered on the second principal component axis. Similarly, ash (0.4664), fibre (0.5313) and moisture (0.303) content were concentrated in the third principal component (PC3) axis, and contributed 12.69% of the total variation. In the fourth principal component (PC4) axis, iron concentration (0.4918), zinc concentration (0.7004) and fibre content (0.3813) contributed 11.72% to the total variance.

**Table 4.6: Principal Component Analysis showing the contribution of grain quality traits to the total variation among the cowpea accessions**

	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<b>PC4</b>	<b>PC5</b>
<b>100 grain weight</b>	0.0052	<b>0.40579</b>	0.25123	0.22905	0.79554
<b>Ash</b>	0.24767	-0.27133	<b>0.46648</b>	0.13522	0.06018
<b>Carbohydrate</b>	-0.24476	<b>0.55776</b>	-0.26242	0.05335	-0.30477
<b>Fat</b>	<b>-0.58362</b>	-0.23556	0.30046	-0.08529	0.09534
<b>Fibre</b>	-0.03323	<b>0.3562</b>	<b>0.53132</b>	<b>-0.38136</b>	-0.22011
<b>Moisture</b>	-0.10247	-0.472	<b>-0.30303</b>	-0.18679	0.23672
<b>Protein</b>	<b>0.64827</b>	0.09441	-0.16623	0.07909	0.03027
<b>Iron</b>	<b>0.32586</b>	-0.09898	0.26449	<b>-0.49188</b>	-0.11517
<b>Zinc</b>	-0.00829	0.16358	-0.29734	<b>-0.70044</b>	0.37795
<b>Eigenvalue</b>	2.102	1.371	1.142	1.054	0.912
<b>%variation</b>	23.36	15.24	12.69	11.72	10.13
<b>% cumulative Variance</b>	23.36	38.60	51.28	63.00	73.13

#### 4.4.4 Correlations among nutritional quality traits of 200 cowpea accessions

The cowpea grain nutritional quality traits are presented in table (Table 4.7). Iron content did not correlate significantly with zinc, ash, fibre and 100 grain weight. Iron content was negatively correlated with Fat ( $r = -0.18$ ,  $p < 0.007$ ) and carbohydrate ( $r = -0.18$ ,  $p < 0.007$ ). On the other hand, iron content was positively correlated ( $r = 0.26$ ,  $p < 0.001$ ) with protein. fat content was negatively correlated with ash content ( $r = -0.13$ ,  $p < 0.05$ ) and protein content ( $r = -0.85$ ,  $p < 0.001$ ). Ash content correlated positively with protein ( $r = 0.14$ ,  $p < 0.05$ ) and negatively with carbohydrate ( $r = -0.23$ ,  $p < 0.001$ ). Protein was negatively correlated with carbohydrate ( $r = -0.29$ ,  $p < 0.01$ ).

**Table 4.7: Pearson's correlation coefficients among nutritional quality traits of 200 cowpea accessions**

	<b>Iron (mg/kg)</b>	<b>Zinc (mg/kg)</b>	<b>% Moisture</b>	<b>% Fat</b>	<b>% Ash</b>	<b>% Protein</b>	<b>% Fibre</b>	<b>% CHO</b>	<b>100 Swt (g)</b>
Iron	1								
Zinc	0.039	1							
Moisture	-0.018	0.014	1						
Fat	<b>-0.189***</b>	-0.022	0.056	1					
Ash	0.121	-0.082	-0.034	<b>-0.137*</b>	1				
Protein	<b>0.269***</b>	0.004	-0.154	<b>-0.858***</b>	<b>0.139*</b>	1			
Fibre	0.094	0.026	-0.120	0.024	0.008	-0.067	1		
CHO	<b>-0.188***</b>	0.061	-0.127	-0.060	<b>-0.239***</b>	<b>-0.299***</b>	0.098	1	
100swt (g)	-0.043	-0.000	-0.116	-0.017	-0.017	0.038	0.098	0.079	1

\*, \*\* and \*\*\* =Significance at 0.05, 0.01 and 0.001 respectively; CHO= carbohydrate; swt= seed weight

#### 4.4.5 Allelic variation based on SNP markers

One of the 170 accessions was not genotyped due to low DNA quantity and quality, respectively. As a result of that, the genetic diversity of 169 cowpea accessions was assessed using 119 SNP markers.

Summary statistics for number of alleles, gene diversity and polymorphic information content (PIC) are presented in Table 4.8. A total of 321 alleles with an average of 3 loci per allele were observed among the accessions. The gene diversity ranged from 0.269 to 0.535 in 7344\_500 SNP and 14929\_258 SNP with a mean of 0.4732. This tends to validate the homozygotic nature of the accessions used being a core collections. This is due to the fact that cowpea is a self-pollinating crop and the level of out crossing is minimal (less than 4%). The allele frequency of all the markers used was generally below 0.95, indicating that they are polymorphic markers. Polymorphism information content (PIC) values ranged from 0.2366 (7344\_500 SNP) to 0.427 in

4749\_1972 and 14929\_258 markers with an average of 0.3713 PIC. Twenty four of the 119 markers were found to be highly informative with high PIC values range from 0.4008 to 0.4273.

The statistics describing the genetic diversity found at each locus were calculated. The average gene diversity for the whole sample was 0.46. The lowest gene diversity (0.26) was detected in one SNP (7344\_500) marker for all accessions. Eighty eight (88) of the SNP markers showed gene diversity of  $> 0.45$ . The highest gene diversity was observed in 6 SNP markers (14929\_258, 16462\_1286, 6065\_457, 13849\_2039, 14462\_1712 and 15534\_890) with the gene diversity of 5 each (Table 4.8).

**Table 4.8: Allelic frequency, gene diversity and polymorphic information content (PIC)**

S/N	Marker	AF	H <sub>e</sub>	PIC	S/N	Marker	AF	H <sub>e</sub>	PIC
1	10969_452	0.5250	0.4988	0.3744	31	13947_415	0.7564	0.3685	0.3006
2	4146_1588	0.7255	0.3983	0.3190	32	14030_764	0.5833	0.4861	0.3680
3	9673_1553	0.5732	0.4893	0.3696	33	14164_1877	0.7962	0.3246	0.2719
4	10115_384	0.6306	0.4659	0.3574	34	1426_521	0.7044	0.4164	0.3297
5	10661_873	0.5385	0.4970	0.3735	35	14462_1712	0.5032	0.5000	0.3750
6	10738_1400	0.5705	0.4901	0.3700	36	14497_540	0.5094	0.4998	0.3749
7	10811_937	0.5287	0.4984	0.3742	37	14542_452	0.5975	0.4810	0.3653
8	11367_1228	0.7278	0.3962	0.3177	38	14619_471	0.5649	0.4916	0.3707
9	11470_272	0.6855	0.4312	0.3382	39	14714_840	0.6923	0.4260	0.3353
10	11622_232	0.6139	0.4740	0.3617	40	14730_1034	0.5355	0.4975	0.3737
11	12119_480	0.5584	0.4932	0.3716	41	14769_1746	0.6730	0.4402	0.3433
12	12122_559	0.6234	0.4696	0.3593	42	14929_258	0.5000	0.5000	0.3750
13	12261_1773	0.7215	0.4019	0.3211	43	14965_280	0.6250	0.4688	0.3589
14	12393_305	0.5250	0.4988	0.3744	44	15054_315	0.6101	0.4758	0.3626
15	12505_1312	0.5535	0.4943	0.3721	45	15183_436	0.5063	0.4999	0.3750
16	12703_553	0.6452	0.4579	0.3530	46	15534_890	0.5032	0.5000	0.3750
17	12933_387	0.6266	0.4680	0.3585	47	15933_118	0.6289	0.4668	0.3578
18	12959_58	0.5226	0.4990	0.3745	48	16043_314	0.5506	0.4949	0.3724
19	1296_808	0.5316	0.4980	0.3740	49	16239_889	0.6139	0.4740	0.3617
20	13017_290	0.6667	0.4444	0.3457	50	16462_1286	0.5000	0.5000	0.3750
21	13034_542	0.5577	0.4933	0.3716	51	16914_262	0.5723	0.4895	0.3697
22	13207_784	0.7152	0.4074	0.3244	52	17023_955	0.5190	0.4993	0.3746
23	13506_333	0.5541	0.4941	0.3721	53	17450_1553	0.6772	0.4372	0.3416
24	13563_863	0.5975	0.4810	0.3653	54	17588_963	0.5732	0.4893	0.3696
25	13586_1058	0.7205	0.4028	0.3217	55	1799_940	0.5223	0.4990	0.3745
26	13707_697	0.6981	0.4215	0.3327	56	2185_132	0.5924	0.4829	0.3663

**Table 4.8: (Cont'd) Allelic frequency, gene diversity and polymorphic information content (PIC)**

S/N	Marker	AF	He	PIC	S/N	Marker	AF	He	PIC
27	13794_319	0.5472	0.4956	0.3728	57	2314_546	0.7468	0.3782	0.3067
28	13849_2039	0.5031	0.5000	0.3750	58	2339_52	0.5897	0.4839	0.3668
29	13863_519	0.5860	0.4852	0.3675	59	234_249	0.5786	0.4876	0.3687
30	13873_544	0.5570	0.4935	0.3717	60	2728_121	0.5478	0.4954	0.3727
61	2974_1109	0.7468	0.3781	0.3066	91	5428_339	0.5613	0.4925	0.3712
62	2997_519	0.7826	0.3403	0.2824	92	5435_569	0.6013	0.4795	0.3645
63	3098_224	0.6026	0.4790	0.3643	93	5448_461	0.7215	0.4019	0.3211
64	3485_771	0.6000	0.4800	0.3648	94	5503_54	0.5849	0.4856	0.3677
65	361_520	0.5125	0.4997	0.3748	95	5552_536	0.6646	0.4458	0.3465
66	3673_401	0.6433	0.4589	0.3536	96	5553_147	0.6415	0.4600	0.3542
67	3720_560	0.7613	0.3635	0.2974	97	5692_1408	0.5223	0.4990	0.3745
68	38_239	0.6346	0.4638	0.3562	98	593_329	0.5605	0.4927	0.3713
69	3838_830	0.7170	0.4058	0.3235	99	6065_457	0.5000	0.5000	0.3750
70	3885_1019	0.7898	0.3320	0.2769	100	6247_659	0.5438	0.4962	0.3731
71	3900_562	0.7170	0.4058	0.3235	101	6673_1242	0.5098	0.4998	0.3749
72	394_316	0.5094	0.4998	0.3749	102	6700_679	0.7179	0.4050	0.3230
73	395_895	0.5355	0.4975	0.3737	103	7344_500	0.8408	0.2678	0.2319
74	411_247	0.5125	0.4997	0.3748	104	7438_464	0.6250	0.4688	0.3589
75	4325_585	0.6456	0.4576	0.3529	105	7857_1368	0.5605	0.4927	0.3713
76	4339_822	0.6101	0.4758	0.3626	106	7906_1032	0.5935	0.4825	0.3661
77	4403_1123	0.5188	0.4993	0.3746	107	7993_539	0.5786	0.4876	0.3687
78	4462_114	0.5064	0.4999	0.3750	108	8011_481	0.5280	0.4984	0.3742
79	4558_472	0.5723	0.4895	0.3697	109	8118_1675	0.6226	0.4699	0.3595
80	4563_661	0.7290	0.3951	0.3170	110	8166_564	0.5871	0.4848	0.3673
81	4749_1972	0.5064	0.4999	0.3750	111	8193_441	0.6090	0.4762	0.3628
82	4778_497	0.5597	0.4929	0.3714	112	8306_119	0.5443	0.4961	0.3730
83	483_1152	0.7296	0.3946	0.3167	113	8605_2122	0.5063	0.4999	0.3750
84	4892_514	0.6625	0.4472	0.3472	114	8969_1386	0.5096	0.4998	0.3749
85	4904_278	0.5290	0.4983	0.3742	115	9134_1559	0.5163	0.4995	0.3747
86	5058_372	0.6646	0.4458	0.3465	116	9147_1655	0.5705	0.4901	0.3700
87	5239_234	0.5506	0.4949	0.3724	117	9678_835	0.8038	0.3154	0.2657
88	5294_469	0.7756	0.3480	0.2875	118	9880_545	0.5385	0.4970	0.3735
89	534_355	0.6968	0.4226	0.3333	119	9955_544	0.5316	0.4980	0.3740
90	5356_124	0.5597	0.4929	0.3714					
						<b>Mean</b>	<b>0.6057</b>	<b>0.4633</b>	<b>0.3548</b>
						<b>STD</b>	<b>0.0847</b>	<b>0.0486</b>	<b>0.0281</b>
							<b>0.50-</b>	<b>0.26-</b>	<b>0.23-</b>
						<b>Range</b>	<b>0.84</b>	<b>0.50</b>	<b>0.37</b>

STD= standard deviation, H<sub>e</sub>= gene diversity (expected heterozygosity)

#### 4.4.6 Genetic distance

The cluster analysis using unweighted pairs average method (UPGMA) is presented in Fig. 4.3. Clustering was based on the calculated genetic distances and clustered the 169 cowpea accessions into 2 main groups ranging from 0.00 to 0.212 genetic distances. The dendrogram further sub-clustered each of the main clusters based on their related genetic distances regardless of their country of origin. For example, two accessions: TVu-1560 from USA and another TVu-10745 from Sierra Leone had 0.00 genetic distance each and sub-clustered in the same branch. Moreover, the sub-clustering corresponded to biochemical data. For example two accessions TVu-320 and TVu-5040 from Nigeria and Niger with genetic distance of 0.17 each were sub-clustered within the main cluster one (Cluster I), both accession had low mean grain iron concentration of less than 50 mg/kg.

Most of the cowpea accession grouped in the main cluster one, originated from Africa with few US cowpea accessions flanked with at least one African accession that showed similar genetic distance. The second main cluster (cluster II) had also various sub clusters comprising cowpea accessions with genetic distances ranging from 0.00 as in TVu-15381 and TVu-15360 to 0.2094 in “Babban wake” a local landrace from Nigeria. The highest genetic distances of 0.212 and 0.209 were observed in TVu-14109 and “Babban wake” from main cluster I and II respectively. Both accessions originated from Chad and Nigeria.

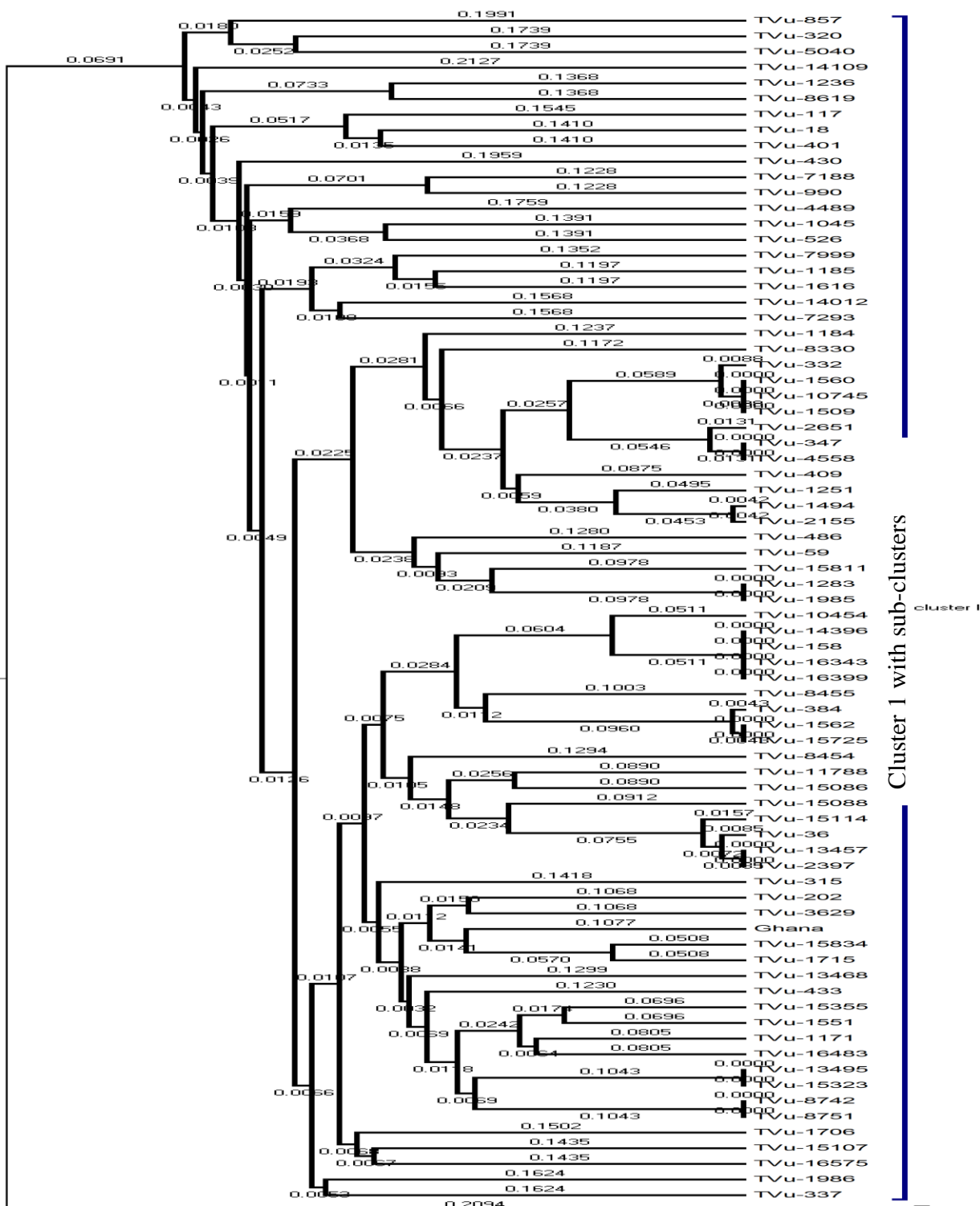


Figure 4.3: Hierarchical dendrogram of 169 cowpea accessions by using similarity coefficients based on the Nei's (1983) original genetic distance calculated from 119 SNP data using the UPGMA method

