

**DEVELOPMENT AND MORPHOLOGICAL CHARACTERIZATION OF  
COWPEA (*Vigna unguiculata* (L.) Walp) LINES FOR DROUGHT  
TOLERANCE**

**BY**

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REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN  
CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.**

**MOROGORO, TANZANIA**

**19 NOV 2013**



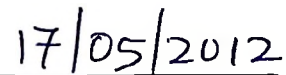
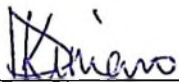
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## ABSTRACT

Development of drought tolerant cowpea varieties is an important aspect in breeding programmes. The main objective of this study was to introgress drought tolerance into farmer's preferred cowpea varieties. A total of 32 cowpea lines were obtained by crossing ITOOK-1263 with Tumaini and ITOOK-1263 with X-Ilonga cowpea varieties in a screen-house experiment laid out in a completely randomized design (CRD) with a split plot arrangement in three replications. The  $F_1$  lines were advanced to  $F_2$  generation and screened for drought tolerance using box method. The results indicate the following lines are drought tolerant ITK-TMA4, ITK-TMA10, ITK-TMA13, ITK-XL1 and ITK-XL9. Heritability estimates were high for all variables in both crosses except for seed yield for plant recorded at 24.41% in a cross between ITOOK-1263 x X-Ilonga. Moisture stress during vegetative stage has more effect than moisture stress at flowering stage for morphological characterization under moisture deficit. The principal components (PC) analysis revealed that the first two PC contributed 99.65% of the total variation among the 18 cowpea genotype evaluated for cross between ITOOK-1263 and Tumaini. The PC1 and PC2 accounted for 93.85 and 5.80 of the total variation respectively. While that of ITOOK-1263 x X-Ilonga contributed 99.78% of the total variation. The PC1 and PC2 accounted for 89.75 and 10.03 of the total variation respectively. Using the PC analysis, this study concludes that the most effective trait responsible for variability among the cowpea genotypes were days to 50% flowering, days to maturity and number of seeds per plant. Further studied are recommended on drought tolerance lines generated in this study, particularly: advancing up to  $F_6$  and evaluation for other abiotic and biotic factors.

## DECLARATION

I, **Didas Rogasian Kimaro**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work, and that it has neither been submitted nor concurrently being submitted in any other institution.

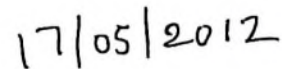


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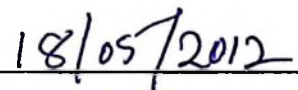
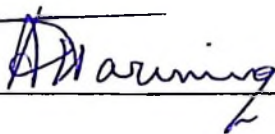
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## **DEDICATION**

This work is dedicated to my beloved parents my late Father Dr. R.T Kimaro and Mrs. Mamelta Kimaro for their inspiration, encouragement and support throughout my studies. They have been instrumental in all my academic achievements for which I am greatly indebted.

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## LIST OF ABBREVIATIONS AND SYMBOLS

a. s. l	Above sea level
ANOVA	Analysis of variance
ARI	Agricultural Research Institute
CABMV	Cowpea aphid-borne mosaic virus
CCMV	Cowpea chlorotic mottle virus
Cm	Centimeter
CMV	Cucumber Mosaic virus
CPMV	Cowpea mosaic virus
CPSMV	Cowpea severe mosaic virus
CRD	Completely Randomized Design
DAS	Days after sowing
F <sub>1</sub>	First filial generation
F <sub>2</sub>	Second filial generation
G	Gram
H <sub>b</sub>	Heritability in broadsense
IBPGR	International Board for Plant Genetic Resources
IITA	International Institute for Tropical Agriculture
ISRA	Institut Senegalais de Recherches Agricoles
ITK	ITOOK-1263
Kg/ha	Kilogram per hectare
NS	Not significant
PCA	Principal Component analysis
$\sigma^2_c$	Variance due to error

$\sigma^2_g$	Genetic variance
SBMV	Southern bean mosaic virus
SUA	Sokoine University of Agriculture
USA	United States of America
TMA	Tumaini
XL	X-Ilonga
%	Percent

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background Information

Cowpea (*Vigna unguiculata* (L.) Walp) is an important food and fodder legume cultivated in the tropics and sub-tropics covering 65 countries in Asia and Oceania, the Middle East, Southern Europe, Africa, southern USA and Central and South America (Singh *et al.*, 1997). It is one of the most ancient crops known to man especially in poor rural households in their drier and sub-humid regions south of the Sahara (Ahenkora *et al.*, 1998, Kitch *et al.*, 1998). It is an important legume crop in coastal and dry savannas of Botswana, Kenya, Mozambique, Somalia, Tanzania, Zambia and Zimbabwe (Amable and Rugambisa, 1992). Cowpea is also cultivated in the drier low hills in Burundi, Kenya, Tanzania and Uganda. It is considered the most economically important traditional legume crop in Africa (Langyintuo *et al.*, 2003).

Cowpea is cultivated for leafy greens, green pods, shelled fresh green peas and shelled dried peas (Ahenkora *et al.*, 1998; Kitch *et al.*, 1998). With more than 25% protein in seeds as well as in young leaves (dry weight basis), cowpea is a major source of protein, minerals and vitamins in daily human diets and is equally important as nutritious fodder for livestock (Singh *et al.*, 2003).

In Tanzania, cowpea is grown in almost all the areas below 1500 m above sea level (Price *et al.*, 1982). It is usually found intercropped with cereals or other crops, although it is sometime grown as a monocrop.

Cowpea production is affected by a number of biotic and abiotic factors including water deficit, the persistently traditional cropping system, insect pests and diseases. Under water deficit conditions 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. However, cowpea has considerable adaptation to high temperatures and drought compared to other crop species. As much as 1000 kg/ha of dry grain has been produced in a Sahelian environment with only 181 mm of rainfall and high evaporative demand (Hall and Patel, 1985).

Cowpea yields are low due to environmental stresses. Abiotic stresses such as drought, heat, untimely or excessive rain, water logging of soils, wind, extreme cold etc cause extensive losses to agricultural production worldwide (Bray, 2000). Tanzania is among the major cowpea producing countries in Africa and suffer serious yield losses in food crops, nearly every year due to drought spells. The estimation of potential yield losses by individual biotic stresses in different environments is 14% (insect pests), 28% (diseases and weeds), and 58% by other factors, while abiotic stresses are estimated at 17% (drought), 20% (salinity), 40% (high temperature), 15% (low temperature) and 8% by other factors (Ashraf and Harris 2005). It is the main environmental stress that is responsible for yield instability and limitations in cowpea production in Tanzania, which affects growth and metabolism. The overall productivity of cowpea is very low with average grain yield in Tanzania of 319kg/ha (Mbwaga *et al.*, 2009) while the potential grain yield lies between 1200 kg/ha and 1500 kg/ha. Breeding for drought tolerance is one of

the most difficult aspects for plant breeders. This is due to lack of reliable screening methods to select drought-tolerant plants and progenies from the segregating populations and the complexity of genetic factors involved in drought tolerance mechanisms (Singh *et al.*, 1999a). One way to eliminate the yield loss due to drought is to identify cowpea lines with adequate levels of resistance to drought.

Cowpea in Tanzania is widely grown in semi arid areas where drought is the major production constraints. Although irrigation is used to reduce water stress in a few regions, still water shortages and high irrigation costs often prevent irrigation at rates required to eliminate drought stress (Van Schoonhoven and Voysest, 1991). Early maturing cultivars can escape the end of season drought that often occurs in semi arid zones. This has helped to increase or at least stabilize cowpeas production in the face of continuing long-term drought spells in this region (Cisse *et al.*, 1995, 1996). Breeders of crops that are propagated from seed are usually concerned with the nature of gene action and the extent to which desirable characters are transmitted from superior plants to their progenies. This calls for a need to examine the pattern of inheritance and estimates of heritability of important characters associated with drought tolerance. A genetic improvement strategy to improve drought tolerance and provide yield stability is an important aspect for plant breeders.

## **1.2 Objectives**

### **1.2.1 Overall objective**

- To introgress drought tolerance into farmer's preferred cowpea lines for Tanzania.

### **1.2.2 Specific objectives**

- (i) To incorporate drought tolerance genes into consumer preferred cowpea varieties and identify the drought tolerant lines using the box screening method.
- (ii) To investigate and estimate heritability for the drought tolerance among segregating genotypes.
- (iii) To conduct morphological characterization of drought tolerant cowpeas lines in response to moisture deficit.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Economic Importance of Cowpeas

Members of the *Phaseoleae* to which cowpea belongs include many of the economically important warm season grain and oilseed legumes, such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*) (Timko *et al.*, 2007). Cowpea is the most economically important indigenous African legume crop and has a wide variety of uses as a nutritious component in the human diet as well as nutritious livestock feed (Langyintuo *et al.*, 2003). It is usually the first crop harvested before the cereal crops are ready and therefore is referred to as "hungry-season crop". With more than 25% protein in dry seeds as well as in young leaves (dry weight basis), cowpea is a major source of protein, minerals and vitamins in daily diets and is equally important as nutritious fodder for livestock (Singh *et al.*, 2003). The high protein content of cowpea grain represents a major advantage for use in infant and children's food (Lambot 2002). The mature cowpea pods are harvested and the green, as well as the dry, haulms are fed to livestock, particularly in the dry season when animal feed is scarce (DeVries and Toenniessen, 2001; Singh *et al.*, 2003) making cowpea a key component of crop-livestock systems.

Cowpea haulms fetch 50% or more of the grain price (dry weight basis). Therefore, cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world, and is a valuable and dependable commodity that produces income for farmers and traders (Singh *et al.*, 2003; Langyintuo *et al.*,

2003). Additionally cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al.*, 2002; Tarawali *et al.*, 2002; Sanginga *et al.*, 2003). The nutritional quality and high consumption levels make cowpea an important food crop contributing to human nutrition, especially in Africa. Cowpea leaves are significant source of  $\beta$ -carotene and ascorbic acid, while cowpea seed only contains negligible amounts of these micronutrients. The freshly harvested cowpea leaves cooked by traditional Kenyan method retain  $\beta$ -carotene well (Imungi and Potter, 1983). While cowpea seeds are known for being protein-rich (about 27% protein) the leaves are an excellent source of vitamins A, D and E (Acland, 1971; Baret, 1990). On average, cowpea seeds contain 23-25% crude protein and 50-67% starch (Singh *et al.*, 2003, Quinn and Myers, 2002).

## **2.2 Major Constraints to Cowpea Production**

Despite its importance, cowpea farmers face several adverse factors in growing the crop and throughout the tropics, diseases and insect pests are major production constraints (Tarawali *et al.*, 2000; Singh *et al.*, 2003). Virus diseases, besides other biological agents such as insect pests, bacteria, fungi and nematodes, have long been associated with yield losses ranging from 10-100% in field grown cowpea crops (Shoyinka *et al.*, 1997), depending on the virus-host vector relationships, as well as prevailing epidemiological factors. The major viruses affecting cowpea in Africa include cowpea chlorotic mottle virus (CCMV), cowpea aphid-borne mosaic virus (CABMV), cowpea mild mottle virus (CPMMV), southern bean mosaic virus (SBMV), cowpea mosaic virus (CPMV), cucumber mosaic virus (CMV), cowpea

chlorotic mosaic virus (CPCMV) and cowpea severe mosaic virus (CPSMV) (Thottappilly and Rossel, 1985; Hampton *et al.*, 1997).

Other diseases such as anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magnus) Bri. & Car.), zonate leaf spot (*Ascochyta phaseolorum* Sacc.), white zonate leaf spot (*Dactuliophora tarri* Leakey), Fusarium wilt (*Fusarium oxysporum* f. sp. *Tracheiphilum* (E.F. Sm.) W.C. Snyder & H.N. Hans.), foot rot (*Fusarium solani* (Mart.) Sacc.) rust (*Uromyces phaseoli* (Pers.) Wint.), scab (*Sphaceloma* sp.), yellow blister (*Synchytrium dolichi* (Cooke) Gaum), gray leaf mold (*Cercospora canescens* Ellis & G. Martini), powdery mildew (*Erysiphe polygoni* DC.) bacterial blight (*Xanthomonas campestris* par. *vignicola* and *Pseudomonas syringae*) are also very important in cowpea production (Edema *et al.*, 1997; Emechebe and Florini, 1997; Wydra and Singh, 1998; Singh *et al.*, 2003).

On the other hand, insect pests represent the most serious constraint to cowpea production throughout Africa. Cowpea is attacked by several insect pests, but those of most economic importance include aphids (*Aphis craccivora* Koch), flower thrips (*Megalurothrips sjostedti* Trybom), pod borers (*Maruca vitrata* Geyer), a complex of podsucking bugs, especially *Clavigralla* spp, and storage the bruchids *Callosobruchus* spp. (Edema and Adipala, 1996; Murdock *et al.*, 1997; Omongo *et al.*, 1997; IITA, 1998; Nampala *et al.*, 1999; Karungi *et al.*, 2000a, b; Singh *et al.*, 2003).

Other factors contributing to low cowpea production in sub-Saharan Africa include parasitic weeds such as *Striga* spp., susceptible local cultivars, low plant population, poor agronomic practices and a lack of improved varieties (Sabiti *et al.*, 2007; Lane *et al.*, 1995). *Alectra vogelii* (Benth.) appear to be the most destructive parasitic weed in cowpea in Africa. Recently released improved cowpea cultivars that are early maturing and more tolerant to key insect pests and diseases especially susceptible to *Alectra* attack experiencing up to 50% yield reductions (Mbwaga *et al.*, 2009).

Drought is the major economic constraints of cowpea production worldwide. High temperature, drought and heat experienced at the critical stages of cowpea growth can lead to substantial reduction in crop yield (Hall, 2004a). Heat injury in legumes including cowpea is mostly associated with pollen infertility, anther indehiscence and lower pod set (Singh, 1996; Thiaw and Hall, 2004). Water stress during flowering can also cause more than 50% reduction in yield due to poor pod formation and seed set, probably caused by limited carbohydrate supply (Turk *et al.*, 1980). Anyia and Herzog (2004a; 2004b) and Sousa *et al.*, (2004) reported a drastic reduction in leaf photosynthesis, thus in dry matter production of cowpea subjected to water stress.

