STABILITY OF YIELD AND ANTIOXIDANT CONTENT OF SELECTED ADVANCED COWPEA (*Vigna unguiculata* [L] Walp.) MUTATION DERIVED LINES

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DECLARATION

I, Nelia Nkhoma hereby declare that the work presented in this dissertation was my own and has never been submitted for a degree at this or any other university

Signature…….

Date……..

APPROVAL

This dissertation of Ms Nelia Nkhoma was approved as fulfilling part of the requirements of the award of Master of Science in Plant Breeding and Seed Systems by the University of Zambia

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ABSTRACT

Zambia has a number of cowpea germplasm which are high yielding and contain antioxidants which are useful for preventing a lot of health problems i.e. Heart attack, Hypertension, Obesity and Cancer. Unfortunately the production suitability of these germplasm in the different areas of the country is not known. Based on this, a study was conducted to evaluate the stability of cowpea yield and antioxidants (total phenolic content and condensed tannin content) in the three agro ecological zones as well as to determine the relationship between the antioxidants and the seed coat colour of the cowpeas. Multilocation field trials involving ten cowpea genotypes were conducted at three different Agro-ecological Regions. A randomized complete block design was employed with 3 replications. Cowpea grain yield and antioxidant contents of the seed were determined and a stability bases analysis tool, Additive Main effect and Multiplicative Model (AMMI) was employed for data analysis. Assessment of genotype x environment (GxE) interaction on cowpea grain yield stability indicated that GxE was not present for yield indicating that genotypes did not respond differently to varying environmental conditions. However, some genotypes had higher yields than others indicating genotype identification to specific environments. Genotypes MS1/8/1/4 and LT11/3/3/12 were adapted to high potential yielding environment and were unstable while BB4/2/4/1 and LT11/5/2/2 were adapted to low yielding environment and were stable environments. Assessment for antioxidants showed that GxE was significant ($p<0.01$) and higher yielding genotypes had low antioxidant contents compared to low yielding genotypes. Genotype LT PRT had higher antioxidant concentration (3.47mg/100mgCE) and stable (IPCA2 0.022) while MS PRT had lower concentrations (0.17mg/100mgCE) and unstable (IPCA1 0.630). The genotypes which had higher antioxidant concentrations had darker seed coat colour (yellowish brown and purplish brown) compared to the ones which had low antioxidant concentration (white). This study identified stable genotypes in both yield and antioxidants. However, further studies for assessing yield stability are necessary and could be achieved by including more seasons and sites to get a better understanding of GxE and yield stability of cowpea in Zambia.

DEDICATION

To the Almighty God, for making everything possible as well as the knowledge, love, good health and protection he has blessed me with throughout my entire life.

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Table of Contents **Page**

List of Tables **Page**

List of Figures **Page**

List of Appendices **Page**

Chapter 1

1. INTRODUCTION

Cowpea *(Vigna unguiculata* (L) Walp) is one of the oldest crops known to man (Martin et al., 1967) with its centre of origin in West Africa (Ng and Padulosi, 1988). It is an important food legume and a valuable component of the traditional cropping systems in the semi-arid tropics covering Asia, Africa, Central and South America (Mortimore et al., 1997; Singh and Tawarali, 1997).

Four cultivar groups of cowpea are recognized (Baudoin and Marechal, 1985): (1) *Unguiculata*, which is the most common form; (2) *Biflora* or *catjang* which is characterized by small erect pods and found mostly in Asia; (3) *Sesquipedalis* or yard-long bean also mostly found in Asia and characterized by its very long pods which are consumed as a green 'snap bean'; and (4) *Textiles*, which is found in West Africa and used for fibers obtained from its long peduncles. Cowpea is a short-day plant with many accessions that are photoperiod sensitive (Ehlers and Hall, 1997). In addition, its seeds are characterized by a wide variation in seed coat color.

Cowpea in Zambia is grown in varying extents across the whole country although yields are usually very low (Muimui, 2001). According to 2004 supplemental survey (CSO/ FSRP, 2004), cowpeas are grown in all Zambian provinces although production is highly concentrated in a few areas. Southern province accounts for the majority of cowpea production with 58%, followed by Central province with 11%, Northern Province 9% and Lusaka province with 6%. The top four provinces account for 83% of total output. This situation has left most of the families in the low income brackets to rely on predominantly cereal grain and starchy tubers diets that are low in protein and minerals (i.e. iron and zinc) and other nutritional factors such as antioxidants. This has lead to high levels of malnutrition especially among expectant mothers and children as well as infant mortality and morbidity. Among the affluent there is high intake of meat especially red meat leading to increased incidence of metabolic syndromes- diabetes mellitus, coronary heart diseases and hypertension.

Cowpea's drought tolerance makes it an important food security crop in Zambia. Anti oxidants are usually secondary metabolites that when consumed help in reducing or preventing cellular damage resulting from free- radicles attack. Antioxidants are essential for inhibiting oxidation of

other phenolic acids, strengthening immune systems, protecting cells from damage caused by unstable molecules and lowering risk of cancer, heart disease as well as memory loss (Bazzano et al 2001; Winham et al, 2007; Lanza et al, 2006; Bobe et al 2008). Scientific evidence is essential for making effective dietary recommendations on the type of cowpea, level of consumption, and design of food processing strategies that maximize the beneficial effects. Such evidence will also provide a basis for genetic and agronomic improvement aimed at optimising composition of beneficial compounds. It is the first step in transforming cowpea into a primary food to address malnutrition in poor populations, and promoting cowpea as a mainstream part of a healthy diet. Such interventions may lead to increased demand for cowpeas and improvement in nutritional and economic well-being of producers and overall health of consumers.

Cowpea is an important food legume grown on 9.8 million hectares of small farms in the dry savannah of tropical Africa and current estimates, (Inaizumai et al., 1999) place world cowpea production at 3 million tons. The crop is of major importance in the livelihoods of millions of relatively poor people in less developed countries of the tropics. Its value lies in its high protein content (23-29%, with potential for perhaps, 35% protein) and up to 50-67% starch. The plant is favoured by farmers because of its ability to maintain soil fertility through its capacity to fix nitrogen, which allows it to grow on, and improve poor soils (pH range 4.5 – 9.0, organic matter less than 0.2%, and a sand content of more than 85%), (Blade *et al.,* 1997).

Cowpea is of major importance to the nutrition and livelihoods of millions of people in poor countries of the tropics (Singh et al., 2003). The species can play a significant role in food security initiatives aimed at addressing problems of food production in these regions. The legume is consumed in several ways. The dried seeds are an important protein source (22 -23 % protein content) (Bressani, 1985), and can be ground into a meal which is used in a number of ways (Nout, 1996; Nielsen et al., 1997). Fresh seeds and immature pods are frozen or canned and consumed as ‘green beans’ in developed countries. The young shoots and leaves are eaten as a leafy vegetable and provide the most widely used pot herbs in tropical Africa which are often dried and can be stored for dry season use. Cowpea is equally important as a nutritious fodder for livestock (Singh and Tawarali, 1997). In West Africa, mature cowpea pods are harvested and the haulms are cut whilst still green; these are stored for use and for sale as a livestock feed

supplement in the dry season (Singh and Tawarali, 1997). The species can also be used as a green manure or cover crop. The seeds are sometimes used as coffee substitutes.

Cowpea also has the ability to be intercropped with cereals such as millet and sorghum. Coupled with these attributes, its quick growth and rapid ground cover have made cowpea an essential component of sustainable subsistence agriculture in marginal lands and drier regions of the tropics, where rainfall is scanty and soils are sandy with little organic matter (Singh *et al., 1993*). However, most of the world's cowpea is grown primarily in dry regions where drought is prevalent among several yield reducing factors (Watanabe *et al.,* 1997).

Being a drought tolerant and hot weather crop, cowpea is well-adapted to the semi- arid regions of the tropics where other food legumes do not perform well (Singh et al., 2003). (Van Rij, 1997) observed that the rainfall requirement for cowpea in Southern Africa can be as low as 300 mm, spread over the growing season.

In the Sahel region, yields of up to 1000 kg ha-1 have been recorded under conditions of limited moisture (181 mm per year) and high temperatures (Hall and Patel, 1985). Yields are reported to range between 2500 kg ha-1 in Southern Africa to 4000 kg/ha at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria (van Rij, 1997). In California, USA, under favourable conditions, yields ranging between 4000-7000 kg ha-1 have been reported (Sanden, 1993).

Although cowpea is an important crop, not many countries have initiated cowpea improvement programmes (Singh et al., 2003). No recent and/or reliable data on global cowpea production can be found since FAO stopped publishing cowpea statistics (FAO, 2001). The production data for cowpea is pooled with that of common bean (*Phaseolus vulgaris*) (FAO, 2006). However, it is estimated that the worldwide area under cowpea is about 14 million ha with over 4.5 million tons of annual production (Singh et al., 2003). More than 60% of the production and 75% of the area is spread over the arid and semi -arid regions of sub-Saharan Africa (Ehlers and Hall, 1997).

The success of most of the cowpea improvement programmes largely depend upon the genetic variability and heritability of desirable traits. It has been shown that most legumes including cowpea have lost many alleles for high productivity, seed quality, and pest and disease resistance in the process of adaptation to environmental stress (Olabisi *et al.,* 2007). Genetic improvement

through mutation breeding therefore offers a possibility for the development of basic genomic resources that could alleviate the crops poor traits. The use of induced mutation has long been recognized as a rapid source of producing genetic variation in crops (Harris, 1979). Mutations whether spontaneous or induced result in changes in base sequences of genes, changes in chromosomes or a change in plasma-genes (Singh, 2007). Induced mutation has helped in widening the genetic base and, therefore, consequently increasing genetic variability that has made it possible to bridge yield gaps, reduce maturity time and improve nutritional quality in many crops (FAO/IAEA, 2004). Other than mutation breeding, use of molecular markers and marker assisted selection are new approaches that expedite the process of plant breeding. Molecular based technologies, in combination with conventional breeding strategies can help to gain a more rapid genetic improvement of cowpea (CGI, 2006). Transgenic (genetically modified) crops that carry genes which could not be introduced otherwise by any conventional method, is another strategy of marker assisted selection. Phenotypically stable genotypes are of great importance because environmental conditions vary from season to season and year to year.

Wide adaptation to particular environments and consistent performance of recommended varieties/hybrids are very important for successful cultivation of cowpea. Although many varieties are recommended for cultivation, the information on their stability is lacking. In the present study, a number of advanced genotypes of cowpea coming out of the breeding programme were evaluated for genotype by environment (G x E) interactions for identifying the high yielding stable genotypes for cultivation and for their utilization in the breeding programme. The genotype by environment (G x E) experimental approach can be a very useful tool for studying plant adaptation, particularly when using a broad range of genotypes and environment. The underlying assumption is that diverse evaluation environments will exert varying selection pressures which will result in differential performance in a diverse group of test genotypes. If environments and genotypes are well characterized by measuring traits associated with differential performance, it becomes possible to use the G x E approach for studying specific adaptation (Berger et al. 2007). Questions of interest to breeders and physiologists include-what makes an environment low or high yielding and do genotypes respond differently to different environments, and if so, why? The historical variety trial conducted over multiple environments is a special case of G × E experiment which provides insight into breeding programmes by

introducing the dimension of time. Thus it becomes possible to assess how varieties change over time, whether the program has produced specific adaptation to regions or different environment.

The main objective of the study was to determine the effect of genotype – environmental (GxE) interactions on yield and protein quality in selected advanced cowpea genotypes.

The specific objectives were

i. To evaluate the stability of yield among the different advanced cowpea genotypes in the different agro ecological zones of Zambia.

ii. To evaluate the stability of antioxidants (total phenolic contents and condensed tannins) traits among the different advanced cowpea genotypes in the three production agro-ecological zones of Zambia.

iii. To determine the relationship between antioxidants content and seed coat colour of different cowpea genotypes.

The results of this study will provide information on adaptability of the new advanced cowpea lines in the three agro ecological zones with regard to selected parameters. This information may assist in determining the usefulness of the possible new varieties vis a vis adaptability and production in the different environments. It is assumed that the results will enhance the efficiency of the crop improvement efforts and also benefit consumers as it will additionally provide information in nutritional factors that hitherto has not been available.

Chapter 2

2. LITERATURE REVIEW

2.1. Origin and diversity of Cowpea

Major diversity in cowpea (*Vigna unguiculata* [L] Walp.) is found in Asia and Africa but the precise origin of cowpea has been a matter of speculation and disagreement for many years. Early observations showed that cowpeas in Asia were very diverse and morphologically different from those in Africa; therefore, both Asia and Africa were thought to be independent centres of origin of cowpea (Johnson, 1970; Summerfield *et al.,* 1974; Tindall, 1983; Coetzee, 1995). However, the absence of wild cowpeas in Asia has brought into question Asia being a centre of origin for cowpea. Current evidence suggests that cowpea originated in Southern Africa although it is difficult to ascertain where in Africa the crop was first domesticated. Several centres of domestication have been suggested such as Ethiopia, Central Africa, South Africa and West Africa. Based on the distribution of diverse wild cowpeas in Eastern Africa stretching from Ethiopia to Southern Africa, the working group meeting of the International Board for Plant Genetic Resources on *Vigna*, held in New Delhi in 1981 recommended as a priority, collection of both wild and cultivated forms of cowpea in southern Africa, Zimbabwe, Transvaal and Natal (Padulosi and Ng, 1997). East and Southern Africa are considered as the primary region of diversity and West and Central Africa to be the secondary centres of diversity. India in particular and Asia in general have been proposed to be the third centre of diversity (Allen, 1983). Recent investigations by the International Institute of Tropical Agriculture (IITA) in collaboration with *Instituto del Germoplasmo* (CNR) Bari, Italy, strongly indicate that the region encompassing Namibia, Botswana, Zambia, Zimbabwe, Mozambique, Swaziland and South Africa have the highest genetic diversity in respect of primitive wild forms of cowpea. Some very primitive species were observed in the Transvaal, Cape Town and Swaziland. Based on this, it has been suggested that Southern Africa may be the origin of cowpea and from there primitive forms moved to other parts in Southern and Eastern Africa, and from there to Asia and West Africa (Flight, 1976).

Since cowpea was known in India before Christ and it has Sanskrit name in early treatise dating back to 150 BC cowpea must have moved from East Africa to Asia more than 2000 years ago

where human selection led to modified forms of cowpea different from Africa. It has been suggested that cowpea probably moved from Eastern Africa to India before 150 BC, to West Asia and Europe about 300 BC and to Americas in 1500 AD. Since Western Asia and Europe do not have desired climatic conditions for cowpea, not much variability and selection occurred as it happened in South Asia and South East Asia where small seeded and vegetable cowpeas were selected. Probably, the wild cowpeas with very small seeds were distributed by birds in East and West Africa much before Christian era and therefore the presence there of great diversity and secondary wild forms. Selections for larger seeds and better growth habits from natural variants in wild cowpeas by humans, must have led to diverse cultivars and their domestication in Asia and in Africa (Flight, 1976).

2.2. Taxonomy

Verdcourt (1970) and Marechal *et al.* (1978) classified cowpea to have come from Fabeles Order, Fabacea family and faboideae subfamily. It is also from phaseoleae tribe, phaseolinae subtribe, *vigna genus* and *unguiculata* species. The genus *Vigna* contains several species that are important in world agriculture. Cowpea (*V. unguiculata*), Mung beans (*V. radiata*), and Urd beans (*V. mungo*). Several other species, i.e., adzuki beans (*V. angularis*), moth beans (*V. aconitifolia*), rice beans (*V. umbellata*), and Bambara groundnut (*V. subterranea*).

2.3. Morphology and botanical characteristics

According to James (2002), all cultivated cowpea varieties are considered warm season and adapted to heat and drought conditions. It is an annual, herbaceous legume. Plant types are often categorized as erect, semi-erect, prostrate (trailing), or climbing. There is much variability within the species. Growth habit ranges from indeterminate to fairly determinate with the non-vining types tending to be more determinate. Cowpea generally is strongly tap-rooted. Root depth has been recorded at 22.5 cm depth 8 weeks after planting. Cowpea seed ranges in size from the very small wild types up to nearly 2-12 mm long and the number of seeds per pod range from 8 to 20. Seed shape is a major characteristic correlated to seed development in the pod. Seeds develop a kidney shape if not restricted within the pod. When seed growth is restricted by the pod the seed becomes progressively more globular (James, 2002).

The seed coat can be either smooth or wrinkled and of various colours including white, cream, green, buff, red, brown, and black (IITA, 2000). Seed may also be speckled, mottled or blotchy. Cowpeas are also referred to as "eyed" (black-eye, pink-eye, purple hull, etc.) based on the colour surrounding the white colored hilum. Germination is epigeal (similar to common bean and lupin) where the cotyledons emerge from the ground during germination. This type of emergence makes cowpea more susceptible to seedling injury, since the plant does not regenerate buds below the cotyledonary node. The trifoliate leaves develop alternately. Leaves are smooth, dull to shiny, and rarely pubescent. Commonly, the terminal leaflet is longer and larger than the lateral leaflets. There is a wide range in leaf size and shape. Cowpea generally is day- length photoperiod sensitive Flowers are borne in multiple racemes on 20 to 50cm flower stalks (peduncles) that arise from the leaf axil. Two or three pods per peduncle are common and often four or more pods are carried on a single peduncle. The presence of these long peduncles is a distinguishing feature of cowpea and this characteristic also facilitates harvest. The open display of flowers above the foliage and the presence of floral nectarines, contribute to the attraction of insects. Cowpea primarily is self pollinating. Cowpea pods are smooth, 15 to 25cm long, cylindrical and generally somewhat curved. As the seeds approach the green-mature stage for use as a vegetable, pod colour may be distinctive, most commonly green, yellow or purple. As the seeds dry, pod color of the green and yellow types becomes tan or brown.

2.4. Growth habit

Seed shape is a major characteristic correlated with seed development in the pod. Seeds develop a kidney shape if not restricted within the pod. When seed growth is restricted by the pod the seed becomes progressively more globular.

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2.5. Utilisation

Seeds can be eaten fresh or dried for storage; leaves can be eaten as a vegetable, or used for forage or silage; and plants can be incorporated as green manure. Indeterminate cultivars are best suited for subsistence farming communities, whereas erect, determinate forms are more suitable for commercial farming in mono-cultural systems (Duke, 1981). Cowpea is a heat-adapted legume that will make maximum growth during short summer periods before late-summer or fall-planting (Miller, 1988). It is also suitable for summer legume cover in orchards and vineyards or under- sown with maize. To prepare poor soil, cultivars with spreading habit and luxuriant growth have been widely used as green manures. Cowpea is a promising multipurpose legume in cropping systems. As a vegetable crop, cowpea can be used at all stages of development. The tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach. Green cowpea seeds are boiled as a fresh vegetable, or may be canned or frozen. Dry mature seeds are also suitable for boiling and canning. In many areas of the world, the cowpea is the only available high quality legume hay for livestock feed. Digestibility and yield of certain cultivars have been shown to be comparable to lucerne (*Medicago sativa*). Cowpea may be used as a green or dry fodder. It also is used as a green manure crop, a nitrogen fixing crop, or for erosion control.

