KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI, GHANA

USING MORPHOLOGICAL AND PHYSIOLOGICAL FACTORS TO EVALUATE SIX COWPEA VARIETIES FOR DROUGHT TOLERANCE

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, FACULTY OF AGRICULTURE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MSc. PLANT BREEDING (AGRONOMY)

BY

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CERTIFICATION

I hereby declare that this submission is my own work and that, to the best of my knowledge, it contains no materials previously published by another person nor materials which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

To my mother, Madam Aichatou Abdallah. This is what your toil has yielded.

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ABSTRACT

A potted experiment was conducted from August to October 2011, in a planthouse at Soil Research Institute (SRI), Kwadaso/Kumasi. The objective was to evaluate the performance of cowpea varieties for drought tolerance using morphological and physiological traits. A completely andomized design replicated three times with two water treatments (control and water-stressed conditions) and six cowpea varieties was used. Data collected included biomass (BM), water use efficiency (WUE), relative water content (RWC), plant height (PHT), number of leaves per plant (NL), stem diameter (SD), root dry mass (RDM) and leaf senescence (LS). There were significant differences among the cowpea genotypes, as regards the water treatments and their interaction for these morpho-physiological parameters. Water stress significantly decreased growth and development of cowpea genotypes, and the variety Dan illa showed no significant difference between the two water regimes in relative water content, plant height, number of leaves per plant and stem diameter. Highly strong positive relationships were obtain between biomass and water use efficiency, and between water use efficiency and number of leaves per plant among water-stressed cowpea varieties with r^2 = 0.92 and r^2 = 0.82, respectively. With relatively better performance under water-stressed condition, as indicated by the drought susceptibility index, the three varieties Dan illa, TN88-63 and Asontem were the genotypes recommended to be used as source for drought tolerance in a cowpea breeding programme.

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CHAPTER ONE

INTRODUCTION

Cowpea [*Vigna unguiculata (L.) Walp.*] is one of the most important food legumes in the tropical and sub-tropical regions where drought is a major production constraint due to low and erratic rainfall (Singh *et al.*, 1997). Of the world total area of about 14 million hectares planted with cowpea, West Africa alone accounts for about 9 million hectares (Singh *et al.*, 2003a). 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.*, 2003b). Despite its inherent capacity to survive levels of drought that would render comparable crops unproductive (Ewansiha and Singh, 2006), significant differences exist among cowpea genotypes in drought tolerance (Mai-Kodomi *et al.*, 1999a).

In the Savanna and Sahel sub regions, cowpea is often cropped in areas with limited rainfall or soil moisture and drought is always a potential problem. However, reports on the response of the crop to drought at different stages of growth are inconsistent although significant progress has been made by many researchers. For example, the simple screening method using the "wooden box technique" has been found suitable for identifying seedling tolerance to drought in cowpea. This method eliminates the influences of the root system on drought tolerance, and permits nondestructive visual identification of shoot dehydration tolerance (Singh *et al.*, 1999a). Also Watanabe *et al.* (1997) reported some genotypic differences in the ability of cowpea to survive imposed drought beginning in the vegetative stage. On the other hand, Turk and Hall (1980) showed that cowpea is highly sensitive to water stress during the flowering and pod-filling stages.

Unlike some other legume crops such as common bean and soybean for which contemporary technological studies for drought tolerance are more advanced (Schneider *et al.*, 1997; Blair *et al.*, 2002) and (Mian *et al.*, 1996, 1998; Specht *et al.*, 2001) respectively, cowpea is well studied for conventional genetics, but poorly characterized at the genomic level. Nevertheless, concerted efforts are being made worldwide to develop drought tolerant cowpea varieties (Turk and Hall, 1980; Hall *et al.*, 1997a) and good progress has been made at the International Institute of Tropical Agriculture (IITA) on breeding for enhanced drought tolerance (Okosun *et al.*, 1998; Singh *et al.*, 1999a, b; Mai-Kodomi *et al.*, 1999a, b).

Recent efforts have focused on the genetic dissection of drought tolerance through identification of markers defining quantitative trait loci (QTL) with effects on specific traits related to drought tolerance (Muchero, 2009). Others have studied the relationship of the drought response and yield components, morphological traits and physiological parameters. Some researchers selected a variety of candidate genes and used differential screening methods to identify cDNAs from genes that may underlie different drought tolerance pathways in cowpea (Iuchi *et al.*, 1996; Diop *et al.*, 2004; El-Maarouf *et al.*, 1999; Matos *et al.*, 2001 and Contour-Ansel *et al.*, 2006). Understanding the genetics of drought tolerance and identification of DNA markers linked to QTLs, with a clear path towards localizing chromosomal regions or candidate genes involved in drought tolerance will help cowpea breeders to develop improved varieties that combine drought tolerance with other desired traits using marker assisted selection (Muchero, 2008).

Drought stress represents the most important abiotic stress affecting cowpea production in the semi-arid zones of Africa where most cowpea is produced. Therefore, developing plants that have an advantage under drought stress conditions is a major challenge for cowpea breeding programmes. Cowpea genotypes possessing the ability to withstand water deficit are potential candidates to ensure sustainable yield in these areas.