Water stress on cowpea has diverse effects on yield depending on development stage at which it occurs (Agboma *et al.*, 2007). Incidence of water stress at seedling stage may lead to higher root dry weights, longer roots, coleoptiles and higher root: shoot ratio (Tekele 2000; Dhanda *et al.*, 2004; Kashiwagi *et al.*, 2004). Water stress taking

place at both pre-flowering and post-flowering stages of development has the most adverse effects on yield (Kebede *et al.*, 2001). Stress during flowering lead to failure of fertilization, because of impairment of pollen and ovule function (Prasad *et al.*, 2008) which in turn results in lower grain yield.

### **2.3 Drought Tolerance Mechanisms**

Several factors and mechanisms operate independently or jointly to enable plants cope with drought stress. Therefore drought tolerance is manifested as a complex trait (Krishnamurthy *et al.*, 1996). Traditionally, drought tolerance is defined as the ability of plants to live, grows, and yields satisfactorily with limited soil water supply or under periodic water deficiencies (Ashley 1993). According to Mitra (2001), the mechanisms that plants use to cope with drought stress can be grouped into three categories viz. drought escape, drought avoidance and drought tolerance.

However, crop plants use more than one mechanism at a time to cope with drought. Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits occur. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water deficit) and remobilization of pre-anthesis assimilates. Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil-moisture. Plants develop strategies for maintaining turgor by increasing root depth or developing an efficient root system to maximize water uptake, and by reducing water loss through reduced epidermal (stomata and lenticular)

conductance, reduced absorption of radiation by leaf rolling or folding and reduced evapo-transpiration surface (leaf area) (Mitra 2001). Drought tolerance is the ability of plants to withstand water-deficit with low tissue water potential. The mechanisms of drought tolerance are maintenance of turgor through osmotic adjustment (accumulation of solutes in the cell), increased cell elasticity and decreased cell size and desiccation tolerance by protoplasmic resistance.

However, all these adaptation mechanisms of the plant to cope with drought have some disadvantages with respect to yield potential. For instance, a genotype with a shortened life cycle usually yields less compared to a genotype with a normal life cycle.

## **2.4 Drought Tolerance Mechanisms in Cowpea**

### **2.4.1 Drought escapes in cowpea**

The increased incidence of drought in some cowpea growing areas has caused a shift to early maturing varieties (Mortimore *et al.*, 1997). Early maturity of cowpea cultivars is desirable and has proven to be useful in some dry environments and years because of their ability to escape drought (Hall and Patel 1985, Singh 1987, 1994). Such early cultivars can reach maturity in as few as 60–70 days in many of the cowpea production zones of Africa. Earliness is important in Africa as early cultivars can provide the first food and marketable product available from the current growing season, and they can be grown in a diverse array of cropping systems. In addition to escaping drought, early maturing cultivars can escape some insect infestations (Ehlers and Hall 1997). The International Institute of Tropical

Agriculture (IITA) and the Institut Senegalais de Recherches Agricoles (ISRA) have been at the forefront in developing early maturing high yielding and pest resistant cultivars. Selection for early flowering and maturity and yield testing of breeding lines under drought conditions has been used successful in developing cowpea cultivars adapted to low rainfall areas (Hall and Patel 1985; Cisse *et al.*, 1997). Early maturity cowpea varieties (i.e., IT84S-2246, Bambey 21) that escape terminal drought have been released and widely adopted by African farmers. However, if exposed to intermittent drought during the vegetative or reproductive stages, these varieties performed very poorly (Singh *et al.*, 1999a). Efforts are therefore being made to breed cowpea varieties with enhanced drought tolerance for early, mid- and terminal season drought stresses.

#### **2.4.2 Mechanisms of drought avoidance and tolerance in cowpea**

In cowpea, two types of drought tolerance have been described at the seedling stage using the wooden box technique (Mai-Kodomi *et al.*, 1999a). The “Type 1” drought tolerant lines stopped growth after the onset of drought stress and maintained uniformity, but displayed a declining turgidity in all tissues of the plants including the unifoliate and the emerging tiny trifoliate for over 2 weeks. In contrast, the “Type 2” drought tolerant lines remained green for a longer time and continued slow growth of the trifoliate under drought stress. The two types of tolerance responses by cowpea seedlings to drought stress indicate that cowpea genotypes evolved different mechanisms to cope with prolonged drought encountered in the semi-arid regions of Africa where the crop is believed to have originated. Closure of stomata to reduce water loss through transpiration and

cessation of growth (for Type 1 drought avoidance) and osmotic adjustment and continued slow growth (drought tolerance in Type 2) have been suggested as the possible mechanisms for drought tolerance in cowpea (Lawan 1983; Boyer 1996). Cowpea is known as dehydration avoider with strong stomatal sensitivity and reduced growth rate (Lawan 1983). This seems to be the mechanism underlying the Type 1 reaction to drought of TVu 11986 and TVu 11979. The Type 2 reaction of Dan Ila and Kanannado appears to be a combination of three mechanisms; stomatal regulation (partial opening), osmotic control and selective mobilization with distinct visible differences in the desiccation of lower leaves compared to the upper leaves and growing tips (Mai-Kodomi *et al.*, 1999a). It seems that the Type 2 mechanism of drought tolerance is more effective in keeping the plants alive for a longer time and ensures better chances of recovery than Type 1 when the drought spell ends. Similarly, Muchero *et al.* (2008) studied 14 genotypes of cowpea at seedling stage and confirmed the existence of significant genetic variation in response to drought stress. Genotypes, IT93 K-503-1 and IT98 K-499-39 were consistently most tolerant whereas CB46 and Bambey 21 were mostly susceptible.

However, the differences in phenotypic responses to seedling-stage drought among the 14 genotypes were not consistently associated to drought tolerance. As for examples, genotypes IT82E-18(232) and Sutiva 2 showed rapid loss of unifoliates but were found at opposite ends of the drought tolerance spectrum. While, genotypes CB27 and Bambey 21 preserved unifoliates but Bambey 21 was highly drought susceptible and CB27 moderately susceptible under similar stress conditions (Muchero *et al.*, 2008). Somehow, these clear phenotypic responses to drought stress

provide an opportunity for detailed studies of specific drought responses and select genotypes to be used as parents to study the inheritance of these specific responses.

## **2.5 Breeding Cowpea for Drought Tolerance**

Breeding for drought tolerance is a major objective for boosting cowpea yield worldwide and particularly in Tanzania. However, attempts to breed cowpea for drought tolerance through conventional methods have met with limited success because drought is physiologically and genetically a complex trait (Agbicodo *et al.*, 2009). Very few reports are available regarding the success of breeding cowpea against this stress. In Tanzania efforts for breeding cowpea for drought tolerance started in 2007 through Tropical Legume II project (Abate, 2009). Currently efforts have been made to incorporate drought tolerance lines into alectra resistant lines. Breeding for adaptation to abiotic stress is extremely challenging due to the complexity of the target environments as well as that of the stress-adaptive mechanisms adopted by plants (Reynolds *et al.*, 2005). From recently studies it is clear that molecular and metabolic response of crops to a combination of drought and heat is unique, and cannot be explained from the response of plants to these stresses individually (Pruneli, 2002, Suzuki *et al.*, 2005). Drought and heat stress represent an excellent example of two different abiotic stress conditions that occur in the field simultaneously (Jiang and Huang, 2001) and these two in combination has a significantly greater detrimental effect on the growth and productivity of the plants and crops compared with each applied individually. For example during heat stress plants open their stomata to cool their leaves but when combined with drought, plants will not open stomata and leaf temperature will increase (Rizhsky, 2004).

Therefore, the acclimation of plants to a combination of different abiotic stresses would require an appropriate response to each of the individual stress. A multidisciplinary approach including breeding, physiology and biotechnology is required for efficient improvement for drought tolerance in cowpea.

## **2.6 Management Strategies to Cope with Water Stress Conditions**

Farmers must combine various management strategies to cope with soil water deficit resulting weather or limited irrigation. These strategies can be translated into various objectives.

- i. Increasing soil stored water at planting time.
- ii. Increasing soil water extraction.
- iii. Reducing the contribution of soil evaporation to total water use.
- iv. Optimizing the seasonal water use pattern between pre and post flowering.
- v. Tolerate water stress and recovery after stress alleviation.
- vi. Application of irrigation at most sensitive growth phase.

To obtain these objectives, tactical decisions concerning soil tillage, type of crop and cultivars, sowing date and density, nitrogen fertilization, irrigation timing have to be made (Debaeke and Aboudrare, 2004).

## **2.7 Effects of Water Stress on Various Stages of Plant Growth.**

### **2.7.1 Effect of water stress on vegetative growth, flowering and pod-filling stages**

Hilel *et al.* (1972) reported that cowpeas subjected to three levels of water stress during one of the three growth stages (vegetative, flowering and pod-filling),

flowering stage was the most sensitive to moisture stress. In a similar study, Turk *et al.* (1980) and Shouse *et al.* (1981) observed that moisture stress at flowering and pod-filling stages had the least effect on seed yield on indeterminate types. They further reported that water use efficiency was decreased when irrigation was withheld during flowering and pod-filling stages but was increased with no irrigation during vegetative stage because there was no decrease in yield. Summerfield *et al.* (1979) reported a contradictory observation where greenhouse grown cowpea was sensitive to water stress only during the vegetative growth stage/phase. It was concluded that high damage was observed when stress was imposed at flowering because results were based on short season cultivars.

### **2.7.2 Effect of water stress on maturity, yield and yield components**

Dadson *et al.* (2005) reported that the effect of water treatments on maturity of cowpeas was similar to the effect on days to flowering. They observed that days to full maturity of cowpeas varied among genotypes and ranged from 61 to 99 days and from 65 to 99 days when plants were grown under non water-stressed conditions. Days to maturity did not appear to be related to seed yield, biological yield or harvest index. This is because cowpea cultivars tend to have narrow range of adaptation as cultivars developed for one zone usually are not very productive in other zones (Hall *et al.*, 2003). Genotypes with lower biological yield tended to have higher harvest index and vice versa (Dadson *et al.*, 2005). Biological yield refers to the total biomass production from the crop, while harvest index refers to the ratio between economical yield over the biological yield. Earliness in maturity of cowpea genotypes is a desirable trait in drier areas where rainfall is scanty and soils are

sandy with little organic matter (Singh *et al.*, 1997). Early maturing cowpea cultivars have proved more useful in some dry environments and years because of their ability to escape drought (Hall and Patel 1985).

Significant reductions in cowpea yield when exposed to water stress at flowering were reported by Anyia and Herzog (2004a; 2004b) and Hamidou *et al.* (2007). It is well established that total plant biomass production depends on the amount of water used for growth (Anyia and Herzog 2004b).

Hamidou *et al.* (2007) reported significant depressive water deficit effect on yield components except number of seeds per pod in cowpea. They however reported higher reduction in number of pods per plant up to 57% and 64% in cowpea when drought was imposed under glasshouse and field conditions respectively. Grain yield reduction due to drought stress that occurs at flowering is mostly attributed to decrease of pod development rather than reduction in size of seeds and number of seeds per pod in several legume crops such as soybean, (Liu *et al.*, 2004), dry bean (Acosta-Gallegos and Shitaba 1989; Acosta-Gallegos and Adams 1991) and common bean (RamirezVallejo and Kelly 1998; Aminian *et al.*, 2007; Ghassemi-Golezani and Mardfar 2008). Most of the studies showed that water stress has a severe effect during flower initiation, flowering, fruit and seed development (Maiti *et al.*, 2006). Water stress prior to flowering or at the beginning of flowering time was reported to delay or totally inhibited the flowering of tillers in pearl millet (Winkel *et al.*, 2007). On cowpea water stress during flowering and pod filling reduce the number of pods per plant due o flower abscission and smaller seed dry

mass (Turk *et al.*, 1980). Reduced seed dry mass, in case of high density water stress during seed filling, may be the result of reduced translocation of carbohydrate to the seed (Turk *et al.*, 1980b).

## **2.8 Screening Cowpea Genotypes using Wooden Boxes**

In an attempt to develop a simple and cheap, but still efficient, screening method to select a drought tolerant plant from large numbers of plants. Singh *et al.* (1999a) developed a simple technique, using wooden boxes. This technique was used to screen cowpea germplasm for drought tolerance at the seedling stage. The more tolerant lines were later evaluated in the field at a mature stage.

Singh and Matsui (1999) compare relationship between box screening and field performance under drought stress. Using box screening method a large number of lines that are drought tolerant have been released (Singh *et al.*, 1999a). The results of box screening indicated that varietal differences for plant responses to drought stress could be assessed at the seedling stage in cowpea. Also, the close correspondence between the results of seedling screening (box method), field screening and pot screening further indicate that the phenomenon responsible for drought tolerance in the seedling stage is also manifested at the reproductive stage in cowpea. Therefore, screening cowpea varieties at the seedling stage appears to be a reliable method to identify drought-tolerant varieties. Since the results of the box screening, field screening and pot screening are similar, box screening is more practical because of the ease of handling, the possibility of using it in controlled environment, and the ability to screen large numbers of lines or plants.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Location

The experiment was conducted in the Department of Crop Science and Production Crop Museum screen house, Sokoine University of Agriculture (SUA) in Morogoro Tanzania located at 6° 5" S 37° 39' E; 524 m a.s.l on the foot of mount Uluguru plateau.

#### 3.2 Materials

Cowpea germplasm for creating population were obtained from ARI Ilonga, Tanzania and IITA, specifically the resistant parent ITOOK-1263 (a germplasm line maintained at IITA) for drought tolerance and a susceptible adapted parent: Tumaini (an improved variety) as well as a local genotype X-Ilonga both maintained at ARI-Ilonga.

#### 3.3 Development of Breeding Population

The F<sub>2</sub> cowpea lines were obtained from two pair of crosses. In one pair of cross; ITOOK-1263 was crossed with Tumaini and in pair two of cross; ITOOK-1263 was crosses with X-Ilonga and F<sub>1s</sub> from both pair of crosses were produced. After harvest of F<sub>1</sub> seeds, planting was done of each F<sub>1</sub> seeds so as to obtain F<sub>2</sub> seeds. Seeds were harvested and planted in the following season for screening for drought tolerance and for heritability studies. Crossing was done by removing the keel with forceps, exposing the stamens and stigma. The stamens were then removed by firmly grasping of the filaments carefully to insure that all 10 stamens were

removed, and no burst occurred and all contact with stigma was avoided. After emasculation pollination was done by brushing matured pollen to a stigma of an opened flower. After pollen transfer wings were returned to the closed position and secured with a 3cm strip of adhesive tape to prevent desiccation and contamination by visiting insects. The crossing was done every day between 6.30-9.30 a.m in a protected structure at Horticulture, SUA.