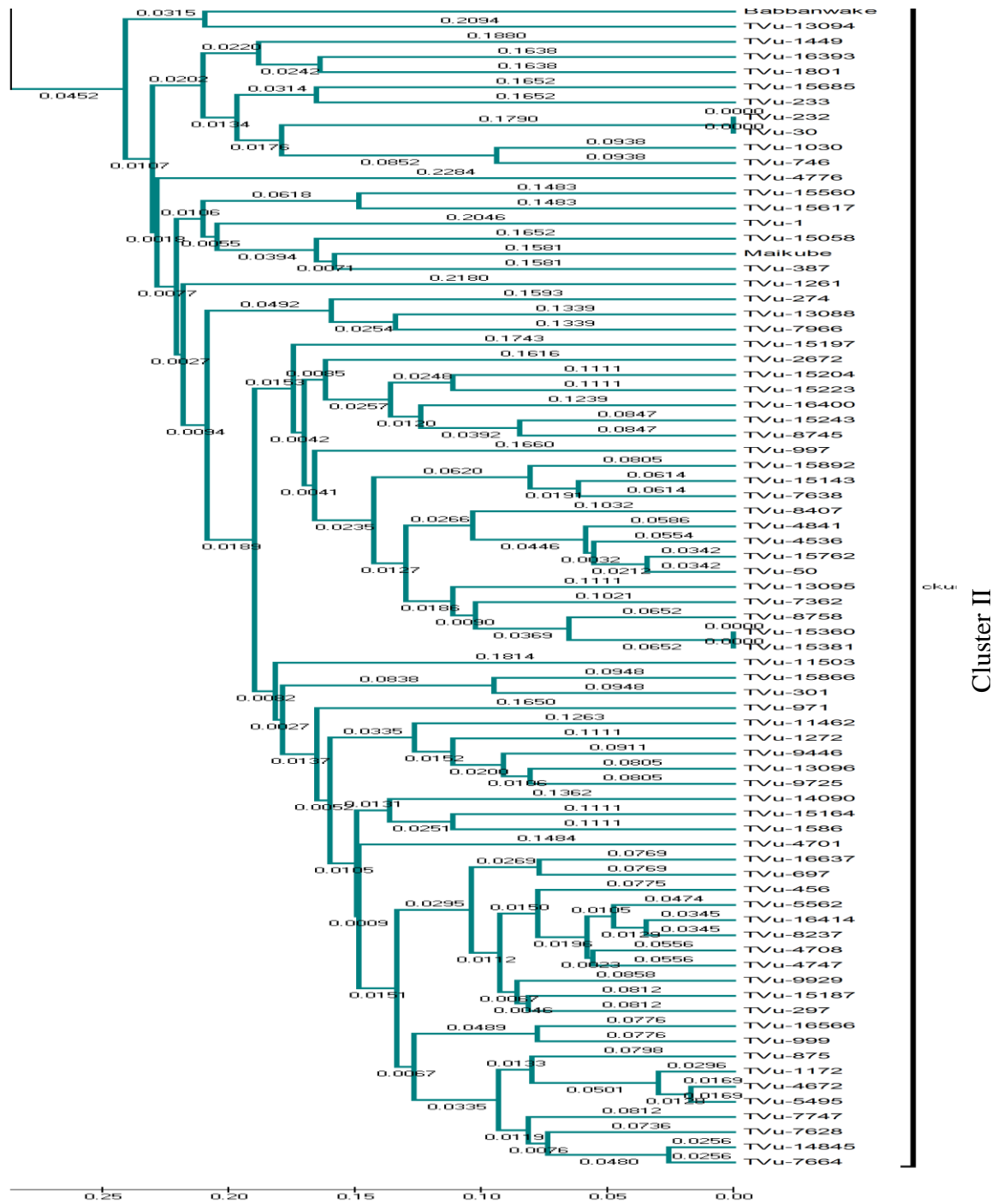


Figure 4.3: (Cont'd) Hierarchical dendrogram of 169 cowpea accessions by using similarity coefficients based on the Nei's (1983) original genetic distance calculated from 119 SNP data using the UPGMA method.

#### **4.4.7 Comparison between Morphological, Biochemical and Molecular Characterization**

Two hundred cowpea accessions were characterized morphologically and biochemically, while only 169 were genotyped. Thirty one accessions discarded due to undesirable traits associated with them.

The 169 cowpea accessions screened for grain quality traits were grouped into three main groups based on morphological and biochemical data and into two main groups based on SNP marker characterization. Morphologically, the 169 accessions were grouped into three maturity groups, two shattering groups, two seed coat textural groups and five seed coat colour patterns. Biochemically, the accessions (169) were categorized into three based on the high, medium and low quantities.

Biochemical data sub-grouped accessions into various sub-clusters with clear extreme values for high and low nutritional values. Similar trend was observed with molecular data. Both biochemical and molecular approaches were able to distinctly differentiate between the accessions and grouped them according to their nutritional value content and genetic distances, respectively.

The three methods: morphological, biochemical and molecular were found to be effective in assessing variability and selection of cowpea grain nutritional value, they could be used concurrently in marker assisted selection.

#### **4.5 Discussion**

The surface layer of the soil had high proportion of sandy soil and low organic matter content thereby making it physically fragile. This is a typical soil characteristic of the Northern guinea savanna zone of Samaru, Nigeria where cowpea is grown well. The particle size distribution corresponded to what was observed by Salako and Kang (2002) who characterized

the soil of Samaru as sandy loam.. The low pH in water recorded showed that the soil is generally acidic in nature, thus favors the root growth of plants.

The 200 cowpea accessions were classified into 3 maturity groups early, medium and late. Almost half (48%) of the accession were late maturing type and only 17% were early maturing. The late maturing group showed prostrating growth habit, while early and medium maturing accessions showed erect and semi erect growth habit, respectively. This may be due to their inherent genetic nature, environmental influence and variation in their response to day length, as most of the accessions are landraces from different countries. This conformed to the findings of Timko & Singh (2008) and Goenaga *et al.* (2008) who associated late maturing of cowpea with photosensitivity response, regardless of the planting time. Five grain eye patterns namely; Holstein, small eye, solid, Watson and Whippoorwill were identified from the 200 accessions. This conformed to classification of Saunders (1959), and reported by Asante (1991).

For 153 African accession, (21accessions =13.73%) of the accessions were found to shatter starting from the first maturing pod to the end of their maturity period. Shattering might be due to the changes in environmental conditions such as low humidity or might be due to their inherent nature. However, the accessions were planted late (23<sup>rd</sup> August, 2012), compared with appropriate cowpea planting time in the Guinea savanna zone of Zaria. The high rainfall received in August and September coupled with early cessation of rainfall may have reduced the level of humidity and induced shattering. This result disagree with Ali *et al.* (2004) findings in Pakistan, who reported that high rates of nitrogen, excessive moisture and low humidity can result in excessive vegetative growth, delayed maturity and pod shattering. However, further research is suggested to find out the reason for shattering, because some shattering accessions like TVu-13088 had high levels of iron, protein and carbohydrate contents.

The significant variation observed among the accessions for percentage crude protein (1.72% - 29.93%) may be due to screening large sample size collected from diverse agro ecological zones of Africa and other parts of the world. This result is not in agreement with the previous results of Horax *et al.* (2004), Ajeigbe *et al.* (2008) and Gupta *et al.* (2010), who reported low variability and small range 20-26%. On the other hand, the results in this study are in close agreement with Tchiagam *et al.* (2011b) who reported up to 31.78% crude protein in cowpea. This low range reported by the previous authors may be attributed to the small number of cowpea accessions and or varieties screened. For example, Ajeigbe *et al.* (2008) examined only 9 cowpea varieties from Nigeria, while Gupta *et al.* (2010) evaluated 23 genotypes from India. The wide variations observed in protein content may be attributed to either genetic or environment or both. The discrepancies in protein content is therefore, attributed to the combination of genotype and environment as reported by Wang & Daun (2004).

Similarly, the high concentrations and substantial variation observed for zinc (10.01 mg/kg to 386.3) and iron (1.01 to 329.15) concentration could also be attributed to the large sample size from diverse agro ecologies. These results are not in agreement with the finding of Asante *et al.* (2009) who reported low variation in cowpea grain zinc concentration with small range of 4 to 13 mg/kg in 44 cowpea accessions. The low range could be attributed to the small number of the accessions and the environment of study. The variations in iron however, conformed to the findings of Asante *et al.* (2009) in cowpea from Ghana and Ribeiro *et al.* (2012) in common bean from Brazil, respectively.

The principal component analysis clustered the 9 grain quality traits into 4 principal component axes that accounted for 63% of the total variation. Grain size, iron concentration, zinc concentration, ash content and moisture contents showed low communalities estimate. This

implied that they were the most variables among the 9 nutritional quality traits studied. Moreover, selection for these traits can be feasible for nutritional enhancement of cowpea. Similar, finding was reported in common beans by Ribeiro *et al.* (2012).

The results showed that iron is not significantly associated with zinc content; this agrees with the findings of Blair *et al.* (2013) in common bean. On the other hand, the result disagrees with the findings of Oluwatosin (1998), who reported a negative correlation in cowpea trial conducted under field condition. This might be due to the environmental differences because, growth promoting bacteria can increase the uptake of iron and zinc by the plants (Barea *et al.*, 2005) and the level of phytoavailability of the genotypes used by Oluwatosin (1998). Protein was negatively correlated with carbohydrate ( $r = -0.29$ ,  $p < 0.01$ ) and fat content ( $r = -0.85$ ,  $p < 0.001$ ). This conformed to observations made by other authors (Omueti & Singh, 1987; Ajeigbe *et al.*, 2008). Protein content was positively correlated with ash ( $r = 0.14$ ,  $p < 0.05$ ) and negatively correlated with fat ( $r = -0.13$ ,  $p < 0.05$ ) and carbohydrate ( $r = -0.23$ ,  $p < 0.001$ ) content indicating that selection for high protein will decrease carbohydrate content and increase ash content which will make the inferior parent superior and nutritional enhancement possible. On the other hand, iron content was positively correlated ( $r = 0.26$ ,  $p < 0.001$ ) with protein which imply that iron could be selected indirectly by selecting for high protein cowpea accession.

No significant correlation was observed between 100 seed weight and protein content and iron content, respectively. These correlations show that breeding for larger grain size (desirable) may not induce a reduction in the grain protein and iron content. Thus, direct selection to increase these two nutrients, via selection for 100 seed weight could be possible. Large grain size and seed coat colour are important consumer preference traits. These results disagree with the findings of

Asante *et al.* (2004) and Moura *et al.* (2012) who reported negative correlations among these traits.

Since traditional selection methods mainly depend on the phenotypic variations and morphological markers are easily influenced by the environment, and some of the morphological markers have epistatic effects (Tan *et al.*, 2012), then selection for grain quality traits of cowpea require genetic markers based on individual nucleotide sequence variation. This improves the reliability and efficiency of the selection process. One hundred and nineteen (119) SNP markers were used to characterize 169 cowpea accessions.

The allele frequency of all the markers used was generally below 0.95, indicating that they were all (100%) polymorphic. Similar results were obtained in cowpea (Ajibade *et al.*, 2000; Gajera *et al.*, 2014) and other crops like wheat (Nagaoka & Ogihara, 1997). It has been reported that the ability to resolve genetic variation may be more directly related to the number of polymorphisms detected by the marker system (Sivaprakash *et al.*, 2004).

The low level of polymorphism (0.24 to 0.38) detected in this results may be attributed to radiation of cowpea from the center of origin (Africa) to other parts of the world via domestication (Li *et al.*, 2001; Tosti & Negri, 2002; Badiane *et al.*, 2004; Diouf. & Hilu, 2005), in addition to its inherent nature of self-pollination mechanism (Badiane *et al.*, 2012).

The small genetic differentiation (0.26-0.45) observed among cowpea accessions comprising the African and non-African (USA and Asia) collections indicates that the entire genetic diversity in the African germplasm might already have spread over cowpea-growing regions in the world as a whole, though not completely within any single region. Another probable reason may be as a result of dispersal mechanisms that might have occurred as revealed by typical patterns of genetic relatedness between world cowpea collections relative to the two

primary gene pools in Africa (Huynh *et al.*, 2013). Moreover, the accessions used are core collection consisting of ~10% of total accessions, which between them capture most of the available diversity in the entire collection (Upadhyaya *et al.*, 2010). These can be thoroughly evaluated and the information so derived can be utilized for improving the efficiency of breeding. This further agrees with (Brown, 1989) who reported that the entries to a core collection should be limited to approximately ~10 %, using the sampling theory of selectively neutral alleles, with a ceiling of 3000 per species. This level of sampling is effective in retaining 70% of alleles of entire collection

Although only three accessions from Asia were included in this study, the majority of the accessions showed a kind of relatedness in either morphological data, biochemical data or molecular data, implying that most of the cowpea accessions from America might have moved from West Africa either as a result of slave-trading (Whit, 2007) or as a result of germplasm exchange (Ehlers & Hall, 1997). However, the results of this work further disagree with that of Huynh *et al.* (2013) who reported clear distinct clusters between African and American cowpea accessions. Another possible cause of relatedness may be due to maintenance methods used in conserving those accessions. Though cowpea is self-pollinating crop, some level of out crossing might probably occur between the accessions. This agrees with the previous findings of Moalafi *et al.* (2010) who reported 4 to 5% outcrossing rates in cowpea. Nevertheless, the effect of pollen movement on population structure is unknown (Kouam *et al.*, 2012). Both domesticated cowpea and its wild progenitor are characterized by a flower structure that should promote inbreeding (Lush, 1979). The clustering of accessions based on their related genetic distances regardless of their country of origin could be due to residual heterozygosity that would be greater for landraces than for elite cultivars, and over time more out- crossing is likely to occur. This is in agreement

with findings of Ortiz *et al.* (2008) in quinoa diversity studies in Peru. However, further work using molecular markers is recommended to check the possible redundancy that may exist among cowpea accessions.

#### **4.6 Conclusion**

The description patterns of genetic distances and clustering of relatively similar individuals into groups and sub-groups reported in this study provides important insights that can improve the efficiency of cowpea germplasm conservation and breeding efforts. The information will enable rational planning by gene bank curators to help reduce duplicates among the accessions and form core collection of their accessions.

For breeding programs, accessions clustered within a related group based on their relative genetic distances may exhibit common adaptive complexes of physiological traits coupled with a relatively restricted range of morphological and underlying genetic variation. Thus, crosses within related accessions are expected to produce a high frequency of relatively similar-looking progeny, while crosses between members of different cluster are expected to produce more variable progeny, perhaps with a relatively low average performance in early generations, in this case selection should be delayed to later generations. Breeding strategies involving series of back crosses using conventional method or 2-3 marker assisted back cross (MAB) with the identified markers would be required to increase the concentration of iron and zinc in cowpea grains.

This study has revealed the existence of genetic diversity within cowpea genome that could be exploited to successfully develop and deploy nutrient-dense cowpea varieties. In addition, significant and positive correlations between iron content and protein content indicate the possibility of improving the concentrations of these elements in cowpea simultaneously. Based on the findings of this study, three cowpea accessions; TVu-13088, TVu-13495 and TVu-

9725 with nutrient-enriched grains have been identified. They could be nominated for further testing in different environment and anti-nutritional factor analysis before recommending for use. Five accessions each for iron, zinc and protein content were identified as good candidates for further improvement;(1) Top 5 iron content accessions: TVu-1330, TVu-13495, TVu-16400, TVu-8751 and TVu-9725 (2) Top 5-zinc content accessions: TVu-1616, TVu-2155, TVu-15251, TVu-301 and TVu-232 (3) High protein content accessions: TVu-13495, TVu-13088, TVu-15187, TVu-347 and TVu-13468

## CHAPTER FIVE

### 5 INHERITANCE OF IRON AND ZINC CONTENT AND OTHER GRAIN QUALITY TRAITS IN COWPEA

#### 5.1 Introduction

Cowpea (*Vigna unguiculata*) is of vital importance to the livelihood of millions of people, especially in West and Central Africa. It provides nutritious grain and an inexpensive source of protein for both rural and urban consumers (Rangel *et al.*, 2004). Cowpea grain contains up to 29% protein, 78% carbohydrate, 329 mg/kg iron and 386 mg/kg zinc (Chapter 4 above) and therefore has a tremendous potential to contribute to the alleviation of malnutrition among rural and urban consumers (Boukar *et al.*, 2010).