The global objective of this study was to evaluate the performance of six cowpea varieties for drought tolerance using some morpho-physiological traits under plant house conditions.

The specific objectives were:

1. To determine the effect of water stress on the vegetative growth of cowpea varieties;

2. To develop drought susceptibility index that could be used for selecting cowpea varieties for drought tolerance.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Cowpea: origin, domestication and distribution

Cowpea [*Vigna unguiculata (L.) Walp*.] (2n = 2x = 22) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al.*, 1974). Cowpea is commonly referred to as "niébé," "wake," and "ewa" in some West African countries, and "caupi" in Brazil. In the United States, other names include "southern peas," "blackeyed peas," "field peas," "pinkeyes," and "crowders." These names reflect traditional seed and market classes that developed over time in the southern United States. The name cowpea probably originated from the fact that the plant was an important source of hay for cows in the southeastern United States and in other parts of the world (Timko *et al.*, 2007). Cowpea most likely originates from Africa, as wild cowpeas only exist in Africa and Madagascar (Steele, 1976). The centre of diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah region of Nigeria, southern Niger, parts of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985). Carbon dating of cowpea (or wild cowpea remains from the Kintampo rock shelter in central Ghana) has been carried out (Flight, 1976) and is the oldest archaeological evidence of cowpea found in Africa. Cowpea is considered to have been domesticated in Africa from its wild ancestral form, *V. Unguiculata subsp*. dekindtiana (Harms) Verdc. (Ng and Marechal, 1985). However, the precise location of origin where cowpea was first domesticated is still under speculation. Ba *et al.* (2004) reported that the crop was probably domesticated by farmers in West Africa while Coulibaly *et al.* (2002) presented some evidence that domestication occurred in northeastern Africa, based on studies of amplified fragment length polymorphism (AFLP) analysis. Cowpea was introduced from Africa to the Indian subcontinent approximately 2000 to 3500 years ago (Allen, 1983). Cowpeas had reached Europe from Asia and have been cultivated in southern Europe at least since the 8th century BC and perhaps since prehistoric times (Tosti and Negri, 2002). From the West Indies, cowpea was taken to the USA in about 1700 BC (Pursglove, 1968). The slave trade from West Africa resulted in the crop reaching the southern USA early in the 18 century however, many US cultivars appear more closely related to germplasm from Asia or southern Europe than West Africa (Fang *et al.*, 2007). Presently cowpea is grown throughout the tropic and subtopic areas around the whole world.

2.2. Description, classification and importance

Cowpeas are generally more robust in appearance than common beans with better developed root systems and thicker stems and branches. Summerfield *et al.* (1974), Kay (1979), Fox and Young (1982) described cowpea as an annual herb reaching heights of up to 80 cm with a strong taproot and many spreading lateral roots in the surface soil. Growth forms vary and include erect, trailing, climbing, or bushy, usually indeterminate growers under favorable conditions. Fruits are pods containing seeds that vary in size, shape, colour and texture. Pods may be held erect, crescent shaped or coiled. They are usually yellow when ripe, but may also be brown or purple. The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 560 cm long peduncles. Flowers are conspicuous, mostly self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in colour. Flowers open in the early day and close at approximately midday.

Vigna has several species, but the exact number varies according to different authors. Cultivated cowpeas have been divided into five cultivar groups based mainly on pod and seed characteristics (Pursglove, 1968; Pasquet, 1999). Cultivar group Unguiculata is the largest and includes most medium and large-seeded African grain and forage-type cowpeas. Cultivar group Melanophthalmus includes “blackeyed pea” type cowpea with large, somewhat elongated seeds with wrinkled seed coats and fragile pods (Pasquet, 1998). Members of cultivar group Biflora (also known as “catjang”) are common in India and characterized by their relatively small smooth seeds borne in short pods that are held erect until maturity. Cultivar group Textilis is a rather rare form of cowpea with very long peduncles that were used in Africa as a source of fiber. Cultivar group Sesquipedialis (known as “yardlong bean,” “long bean,” “Asparagus bean,” or “snake bean”) is widely grown in Asia for production of its very long (40 to 100 cm) green pods that are used as “snap” beans.

Members of the Phaseoleae (which cowpea belongs to) 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 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 "hungryseason 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.*, 2003b). The high protein content of cowpea grain represents a major advantage for use in infant and children's food (Lambot, 2002). The mature pods are harvested and the haulms are cut while still green and rolled into small bundles containing the leaves and vines. These bundles are stored on rooftops for use as feed supplement in the dry season, 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, 2002; 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). Figure 2.2 summarizes the potential contributions of cowpea described by Tarawali *et al.* (2002).