Cowpea seed is a nutritious component in the human diet, as well as a nutritious livestock feed. Nutrient content of cowpea seed is 24.8% protein, 1.9 % fat, 6.3% fiber, 63.6% carbohydrate, 0.00074% thiamine, 0.00042% riboflavin and 0.00281% Niacin. The protein in cowpea seed is rich in essential amino acids lysine and tryptophan, compared to cereal grains; however, it is deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins.

2.6. Environmental Requirements:

2.6.1. Climate

Cowpea is a warm-season crop well adapted to many areas of the humid tropics and temperate zones. It tolerates heat and dry conditions, but is intolerant of frost. Germination is rapid at temperatures above 28°c and colder temperatures cause slow germination.

Cowpea is grown under both irrigated and non-irrigated production regimes. The crop responds positively to irrigation but will also produce well under dry land conditions. Cowpea is more drought tolerant than common bean. Drought tolerant is one reason that cowpea is such an important crop in many underdeveloped parts of the world. If irrigation is used, more vegetative growth and some delay in maturity may result. Application rates should insure that the crop is not overwatered, especially in more northern latitudes, as this will suppress growth by lowering soil temperatures. The most critical moisture requiring period is just prior to and during bloom.

2.6.2. Soil Fertility and Lime Requirements

Cowpea tolerates a wide variety of soils and soil conditions, but performs best on well-drained sandy loams or sandy soils with a soil pH of 5.5 to 6.5. Cowpea, like all legumes, forms a symbiotic relationship with a specific soil bacterium (*Rhizobium* spp.). *Rhizobium* makes atmospheric nitrogen available to the plant by a process called nitrogen fixation. Fixation occurs in root nodules of the plant and the bacteria utilize sugars produced by the plant. Although cowpea *Rhizobium* is normally widespread, seed inoculation with *Rhizobium* specific to cowpea would be beneficial in areas where it is not present.

Excess nitrogen (N) promotes lush vegetative growth, delays maturity, may reduce seed yield and may suppress nitrogen fixation. The plant will perform well under low N conditions due to a high capacity for N fixation.

2.6.3. Diseases and their Control

Cowpea is susceptible to a wide variety of pests and pathogens that attack the crop at all stages of growth. Common diseases in Zambia include: scab, blight, cercospora leaf spot, web blight,

mosaic virus and bacterial blight (Kaitisha *et al.,* 2002). The common pests include aphids (*Aphis cracivora)*, flower beetles (*Euphoria sp*.), pod borer (*Maruca vitrata*), bean fly (*Ophiomyia phaseoli*), leaf hopper (*Empoasca dolichi*) and cowpea bruchids (*Callosobruchus maculates)*.

Fungal diseases

Southern blight is caused by a fungus that attacks roots and stems of cowpeas. The first visible symptom of southern blight is a progressive, yellowing and wilting of the foliage beginning on the lower leaves. The plant dies within a few days after the rust symptoms appear. A brownish vascular discolouration inside the stem may extend several inches above the soil line. During warm, moist conditions, the coarse, white mycelium of the fungus makes characteristic fan-shaped patterns of growth on the stem at the soil line. In these white-mat of the fungus, numerous smooth, round, light-tan to dark-brown mustard seed-like bodies called sclerotia are formed.

Another important category of diseases is that of fungi and includes the important fusarium wilt (Armstrong, 1941). Fusarium wilt is caused by *Fusarium oxysporum*) and usually causes the lower leaves on one side of the plant to turn chlorotic. Infected plants usually are stunted and later on wilted as the organism develops in the assimilate and water conducting tissues. Brick red tissue can be observed in the stem when it is split lengthwise. The best control of Fusarium wilt is the use of resistant varieties. When resistant varieties are not used, it is important that root-knot nematode control practices be followed since nematodes increase plant susceptibility to Fusarium wilt. Bacterial diseases are not very common in Zambia.

Diseases can be reduced by:

- Treating high quality seed with fungicides labeled for cowpeas.
- Avoiding throwing soil against plant stems during cultivation.
- A four or five year rotation with other crops.
- Seeding into warm, well-prepared soils.
- Planting certified seed of resistant varieties.
- Controlling weeds.

- The removal of virus-affected plants.

Viral diseases

Several viruses attack cowpeas including Mosaic virus. A characteristic symptom of the mosaic virus disease is an intermixing of light and dark-brown areas. Mottled areas are irregular in outline and may follow the main veins. Infected leaves are generally smaller than healthy ones, and often there is a slight puckering and curling of leaf edges. Infected plants usually are more dwarfed and bushy and yields are reduced. Mosaic diseases also result in malformed pods. Plants infected during seedling stages may be barren. A number of methods are used to manage viral infections. One cost effective way to prevent large yield losses from virus diseases is to grow tolerant varieties.

2.6.4. Insects and other predators and their control

Root-knot nematodes cause the root to appear knotted and galled. Above ground nematode symptoms appear as nutrient deficiencies, with stunting and often wilting because the root system is incapable of absorbing adequate amounts of water and nutrients. It is common to confuse nematode root symptoms with the nodules of nitrogen fixing bacteria. Nodules are attached to sides of roots, and galls are within the roots. Root-knot nematodes are additionally harmful to the cowpea because root injuries make the plants much more susceptible to attack by Fusarium wilt. In addition to detecting the presence of nematodes by observing galled roots, they can be detected by a soil test for nematodes. If nematodes are present certain practices help reduce nematode populations. These practices include crop rotation, fallowing, sanitation; weed control, and planting resistant varieties.

Cowpea bruchids (*Callosobruchus maculates*) are the major storage pests in Zambia and Africa as a whole (Profit, 1997). The damage is restricted to eating quality only where it makes it not easy to sell cowpea grains when riddled with bruchid holes. However, germination is not affected (Farming Systems Research and Extension unit, 1999). Damaged grains are full of small holes and dead beetles may be found inside the grains. For control, farmers mix cowpea grains

with ash. In biological control, a larval parasitoid wasp (*Dinarrmus basalis*) and egg parasitoid wasps (*Uscana lariophoga*) can also be used.

Aphids (*Aphis cracivora)* are small, green, soft-bodied insects that feed by piercing the plant tissue and withdrawing plant juices. Infestations of this pest develop on leaves and the fruiting stems. Their feeding, especially on the fruiting stem reduces the amount of plant nutrients available for pod and pea development. Infested foliage turns yellow and dies. Aphids excrete large quantities of a sugary substance called honey dew which supports the growth of sooty mold. Sooty mold, a fungus, is dark in color, which reduces the amount of sunlight that reaches the plant.

2.6.5. Harvesting

Harvesting can be carried out manually (hand harvesting) or by using a combine harvester in the case of large-scale production. The upright cultivars are easy to harvest by machine. Cowpea grown as a dried seed product can be direct combined, using a platform head or a row crop head. In the case of cowpeas grown for vegetable purposes, young leaves are mainly picked by hand. Older leaves accumulate dust or get spattered with mud from raindrops if not harvested. Harvesting of cowpea in most cases should coincide with the onset of dry season when the dry pods can remain about a week awaiting harvesting without spoilage. However, to avoid field weathering or shattering, dry pods should not be left in the field longer than 2 weeks after full pod maturity.

2.6.6. Drying and Storage

The storage life of cowpea depends on its moisture content before storage. The lower the moisture content, the better the quality of seeds in storage. In developed countries; one alternative is the use of cold storage. An exposure to -18 ^{o} C during 6 to 24 hours can reduce pest numbers by more than 99 %. The grain can be stored short term at around 12 % moisture or less, with 8 to 9% recommended for long-term storage. Cowpea leaves are dried to store them for the dry season. Sun-dried leaves may store for up to a year because dried, cooked leaves are not damaged as much by insects as dried seeds. However, most farmers small scale farmers in Zambia store cowpea in their clay pots for future use in dry season.

2.6.7. Production systems

Studies have shown that traditionally in West and Central Africa and Asia large portions of the fields had mixed cropping involving millet, sorghum, cowpea and groundnut with occasional fields of maize, cassava and Bambara groundnut. The major crop mixtures were millet-cowpea (22%), millet-sorghum-cowpea (15%), millet-cowpea-groundnut (12%) and sorghum-cowpea-groundnut (6%). Cowpea was thus a predominant component of all crop mixtures. The planting pattern differed from farmer to farmer, but cowpea was generally planted in alternate rows or within the cereal rows, occupying 33 to 50% of the land area in each field. There was great diversity in the varieties grown; early-maturing varieties, grown for grain; and late-maturing varieties, grown for fodder. Both types were planted in the same field. One of the most commonly practiced rotations was millet-grain, cowpea-millet-fodder cowpea (Singh, 1993).

2.7. Production: Global and National

While cowpea is grown throughout West and Central Africa, its adaptation to drought makes it especially important for the rest of Sub-Saharan region. In Nigeria, (Singh et al., 1997) reported on-farm trials yields of 2.8 t ha^{-1}, while in Burkina Faso, reported average yields are about 83% less than experimental on-farm trial yields (SAFGRAD, 1998). Nambou et al., (1999), reported 2.0 t ha^{-1} in Togo, compared with 0.24 t ha^{-1} at farm level and in Ghana, the estimated researcher-managed on-farm yields of 1.8 t ha^{-1} is more than double the average farm level yields (SARI, 1999). Reasons for the low yields in most countries include use of low yielding traditional varieties, poor soil fertility, unfavorable weather, and insect pests and diseases. Between 1990 and 1999, West and Central Africa annually produced 2.6 million tons on 7.5 million ha, or about 59% of the world's harvested area. Nigeria, the largest cowpea producer in the world, accounted for about 65% of the region's supply and Niger, the second largest producer in the region, and third in the world, accounted for 15%. The remaining 20% was produced in Burkina Faso, Mali, Benin, Ghana, Cameroon, Togo, Senegal, Chad, Côte d'Ivoire, and Mauritania. Production costs for cowpea vary depending on the technology used in particular, varieties, fertilizer, and tillage and pest management). Examples drawn from Bean/Cowpea CRSP studies and other sources show that labour often accounts for over 70% of the total cost of production. Cowpea production appears generally profitable, but returns vary widely from place to place.

2.8. Cowpea Improvement

2.8.6. Historical perspective

Major achievements in cowpea breeding in Africa include the development of productive early maturing cultivars, with a range of preferred seeds types and resistance to critical pests and diseases (Singh and Ntare, 1985). Such cultivars mature in 60- 70 days and can produce grain yields of 2000 kg ha-1 (Ehlers and Hall, 1997).

However, the adoption of these early cultivars has not been rapid; yields are still considerably low (300 kg ha^{-1}) (Ehlers and Hall, 1997). This can be attributed among other reasons to: (1) ineffective extension systems; (2) lack of the high density sole- cropping and crop husbandry practices that are required for these modern cultivars to achieve high grain yield (Ehlers and Hall, 1997). Farmers still prefer using locally adapted cultivars and low planting densities in traditional intercropping systems with cereal crops; (3) resource poor farmers in the marginal areas of Africa who attempt to grow crops under diverse environmental conditions, which are risk prone and are characterized by environmental stresses such as inadequate moisture availability and nutrient deficiencies and (4) the poor quality of seed used by farmers could also be a major limiting factor contributing to low yields.

The low productivity has been attributed to water deficits, the persistent traditional cropping system, pests, and diseases. Under adequate soil moisture conditions, the indeterminate cowpea flowers over a long period. As a consequence it produces more seed, and yield loss is limited. On the contrary, under water deficit conditions as is often the case in the semi-arid zone, the flowering period is cut short while the seed mature earlier. Moreover, the formation of new floral nodes and flowers are delayed (Turk et al., 1980) and/ or aborted, thus leading to low productivity.

It is postulated that environmental stresses in cowpea production areas during plant growth can interact with seed developmental processes and ultimately influence seed quality and yield. Progress in cowpea breeding for dry environments has been achieved by yield testing large collections over several locations and years (Hall et al., 1997). Watanabe (1998) evaluated 900 accessions of cowpea offered by the Genotypic Resources Unit of IITA in the field. These empirical approaches are slow, laborious, and expensive because of the need to assess the yield

of large number of lines across several locations and years, and the substantial variation from the effects of environment, error, and genotype – environment interactions (Blum, 1988). Shackel et al. (1982) have argued that when selecting genotypes with increased drought resistance, it is reasonable to propose that the evaluation be made under water limited conditions; but, because of the inconsistencies they observed under water deficit conditions, they concluded that an irrigated condition might be a more reliable indicator of genotypic differences than measurements of plants under drought. Grantz (1979) has further observed that the problem with selecting in an environment that causes water stress is that the time of anthesis, partitioning of carbohydrates, and time of maturity are influenced by drought. Environmentally induced variations under stress conditions therefore make it difficult to detect genotypic differences. The approach of Blum (1983), which combines selection for yield potential in favorable conditions with selection under controlled, repeatable stress environment for the expression of several traits is most effective (Fussell et al., 1991).

2.8.7. Agro-Ecological Regions of Zambia

Zambia is divided into three main agro-ecological regions which are defined on the basis of climatic characteristics of which rainfall is the dominant factor. These regions were mainly established using the following;

- Length of growing season at 70% probability,
- Occurrence of drought in rainy season at 70% probability,
- Mean monthly temperature,
- Amount of sunshine in the rain season,
- Occurrence of frost in the dry season.

2.8.7.1. Region I

The region covers the major valleys such as Gwembe, Lunsemfwa and Luangwa which lie between 300 and 900 meters above sea level. It includes the southern parts of Western and Southern Provinces with elevations between 900 and 1200m (Zambia Seed Technology Handbook, 1995).

The mean annual rainfall in this region is low and does not exceed 800mm. Rainfall is generally well distributed and the length of the growing season , at 70% probability, ranges from 80 to 120 days and this is the shortest growing season in Zambia. The growing season may contain as many as five 10 day dry period of less than 30mm rainfall and this is the driest and most prone to drought (Zambia Seed Technology Handbook, 1995).

It is characterized by relative high temperatures and during the growing season, mean daily temperatures may vary from 20 ^{C} to 25 ^{C}. The means are as high as 38 ^{C} in October and in the cold season may expect mild to severe frost (Zambia Seed Technology Handbook, 1995).

There are three main vegetation types; Mopane, woodland and acacia woodland and the deciduous thickets. The mopane is one storeyed woodland with an open deciduous canopy found on the hot river valleys of Luangwa, Kafue and Zambezi (Zambia Seed Technology Handbook, 1995). The type of soils found in region I are;

i. Loamy and clay soils with coarse to fine loam top soils.

The soils are slightly acidic to alkaline with minor fertility problems and high potential for agricultural production.

ii. Reddish coarse sandy soils either medium to very strong acidic found in pan dambo areas.

The soils developed on Kalahari sands and have major limitations to crop production some of which include aluminium toxicity due to low pH, low available water capacity and low nutrient reserve.

iii. Poorly drained sandy soils occurring on the western side of Zambezi River in Western Province.

The soils have severe wetness, acidic and generally have low fertility.

iv. Shallow and gravel soils.

Found in rolling to hilly areas including escarpment zones. They are not suitable for cultivation because of their depth limitations.

2.8.7.2. Region II

The region covers the central part of Zambia extending from east to west. It is subdivided in to a sub region IIa comprising the sand veldt plateau of Central, Eastern, Lusaka, and Southern Provinces and a sub region IIb comprising the Kalahari sand plateau and the Zambezi flood plain in Western Province. The general elevation is between 900 to 1300 metres above sea level (Zambia Seed Technology Handbook, 1995).

The region receives medium annual rainfall of between 800 and 1000mm. Rainfall is generally well distributed and the length of the growing season at 70% probability ranges from 100 to 140 days. The growing season may contain one to three 10- day dry period of less than 30mm rainfall.

The mean daily temperatures during the growing season range from 23 ^{C} to 25 ^{C}. The maximum temperatures may reach 32 ^{C} in October with minimum temperatures as low as 10 ^{C} in July (Zambia Seed Technology Handbook, 1995). Severe frost may be experienced in some parts of the region during the period of June to August. The vegetation is varied, from miombo woodland to munga woodland until Kalahari woodland. The main soils have slight to severe chemical and physical limitations to crop production. The limitations include low water holding capacity, shallow rooting depths, low organic matter, low nutrient reserves and acidity, capping and coarse textured top soils which increase the erosion hazard (Zambia Seed Technology Handbook, 1995). The four types of soils are;

a) Moderately leached clayey to loamy soils with medium to strong acidity.
b) Slightly leached clayey soils, red to reddish in colour with slight to medium acidity.
c) Coarse sandy loams in large valley dambos with medium to strong acidity
d) Kalahari soils on Kalahari sand

2.8.7.3. Region III

The region covers Nothern, Luapula, Copperbelt and North western Provinces and some parts of Serenje and Mkushi. The region is part of the degraded Central African plateau with attitudes ranging from 1100 to 1700 meters above sea level, except for Luapula Valley with land below 1000 meters above sea level (Zambia Seed Technology Handbook, 1995).

The mean annual rainfall in the region exceeds 1000mm and the length of the growing season varies from 120 days to 150 days. Local variations in amount and distribution are expected within a region. The mean monthly temperatures during the growing season vary from 16 ^{C} to 28 ^{C}. A few isolated areas experience critical frost problems averaging 17 days per year and the rest of the region experiences insignificant problems of frost (Zambia Seed Technology Handbook, 1995). The vegetation is broadly divided into miombo and mixture of chipya and dry evergreen forest (Storrs, 1995). The soils consist of highly weathered and leached type that are characterised by low pH of less than 4.5 and very low reserves of primary minerals. They are usually deficient in phosphorus, nitrogen and major plant nutrients and micronutrients. The low pH and the associated high levels of aluminium and manganese are often toxic to plant growth. Though the soils have serious chemical limitations to plant growth, the physical properties are favorable (Zambia Seed Technology Handbook, 1995). These include micro- structural stability, deep and well drained soils and high biological activity as listed below;

a) Red to brown clayey to loamy soils with very strong acidity
b) Shallow and gravel soils occurring in rolling to highly areas
c) Clayed soils, red in colour and moderately to strongly leached
d) Poorly to very poorly drained floodplain soils of variable texture and acidity
e) Coarse sandy soils with very strong acidity found in pan dambos on Kalahari sands
f) Soils of the rift valley with variable textures.