Humans require at least 22 mineral elements for their wellbeing (Welch & Graham, 2004; Graham *et al.*, 2007). These can be supplied by an appropriate diet. Furthermore, it is estimated that over 60% and 30% of the world's population are iron (Fe) and zinc (Zn) deficient (White and Broadley, 2009). This situation is attributed to crop production in areas with low mineral phytoavailability and consumption of staple crops with inherently low tissue mineral concentrations (White & Broadley, 2005). The problem of iron and zinc malnutrition can be addressed through dietary diversification, mineral supplementation and increasing mineral concentrations in edible crops. However, strategies to increase dietary diversification, mineral supplementation and food fortification have not always been successful, due to concern of yield drag. For this reason, biofortification of crops through breeding varieties with an increased mineral elements concentration is recommended as an immediate strategy not only to increase mineral concentrations in edible crops but also to improve yields on infertile soils (White & Broadley, 2005). To set an enhanced breeding program, it is essential to know the proportion of phenotypic variation of a trait that is heritable (Kearsey & Pooni, 1996). This is because the

selection efficiency of a trait is mainly dependent on the magnitude of genetic variation and heritability of such trait (Falconer & Mackay, 1996).

Generation mean analysis provides information on the relative importance of additive and dominance effects in populations created from two inbred lines. It involves measuring the means of different generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1P_1$ ,  $BC_1P_2$ ) derived from two contrasting parents and interpreting the means in terms of the different genetic effects (Bernardo, 2002). This is because the actual means of single loci are unobservable, generation means estimate the pooled genetic effects across loci. Bi-parental mating system has been used to study gene action controlling important agronomic and quality traits in various crops. For example, Tchiagam *et al.* (2011b) reported 5 genes controlling sucrose accumulation in cowpea grain; Non-allelic interactions mainly additive x additive and additive x dominance were reported in rice grain size (Kiani *et al.*, 2013). Dominance gene effect was also found to be more important than additive, in the inheritance of protein content in chickpea (Kumhar., 2013), while Tchiagam *et al.* (2011a) associated high percentage (%) of protein with recessive gene in cowpea.

It is therefore, understandable that knowledge of the genetic factors responsible for the inheritance of iron, zinc and other agronomic traits is essential for breeding program. Moreover, information regarding genetic control of iron and zinc in cowpea is scarce. Therefore, the objectives of this study were to:

1. Determine the mode of inheritance of iron and zinc concentration in cowpea
2. Determine the mode of inheritance of grain size in cowpea
3. Determine the relationship between zinc concentration and yield related components

## 5.2 Material and Methods

### 5.2.1 Parent Materials

Six distinct cowpea accessions selected for inheritance of grain quality traits are presented in Table 5.1. Two parents: **P<sub>1</sub>=TVu-14845** and **P<sub>2</sub>=TVu-15251** are characterized by having low zinc concentration (15.15 mg/kg) and high zinc concentration (52.03 mg/kg), respectively. The grains of the 2 parents are morphologically distinct: with white grain coat, rough texture, black eye for P<sub>1</sub> and brown grain coat and rough texture for P<sub>2</sub>.

Similarly, 2 parents **P<sub>1</sub>=TVu-1** (smooth coat texture) and **P<sub>2</sub>=TVu-999** (brown grain colour and smooth grain coat texture) were used for inheritance of cowpea grain iron concentration.

**Table 5.1 Cowpea parental materials, origin and contrasting characteristics**

Parents	Origin	Contrasting characteristics
1. TVu-1	A single plant selection from local variety from Nigeria	Low iron content (1.50 mg/kg), smooth and brown grained and small seeded.
2. TVu-15251	A single plant selection from local variety from Chad	High iron and zinc (84.27 & 52.03 mg/kg), brown grain coat, rough texture and medium grained.
3. TVu-999	A single plant selection from landrace from Ghana	High iron content (98.05 mg/kg), Light brown coat and smooth texture and medium grained.
4. TVu-14845	A single plant selection from local variety from Mali	Low zinc content (15.15 mg/kg), white grain coat, rough texture, black eye with grey hilum and small grained.

### 5.2.2 Population development

The 6 basic generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1P_1$  and  $BC_1P_2$ ) were developed in the screen house of the Institute for Agricultural Research (IAR), Zaria ( $11^\circ 17.0' N$  and  $7^\circ 36.01'E$ ). Pots (Height = 21cm, Width = 23cm.) were filled up with sandy-loam soil collected from the field, after the analysis of soil trace elements (iron and zinc). Three seed was plant per pot and weeds were hand removed from the pots and no fertilizer was applied. Two and three liters of water per pot were applied for every 24 hours at vegetative stage and flowering to maturity stages, respectively.

Crosses were generated based on the combined procedures of Ehlers & Hall (1997) with slight modification. To ensure the success of the crosses and minimize contamination, capping method was employed throughout. The female plants were emasculated in the evening and pollinated in the morning. The process of emasculation was carefully done with sharply pointed forceps sterilized with alcohol between crosses to prevent contamination by unwanted pollen. Each cross was tagged immediately, names of parents that were involved in the cross and date of the cross. In the presence of adequate flowers from both parents, emasculation and pollination were simultaneously carried out twice a day; early morning and evening time when the sun was about to set. In a situation whereby the flowers of the paternal ( $P_2$ ) parent is ready and maternal ( $P_1$ ) parent is not, the paternal flowers were collected early in the morning, preserved in the fridge for 11 hours and then used to pollinate the maternal parent in the evening.

#### **Zinc set (TVu-14845 and TVu-15251)**

Crosses were made between low zinc (TVu-14845) and high zinc (TVu-15251) parents between 2013 and 2014. One hundred and seventy eight (178)  $F_1$ s were generated and classified into 4 groups: for (1&2 portion) the 2 parents  $P_{TVu-14845}$  and  $P_{TVu-15251}$  were backcrossed to the 80

F<sub>1</sub> plants (40 to each parent) and generated 75 BC<sub>1</sub>P<sub>1 TVu-14845</sub> and 82 BC<sub>1</sub>P<sub>2TVu-15251</sub>, respectively. (2)The third portion (40 F<sub>1</sub> grains) was advanced to harvest F<sub>2</sub> seeds and retained the (4) fourth portion as F<sub>1</sub>. The F<sub>1</sub> backcrosses to respective parents (BC<sub>1</sub>P<sub>1 TVu-14845</sub> and BC<sub>1</sub>P<sub>2TVu-15251</sub>) were advanced to F<sub>2</sub> generations backcrosses to get more seeds for elemental analysis (November, 2013 and February, 2014).

### **Iron set (TVu-1 and TVu-999)**

A cross between TVu-1 and TVu-999 was started in March, 2013. The seeds from the direct crossing were classified into 4 groups. The 2 portions of the grains (80 grains) were backcrossed to their parents (P<sub>TVu-1</sub> and P<sub>TVu-999</sub>) and generated BC<sub>1</sub>P<sub>1TVu-1</sub> and BC<sub>1</sub>P<sub>1TVu-999</sub>, respectively. The third portion of the seeds from the direct crossing (F<sub>1</sub>) was advanced to F<sub>2</sub>, while the fourth portion was retained for subsequent evaluation (November, 2013 to February, 2014).

### **5.2.3 Evaluation of Iron and Zinc concentration from the two sets of six generations**

The 2 sets of the 6 basic generations (P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>) derived from a cross between TVu-1 x TVu-999 (Iron) and TVu-14845 x TVu-15251(Zinc) were then evaluated for iron and zinc concentrations respectively, following the HarvestPlus protocol described in sub-section 4.2.3.2 of chapter four above.

### **5.2.4 Data collection**

Data were taken from 30 plants each from non-segregating populations (F<sub>1</sub> and their parents), 60 plants each for backcrosses to respective parents (BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>) and 150 plants for each of the F<sub>2</sub> generations derived from 2 crosses: TVu-1 x TVu-999 and TVu-14845 x TVu-15251).

For agronomic data the following traits were measured on individual plants from each generation, in accordance with cowpea descriptors of International Board for Plant Genetic Resources (IBPGR, 1983) and Mahalakshmi *et al* (2007).

**Days to 50% flowering:** This was recorded as the number of days from sowing to when half of the plants from each generation: 30 for non-segregating population, 60 for back crosses and 150 for F<sub>2</sub> population had flowered.

**Plant height (cm):** Plant height was taken as the perpendicular height of the plant from the top soil level (in the pot) to the end of the top most leaf of the plant.

**Hundred (100) grain weight (g):** One hundred grains were randomly picked at 12% moisture content from individual plants of each generation and weighed.

**Grain coat colour:** was assessed based on visual examination, by spreading sub-samples from individual plants from each generation on a white sheet of paper. The various colour observed were than recorded against each plant and generation.

**Grain coat texture:** visual inspection and hand rubbing of the randomly picked grains, from individual plant of each generation was used to assess the grain coat texture.

**Elemental analysis:** for iron and zinc concentrations from 2 sets of crosses (TVu-1 x TVu-999) and (TVu-14845 x TVu-15251) were estimated as described in sub-section 4.3.2.3 chapter four.

**Yield related traits** data were recorded from the F<sub>2</sub> population derived from a cross involving low zinc parent (TVu-14845) and high zinc content (TVu-15251) parent according to the procedure of Mahalakshmi *et al.* (2007)

**Number of pod per plants:** The total harvestable pod per individual plant were counted after harvest

**Mean Number of Seed per pod:** Due to the limited number of pods, five pods were taken at random, number of seed per pod was counted and mean was of the five pod was then estimated by dividing the total seed counted by the number of pod (5).

**Mean pod length (cm):** A thread was used to measure the length of a pod and corresponding length was then measured on a ruler. The average pod length was then estimated from five randomly picked pods.

**Hundred (100) seed weight (g):** One hundred seed was taken at random from the total seed threshed from individual plant.

### 5.3 Data Analysis

With the exception of degree of dominance, all other analyses were run using SAS software (version 9.3). The iron and zinc concentrations determined for the 2 set of crosses were subjected to analysis of variance to test for the equality of the means using proc ANOVA. Bartlett's homogeneity variance test was run for the  $F_2$  phenotypic values of each trait, using DIST macro of SASQuant, developed by Gusmini *et al* (2007). This is to verify the assumption of equal variance needed to pool data for a specific source. Genetic parameters, m (mid-parent), a (additive component) and d (dominance component), variance components and broad and narrow sense heritability were estimated using SAS program (SASQuant) developed by Gusmini *et al.* (2007). Hayman's mean separation analysis procedure (Hayman, 1958; Gamble, 1962) based on the following linear model:

$$Y = m + \alpha a + \beta d + \alpha^2 aa + 2\alpha\beta ad + \beta dd$$

$Y$  = the observed mean,  $\alpha$  and  $\beta$  are the coefficients for  $a$  and  $d$ , respectively,  $m$  = mean of the  $F_2$  (i.e., the base population),  $a$  = pooled additive effects,  $d$  = pooled dominance effects,  $aa$  = additive x additive gene interaction effects,  $ad$  = additive x dominance gene interaction effects and  $dd$  = dominance x dominance gene interaction effects

The notation of Gamble (1962)  $m$ ,  $a$ ,  $d$ ,  $aa$ ,  $ad$ ,  $dd$  were used and weighted using the inverse of the variance. The best fitting model among the two models: model 1 [ $m$ ], [ $a$ ] and [ $d$ ] and the second model comprises of the epistatic effects, [ $aa$ ], [ $ad$ ], [ $dd$ ] were used to determine the significant additive or dominant effect.

Broad and narrow sense heritability were estimated as follows:

$$\text{Broad sense heritability } (H_b^2) = [ V_{F_2} - ( V_{P_1} + V_{P_2} + V_{F_1} ) / 3 ] / V_{F_2}$$

$$\text{Narrow sense heritability } (H_n^2) = [ 2V_{F_2} - ( V_{BC_1P_1} + V_{BC_1P_2} ) ] / V_{F_2}$$

Where,  $V$  = variance for  $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1P_1$  and  $BC_2P_2$  generations.

Gene factors controlling the iron, zinc, seed weight and plant height were calculated following the method of Wright (1952) using SASQuant program of Gusmini *et al.* (2007)

The degree of dominance (deviation from the mid-parent value) and direction of dominance in the two sets (zinc and iron) and their respective 100 grain weight and plant heights were estimated by hand in accordance with the method of Falconer & Mackay (1996) as follows:

$$D (\text{degree of dominance}) = d/a$$

Where,  $d$  = heterozygote = means of  $F_1 - 1/2 (P_1 + P_2)$

Where means of  $P_1$ ,  $P_2$  and  $F_1$  are used in the two crosses.

## 5.4 Results

### 5.4.1 Generation Means for Zinc Concentration, Grain weight and Plant Height

The segregating and non-segregating populations derived from the 3 set of crosses are presented in Table 5.2. The first two sets of crosses were developed from a single family each and used independently, in the inheritance studies of cowpea grain zinc concentration and iron concentration. As expected, the number of F<sub>2</sub> plant was relatively higher than that of their corresponding back crosses (Table 5.1).

**Table 5.1: Number of progenies generated from two set of crosses for iron, zinc and grain coat colour studies**

Cross	F <sub>1</sub>	F <sub>2</sub>	BC <sub>1</sub> P <sub>1</sub>	BC <sub>1</sub> P <sub>2</sub>	Population generated used for:
TVu-14845 x TVu-15251	178	221	86	94	Inheritance of zinc content, seed size and plant height
TVu-1 x TVu-999	189	234	88	91	Inheritance of iron content, seed size and plant height

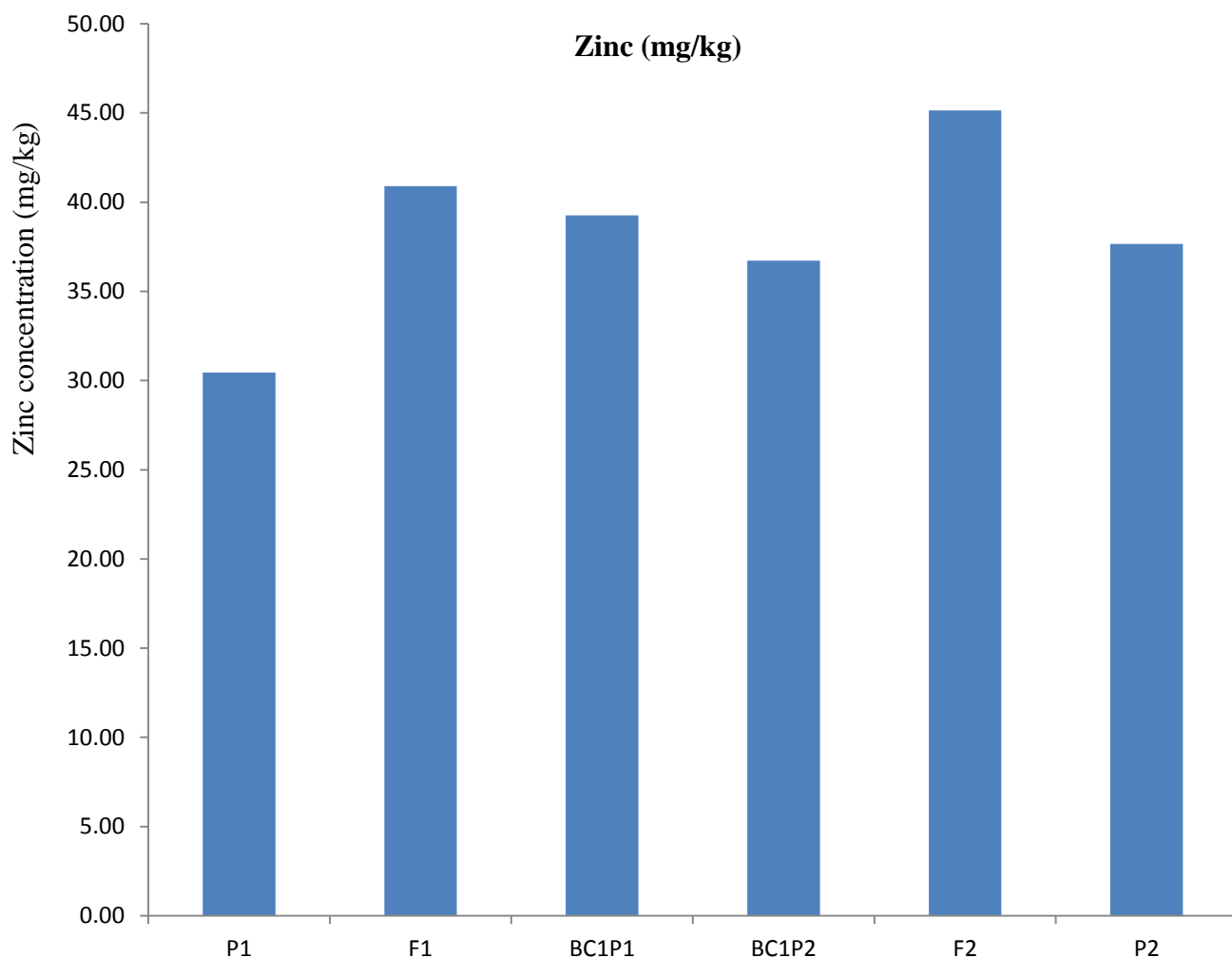
The results of analysis of variance (ANOVA) for the six basic generations are presented in Table 5.2. Highly significant ( $P < 0.001$ ) difference were detected among generation means for grain zinc concentration, 100 grain weight and plant height, respectively.