2.3. World Production

Production of a cultivar group Sesquipedialis (or yardlong) bean is widespread throughout Asia and is thought to be grown on about 300,000 ha. Dry grain production is the only commodity of cowpea formerly estimated on a worldwide basis. The United Nations Food and Agricultural Organization (FAO) estimates that nearly 4 million metric tons (mt) of dry cowpea grain is produced annually on about 10 million ha worldwide (www.faostat.fao.org/faostat). Cowpea grain production estimates by Singh *et al.* (2002) are

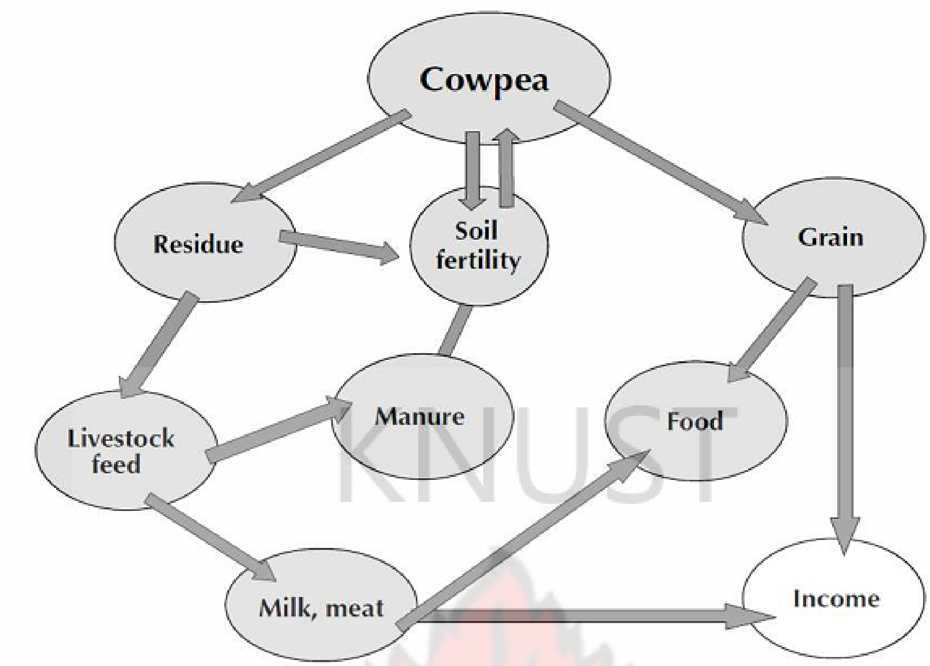

Figure 2.1. Schematic representation of the potential contributions of cowpea in crop livestock systems in the dry savannas. (After Tarawali *et al.*, 2002).

slightly higher than FAO estimates, with worldwide production of 4.5 million (mt) on 12 to 14 million ha. About 70 % of this production occurs in the drier Savanna and Sahelian zones of West and Central Africa, where the crop is usually grown as an intercrop with pearl millet [*Pennisetum glaucum (L.) R.Br*.] or sorghum [*Sorghum bicolor (L.) Moench*] and, less frequently, as a sole crop or intercropped with maize (*Zea mays L*.), cassava (*Manihot esculenta Crantz*), or cotton (*Gossypium sp*.) (Langyintuo *et al.*, 2003). Other important production areas include lower elevation areas of eastern and southern Africa and in South America (particularly in northeastern Brazil and in Peru), parts of India, and the southeastern and southwestern regions of North America. Nigeria is the largest producer and consumer of cowpea grain, with about 5 million ha and over 2 million mt production annually, followed by Niger 650,000 mt) and Brazil (490,000 mt) (Singh *et al.*, 2002).

Trade in dry cowpea grain and cowpea hay is important to the economy of West Africa in particular, with substantial quantities of cowpea grain being traded at the local and regional level (Singh 2002; Langyintuo *et al.*, 2003). The large urban centers of coastal West Africa are huge markets for cowpea produced further inland where climates are drier and favorable to production of high-quality grain. The United States produces about 80,000 mt, in several southern states (Alabama, Arkansas, Georgia, Louisiana, Missouri, and Tennessee) and in Texas and California (Fery, 2002).

A long-term drought in the Sahelian zone of West Africa has caused many farmers in this part of Africa to shift most of their production to cowpea because of its drought tolerance (Duivenbooden *et al.*, 2002). As a result of this shift in production and the adoption of new varieties and improved production systems, worldwide cowpea production has gone from an annual average of about 1.2 million mt during the decade of the 1970s to 3.6 million mt per annum (during the five-year period spanning from 1998 to 2003) according to the FAO (http://faostat.fao.org/faostat). The drier zones of northern Nigeria and Niger have the largest area of cowpea production in the world but yields are only between 100 to 500 kg/ha, despite its 5 times higher biological potential (Carsky *et al.*, 2001). Niger is the second largest producer of cowpea after Nigeria yet it has the lowest average grain yield of 110 kg/ha (Table 1). This is probably due to the fact that the whole country is located in the Sahel where rainfall is rather low. Moreover, drought conditions weaken the plants making them more vulnerable to disease infestations and insect pests' attacks. As an African crop grown in resource-poor areas, few countries have cowpea improvement programs and the continent has very low average grain yield compared to, for instance, the United States (Table 1). Rapidly growing populations with high per-capita cowpea consumption in the West and Central African regions have fueled demand for cowpea grain during this period, and the trend is expected to continue.