2.9. Determinants of Plant performance

2.9.6. Genotype

A genotype is an individual's collection of genes. The term also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the genes' DNA is used to make protein and RNA molecules (Genetic Home Reference, 2013). The expression of the genotype contributes to the individual's observable traits, called the phenotype.

2.9.7. Environment

Environment is generally considered as the physical and biological factors along with their chemical interactions that ultimately affect the survival of an organism (Anon, 2013). It also refers to the surrounding of a physical system that may interact with the system by exchanging mass, energy or other properties. The environment therefore, is an environment that encompasses the interaction of all living species.

2.9.8. Genotype Environmental Interactions

There is rather general agreement amongst plant breeders that interactions between genotype and environment have an important bearing of better varieties. However, it is much more difficult to find agreement as to what we ought to know about genotype- environment interactions and what we should do about them (Allard and Bradshaw, 1964). Others believe that improvements in efficiency are unlikely as long as yield and quality are considered.

There is a lot literature on genotype- environmental interactions. It ranges from field variety trials to studies on mechanisms. Many genotypes and environments are considered and different possible types of interactions are very great (Allard and Bradshaw, 1964). For example if there are only 2 genotypes and 3 environments, and a single criterion of classification like Yield, 60 types of interactions are possible. Even though only a small proportion of the possible number of interactions may have any importance for the breeder, the chance that they can be analysed and explained in terms of basic causes are small. Estimates of the magnitude of genotype-environmental interactions, which must be made relative from small samples, provide little more than gross approximations of the total potential of such interactions (Allard and Bradshaw, 1964).

Genotype- environment (GE) interaction is said to exist when phenotypic response invoked by a change in environment is not the same for all genotypes (Comstock and Moll, 1963). Mean yield is the most common description of a genotype's performance but it is generally inadequate, as it does not fully indicate consistency of performance. A combined analysis of variance is commonly used to identify the existence of GE interaction. Francis and Kannenberg (1978) came up with a descriptive method for grouping genotypes on the basis of mean yield and coefficient of variation across environments to see the consistence of performance. This Genotype-Grouping technique is also used to expose differential fertility levels, to check for varieties which respond to increasing fertility for greater yield variance across different environments. The mean yield are plotted against coefficient of variation (CV) and the grand mean yield, thus divide the figure into four groups as follows;

- Group I – high yield, small variation
- Group II – high yield , large variation
- Group III – low yield, small variation
- Group IV – low yield , large variation

Variations of the environments are divided into Predictable and Unpredictable (Allard and Bradshaw, 1964). The Predictable includes all permanent characters of the environment, such as general features of the climate and the soil type as well as those characteristics of the environment which fluctuate in a systematic manner like day length. It also includes those aspects of the environment that are determined by man and can therefore be fixed more or less at will, such as planting date, sowing density, methods of harvest and other agronomic practices (Allard and Bradshaw, 1964). The unpredictable includes fluctuations in weather, such as amount and distribution of rainfall and temperature and other factors such as established density of the crop.

In predictable environmental variations, large environmental differences such as difference between the oceanic and continental climate present no problem but differences which are small or difficult to measure without elaborate test or apparatus may be troublesome (Allard and Bradshaw, 1964). The crop itself would be the best indicator of the importance of these predictable variations as modern techniques of plant analysis for nutrient deficiencies would

show. Large variety x location interactions, when a crop is tested throughout the region indicates that the region includes a number of different and special environments. Similarly, large variety x treatment interactions, such as interactions between genotypes and fertility levels, sowing dates and so forth, indicate that the treatments induce special environments (Allard and Bradshaw, 1964).

Significant variety x location or variety x treatment interactions indicate that appropriate breeding program should develop a number of varieties, each particularly adapted to one of the special environments (Allard and Bradshaw, 1964). Such a course of action is feasible because there is no limit to the variability available enabling plants to adapt to special conditions of temperature, photoperiod, soil fertility, method of harvesting etc. Sometimes, environmental factor may be the one with adverse effects, which could be remedied if suitable agronomic or other steps are taken e.g. Correction of salinity (Allard and Bradshaw, 1964). However, it may also be easier to alter the genotype of the crop instead, for instance to cure the genotype rather than environmental.

In unpredictable environmental variations, the implications of variety x year interactions are very different from variety x location or variety x treatment interactions. This is because year-to-year fluctuations cannot be predicted in advance and the breeder can hardly aim the programme at developing varieties suited to special circumstances which cannot be seen (Allard and Bradshaw, 1964). In varietal trials it is common to find large variety x year and large variety x year x location interactions. Within a region, where it is likely that a set of varieties would be adapted, it's essential that tests be conducted in a series of locations preferably over a series of years. Some authors, however, have applied the yield stability concept with respect to consistency in time of genotype performance, using the adaptation concept in relation to consistency in space (Barah et al., 1981; Lin and Binns, 1988; Evans, 1993). It has also been widely acknowledged (Ghaderiet al., 1980; Becker, 1984; Lin and Butler, 1988; Bowman, 1989; Annicchiarico, 1992, 1997b; Romagosa and Fox, 1993; Piepho et al., 1998) that only genotype x location (GL) interaction, rather than all kinds of GE interaction, is useful for depicting adaptation patterns, as only this interaction can be exploited by selecting for specific adaptation or by growing specifically adapted genotypes. Great precision in the conduct of any one trial at any one location is unnecessary and may be wasteful (Allard and Bradshaw, 1964). In a study of spring oat

varieties carried out in Great Britain, it was concluded one replication was adequate and two replications quite sufficient in any one place in any year. These findings have a bearing on testing in the development stages of breeding programmes, during which the attempts to select superior genotypes from genetically variable populations. If testing is carried out, at a number of places, chances of identifying genotypes adapted to several environments are improved. At the same time, difficulties of testing selected materials at many locations are formidable (Allard and Bradshaw, 1964).

2.9.9. Concepts of Stability

Stability is the ability of a genotype to produce or perform under stressful conditions and yet be able to respond (Lin et al, 1986). Tollenaar and Lee (2002) defined stability as a measure of the ability of a genotype to maintain relative performance across wide environments. Genotypes that show little interaction with environments are called stable. Stability is either a static or dynamic where in static, performance of the genotype remains unchanged regardless of the environmental conditions and in dynamic, performance of a genotype changes in a predictable manner across a wide range of environmental conditions (Tollenaar and Lee, 2002). Thus static stability is an absolute measure, while dynamic stability is a relative measure.

Stability statistics fall into four groups depending on whether they are based on the deviations from the average genotype effect or on the genotype by environment (GE) term, and whether or not they incorporate a regression model on an environmental index (Lin et al., 1986). These groups of stability statistics are related to four concepts which are; Type 1, genotype is considered stable if its among- environment variance is small, Type 2, genotype is considered to be stable if its response to environments is parallel to the mean response of all genotypes in the trial, Type 3, genotype is considered to be stable if the residual MS (error) from the regression model on the environmental index is small and Type 4, genotype is considered stable when the mean square within locations and years is small.

- *Type 1 Stability*

Type 1 is analogous to the concept of homeostasis and is of a biological concept. Despite the type being theoretically sound, most breeders do not often use it because the yields it gives are low (Lin et al., 1986). Type 1 stability is associated with poor response and low yield in environments that are high yielding for other cultivars. Another reason for breeders' non-preference for Type 1 is, although a high level of performance under a wide range of environments may be desirable, it is difficult to achieve in practice. The usefulness of Type 1 stability depends on the range of environments under which the experiment is conducted. If the range is very large like a collection of sites from different continents, Type 1 stability may not be very meaningful, but if the geographical range is restricted like the agro ecological zone of the same country, it could be important (Lin et al., 1986).

- Type 2 Stability:

This can be interpreted as Type 1 depending on how stable the genotype is defined. Type 2 stability is a relative measure depending on the genotypes included in the experiment. Its scope of inference is not generalized but specific to the test set (Lin et al., 1986). For a genotype to be considered stable by this definition, is so only with respect to the other genotype in the test but without any assurance that it will appear stable when assessed with another set of genotypes (Lin et al., 1986).

- Type 3 Stability:

It is recommended to be used because of, the variability of any genotype with respect to environment that can be subdivided to predictable part corresponding to regression and an unpredictable part corresponding to deviation MS (Lin et al.,1986). However, because the regression part can be predicted and to some extent controlled, it is no longer profitable to consider this component of GE interaction as a measure of stability. Hence stability should be reserved to describe measurements of unpredictable irregularities in the response to environment as provided by deviation from regression (Lin et al., 1986).

For a useful predictable model, the independent variable must be measured prior to the experiment and the deviation MS from regression may be a deterministic property that can be

associated with genotypes but environmental index cannot be measured prior (Lin et al., 1986). Because of the model is purely empirical, the deviation MS does not have a deterministic property and just indicates how good is the fit but has no direct bearing on the genotype's stability.

- Type 4 Stability

This is based on the genotypes and years within a location mean square and it is part of G x L x Y. The derivation the parameter, starts with the separation of the environmental variation in to predictable (G x L) and unpredictable (G x Y) (Lin and Binns, 1991). The parameter to use in stability must be heritable or genetic and if the characteristic measured by the parameter is non-genetic, then the variation is fruitless.

Type 1 and Type 4 measures are moderately repeatable in most instances, and tend to have higher repeatability/heritability than Type 2 measures; Type 3 stability has low or negligible repeatability (Léon and Becker, 1988; Lin and Binns, 1991; Eskridge and Mumm, 1992; Zavala-Garcia et al., 1992; Jalaluddin and Harrison, 1993; Helms, 1993; Sneller et al., 1997; Schut and Dourleijn, 2000). The repeatability values can vary largely depending on the crop and the data set (Jalaluddin and Harrison, 1993; Annicchiarico, 1997), but they remain distinctly lower than those for genotype mean yield across environments (Becker, 1987; Pham and Kang, 1988; Eskridge and Mumm, 1992; Jalaluddin and Harrison, 1993; Annicchiarico, 1997).

Therefore, Type 3 is the least attractive among the three concepts of stability because it is difficult to justify. Type 2 stability is useful for comparing a specific set of genotypes, but by a relative measure, it does not have a sufficiently broad inferential base for general assessment (Lin et al., 1987). In contrast, Type 1 has a broad inferential base because its stability definition does not depend on other genotypes included in the test and it is also unambiguous. However, it does not provide information on the response pattern over the test environment that is very vital for cultivar recommendations (Lin et al., 1986). Type 1 and 4 are genetic and can be inherited additively in F1 hence improvement of genotypes stability through crossing is theoretically possible. Type 1 being a simple variance estimate across locations, measures the homeostatic property in terms of overall environmental variation while Type 4 which looks at the year within location MS averaged over locations, measures homeostatic property only with respect to

unpredictable variation excluding the predictable part (Location) that is controlled. Moreover, Type 4 is not tied to a range of genotypes which are included in the test.

2.9.10. Mechanisms promoting stability

It is important to emphasise that the stability of concern does not imply general consistency of phenotype in varying environments. It implies stability in those aspects of phenotype, especially yield and quality that are important economically (Allard and Bradshaw, 1964). Such stability may in fact depend on holding some aspects of morphology and physiology in steady state and allowing others to vary. Thus the required varieties will show low genotype- environment interaction for agriculturally important characters, particularly yield, and not necessarily for other characters. A variety which can adjust its genotypic or phenotypic state in response to transient fluctuations in environment in such ways that it gives high and stable economic return for the place and year is termed well buffered or homeostatic (Allard and Bradshaw, 1964).

There are two ways in which a variety can achieve stability, Individual and Population buffering. In individual buffering, the individuals themselves may be well buffered so that each member of the population is well adapted to a range of environments (Allard and Bradshaw, 1964). Homogenous populations, such as pure line varieties or single crosses, depend heavily on individual buffering to stabilize productivity.

Population buffering refers to buffering above and beyond that of individual constituents of populations i.e., buffering which arises in interaction among coexisting genotypes. The most precise information of population buffering comes from comparisons between pure line varieties grown singly and in mixture (Allard and Bradshaw, 1964). Simmonds (1999) reviewed this topic and found that mixed populations are nearly always more stable in yield than their components. In wheat, for example, coefficients of variability over seasons were about two thirds as large for mixtures (7.3%) as for homogeneous populations (11.6%). In mean yielding ability the average advantage of mixtures over the means of components was of the order of 3 to 5% but many mixtures out yielded the higher component when tested over several years (Allard and Bradshaw, 1964).

2.9.11. Phenotypic Stability

Absolute phenotypic stability would be expressed by b= 0.0. The simple linear regression used to describe various types of variety adaptability to a range of environment can also be used as a quantitative measure of phenotypic stability (Finlay and Wilkinson, 1963). However, in adaptation analysis, Finlay and Wilkinson came up with the two important indices which are the regression coefficient and the variety mean yield over all environments. To summarize,, regression coefficients approximating to 1. 0 indicate average stability. When this is associated with high mean yield, varieties have general adaptability; when associated with low mean yield, varieties are poorly adapted to all the environments (Finlay and Wilkinson, 1963). Regression values increasing above 1.0 describe varieties with increasing sensitivity to environmental change (below average stability), and greater specificity of adaptability to high-yielding environments (Finlay and Wilkinson, 1963). Regression coefficients decreasing below 1.0 provide a measure of greater resistance to environmental change (above average stability), and therefore increasing specificity of adaptability to low-yielding environments. At this stage when you are looking at the state of knowledge about critical concepts, you would do well to look at a number of different authors on same topic. For example Mandel (1971) Dias (2003) and Crossa et al. (2002) provided comparison to Finlay and Wilkinson (1963) and Eberhart and Russell (1963); Evolution is shown of current approach and its relevance to the study. One of the interesting features is that the variability (between varieties) in phenotypic stability (regression coefficient) is inversely proportional to the mean yield. The varieties with general adaptability (highest mean yields over all environments) all possess slightly above-average phenotypic stability (Finlay and Wilkinson, 1963). The ideal variety having general adaptability is the one with maximum yield potential in the most favorable environment, and maximum phenotypic stability. The varieties with the high phenotypic stability all have low mean yields. They are so stable, in fact, that they are unable to exploit high-yielding environments. On the other hand, varieties can be too sensitive to environmental change, although with low mean yields of the varieties with high regression coefficients. The generally adapted varieties balance between the extremes, the actual point of balance depends on the particular genotype and range of environments (Finlay and Wilkinson, 1963).

Other statistical methods include; Multivariate statistical methods, multivariate ANOVA, multiple regression, Principal Component Analysis (PCA), Factor analysis, clustering and ordination and Additive main Effect and Multiplicative Interaction (AMMI) model. Multivariates statistical methods explore multidirectional parameters and extract more information on the components of phenotypic variability (Hussein, 2000).

The AMMI model is effective for gaining accuracy in G x E studies (Gauch, 1992) because it analyses the interaction effect in a more statistical robust procedure. With the AMMI model, main effects (genotypes and environments) are first accounted for by a regular analysis of variance; thereafter, the interaction G x E is analyzed by principal component analysis (Gauch, 1992; Dias, C.T., 2003) leading to a more exhaustive data analysis, accurate yield estimates and reliable selections. AMMI biplots make it easy to visualize and identify stable genotypes (Crossa at al 2002).

2.10. Nutritional characteristics

2.10.6. Significant parameters

2.10.6.1. Proteins

Proteins are large biological molecules consisting of one or more chains of amino acids (Bailey B., 2012). Proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity.

Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the

cytoskeleton, which form a system of scaffolding that maintains cell shape. Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle. Proteins are also necessary in animals' diets, since animals cannot synthesise all the amino acids they need and must obtain essential amino acids from food. Through the process of digestion, animals break down ingested protein into free amino acids that are then used in metabolism.

Cowpea contains between 20% and 30% protein and it is rich in amino acids, lysine, tryptophan, compared to cereal grains (Nidhi, 2009). These proteins are deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins (Nidhi, 2009). The anti-nutritional factors which are found in cowpea such as trypsin inhibitors, lectins and tannins decrease protein digestibility and reduce protein quality (Gatehouse and Boulter, 1983)

2.10.6.2. Secondary metabolites (Phenolic Compounds and organic Acids)

1) Secondary metabolites

These are organic compounds that are synthesised from primary metabolites such as glucose and involved in the growth, development, or reproduction of an organism (Sams et al, 2011). They are produced by plants and, fungi. Secondary metabolites play an important role in plants such as in defense mechanisms against herbivores, visual cues and responses to environmental stresses and signaling. They include

- Glycosides e.g. glucosinolates;
- Alkaloids atropine e.g. cocaine
- Fatty acid synthases;
- Some antibiotics.

Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens, herbivores. They are also involved in

signaling within the plant. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years (Sahelian, 2009). Humans also use secondary metabolites as medicines, flavourings and recreational drugs.

2.10.6.3. Chemistry of plant phenolic compounds

Plant phenolic compounds are diverse in structure but are characterised by hydroxylated aromatic rings (e.g. flavan-3-ols). They are categorized as secondary metabolites, and their function in plants is often poorly understood. Many plant phenolic compounds are polymerized into larger molecules such as the proanthocyanidins (PA; condensed tannins) and lignins (www.raysahelian.com/phenolic.html 20 Sept 2012).

Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides. Antioxidants are an important part of the defense system of the human body and help to cope with oxidative stress caused by reactive oxygen species. There is a growing interest in the antioxidant activity of phenolics and condensed tannin contents of plant extracts due to their potential role in disease prevention and health promotion. Estimation of total phenolic contents (TPC) and condensed tannin contents is a common-bench assay and first step used during evaluation of antioxidant activity of plant extracts and natural products isolated therefrom.

Phenolic contents of cowpea are comparatively greater than those observed for seed extracts of chickpea and lentil cultivars. According to literature data, the total phenolic content is directly associated with antioxidant activity. It is evident that condensed tannin contents of cowpea are also greater than those of seed extracts of chickpea and lentil. Condensed tannins are located mainly in the testa and play an important role in the defense system of seeds that are exposed to oxidative damage by many environmental factors. It is well-known that phenolic content as well as condensed tannin contents vary depending on several factors such as different genotype, growing condition, agronomic practices employed, season, maturity, post-harvest storage and processing conditions and solvent used for extraction.