**Table 5.2: Analysis of variance for the zinc concentration, seed weight and plant height in a cross between TVu-14845 and TVu-15251**

Source of variation	DF	Zinc (mg/kg)	SWT(g)	PLHT(cm)
Generations	5	476.903***	490.0***	1220.655***
Error	354	69.249	17.089	76.956

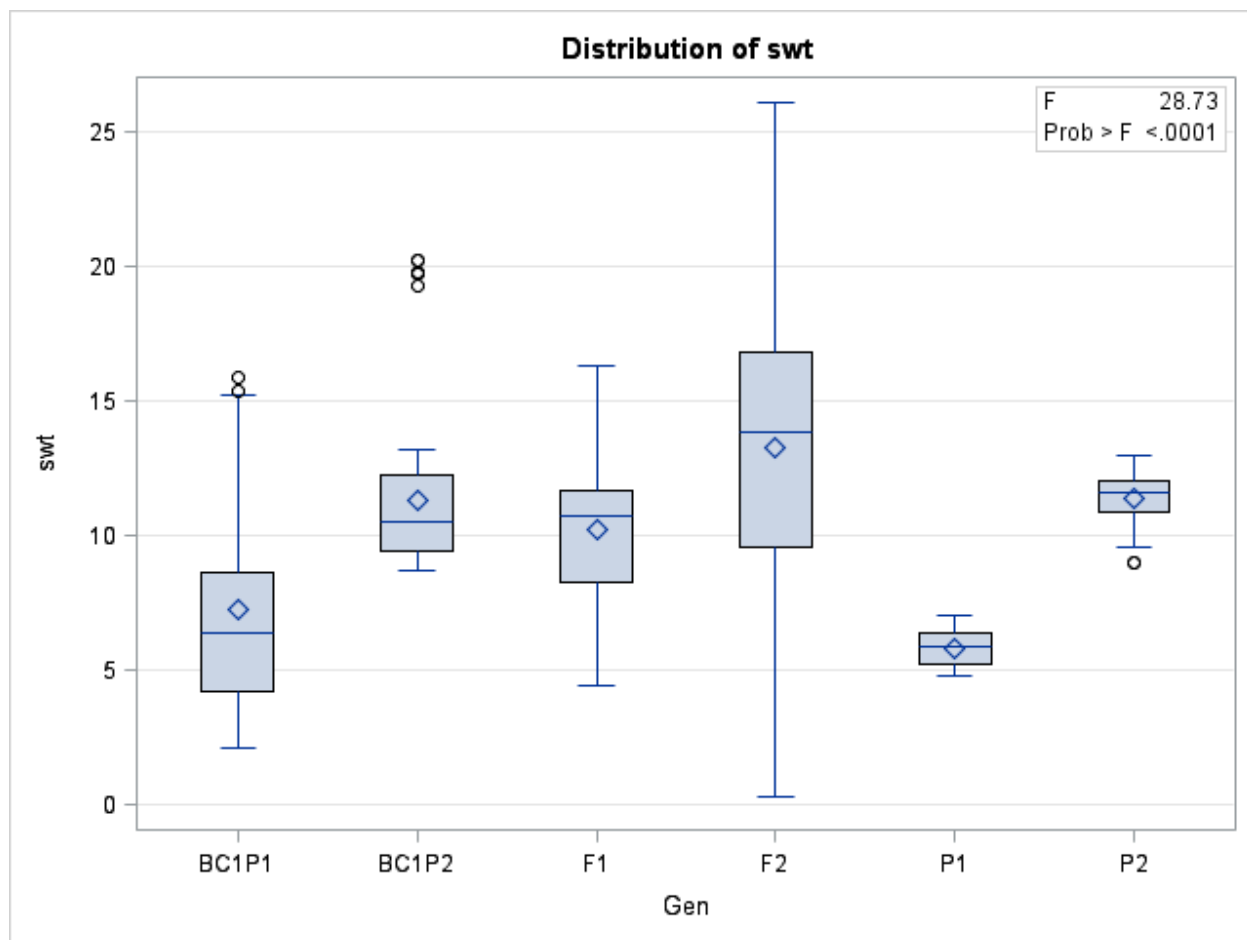
\*\*\*; Significant at  $P < 0.0001$ ; SWT= 100 grain weight (g); PLHT= Plant height (cm); DF = degree of freedom

Population mean distribution for zinc content is presented in Fig 5.1. Distribution of zinc content in the two backcrosses ( $BC_1P_1$  and  $BC_1P_2$ ), was skewed toward high zinc parent (TVu-15251). Individual backcrosses and some  $F_2$  plants produced high grain zinc content, higher than the superior parent (TVu-15251), indicating transgressive segregation among some individual  $F_1$  and  $F_2$  populations.



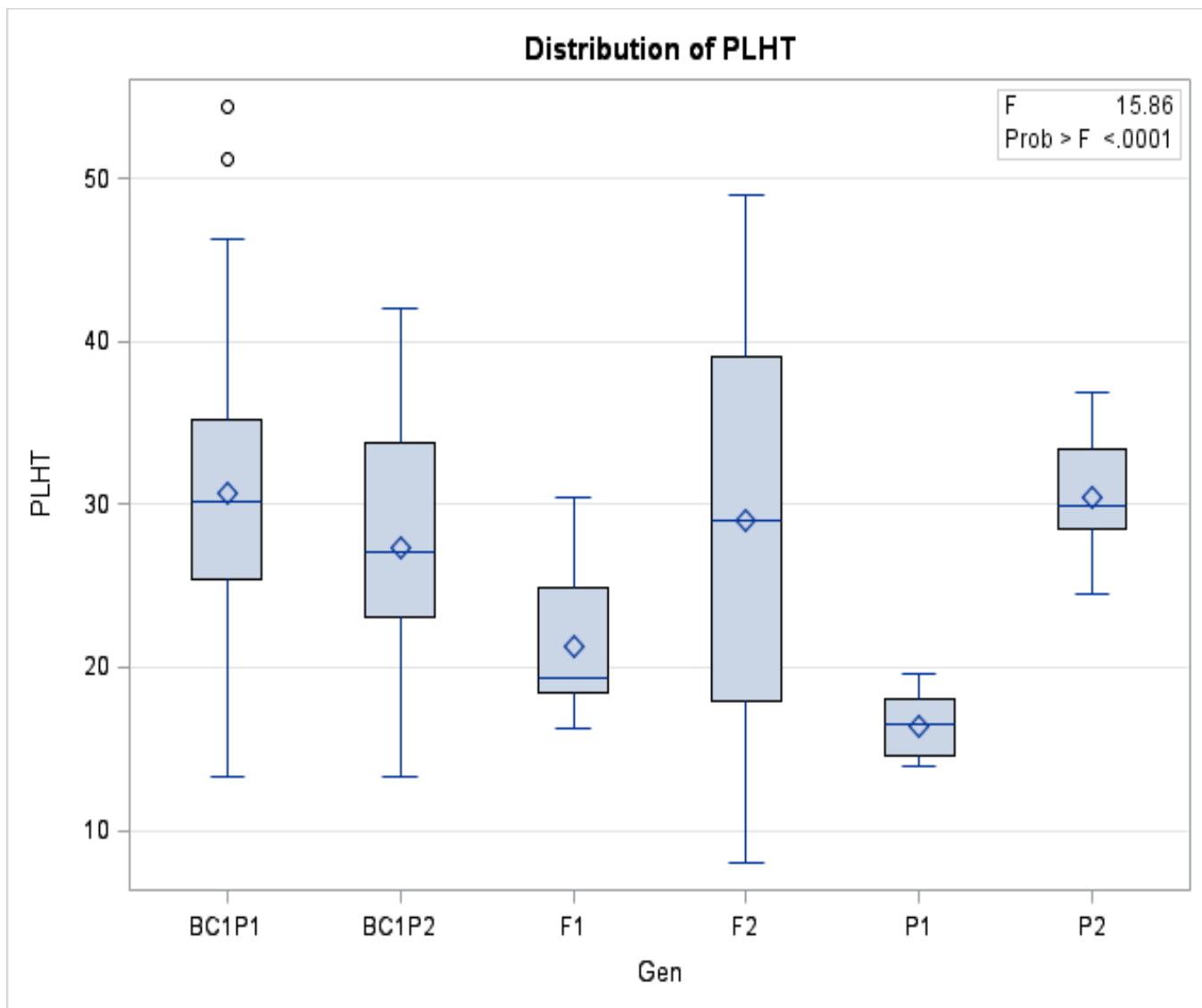
**Figure 5.1: Population mean distribution for zinc concentrations among six basic generations derived from low zinc content (TVu-14845) and high zinc content (TVu-15251) parents**

The distributions of seed weight among segregating and non-segregating populations derived from a cross involving small seeded parent (TVu14845) and large seeded parent (TVu-15251) are presented in Figure 5.2. The seed weight in parent two (TVu-14845) was almost two times heavier than that of parent one (TVu-14845), indicating the distinctness of the two parents. As expected, F<sub>2</sub> showed wider variability and the two backcrosses to parent one and two was skewed toward the low and high seed weight parents (BC<sub>1</sub>P<sub>1</sub> to TVu-14845 and BC<sub>1</sub>P<sub>2</sub> to TVu-15251), respectively.



**Fig 5.2: Distribution of 100-seed weight (swt) in six generations derived from crosses involving small seeded parent (TVu-14845) and large seeded parent (TVu-15251)**

The distribution of mean plant height among segregating and non-segregating populations generated from a cross between short parent plant (TVu14845) and tall parent plant (TVu-15251) are shown in Figure 5.3. The two plants showed contrasting height having means of 13.40 cm and 30.30 cm for parent one and two respectively. More variability and transgressive segregation was observed among F<sub>2</sub> population.



**Fig 5.3: Distribution of plant height (PLHT) in six generations derived from crosses involving short parent (TVu-14845) plants and tall parent (TVu-15251) plants**

Means for parental,  $F_1$ ,  $F_2$  and backcross generations derived from a cross involving TVu-14845 and TVu-15251 cowpea parents are listed in Table 5.3. The parent with high zinc concentration (TVu-14845) had significantly higher mean performance compared to the low zinc content parent (TVu-15251). The means of grain zinc concentration of non-segregating population ( $F_1$  39.90) and segregating populations ( $F_2$  37.30;  $BC_1P_1$  40.70 and  $BC_1P_2$  37.50) were significantly higher than that of the mid-parent (33.95). Similar trend was equally observed in 100 seed weight and plant height Table 5.3.

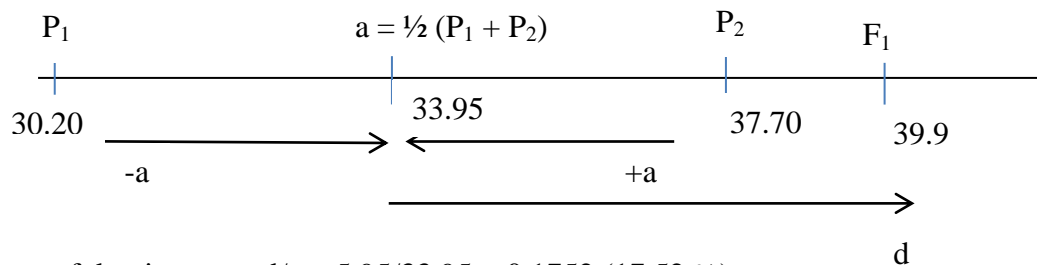
**Table 5.3: Means of families derived from a cross involving TVu-14845 (low zinc) and TVu-15251 (high zinc) cowpea parents**

Generation	Traits		
	[Zinc] mg/kg	100 SWT (g)	Plant height (cm)
$P_1$	30.20	5.80	13.40
$P_2$	37.70	11.40	30.30
$F_1$	39.90	10.30	18.60
$F_2$	37.30	13.30	29.00
$BC_1P_1$	40.70	7.30	30.70
$BC_1P_2$	37.50	11.40	25.50
<b>MP</b>	<b>33.95</b>	<b>8.60</b>	<b>21.85</b>
<b><math>d = F_1 - 0.5 (P_1 - P_2)</math></b>	<b>5.95</b>	<b>1.7</b>	<b>- 3.25</b>
<b><math>D = d/a</math></b>	<b>0.1725</b>	<b>0.1976</b>	<b>0.1487</b>

MP= mid-parent mean; [Zinc] = seed zinc concentration; D= degree of dominance; means of  $F_1$ ,  $P_1$  and  $P_2$  and  $d$  = heterozygote;  $d$  =heterozygote

The degree of dominance for zinc content, grain weight and plant height were estimated according to Falconer & Mackay (1996) to determine the direction of dominance in each trait.

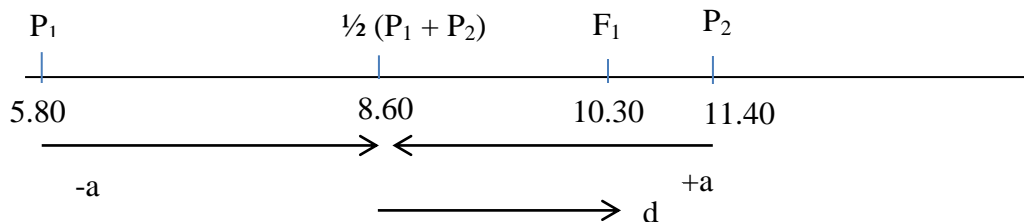
Degree of dominance for zinc concentration



Degree of dominance =  $d/a = 5.95/33.95 = 0.1752$  (17.52 %)

Where  $d = F_1 - \frac{1}{2} (P_1 + P_2) = 5.95$  from the table above and  $a = \frac{1}{2} (P_1 + P_2) = 33.95$

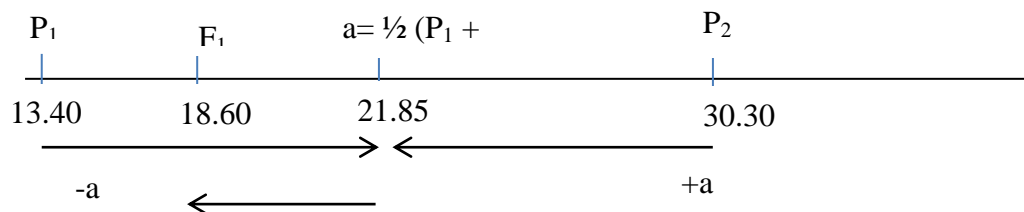
Dominance in seed weight



Degree of dominance =  $d/a = 1.70/ 8.60 = 0.1976$  (19.76 %)

Where  $d = F_1 - \frac{1}{2} (P_1 + P_2) = 1.70$  and  $a = \frac{1}{2} (P_1 + P_2) = 8.60$

Dominance in plant height



Degree of dominance =  $d/a = - 3.25/21.85 = - 0.1487$  (14.87 %)

The nature of gene action involved in the control of the zinc concentration, 100 grain weight and plant height were investigated by the analysis of generation means and presented in Table 5.4. The generation means analysis indicated that the additive-dominance model was adequate to explain the gene action involved in the inheritance of zinc concentration in cowpea grain. The negative dominance x dominance [dd] and positive dominance suggest the presence of dominance effects toward the high zinc content parent plant.

For grain weight involving a cross between TVu-14845 and TVu-15251 (Table 5.4), fitted the first model with highly significant additive [a] and dominance [d] effects, both having negative signs. The dominance [dd] gene action was also significant ( $P < 0.05$ ) and positive in the direction of the high zinc content parent (TVu-15251).

Positive significant additive [a], additive x dominance [ad] gene action and negative dominance by dominance [dd] gene actions were detected for plant height (Table 5.4). The significant and negative dominance x dominance [dd] gene action would suggest the presence of dominance effects at heterozygous loci for shorter parent plants. The negative sign for the dominance [d] and dominance x dominance [dd] gene action indicated the contribution of alleles from the shorter parent (TVu-15251) plant.

**Table 5.4 Estimate of gene effects ( $\pm$ SE means) for zinc, 100 grain weight and plant height in a cross between TVu-14845 and TVu-15251**

Genetic component	Zinc concentration (mg/kg)	100 grain weight (g)	Plant height (cm)
M	37.30 $\pm$ 0.76 ***	13.29 $\pm$ 0.44***	28.99 $\pm$ 0.94***
A	3.21 $\pm$ 2.31	-4.10 $\pm$ 0.87***	5.21 $\pm$ 2.06**
D	13.07 $\pm$ 9.19	-14.30 $\pm$ 4.18 ***	-6.65 $\pm$ 9.04
Aa	7.13 $\pm$ 7.66	-15.93 $\pm$ 3.52***	-3.42 $\pm$ 7.90
Ad	6.96 $\pm$ 2.94*	-1.31 $\pm$ 1.03	13.64 $\pm$ 2.64***
Dd	-15.72 $\pm$ 15.34	16.52 $\pm$ 6.52*	-28.21 $\pm$ 14.31*

\*, \*\* and \*\*\* are significant difference at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively; a =additive gene effect; d = dominance gene effect; aa = additive by additive gene effect; ad = additive by dominance gene effect; dd = dominance by dominance gene effect.