### **3.4 Experimental Design and Layout**

The experiment was arranged as a split plot in completely randomized design (CRD) in a screen house. The three water regimes were the main plot (factor A) and cowpea genotypes were the subplots (factor B).

The arrangement was as follows:-

Main plot 1 - Well watered throughout the growing period (control).

Main plot 2 - Water was withheld from full establishment (21 DAS) until flowering period, there after water was applied normally up to maturity.

Main plot 3 - Plants were watered until flowering. Thereafter, water was withheld Until pod filling and then re-watered again to maturity.

Sub plots constitute of 16 cowpea lines and parents.

### **3.5 Box Screening for Shoot Dehydration Tolerance**

Singh *et al.* (1999a) described a simple wooden box screening method showing good correlation with drought tolerance at vegetative and reproductive stages to select drought-tolerant plants or progenies in cowpea.

Wooden boxes of 150 cm length, 70 cm width, and 15 cm depth were kept on benches in a screen house. The boxes were lined with polyethylene sheets and filled with a sterilized soil. The boxes were filled with sterilized forest soil to 12 cm depth, leaving about 3 cm space on the top for watering. The polyethylene lining along the sides and bottoms of the boxes ensured even distribution of water. A spirit level was used to ensure a flat soil surface on the boxes after watering. Equidistant holes were made in straight rows 30 cm apart with a hill-to-hill distance of 25 cm within the rows. Holes of 2 cm depth were made. Two good seeds were sown in each hole and after germination, thinned to one plant per hill. Gap-filling was done one week after germination for some of the seeds which failed to germinate. Each box contained both parents and 16 F<sub>2</sub> cowpea lines with 18 plants. Three boxes constitute one replication. The 18 cowpea plants were the subplot and three moisture regimes were the main plot. The boxes were watered daily using a small watering can until the partial emergence of the first trifoliolate leaf for all treatment, thereafter applied as per required treatment. Wilted plants in each variety were counted daily until re-watering again depending on the main plot. Based on the days taken to wilting and recovery, the varieties were rated as drought tolerant or susceptible.

Urea fertilizer was applied as per requirement in order to initiate vegetative growth as the sterilized soil doesn't contain nutrient enough for support cowpea growth. Control of insect and diseases was done by spraying Thionex (40mls/20litres of water) and Ridomil Gold.

### **3.6 Data Collection**

#### **3.6.1 Drought score (1-9) procedure**

Data for drought tolerance and susceptible lines was recorded for two phases. Phase one involved treatment number two where water was withheld from 21 days after sowing (DAS) after full establishment until flowering. Phase two involved genotypes from treatment number three where water was withheld from flowering to maturity. Each plant was scored using the International Board for Plant Genetic Resources (IBPGR, 1983) descriptors for cowpea. They were scored on a 1-9 scale.

Where; 3 = resistant,

5= medium susceptibility (plant alive with most of the leaves yellow/or Wilting);

7= high susceptibility (plant dead and dry).

The score of lines in boxes were averaged for three replication in each main plot. Using the averages of the evaluation, the tested lines were then classified into three categories of susceptibility. (1) With a mean ranging from 3 to 4.9, the lines were classified as having a low susceptibility. (2) With a mean ranging from 5 to 5.5, the lines were classified as medium susceptibility (3) With a mean ranging from 5.6 to 7; the lines were classified as highly susceptible.

#### **3.6.2 Data for estimates of broadsense heritability and yield components**

The following data were collected days to 50% flowering, days to maturity, 100 seed weight, number of pods per plant, pod length, number of seeds per pod, number of seeds per plant and seed yield per plant.

**Days to 50% flowering**

Days to flowering was measured as a number of days from planting to the time at which 50% plants had one or more flowers.

**Days to maturity**

Days to maturity was measured as the number of days from sowing to the time when at least 50% of the pods has turned its color to brown.

**100 seed weight (g)**

Weight of 100 seeds obtained from each plant was weighed and recorded.

**Number of pods per plant**

Counting of pods was done on each individual plant harvested, and total numbers of pods per plant were recorded.

**Pod length (cm)**

Pod length was measured using ruler where by each pod was measured in a plant and average was recorded per each plant.

**Number of seeds per pod**

Counting of seeds was done from all pods, and then average number of seeds per pod was determined by the following relationship: Total number of seeds per plant/Number of pods per plant = Number of seeds per pod

**Number of seeds per plant**

Number of all seeds per plant was counted and recorded per each individual plant.

**Seed yield per plant (g/plant)**

The seeds harvested in each plot were weighed and the seed yield per plant was calculated as follows:

Seed yield / plant = Seed weight of harvested plants/Number of harvested plants.

**3.6.3 Data for morphological characterization**

The following data were collected for morphological descriptors of cowpea lines under moisture deficit; leaf senescence maintenance of stem greenness, leaf area and number of leaves.

**Leaf senescence**

In each plant leaf senescence was measured using rating scale of 0-10 (Muchero *et al.*, 2008). Score was done after three weeks of stress.

Whereas; 0-1 = 0 to <5% senescence,

2-3 = 5-10% damage,

3-4 = 10-15% damage,

5-6 = 15-25% damage,

7-8 = 25-50% damage,

9-10 = >50% senescence.

### Maintenance of stem greenness

Stem greenness was scored on a scale of 0 to 5, as described by (Muchero *et al.*, 2008) where as 0=being completely dried and dead.

1= being completely yellow

2=yellow and light brown leaves with severe wilting

3=yellowish grey with moderate wilting

4=green and

5= being completely green

### Leaf area (cm)

Sample of leaf of each plant was measured in a scanner by using leaf area software installed in computer. A leaf was kept on a scanner connected to computer, scanned in a specified scale and recorded in centimeter.

### Number of leaves

Counting of number of leaves in each plant was done and recorded.

### 3.7 Heritability Estimate

Parental and  $F_2$  variances were calculated and heritability in the broad sense was estimated following Mahmud and Kramer (1951).

$$H^2_{bs} = \frac{\sigma^2_{F_2} - \sqrt{\sigma^2_{P_1} \cdot \sigma^2_{P_2}}}{\sigma^2_{F_2}} \times 100 \dots\dots\dots(i)$$

Where  $H^2_{bs}$  = Heritability estimate in the broad sense

$\sigma^2_{P_1}$  and  $\sigma^2_{P_2}$  are variance of parent 1 and 2 respectively.

$$\sigma^2 F_2 = \text{Variance of } F_2$$

### 3.8 Data Analysis

Data was analyzed by using SAS (Statistical Analysis Software). The following model was used:

$$X_{ij} = \mu + R_i + W_j + (RW)_{ij} + V_k + (WV)_{jk} + (RWV)_{ijk} \dots\dots\dots(ii)$$

Where:  $X_{ij}$  = Response

$\mu$  = overall mean of all observation

(R)  $i = i^{\text{th}}$  Replication effect

(W)  $j = j^{\text{th}}$  Water stress effect,

(RW)  $ij$  = Water stress error

(V)  $k = k^{\text{th}}$  Variety effect

(WV)  $jk$  = Interaction of water and genotypes

(RWV)  $ijk$  = error term for each observation

### 3.9 Principal Component Analysis (PCA)

Principal Component (PC) was analyzed by Genstat software, using the data from the yield traits. Cluster analyses were used to determine the suitability of the features to characterize the variations of the observations and to determine the natural group from the genotypes studied.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Identification of Drought Tolerant Lines

The response to drought among cowpeas lines were observed in this study: Cowpea genotypes in main plot one grow normally until reach maturity and produced seeds. Cowpea genotypes in main plot two (vegetative stage) and main plot three (flowering stage) had the following observation. In the first type cowpea plants produced flower and seeds before reaching the permanent wilting stage. Although some of the plants did not recover after the stress period, viable seeds were produced, thus ensuring a next generation (drought escape). The other type was to withstand the period of drought stress and being able to recover to such an extent that the plants were able to produce seed after being re-watered (drought tolerance). The recovery of lines under investigation was from apical meristem recovery few days after re-introduction of water. The third type of plants did not produce seeds; they started wilting after few weeks of withheld water and eventually died. They did not recover after re-watering. These plants were therefore classified as susceptible to drought conditions.

According to the drought score methodology used in this study, lines that score 4.5 and 4.7 were regarded as the most resistant lines (Table 1). Lines that had a score 5.0 and 5.3 were regarded as with medium susceptibility and those that scored 5.7 and 6.0 were regarded as susceptible (Table 1). Some lines were observed to show low wilting score, but did not produced higher yield. Most of the lines that demonstrated drought tolerance also produced more seeds per plants with the highest of 129 with the exception of line which have only 92 seeds per plant (Table 1).

**Table 1: Mean performance of cowpea genotypes for drought tolerance and yield components for a cross between ITOOK-1263 and Tumaini**

Cowpea genotype	Pod/plant	Seed/plant	Seed/pod	Seed yield/plant	Drought Score
ITK-TMA1	9	103	10	9.8	5.7
ITK-TMA2	10	129	12	15.1	4.7
ITK-TMA3	8	107	10	10.6	5
ITK-TMA4	8	101	10	9.1	5.3
ITK-TMA5	10	122	12	13.1	5
ITK-TMA6	9	107	11	11.5	5
ITK-TMA7	9	108	12	9.5	5.7
ITK-TMA8	9	104	9	10.3	5.3
ITK-TMA9	8	92	10	8.5	4.5
ITK-TMA10	8	122	9	9.7	6
ITK-TMA11	10	122	12	11.5	4.7
ITK-TMA12	10	121	12	11	5
ITK-TMA13	8	91	9	9.9	5.7
ITK-TMA14	11	124	11	9.7	4.7
ITK-TMA15	10	113	11	9.8	5
ITK-TMA16	10	120	11	11.2	5
ITOOK-1263	15	168	14	16.5	3.6
Tumaini	14	154	15	13.8	4.3
Mean	9.7	117.1	11.1	11.1	5
LSD (0.05)	3.92	46.48	4.56	4.95	-
CV %	43.39	42.36	44.62	45.77	-
Moisture	ns	ns	ns	ns	-
Genotype	1.93	2.11	ns	1.86	-
Moisture x Genotype	ns	ns	ns	ns	-

Note: ITK-TMA- Cross between ITOOK-1263 x Tumaini

For the second set of cross involving ITOOK-1263 (drought tolerance parent) and X-Ilonga (local landrace), the same drought score methodology was used to identify resistance lines and susceptible lines. In this cross 7 lines demonstrated to be tolerant with the score of 3.6, 4.0, 4.3 and 4.7 (Table 2).

**Table 2: Mean performance of cowpea genotypes for drought tolerance and yield components for a cross between ITOOK - 1263 and X-Ilonga**

Cowpea genotype	Pods/plant	Seed/pod	Seed/plant	Seed yield/plant	Drought Score
ITK-XL1	8	10	112	11.3	5
ITK-XL2	11	12	145	15.5	3.6
ITK-XL3	11	13	146	14.5	4.7
ITK-XL4	9	11	116	10.3	5
ITK-XL5	10	12	128	13.4	4.3
ITK-XL6	10	12	130	14.1	4.7
ITK-XL7	9	11	113	11.5	5.3
ITK-XL8	10	12	129	11.8	4.3
ITK-XL9	7	9	101	7.7	5.7
ITK-XL10	9.8	12	125	13	4.7
ITK-XL11	8	10	108	12	6.3
ITK-XL12	8	10	110	11	5
ITK-XL13	9	11	109	10.7	6
ITK-XL14	11	11	138	13.9	4.3
ITK-XL15	10	13	125	12.9	5.3
ITK-XL16	11	14	153	14.4	4
ITOOK-1263	12	13	153	15.3	3.6
X-Ilonga	10	13	130	14.4	3.6
Mean	7.98	11.6	126.2	12.6	4.74
LSD (0.05)	3.54	47.21	4.22	5.12	-
CV %	38.33	39.33	38.52	42.36	-
Moisture	ns	ns	ns	7.35	-
Genotype	2.17	1.76	ns	ns	-
Moisture x Genotype	ns	ns	ns	ns	-

Note: ITK-XL- Cross between ITOOK-1263 x X-Ilonga.

Lines with the score of 5.0 and 5.3 were regarded as medium susceptible. While 4 lines had a highest scores of 5.7, 6.0 and 6.3 and these are susceptible (Table 2). In terms of other variables most of the resistant lines performed better than others (Table 2).

## 4.2 Estimate Heritability for Drought Tolerance among Segregating Genotypes

### 4.2.1 Estimates of broadsense heritability for ITOOK-1263 x Tumaini

Estimates of broad sense heritability for days to flowering, days to maturity, 100 seed weight, numbers of pods per plant, pod length, number of seed per pod, number of seeds per plant and seed yield per plant are presented in Table 3. All variables estimated have high values of broadsense heritability.

**Table 3: Variance of parent 1 ( $\sigma^2_{p_1}$ ), variance of parent 2 ( $\sigma^2_{p_2}$ ), variance of F2 ( $\sigma^2_{f_2}$ ) and heritability estimate for cross between ITOOK-1263 and Tumaini**

Trait	Variance			Broad sense heritability ( $h_m^2$ ) (%)
	$\sigma^2_{p_1}$	$\sigma^2_{p_2}$	$\sigma^2_{f_2}$	
Days to 50% flowering	75.94	106.11	702.85	87.22
Days to maturity	80.78	105.53	1156.71	92.02
100 seed weight (gm)	1.42	4.36	109.68	97.73
Pod Length	1.28	1.44	33.49	95.93
Number of seeds per pod	0.86	2.36	30.47	95.33
Number of pods per plant	3.44	1.78	22.47	89.00
Number of seeds per plant	543.25	559.5	3636.37	85.11
Seed yield per Plant (seed weight)	4.82	8.55	39.95	83.93

Key:  $\sigma^2_{p_1}$  – Variance of ITOOK-1263,

$\sigma^2_{p_2}$  – Variance of Tumaini

$\sigma^2_{f_2}$  - Variance of F<sub>2</sub> genotypes

#### 4.2.2 Estimates of broadsense heritability for ITOOK-1263 and X-Ilonga

Estimates of broad sense heritability for days to flowering, days to maturity, 100 seed weight, numbers of pods per plant, pod length, number of seed per pod, number of seeds per plant and seed yield per plant are presented in Table 4. Except for seed yield per plant which had the lowest value, all the remaining variables estimated have high values of broadsense heritability.