Table 2.1. Average yield (t ha^{-1}) of cowpea production in selected countries in West and Central Africa (1990-1999) and the United States (Langyintou *et al.*, 2003)

Countries	Average yield (t ha^{-1})	Countries	Average yield (t ha^{-1})
Nigeria	0.494	Benin	0.635
Niger	0.110	Mauritania	0.331
Mali	0.244	Cote d'Ivoire	0.500
Burkina	0.777	Chad	0.489
Ghana	0.663	Cameroon	0.827
Togo	0.284	Africa	0.475
Senegal	0.341	United States	1.950

2.4. Cultural practices

2.4.1. Choice of cultivars, plant population and spacing

In cowpea, cultivar choice will affect plant population. Cultivars with erect growth forms have a higher plant population than prostrate or semi prostrate types, because the erect forms performs much better in narrow rows (Weber *et al.*, 1996). Plant population requirement of cowpea with respect to growth type, seed rate/ha and inter/intra row spacing is summarized in Table 2.2. The environmental potential of the land to be used, will determine the most favorable plant population for cowpea (Coetzee, 1995).

Table 2.2. Seed rate/ha based on recommended plant spacing (Dugje *et al.*, 2009).

Cowpea type	Maturity	Spacing (cm)	Quantity of seeds/ha
Erect	Extra-early	50 × 20	25 kg (10 *mudus*)
Semi-erect	Early/medium	75 × 20	20 kg (8 *mudus*)
Prostrate (creeping)	Medium/late	75 × 30	16 kg (7 *mudus*)
Prostrate	Late	75 × 50	12 kg (5 *mudus*)

2.4.2. Fertilization

Cowpea, like all legumes, forms a symbiotic relationship with a specific soil bacterium (*Rhizobium sp*.), which makes atmospheric nitrogen available to the plant via nitrogen fixation. Nitrogen fixation occurs in root nodules and the bacteria utilize sugars produced by the plant. Although cowpea *Rhizobium* is normally widespread, seed inoculation with *Rhizobium* specific to cowpea would be beneficial in areas where it is not present. It is, however, important to use *Rhizobium* of the cowpea type (Eaglesham *et al.*, 1977).

Excess nitrogen (N) promotes lush vegetative growth, delays maturity, may reduce seed yield and suppress nitrogen fixation. The plant will perform well under low N conditions due to a high capacity for N fixation. A starter N rate of 27 $kg.ha^{-1}$ is sometimes required for early plant development on low-N soils (Rupela and Saxena, 1987; Bluementhal *et al.*, 1992).

2.5. Environmental requirements of cowpea

Cowpea is grown between 35^o N and 30^o S of the equator. Temperature and photoperiod interact with genotype and other aspects of the environment to determine yield potential of seed legumes through their effects on duration of the vegetative and reproductive growth stages (Hadley *et al.*, 1983; Wien and Summerfield, 1984). High temperature adversely affects productivity of many crops, and these adverse conditions are often influenced by planting date (Hall, 1992). In turn, sensitivity to photoperiod can be moderated by temperature. Developing improved germplasm for hot environments requires an understanding of genetic variation for these responses (Patel and Hall, 1990).

Many cowpea genotypes exhibit heat-induced suppression of floral bud development, which results in a two-week delay in flowering when plants are grown in very hot field environments under long days (Warrag and Hall, 1984a, b; Patel and Hall, 1990). Two weeks or more of consecutive or interrupted hot nights during the first four weeks after germination can cause complete suppression of the development of the first five floral buds on the main stem of sensitive genotypes (Ahmed *et al.*, 1992). This damage reduces pod set, number of seeds per pod, and thus seed yield.

Plant or crop development is the progression from sowing to maturity through a series of discrete and clearly defined stages (Squire, 1990). It is nodulated primarily by temperature and photoperiod (Roberts and Summerfield, 1987; Squire, 1990). Understanding the environmental influence of development is important. Firstly, because the time from sowing to flowering and maturity determines the duration of biomass accumulation and, secondly the duration of different developmental phases affects the partitioning of the biomass and therefore the ratio of seed to vegetative yield (Mutters *et al.*, 1989).

To maximize biomass accumulation, the life cycle, and particularly the timing of reproductive development and growth, must be timed to match the available resources (Ludlow and Muchow, 1990; Lawn and Imrie, 1991). In the semi-arid environment of Sub-Saharan Africa, this means ensuring maximum biomass accumulation when moisture is adequate and temperatures are favorable.

The response to photoperiods may be influenced by temperature with a particular genotype exhibiting different degree of temperature x photoperiod interaction (Miller *et al.*, 1958). Breeders of seed legumes, commonly characterize their germplasm into early and medium. For many genotypes, however, these categories are environmentally specific mostly due to different responses to photoperiod and temperature (Huxley and Summerfied, 1996).

Photoperiod effects remain unseen under short day lengths. Most differences in duration of vegetative stage occur under long photoperiods. Genotype specific photoperiod x temperature interaction effects on floral bud development (and hence flowering) further reduce the predictability of flowering date and adaptation from one environment to other (Hadley *et al.*, 1983).