The magnitude of the phenotypic variances for zinc concentration, 100 grain weight and plant height were generally higher than the genotypic variances (Table 5.5) showing a greater influence of the environment on these traits. Similarly, broad sense heritability, narrow sense heritability and number of effective factors are presented in Table 5.5. High broad sense heritability:  $h_b^2=0.79$ , 0.86 and 0.93 were recorded in grain zinc concentration, 100 grain weight and plant height, respectively.

Overestimated narrow sense heritability (1.03 and 1.22) was observed in 100 grain weight and plant height, respectively. Number of effective factors based on Wright method was 0.2 each for zinc concentration and 100 grain weight, while 0.3 was observed for plant height (Table 5.5).

**Table 5.5 Genetic variances, heritability and effective factors for zinc content seed weight and plant height in a cross involving TVu-14845 and TVu-15251**

Trait measured	(V <sub>G</sub> )	(V <sub>A</sub> )	(V <sub>D</sub> )	(V <sub>E</sub> )	(V <sub>P</sub> )	(h <sup>2</sup> <sub>b</sub> )	(h <sup>2</sup> <sub>n</sub> )	EF
Zinc mg/kg	68.51	1.71	66.80	18.32	86.83	0.79	0.02	0.2
100 gw (g)	25.41	36.11	-10.70	4.22	29.63	0.86	1.22	0.2
Plant height (cm)	122.41	136.64	-14.23	9.80	132.22	0.93	1.03	0.3

V<sub>G</sub>= Genotypic variance; V<sub>A</sub>=Additive variance; V<sub>D</sub> =Dominance variance; V<sub>E</sub>=Environmental variance; V<sub>P</sub>= Phenotypic variance; h<sub>b</sub><sup>2</sup> = Broad sense heritability and h<sub>N</sub><sup>2</sup>= Narrow sense heritability; gw = grain weight; EF = effective factor [Wright (1952)]

#### 5.4.2 Generation means for seed iron concentration, seed weight and plant height in a cross between low iron (TVu-1) and high iron (TVu-999) parents

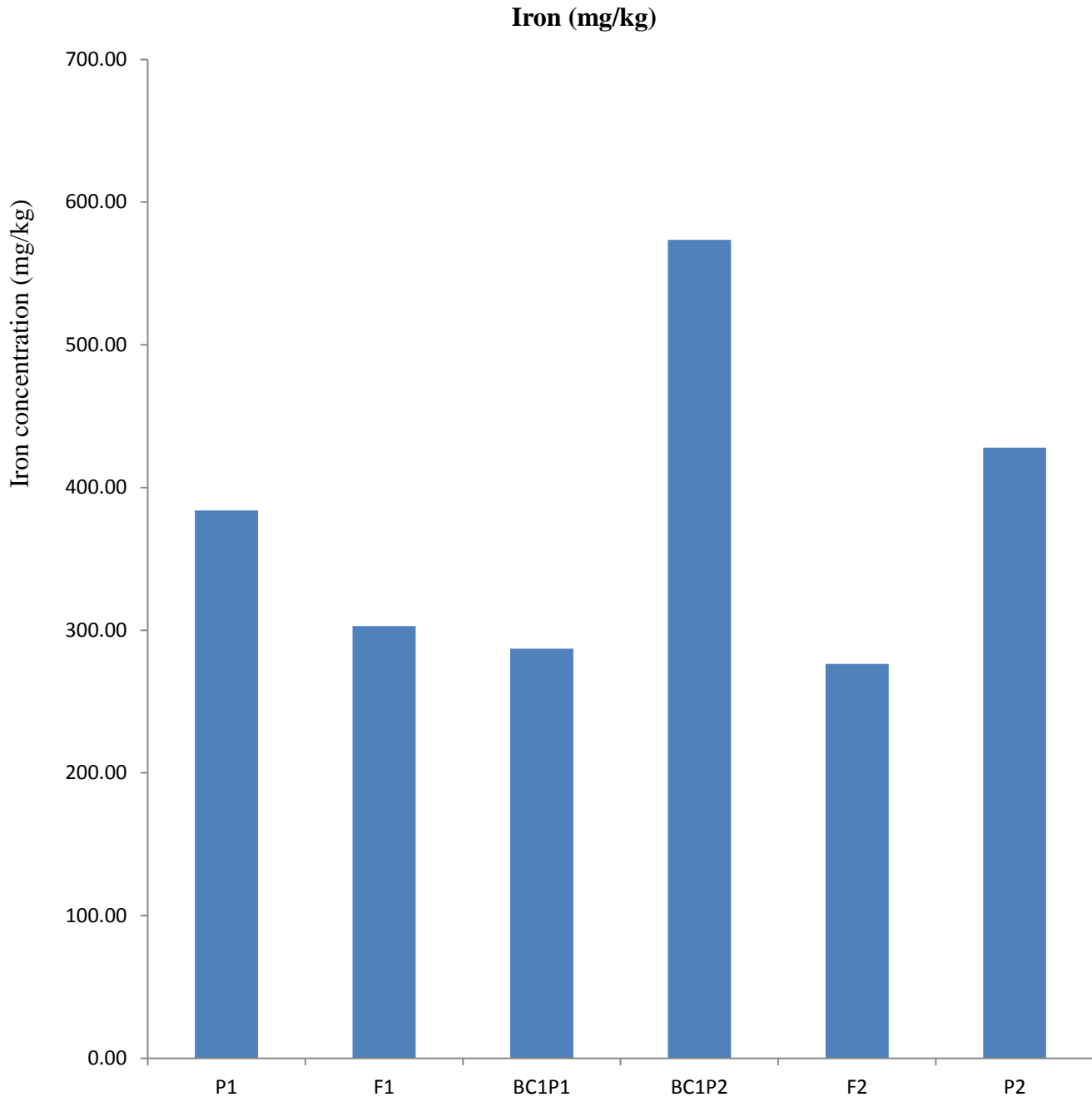
Analysis of variance results for the six basic generations showed highly significant difference ( $P < 0.001$ ) for iron concentration, 100 grain weight and plant height (Table 5.6)

**Table 5.6 Mean squares for iron concentration, 100-grain weight and plant height in a cross between TVu-1 and TVu-999**

Source of variation	DF	Iron (mg/kg)	SWT(g)	PLHT(cm)
Generations	5	82941.057***	139.215***	1144.157***
Error	199	10605.52	26.585	209.740

\*\*\*= significant at  $P < 0.001$ ; SWT= 100 grain weight and PLHT= Plant height

Distribution of generation means of iron concentrations among segregating and non-segregating population derived from a cross involving low iron (TVu-1) and high iron (TVu-999) parents is presented in Figure 5.4. Parent two (TVu-999) had higher iron concentration (422.89 mg/kg) than that of parent one TVu-1 (383.83 mg/kg). Population means distribution for iron concentration showed that back cross (BC<sub>1</sub>P<sub>2</sub>) to high iron content parent (TVu-999) produced higher grain iron content than that of back cross with low iron content parent (TVu-1).



**Figure 5.4: Population mean distribution for iron concentrations among six basic generations derived from low iron content (TVu-1) and high iron content (TVu-15251) parents**

Analysis of variance results for the six basic generations showed highly significant difference ( $P < 0.001$ ) for iron concentration, 100 grain weight and plant height respectively (Table 5.6)

**Table 5.6: Mean squares for iron concentration, 100-grain weight and plant height in a cross between TVu-1 and TVu-999**

Source of variation	DF	Iron (mg/kg)	SWT(g)	PLHT(cm)
Generations	5	82941.057***	139.215***	1144.157***
Error	199	10605.52	26.585	209.740

\*\*\*= significant at  $P < 0.001$ ; SWT= 100 grain weight; PLHT= Plant height and DF=degree of freedom.

Means for parental,  $F_1$ ,  $F_2$  and backcross generations for the cross involving TVu-1 and TVu-999 cowpea are presented in Table 5.7. As expected, the parent with high iron concentration (TVu-999) had higher mean (419 mg/kg) iron concentration compared to the low iron parent TVu-1 with 384 mg/kg. The means for iron concentration of both  $F_1$  (300.02) and  $F_2$  (353.00) were lower than the low iron parent (TVu-1). The  $BC_1P_1$  was closer to the low iron content of the parent, while  $BC_1P_2$  population had significantly higher mean concentration of iron than the high iron content parent (TVu-999) and mid-parent. For 100 grain weight, both  $F_1$  and  $F_2$  produced large grains that resembled the weight of the largest grained parent (TVu-999). Backcross to respective parents yielded large grain, though, a bit larger in  $BC_1P_2$ . For plant height, the  $BC_1P_1$  population produced the tallest plant with 60 cm population mean. This is taller than the tallest means of the parent plant and the mid-parent means.

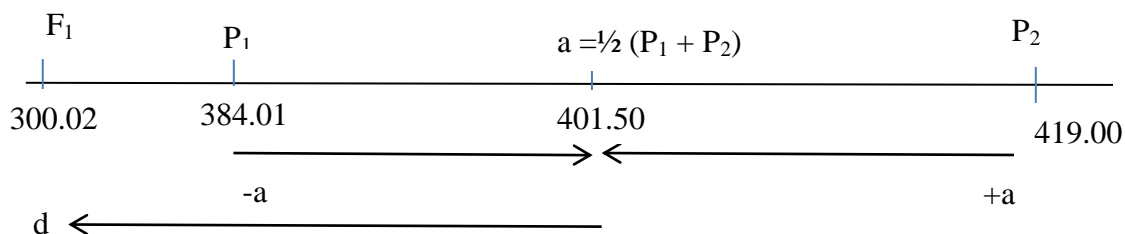
**Table 5.7 Means of families derived from a cross involving TVu-1 (low iron) and TVu-999 (high iron) cowpea parents**

Generation	Traits		
	Iron content (mg/kg)	100 grain weight (g)	Plant height (cm)
P <sub>1</sub>	384.01	5.90	40.70
P <sub>2</sub>	419.00	12.40	21.30
F <sub>1</sub>	300.02	12.60	31.80
F <sub>2</sub>	353.00	11.20	25.00
BC <sub>1</sub> P <sub>1</sub>	332.01	9.10	60.00
BC <sub>1</sub> P <sub>2</sub>	458.00	8.30	24.10
<b>MP</b>	<b>401.51</b>	<b>9.15</b>	<b>31.00</b>
<b>Heterozygote (d)</b>	<b>-101.50</b>	<b>3.45</b>	<b>0.80</b>
<b>D = d/a</b>	<b>0.2527</b>	<b>0.3770</b>	<b>0.0258</b>

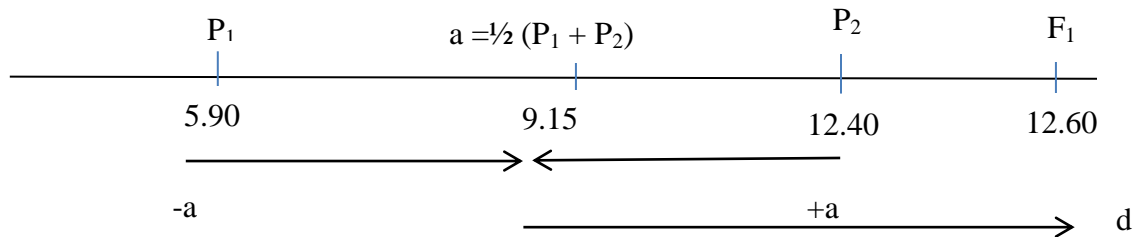
MP= mean of mid parent; d = heterozygote; D = degree of dominance

### Degree of dominance for grain iron content, grain weight and plant height

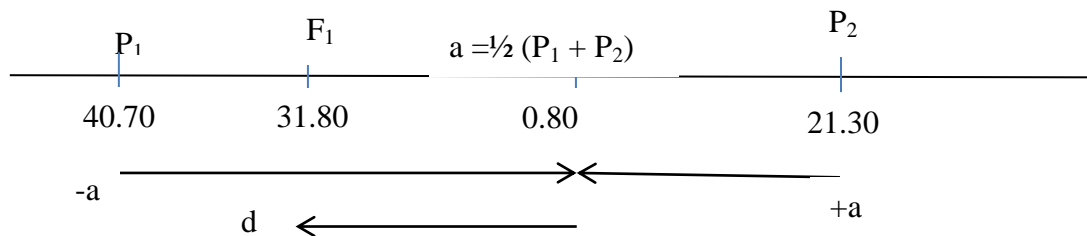
Degree of dominance for iron content



Degree of dominance for iron content =  $d/a = -101.48/401.50 = 0.2527$  (25.27 %)

**Degree of dominance for seed weight**

Degree of dominance =  $d/a = 3.45/9.15 = 0.3770$  (37.70 %)

**Degree of dominance for plant height**

Degree of dominance =  $d/a = 0.80/31 = 0.0258$  (2.58 %)

The genetic effect of iron concentration and 100 seed weight are presented in Tables 5.8. The generation means analysis indicated negative additive [a] gene action is significant to explain the nature of gene action for iron concentration. Additive by dominance [ad] dominance gene action was also significant but in the negative direction, suggesting the contribution of alleles from the low iron parent (TVu-1). The heterozygote ( $d = 300.02 < 384.01$  low iron content parent) on the dominance scale further, indicates the low iron parent is dominant over high iron content parent (TVu-999).

Significant negative additive x additive [aa] and positive additive x dominance [ad] and dominance x dominance [dd] gene actions were identified as important for grain weight

inheritance in cowpea. This indicates duplicate gene action in grain weight inheritance in a cross between small grained (TVu-1) and large grained (TVu-999) parents.

**Table 5.8 Estimate of gene effects ( $\pm$ SE means) for zinc, 100 grain weight and plant height in a cross between TVu-1 and TVu-999**

Components	Iron concentration (mg /kg)	100 grain weight(g)	Plant height (cm)
m	353.07 $\pm$ 8.15***	11.16 $\pm$ 0.68***	24.97 $\pm$ 1.68***
a	-125.20 $\pm$ 29.39***	0.81 $\pm$ 0.81	3.30 + 3.16
d	65.31 + 110.77	-6.31 +5.14	4.01 + 16.63
aa	167.60 +91.35	-9.73 + 4.33*	3.22 + 13.04
ad	-107.70 +35.27**	4.07 + 1.09***	-6.40 + 4.65
dd	-344.80 + 188.95	18.25 + 7.57*	19.46 + 26.54

\*, \*\* and \*\*\* are significant difference at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively; a =additive gene effect; d = dominance gene effect; aa = additive by additive gene effect; ad = additive by dominance gene effect; dd = dominance by dominance gene effect.

The magnitude of the phenotypic variances for all the traits (iron content, 100 grain weight and plant height) were generally higher than their corresponding genotypic variances (Table 5.9) indicating greater influence of the environment on these traits. Negative additive variance [ $V_A$ ] and negative dominance variance [ $V_D$ ] were recorded in seed iron concentration and 100 grain weight respectively. Exaggerated negative narrow sense heritability (-2.4) was also recorded in cowpea iron concentration. Broad sense heritability estimates were generally medium to high; 0.54, 0.89 and 0.70 for iron content, grain weight and plant height respectively. Almost one (0.8) effective factor was recorded for iron concentration in cowpea grain, and 0.2 effective factors each for 100 grain weight and plant height respectively.

**Table 5.9 Genetic variances, broad and narrow sense heritability and number of effective factors in a cross involving TVu-1 and TVu-999**

Trait measured	(V <sub>G</sub> )	(V <sub>A</sub> )	(V <sub>D</sub> )	(V <sub>E</sub> )	(V <sub>P</sub> )	(h <sup>2</sup> <sub>b</sub> )	(h <sup>2</sup> <sub>n</sub> )	EF
Iron mg/kg	3606.50	-15923	19529	3028.30	6634.80	0.54	-2.40	1.0
100 gw (g)	41.14	72.66	-31.52	4.93	46.07	0.89	1.58	0.2
Plant height (cm)	197.60	263.27	-65.68	84.07	281.67	0.70	0.93	0.2

V<sub>G</sub>= Genotypic variance; V<sub>A</sub>=Additive variance; V<sub>D</sub> =Dominance variance; V<sub>E</sub>=Environmental variance; V<sub>P</sub>= Phenotypic variance; h<sub>b</sub><sup>2</sup> = Broad sense heritability and h<sub>N</sub><sup>2</sup>= Narrow sense heritability; gw = grain weight; EF= effective factor [Wright (1952)]

The phenotypic variances of the six generations derived from zinc and iron set of crosses are presented in Table 5.10. The two backcrosses (BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>) had larger phenotypic variances in both crosses. Backcross one to high zinc parent (BC<sub>1</sub>P<sub>TVu-14845</sub>) had the largest phenotypic variance (129.69) to both parents. Similar trend was also detected in a cross between low and high iron (TVu-1 x TVu-15251) parents. The largest phenotypic variances observed for BC<sub>1</sub>P<sub>2</sub> to their respective high zinc and iron parents indicate that dominance is for the lower iron and zinc content parents.