2.10.6.4. Anti- nutritional factors limiting utilisation in cowpea

A major limiting factor to the utilisation of cowpeas as food is the presence of anti- nutritional factors such as trypsin inhibitors, oligosaccharides and phenolic compounds (Chavan et al 1989). Phenolic compounds (tannins in particular) are an important group of such antinutritional factors. They are able to form complexes with food nutrients such as minerals and protein, thus rendering them less soluble or less susceptible to enzymatic degradation and less available for absorption (Towo et al, 2003). Processes such as dehulling, soaking, heating and fermentation are known to reduce the presence of the antinutritional factors (Vijayakumari et al., 1998; Egounlety and Aworh, 2003). However, phenolic compounds have a beneficial role as well. They are naturally concentrated in the seed coat (Preet and Punia, 2000) where they play a major role in the physical and chemical defense system of the seeds when exposed to environmental factors such as oxidative damage and microbial infections thus contributing to antioxidant and antimicrobial activity (Troszynska et al., 2002).

Different varieties of cowpea are known to contain phenolic compounds which are, ubiquitous in plants and essential part of the human diet and are of considerable interest due to their antioxidant properties. These compounds posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high- molecule weight polymer.

Flavonoids which bear the C_6-C_3-C_6 structure, account for more than half of the over eight thousand different phenolic compounds. The antioxidant activity of phenolic compounds depends on the structure in particular, the number of positions of the hydroxyl groups and the nature of substitutions on the aromatic rings.

Fruits, vegetables and beverages are the major sources of phenolic compounds in human diets. The food and agricultural products processing industries generate substantial quantities of phenolics – rich by –products, which could be valuable natural resources of antioxidants. Some by- products have proven to be effective sources of phenolic antioxidants. When tested in edible oils, and in fish, meat and poultry products, phenolic - rich extracts have shown antioxidant activities compared to these of synthetic antioxidants.

Medicinal plant species express variability of quantitative and qualitative traits, and due to it, they have adapted to and grow under different agro ecological conditions. It should be emphasised that medicinal plant genotypes with a lower yielding potential, similar to other plant species, often have a broader adaptability and better stability of the yield than high yielding genotypes.

Despite the good nutritional content of cowpeas, their consumption is limited due to the presence of anti- nutritional factors. The anti- nutritional factors found in cowpeas include inhibitors of enzymes such as trypsin, raffinose group of oligosaccharides and polyphenols (Chavan et al., 1989). Trypsin inhibitor is known to inhibit the action of the enzyme trypsin. It does not necessarily interfere with the ultimate digestion of proteins but it may retard the liberation of the amino acid methionine from the protein (Richardson, 1977). Thus methionine cannot be used effectively for protein synthesis (Aykroyd and Doughty, 1964). Oligosaccharides are not digested by monogastric animals and they are thus fermented by microbes in the colon, which results in the production of flatus and other discomfort (Onyenekwe et al., 2000).

2.10.6.5. Tannins

Tannins refer to substances of vegetable origin capable of transforming fresh animal hide into leather (Hahn et al., 1984). Tannins are rich in phenolic hydroxyl groups. They are divided into two classes, namely: Hydrolysable tannins and Condensed (Non- hydrolysable) tannins (Waterman & Mole, 1994). Hydrolysable tannins are phenolic carboxylic acids esterified to sugars such as glucose. They are called hydrolysable tannins because they break down into sugars and a phenolic acid (Gallic or allergic acid) upon hydrolysis with acid, alkali or hydrolytic enzymes (tannase) (Hahn et al., 1984). Condensed (Non-hydrolysable) tannins are polymers of flavan-3-ol units and are also known as proanthocyanins (or proanthocyanidins) (Butler et al., 1984) because they yield anthocyanins upon heating in acidic media (Santos- Buelga and Scalbert, 2000).

2.10.6.6. Phenolic compounds

Most research into the phenolics of cowpeas also includes an estimation of total phenol content. Values reported for the total phenol content of cowpeas are highly variable ((Butler et al., 1984). Factors such as the assay method and conditions (e.g. type of extraction solvent), type of standard used and type of cowpea sample used (e.g. variety, colour and maturity) all influence the levels of total phenols obtained (Chang et al., 1994; Nwokolo and Ilechukwu, 1996). Dark coloured seeds generally contain larger amounts of phenols than white or cream-coloured seeds (Nwokolo and Ilechukwu, 1996).

Cowpeas contain phenolic compounds in the three main groups namely, flavonoids (Quercetin et al., 1999; Lattanzio et al., 2004; Ng, 1997; Chang and Wong, 2004; Duenas et al., 2005), phenolic acids (coumaric, ferulic, caffeic, hydroxybenzoic, syringic, sinapic and protocatechuic acids) (Cai et al., 2003; Sosulski and Dabrowski, 1984) and tannins (Morrison et al 1995; Lattanzio et al., 1997; Egounlety and Aworh, 2003). These phenolic compounds are mainly concentrated in the seed coat (Preet and Punia, 2000). Cai et al. (2003) analysed 17 cowpea varieties and observed that protocatechuic acid was the major bound phenolic acid. Analyses of tannins in cowpeas have been done using specific methods for condensed tannins such as the vanillin-HCl method (Chang et al., 1994; Morrison et al., 1995; Oigiangbe and Onigbinde, 1996; Oluwatosin, 1999; Egounlety and Aworh, 2003).

2.10.6.7. Activity mechanisms and structure-activity relationships of Phenols as antioxidants

Generally the efficacy of phenolic compounds as antioxidants depends on a number of factors such as the number of hydroxyl groups bonded to the aromatic ring, the site of bonding, mutual position of hydroxyls in the aromatic ring (Sroka and Cisowski, 2003) and their ability to act as hydrogen or electron donating agents and free radical scavengers. All polyphenols are capable of scavenging singlet oxygen and alkyl radical through electron donating properties, thus generating a relatively stable phenoxyl radical (Santos-Buelga and Scalbert, 2000).

A relationship exists between the efficacy of phenolic compounds as antioxidants and their chemical structure. The configuration and total number of hydroxyl groups substantially

influence the mechanism of antioxidant activity (Heim et al., 2001). The phenolic ring with hydroxyl groups are the main structural features required for antioxidant activity. In order for phenolic compounds to act as antioxidants, their hydroxyl groups have to be in free form. This is because the attachment of an external group to the hydroxyl groups reduces the antioxidant power of the phenolic compounds as they lack hydrogen atom for donation (Farag et al., 2003).

Flavonoids are known to stabilise radicals by donating hydrogen and electrons from the hydroxyl groups in the B-ring to hydroxyl, peroxyl and peroxynitrite radicals, thus giving rise to relatively stable flavonoid radicals (Cao et al., 1997). Flavonoids therefore generally function as primary antioxidants and superoxide radical anion scavengers. The aglycones are more effective than glycosides. The position and the degree of hydroxylation of the B ring determine the antioxidant activity of flavonoids (Madhavi et al., 1996). Flavonoids are also known to have the ability to sequest (or chelate) and thus reduce the activity of oxidative inducing metals such as copper and iron (Soleas et al., 1997). Tannins inhibit lipid oxidation by scavenging the initial free radicals or the lipid peroxy radicals. They are also excellent chelators of metals ions such as copper and iron (Soleas et al., 1997).

The antioxidant activity of phenolic acids depends on the degree of hydroxylation. The derivatives of cinnamic acids are generally more effective than the derivatives of benzoic acid (Marinova and Yanishlieva, 2003). The presence of the CH=CH-COOH group in cinnamic acid derivatives ensures greater efficiency than the COOH group in benzoic acids (Madhavi et al, 1996). The double bond has been reported to participate in stabilising the phenoxyl radical by resonance (Cuvelier et al., 1992; Marinova and Yanishlieva, 2003). Phenolic acids are known to be scavengers of oxygen species. The position of the hydroxyl groups in the aromatic ring is important in the efficiency of phenolic acids as antioxidants (Sroka and Cisowski, 2003). For phenolic acids for instance, the presence of OH group in the para position is important for high antioxidant activity (Pannala et al., 1998; Pannala et al., 2001). For flavonoids, structural features such as the attachment of the 3-OH group to the 2,3 double bond and adjacent to the 4-carbonyl in the C ring (Rice-evans et al., 1997), a 3,4 dihydroxy arrangement in the B ring and the meta 5,7 dihydroxy arrangements in the A ring (Rice-Evans et al., 1997) are important for high antioxidant activity.

2.10.7. Inheritance of phenolic compounds

According to Pree and Punia (2000), Warrington et al. (2002), Cai et al. (2003) and Nzaramba (2004) a large genetic variability for the phenolic compounds content and antioxidant capacity exists in cowpea, with pigmented varieties as preferred parental material. The genetic analysis of the phenolic compounds and antioxidants of *V. unguiculata* seeds is important for nutraceutical and functional applications (Siddhuraju and Becker, 2007). Manach et al. (2004) noted that environmental and genetic factors have a major effect on polyphenols content. So far, a comprehensive assessment of the inheritance of polyphenols and antioxidants of dehulled cowpea seeds has not been reported (Phillips and Mcwatters, 1991; Siddhuraju and Becker, 2007). Knowledge of the genetic basis and heritability of these health beneficiary phytochemical profiles is essential for efficient development of new cultivars for food processing industries and breeders. The choice of an efficient breeding procedure depends to a large extent on knowledge of the genetic system controlling the characters to be selected (Allard, 1960).

Cowpea and other medicinal plant species express variability of quantitative and qualitative traits, and due to it, they have adapted to and grow under different agro ecological conditions. Crops and other medicinal plant genotypes with a lower yielding potential, similar to other plant species, often have a broader adaptability and better stability of the yield than high yielding genotypes.

Chapter 3

3. MATERIALS AND METHODS

3.6. Location

The experiment was carried out in the three agro ecological zones of Zambia during 2011/2012 the growing season; region I was at the National Irrigation Research Station in Nanga, Southern Province, region II at Natural Resource Development College Irrigation area in Lusaka and region III at Copperbelt Research Station in Mufulira.

Nanga is located at latitude15^{o} 46' and longitude 27^{o} 55'at an altitude of 978 metres above sea level. The majority of the soils in the study area are well drained, deep, dark yellowish brown to strong brown clayey soils which are the Nakambala series with pH of 6.5.

In terms of classification, to the family level (International Classification) the soils consists of Typic Kanhaplustalf, fine, kaolinitic, iso-hyperthermic (USDA, 1975). The main physical property is sandy clay loam with a moderate to strong structure. Soil water permeability is medium, with a well drained condition. Plant root penetration is moderate to good. The main chemical characteristics are dominated by a medium acid soil reaction state, combined with high ability to hold plant nutrients and moderate high soil fertility status. Both the physical and chemical properties are considered as attributes from the geological contribution of the limestone dolomite parent materials prevalent in the area. The weather conditions were as shown in Table 1.

The Natural Resources Development College (NRDC) Irrigation area is located at latitude 28^{o} 20' and longitude 15^{o} 22' at an altitude of 1250 meters above sea level. The soil types are mainly sandy clay loam with pH 6. The detailed soil descriptions are moderately well drained to imperfectly drained, moderately shallow, brown to yellowish brown, coarse to fine loamy soils and classified as Orthi- eutric Leptosols (Exploratory soil map of Zambia, Scale 1: 1000 000). The weather conditions were as shown in Table 2.

Copperbelt Research Station is situated in Kalulushi District but it is commonly called Mufulira (because it is near Mufulira). It is located at latitude 12^{o} 36' and longitude 28^{o} 07'at an altitude of 1227 meters above sea level. The soil types are mainly acidic sandy loam Mufulira series with pH of 4.5 low base saturation. The detailed soil descriptions are deep yellowish brown to strong brown soils, sandy loam top soil over lain by sandy clay loam sub soil and classified as Acric Haplustox, clayey, kaolinitic, Iso (hyper) thermic acid (USDA,1975). The weather conditions were as shown in Table 3.

Table 1. *Weather pattern experienced at Nanga in 2011/2012 growing season*

Month	Max temp	Min temp	Ground min temp	Mean dew point	Rainfall total	Total rain days	Max mean temp	Mean RH	Total sunshine
January	-	-	-	-	267.7mm	20	-	-	-
February	31.8	18.9	18.8	18.8	240.8mm	18	25.7	-	-
March	31.1	18.2	18.3	19.2	186.1mm	11	24.6	-	-
April	29.7	13.8	13.5	16.4	Trace	01	21.8	-	-
May	29.1	13.5	13.5	12.8	Trace	Nil	21.3	-	-
June									
July									

***Table 2**. Weather pattern experienced at NRDC in 2011/2012 growing season*

Month	Max Temp	Min Temp	Ground Min Temp	Mean Dew Point	Rainfall Total	Total Rain Days	Mean Temp	Mean RH	Total Sunshine
January	26.4	17.1	17.0	17.3	218.9mm	17	21.7	-	-
February	26.3	16.9	16.5	16.2	185.9mm	15	21.6	-	-
March	26.6	16.6	16.3	16.2	110.9mm	11	21.6	-	-
April	26.2	15.0	15.0	14.1	39.7mm	02	20.6	-	-
May	24.7	12.8	12.4	11.8	2.8mm	Nil	18.7	-	-
June									
July									

Table 3. *Weather Pattern experienced in Mufulira in 2011/2012 growing season*

Month	**Max temp**	**Min temp**	**Ground min temp**	**Mean dew point**	**Rainfall total**	**Total rain days**	**Max mean temp**	**Mean RH**	**Total sunshine**
January	-	-	-	-	356.7mm	25	-	-	-
February	28.8	16.9	16.8	16.8	340.8mm	22	22.9	78.0	05.7
March	28.1	17.2	17.3	18.2	410.1mm	22	22.7	83.0	04.9
April	27.7	11.8	11.5	14.4	03.7mm	02	19.7	72.5	08.2
May	28.1	06.6	06.2	10.8	Trace	Nil	17.3	65.0	09.4
June									
July									

3.7. Plant Materials

Ten genotypes which included three parents and seven progeny lines derived from induced mutation which are in the M6 generation were used in the study (see Table 4). The mutation bred materials were a result of plant improvement work of the Plant Science Department, School of Agricultural Sciences, University of Zambia while the parental lines are released varieties from Msekera Research Station in Chipata.

Table 4. *Cowpea (Vigna unguiclata) plant materials used in the study*

Name	Code	Pedigree	Key Characteristics
Lutembwe Parent	LT PRT	[x]Msekera	Long thick pods, medium maturing
Msandile Parent	MS PRT	[x]Msekera	Deep green leaves, late maturing
Bubebe Parent	BB PRT	[x]Msekera	Long trifoliate leaves, medium maturing
Lutembwe derived mutant	LT11/3/3/12	[y]UNZA	Stay green characteristic, climber
Lutembwe derived mutant	LT11/5/2/2	[y]UNZA	Broad leaves, stay green, late maturing
Lutembwe derived mutant	LT11/3/8/4/1	[y]UNZA	Long slender small leaves, late maturing
Lutembwe derived mutant	LT3/8/4/6	[y]UNZA	Broad leaves, climber, early maturing
Msandile derived mutant	MS1/8/1/4	[y]UNZA	Long trifoliate leave, very prolific
Bubebe derived mutant	BB4/2/4/1	[y]UNZA	Long slender small leaves, climber
Bubebe derived mutant	BB10/4/2/3	[y]UNZA	Narrow leaves, still segregating

[X]: Msekera Research station, Chipata

[Y]: University of Zambia, Department of Plant Sciences, Lusaka

3.8. Crop Management

3.8.6. Land preparation and plot sizes

Land was prepared by first clearing the land due to the tall grass in the chosen areas. The land was then ploughed using a tractor and ridges were made thereafter with the use of hand hoe. The whole plot sizes in each site were 24m x 20metres ($480m^2$).

Planting was done by hand at the depth of 2 cm. The other dimensions were as follows; 36 plots in one site, each with 2 rows which were 2 metres long. The spacing was 60 x15cm and 2 seeds were planted per station. The experiment was replicated 3 times.

3.8.7. Agronomic practices

Standard cowpea production practices were followed in managing the crop (Davis et al 1991). Cowpea is very susceptible to aphids and to combat that, the experiment was sprayed three times using phoskill at the recommended rate. For fertilization basal fertilizer (D compound, 10- 20- 10 NPK) was applied at planting. The crop was rain fed and no supplementary irrigation was done. Maturity was at 90 days. The crop was considered mature and ripe and was hand harvested 28 weeks from the day of planting.

3.9. Parameters measured

i. **Yield characteristics**; the following were measured:

- Yield (total seed weight)
- Seed size
- Seed coat colour
- Leaf colour.

Days to 50% flowering were considered as the number of days from planting until half of the plants in the plot were at 50 percent flowering. Date of maturity and days to first harvesting were done by observing the changes in the phenotypic features of the plant.

3.10. Analytical methods

Proximate analysis was carried out on all the genotype samples of cowpea to determine moisture content, ash contents and crude protein content. For determination of moisture and ash, the Oven Drying method was used at different temperatures of 105^{o}c (2 hours) and 500^{o}c (4hours) respectively. Loss of weight of the sample was used to calculate the moisture and ash contents. Crude protein determination was done using a standard method called kjeldahl method (Johann Kjeldahl 1883) with a conversion factor of 6.25.

3.10.6. Total phenolics

Total phenolic content was determined by Folin - Ciocalteu method (Singleton 1965; Waterman 1994). Extracts of 0.5g of ground cowpea was mixed with 80% aqueous methanol of 20ml volume (V1). Standard catechin solutions of concentrations 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.6 and 2.0 were prepared. 0.5ml of phenolic extract and each catechin solutions were place in 50ml volumetric flasks containing 10ml distilled water and mixed thoroughly. 2.5ml folin – ciocaltea reagent was added to each volumetric flask and mixed, followed by 7.5ml of 20% (w/v) sodium carbonate solution within one to eight minutes after addition of the folin - -ciocalteu reagent (and timing was done after addition of the sodium carbonate solution). Absorbance of the solutions was measured after 2 hours at 760nm using a spectrophotometer.

A standard curve of catechin concentration on the x-axis against mean absorbance on y-axis was plotted using Microsoft Excel and fitted on a linear regression using equation y= mx + c. Phenolic concentrations were then worked out using x= (y-c)/m and denoted as (X1). Amount of catechin equivalent in mg (X2) in the volume V1 of the original sample was determined using the formula X2= (V1 x X1)/1. The amount of total phenolics in mg catechin was expressed in equivalents per 100mg of sample.

3.10.7. Condensed tannin

Condensed tannin were determined by the Vanillin & Vanillin or HCl Tannin Determination method (Broadhurst and Jones 1978) (modified by Chang et al., 1994; Morrison et al., 1995; Oigiangbe and Onigbinde, 1996; Oluwatosin, 1999; Egounlety and Aworh, 2003).