Patel and Hall (1990) indicated that cowpea can yield satisfactorily under greater diversity of climatic, soil, and cultural conditions than other leguminous crops. The factors responsible for the broad adaptation of cowpea are poorly understood (Hall *et al.,* 1997c). The growing period of cowpea plants is too long to permit its growth for grain where they are not adapted (Russell, 1980). A mean temperature of 27 ºC is optimum for good pod formation and seed yield. It performs better in regions with rainfall of 250 - 1000 mm per annum (Marfo and Hall, 1992). The crop is much more tolerant to high heat and extended drought periods than Phaseolus beans, which are largely confined to higher elevations (Massey *et al.*, 1998).

2.6. Biotic and abiotic constraints

2.6.1. Biotic stress

2.6.1.1. Diseases

Cowpea is susceptible to a wide variety of pests and pathogens that attack the crop at all stages of growth (Allen, 1983). For instance cowpea wilt caused by *Fusarium oscysporium*, cowpea root rust caused by a nematode (*Meloidogyne ssp*) and cowpea bacterial blight caused by *Xanthomonas vignicola*. The two types of parasitic weeds that attack cowpea are Striga and Alectra but Striga has a more devastating effect than Alectra. *Striga gesnerioides* is widespread in areas with low rainfall and poor soil fertility, conditions that are common throughout the northern Guinea and Sudan savanna zones. It causes yellowing between the veins of cowpea leaves, resulting in the death of infested plants. The problem becomes worse when soil moisture is limiting. Losses due to pest attacks or diseases can be as high as 90 % (IITA, 2000).

2.6.1.2. Insects

Some of the major insect enemies of cowpea are cowpea weevil (*Callosobruchus maculatus*), cowpea cuculus (*Chalcodermus sermus*), and the southern cowpea weevil (*Mylabris quadrimaculatus*).

2.6.2. Abiotic stress

2.6.2.1. Drought stress

The effects of the environment on plant growth may be divided into enforced damage effects (stress), caused by the environment, and adaptive responses, controlled by the plant (resistance) (Fitter and Hay, 1987). Damage, which may be manifested as death of all or part of the plant, or merely as reduced growth rate due to physiological malfunction, is a common phenomenon and the agents are various: temperature, water availability, soil chemistry, physical properties and others such as air pollution, wind and diseases. However, the most important environmental agents affecting plant growth in the semi-arid tropical zone is drought.

Linsley *et al.* (1959) defined drought as a sustained period of time without significant rainfall. Katz and Glantz (1977) suggested that there were meteorological and agricultural definitions of drought. A meteorological drought could be defined as that time period when the amount of precipitation is less than some designated percentage of the long term mean. An agricultural drought, on the other hand, could be defined in terms of seasonal vegetation development.

Levitt (1980) reported that drought stress occurs when water uptake from soil cannot balance water loss through transpiration. The subsequent cellular water loss is referred to as dehydration. Drought may start at any time, last indefinitely and attain many degrees of severity. It can occur in any region of the world, with an impact ranging from slight personal inconvenience to endangered nationhood (Hounam *et al.*, 1975).

Agricultural drought occurs when there is not enough moisture available at the right time for the growth and development of crops. As a result, yields and/or absolute production decline (Glantz, 1987). As transpiration occurs as a result of the high temperature common in tropical areas, especially during drought periods, the leaf water potential is reduced. This reduced water potential is then carried down to the roots through the xylem. The soil water potential then decreases because of osmosis into the roots (Raven *et al.*, 1992; Eichhorn, 1992). As a result of a smaller water potential gradient between the root and the soil, less water is absorbed and this limits the vegetative growth resulting in low plant yields. Drought does not only affect the yield, but also the quality of the grain and also the appearance of the plant.

Eighty-five percent of the world's cowpea is concentrated in the savannah zone of West Africa between 10º and 20º N latitude (FAO, 2004). Droughts occur frequently in this area, most commonly due to erratic start or early cessation of the rainfall during the growing season, or occasionally, due to almost no rainfall during the normal growing season for several years in succession (Hall, 2007).

Hiler *et al.* (1972) working on drought stress of cowpea found that the flowering stage is the most susceptible to severe imposed stress (-14 to -28 bars leaf water potential). Meanwhile

Summerfield *et al.* (1974) found that stress during the vegetative stage irreversibly reduced leaf area and caused significant yield decline.

Water stress is arguably the most important environmental variable affecting plant growth and drought as one of the most important factors threatening food security in the world (Baker, 1989). The frequency and severity of drought may increase in the future as global warming intensifies.

Furthermore drought stress is highly variable in time (over seasons and years) and space (between and within sites), and is extremely unpredictable. This makes it very difficult to identify a representative drought stress condition (Visser, 1994). The unpredictable and variable forms in which drought stress will manifest itself, makes selection of promising individual plants and breeding for drought tolerance extremely difficult.

Drought tolerance has been shown to be a highly complex trait, influenced by many different genes and should not be regarded as a unique heritable trait, but as a complex of often fully unrelated plant properties (Visser, 1994). Drought can hardly be separated from other important abiotic stresses such as temperature and salinity. Due to these interrelations, no single mechanism exists by which multiple stresses are alleviated. A better understanding of how drought stress affects crop growth and development processes are fundamental. The understanding of the mechanisms of plant adaptations to drought would help breeders to improve drought tolerance of crop plants more effectively. Improved tolerance could sustain productivity and help extend cultivation of certain crops into areas that are currently unsuitable for crop production.