**Table 5.10 Phenotypic variances of the generations derived from zinc and iron set of crosses**

Generation	Zinc (TVu-14845 x TVu-15251)	Iron (TVu-1 x TVu-999)
P <sub>1</sub>	18.33	249.96
P <sub>2</sub>	7.08	2743.80
F <sub>1</sub>	23.94	5923.20
F <sub>2</sub>	86.83	11655.00
BC <sub>1</sub> P <sub>1</sub>	<b>42.26</b>	<b>6741.00</b>
BC <sub>1</sub> P <sub>2</sub>	<b>129.69</b>	<b>24147.00</b>

### 5.4.3 Yield performance among F<sub>3</sub> population evaluated under screen house conditions

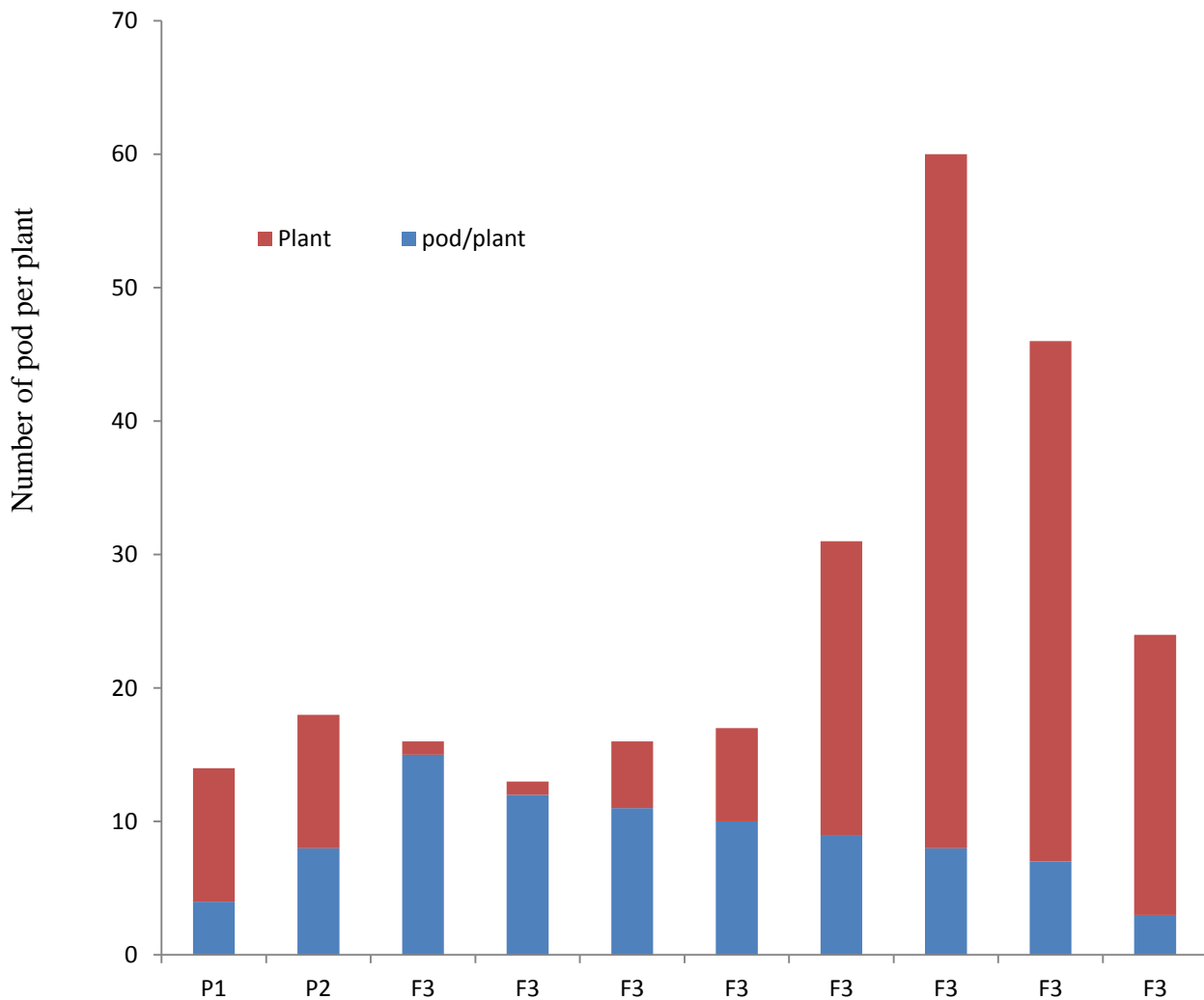
Means number of pod per plant; pod length; seed per pod and 100 seed weight per individual plants of 148 F<sub>3</sub> population and their respective parents (TVu-14845 and TVu-15251) evaluated under screen house condition are presented in Table 5.11. Number of pod per plant among F<sub>3</sub> populations ranges from 7 to 15 pods per plant. For 100 seed weight, high zinc content parent (TVu-15251) produced relatively large seed (11.596 > 5.575) compared with low zinc content parent. Similar trend was observed for number of seeds per pod, with low zinc parent (TVu-14845) having the highest number of seed per pod (11) compared to the high zinc content parent (TVu-15251) with 6 seeds per pod.

**Table 5.11 Distribution means of yield related traits among 148 F<sub>3</sub> cowpea populations evaluated under screen house conditions.**

Generation	Number of pod per plant	Pod length	Number of seed per pod	100 seed weight (g)
P <sub>1</sub>	4	9.731	11	5.575
P <sub>2</sub>	8	6.825	6	11.596
F <sub>3</sub>	8	10.185	10	14.448
Range	7 – 15	1.3 - 16.5	1 – 15	3.28 - 26.13

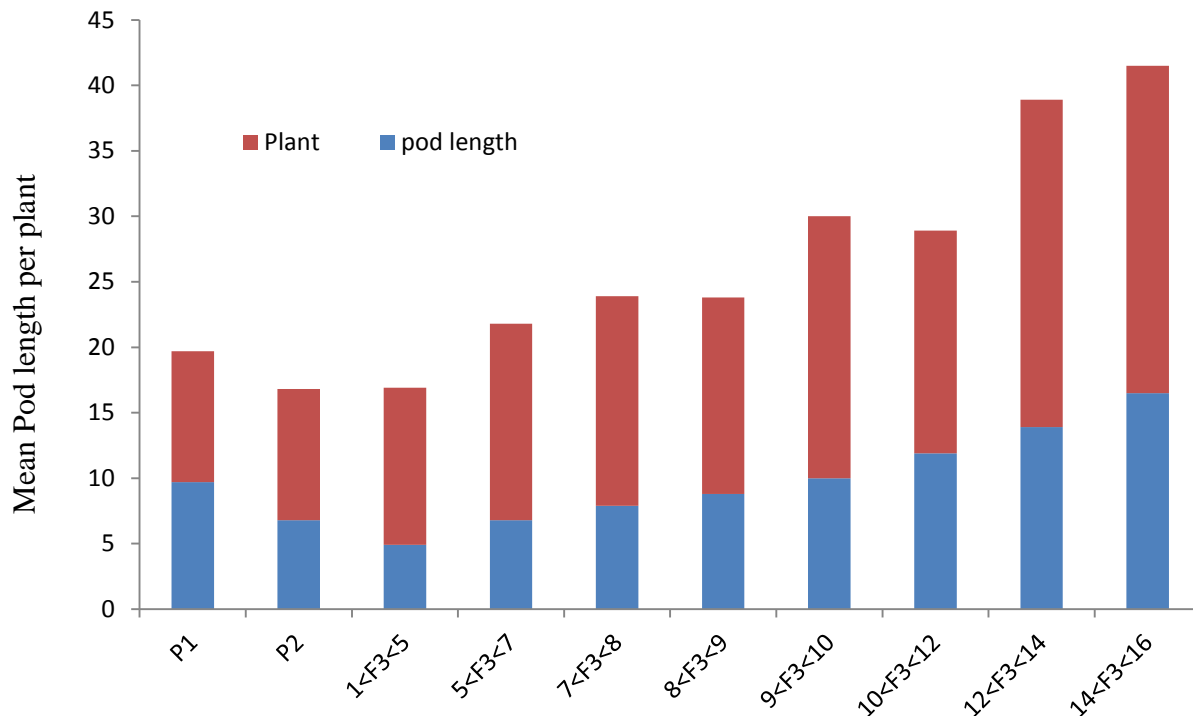
P<sub>1</sub>= TVu-14845, P<sub>2</sub>= TVu-15251

The distribution of number of pod per plant (Figure 5.5) showed that high pod yielding parent plant (TVu-1521) produced 2 fold pod yield increase (8 > 4 pods per plant) compared to the low pod yielding parent (TVu-14845). The distribution of pod per plant among F<sub>3</sub> plants skewed toward high pod yielding parent (TVu-15251), with most individual plants producing 7-8 pods per plants, indicating transgressive segregation.



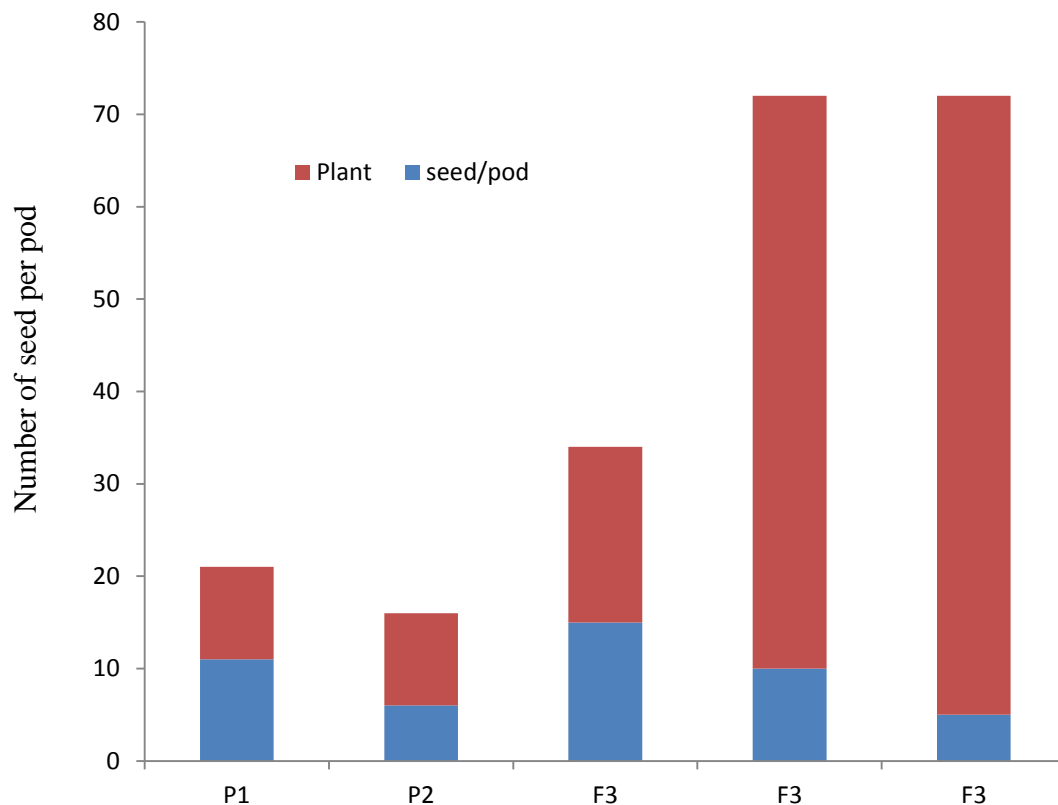
**Figure 5.5: Frequency distribution of number of pod per plant among 148 F<sub>3</sub> Population.**

Majority of the plants (134) produced long pod, ranging from 6.0 -16.5 cm, though some seed chambers were empty seeded (Figure 5.6). This also indicates the skewedness of the population toward parent two (TVu-14845) that produced long pods.



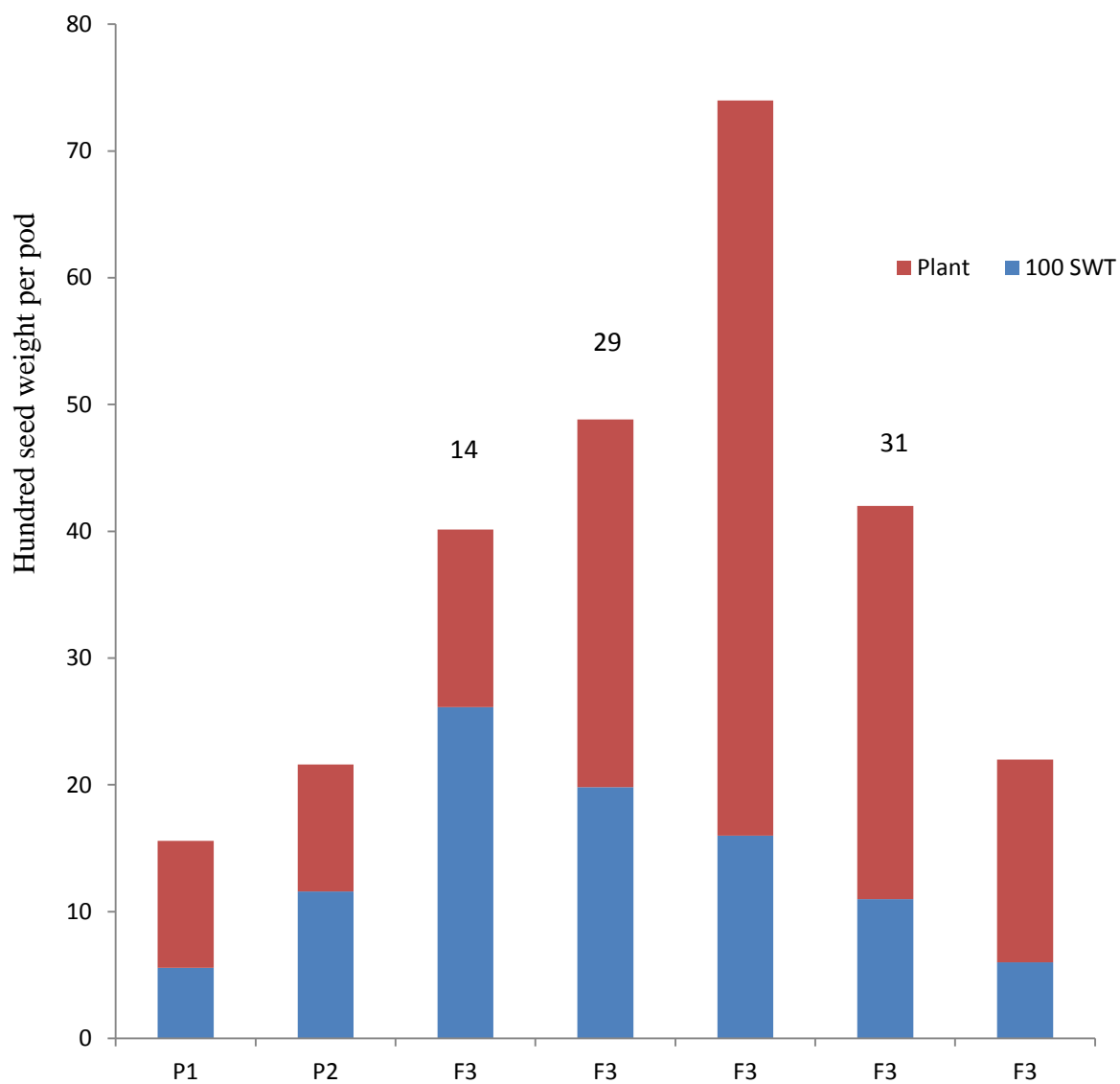
**Figure 5.6: Frequency distribution of mean pod length per pod among 148 F<sub>3</sub> Population**

The distribution of mean number of seed per pod per plant among F<sub>3</sub> population showed a transgressive segregation with 19 plants producing more number of seed per pod than the best seed producing parent (TVu-14845) and 5 plants produced few number of seed per pod lower than the low producing seed per pod plants (TVu-15251). Majority of the F<sub>3</sub> population (62 individual plants) produced 6 -10 seeds per pod (Fig 5.7).



**Figure 5.7: Frequency distribution of number of seed per pod among 148 F<sub>3</sub> Population**

The distribution of seed weight among 148 F<sub>3</sub> is presented in Figure 5.8. The populations also revealed significant number of (43) of individual plants showed transgressive segregation producing large seeds per plant that ranged from 19.82 to 26.13 g.



**Figure 5.8: Frequency distribution of seed weight (g) among 148 F<sub>3</sub> Population derived from a cross between TVu-14845 and TVu-15251 grown under screen house condition**

#### 5.4.4 Correlation between Zinc concentration and yield related traits in cowpea evaluated under screen house conditions

The zinc content and yield related traits (number of pod per plant; pod length; seed per pod; and 100 seed weight) were included in the correlation analysis (Table 5.12). Weak negative significant ( $P < 0.05$ ) correlation was recorded between zinc concentration and number of pod per plant. Pod size was positively correlated with number of seed per pod and 100 seed weight respectively. But no significant correlation was observed between zinc concentration and 100 seed weight. However, zinc content showed negative association with all yield related traits studied.