Three replicates of 0.3g ground cowpea of each genotype were placed in a centrifuge tube and 8ml of 1% HCL were added in methanol to each tube at 1 minute intervals. Samples were centrifuged at 2000 rpm for four minutes; 1 ml of aliquot from supernatant of each sample was placed in separate test tube together with blank ones and placed in to the water bath for 20 minutes; 5ml of vanillin reagent was added to the first sample tube in each pair of the sample at 1minute intervals.5ml of the 4%HCL in methanol was added to second tube in each pair (blank) at 1 minute intervals. Absorbencies of each sample and blank were read after 20 minutes on the spectronic 20 set at 500nm. A standard curve was run using regression to determine the slope of the line and r^2 values to calculate the catechin equivalents of the condensed tannins.

3.10.8. Organoleptic Test of the leaves (Palatability)

Due to the small size of yield, palatability of cowpea leaves was only carried out on the parental lines using the method of Dadzie and Orchard (1997) modified by Kiya et al, (2007). The parameters measured in the leaves were Cooking Time, Taste and Texture. The cowpea leaves were prepared in three different forms where farmers had to test and give their opinion on what they thought about the genotype. A portion of each genotype (line) was prepared using cooking oil, groundnut powder and plain (with only salt). Segregation according to gender was done when carrying out the tests and results were ranked according to performance.

A scoring scale was developed to help come up with proper assessment of the cowpea. The subjective scale included the following; **1** – Very Poor, **2** – Poor, **3**- Fair, **4** – Good and **5** – Very good.

3.11. Experimental Design

The experimental design used was a Randomized Complete Block Design (RCBD) with three replications (Gomez and Gomez 1976). In each block, there were twelve plots which totaled thirty six (36) plots for the whole experiment.

3.12. Data Analysis

GENSTAT 14th version and IRRISTAT 5.0 computer packages were used in analysis of variance (ANOVA), for multi variate analysis. Additive Main Effect Multiplicative Interaction Model was used (AMMI) was used in partitioning of genotype and environment and construction of biplots.

The mathematical model for randomized complete block design described by Gomez and Gomez (1984) was used;

$$Xij = \mu + Ti + \beta ji + Єij$$

Where;

Xij = is the ij^{th} observation which could be any observation,

μ = is the population mean,

Ti = the effect of the i^{th} genotype,

Bji = is the j^{th} block effect,

Єij = is the environmental effect peculiar to the ij^{th} individual (random error)

Further characterization of the G x E interaction was done using the Additive Main effect and Multiplicative Interaction (AMMI) model (Gauch, 1992). Stable varieties were identified according to the interpretation given by Declay (1996) and Chapman et al. (1997) that ordinates for two principal components plotted against each other, entries near the centre are said to be average in performance and stable.

The AMMI analysis Model:

The graphic representation of genotypes and environments by AMMI analysis results from a model of main additive effects and multiplicative interaction. This model is expressed mathematically by;

$$Y_{ger} = \mu + \alpha_g + \beta_e + \Sigma_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where;

Y_{ger} = is the yield of genotype g in the environment e for replication r,

μ = is the grand mean

α_g = is the deviation of the genotype g from the grand mean,

β_e = is the deviation of the environment e from the grand mean

λ_n = is the singular value for the interaction principal component axis (IPCA) n,

γ_{gn} = is the genotype eigenvector value for IPCA axis n, (square root of the eigen value which is also the sum of squares divided by the number of replications),

δ_{en} = $_{is}$ the environment e eigenvector vector value for IPCA axis n,

ρ_{ge} = is the residual and ε_{ger} is the error term if the experiment is replicated.

The eigen vectors scaled as unit vectors are unit less,

μ, αg, βe are additive parameters and enter the model additively while λ_n γ_{gn} and δ_{en} are multiplicative parameters and enter the model multiplicatively.

The model is fitted sequentially, combining the ANOVA and the PCA. Thus the residuals from the fitting of the main effects by ANOVA are modeled, in a second step, by PCA. The scores or coordinates of the genotypes and environments are produced on the principal interaction axes, conventionally called IPCA, that permit their representation together in a biplot graph.

The interpretation of a biplot assay is that if main effects have IPCA score close to zero, it indicates negligible interaction effects and when a genotype and an environment have the same sign on the IPCA axis, their interaction is positive; if different, their interaction is negative. The IPCA 1 versus IPCA 2 biplot explains the magnitude of the interaction of each genotype and

environment. The genotypes and environments that are farthest from the origin being more responsive fit the worst. Genotypes and environments that fall into the same sector interact positively; negatively if they fall into opposite sectors (Anandan et al., 2009).

Chapter 4

4. RESULTS

4.1 General Characteristics of the selected Sites

The 2011/2012 growing season was generally normal and received good rains which led to favorable yields. The selected sites where experiments were carried from were considered as individual environments and had soil pH which varied from 4.5 in Mufulira to 6.5 in both Nanga and NRDC. The soil types also varied from sandy loam in Mufulira to sandy clay loam in Nanga and clay loam in NRDC as summarized in Table 5.

Table 5*.Summary of attributes for different sites used in the study*

Attributes	**Site of Experiments**		
	NANGA	MUFULIRA	NRDC
Soil Type	Sandy clay loam	Sandy loam	Clay loamy
Soil pH	6.5	4.5	6.5
Rainfall (mm)	650mm	1110mm	840mm
Altitude	978m	1227m	1250m

4.2 Morphological Characteristics

4.2.1 Seed Coat Colour

In this study, the seed coat colours of the different genotypes were vividly different and varied from cream colour to purple (Table 6). Msandile parent and its mutant- derived genotypes were white in colour, Lutembwe (LT) genotypes, yellowish brown and Bubebe (BB) genotypes were purplish brown. These seed coat colours were related with the antioxidant contents of the genotypes.

Table 6. *Seed coat colour characteristics of the cowpea (Vigna unguiclata) genotypes*

Genotype	[z]Leaf colour Intensity	[y]Main seed colour	[x]Presence of seed secondary colour	[w]Seed secondary colour	[v]Pattern of secondary colour
MS PRT	5	1	9	4	1
LT11/3/8/4/1	3	2	1	0	0
LT11/5/2/2	7	2	1	0	0
BB10/4/2/3	5	5	1	0	0
LT PRT	3	2	1	0	0
BB PRT	5	5	1	0	0
LT3/8/4/6	3	2	1	0	0
MS1/8/1/4	7	1	9	4	1
BB4/2/4/1	5	5	1	0	0
LT11/3/3/12	7	2	1	0	0

[z]Scale: leaf colour intensity (3= light 5= medium 7= dark)
[y]Scale: main seed colour characteristics (1= White 2=Yellowish brown 3= Brown 4= Reddish brown 5= Purplish brown).
[x]Scale: Presence of seed secondary colour (1= Absent 2= Present)
[w]Scale: Seed Secondary colour (1= Brown 2= Reddish brown 3= Purplish brown 4= Black)
[v]Scale: Pattern of secondary colour

4.2.2 Seed Shape and Size

The shapes of the genotypes in the study were all elliptic and were generally smaller types of seed except for the Msandile genotypes which were longer than the others (Table 7).

Table 7. *Seed shape and size*

Genotype	Seed shape	Seed Length	Seed width
MS PRT	1	3	7
LT11/3/8/4/1	1	3	5
LT11/5/2/2	1	3	7
BB10/4/2/3	1	3	7
LT PRT	1	3	7
BB PRT	1	3	7
LT3/8/4/6	1	3	5
MS1/8/1/4	1	3	7
BB4/2/4/1	1	3	5
LT11/3/3/12	1	3	5

Scale: Seed shape
1= Elliptic 2= Kidney shaped 3= Curved

Scale: Seed Length
3= Short 5= Medium 7= Long

Scale: Seed Width
3= Narrow 5= Medium 7= Broad

4.3 Organoleptic Test for parental lines

Preference of the genotype leaves for the parents varied. Lutembwe was the most preferred because it had both very good leaf texture and taste although the time for preparation was the same with Bubebe. It took 19 minutes to prepare in cooking oil, 14 minutes in groundnut powder and 11 minutes when prepared plainly with just salt (Table 8). Bubebe leaves were the least preferred with a fair taste and leaf texture.

***Table 8**. Organoleptic results for parental lines*

VARIETY	LEAF TEXTURE	TASTE	COOKABILITY TIME (MINUTES)
Lutembwe			
cooking oil	5.0	5.0	19
Groundnut powder	4.5	5.0	14
Plain	5.0	5.0	11
Msandile			
cooking oil	4.0	3.0	20
Groundnut powder	4.0	4.5	16
Plain	4.0	4.5	12
Bubebe			
cooking oil	3.5	3.0	19
Groundnut powder	4.0	3.5	14
Plain	3.5	3.5	11

***1** – Very Poor, **2** – Poor, **3**- Fair, **4** – Good and **5** – Very good*

4.4 Grain Yield

The mean performance of the genotypes varied from one site to the other (Table 9). Agro-ecological region II, NRDC had the highest grain yields. Genotype MS1/8/1/4 out yielded the other genotypes with 975kg/ha while genotypes MS PRT, LT11/3/8/4/1, LT11/5/2/2, BB4/2/4/1, BB PRT and LT3/8/4/6 had the lowest yields.

Agro- ecological region I , Nanga was second in grain yield performance where genotype LT11/3/3/12 out yielded the other genotypes with 525kg/ha, followed by genotype MS1/8/1/4 with 479.8kg/ha and genotype LT11/5/2/2 had the lowest grain yield of 314kg/ha.

Agro- ecological region III, Mufulira, performed poorly in terms of grain yield where the highest genotype was LT11/3/3/12 with 75kg/ha and the lowest genotype was BB PRT with 17.5kg/ha.

ANOVA (general) indicated significant ($p< 0.01$) interactions among cowpea genotypes and test locations (Appendix 1). The significant interactions suggested that test of the selected genotypes under these environments were important for genotypic comparisons and to determine average stability of each genotype. However, the main focus was on AMMI ANOVA

Table 9. *Mean yield (kg/ha) of ten cowpea genotypes grown in the three different locations 2011/2012 season*

VARIETY (TRT)	**ENVIRONMENT**			**TRT MEANS**
	NANGA	**NRDC**	**MUFULIRA**	
MS PRT	469.2	700.0	55.0	408.1
LT11/3/8/4/1	474.0	700.0	62.5	179.8
LT11/5/2/2	314.0	700.0	37.5	117.2
BB4/2/4/1	372.0	700.0	72.5	148.2
LT11/3/3/12	525.5	825.0	75.0	475.2
BB10/4/2/3	404.2	775.0	67.5	415.6
LT PRT	441.0	825.0	35.0	433.7
BB PRT	320.8	700.0	17.5	346.1
LT3/8/4/6	388.0	700.0	35.0	141.0
MS1/8/1/4	479.8	975.0	45.0	499.9
SITE MEAN	**419.1**	**480.0**	**50.2**	**316.5**
SITE INDEX	**3.867**	**2.533**	**3.100**	**3.167**

4.4.2 Multivariate (AMMI) Model Analysis

The AMMI ANOVA analysis revealed significant ($p< 0.01$) differences among genotypes and among environments but genotype x environment interaction effects for grain yield was not significant (Table 10).

Table 10. *ANOVA for the AMMI Analysis of Grain Yield across the three Environments*

Source	df	SS	MS	F	F_prob
Total	89	8733126	98125	*	*
Treatments	29	8164623	281539	42.02	0.00000
Genotypes	9	206862	22985	3.43	0.00265
Environments	2	7811938	3905969	90.04	0.00000
Block	6	260290	43382	6.47	0.00005
Interactions	14	145823	10416	1.55	0.12985
IPCA	10	101281	10128	1.51	0.16595
IPCA	8	44542	5568	0.83	0.58003
Residuals	-4	0	0	0.00	*
Error	46	308212	6700	*	*

The first Interaction Principal Component (IPCA 1) and the second (IPCA 2) accounted for 64% and 36% respectively of the IPCA mean squares. AMMI predicted yield means ranged from 345.9kg/ha to 499.9kg/ha across environments (Table 11). There were no significant differences for the two principle component analysis. AMMI biplot (IPCA1) (Figure 1) for grain yield showed seven genotypes and two environments dispersed away from the centre of biplot showing large variability. Three genotypes (LT11/3/8/4/1, BB4/2/4/1 and MS1/8/1/4) and one environment (Nanga) were clustered near the centre of biplot indicating an average performance of the genotypes and environment. The other AMMI biplot (IPCA2) (Figure 2) for grain yield had six genotypes and all the three environments away from the centre while those clustered near the centre were three genotypes (MS1/8//1/4, LT3/8/4/6 and BB10/4/2/4/1).

Table 11*.Genotype yield Mean and IPCA scores for the ten genotypes in the three environments*

Genotype	Yield Mean	IPCAg[1]	IPCAg[2]
BB23	415.6	0.54516	1.95308
BB41	392.2	2.93070	3.30291
BBP	346.1	1.68478	3.69062
LT22	345.9	3.36718	4.67594
LT312	475.2	-0.97373	-5.17053
LT41	439.5	0.62168	-3.49634
LT46	381.5	1.83402	0.15149
LTP	433.7	-3.51989	-0.74885
MS14	499.9	-11.07214	1.18249
MSP	408.1	4.58224	-5.54080

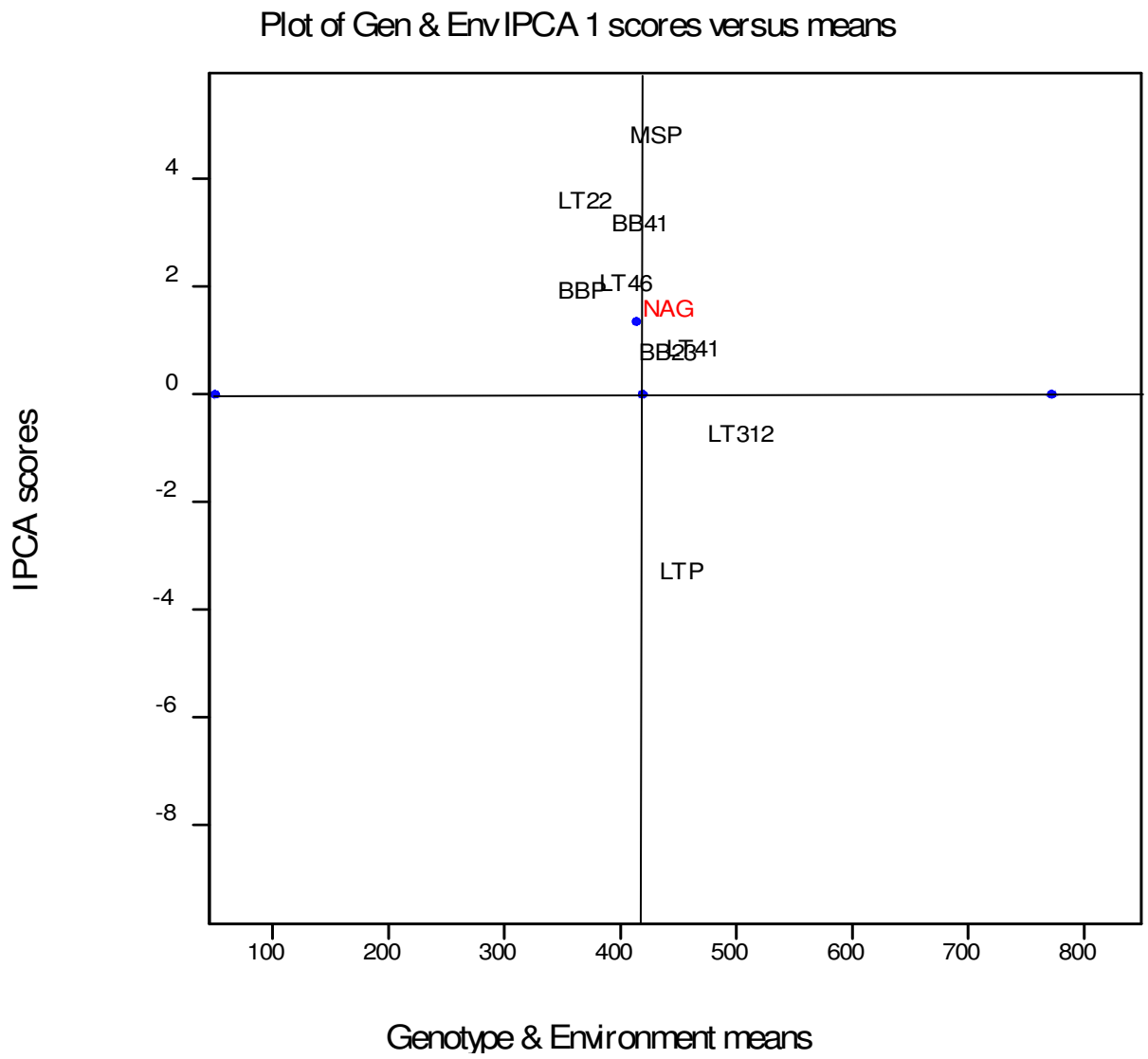

Figure 1. *Biplot of genotype and environment IPCA1 scores with mean yield of ten genotypes and three environments*