Several factors and mechanisms operate independently or jointly to enable plants to 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, grow, and yield 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: drought escape, drought (dehydration) avoidance and drought (dehydration) tolerance. However, crop plants may 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.

Dehydration 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 (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evapotranspiration surface (leaf area) (Mitra, 2001).

Dehydration 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. The mechanisms that confer drought avoidance by reducing water loss (such as stomatal closure and reduced leaf area) decrease carbon assimilation due to reduction in physical transfer of carbon dioxide molecules and increase leaf temperature thus reducing biochemical processes, which negatively affects yield. Plants try to maintain water content by accumulating various solutes that are nontoxic (such as fructans, trehalose, polyols, glycine betaine, proline and polyamines) and do not interfere with plant processes and that are, therefore, called compatible solutes (Yancey *et al.*, 1982). However, many ions concentrated in the cytoplasm due to water loss are toxic to plants at high concentrations leading to what is termed a glassy state. In this condition whatever liquid is left in the cell has a high viscosity, increasing the chances of molecular interactions that can cause proteins to denature and membranes to fuse (Hartung *et al.*, 1998).

Consequently, crop adaptation to water stress must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity. Drought escape, avoidance, and tolerance mechanisms have been described in cowpea. However, the drought response pathways associated with these mechanisms are not yet understood, and the degree to which these adaptations operate jointly or separately to allow the crop to cope with drought still needs to be established.

2.6.2.2. High temperature

Resistance to the stress caused by high temperatures requires that limiting plant processes are not irreversibly damaged. All plant processes are irreversibly damaged if high enough temperatures are imposed for sufficient time (Hall and Patel, 1985). Consequently, the key questions for breeders are:

- What aspects of high temperatures (considering temperature levels and duration at different times during the season and day) cause significant reductions in productivity in different climates?
- What plant processes and stages of development are most sensitive to high temperatures and are responsible for reduction in productivity?

For cowpeas, considering the natural variation in temperatures that occur in the tropics and subtropics and studies on cowpea response to temperature (Warrag and Hall, 1984a, b), it can be concluded that high temperatures at night can be much more damaging to grain yield of cowpeas than high temperatures during the day. Growth chamber and field studies demonstrated that the temperatures that commonly occur at night in the tropics can cause male sterility (Warrag and Hall, 1984b) and substantially reduce grain yield by increasing floral abscission and decreasing the pods/m². Studies have been conducted with cowpea plants subjected to higher night temperatures during flowering using enclosures in field conditions (Nielsen and Hall, 1985a, b), and with almost isogenic pairs of heat-resistant and heat susceptible lines grown in field environments with contrasting temperatures (Ismail and Hall, 1998). These studies showed that increases in night temperature caused 4 – 14 % decreases in both pod set and grain yield for each ºC above a threshold of 16 ºC. The main mechanism for these effects on cowpea is that high temperatures occurring in the late night during flowering can cause pollen sterility and indehiscence of anthers (Hall, 1992; 1993). For cowpea, the heat-stress problem mainly has been solved by breeding; however, crop management also can be important in some cases.

Breeding for heat tolerance, involved subjecting progeny to very high night temperatures and long days in either field or glasshouse conditions, which only could be done in the summer, and selecting plants with the ability to abundantly produce flowers and set pods (Hall, 1992; 1993). Long days must be used because under short days the detrimental effects of heat on reproductive development of cowpea are either much smaller or may not occur (Ehlers and Hall, 1998). In the F2 generation, plants were selected that abundantly produced flowers and set pods. This virtually fixed the recessive gene that provides tolerance to heat-induced suppression of floral bud development (Hall, 1993). Tolerance to heat during pod set was more difficult to incorporate because, even though it appeared to mainly involve a single dominant gene, the realized heritability was low at about 0.26 (Marfo and Hall, 1992) and it is likely that inheritance also depends on some minor genes (Hall, 1993).

2.7. Breeding for drought tolerance in cowpea

Success in breeding for drought tolerance in cowpea has not been as pronounced as for many other traits (Singh *et al.*, 1997). This is partly due to the lack of simple, cheap, and reliable screening methods to select drought tolerant plants and progenies from the segregating populations. The complexity of factors involved in drought tolerance could also have contributed to this. Nevertheless, cowpea genotypes with contrasting response to drought have been identified. Researchers have proposed two approaches for screening and breeding for drought tolerance in plants. The first is the empirical or performance approach that utilizes grain yield and its components as the main criteria, since yield is the integrated expression of the entire array of traits related to productivity under stress. The second is the analytical or physiological approach that identifies a specific physiological or morphological trait that will contribute significantly to growth and yield in the event of drought. Modest progress in cowpea breeding for dry environments has been achieved by selecting for yield in breeding lines over several locations and years (Turk *et al.*, 1980; Hall and Patel, 1985; Selvaraj *et al.*, 1986; Cisse *et al.*, 1997; Hall *et al.*, 1997b).