**Table 4: Variance of parent 1 ( $\sigma^2p_1$ ), variance of parent 2 ( $\sigma^2p_2$ ), variance of F2 ( $\sigma^2f_2$ ) and heritability estimate for cross between ITOOK-1263 and X-Ilonga**

Trait	Variance			Broad sense heritability ( $h_m^2$ ) (%)
	$\sigma^2p_1$	$\sigma^2p_2$	$\sigma^2f_2$	
Days to 50% flowering	194.25	162	683.6	74.05
Days to maturity	207.25	176.69	1066.4	82.06
100 seed weight (gm)	1.15	4.21	17.7	87.57
Pod Length	0.31	1.94	28.8	97.32
Number of seeds per pod	1.44	2.19	25.9	93.17
Number of pods per plant	0.5	1.78	18.2	94.83
Number of seeds per plant	252.19	569.78	3187.15	88.1
Seed yield per Plant (seed weight)	3.89	186.18	35.6	24.41

Key:  $\sigma^2p_1$  – Variance of ITOOK-1263,

$\sigma^2p_2$  – Variance of X-Ilonga

$\sigma^2f_2$  - Variance of F<sub>2</sub> genotypes

### **4.3 Morphological Descriptors of Cowpea under Moisture Deficit.**

#### **4.3.1 Leaf senescence**

Moisture regimes were not significantly different at ( $P \leq 0.05$ ) in both crosses involving ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). Genotype and interaction between moisture and genotypes were not significant difference for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). The response of leaf senescence among lines differed. Most of the lines in a cross between ITOOK-1263 x X-Ilonga showed delayed senescence for a longer period than those at the other cross ITOOK-1263 x Tumaini (Table 5 and Table 6) respectively. Plants that were stressed at flowering stage survived much longer than those at Vegetative (seedling growth stage). This trait was assessed easily by visual score.

#### **4.3.2 Stem greenness**

Moisture regimes were not significantly different ( $P \leq 0.05$ ) under investigation for both crosses between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). Additionally there was no significant difference ( $P \leq 0.05$ ) for genotypes and interaction between moisture and genotypes for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6).

After exposure to drought stress for up to 30 days, for lines stressed at seedling stage some cowpea genotypes maintained stem greenness much more than others. Plant

**Table 5: Effects of moisture stress for morphological descriptors at different growth stages for cross between ITOOK-1263 x Tumaini**

Genotype	Trait															
	Leaf Senescence				Stem greenness				Leaf Area				Number of leaves			
	Moisture stress at		Flowering		Moisture stress at		Flowering		Moisture stress at		Flowering		Moisture stress at		Flowering	
Control	Vegetative	Control	Flowering	Control	Vegetative	Control	Flowering	Control	Vegetative	Control	Flowering	Control	Vegetative	Control	Flowering	
ITK-TMA1	2.3	9.0	6.3	5.0	0.7	2.7	129.0	88.1	157.2	47.3	13.7	30.0				
ITK-TMA2	2.0	6.7	6.3	5.0	2.3	3.3	214.3	112.6	189.5	48.3	18.3	32.0				
ITK-TMA3	2.7	6.7	7.7	5.0	2.7	2.3	172.4	120.0	120.4	41.3	20.3	30.7				
ITK-TMA4	2.3	7.0	7.3	4.7	2.3	2.0	160.1	85.4	141.4	41.0	19.7	32.0				
ITK-TMA5	2.7	6.0	7.0	4.7	3.0	2.3	85.3	76.0	138.7	39.0	20.0	32.0				
ITK-TMA6	2.3	7.0	7.7	4.7	2.3	2.3	138.9	68.0	135.7	45.3	18.0	32.0				
ITK-TMA7	2.7	8.0	7.3	4.7	2.0	2.0	150.6	72.0	138.4	34.3	15.7	31.7				
ITK-TMA8	2.3	6.3	8.3	4.7	3.3	1.0	173.4	87.1	112.5	47.0	21.7	26.7				
ITK-TMA9	2.7	5.7	8.3	5.0	3.3	1.0	209.2	88.7	131.8	40.7	18.0	28.3				
ITK-TMA10	2.3	7.3	8.3	4.7	2.0	1.0	153.4	68.6	137.7	51.7	16.7	26.0				
ITK-TMA11	2.3	6.0	8.0	4.7	3.7	2.0	239.9	94.8	82.8	44.7	19.0	28.0				
ITK-TMA12	2.3	6.7	7.0	4.7	3.0	2.3	176.9	70.1	177.8	42.7	21.0	34.3				
ITK-TMA13	2.0	6.7	8.0	5.0	2.3	1.0	137.9	72.4	165.1	47.7	20.3	27.0				
ITK-TMA14	2.7	7.0	6.3	5.0	2.3	3.7	135.4	90.2	141.6	42.3	18.3	34.7				
ITK-TMA15	2.3	6.7	7.0	5.0	2.3	2.7	190.9	73.9	170.9	42.7	19.0	33.3				
ITK-TMA16	2.7	6.3	6.7	4.7	2.3	3.0	89.4	80.3	181.1	41.3	20.0	38.7				
LSD			1.43			1.27			51.90			6.48				
CV %			27.61			43.11			41.46			22.48				
Moisture			ns			ns			ns			ns				
Genotype			ns			ns			1.90			2.42				
Moist x Geno			ns			ns			ns			ns				

**Table 6: Effects of moisture stress for morphological descriptors at different growth stages for cross between ITOOK-1263 x X-Ilonga.**

Genotype	Trait											
	Leaf Senescence			Stem greenness			Leaf Area			Number of leaves		
	Moisture stress at		Flowering	Moisture stress at		Flowering	Moisture stress at		Flowering	Moisture stress at		Flowering
ITK-XL1	2.3	6.0	3.3	5.0	2.3	4.3	246.4	83.1	250.8	46.0	21.0	45.0
ITK-XL2	2.0	6.7	3.7	5.0	2.3	4.7	249.8	102.5	232.5	41.7	17.0	43.3
ITK-XL3	2.0	6.3	4.3	4.7	3.0	3.7	242.5	105.2	211.3	43.0	14.0	40.7
ITK-XL4	2.0	6.7	5.3	4.7	2.7	2.7	158.4	97.9	162.9	45.0	14.7	34.7
ITK-XL5	1.7	5.3	5.7	5.0	3.7	2.7	157.7	195.1	129.1	43.7	17.3	27.3
ITK-XL6	1.7	6.7	6.7	5.0	2.7	2.7	250.3	127.4	220.6	40.3	13.3	35.3
ITK-XL7	1.7	7.7	6.0	4.7	2.0	2.3	221.8	117.3	151.3	42.7	10.0	27.7
ITK-XL8	1.7	6.3	5.0	4.7	2.3	3.7	148.7	164.2	173.1	41.3	13.0	32.3
ITK-XL9	2.3	9.0	5.0	5.0	0.0	4.0	244.6	107.4	270.6	39.0	5.7	43.7
ITK-XL10	2.0	7.0	6.0	5.0	2.3	3.3	207.0	145.5	212.7	39.0	14.0	37.0
ITK-XL11	1.7	8.0	5.0	5.0	1.0	3.3	210.2	108.9	194.2	40.3	14.0	34.3
ITK-XL12	2.0	5.7	6.0	5.0	2.7	2.3	222.3	135.2	170.8	45.0	22.0	30.7
ITK-XL13	2.0	6.7	4.3	5.0	1.7	3.0	237.8	92.2	155.6	38.7	17.7	29.3
ITK-XL14	2.3	4.0	4.7	5.0	4.0	3.0	216.5	93.9	135.0	42.0	21.7	26.7
ITK-XL15	2.3	6.7	4.3	5.0	2.0	3.0	181.8	102.1	198.8	44.7	21.0	34.0
ITK-XL16	2.7	4.0	5.0	4.7	4.3	3.0	184.7	143.6	210.7	50.3	27.7	36.0
LSD			1.61			1.25			61.02			7.72
CV %			38.17			39.27			35.95			26.57
Moisture			ns			ns			ns			ns
Genotype			ns			ns			1.84			2.54
Moist x Geno			ns			ns			ns			ns

greenness was higher under control compared to other moisture regimes (Table 5) and (Table 6) respectively. However, under water stress onset of senescence occurred early among some lines while others kept their green stem for a longer period of time than others. Some genotypes have a yellowish tinge to its color which confounded visual scoring of stem greenness, therefore score lowest under this study. Genotypes which score higher for stem greenness they also had the highest score for drought tolerance. For flowering stage most of the cowpeas lines changes in stem color started few days after withholding water from green to yellow within few days. Lines such as ITK-TM3, ITK-TMA6, ITK-TMA8, ITK-TM14, ITK-TMA16, ITK-XL1, ITK-XL13 and ITK-XL16 maintained green stem for much longer period than the rest line. The lines that were obtained from cross between ITOOK-1263 and X-Ilonga (Table 6) had higher score for stem greenness as compared to the lines obtained from a cross between ITOOK-1263 and Tumaini (Table 5).

#### **4.3.3 Leaf area**

Moisture regimes were not significantly different ( $P \leq 0.05$ ) for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). However there were significant difference for genotypes ( $P \leq 0.05$ ) for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). The interaction between moisture and genotype were not significantly different for crosses between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). The results showed that leaf area were significantly decreased with increasing moisture stress. Most of the genotype subjected to stress during the vegetative phase had the lowest leaf area as compared to those during flowering phase that were stressed and

the continuously watered controls (Table 5) and (Table 6) respectively. The highest leaf area recorded was 239.9 cm<sup>2</sup> and the lowest was 85.3cm<sup>2</sup> for lines in the control (Table 5) while, the highest leaf area for lines at seedling stage was 120 cm<sup>2</sup> and the lowest was 68 cm<sup>2</sup> (Table 5). The highest leaf area recorded for lines stressed at flowering stage was 189.5 cm<sup>2</sup> and the lowest was 82.8 cm<sup>2</sup> for lines obtained from a cross between ITOOK-1263 and Tumaini (Table 5). For lines obtained from a cross between ITOOK-1263 and X-Ilonga the lowest and highest leaf area recorded were 148.7cm<sup>2</sup> and 250.3 cm<sup>2</sup>, for lines grown under control treatment (Table 6). For vegetative (seedling) stage 83.1 cm<sup>2</sup> and 164.2 cm<sup>2</sup> were the lowest and highest leaf area recorded (Table 6). For plants stressed at flowering stage the lowest was 135 cm<sup>2</sup> and highest was 250.8 cm<sup>2</sup> (Table 6). From this study cowpeas lines obtained from a cross between ITOOK-1263 x X-Ilonga was not affected much with the onset of drought as compared from those obtained from the cross between ITOOK-1263 x Tumaini.

#### 4.3.4 Number of leaves

Moisture regimes were not significantly different ( $P \leq 0.05$ ) for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). However there were significant difference ( $P \leq 0.05$ ) in genotypes for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6) respectively. The interaction between moisture regimes and genotypes were not significant difference ( $P \leq 0.05$ ) for cross between ITOOK-1263 x Tumaini (Table 5) and ITOOK-1263 x X-Ilonga (Table 6). The result shows that the number of leaves per plant decreased with increasing moisture stress, however most of genotypes that were screened at seedling stage (vegetative) had the lowest number of leaves per plant as compared under those at flowering phase (Table 5) and (Table 6)

respectively. In this study there's reduction in leaf production in vegetative phase. It has been observed that cowpea suffered in drought much more in seedling stressed stage than other stages of growth. Under water stress very few or no more new emerged leaves occurred as a result of drought for most of the lines. Lines that were stressed at flowering stage showed changes in leaf color from green to yellow after few days of withholding water and rapidly dropped off of unifoliate leaves.

**Table 7: Mean performance of cowpea genotypes for leaf senescence, stem greenness, leaf area and number of leaves for cross between ITOOK-1263 and Tumaini**

<b>Genotype</b>	<b>LS</b>	<b>STGR</b>	<b>LA</b>	<b>NLVS</b>
ITK-TMA1	6.1ab	2.67b	120.33cd	30.33bc
ITK-TMA2	5.33ab	3.44ab	161.01abc	31.67bc
ITK-TMA3	5.67ab	3.22ab	126.49bcd	27.89c
ITK-TMA4	5.56ab	3.0ab	140.08bcd	30.89bc
ITK-TMA5	5.67ab	3.22ab	95.51d	28.78bc
ITK-TMA6	5.78ab	3.11ab	114.20cd	30.67bc
ITK-TMA7	6.00ab	2.78b	120.36cd	27.22c
ITK-TMA8	5.89ab	3.0ab	124.34bcd	31.78bc
ITK-TMA9	5.56ab	3.11ab	143.23bcd	30.22bc
ITK-TMA10	5.89ab	2.44b	119.90cd	31.44bc
ITK-TMA11	5.56ab	3.44ab	139.18bcd	30.56bc
ITK-TMA12	5.44ab	3.11ab	141.60bcd	30.89bc
ITK-TMA13	5.67ab	2.67b	120.70cd	29.00bc
ITK-TM14	5.67ab	3.33ab	111.29cd	27.67c
ITK-TMA15	5.33ab	3.33ab	141.88bcd	30.56bc
ITK-TMA16	6.22 a	3.00ab	113.58cd	27.67c
ITOOK	3.89c	4.22a	201.94a	43.11a
Tumaini	4.78bc	3.67ab	175.57ab	35.00b

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ ) according to Student Newman-Keuls Test at three moisture regime

**Table 8: Mean performance of cowpea genotypes for leaf senescence, stem greenness, leaf area and number of leaves for cross between ITOOK-1263 and X-Ilonga**

Genotype	LS	STGR	LA	NLVS
ITK-TMA1	3.89a	3.89a	193.44bcd	34.11ab
ITK-TMA2	4.33a	3.89a	194.44bcd	34.00ab
ITK-TMA3	4.33a	3.56a	186.32bcd	34.11ab
ITK-TMA4	5.11a	3.33a	139.71b	26.67b
ITK-TMA5	4.44a	3.44a	160.64ab	27.89b
ITK-TMA6	5.00a	3.33a	199.40ab	30.56b
ITK-TMA7	5.11a	3.00a	163.46ab	26.78b
ITK-TMA8	4.56a	3.33a	162.01ab	29.11b
ITK-TMA9	5.0a	3.00a	207.51ab	33.44ab
ITK-TMA10	5.11a	3.44a	185.09ab	31.44b
ITK-TMA11	5.00a	3.11a	171.12ab	28.89b
ITK-TMA12	4.33a	3.33a	176.12ab	29.89b
ITK-TMA13	4.22a	3.22a	161.87ab	27.11b
ITK-TMA14	4.11a	3.78a	148.50b	27.22b
ITK-TMA15	4.67a	3.22a	160.91ab	27.67b
ITK-TMA16	4.33a	3.78a	176.33ab	30.67b
ITOOK	3.89a	3.67a	268.38a	44.00a
X-Ilonga	4.11a	3.33a	212.93ab	36.22ab

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test at three moisture regime.