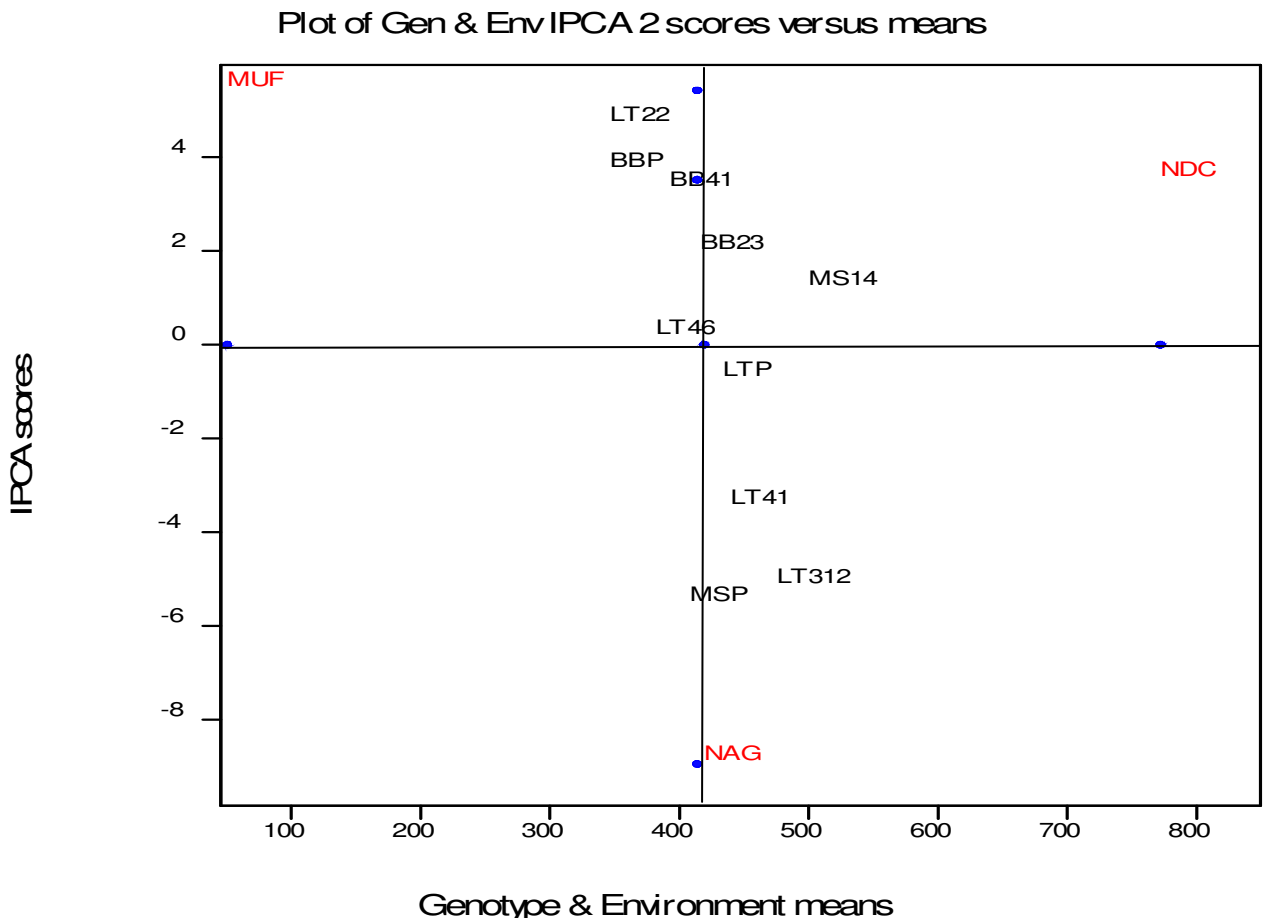

Figure 2. *Biplot of genotype and environment IPCA2 scores with mean yield of ten genotypes and three environments*

Genotype IPCA1 scores ranged from -11.0 (MS1/8/1/4) to 4.5 (MS PRT). Seven genotypes (BB10/4/2/3, BB4/2/4/1, BB PRT, LT11/5/2/2, LT11/3/8/4/1, LT3/8/4/6 and MS PRT) had positive IPCA 1 scores (0.5 to 4.5) and three genotypes (LT11/3/3/12, LT PRT and MS1/8/1/4) had negative IPCA1 scores (Table 11). The genotypes with negative IPCA1 scores were higher yielding than the positive score genotypes. Figure 3 show that Genotype x Environment interaction did not have significant influence on the genotypes. Genotype IPCA2 scores ranged from -5 (MS PRT) to 4.6 (LT11/5/2/2). Six genotypes (BB10/4/2/3, BB4/2/4/1, BB PRT, LT11/5/2/2, MS1/8/1/4 and LT3/8/4/6) had positive IPCA 2 scores (0.1 to 4.6) and four genotypes ((LT11/3/3/12, LT PRT, LT11/3/8/4/1 and MS PRT) had negative IPCA2 scores (Table 11) (Figure 4).

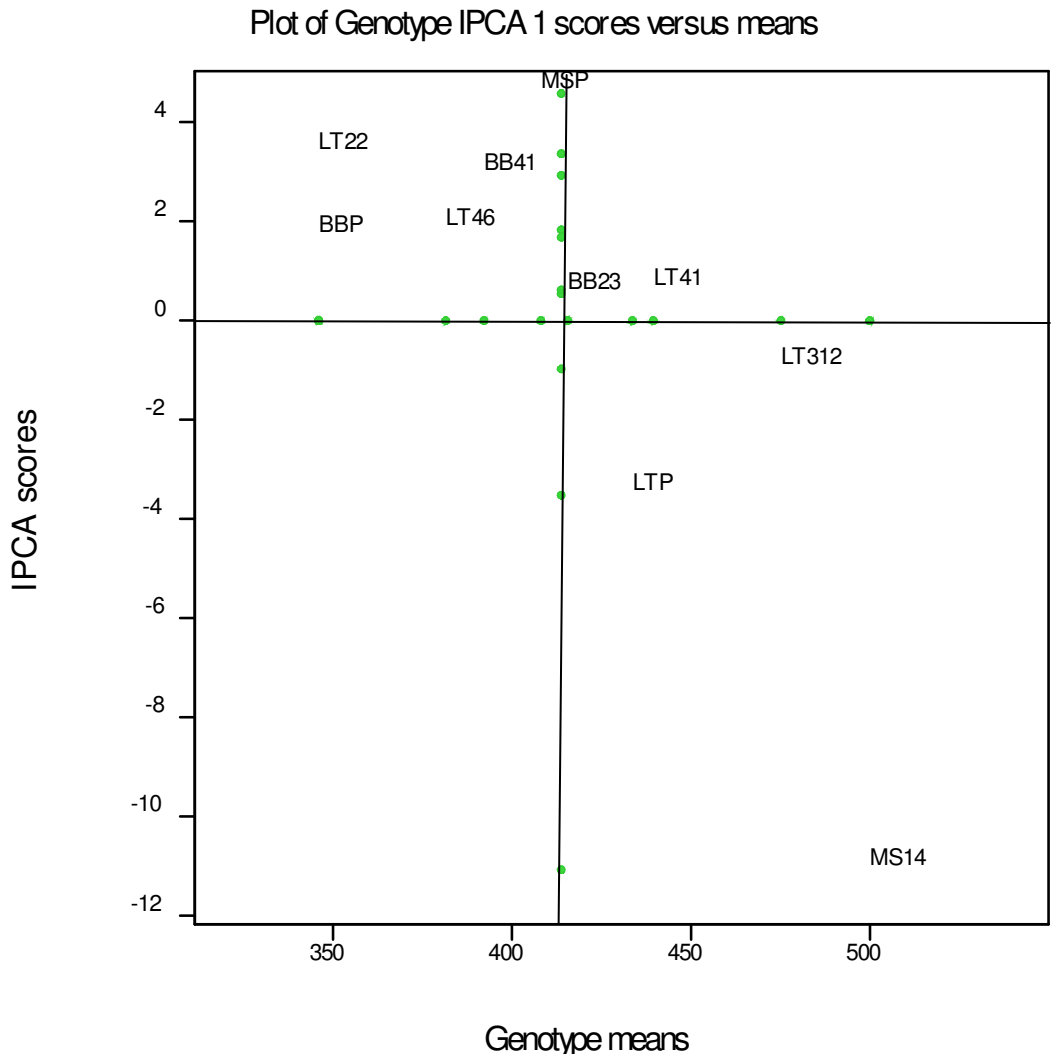

Figure 3. *Biplot of genotype IPCA1 scores with mean yield of ten genotypes and three environments*

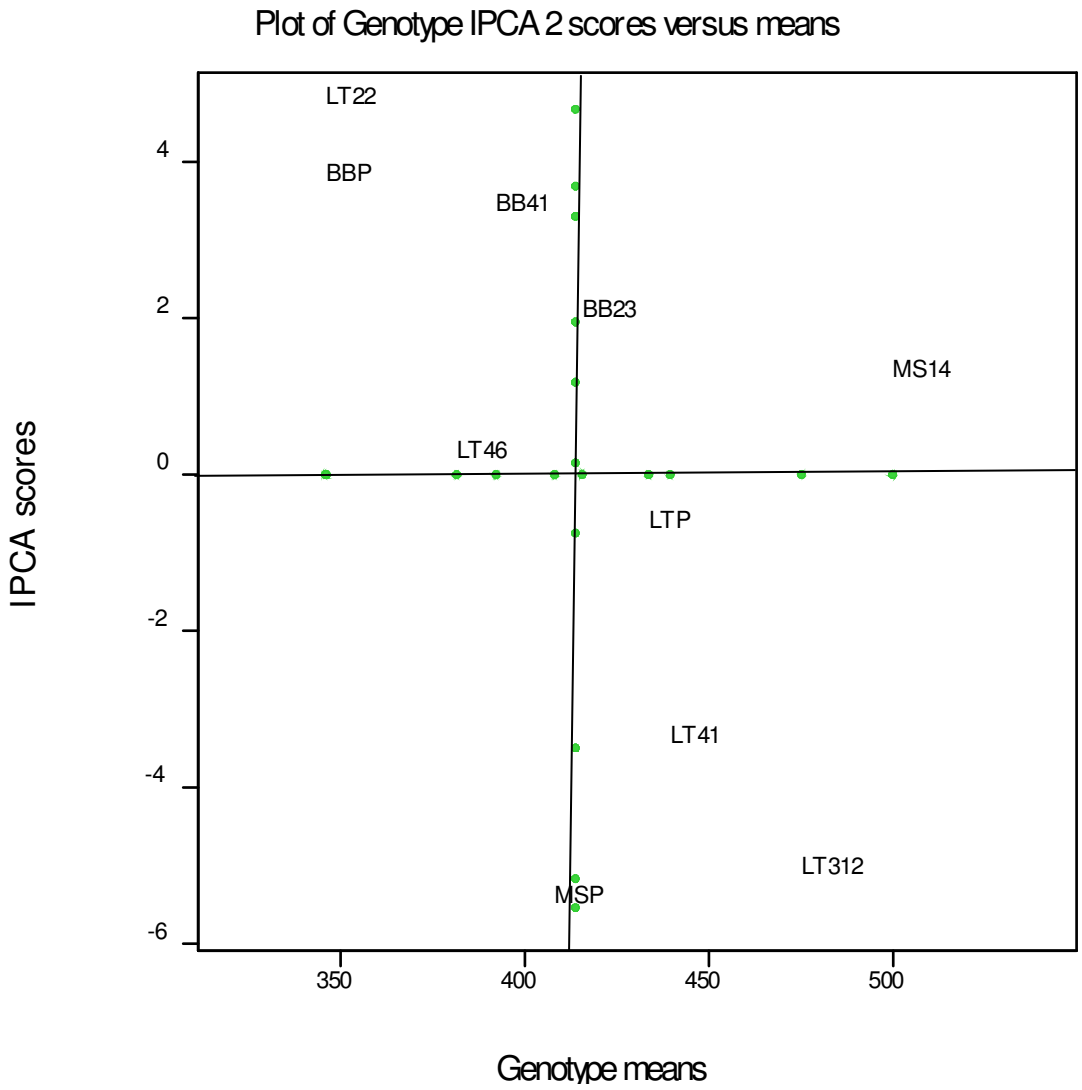

Figure 4. *Biplot of genotype IPCA2 scores with mean yield of ten genotypes and three environments*

Estimates for environmental IPCA 1 scores (Figure 5) showed that Mufulira had the largest positive IPCA score (8.0) while NRDC had the largest negative IPCA score (-10.0). Nanga had a positive IPCA score of 2 indicating the stable environment. Estimates for environmental IPCA 2 scores versus means, Mufulira had a largest positive score (6.0), NRDC was second (3.0) and Nanga had the largest negative score (-9.0) (Figure 6).

In summary ,following the IPCA scores, LT3/8/4/6 was the most stable genotypes with IPCA 1 score of 1.83 and IPCA 2 score of 0.15 (Table 11). It was closely followed by BB10/4/2/3 with IPCA 1 score of 0.54 and IPCA 2 score of 1.95. The higher the IPCA scores the lower the yields while the lower IPCA scores had average mean yields. For environments, Nanga was the most stable environment (IPCA 1 score 1.35) (Table 12).

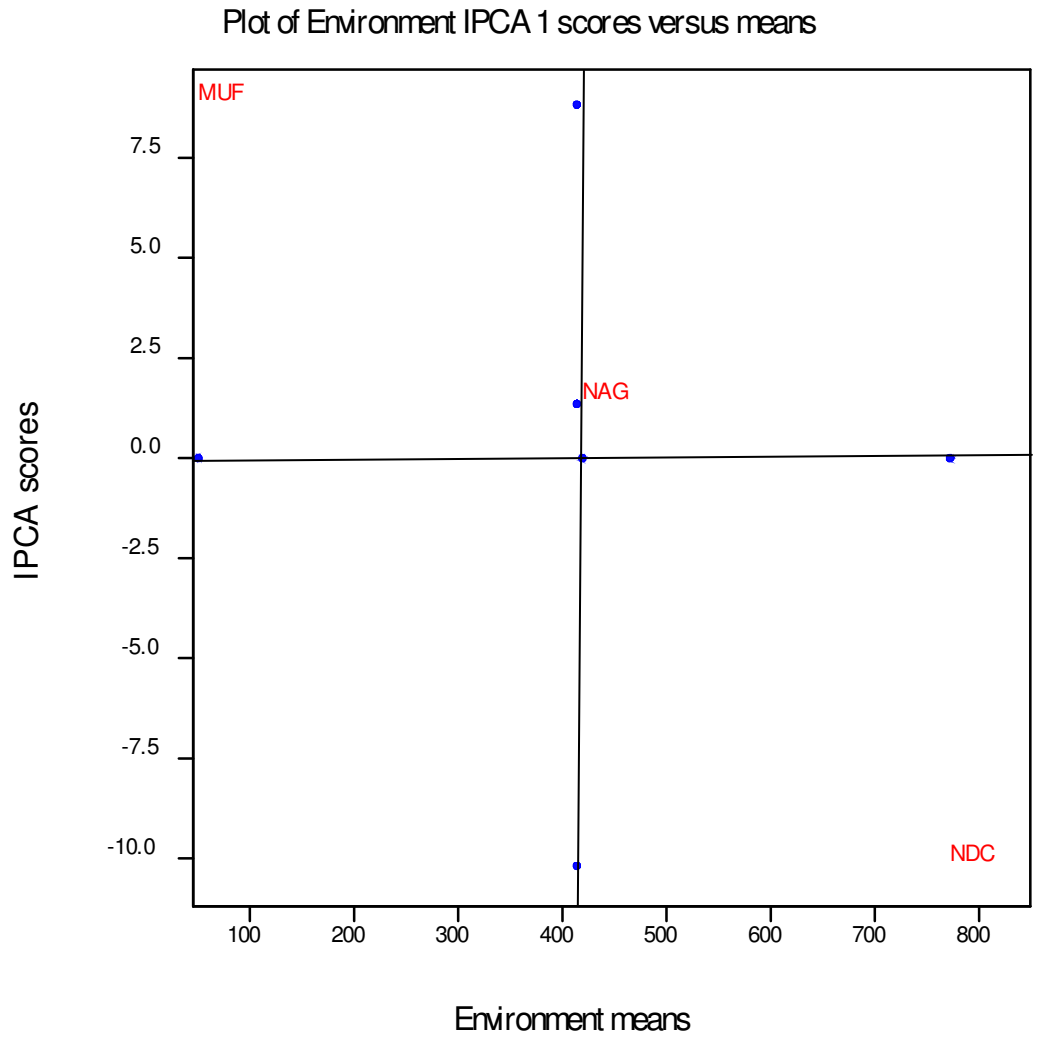

Figure 5. *Biplot of environment IPCA1 scores with mean yield of ten genotypes and three environments*

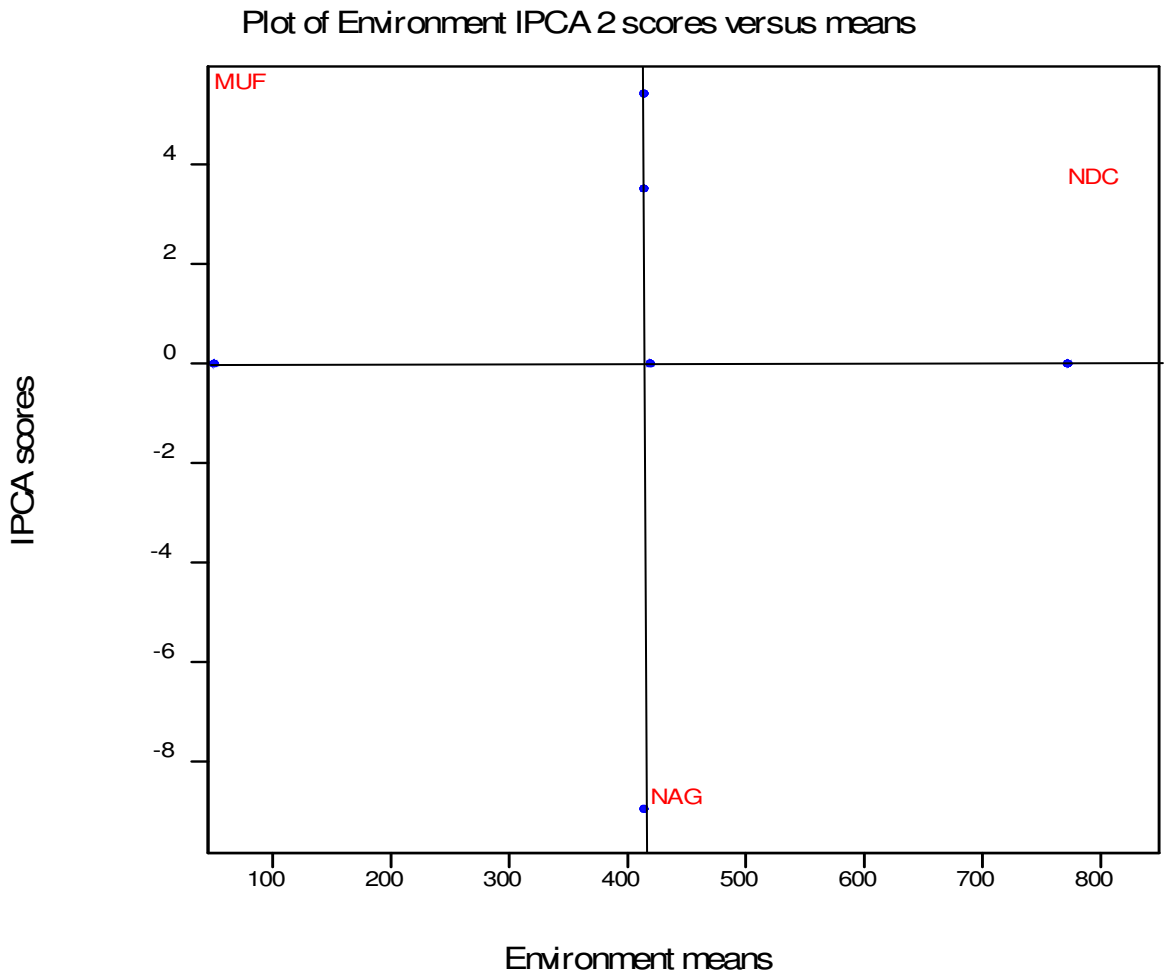

Figure 6. *Biplot of environment IPCA2 scores with mean yield of ten genotypes and three environments*

Table 12.*Environmental means and IPCA scores of the ten genotypes*

Environment	Yield Mean	IPCA[1]	IPCA[2]
Mufulira	50.3	8.83605	5.42735
Nanga	419.1	1.35371	-8.94529
NRDC	771.9	-10.18977	3.51794

4.4.3 AMMI Ranking (Stability)

The first four genotypes in each environment using AMMI were selected and the highest yielding genotype across the three environments was LT11/3/3/12. This genotype performed well in both favorable and unfavourable environments. The other genotypes which performed well across the three environments were MS1/8/1/4, LT11/3/8/4/1, BB4/2/4/1, BB10/4/2/3, LT PRT and MS PRT (Table 13).