However, these empirical approaches are slow, laborious, and expensive because of the need to assess the yield of a large number of lines across several locations and years, and the substantial variation from the effects of environment, and genotype – environment interactions (Blum, 1985). As suggested by Blum (1983) and Fussell *et al.* (1991), the approach which combines selection for yield potential in favorable conditions with selection for the expression of physiological traits thought to be associated with drought tolerance under controlled, repeatable stress environments might be the most effective. This therefore requires the identification of specific traits associated with drought tolerance under adequate water management that are easy and reliable to measure (Fischer and Wood, 1979).

2.7.1. Morphological, physiological and biochemical indicators for drought response

Data on changes of morphological, biochemical and physiological traits in response to drought are available for some cultivars of *Vigna unguiculata* (Turk *et al.*, 1980; Ogbonnaya *et al.*, 2003; Matsui and Singh, 2003; Slabbert *et al.*, 2004). These traits include water use efficiency (WUE), leaf water potential, relative turgidity, leaf gas exchange, relative water content (RWC), diffusion pressure deficit, chlorophyll stability index, and carbon isotope discrimination (Bates *et al.*, 1981; Turk and Hall, 1980; Morgan *et al.*, 1991; Hall *et al.*, 1990; 1997b; Anyia and Herzog, 2004; Souza *et al.*, 2004).

While comparing physiological responses of *Phaseolus vulgaris* and *Vigna unguiculata* to drought, Cruz de Carvalho *et al.* (1998) demonstrated that stomatal conductance to water vapour (gs, mol H_2O m^{-2} s^{-1}) and net assimilation rates (A, mmol CO_2 m^{-2} s^{-1}) measured during and after a water stress treatment were reliable physiological parameters to use in early screening for drought tolerance in these species. Stomatal closure in the cowpea cultivar EPACE-1 was not related to any change in relative water content (RWC) indicating that early stomatal responses to substrate water depletion are not triggered by changes in leaf water content. Therefore, RWC alone cannot be used as a drought indicator for cowpea. This also suggests the possible existence of a root to leaf communication, independent of the leaf water status that informs the shoot about changes in the root zone.

Following exposure of six cowpea varieties to drought in the upper 20 cm rooting zone, Kulkarni *et al.* (2000) compared the rate of abscisic acid (ABA) synthesis relative to total root mass and inherent variation per unit root mass. The authors observed that the intrinsic ABA synthesizing capacity rather than the root mass is responsible for the total ABA produced in the roots of the dry soil zone. The relationship between stomatal conductance and total root ABA was assessed and found to be negative ($r = > 0.90$, $n = 24$, $P = 0.05$) suggesting that the intrinsic capacity of cowpea varieties for ABA synthesis could play an important role in regulating stomatal conductance in a drying soil and provide useful selection criteria for tolerance to drought stress in cowpea. In support to these results, stomatal regulation was reported to be the common strategy used by the five different cowpea genotypes to avoid dehydration both under glasshouse and field conditions (Hamidou *et al.*, 2007). These authors measured the physiological, biochemical and agronomic responses to water deficit at flowering stage in five cowpea genotypes, Gorom local, KVX61-1, Mouride, Bambey 21 and TN88-63 that were grown in the glasshouse and the field. The five cowpea genotypes are known to differ in their susceptibility to water stress. Water deficit significantly increased the canopy temperature and the proline content of the five genotypes while gaseous exchanges and starch content decreased significantly.

Yield components of the five genotypes, with the exception of seed number per pod, were also significantly affected. Number of pods and number of seeds per plant decreased after drought treatment by 57 % in the glasshouse and by 64 % in the field when compared to non-stressed plants. Genotypic differences were observed for both of the yield components. Genotype TN88-63 was more productive than the other four genotypes under glasshouse conditions, while under field conditions, Mouride and Gorom local proved to be more productive than KVX61-1, which in turn performed better than Bambey 21.

As an alternative to all the above investigations which focus on some specific physiological, biochemical and agronomic traits, an integrated approach which combines cellular water relations, rooting characteristics, leaf area and biochemical and morphological changes to screen cowpea for drought tolerance has been proposed by Slabbert *et al.* (2004). The different screening techniques that were tested included: the antioxidative response in the form of superoxide reductase (SOD), glutathione reductase (GR), ascorbate peroxidase (AP), proline accumulation, 2,3,5 - triphenyltetrazolium chloride (TTC) assays, early drought screening at the seedling stage (wooden box technique), cell membrane stability (CMS), relative water content (RWC), leaf water potential (LWP), leaf area, chlorophyll a and b and carotenoid content and chlorophyll fluorescence (JIP test). Contrary to the results of Cruz de Carvalho *et al.* (1998), RWC was a good parameter to discriminate genotypes under water stress in cowpea (Slabbert *et al.*, 2004). An important morphological trait that may contribute to drought adaptation is the delayed leaf senescence (DLS) trait (Gwathmey *et al.*, 1992). This trait enhances plant survival after a mid-season drought damages the first flush of pods, which enables a substantial second flush of pods to be produced. Cultivars with DLS also have enhanced production of forage because their leaves remain green and attached to the plant until harvest. The DLS trait allows the crop to stay alive through mid-season drought and recover when rainfall resumes. Most importantly, DLS can be easily measured by visual observation using an appropriate scale.