#### 4.4 Effects of Moisture Stress on Yield and Yield Components, Days to 50%

##### Flowering and Days to Maturity among Segregating Cowpea Genotypes

The yield characteristics observed in this study for both set of crosses were hundred seed weight, pod length, number of pods per plant, number of seeds per pod, number of seeds per plant and seed weight. All of the parameters analyzed were not statistically difference under different moisture regimes at ( $P \leq 0.05$ ) for a cross between ITOOK-1263 x Tumaini (Table 9) and ITOOK-1263 x X-Ilonga (Table 10).

Table 9: Mean sum of squares (MS) and experimental errors (MSe) for the traits evaluated derived from general

linear model for cross between ITOOK-1263 x Tumaini

Source of Variation	Days to Flowering	Days to Maturity	100 Seed Weight	Pod Length	Number of Seeds/pods	Number of pods/plant	Number of seeds/plant	Seed weight
Moisture	3851.49*	6682.06*	223.51ns	485.14ns	512.68ns	375.88ns	86427.74ns	817.49ns
Genotype	866.47ns	1236.29ns	28.14ns	22.93ns	23.48ns	34.09*	5225.12*	52.25*
Moisture X Genotype	531.30ns	851.26ns	17.95ns	18.97ns	18.08ns	15.25ns	2038.89ns	25.45ns
Errors	711.01	1146.85	18.23	28.74	23.82	17.64	2474.26	28.09

\* Significant at 5% levels of probability

Ns- Not significant at 5% levels of probability

**Table 10: Mean sum of square (MS) and experimental errors (MSe) for the traits evaluated derived from general linear model for cross between ITOOK-1263 x X-Ilonga**

Source of Variation	Days to Flowering	Days to Maturity	100Seed weight	Pod Length	Number of Seed /pods	Number of pods/Plant	Number of Seeds/plant	Seed Weight
Moisture	2356.35*	4219.13*	119.04ns	390.03ns	309.12ns	253.45ns	44838.25ns	221.43ns
Genotype	945.98ns	1436.84ns	26.79*	21.75ns	24.26ns	31.18*	4501.98*	43.37ns
Moisture X Genotype	643.51ns	1008.19ns	17.74ns	24.10ns	23.98ns	13.67ns	2532.17ns	36.41ns
Errors	652.68	1002.47	15.31	23.09	20.48	14.39	2552.47	30.12

\* Significant at 5% levels of probability

Ns – Not significant at 5% levels of probability

Genotypes were significantly different ( $P \leq 0.05$ ) for number of pod per plant and number of seeds per plant for cross between ITOOK-1263 x Tumaini (Table 9). However genotypes were not significantly different ( $P \leq 0.05$ ) for hundred seed weight and pod length (Table 9). For cross between ITOOK-1263 x X-Ilonga genotypes were significantly different ( $P \leq 0.05$ ) for hundred seed weight, number of pods per plant and number of seeds per plant (Table 10). However genotypes were not significantly different ( $P \leq 0.05$ ) for pod length (Table 10). The interaction between moisture regimes and genotypes were not significant ( $P \leq 0.05$ ) for all variables analyzed in cross between ITOOK-1263 x Tumaini (Table 9) and ITOOK-1263 x X-Ilonga (Table 10).

The following lines ITK-TMA2, ITK-TMA5, ITK-TMA11, ITK-TMA12, ITK-TMA14 and ITK-TMA16 produced more number of pods per plant and number of seeds per plant than other lines in cross between ITOOK-1263 x Tumaini (Table 11). The number of pods per plant produced were between 10 and 12 while the number of seeds per plant were between 120 and 129 (Table 11). For a cross between ITOOK-1263 x X-Ilonga the following lines ITK-XL2, ITK-XL3, ITK-XL6, ITK-XL14 and ITK-XL16 produce more pods per plant and seeds per plant than other lines (Table 12). The number of pods per plant was between 10 and 12 while the number of seeds per plant was between 130 and 155 (Table 12). The highest mean numbers of seed per plant were 129 for progenies from ITOOK-1263 vs. Tumaini (Table 1) and 153 for progenies originating from ITOOK-1263 vs. X-Ilonga (Table 2).

**Table 11: Mean performance of cowpea genotypes for days to 50% flowering, days to maturity, yield and yield components for cross between ITOOK-1263 and Tumaini**

Genotype	Trait						
	DT50%F	DTM	100SWT	NPP	PODL	NSPOD	NS/PL
ITK-TMA1	48.67c	63.33c	8.87b	8.67bc	10.44b	10.11b	103.44b
ITK-TMA2	56.33abc	73.11abc	10.23b	10.11bc	13.11ab	12.33ab	128.89b
ITK-TMA3	49.78c	65.00bc	7.94b	9.11bc	10.67b	9.89b	107.22b
ITK-TMA4	46.11c	60.66c	8.04b	8.33bc	10.84b	10.33b	100.89b
ITK-TMA5	49.11c	65.89bc	10.00b	9.78bc	12.42ab	11.67ab	122.00b
ITK-TMA6	50.67bc	65.22bc	9.18b	8.56bc	10.89b	10.78b	113.89b
ITK-TMA7	48.11c	62.67c	8.41b	8.89bc	11.01b	10.00b	107.44b
ITK-TMA8	48.33c	63.00c	7.40b	8.87bc	10.89b	10.33b	107.78b
ITK-TMA9	50.78bc	65.56bc	7.41b	8.11bc	10.44b	10.44b	104.11b
ITK-TMA10	44.33c	57.44c	6.54b	7.56c	9.33b	8.67b	92.22b
ITK-TMA11	55.89abc	73.00abc	8.13b	10.11bc	12.33ab	11.78ab	122.33b
ITK-TMA12	57.56abc	74.78abc	7.93b	10.22bc	12.22ab	11.44b	121.22b
ITK-TMA13	40.11c	52.33c	7.16b	7.56c	9.33b	8.89b	91.11b
ITK-TM14	51.78bc	68.56abc	8.10b	10.78bc	12.33ab	11.11b	123.67b
ITK-TMA15	49.11c	63.67c	7.1b	9.56bc	11.00b	10.22b	113.11b
ITK-TMA16	54.89abc	72.11abc	8.4b	10.44bc	12.33ab	11.67ab	119.78b
ITOOK	75.11ab	96.11ab	14.36a	16.0a	16.17a	16.11a	201.67a
Tumaini	79.44a	98.22a	10.07b	11.78b	12.78ab	11.11b	132.78b

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test at three moisture regimes.

Note: DTF-Days to 50% flowering, DTM-Days to maturity, 100SWT-Hundred seed weight, NPP-Number of pods per plant, PODL-Pod Length, NSPOD-Number of seeds per pod, NS/PL- Number of seeds per plant

**Table 12: Mean performance of cowpea genotypes for days to 50% flowering, days to maturity, yield and yield components for cross between ITOOK-1263 and X-Ilonga**

Genotype	Trait						
	DT50%F	DTM	100SWT	NPP	PODL	NSPOD	NS/PL
ITK-XL1	43.89abcd	56.78cd	7.9bcd	7.89bc	10.44b	10.00bc	111.89bc
ITK-XL2	56.22abcd	73.44abcd	10.41bc	11.33b	13.44ab	12.11abc	145.44bc
ITK-XL3	66.22abc	86.56abc	10.46bc	11.33b	14.11ab	13.22abc	146.44bc
ITK-XL4	54.89abcd	69.89abcd	7.92bcd	8.89bc	11.11b	10.56bc	116.11bc
ITK-XL5	60.78abcd	78.11abcd	9.6bcd	9.78bc	12.89ab	12.22abc	128.11bc
ITK-XL6	57.78abcd	74.89abcd	9.45bcd	10.00bc	12.17ab	11.56bc	130.11bc
ITK-XL7	54.44abcd	69.22abcd	7.7bcd	8.56bc	11.44b	10.44bc	112.56bc
ITK-XL8	59.11abcd	76.11abcd	9.2bcd	9.89bc	13.11ab	11.89abc	129.33bc
ITK-XL9	37.44d	50.11d	6.64d	7.44c	10.11b	9.33c	100.56c
ITK-XL10	61.89abc	78.11abcd	9.56bcd	9.78bc	12.56ab	12.00abc	125.44bc
ITK-XL11	48.67cd	63.44bcd	8.94bcd	8.22bc	11.22b	10.22bc	108.33bc
ITK-XL12	50.11cd	65.00bcd	7.20cd	8.33bc	11.56b	10.67bc	109.56bc
ITK-XL13	50.67bcd	65.56bcd	7.40cd	8.56bc	11.44b	10.56bc	109.33bc
ITK-XL14	66.78abc	86.56ab	11.00ab	11.11b	14.11ab	13.00abc	138.44bc
ITK-XL15	55.67abcd	73.44abcd	9.41bcd	9.56bc	12.00ab	12.56abc	124.78bc
ITK-XL16	67.11abc	86.67ab	9.73bcd	11.11b	14.11ab	13.89ab	153.22ab
ITOOK	74.44ab	96.22a	14.13a	15.44a	16.33a	16.11a	196.00a
X-Ilonga	78.22a	97.89a	9.38bcd	11.89bc	12.00ab	11.11bc	126.44bc

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test at three moisture regimes.

Note: DT50%F-Days to 50% flowering, DTM-Days to maturity, 100SWT-Hundred seed weight, NPP-Number of pods per plant, PODL-Pod Length, NSPOD-Number of seeds per pod, NS/PL- Number of seeds per plant

The effects of moisture stress for days to 50% flowering and days to maturity among segregating genotypes were observed in this study. There were significantly different ( $P \leq 0.05$ ) for moisture regimes for days to 50% flowering and days to maturity for cross between ITOOK-1263 x Tumaini (Table 9) and ITOOK-1263 x

X-Ilonga (Table 10). Genotypes and interaction between moisture regimes and genotypes were not significantly different ( $P \leq 0.05$ ) for both crosses (Table 9) and (Table 10) respectively. The following lines ITK-TMA1, ITK-TMA8, ITK-TMA7, ITK-TMA4, ITK-TMA10 and ITK-TMA13 for cross between ITOOK-1263 x Tumaini flowered early among all genotypes under investigation (Table 11). The same lines also matured early. Early flowering are those lines in which flowering occurred between 40 and 49 days (Table 11) while early maturing lines took between 52 and 64 days to mature (Table 11). While for cross between ITOOK-1263 x X-Ilonga, genotypes ITK-XL1, ITK-XL9 and ITK-XL11 were the genotypes that had both early flowering and maturity as well among all genotypes observed for this cross (Table 12). Early flowering occurred between 37 and 49 days while early maturity took between 50 and 64 days (Table 12).

#### **4.5 Principal Component Analysis (PCA)**

##### **4.5.1 Principal Component Analysis (PCA) for cross between ITOOK-1263 x Tumaini**

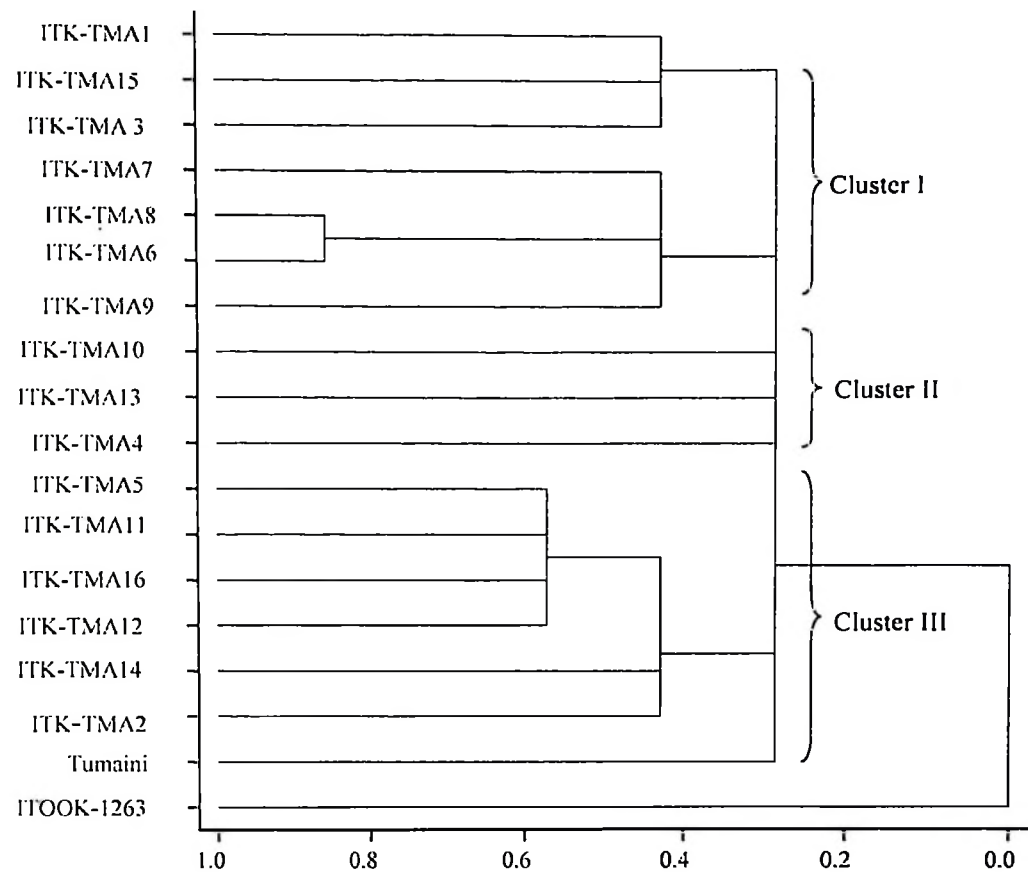
The results of the Principal Component Analysis for a cross between ITOOK-1263 x Tumaini for 18 cowpea genotypes are presented in Table 13. The first two components contributed 99.65 of the variability among 18 cowpea genotypes evaluated. The PC1 and PC2 accounted for 93.85 and 5.80 of the total variation respectively. The contribution of the traits towards the diversity of the cowpea genotypes revealed that in the first principal component axis, the traits with the highest were number of seeds per plant, days to maturity and days to 50% flowering.