Genotype LT11/3/3/12 (75kg/ha) was ranked first in Mufulira and seconded by BB4/2/4/1 (72kg/ha) while the lowest genotype was BB PRT (17.5kg/ha) (Table 14).At Nanga, genotype LT11/3/3/12 (525kg/ha) was ranked first and followed by genotype MS1/8/1/4 (479kg/ha) while genotype LT11/5/2/2 (314kg/ha) was least (Table 15). At NRDC, genotype MS1/8/1/4 (975kg/ha) was ranked first, followed by genotype LT11/3/3/12 (825kg/ha) and genotype LT11/5/2/2 (686kg/ha) was least (Table 16).

Table 13. *Grain yield ranking of the first four genotypes across the three different environments in 2011/2012 season*

Number	Environment	Mean	Score	1	2	3	4
1	MUF	50.3	8.836	LT312	BB41	BB23	LT41
2	NAG	419.1	1.354	LT312	MS14	LT41	MSP
3	NDC	771.9	-10.190	MS14	LT312	LTP	LT41

***Table 14**. Grain yield ranking of the ten genotypes from Mufulira in 2011/2012 season*

Genotype	AMMI Estimates	Genotype	Rank	Ranked no.
BB23	67.50	LT312	75.00	1
BB41	72.50	BB41	72.50	2
BBP	17.50	BB23	67.50	3
LT22	37.50	LT41	62.50	4
LT312	75.00	MSP	55.00	5
LT41	62.50	MS14	45.00	6
LT46	35.00	LT22	37.50	7
LTP	35.00	LT46	35.00	8
MS14	45.00	LTP	35.00	9
MSP	55.00	BBP	17.50	10

Table 15. *Grain yield ranking of the ten genotypes from Nanga in 2011/2012 season*

Genotype	AMMI-estimates	Genotype	Rank	Ranked no.
BB23	404.3	LT312	525.5	1
BB41	372.0	MS14	479.8	2
BBP	320.8	LT41	477.0	3
LT22	314.0	MSP	469.3	4
LT312	525.5	LTP	441.0	5
LT41	477.0	BB23	404.3	6
LT46	388.0	LT46	388.0	7
LTP	441.0	BB41	372.0	8
MS14	479.8	BBP	320.8	9
MSP	469.3	LT22	314.0	10

Table 16. *Grain yield ranking of the ten genotypes from NRDC in 2011/2012 season*

Genotype	AMMI-estimates	Genotype	Rank	Ranked no.
BB23	775.0	MS14	975.0	1
BB41	732.0	LT312	825.0	2
BBP	700.0	LTP	825.0	3
LT22	686.1	LT41	778.9	4
LT312	825.0	BB23	775.0	5
LT41	778.9	BB41	732.0	6
LT46	721.4	LT46	721.4	7
LTP	825.0	BBP	700.0	8
MS14	975.0	MSP	700.0	9
MSP	700.0	LT22	686.1	10

4.5 ANTIOXIDANT CONCENTRATION

The mean catechin equivalents on dry matter basis of total phenolic contents and condensed tannins contents for the three environments ranged from 1.07 ± 0.00 to 3.77 ± 0.02mg/100mg and from 0.17 ± 0.01 to 0.45 ± 0.02mg/100mg respectively. Genotype LT PRT had the highest antioxidant concentrations (3.77 ± 0.02mg/100mgCE total phenolics and 0.45 ± 0.02mg/100mgCE condensed tannins) across the three environments while genotype MS PRT had the lowest antioxidant concentrations(1.07 ± 0.00mg/100mgCE total phenolics 0.17 ± 0.01 mg/100mgCE condensed tannins) and across the three environments.

At Mufulira, [the total phenolic content for the ten cowpea genotypes ranged from 1.07 ± 0.00 to 3.77 ± 0.02mg/100mg while for the condensed tannin content, it ranged from 0.71 ± 0.01 to 0.45 ± 0.02mg/100mg]. Genotype LT PRT had the highest total phenolic contents of 3.77 ± 0.02mg/100mg catechin equivalent (CE) and highest condensed tannins contents of 0.45 ± 0.02mg/100mg CE. The genotype with lower concentration for total phenolics and condensed tannins in this environment was MS PRT with 1.07 ± 0.01mg/100mg and 0.17 ± 0.01mg/100mg catechin equivalent respectively (Table 17).

At NRDC, [the range of total phenolics was from 1.56±0.01 to 3.07±0.02mg/100mgCE while condensed tannins were from 0.19±0.00 to 0.31±0.00mg/100mgCE]. The highest genotype for total phenolics was BB10/4/2/3 (3.07±0.02mg/100mgCE) while the lowest was MS1/8/1/4 (1.56±0.01mg/100mgCE). The highest genotype for condensed tannins was LT11/3/3/12 (0.31±0.00mg/100mgCE) while the lowest was MS PRT (0.19±0.00mg/100mgCE) (Table17).

At Nanga, [the range of total phenolics was from 1.47±0.00 to 3.23±0.01mg/100mg while condensed tannins were from 0.25±0.00 to 0.38±0.02mg/100mgCE]. The highest genotype for total phenolics was LT11/3/3/12 (3.23±0.01mg/100mgCE) while the lowest was MS1/8/1/4 (1.47±0.00mg/100mgCE). The highest genotype for condensed tannins was BB10/4/2/3 (0.38±0.02mg/100mgCE) while the lowest was LT11/3/8/4/1 (0.25±0.00mgCE) (Table 17).

Table 17*.Catechin equivalent (CE) concentration (mg/100mg) of total phenolic content and condensed tannins content for ten cowpea genotypes across three different environments in 2011/2012 season*

VARIETY	TOTAL PHENOLICS			CONDENSED TANNINS		
	Nanga	**NRDC**	**Mufulira**	**Nanga**	**NRDC**	**Mufulira**
MS PRT	2.04±0.16	1.74±0.01	1.07±0.00	0.31±0.01	0.19±0.00	0.17±0.01
LT11/3/8/4/1	2.60±0.00	2.79±0.00	2.84±0.01	0.25±0.00	0.22±0.01	0.36±0.03
LT11/5/2/2	2.25±0.00	2.19±0.00	2.91±0.01	0.30±0.02	0.21±0.01	0.36±0.01
BB4/2/4/1	2.74±0.01	3.07±0.02	3.10±0.01	0.30±0.00	0.22±0.01	0.36±0.02
LT11/3/3/12	3.23±0.01	2.55±0.01	3.47±0.01	0.36±0.00	0.31±0.00	0.29±0.01
BB10/4/2/3	2.62±0.00	3.01±0.02	3.11±0.01	0.38±0.02	0.22±0.01	0.28±0.01
LT PRT	3.10±0.00	2.58±0.01	3.77±0.02	0.37±0.03	0.30±0.00	0.45±0.02
BB PRT	2.34±0.01	2.79±0.00	3.08±0.00	0.45±0.01	0.23±0.01	0.25±0.01
LT3/8/4/6	2.45±0.00	2.97±0.02	2.42±0.01	0.42±0.01	0.21±0.01	0.31±0.01
MS1/8/1/4	1.47±0.00	1.56±0.01	1.20±0.00	0.30±0.01	0.23±0.00	0.19±0.01

4.5.1 Antioxidant and Seed coat Colour

The antioxidant concentrations of the genotypes for both total phenolics and condensed tannins varied according to seed coat colour across the three environments. It was observed that genotypes with dark seed coats were high in both total phenolics and condensed tannins compared with the light seed coats (Table 18). Lutembwe (LT) genotypes which were yellowish brown in colour and Bubebe (BB) genotypes, purplish brown in colour had higher antioxidant values compared with Msandile (MS) genotypes which were white in colour.

Table 18*.Antioxidant concentrations (mg/100mg) of the genotypes according to the seed coat colour across the three environments in 2011/2012 season*

VARIETY	TOTAL PHENOLICS			CONDENSED TANNINS			
	Nanga	**NRDC**	**Mufulira**	**Nanga**	**NRDC**	**Mufulira**	**Seed Coat Colour**
MS PRT	2.04±0.16	1.74±0.01	1.07±0.00	0.31±0.01	0.19±0.00	0.17±0.01	White
LT11/3/8/4/1	2.60±0.00	2.79±0.00	2.84±0.01	0.25±0.00	0.22±0.01	0.36±0.03	Yellowish-brown
LT11/5/2/2	2.25±0.00	2.19±0.00	2.91±0.01	0.30±0.02	0.21±0.01	0.36±0.01	Yellowish-brown
BB4/2/4/1	2.74±0.01	3.07±0.02	3.10±0.01	0.30±0.00	0.22±0.01	0.36±0.02	Purplish-brown
LT11/3/3/12	3.23±0.01	2.55±0.01	3.47±0.01	0.36±0.00	0.31±0.00	0.29±0.01	Yellowish-brown
BB10/4/2/3	2.62±0.00	3.01±0.02	3.11±0.01	0.38±0.02	0.22±0.01	0.28±0.01	Purplish-brown
LT PRT	3.10±0.00	2.58±0.01	3.77±0.02	0.37±0.03	0.30±0.00	0.45±0.02	Yellowish-brown
BB PRT	2.34±0.01	2.79±0.00	3.08±0.00	0.45±0.01	0.23±0.01	0.25±0.01	Purplish-brown
LT3/8/4/6	2.45±0.00	2.97±0.02	2.42±0.01	0.42±0.01	0.21±0.01	0.31±0.01	Yellowish-brown
MS1/8/1/4	1.47±0.00	1.56±0.01	1.20±0.00	0.30±0.01	0.23±0.00	0.19±0.01	White

4.5.2 AMMI for the Antioxidants

The AMMI ANOVA (Appendices 1 and 2) analysis revealed significant differences ($P<0.001$) for both total phenolic contents and condensed tannins contents across the three different environments implying large variability in genotypes and environments. Genotype LT PRT (IPCA2 0.022) was the most stable followed by genotype BB10/4/2/3 (IPCA1 0.037) for total phenolic contents and the least stable genotype was MS PRT (IPCA1 0.63) across the three environments. For the condensed tannins content, the most stable genotype was BB4/2/4/1(IPCA1 0.025) while genotype MS PRT (IPCA1 0.299) was the least stable across the three environments (Appendices 3 and 4).

The AMMI results were also shown as scatter plots (biplots) which allowed visualisation of relationships between the eigen values for the first Principal Component axis (PCA1) and the genotype and environment means. The model explained 91.32% (PC1) and 7.49% (PC2) for total phenolic content while condensed tannins had 86.8% (PC1) and 12.84% (PC2) (Figures 7 and 8).

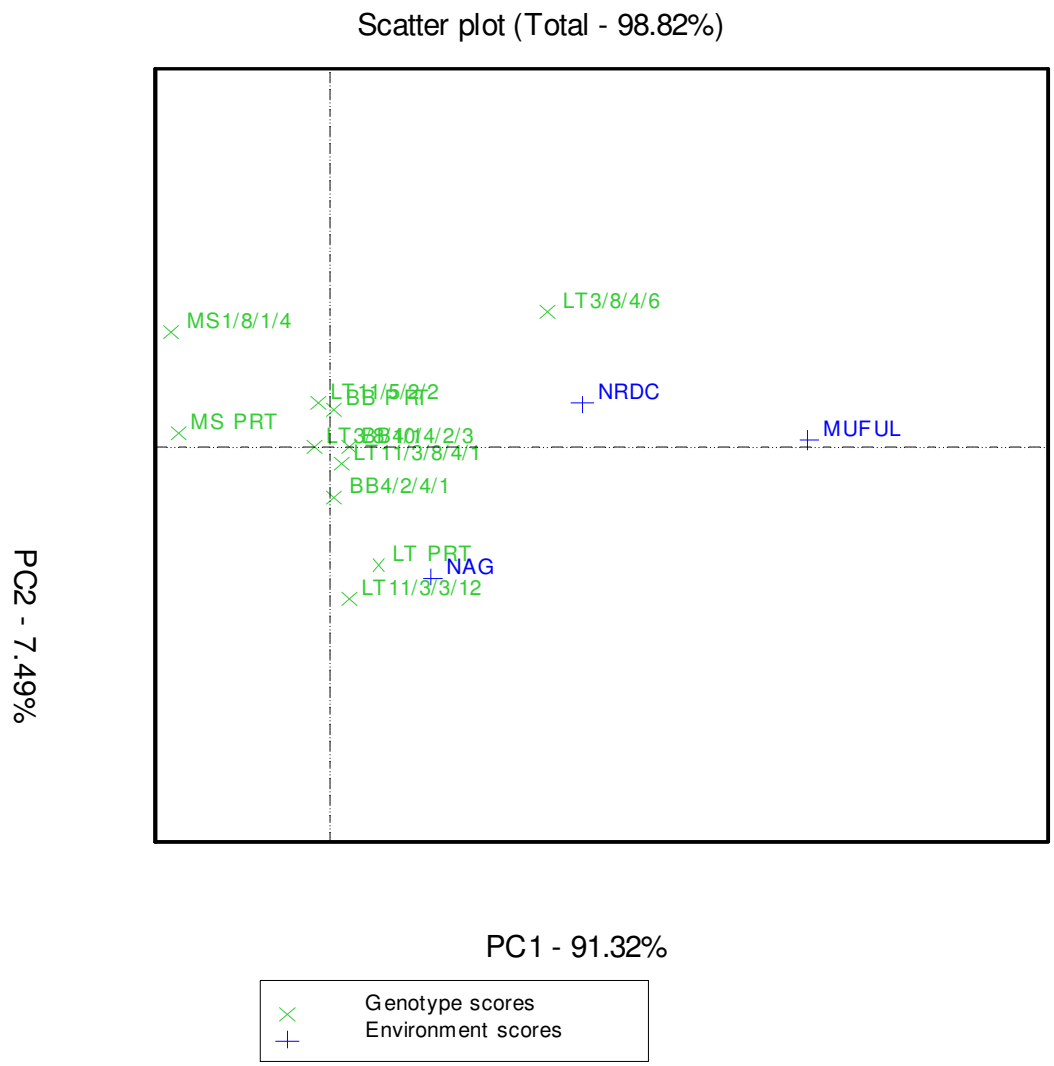

Figure 7*. Scatter plot for total phenolic content of Genotype and Environment PC1 scores with PC2 of ten genotypes and three environments*

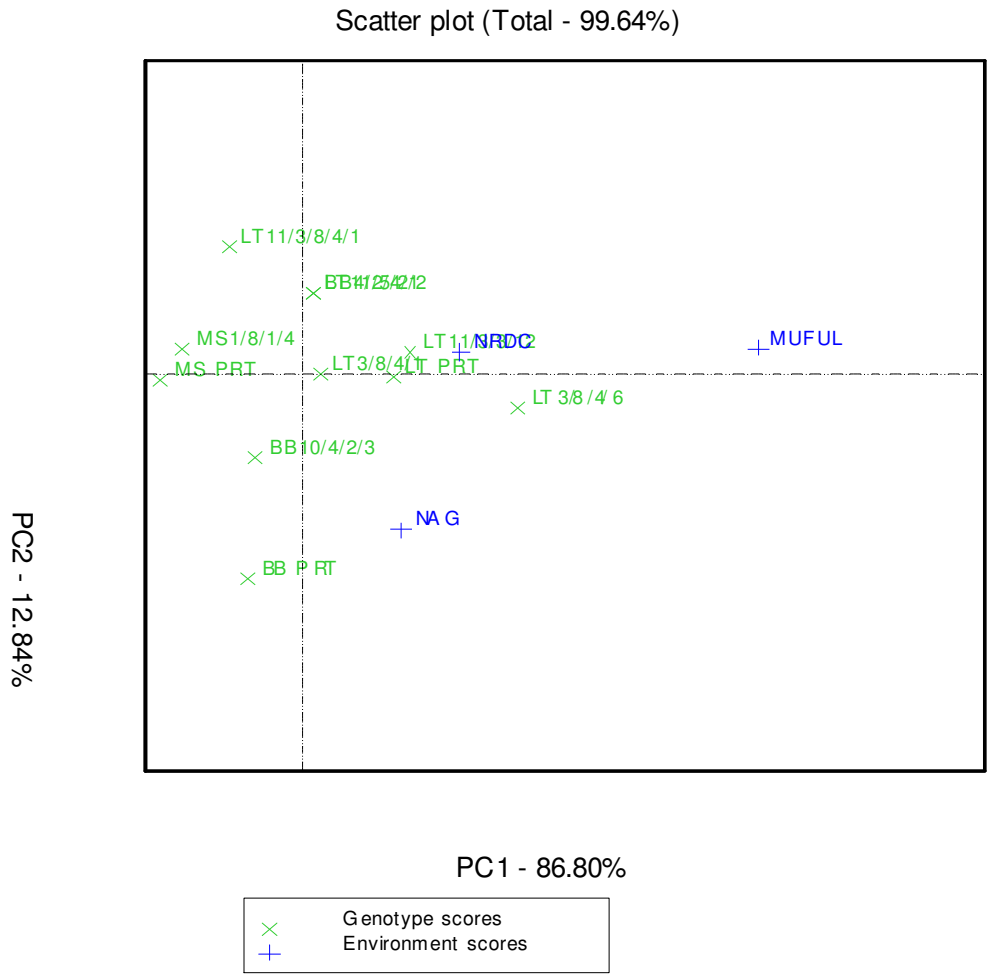

Figure 8. *Scatter plot for condensed tannins content of Genotype and Environment PC1 scores with PC2 of ten genotypes and three environments*

4.6 CRUDE PROTEIN CONTENT

The mean crude protein content across the three environments ranged from 21.25 % to 25.36% (Table 19). AMMI Analysis of Variance showed that the values were significantly different (P<0.00) among genotypes and across environments (Appendix 5). The genotype with highest protein content was obtained from Mufulira, LT3/8/4/1(25.36%) while the lowest genotype was from Nanga, MS PRT (21.25%).