**Table 5.12 Phenotypic correlation coefficients between zinc concentration and yield related traits measured from F<sub>3</sub> population derived from a cross between TVu-14845 and TVu-15251**

	Pod/plant	Pod length	Seed/pod	100 seed weight (g)	Zinc (mg/kg)
Pod /plant	1				
Pod length	0.0071	1			
Seed/pod	-0.0069	<b>0.419***</b>	1		
100 seed weight	0.0335	<b>0.272***</b>	0.137	1	
Zinc	<b>-0.1806*</b>	-0.062	-0.114	-0.074	1

\*,\*\*\*= significant difference at 0.05 and 0.001 respectively

#### 5.5 Discussion

Differential performance of genotypes in segregating populations indicates the presence of heritable variation. The large degrees of variation among the different populations for iron and zinc concentrations in the two different crosses strongly suggest the existence of genetic differences for these traits which can be utilized in breeding for high iron and zinc cowpea. Similar results have been reported for genotypic differences of zinc in rice grain (Graham *et al.*,

1999; Cakmak *et al.*, 2004; Gregorio *et al.*, 2000), maize grain (Bänziger & Long, 2000) and cowpea grain by Asante *et al.* (2009) and Carvalho *et al.* (2012)

The transgressive segregates observed in some individual  $F_2$  and backcross populations in zinc concentration and the deviation of  $F_1$  population toward the high zinc content parent with mean zinc concentration ( $39.90 > 37.70$ ) higher than that of the high zinc content parent, suggests that high zinc content parent (TVu-15251) contributed more of the alleles. Also the high zinc content parent was partially dominant (0.17 degree of dominance) to low zinc content. Similar results were observed in common beans by Islam *et al.* (2004) and Blair *et al.* (2008). Similarly, the two fold increase in cowpea grain zinc concentration observed among some  $F_2$  individual (82.54 mg/kg) compared with the highest zinc content parent (37.70 mg/kg) conform to a similar report of up to 37.3% increases zinc concentration in common bean by Ribeiro *et al.* (2014), when the crossing and selection among segregation progenies in early hybrid generations were done in a controlled environment in Zaria.

The marginal over dominance (0.17) observed for zinc concentration in cowpea in favor of the high zinc content parent, could be due to allelic effects, where allele affects the components in opposite directions and there is degree of dominance on the scale in which the components combine to give fitness (Wallace, 1968). Moreover, different stages of life cycle (maturity period) observed among the population, coupled with seasonal variation encountered by the same  $F_3$  population could also be another reason for over dominance (Falconer & Macky, 1996). This population undergoes seasonal variation from optimum to stressed (extremely cold) temperatures (November 18<sup>th</sup>, 2013 to February, 2014).

For iron concentration in cowpea,  $F_1$  population produced seeds with low iron concentration, lower than that of the lowest iron content parent (TVu-1), implying that the low

iron content parent is dominant over the high iron content parent (TVu-999). However, transgressive segregation was observed among some  $F_2$  population with two fold iron concentration increase over the high iron content parent ( $1070.23 \text{ mg/kg} > \text{TVu-999}=419 \text{ mg/kg}$ ). My preliminary findings indicate an increase in iron concentration. This is the first attempt to study the genetic pattern of iron and zinc in cowpea; the results need to be validated. Furthermore, this result implies that cowpea breeding for enhanced nutrition could be possible. However, similar reports of 94.0% increases in iron concentration in the common bean grain have been reported by Blair *et al.* (2008) and Ribeiro *et al.* (2012). The deviation from the mid-point to less than the concentration of low iron content parent implies that low iron content parent (TVu-1) contributes more alleles to the inheritance of iron concentration. The marginal over dominance could be due to seasonal variation leading to changes in the environmental differences. For example maximum temperature before flowering, rainfall after flowering, presence of zinc in 30 – 60 cm soil depth and relative humidity (RH) after flowering could attribute to significant variation. This conforms to the findings of Joshi *et al.* (2010) who reported that environmental conditions were significant and accounted for around 59% of variation in grain mineral concentration in wheat.

The low iron concentration observed among  $F_1$  seeds which resemble the low iron content parent is an indication of significant maternal effect. This further implies that iron concentration is dependent on the seed coat and selection should begin on the  $F_3$  seeds (embryos in the  $F_2$  generation) when segregation was verified  $F_3$ . Thus, a breeding program should investigate the existence of maternal effect on iron concentrations in germplasm subjected to selection. The embryo generation should be considered for the selection process and the progression of

segregating populations in genetics and breeding programs. Similar findings were reported in common bean by Rosa *et al.* (2010).

On the other hand, the expression of low zinc concentration was overshadowed by the paternal parent in the  $F_1$  seeds derived from low and high zinc parents. The phenotypes of these seeds were similar in terms of zinc concentration and represented the expression of the genotype of the  $F_1$  generation. Therefore,  $F_3$  seeds showed embryos in the  $F_2$  generation. For this reason, selecting for a high zinc concentration in the cowpea seeds should begin with the  $F_2$  seeds, a generation where ample genetic variability is observed. Similar findings were reported in copper concentration in common bean (Samineni *et al.*, 2011)

In addition to additive [a] and additive by dominance [ad] gene effects observed in the two set of crosses for zinc and iron concentrations, epistatic gene effects had high contributions in controlling zinc content in cowpea grain. The observed positive dominance [d] and negative dominance by dominance [dd] gene effects for both iron and zinc contents indicate the duplicate gene action. This is in accordance with Mather & Jinks (1982) who stated that gene action is considered to be duplicating when dominance [d] and dominance by dominance [dd] gene effects have different signs (positive and negative or vice versa). Furthermore, the observed negative dominance by dominance [dd] in both iron and zinc population implies that unidirectional dominant and reductive alleles were involved in dominant phenotype (Ribeiro *et al.*, 2012)

Effective factors; 0.2 and 0.8 estimated for zinc and iron concentrations, respectively indicate that in addition to minor genes, one important gene is involved in the control of iron concentration in cowpea seed, while many genes interact in the inheritance of zinc concentration. These minor genes could be in duplicate recessive form, where when one gene exists in recessive form, the zinc concentration may not be inherited. These results do not agree with the findings of

Gelin *et al.* (2007), who did not identify a QTL for iron in common bean, perhaps due to limited map coverage. However, Foster-Hartnett *et al.* (2002) and Cichy *et al.* (2005) reported a monogenic inheritance for grain zinc concentration in common bean and recently, Blair *et al.* (2008) and Blair *et al.* (2011) reported 6 QTL for zinc concentration in the same common beans using different genotypes with that of Foster-Hartnett *et al.* (2002) and Cichy *et al.* (2005). These discrepancies could be due to species differences (*Phaseolus vulgaris* and *Vigna unguiculata*) and environmental differences as well. This is because within common bean alone discrepancies exist between Foster-Hartnett *et al.* (2002) and Blair *et al.* (2008) findings, which may be due to genotypic differences and environmental effects. Further genetic study to explore the inheritance pattern of zinc in cowpea grain is therefore recommended.

The high broad sense heritability observed for both iron and zinc concentrations indicate that a progress could be made. This result did not agree with the finding of Oluwatosin (1998) and Joshi *et al.* (2010) who reported low heritability for iron and zinc concentrations in cowpea and wheat grains respectively. The discrepancies between these results and that obtained by Oluwatosin (1998) could be due to the genetic make-up of the cowpea genotypes used and environments where the evaluation was conducted. In this study, the populations were evaluated under confined area (Screen house), with known status of soil iron and zinc content, while Oluwatosin's trials were conducted under field conditions in three locations without details of soil nutritional content. The same multilocation evaluation applied for wheat trial by Joshi *et al.* (2010).

The overestimated narrow sense heritability values recorded for iron content as well as grain weight and plant height could be alluded to epistasis or developmental factors or true micro-environmental variations or sampling error. Similarly, values in early generation tend to be

overestimated due to an upward bias from repulsions phase of linkage. This linkage is broken in a later generations due to recombination and low degree of dominance (Samineni *et al.*, 2011) However, Kearsley & Pooni (1996) reported that developmental factor such as plant height and grain weight cannot be separated from the genuine within the family variations. Earlier reports also indicated that it could be caused by environmental influence and some errors during sampling. Similar findings were reported in other traits in chickpea (Samineni *et al.*, 2011) and rice (Kiani *et al.*, 2013).

Signs associated with different estimates of epistasis indicate the direction in which gene effects influence the population means. Mather and Jinks (1982) proposed the association or dispersion of genes in the parents based on signs associated with epistatic gene effects such as additive by additive [aa] and additive by dominance [ad]. These signs were in opposite directions and significant in the control for 100 seed weight in a cross between low iron (small seeded) and high iron (medium seeded) parents. A negative sign for any of these parameters indicates an interaction between increasing and decreasing alleles, thus providing some evidence for the existence of dispersion in the parental genotypes which hinders early selection for such traits. Similarly, signs of these parameters were in opposite direction for seed weight which suggests the contribution of alleles from the larger grain parent in the expression of the dominant phenotype. On the other hand, significant negative additive [a] and negative dominance [d] gene actions were observed with epistasis interaction of negative [aa] in a cross between TVu-14845 and TVu-15251 respectively. Having the same negative signs indicates large influence of recessive parent. (Mather & Jinks, 1982)

Positive or negative form of additive  $\times$  additive [aa] interaction show association and dispersion of alleles in parents, respectively. Therefore, negative and significant values of additive

by additive [aa] interaction for 100 grain weight in the two crosses showed alleles dispersion in parents for 100 seed weight per plant. The contribution of additive gene effect relative to dominance genes toward cowpea grain weight was identified, characterizing an additive allelic interaction. This result is vital for breeding programs. When an additive allelic interaction is predominant, selection is facilitated, because superior parent (Tvu-14845 and TVu-999) will produce superior ancestry, as observed in the segregating population of this result. Similar results were reported by many authors (Lopes *et al.*, 2003; Adeyanju *et al.*, 2012; Egbadzor *et al.*, 2013) for *Vigna unguiculata*. The negative dominance [d] gene action for seed weight further indicates reductive alleles involving dominant phenotype otherwise increasing alleles including dominant phenotype. This result shows an increase or contribution of alleles from the large seeded parent from each of the cross.

Plant height showed negative significant additive [a] and positive additive by dominance [ad] gene action in a cross between short and tall plants of TVu-14845 and TVu-15251. This implies that plant height was predominantly under complementary gene action in this study. These results are in agreement with the findings of Adeyanju *et al.* (2012) who reported complementary and duplicate gene action for plant height in two different crosses of cowpea. The occurrence of duplicate gene action would limit selection in early generations for plant height. It is therefore good to make a selection in later generations when reasonable homozygosity is attained.

The occurrence of high narrow sense heritability in grain size indicates that the selection for grain size can be made in early generations. High narrow sense heritability observed for 100 seed weight, conformed to Tchiagam, *et al.* (2011b) but did not agree with other authors (Adeyanju *et al.*, 2012; Egbadzor *et al.*, 2013). These differences could be attributed to the types

of cowpea genotype used, environmental conditions and more importantly developmental factor such as grain size which varied within a pod of a plant. To improve grain size in cowpea, it is important to pay attention to the nature of the genotype, true micro-environmental variations and sampling error, especially, when dealing with duplicate structures like pod size and grain size (Kearsey & Pooni, 1996). Selection for seed weight and plant height in cowpea is possible. The fixation of these characters could be observed in advanced generations.

The significant variation in mean number of pods per plant, seeds per pod, pod length and 100 seed weight among  $F_3$  population with various degree of segregation in all the yield related traits observed, conform to the findings of many authors (Ariyo, 1995; Rangaiah *et al.*, 1999; (Romanus *et al.*, 2007).

Negative significant correlation observed between zinc concentration and number of pod per plant, implies that increase in number of pods per plant may lead to decrease in zinc concentration in cowpea seeds. It further suggests that selection for high pod loads per plants may indirectly lead to selection for low zinc content. This could be due to environmental factors such as availability of soil mineral elements that may lead to plant uptake and subsequent accumulation in the seed and other parts of the plants. This conform to similar findings of Joshi *et al.* (2010) who observed high grain concentration in wheat grains, grown in the soil that contained high zinc content and replicated in a low zinc content soil. The correlation further shows that breeding for higher pod yield (desirable for farmers) can induce a reduction in the grain zinc content. Thus, indirect selection to increase either of the traits, via selection for one, should be avoided. Similar findings were reported in cowpea by Moura *et al.* (2012)

The non-significant correlation observed between zinc concentration and seed weight in cowpea, conformed to similar reports of Moraghan & Grafton, 2001) in common bean. The strong

positive correlation observed between pod length and number of seeds per pod and 100 seed weight; suggest that pod size increases with increase in number and size of the seeds contained within a pod. The correlation also indicates the reliability of these traits in selecting for large seeded plants.

## **5.6 Conclusion and recommendations**

Substantial variation of grain nutritional traits among cowpea observed: Fe (1.0 - 329mg/kg), Zn (10.01 - 386 mg/kg) and Protein (1.72-29.93%). Cowpea genotypes with less than 10% protein content cleared the consumers' perception that all cowpea have high proteins. Differential performance of genotypes among segregating populations and large degrees of variation among the different populations for iron and zinc concentration strongly suggest the existence of genetic variability that can be utilized for cowpea enhancement. The marginal over dominance and transgressive segregates observed in some individual of  $F_1$ ,  $F_2$  and backcross populations in zinc and iron concentration with two fold increase mean zinc concentration over the high zinc content parent suggests that progress could be made at early generation selection for zinc and later generation for iron.

Genetic pattern of iron and zinc content elucidated and possibility of enhancement of Fe and Zn in cowpea is realized. The results of this study indicated that the inheritance of iron and zinc accumulation in cowpea grains was found to be epistasis with mainly additive-dominance gene effects. Inheritance of iron and zinc is polygenically controlled. This can be explained by the fact that both iron and zinc have similar mechanisms of uptake and accumulation to grain. This is promising for plant breeders who are interested in micronutrient enhancement, given that if they have similar mechanism for uptake and inheritance it may be easy to select for these traits both

phenotypically and through marker assisted selection. Plant height was predominantly under complementary gene action in both crosses.

The weak negative correlation between zinc concentration and number of pods per plant may decrease the pod yield of a cowpea genotype; as such direct selection for zinc content by selecting high yield genotype should be avoided. For selection of a cowpea genotype to be effective in at least maintaining the yield of that genotype, it is important that yield components should not be significantly correlated with zinc content. The number of seed per pod, 100 seed weight and pod length which showed relatively non significantly correlated, could be considered the best predictors of seed yield in cowpea.

Five promising accessions could be used as donor parents in future study or consumptions:

(1) Iron content: TVu-1330, TVu-13495, TVu-16400, TVu-8751 and TVu-9725

(2) Zinc content: TVu-1616, TVu-2155, TVu-15251, TVu- 301 and TVu-232

(3) Protein: TVu-13495, TVu-13088, TVu-15187, TVu- 347 and TVu-13468

## CHAPTER SIX

### 6 GENERAL DISCUSSION

#### 6.1 Participatory Rural Appraisal (PRA)

The participatory rural appraisal conducted revealed that farmers identified cowpea production constraints that did not differ significantly between Sudan-savanna zone (Bunkure and Wudil) and Sudano-Sahelian transition zone of Bichi local government area. Farmers in both Sudan savanna and Sudano-sahelian transition zones emphasized on seed related issues as the main production constraints: inadequate supplies of improved cowpea varieties especially at planting time, lack of insect resistant variety, diseases and drought tolerant varieties. As part of the management strategies, farmers adopt use of various insecticides at different formulations, which affect the environment and alter the ecosystem thereby, reducing the beneficial insects that aid pollination. This implies that breeding emphasis should focus more on host plant resistance in addition to ongoing pod borer resistant cowpea project in Africa by AATF. Furthermore, tackling biotic constraints like that of insects needs a multidisciplinary team of cowpea breeders, entomologist, pathologists, soil scientists, agronomist and socio-economist. The team could work more successfully if farmers' participation is encouraged.

Farmers and consumers identified seed quality traits as important criteria for making choices for cowpea varieties. Majority of the processors mainly women preferred reduced cooking time, ease of removal of the hilum and testa and less oil consumption. End users, on the other hand, considered protein, grain colour and size as their most preferred traits. Women consumers had more knowledge of nutritional values (iron and zinc content) of cowpea than men. Different cowpea characteristics desired by different cowpea users (farmers, processors and consumers) requires the involvement of various cowpea stakeholders to actively participate in the

process of variety development. This will lead to the development of cowpea varieties for specific locations and particular needs of consumers. Farmers in the study areas indicated their willingness to pay premium price for improved cowpea varieties that will satisfy their needs. Farmers in Bunkure and Wudil preferred insect resistant varieties in addition to grain attributes, while Bichi farmers were more concerned about early maturing variety that will escape terminal drought. Consumers especially women showed their willingness to pay for premium price for nutrient added value cowpea. These preferences for cowpea pose challenges to breeder to think of forming a multidisciplinary team that will include medical personnel, nutritionist and food industry toward solving consumer's problems.