The second principal component axis weighed highest in days to maturity, number of seeds per plant and days to 50% flowering.

**Table 13: The eigenvector for principal component analysis for 18 cowpea genotypes for a cross between ITOOK-1263 x Tumaini**

<b>Traits</b>	<b>PC1</b>	<b>PC2</b>
Days to 50% flowering	-0.33777	0.54280
Days to maturity	-0.43486	0.63235
Hundred seed weight	-0.05078	-0.03371
Number of pods/plant	-0.06817	-0.00155
Pod length	-0.06176	-0.00422
Number of seed per pod	-0.05986	-0.02936
Number of seed per plant	-0.82595	-0.55089
Percentage variation	93.85	5.8

On the basis of Multivariate cluster analysis classifying 18 cowpea genotypes 9 groups has been suggested for a cross between ITOOK-1263 x Tumaini. Indeed there is not any standard procedure to determine the final number of cluster exists (Johnson, 1998) instead many criteria and guideline have been developed. For this reason the set of varieties run for different number of clusters. The dendogram produced by cluster analysis grouped the genotypes with the days to maturity and days to flowering in the same clusters (ITK-TMA4, ITK-TMA10 and ITK-TMA13) in cluster II (figure 1). These lines have shorter days to 50% flowering and days to maturity. Genotypes were grouped into three clusters (Table 14). Lines ITK-TMA2 and ITK-TMA14 grouped in cluster III produced high number of seeds per plant (figure 1). Genotypes in this cross were grouped in three clusters (Table 14).



**Figure 1: Dendrogram cluster analysis for 18 cowpea genotypes based on yield traits for cross between ITOOK-1263 x Tumaini**

**Table 14: Cluster distribution of 18 cowpea genotypes based on 7 yield traits**

Cluster	Number of genotypes	Name of genotypes
Cluster I	7	ITK-TMA1, ITK-TMA15, ITK-TMA3, ITK-TMA7, ITK-TMA9, ITK-TMA6, ITK-TMA8
Cluster II	3	ITK-TMA10, ITK-TMA13, ITK-TMA4
Cluster III	7	ITK-TMA14, ITK-TMA2, ITK-TMA5, ITK-TMA11, ITK-TMA16, ITK-TMA12, Tumaini

#### 4.5.2 Principal Component Analysis (PCA) for cross between ITOOK-1263 x X-Ilonga

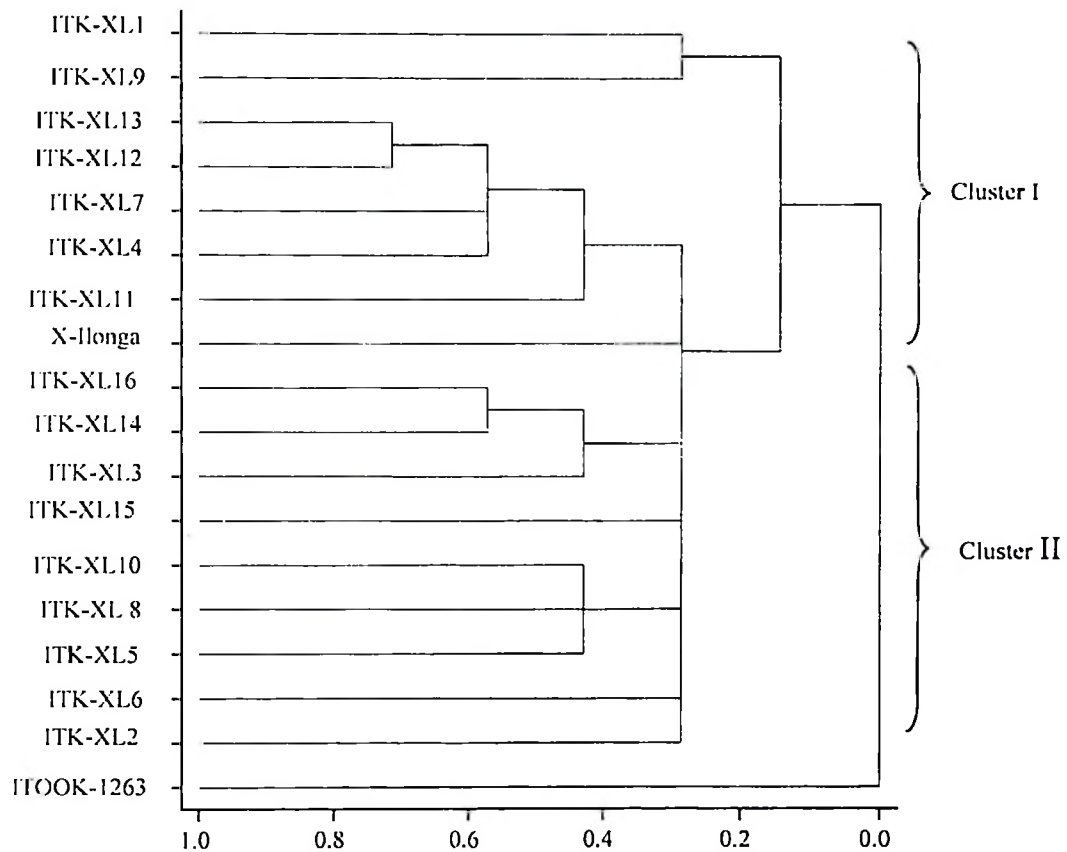
The results of the Principal Component Analysis for a cross between ITOOK-1263 x X-Ilonga for 18 cowpea genotypes are presented in Table 15. The first two components contributed 99.78 of the variability among 18 cowpea genotypes evaluated. The PC1 and PC2 accounted for 89.75 and 10.03 of the total variation respectively. The contribution of the traits towards the diversity of the cowpea accessions revealed that in the first principal component axis, the traits with the highest loadings were number of seeds per plant, days to maturity and days to 50% flowering. The second principal component axis weighed highest in days to maturity, days to 50% flowering and number of seeds per plant.

**Table 15: The eigenvector for principal component analysis for 18 cowpea genotypes for a cross between ITOOK-1263 x X-Ilonga.**

Traits	PC1	PC2
Days to flowering	-0.33552	0.56053
Days to maturity	-0.42538	0.62588
Hundred seed weight	-0.06064	-0.02272
Number of pods/plant	-0.06884	-0.01675
Pod length	-0.05494	-0.02254
Number of seed per pod	-0.06288	-0.01344
Number of seed per plant	-0.83132	-0.54093
Percentage variation	89.75	10.03

On the basis of Multivariate cluster analysis classifying 18 cowpea genotypes 8 groups has been suggested for a cross between ITOOK-1263 x X-Ilonga. The dendrogram produced by cluster analysis grouped the genotypes with the days to maturity and days to 50% flowering in the same clusters (ITK-XL1 and ITK-XL9) (figure 2). These lines have shorter days to 50% flowering and days to maturity.

Lines ITK-XL2, ITK-XL3 and ITK-XL16 produced high number of seeds per plant and grouped in cluster II (figure 2). Genotypes in this cross were grouped into two clusters (Table 16).



**Figure 2: Dendrogram cluster analysis for 18 cowpea genotypes based on yield traits for cross between ITOOK-1263 x X-Ilonga.**

**Table 16: Cluster distribution of 18 cowpea genotypes based on 7 yield traits**

Cluster	Number of genotypes	Name of genotypes
Cluster I	8	ITK-XL1, ITK-XL9, ITK-XL13, ITK-XL12, ITK-XL7, ITK-XL4, ITK-XL11, X-Ilonga
Cluster II	9	ITK-XL3, ITK-XL14, ITK-XL16, ITK-XL15, ITK-XL10, ITK-XL8, ITK-XL5, ITK-XL6, ITK-XL2

## CHAPTER FIVE

### 5.0 DISCUSSIONS

#### 5.1 Identification of Drought Tolerant Lines

Results from this study confirmed that despite its inherent and comparative tolerance to drought cowpea exhibits significant variation in response to drought stress. Evidence of water stress on plants was first observed among some cowpea lines sown in the water stressed boxes from about two weeks after suspension of irrigation. In some other accessions wilting became apparent in a few weeks or several days later. Only few lines demonstrated good survival under water stress conditions after withholding water for four weeks. Some of the cowpeas lines responded differently as the onset of drought continued. Few lines flowered early and produced viable seeds. This mechanism was commonly adopted in cowpea genotypes under moisture deficit as drought escape.

Some of the lines that were more sensitive to water stress started to show yellowing of lower leaves within two weeks of their being exposed to drought. The leaves of most of the cowpea lines in the water stressed boxes were paraheliotropic and orientated in a parallel position to the sun's rays a situation commonly observed in cowpea leaves in order to reduce evapo transpiration (Shackel & Hall 1979). The leaves are generally cooler when they assume this position. This was a drought avoidance mechanisms adopted by some of the cowpea plants for water conservation during the water stressed period. Some of the lines withstood the period of drought stress and recovered from apical meristem after re-introduction of water. The ability of plants to withstand drought stress and recover after watering was the mechanism

of drought tolerance. This observation was similar to those reported by Muchero *et al.* (2008), who observed and confirmed the existence of significant genetic variation in response to drought stress in 14 genotypes at seedling stage. The variation was due to the ability to withstand drought stress and recover and rapid loss of unifoliates. The drought tolerant genotypes recovered and maintained growth from the apical meristem while the intermediate genotypes lost apical meristem viability and tended to put out recovery growth from basal meristem. The susceptible genotypes showed neither apical nor basal meristem recovery growth from drought stress. The recovery of cowpea plants was due to leaf expansion and most probably due to an increase in water uptake. Plant recovery from water stress after re-watering is an important aspect of drought tolerance (Huang *et al.*, 2007).

The drought tolerances observed under this study are similarly to those reported by Mai-Kodomi *et al.* (1999a). In his observation he reported two types of drought tolerance. In this study lines that were stressed at flowering stage resulted in senescence, having high percentage of flower abortion and as a result only few flowers formed pods and produced few pods after re-watering at pod filling stage most of them did not recover. Maiti *et al.* (2006) reported that water stress has a severe effect during flower initiation, flowering, fruit and seed development, but less during the vegetative growth stages. Winkel *et al.* (2007) reported water stress prior to flowering and at the beginning of flowering time delay or totally inhibited the flowering of tillers in pearl millet. Upendra *et al.* (2008) reported water stress at flowering delayed tassel initiation and pollination in maize and other crops. By inducing drought stress at the flowering stage some difficulties in scoring was

experienced in some lines to distinguish senescence from the effect of drought. This difficult of scoring at flowering stage was earlier reported by Magloire (2005). In other studies on drought tolerance in cowpea, Watanabe *et al.* (1997) found some germplasm lines with better levels of drought tolerance. The genetic variation reported was due to few numbers of leaves, shorter stem, shedding-off leaves and leaf rolling. These enabled kept plants to respire less and avoid water loss and therefore survived much longer in moisture deficit. Fatokun *et al.* (2009) also reported a variation for drought tolerance among 142 cowpea genotypes lines studied. It was observed that some genotypes were able to flower and produce one pod with seeds per plant before drying up. This early flowering in cowpea was construed as a mechanism for drought escape. In breeding cowpea for drought tolerance genotypes that had early flowering and maturity are important because they can escape the period of drought.

These observations suggest that it is possible to screen and select cowpea genotypes with better levels of drought tolerance at seedling stage and flowering stage. The lines having resistance to drought can be utilized in breeding programme for developing drought resistant lines after testing in field conditions.

## **5.2 Estimation of Heritability of Drought Tolerance among Segregating Cowpea Genotypes**

### **5.2.1 Days to 50% flowering**

Estimates of broadsense heritability of days to 50% flowering in lines originating from a cross between IT00K-1263 x Tumaini were found to high i.e. 87.22%. For

lines of cross between ITOOK-1263 x X-Ilonga Heritability were found to be high 74.05%. Fery and Singh (1997) showed high broadsense heritability estimates of 75% for days to 50% flowering. These results are not in agreement with those found by Agbicodo *et al.* (2009) who reported broadsense heritability of 32% and 26% for days to flowering. When traits or characters are highly heritable in the broadsense (i.e. more than 70%), it means that such trait or characters could be selected for in the early generation assuming there is additive gene action.

Breeding for phenological traits such as early flowering has been very successful and a number of improved cowpea varieties (like Bambey 21 and Vuli1) (Muchero *et al.*, 2008) which can reach maturity at 60 days after planting have been released. Lines such as these can escape the effects of terminal drought and still produce appreciable grain yield. Hence positive association between reduction of days to flowering and grain yield is desirable. In this study lines that are early flowering produced fewer pods and seeds.

### **5.2.2 Days to maturity**

Broadsense heritability for days to maturity for crosses between ITOOK-1263 x Tumaini were high with 92.02 % while for lines of cross between ITOOK-1263 x X-Ilonga Heritability were high 82.06 %. Fery and Singh (1997) showed high broadsense heritability estimates of 79% for pod maturity. Apte *et al.* (1987) reported broadsense heritability estimates of 34.9% (low). Faisal and Suliman (2010) reported a broadsense heritability of 78.3% for days to maturity. The high broadsense heritability estimate in this study may be due to a small population used

under investigation. The implication of high  $H_b$  estimates for days to maturity suggests that, singular selection for trait in an early generation is possible.