BB PRT was the most stable genotype across the three environments (IPCA1 0.019) while the least stable genotype was BB4//2/4/1(IPCA2 0.66) (Appendix 6)

Table 19*.Crude Protein Content (%) of ten Cowpea genotypes across three environments for 2011/2012 season*

VARIETY (TRT)	**ENVIRONMENT**		
	NANGA	**NRDC**	**MUFULIRA**
MS PRT	21.25	23.82	23.01
LT11/3/8/4/1	23.08	23.10	25.36
LT11/5/2/2	21.70	23.09	23.66
BB4/2/4/1	22.62	22.84	24.60
LT11/3/3/12	24.04	22.47	24.21
BB10/4/2/3	24.02	22.06	24.11
LT PRT	23.43	23.90	21.74
BB PRT	22.11	22.92	23.55
LT3/8/4/6	23.55	22.01	24.88
MS1/8/1/4	23.46	24.09	24.28
SITE MEAN	**22.93**	**23.01**	**23.93**
MAX	**24.04**	**24.09**	**25.36**
MIN	**21.25**	**22.01**	**21.74**

4.7 Relationships among grain yield, total phenolic content, condensed tannin content and crude protein content in the ten cowpea genotypes

Results from simple linear correlation of yield and other quantitative traits are shown in Table 20 below. Yield was negatively correlated with the total phenolics, condensed tannins and crude protein. Yield and total phenolic content had low negative correlation of, $r = -0.279$, yield and condensed tannins with, $r = -0.349$ ($p < 0.048$) and yield and crude protein had a correlation of $r = -0.3432$. There was positive strong correlation between total phenolics and condensed tannins ($r = 0.485$) and this was highly significant ($p < 0.007$). Crude protein and the antioxidants showed no relatedness with correlation $r= 0.044$ and 0.126.

Table 20. *Correlation coefficients for yield, total phenolic, condensed tannins and crude protein contents of the ten cowpea genotypes grown in the three different environments in 2011/2012 season*

	Yield	Total Phenolics	Condensed Tannins	Crude Protein
Yield	X	-0.2796(p<0.1346)	-0.3495*(p<0.0483)	-0.3432(p<0.063)
Total Phenolics		x	0.4846*(p<0.0067)	0.1264(p<0.5058)
Condensed Tannins			X	0.0444(p<0.8158)
Crude Protein				x

Chapter 5

5. DISCUSSION

The total mean yield obtained from the current study were lower than those reported before and were not agreeable with Van Rij (1997) of IITA, Nigeria who reported that yield of cowpea range between 2500 kg/ha in Southern Africa to 4000 kg/ha in West Africa and above 6000 kg/ha in California. This could be attributed to crop management and suitability of the environment during the growing season, the rains were generally poor. Additionally inherent genetic differences in the ability to partition assimilate of the tested genotypes could have contributed as well. Abalo et al. (2003) stated that one should rely more on crop management and suitability of the environment than on genotype differences alone to attain high yields. The differences in mean yield among the tested genotypes in the different environments suggested specific adaptation stability which requires location specific recommendation. Genotypes LT11/3/3/12, MS1/8//1/4 and LT11/3/3/12 displayed the higher yield levels of 525, 975 and 75kg/ha at Nanga, NRDC and Mufulira respectively. The significance of genotype by environment interaction in selection for wide adaptation (stability) has been reviewed by other workers (Becker and Leon, 1988; Crossa et al., 1990; Cooper and Dalecy, 1994.

In this study, ANOVA for AMMI model showed that there was variability in the main effects which are genotypes (G) and Locations (L)/ environments. However, there were no significant interactions among the genotypes and the environments (GxE) Table (10). Genotypes which had lower IPCA scores and clustered near zero or the centre are said to be stable (Chapman et al (1997). LT3/8/4/6 was the most stable and had IPCA2 of 0.151 for genotype and environment scores versus means across the environments (Table 11). Therefore, LT3/8/4/6 (IPCA2 0.151) BB10/4/2/3(IPCA1 0.545) and LT11/3/8/4/1(IPCA1 0.621) were the most stable genotypes across environments however, their yields were very low. Abalo et al., (2003) and Asio, (2004) also indicated that yield stability could only be expected from low yielding genotypes of which do not exploit favorable environments. This implies that the stable the genotype is, the low yielding it becomes. However a farmer always looks for high yielding varieties for economic gain. Genotype MS1/8/1/4 recorded the highest mean yield 975kg/ha at NRDC (Table 9) but it was highly unstable with IPCA1 -11.072 (Table11). The genotypes with negative IPCA1 scores were the highest yielding than those with positive scores. This implies that they are specifically

adapted to high potential areas and performed well under optimal conditions. Zobel et al. (1988) and Crossa et al. (1997) reported that the greater the IPCA score, the more specifically adapted the genotypes to certain agro- ecology environments. Gauch and Zobel (1996) reported that AMMI estimates have a profound effect in producing sharper and stratified ranking pattern. Based on this, genotypes LT11/3/3/12 and MS1/ 8/ 1/ 4 were ranked as the best yielders using AMMI across the three different environments (Agro- ecological zones).

The study revealed significant differences in the main effects (genotypes and location) as well as interaction of these genotypes by the environment (GxE) of the antioxidant concentrations. GxE effect was present for the total phenolic contents and in the condensed tannins. The highest concentrations of total phenolics were in Lutembwe (LT) and progenies; LT PRT with 3.47 mg/100 mgCE and LT11/3/3/12 with 3.10 mg/100 mgCE while the lowest were from Msandile (MS) types of genotypes; MS PRT (0.17 ± 0.01 mg/100 mgCE) and MS1/8/1/4 (0.19 ± 0.01 mg/100 mgCE). The most stable genotype was LT PRT (IPCA2 0.022) across the environments which was also high yielding and the most unstable genotype was MS PRT (IPCA1 0.630) and low yielding. In this case, the stable genotypes in total phenolics were also high yielding genotypes. This agreed with Pacheco et al. (2005) who said that for cultivar recommendation purposes, stable genotypes should also have desirable characteristics. This implies that the high yielding genotypes could be selected for total phenolics stability across the country. Genotypes with very high IPCA scores (i.e. MS PRT and BB4/2/4/1) were specifically adapted in specific regions for them to perform up to their potential. This is in agreement with Zobel et al (1988) and Crossa et al (1997) who reported that the greater IPCA scores the more specifically adapted the genotypes were in certain agro- ecological environments (Appendix 3).

The concentrations of condensed tannins of the ten genotypes for the different environments varied. Nanga had an average concentration of 0.34 ± 0.01mg/100mgCE, NRDC with 0.25 ± 0.01mg/100mgCE and Mufulira with 0.31 ± 0.01mg/100mgCE. The highest concentrations in condensed tannins was in genotype BB PRT (0.45 ± 0.01mg/100mgCE) while the least was in genotype MS PRT (0.17 ± 0.01mg/100mgCE) across the environments. The most stable genotypes were LT11/3/3/12 and LT11/3/8/4/1 with IPCA1 scores of 0.0243 and 0.0246 respectively and were also clustered near the centre on the biplot (Figure 12). The unstable genotype which had very high IPCA scores in condensed tannins was MS PRT (0.299) and it

was also plotted away from the centre of the biplot. This observed response agreed with Delay, (1996) and Chapman et al (1997) who suggested that when ordinates for two principal components are plotted against each other, the one near the centre is average in performance and the most stable.

The results for antioxidants content showed that antioxidant content was related to the seed coat colour. Genotypes with dark seed coat colour had higher antioxidant concentrations compared to the lighter ones. Genotypes LT11/3/3/12, LT PRT, BB PRT, BB4/2/4/1 and BB10/4/2/3 had higher concentrations of both total phenolics and condensed tannins. These genotypes were yellowish brown and purplish brown. On the contrary, MS PRT and MS1/8/1/4 which were white in colour had very low concentrations of both total phenolics and condensed tannins. This is in agreement with findings of Shindano et al., (2012) who reported that the lowest and highest condensed tannin contents were found in Msandile (white coat black eye peas) 290 mg/100g and purple (520mg/100g) varieties, respectively. These workers further observed that when the varieties were categorised according to seed coat colour, white coloured seeds generally had lowest tannin content followed by brown colour and purple seed had highest condensed tannin contents. Similarly Chang et al. (1994) reported higher concentration of phenolic compounds in coloured cowpea varieties than the white varieties. The dark coloured seed coat of lima beans, pigeon peas, African yam bean and jack bean were also found to contain significantly higher tannin content than the lighter coloured seed coats by Oboh et al. (1998). However, Mokgope (2007) reported that the seed coats of the cream- coloured Bechuana white cowpea variety contained higher levels of total phenols than the purple coloured agriblue variety using both the Ferric Ammonium Citrate (FAC) and Folin- Ciocalteu (FC) methods.

ANOVA AMMI revealed highly significant differences in the crude protein contents ($p < 0.01$) of the genotypes across the three environments. The range of the crude protein ranged from 21.25% to 25.36% in all the environments with the highest being LT3/8/4/1(Mufulira) and the lowest was MS PRT (Nanga). This is within the average range of crude protein content which were reported by Aletor and Aladetimi (1998). Mokoboki (2000) reported that a crude protein in cowpea is an important determinant of nutrition and forage quality, especially in crop residues. The most stable genotype in crude protein content was BB PRT (IPCA1 0.019). This genotype had average yield and high concentrations of antioxidants. It is in agreement with Ebehart and

Russel (1966) who recommended that breeders desire to develop varieties that are not only stable but also have above average performance in other traits. This implies that genotype BB PRT could be selected for breeding for crude protein in all the three environments and also gives high yields. In this study, the three selected environments which were situated in the three agro-ecological zones of Zambia played an important role. Variations in the yield as well as the antioxidant concentrations were seen in these environments although G x E was not significant for the yield. The concentrations of both total phenolics and condensed tannins were higher in the genotypes from agro- ecological region III, Mufulira which recorded the lowest grain yield. This could have been attributed to the acidic nature of the soil types which are found in this region. In region I and II where the soils are less acidic, the phenolic contents were lower with higher grain yields.

Correlation analysis revealed the inverse relationship of the yield with the antioxidants as well as crude protein. There was negative correlation (albeit low) of $r = -0.279$ ($P < 1.346$) for yield and total phenolic contents, $r = -0.349$ ($P<0.048$) for yield and condensed tannins and $r = -0.343$ ($P< 0.063$). Condensed tannin content showed negative significant correlation with the yield showing the antagonism which exist between the two. Adams (1976) reported the existence of the negative correlation of tannins in several plants which posed as an obstacle to yield improvements. This was evident in the environments where the yields were high and the antioxidant contents were low e.g. NRDC (Agro –ecological region II). Ibrahim (1986) reported that there is interference by phenolics on chlorophyll activity and ion uptake mechanisms which inhibit the growth of crop seedlings. There was a significant positive relationship ($r = 0.484$, $P< 0.0067$) between total phenolic content and condensed tannin content. This meant that the higher the total phenolic contents the genotype contained, the higher the condensed tannins. This was evident in genotype LT PRT which had high concentrations of total phenolics (3.77 mg/100mg) and high concentrations of condensed tannins (0.45 mg/100mg) as well as low concentrations of total phenolics (1.07 mg/100mg) and condensed tannins (0.17 mg/100mg) of MS PRT. Crude protein content and antioxidants had no effect on each other and this was shown by a much lower positive correlation of $r = 0.044$.

Chapter 6

6. CONCLUSION AND RECOMMENDATIONS

The study showed that cowpea genotypes did exhibit substantial variation on yield and antioxidant concentrations in the different agro- ecological zones of Zambia. This provides an opportunity to identify stable genotypes with high performance. The highest yielding genotype across the three environments was LT11/3/3/12. However, there were specific genotypes which performed well in specific zones or regions (niche environments); LT11/3/3/12 was highest yielding genotype in Nanga and Mufulira (agro ecological region I and III), while MS1/8/1/4 was the highest at NRDC (region II). In terms of adaptation, genotypes LT PRT, MS1/8/1/4 and BB10/4/2/3 showed very high yield potential and therefore would be recommended for production in high potential environment (Nanga). The genotypes LT3/8/4/6, LT11/5/2/2, BB4/2/4/1 and LT11/3/8/4/1 would be deployed only in low potential environments (NRDC and Mufulira) to which they were specifically adapted.

For total phenolic and condensed tannin concentrations, the most stable genotype across the environments was LT PRT. For specific adaptation in both total phenolic contents and condensed tannin content LT11/3/3/12, BB10/4/2/3, as well as LT PRT would be recommended for deployment in agro-ecological region I, II, and III respectively. The study identified some genotypes that displayed both high yield and antioxidant stability. Genotypes LT PRT and BB10/4/2/3 were stable across the three environments and had high yields and they could be recommended for release across all the environments. Correlation studies revealed that total phenolic contents, condensed tannin contents and crude protein contents had negative influence on the yield of the cowpea genotypes across the three environments. Biplots generated by AMMI model gave more valuable and hidden useful information which gave an overall picture of the genotypes behavior in the three selected different environments.

The study revealed low but positive correlation between total phenolic contents and condensed tannin contents. This indicates the possibility of getting genotypes which would combine both traits through selection from a large population. The antioxidant contents were related to the

seed coat colour where dark coloured seeds (Yellow brownish and purplish brown) had more antioxidants than lighter ones (white). In this case Lutembwe (LT) and its mutant derived lines and Bubebe (BB) and its mutant derived line had higher antioxidants than Msandile (MS) and its mutant derived lines.

This study was carried out from one selected area in the agro- ecological zone and was done in one growing season (one year). This suggest further research to be conducted for confirmation of stability of cowpea genotypes across the country, by increasing the sites in the different agro-ecological zones as well as increasing the number of years.

Chapter 7

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Chapter 7

7. APPENDICES

Appendix 1. *ANOVA Table for the grain yield of the ten genotypes across the three environments*

Source	Df	SS	MS	F	F_PROB
Replication	2	4694	2347	0.29	
Genotype x Location	9	1882556	209173	25.81	0.001
Location	3244750	1622375	200.18	0.001	
Genotype x Location	18	3029275	168293	20.77	0.001
Residual	58	470058	8104		

Appendix 2. *ANOVA Table for the AMMI Model of the Total Phenolic concentrations of the ten genotypes across the three environments*

Source	Df	SS	MS	F	F_prob
Total	89	36.97	0.4154	*	*
Treatments	29	36.32	1.2523	105.46	0.00000
Genotypes	9	16.95	1.8828	158.55	0.00000
Environments	2	1.91	0.9541	549.72	0.00000
Block	6	0.01	0.0017	0.15	0.98907
Interactions	18	17.46	0.9702	81.70	0.00000
IPCA	10	12.19	1.2188	102.64	0.00000
IPCA	8	5.28	0.6595	55.53	0.00000
Residuals	0	0.00	*	*	*
Error	54	0.64	0.0119	*	*

Appendix 3. *ANOVA Table for the AMMI Model of the Condensed Tannins concentrations of the ten genotypes across the three environments*

Source	df	SS	MS	F	F_prob
Total	89	0.4828	0.00542	*	*
Treatments	29	0.4661	0.01607	83.03	0.00000
Genotypes	9	0.1885	0.02095	108.20	0.00000
Environments	2	0.1235	0.06174	59.62	0.00000
Block	6	0.0062	0.00104	5.35	0.00022
Interactions	18	0.1541	0.00856	44.24	0.00000
IPCA	10	0.1037	0.01037	53.59	0.00000
IPCA	8	0.0504	0.00630	32.55	0.00000
Residuals	0	0.0000	*	*	*
Error	54	0.0105	0.00019	*	*

Appendix 4. *Principal Component Axis values for the Total Phenolic concentrations of the ten genotypes across the three environments*

Genotype	NG	Gm	IPCAg[1]	IPCAg [2]
BB PRT	1	2.712	0.14770	0.31877
BB10/4/2/3	2	2.613	0.03690	-0.06101
BB4/2/4/1	3	2.727	0.51153	-0.31686
LT PRT	4	3.027	-0.26984	0.02235
LT11/3/3/12	5	2.463	-0.11819	-0.85789
LT11/3/8/4/1	6	2.623	0.24417	-0.13472
LT11/5/2/2	7	2.039	-1.02850	0.25983
LT3/8/4/6	8	2.700	0.16461	0.16873
MS PRT	9	2.798	0.63063	0.51168
MS1/8/1/4	10	1.432	-0.31901	0.08913

Appendix 5. *Principal Component Axis values for the Condensed Tannins concentrations of the ten genotypes across the three environments*

Genotype	NG	Gm	IPCAg[1]	IPCAg[2]
BB PRT	1	0.3367	-0.19393	0.10077
BB10/4/2/3	2	0.3322	0.05744	0.08410
BB4/2/4/1	3	0.2889	0.02525	-0.12878
LT PRT	4	0.3178	0.08481	0.15346
LT11/3/3/12	5	0.2878	0.02434	0.16175
LT11/3/8/4/1	6	0.2444	0.02463	-0.15602
LT11/5/2/2	7	0.2333	-0.17113	-0.09504
LT3/8/4/6	8	0.3633	-0.02262	0.03221
MS PRT	9	0.3544	0.29957	-0.07069
MS1/8/1/4	10	0.2411	-0.12836	-0.08177

Appendix 6. *ANOVA Table for the AMMI Model of the Crude Protein concentrations of the ten genotypes across the three environments*

Source	DF	SS	MS	F	F_prob
Total	89	86.35	0.970	*	*
Treatments	29	83.71	2.887	71.48	0.00000
Genotypes	9	31.56	3.507	86.85	0.00000
Environments	2	19.03	9.515	124.64	0.00000
Block	6	0.46	0.076	1.89	0.09926
Interactions	18	33.12	1.840	45.56	0.00000
IPCA	10	26.89	2.689	66.60	0.00000
IPCA	8	6.22	0.778	19.26	0.00000
Residuals	0	0.00	*	*	*
Error	54	2.18	0.040	*	*

Appendix 7*. Principal Component Axis values for Crude Protein concentrations of the ten genotypes across the three environments*

Genotype	NG	GM	IPCAg[1]	PCAg[2]
BB PRT	1	23.02	0.01965	0.55061
BB10/4/2/3	2	24.15	0.61971	0.06276
BB4/2/4/1	3	23.47	0.29718	0.66011
LT PRT	4	23.00	0.48432	-0.49057
LT11/3/3/12	5	24.02	-0.04529	-0.52395
LT11/3/8/4/1	6	23.25	-0.07703	-0.34098
LT11/5/2/2	7	22.55	-0.41570	0.21154
LT3/8/4/6	8	23.54	0.60423	-0.10669
MS PRT	9	22.35	-1.30711	-0.07985
MS1/8/1/4	10	24.04	-0.17997	0.05701