## **6.2 Variation among cowpea accessions for grain quality traits**

The significant variation observed among the accessions for percentage crude protein, zinc content and iron content, implies that genetic variability for nutritional value particularly iron and zinc exist in cowpea and could be exploited. Many of these variations can be generated by conventional breeding methods to address the nutritional needs in developing countries. Modifying rank summation index of Mulamba and Mock (1978) identified three accessions: TVu-13088, TVu-13495 and TVu-9725 that contain the largest number of desirable nutritional attributes. These accessions can be nominated for anti-nutritional testing and yield stability testing across cowpea growing areas before recommendation for release for infant diets formulation and general use. For nutrient specific, five accessions each for high protein content, high zinc content and high iron content were identified which can also be recommended for use after validation.

The small genetic differentiation (0.26-0.45) between African cowpea accessions and USA accessions provides important insights that can be used to improve the efficiency of cowpea germplasm preservation and selection for parental lines when initiating a breeding work. The

information will enable rational planning by gene bank curators to help reduce duplicates among the accessions and form core collection of their accessions. Clustering of accessions based on closely related genetic distances implies that, accessions that are within a related genetic distance may exhibit common adaptive complexes of physiological traits coupled with a relatively restricted range of morphological and underlying genetic variation. Thus, crossing related accessions is expected to produce high frequency of relatively similar-looking progeny, while crossing between members of different cluster is expected to produce more variable progeny, perhaps with a relatively lower average performance in early generations, in this case selection should be delayed to later generations. Breeding strategies involving series of back crosses using conventional method is therefore recommended.

The negative correlation observed between protein and carbohydrate as well as protein and fat content indicates that selection for high protein will decrease carbohydrate content and fat content. Therefore, indirect selection for these traits should be avoided. On the other hand, the positive significant correlation observed between protein content and iron content, protein content and ash content implies the possibility of improving iron content by selecting for high protein or ash content. The non-significant correlation observed between 100 seed weight and protein content and iron content, respectively, indicates that direct selection for large grain size through selecting protein or iron is possible.

### **6.3 Inheritance of iron and zinc concentration and other seed quality traits in cowpea**

Iron and zinc content in cowpea grain fitted into the additive [a] and additive by dominance [ad] model. The resemblance of  $F_1$  to low iron content parent might be due to dominance, overdominance or epistasis. It may also be an indication of maternal inheritance of iron content in cowpea grain, as the expression of high iron content was masked in  $F_1$ , and started

manifesting in the subsequent generations ( $F_2$  &  $F_3$  seeds). This implies that iron concentration may be dependent on the seed coat, and selection for high iron in the cowpea grain should begin from the  $F_3$  seeds (embryo in  $F_2$  generation). On the other hand, the non-resemblance of the  $F_1$  population to the maternal parent (low zinc content) suggest that selection for high zinc content can begin with the  $F_2$  seeds.

Seed weight is predominantly under complementary gene action in this study, suggesting the possibility of considerable amount of heterosis for seed weight. The implication of this to breeding is that selection is facilitated as superior (large seeded) parent produced superior progenies (transgressive segregates) observed in this study. Seed weight in a cross between TVu-14845 and TVu-15251 showed significant negative additive [a] and negative dominance [d] suggesting large influence of recessive parents. High narrow sense heritability estimates for grain weight further indicates that selection can be made in early generation.

Plant height is under the control of duplicate gene action in a cross between short and tall plant and complementary in a cross between tall and short plant respectively. The prevalence of duplicate gene action would limit selection in early generations for plant height. It may therefore be necessary to delay selection in early selfing generations until homozygosity is reached. Therefore, it is recommended to make maternal parent plant to be taller when making crosses, as heterosis is expected there, due to complementary gene action.

#### **6.4 Challenges**

Integrating preferences of different cowpea stakeholders (farmers, processors, and vendors) into breeding objectives needs a multidisciplinary team of scientists. Few polymorphic markers (25 SNP for zinc and 10 out of 25 for iron) identified, constrained the identification of QTL associated with iron and zinc, despite the development of mapping population.

## 6.5 Recommendation

PRA should be considered as pre-breeding and part of breeding activity, as it will be used to set breeding objectives that will solve farmers and consumer's needs. This will facilitate adoption of new varieties when developed. This will help in proper designing, planning, implementation, monitoring and evaluation of breeding activities and impact assessment. Farmers' preference varied according to agro-ecological savanna and blanket recommendations could mask the preference choices in a particular savanna. This calls for the need for breeding varieties that are trait and site specific, in addition to high yielding.

Variability study have identified some promising accessions that combine reasonable (within the recommended dietary intake level) amount of protein, iron and zinc content and other set for nutrient specific. I therefore, recommend further anti-nutritional testing and yield stability testing before recommending to consumers as alternatives to animal protein which is currently beyond the reach of many consumers.

The study also raises several questions of a physiological and biochemical nature of minerals uptake by the plant, indicating that processes that culminate in the mineral storage in cowpea seeds are not well studied. Additional studies are needed to understand the integration of all those processes and their implications to micronutrient enhancement in crops like cowpea. Further research to explore more polymorphic SNP markers that could be used to identify QTL associated with iron and zinc concentrations and subsequent MAS is also encouraged. This will facilitate the development and deployment of enhance cowpea with iron and zinc content.

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## APPENDICES

### Appendix 3.1: Checklist for Focus Group Discussion (FGD) guiding questions

#### **Section A: Meeting with Farmers/growers**

1. How do you access agricultural information? (Be brief)
2. What are the social structures (relationships & membership of farmers to Researchers, Agricultural organization & Seed Companies)?
3. Identify the dominant crop grown in the area and why?
4. When does the cowpea grain is considered as the most important food crop in a year? (Calendar)
5. What are the problems that constrained cowpea production in the area? (Draw a table and allow farmers to rank in order of their preference)
6. What are the percentages of the area under cowpea cultivation, farmers that grow cowpea for food, feed or cash? (Draw a table)
7. Which of the varieties preferred by the consumers? Name & Seed size, colour & texture
8. Where do farmers get cowpea seed for planting? (Help to set research objectives)
9. How many varieties of cowpea do you grow? (Improved or local)
10. What attributes of cowpea do you prefer (early/late, high grain or fodder yield, Size, colour, texture, taste)?
11. Why do you cultivate cowpea; for food, feed or income? Which of the variety do you preferred most and why?

12. Do you process cowpea? [If yes] What are the attributes do you like most and why? any special preference traits that you need in a new variety?

14. In what form do you market your cowpea? Grain, prepared products etc.

#### Section B: Meeting with the Processors/ Consumers

15. Which of the cowpea attributes do you prefer most and why? Seed; sizes, colour, coat, texture, sweet or sweet less grain

16. Which of the cowpea consumable products (moin-moin, Akara etc) do you like best? and why?

17. How frequent do you consume your favourite cowpea products and of what quantity?

18. What difficulties do you encounter after eating any of the cowpea consumable products? Flatulence, stomach disorder etc

19. What are the constraints to cowpea processing? Time to imbibe water when soaking, hard-to-cook etc,

20. How does seed coat texture, colour and size of the cowpea affect coast of production, processing and marketing of the prepared products? Oil consumption, time to cook, milling difficulties etc

21. Which of the consumable products preferred most by the consumers?

22. What other importance of cowpea do you know apart from quenching hunger? Nutritional content of cowpea, present of micronutrients etc.....

Appendix 3.2: Farmers' Perceptions and Preferences for Cowpea Grain Quality Traits  
Questionnaire 2012

This inquiry is undertaken by a Plant Breeding student to collect information. I assure you that the information to be provided will be recorded completely, accurately, and be treated as

**CONFIDENTIAL**

**Section A: Farmer's identification**

1. State/savanna..... 5. L.G.A./District.....
2. Extension area..... 6. Village.....
3. Farmer's Name..... 7. Farmer's sex .....   
(Assign code 1 for male and code 2 for female)
4. Farmer's age.....   
(Assign code 1 for below 40 and code 2 for above 50 years)

**Section B: Constraint to production and farmers' preferences**

1. What is the main constraint to cowpea production in this area? (a) Striga  (b) Drought  (c) Insects  (d) Diseases  (e) others.....
2. Nature of the major constraint to cowpea production in your area
- (a) Drought: (a) terminal  (b) erratic rainfall
- (b) Major insect (a) Aphid  (b) Thrips  (c) *Maruca*
- (c) Major disease caused by: (a) Virus (b) Fungi (c) Nematode
- (d) Others.....
3. If drought is the main constraint, at which stage is becoming more devastating? (a) seedling  (b) pre-flowering  (c) during flowering
- (d) During grain filling
4. Do you have access to quality planting seed? (a) Yes  (b) No

5. Is the seed affordable, at the required time? (a) Yes  (b) No
6. If yes, how do you get access to the seeds? (a) Agro-dealers  (b) Government-subsidy  (c) NGOs  (d) Others.....
7. Why do you cultivate cowpea? (a) Food  (b) Feed  (c) Income  (d) All
8. Which percent do you sell? (a) below 50%  (b) Above 50%
9. Which of the grain attributes do consumer pay premium price in your locality?  
(a) White & large seeded  (b) White & small seeded  (c) Brown & large seeded   
(d) Others specify.....
10. Do you consider end users' preferences when sourcing a variety? (a) Yes   
(b) No  (c) sometimes
11. Which of the attributes of cowpea do you like most? (a) earliness  (b) lateness   
(c) high grain yield  (d) high fodder yield  (e) a, c & d

### Appendix 3.3: Cowpea Processing and Consumption Constraints and Preferences for Grain Quality Traits

This inquiry is undertaken by a Plant Breeding student to collect information. I assured you that the information to be provided will be recorded completely, accurately, and be treated as

**CONFIDENTIAL**

#### Section A: Processor/Consumer's identification

1. State/savanna zone.....
2. Extension area.....
3. Name.....
4. Interviewee's age.....  
(Assign 1 for 15-30yeras, 2 for 30-40yrs and 3 for 50 years and above)
5. L.G.A./District.....
6. Village area .....
7. Sex.....  
Assign code 1 for male and code 2 for female)
12. Does seed coat colour determine your acceptability of cowpea?(a)Yes  (b) No
13. If yes, which colour is the most acceptable by the consumers? (a) Red  (b) White  (c) Brown  (d) others.....
14. Does grain texture affect processing/ preparation time? (a) Yes  (b) No
14. How does the texture affect preparation time? (a) Delays imbibitions when soaking  (b) Difficulty in dehulling  (c) Difficulty in milling  (d) others  specify.....
14. Which of the texture has short time to preparation? (a) Smooth  (b) Rough
15. Do you store cowpea grain for a long time? (a) Yes  (b) No
16. Which of the texture hasten hard-to-cook phenomenon when cowpea is stored for a long time? (a) Rough  (b) smooth
17. Which of the grain colour do you prefer most? (a) White  (b) Red  (c) Brown  (d) others specify.....

18. Which of the cowpea consumable products do you like most?

- (a) Akara/kossai  (b) Dumplings  (c) Cowpea with rice   
 (c) Moin moin  (d) others.....

19. How frequent do you eat cowpea or cowpea products?

Cowpea consumable products	everyday	3 days in a week	Once in a week	Occasionally (> 2 week)	Not at all
cooked cowpea					
Cowpea dumpling (Danwake)					
Cowpea mixed with rice					
Moin moin					
Akara / Kossai					
Cowpea for soup					
Others specify.....					

(Score in order of your preference 1=most, 2=moderately and 3=least preferred)

20. If you are not frequently eating cowpea/ cowpea products why? (a) Flatulence  (b) stomach disorder  (c) others specify.....

21. How frequent do you feed your children (under age 5) with cowpea/ cowpea product? (a) Frequently  (a) Not frequently  (c) Not at all

22. Do you encourage pregnant and nursing mothers to eat cowpea/ its products? (a) Yes   
(b) No

23. Which of the cowpea grain do you like most? (a) Sweet  (b) Sweet less

## Appendix 4.1 Selection criteria for cowpea Parental lines (Mulamba and Mock, 1978)

Accession	Mat urity	Grai n size	Zinc	Iron	% prote in	% Fibr e	% CH O	% F at	Gro wth habit	Sha tteri ng	Grain colour	Grain Text ure	sum total score	Remarks
TVu-1	2	2	3	3	1	3	1	3	2	1	2	1	2.0 (24)	S
TVu-15225	2	2	3	2	1	3	2	3	3	1	2	2	2.2 (26)	NS
TVu-13088*	1	3	2	2	1	2	2	3	1	1	3	2	1.9 (23)	NS
TVu-16400	2	1	3	1	2	3	1	3	1	1	2	2	1.8 (22)	S
Babban wake	3	1	3	3	1	3	1	2	3	1	1	1	1.9 (23)	NS
TVu-999	2	2	3	2	1	3	2	3	2	1	3	3	2.3 (27)	S
TVu-8742	1	2	3	3	1	2	2	3	1	1	2	2	1.9 (23)	S
Sampea-13	1	2	3	3	1	2	2	2	1	1	1	1	1.7 (20)	S
TVu-14845	2	2	3	3	3	2	2	3	1	1	1	1	2.0 (24)	S
TVu-875	2	2	3	3	3	2	2	3	2	1	1	2	2.2 (26)	NS
TVu-997	2	2	3	1	1	3	2	3	3	1	2	2	2.1 (25)	NS
TVu-15251	2	2	3	2	1	1	2	3	3	1	1	1	1.8 (22)	S

Number in the bracket is the sum total, the lower the sum total score the better the cowpea lines; S= selected for making crosses; NS=

not selected

**Scales:** 1= High protein or Fe or Zn content or / early maturing or erect or white colour, 2= Medium protein or Fe or Zn content or shattering or brown or Semi erect and 3= Low protein or Fe or Zn content, prostrating, late maturing or small grained.

- a. Maturity period – 1= early (70-75days), 2= Medium (80-95 days) and 3= late maturing (above 100 days)
- b. Growth habit – 1- Erect, 2= Semi-erect and 3= prostrating type
- c. Shattering- 1= Normal, 2= Low shattering and 3= High shattering
- d. Grain size (100 grain weight g) - 1= Greater than 20g, 2= 15-19g and 3= less than 10g
- e. Protein content - 1= 21-29%, 2=10-20% and 3= less than 10%
- f. Zinc content (mg/kg)- 1= greater than 50, 2= 21-40 and 3= below 20
- g. Iron content (mg/kg)- 1= greater than 100, 2= 50-99 and 3= less than 50
- h. Fibre content (%)- 1= 3.1- 4.9%, 2= 2.1-3.0% and 3= 1.0-2.0%
- i. Carbohydrate- 1= 71-78%, 2= 60- 70% and 3= less than 59%
- j. Fat – 1= 11-22%, 2= 6-10% and 3= 1-5%
- k. Grain coat colour- 1= White, 2= Brown and 3= others
- l. Grain coat texture – 1= Rough and 2= Smooth

Appendix 4.2: List of SNP markers used for characterizing 169 cowpea accessions

S/N	DNA \ Assay	S/N	DNA \ Assay	S/N	DNA \ Assay	S/N	DNA \ Assay	S/N	DNA \ Assay
1	10969_452	26	13707_697	51	16914_262	76	4339_822	101	6673_1242
2	4146_1588	27	13794_319	52	17023_955	77	4403_1123	102	6700_679
3	9673_1553	28	13849_2039	53	17450_1553	78	4462_114	103	7344_500
4	10115_384	29	13863_519	54	17588_963	79	4558_472	104	7438_464
5	10661_873	30	13873_544	55	1799_940	80	4563_661	105	7857_1368
6	10738_1400	31	13947_415	56	2185_132	81	4749_1972	106	7906_1032
7	10811_937	32	14030_764	57	2314_546	82	4778_497	107	7993_539
8	11367_1228	33	14164_1877	58	2339_52	83	483_1152	108	8011_481
9	11470_272	34	1426_521	59	234_249	84	4892_514	109	8118_1675
10	11622_232	35	14462_1712	60	2728_121	85	4904_278	110	8166_564
11	12119_480	36	14497_540	61	2974_1109	86	5058_372	111	8193_441
12	12122_559	37	14542_452	62	2997_519	87	5239_234	112	8306_119
13	12261_1773	38	14619_471	63	3098_224	88	5294_469	113	8605_2122
14	12393_305	39	14714_840	64	3485_771	89	534_355	114	8969_1386
15	12505_1312	40	14730_1034	65	361_520	90	5356_124	115	9134_1559
16	12703_553	41	14769_1746	66	3673_401	91	5428_339	116	9147_1655
17	12933_387	42	14929_258	67	3720_560	92	5435_569	117	9678_835
18	12959_58	43	14965_280	68	38_239	93	5448_461	118	9880_545
19	1296_808	44	15054_315	69	3838_830	94	5503_54	119	9955_544
20	13017_290	45	15183_436	70	3885_1019	95	5552_536		
21	13034_542	46	15534_890	71	3900_562	96	5553_147		
22	13207_784	47	15933_118	72	394_316	97	5692_1408		
23	13506_333	48	16043_314	73	395_895	98	593_329		
24	13563_863	49	16239_889	74	411_247	99	6065_457		
25	13586_1058	50	16462_1286	75	4325_585	100	6247_659		