### **5.2.3 Hundred (100) seed weight**

Broadsense heritability estimates for hundred seed weight were mostly found to be high in this study in crosses between ITOOK-1263 x Tumaini with 97.73% and for ITOOK-1263 x X-Ilonga were high with 87.57%. In similar observations, Damarany (1994) reported 83.3% while 96% was obtained by Ajibade and Morakinyo (2007). The high broadsense heritability estimates for this study suggests that priority while selecting genotypes for yield improvement in cowpea. Traits with relatively medium to high heritability or additive gene variance have been reported to respond highly to selection and cross breeding.

### **5.2.4 Number of pods per plant**

Estimates of broadsense heritability for number of pods per plant were found to be high in crosses between ITOOK-1263 x Tumaini at 89.00% while in a crosses between ITOOK-1263 x X-Ilonga were found to be high at 94.83%. These results are in agreement with earlier studies by Agbicodo *et al.* (2009) which showed a broadsense heritability estimates for the number of pods per plant of 79% in Ibadan; however moderate heritability estimates of 51% and 63% were reported. Agbicodo *et al.* (2009) concluded that number of pods per plant is genetically the most stable component among the traits evaluated in optimum and drought conditions. Fery (1985) confirmed that variation in number of pods per plant among varieties was due to genetic factors, and estimated that a heritability of 53.1 percent accounted for the observed differences in the varieties used.

Number of pods per plant is the most stable genetic component and a key selection criterion to determine pod yield under optimum and drought conditions. The high  $H_b$  for number of pods per plant in this study suggests that it is a genetically most stable component for selection under drought conditions. High number of pods per plant is an indication of high yield so number of pods per plant has been used in estimating heritability for yield component.

#### **5.2.5 Pod length**

Heritability estimates for pod length in cross between ITOOK-1263 x Tumaini were found to be high at 95.93% while in a cross between and ITOOK-1263 x X-Ilonga Heritability were found to be high 97.32%. Fery (1985) showed that pod length was highly heritable with average heritability estimate of 75.2 percent. Chiulele (2003) also reported similar findings with heritability estimate of 70.2 %. A broadsense heritability of 71.0% and 66.0% was reported by Uguru (2004). When characters or traits are highly heritable (i.e. more than 80%) it means that such traits or characters could be selected for in the early generation assuming there is significant additive gene action.

#### **5.2.6 Number of seeds per pod**

Broadsense heritability for the number of seeds per plant in crosses between ITOOK-1263 x Tumaini and ITOK-1263 x X-Ilonga were found to be high at 95.33% and 93.17% respectively. These results are not in agreement with Agbicodo *et al.* (2009) who reported low heritability estimates for the number of seeds per pod of 33%, 37% and 29%. Chiulele (2003) reported 83.0% (high) heritability estimates for the number of seeds per pod. Similar observation was made by Fery (1985) who

reported a moderate to high heritability estimates of up to 75.2%. Abayomi and Abidoeye (2004) also reported high broadsense heritability of number of seeds per pod of 69.5%. Traits with high heritability or additive gene variance have been reported to respond highly to selection and cross breeding (Falconer and Mackay, 2008), and that early selection for this trait would be effective in crop improvement. High heritability estimate implies that effective selection in early generation is possible.

#### **5.2.7 Number of seeds per plant**

Broadsense heritability estimates for the number of seeds per plant in crosses between ITOOK-1263 x Tumaini were high 85.11% as well as in a crosses between ITOOK-1263 x X-Ilonga with an estimated value of 88.1%. These results are in agreement with those found by Chiulele (2003) with broadsense heritability of 81.2% and 72.6%. Similar results were reported by Agbicodo *et al.* (2009). On other hand Uguru (2004) reported a broadsense heritability of only 49% and 56% for number of seeds. Number of seeds per plant is one of the yield components. Plants that have higher number of seeds per plant in moisture stress with a high broad sense heritability estimate can be suitable for selection in breeding programme.

#### **5.2.8 Seed yield per plant (seed weight)**

Estimates for broad sense heritability for seed weight were high for cross between ITOOK-1263 x Tumaini with an estimated value of 83.93% while in a cross between ITOOK-1263 x X-Ilonga the values were low with 24.41%. In a similar study Agbicodo *et al.* (2009) reported a high heritability estimate of seed weight 74% and moderate estimate of 66%. Traits that have a high heritability are suitable

for selection in early generation in breeding programme. The low heritability for seed weight from crosses between ITOOK-1263 x X-Ilonga shows that non-genetic factors play a greater role in the overall performance. This suggests that, single plant in early generation would not be effective as it is difficult to select genetically superior individuals so it is suggested that selection for this trait should be done in later generations.

### **5.3 Morphological Descriptors for Cowpea under Moisture Deficit.**

#### **5.3.1 Leaf senescence**

Few cowpea lines in the water stressed boxes flowered late or produced no pods. These plants however remained green for much longer time. This characteristic of cowpea plants retaining their leaves in green state when water stressed for such length of time has previously been reported by Hall *et al.* (1997). Gwathmey & Hall (1992) referred to this trait as delayed leaf senescence (DLS). Their study revealed that plants which, exhibit this trait have capacity to survive mid season drought caused by intermittent rainfall and are able to flower and set pod when rains stabilize later. They also reported that plants with DLS showed 10 to 14 percent higher water use efficiency than non-DLS lines. In their DLS lines flowering and pod formation continued for a longer when exposed to drought. In this study plants differed in response to Delayed leaf senescence. Some genotypes have managed to survive much longer than others. In other studies it was shown that cowpea genotypes exhibiting seedling drought tolerance were more tolerant to terminal drought under field conditions than genotypes exhibiting seedling sensitivity to drought (Singh *et al.*, 1999a; 1999b; Muchero *et al.*, 2008; 2009b).

A similar trait coined “stay-green” has been reported in sorghum (*Sorghum bicolor* L. Moench) with post-flowering drought tolerance mediated by the “stay green” trait (Subudhi *et al.*, 2000). Stay-green is a drought tolerance mechanism exhibited in some sorghum genotypes subjected to post-flowering drought stress. The trait allows tolerant genotypes to maintain green leaf area during the grain-filling stage, thereby allowing more productivity (Crasta *et al.*, 1999). However, it is not clear yet whether the stay-green trait in sorghum, which is a post-flowering phenomenon and the delayed drought-induced leaf and stem senescence traits observed in cowpea at the seedling stage are regulated by similar mechanisms.

The prolonged lifespan which was observed on DLS plants would also add to the plants ability to tolerate better a terminal drought. In sorghum, genotypes with delayed senescence (stay green) remain physiologically active during the late stages of grain filling and this enhances the plants stress tolerance through increasing assimilate supply for grain filling and maintaining root function or moisture uptake or even both (Van Oosterom *et al.*, 1996). Rosenow *et al.* (1983) have suggested DLS as an indirect selection criterion in breeding for post-flowering drought tolerance in crops.

### **5.3.2 Stem greenness**

Stem greenness is an indicator for drought tolerance. Muchero *et al.* (2008) and Agbicodo *et al.* (2009) emphasized that stem greenness, survival and recovery dry weights were useful traits to screen cowpea genotypes for their ability to withstand drought stress at the seedling stage. Stem greenness was an excellent predictor of

seedling survival to drought. In this study lines that received water throughout the cropping season were greener than those which were stressed at seedling and flowering stages. Lines that were stressed at flowering stage kept their stem green during irrigation period and once water was withheld they started changing in stem color from green to completely yellow and some of them dried and died. For those lines that were stressed at seedling stage the stem color changes started two weeks after withholding water from slightly yellow to moderate and completely yellow dried and dead for some lines. But most of them recovered after re-watering and continue with normal growth.

### **5.3.3 Leaf area**

The results indicate that leaf area was much reduced under moisture stress. The reduction in leaf area observed under this study might be associated with decreased leaf growth and or increased leaf senescence and could be one of drought avoidance mechanisms. Similarly, reduction in leaf production and or increase in leaf senescence and abscission due to water stress have been reported (Abayomi and Abidoye, 2004). Leaf area for lines that were stressed at seedling stage was low as compared to others. Water stress during the vegetative stage was reported to reduce leaf area (Gardner *et al.*, 2005, Turk and Hall, 1980b, Winkel *et al.*, 2007). The reduction in leaf area by water stress was found to be the result of the reduction in the leaf expansion rate due to the sensitivity of cell enlargement to water stress. Other workers have also shown that water deficit during the vegetative phase causes leaf and plant growth reductions (Kerbaui, 2004). This is in agreement with Hilel *et al.* (1972) who observed that leaf area in cowpea was decrease by water stress.

These results imply that moisture stress has a significant effect on leaf expansion and growth. Decrease in leaf area consequently resulted from decreased leaf production and or increase in leaf senescence and abscission due to moisture deficit.

#### **5.3.4 Number of leaves**

The number of leaves was the most important parameter that affected leaf area. Cowpea lines that were stressed at seedling stage had low number of leaves as compared to others, and shed off most of leaves in order to reduce evapotranspiration. Very few, and for some lines no new emerged leaves was noted in this study. Plants tend to reduce the number of leaves under moisture deficit conditions. Abidoye (2004) reported significantly leaf production in cowpea genotypes when grown under moisture deficit. The reduction in the number of leaves was due to reduce loss of water (Sullivan and Nguyen, 1997). Other workers have also shown that water deficit at vegetative (seedling) stage causes leaf and plant growth reduction and abscission. The other growth stage are not important in number of new leaves emerged as they were able to produce more and more new leaves when they received water. However, it has been noted that withholding moisture at flowering stage cowpea cause shedding off of most of leaves.

#### **5.4 Effects of Moisture Stress on Yield and Yield Components, Days to Flowering and Days to Maturity**

Most of the yield parameters were affected by moisture stress. Water stress resulted in greater reduction in the number of pods per plant, number of seeds per plant and seed yield per plant. These results imply that under moisture stress cowpea plants

reduce the number of pods per plant while it maintained size of seeds and number of seeds per pod and pod length. Hamidou *et al.* (2007) also reported significant depressive water deficit effect on yield components. Hall and Patel (1985) observed that cowpea plants under high moisture regimes produced more pods per plant than those under deficient moisture. Ndunguru *et al.* (1995) also showed that limited moisture supply reduced number of pods per plant in groundnut.

The results indicated that some cowpea genotypes flowered and matured early. Earliness in maturing of cowpea is desirable in dry areas where farmers experience irregular and unreliable rainfall during cropping season. Drought occurs shortly after planting for some weeks. It may also occur mid season when plants have yet to flower or during the reproductive phase. Early maturity cowpea varieties (like IT84S-2246 and Bambey 21) that escape terminal drought have been released and widely adopted by African farmers (Singh *et al.*, 1997). Fatokun *et al.* (2009) reported early maturing lines Sanzi and IT88D-345 which are escapes were able to flower and produce seeds.

### **5.5 Principal Component Analysis (PCA)**

The main reason for plant collection is to obtain natural variability that can be useful for providing germplasm pools for crop improvement. The PCA is perhaps the most useful statistical tool for screening multivariate data (Johnson, 1998). Information obtained through principal component analysis (PCA) may assist the plant breeders to indentify a limited number of highly differentiated populations for use in hybridization and selection programs. From the principal component analysis results conducted in this study the most effective characters for distinguishing

among the cowpea genotypes included days to flowering, days to maturity and number of seeds per plant in both in cross between ITOOK-1263 x Tumaini and ITOOK-1263 x X-Ilonga. All these yield traits weighed high towards total divergence among the cowpea genotypes. The variability of some cowpea cultivars have been described based on the aforementioned traits (Ubi *et al.*, 2001; Ishiyaku *et al.*, 2005; Omoigui *et al.*, 2006). Using the PCA some of these characters have also been used extensively to characterize Bambara groundnut (Oyiga *et al.*, 2010) and *Cucurbita* (Aruah *et al.*, 2010) accessions in Nigeria. The hierarchical cluster analysis conducted on the yield traits grouped the genotypes into two clusters, indicating sufficient variability to warrant selection.

The cluster means shows that cluster II comprised of early flowering cowpea genotypes that recorded low yield of all other yield traits assessed for cross between ITOOK-1263 x Tumaini. Conversely, the cowpea genotypes in cluster III were essentially late flowering but prolific in the production of number of pods per plant, hundred seed weight, pod length and number of seeds per pod. For cross between ITOOK-1263 x X-Ilonga cluster means shows that cluster I comprised of early flowering and mature cowpea genotypes that recorded low yield of other yield traits under investigation, while cluster II comprised of cowpea genotypes that were late flowering and mature but excellent in the production of pods per plant, hundred seed weight, pod length and number of seeds per pod. The greater diversity in the present materials will offer a good scope for cowpea improvement programmes of the yield traits.

## CHAPTER SIX

### 6.0 CONCLUSIONS AND RECOMMENDATION

#### 6.1 Conclusions

From the results in this study, differences have been observed for drought tolerance under different moisture regime. The box screening method has provided a simplified approach to the study of drought tolerance in cowpea and may lead to faster progress in breeding for drought tolerance in other crops.

The genetic variation observed among the segregating cowpea lines for drought tolerance in this study confirmed that cowpea can withstand the period of drought stress by different mechanisms. i.e. drought escape, drought avoidance and drought tolerance.

For future cowpea breeding programmes for drought tolerance the following lines should be selected ITK-TMA4, ITK-TMA10, ITK-TMA13, ITK-XL1 and ITK-XL9. These lines flowered and matured early, very important characteristics in breeding for drought tolerance. Lines ITK-TMA2 and ITK-TMA14, ITK-XL2, ITK-XL3 and ITK-XL16 should also be selected; they produced high number of seeds per plant.

Heritability for most of the trait in this study was high. This shows that selection in early generation is effective. It is possible to select genetically superior individuals.

Moisture stress during vegetative stage has more effect than moisture stress at flowering stage for morphological characterization under moisture deficit.

Using principal component analysis the most effective trait responsible for variability among the cowpea genotypes in this study are days to 50% flowering, days to maturity and number of seeds per plant.

## **6.2 Recommendation**

Further studies on drought tolerance lines generated in this study, particularly: advancing up to F6 and evaluation of other abiotic and biotic factors. The use of molecular markers or identification of genes associated with seedling drought tolerance for use in marker-assisted selection should be done for breeding for drought tolerance.

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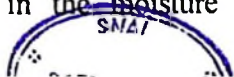
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## APPENDICES

## Appendix 1: Analysis of Variance for Cross ITOOK-1263 and Tumaini

## ANOVA TABLE FOR DAYS TO FLOWERING

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	1 882.56	941.28		
Moisture	2	7 702.98	3 851.49	5.42*	0.0058
Genotype	17	14 729.96	866.47	1.22	0.2630
Moisture x Genotype	34	18 064.34	531.30	0.75	0.8331
Errors	106	75 367.44	711.01		
Total	161	118 070.77			

\* Significant at P value  $\leq 0.05$ 

## ANOVA TABLE FOR DAYS TO MATURITY

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	2 753.39	1 376.69		
Moisture	2	13 364.12	6 682.06	5.83*	0.0040
Genotype	17	21 016.88	1 236.29	1.08	0.3846
Moisture x Genotype	34	28 942.91	851.26	0.74	0.8387
Errors	106	121 565.94	1 146.85		
Total	161	188 097.96			

\* Significant at P value  $\leq 0.05$ 

## ANOVA TABLE FOR 100 SEED WEIGHT

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	30.18	15.09		
Moisture	2	447.02	223.51	12.26***	<0.0001
Genotype	17	478.36	28.14	1.54	0.0936
Moisture x Genotype	34	610.39	17.95	0.98	0.5028
Errors	106	1 932.56	18.23		
Total	161	3 492.30			

\*\*\* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR POD LENGTH**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	34.76	17.38		
Moisture	2	970.28	485.14	16.88***	<.0001
Genotype	17	389.89	22.93	0.80	0.6919
Moisture x Genotype	34	645.17	18.97	0.66	0.9164
Errors	106	3 046.41	28.74		
Total	161	5 090.95			

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF SEEDS PER POD**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	64.35	32.17		
Moisture	2	1 025.37	512.68	21.52***	<.0001
Genotype	17	399.26	23.48	0.99	0.4795
Moisture x Genotype	34	614.72	18.08	0.76	0.8195
Errors	106	2 524.98	23.82		
Total	161	4 635.38			

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF PODS PER PLANT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	27.37	13.68		
Moisture	2	751.75	375.88	21.31***	<.0001
Genotype	17	579.59	34.09	1.93*	0.0225
Moisture x Genotype	34	518.39	15.25	0.86	0.6796
Errors	106	1 869.97	17.64		
Total	161	3 747.31			

\* Significant at P value  $\leq 0.05$

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF SEEDS PER PLANT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	12 602.92	6 301.46		
Moisture	2	172 855.47	86 427.74	34.93***	<.0001
Genotype	17	88 827.10	5 225.12	2.11*	0.0112
Moisture x Genotype	34	69 322.51	2 038.89	0.82	0.7363
Errors	106	262 271.75	2 474.26		
Total	161	605 931.46			

\* Significant at P value  $\leq 0.05$ ,

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR SEED YIELD PER PLANT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	102.76	51.38		
Moisture	2	1 634.98	817.49	29.10***	<.0001
Genotype	17	888.31	52.25	1.86*	0.0297
Moisture x Genotype	34	865.19	25.44	0.91	0.6188
Errors	106	2 977.71	28.09		
Total	161	6 467.62			

\* Significant at P value  $\leq 0.05$

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR LEAF SENESCENCE**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	31.81	15.91		
Moisture	2	617.92	308.96	131.25***	<.0001
Genotype	17	42.89	2.52	1.07	0.3907
Moisture x Genotype	34	81.85	2.41	1.02	0.4490
Errors	106	249.52	2.35		
Total	161	1 024.00			

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR STEM GREENNESS**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	7.94	3.97		
Moisture	2	186.01	93.01	50.28***	<.0001
Genotype	15	25.59	1.50	0.81	0.6742
Moisture x Genotype	30	51.54	1.51	0.82	0.7423
Errors	94	196.06	1.84		
Total	143	467.14			

\* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR LEAF AREA**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	3 651.33	1 825.67		
Moisture	2	211 266.02	105 633.01	34.24***	<.0001
Genotype	17	99 424.92	5 848.52	1.90*	0.0259
Moisture x Genotype	34	94 584.51	2 781.89	0.90	0.6248
Errors	106	326 982.68	3 084.74		
Total	161	735 909.46			

\* Significant at P value  $\leq 0.05$

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF LEAVES EMERGED**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	43.11	21.56		
Moisture	2	17 936.33	8 968.17	186.44***	<.0001
Genotype	17	1 982.00	116.59	2.42*	0.032
Moisture x Genotype	34	2 408.11	70.83	1.47	0.0702
Errors	106	5 098.89	48.10		
Total	161	27 468.44			

\* Significant at P value  $\leq 0.05$

\* \* \* Significant at P value  $\leq 0.01$

**Appendix 2: Analysis of Variance Table for Cross ITOOK-1263 and X-Ilonga****ANOVA TABLE FOR DAYS TO FLOWERING**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	721.33	360.67		
Moisture	2	4 712.70	2 356.35	3.61*	0.0304
Genotype	17	16 081.61	945.97	1.45	0.1286
Moisture x Genotype	34	21 879.29	643.51	0.99	0.5010
Errors	106	69 184.00	652.68		
Total	161	112 578.94			

\* Significant at P value  $\leq 0.05$ **ANOVA TABLE FOR DAYS TO MATURITY**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	1 009.33	504.67		
Moisture	2	8 438.26	4 219.13	4.21*	0.0174
Genotype	17	24 426.28	1 436.84	1.43	0.1356
Moisture x Genotype	34	34 278.63	1 008.19	1.01	0.4728
Errors	106	106 262.00	1 002.47		
Total	161	174 414.50			

\* Significant at P value  $\leq 0.05$ **ANOVA TABLE FOR 100 SEED WEIGHT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	90.79	45.39		
Moisture	2	238.07	119.04	8.44***	0.0004
Genotype	17	455.54	26.79	1.90*	0.0256
Moisture x Genotype	34	603.09	17.74	1.26	0.1890
Errors	106	1 495.48	14.11		
Total	161	2 882.98			

\* Significant at P value  $\leq 0.05$ \* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR POD LENGTH**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	43.86	21.93		
Moisture	2	780.06	390.03	16.89***	<.0001
Genotype	17	369.79	21.75	0.94	0.5279
Moisture x Genotype	34	819.55	24.10	1.04	0.4200
Errors	106	2 447.64	23.09		
Total	161	4 460.90			

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF SEED PER POD**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	23.75	11.88		
Moisture	2	618.23	309.12	15.09***	<.0001
Genotype	17	412.40	24.26	1.18	0.2897
Moisture x Genotype	34	815.32	23.98	1.17	0.2679
Errors	106	2 170.91	20.48		
Total	161	4 040.62			

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF PODS PER PLANT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	40.34	20.17		
Moisture	2	506.90	253.45	17.62***	<.0001
Genotype	17	530.10	31.18	2.17*	0.0090
Moisture x Genotype	34	464.88	13.67	0.95	0.5530
Errors	106	1 524.99	14.39		
Total	161	3 067.22			

\* Significant at P value  $\leq 0.05$

\* \* \* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF SEEDS PER PLANT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	1 256.46	628.23		
Moisture	2	89 676.49	44 838.25	17.57***	<.0001
Genotype	17	76 533.66	4 501.98	1.76*	0.0426
Moisture x Genotype	34	86 093.95	2 532.17	0.99	0.4923
Errors	106	270 561.54	2 552.47		
Total	161	524 122.10			

\* Significant at P value  $\leq 0.05$ \*\*\* Significant at P value  $\leq 0.01$ **ANOVA TABLE FOR SEED YIELD PER PLANT**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	70.25	35.12		
Moisture	2	442.87	221.43	7.35***	0.0010
Genotype	17	737.31	43.37	1.44	0.1327
Moisture x Genotype	34	1 238.12	36.41	1.21	0.2307
Errors	106	3 193.09	30.12		
Total	161	5 681.64			

\*\*\* Significant at P value  $\leq 0.01$ **ANOVA TABLE FOR LEAF SENESCENCE**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	20.83	10.41		
Moisture	2	444.46	222.23	74.27***	<.0001
Genotype	17	28.12	1.65	0.55	0.9184
Moisture x Genotype	34	111.76	3.29	1.10	0.3495
Errors	106	317.17	2.99		
Total	161	922.35			

\*\*\* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR STEM GREENNESS**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	2.11	1.05		
Moisture	2	118.78	59.39	32.81***	<.0001
Genotype	17	12.06	0.71	0.39	0.9847
Moisture x Genotype	34	68.78	2.02	1.12	0.3269
Errors	106	191.89	1.81		
Total	161	393.61			

\*\*\* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR LEAF AREA**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	29 678.69	14 839.34		
Moisture	2	260 619.84	130 309.92	30.58***	<.0001
Genotype	17	133 564.99	7 856.76	1.84*	0.0317
Moisture x Genotype	34	135 514.37	3 985.72	0.93	0.5758
Errors	106	451 907.55	4 263.28		
Total	161	1 011 285.44			

\* Significant at P value  $\leq 0.05$

\*\*\* Significant at P value  $\leq 0.01$

**ANOVA TABLE FOR NUMBER OF LEAVES**

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum Squares	F value	P value
Replication	2	680.83	340.41		
Moisture	2	6 626.09	3 313.04	48.52***	<.0001
Genotype	17	2 942.86	173.11	2.54*	0.0020
Moisture x Genotype	34	2 440.13	71.77	1.05	0.4105
Errors	106	7 238.51	68.29		
Total	161	19 928.42			

\* Significant at P value  $\leq 0.05$

\*\*\* Significant at P value  $\leq 0.01$

**Appendix 3: Mean Performance of moisture regimes for different growth stages for days to 50% flowering, days to maturity, yield and yield components for cross ITOOK-1263 and Tumaini**

Moisture regimes at	Trait						
	DT50%F	DTM	100SWT	NPP	PODL	NSPOD	NS/PL
Control	59.44a	79.2a	10.94a	12.72a	14.94a	14.42a	163.26a
Vegetative	55.70a	70.43a	7.78b	8.28b	10.64b	9.81b	99.46b
Flowering	43.70b	57.13b	7.16b	8.04b	9.18b	8.57b	89.54b

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test

Note: DTF-Days to 50% flowering, DTM-Days to maturity, 100SWT-Hundred seed weight, NPP-Number of pods per plant, PODL-Pod Length, NSPOD-Number of seeds per pod, NS/PL- Number of seeds per plant

**Appendix 4: Mean Performance of moisture regimes for different growth stages for days to 50% flowering, days to maturity, yield and yield components for cross ITOOK-1263 and X-Ilonga**

Moisture regimes at	Trait						
	DT50%F	DTM	100SWT	NPP	PODL	NSPOD	NS/PL
Control	65.31a	85.04a	10.22a	12.17a	15.07a	14.37a	159.35a
Vegetative	56.29ab	69.44b	9.95a	9.67b	12.58b	11.18b	123.68b
Flowering	52.44b	69.52b	7.52b	7.85c	9.70c	9.68b	102.31c

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test

Note: DTF-Days to 50% flowering, DTM-Days to maturity, 100SWT-Hundred seed weight, NPP-Number of pods per plant, PODL-Pod Length, NSPOD-Number of seeds per pod, NS/PL- Number of seeds per plant

**Appendix 5: Mean Performance of moisture regimes for different growth stages leaf senescence, stem greenness, leaf area and number of leaves for cross ITOOK-1263 and Tumaini**

Moisture regimes at	LS	STG	LA	NLV
Control	2.81c	4.67a	168.07a	43.24a
Vegetative	6.63b	2.48b	83.99b	17.52c
Flowering	7.22a	2.31b	149.81	31.79

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test

**Appendix 6: Mean Performance of moisture regimes for different growth stages leaf senescence, stem greenness, leaf area and number of leaves for cross ITOOK-1263 and X-Ilonga**

Moisture regimes at	LS	STG	LA	NLV
Control	2.81c	4.67a	168.07a	43.24a
Vegetative	6.63b	2.48b	83.99b	17.52c
Flowering	7.22a	2.31b	149.81	31.79

Means followed with the same letters are not significantly difference at ( $P \leq 0.05$ )

according to Student Newman-Keuls Test

**Appendix 7: Effect of moisture stress on flowers and pod set for cross  
ITOOK-1263 x Tumaini**

<b>Cowpea genotypes</b>	<b>Numbers of flowers</b>	<b>Number of flowers abortion</b>	<b>Number of pod set</b>	<b>% flower abortion</b>
ITK-TMA1	22	12	10	54.5
ITK-TMA2	23	12	11	52.17
ITK-TMA3	19	6	13	31.57
ITK-TMA4	23	10	13	43.47
ITK-TMA5	21	8	13	38.09
ITK-TMA6	19	5	14	26.31
ITK-TMA7	23	8	15	34.78
ITK-TMA8	19	7	12	36.84
ITK-TMA9	23	10	13	43.47
ITK-TMA10	27	13	14	48.14
ITK-TMA11	21	7	14	33.33
ITK-TMA12	18	5	13	27.7
ITK-TMA13	25	12	13	48
ITK-TMA14	28	14	14	50
ITK-TMA15	29	14	15	48.27
ITK-TMA16	29	15	14	51.72
ITOOK-1263	30	15	15	50
TUMAINI	27	13	14	48.14

**Appendix 8: Effect of moisture stress on flowers and pod set for cross  
ITOOK x X-Ilonga**

<b>Cowpea genotypes</b>	<b>Number of flowers</b>	<b>Number of flowers abortion</b>	<b>Number of pod set</b>	<b>%flower abortion</b>
ITK-XL1	19	6	13	31.57
ITK-XL2	24	12	12	50
ITK-XL3	24	11	13	45.83
ITK-XL4	22	11	11	50
ITK-XL5	21	9	12	42.85
ITK-XL6	20	7	13	35
ITK-XL7	24	12	12	50
ITK-XL8	20	9	11	42.85
ITK-XL9	23	11	12	47.82
ITK-XL10	27	14	13	51.85
ITK-XL11	20	8	12	40
ITK-XL12	19	7	12	36.84
ITK-XL13	25	13	12	52
ITK-XL14	26	15	10	57.69
ITK-XL15	24	14	10	58.33
ITK-XL16	28	13	15	46.42
ITOOK-1263	30	14	16	46.67
X-ILONGA	28	15	13	53.57