2.7.1.1. Water Use Efficiency (WUE)

Water-use efficiency (WUE) is defined as a ratio of biomass accumulation, expressed as carbon dioxide assimilation, total crop biomass, or crop grain yield, to water consumed, expressed as transpiration, evapotranspiration (ET), or total water input to the system (Sinclair *at al.*, 1984). The time-scale for defining water-use efficiency can be instantaneous, daily, or seasonal.

Shamsi *at al.* (2010) while investigating the role of water deficit stress and water use efficiency (WUE) on bread wheat cultivars concluded that grain yield increased more intensely as water utilization increased in the unit area resulting in an increase in WUE. Under moist stress condition at stages after stem elongation (I1), booting (I2), and grain-filling (I3), a decrease in WUE in the unit area reduced yield compared to the control condition (I4) which resulted in a decrease in WUE. These results are in agreement with reports of Lie *et al.* (2000) who demonstrated that weight of wheat grains was dependent on speed and duration of grain growth period affected by assimilation. Drought stress decreases the rate of assimilation production due to closing of stomata. Final grain weight was higher for well-irrigated plants than that of those under drought stress condition due to longer duration of grain-filling period.

For WUE, conflicting results have been reported, by researchers like (Johnson *et al.*, 1990 and Karam *et al.*, 2003) who reported an increase in WUE as water utilization rate decreased while Shangun *et al.* (2000); Oktem *et al.* (2003) reported an increase in WUE as water utilization rate increased. Such differences are due to different climatic and soil conditions, different methods of exercising water treatment, and different cultivars used in the different experiments. But, generally, any managerial efforts of reducing water loss through pathways other than transpiration, increasing leaf area index of crops increased surface absorbing sunlight, decreased evaporation rate from soil surface, and therefore raised WUE (Andrade *et al.*, 2002). Also, Blum (2005) reported that genotypic variations in WUE are normally expressed mainly due to variations in water use (WU; the denominator). Reduced WU, which is reflected in higher WUE, is generally achieved by plant traits and environmental responses that reduce yield potential (YP). Improved WUE on the basis of reduced WU is expressed in improved yield under water-limited conditions only when there is need to balance crop water use against a limited and known soil moisture reserve. However, under most dryland situations where crops depend on unpredictable seasonal rainfall, the maximization of soil moisture use is a crucial component of drought resistance (avoidance), which is generally expressed in lower WUE.

2.7.1.2. Relative water content (RWC)

Leaf water status is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer and Boyer, 1995). Water content and water potential (Yψ) have been widely used to quantify the water deficits in leaf tissues. Leaf water content is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues. On the other hand, water potential measures the energetic status of water inside the leaf cells (Slatyer and Taylor, 1960).

The relative water content technique, formerly known as relative turgidity, was originally described by Weatherly (1951) and has been widely accepted as a reproducible and meaningful index of plant water status (Barrs, 1968). Almost a decade ago, Gonzales and Gonzales-Vilar (2001) reported that the relative water content (RWC) stated by Slatyer (1967) is a useful indicator of the state of water balance of a plant essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. It expresses the water content in per cent (or sometimes, in decimal form) at a given time as related to the water content at full turgor.

2.8. Advances in cowpea breeding for drought tolerance

Attempts to improve drought tolerance of crops through conventional breeding programs have met with limited success because drought tolerance is physiologically and genetically a complex trait. The use of molecular markers to identify and locate different genes and genomic regions possessing factors which influence drought tolerance in cowpea will help to gain insight into the complex trait of drought tolerance. In addition, these markers can be used to select for multiple traits and combine genes underlying these traits in cultivars with improved drought tolerance. These properties and prospects have initiated an increased interest in the application of Marker-Assisted Selection (MAS) for improving drought tolerance in many crops including cowpea. For better understanding of different biochemical and physiological pathways involved in drought tolerance in cowpea, three main approaches using molecular marker tools can be used.

The first approach assumes no prior knowledge about genes and is based on the so-called quantitative trait loci (QTL) method. On the most recent genetic map of cowpea (Ouedraogo *et al.*, 2002), consisting of 11 linkage groups (LGs) spanning a total of 2670 cM, with an average distance of approximately 6 cM between markers, no genes/QTLs related to drought tolerance were mapped. However, different recombinant inbred lines (RILs) are being currently screened at IITA for mapping and identification of QTLs with effects on drought tolerance across populations. The development of a set of Expressed Sequence Tag (ESTs) from drought-stressed and non-stressed drought-sensitive and tolerant cowpea lines will be helpful in genotyping.

The ESTs are utilized to develop other molecular markers such as simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and conserved ortholog set (COS) markers. The COS markers would facilitate cross-legume studies and allow better integration of cowpea into legume functional genomics. Currently cowpea genomics is receiving increased attention, which has resulted in projects that are producing large sets of ESTs and other genome sequences which have recently applied an Illumina Goldengate SNP array with 1536 SNPs [University of Californa Riverside (UCR)] to several RIL populations and diverse array of genotypes. This is an opportunity for the cowpea community to use a common set of markers in a wide collection of crosses and germplasm for construction of a densely populated consensus genetic map and for connecting genetics and QTLs/genes in cowpea. All the efforts in improving genetic maps and increasing available sequence data are only useful for QTL analysis if drought tolerance parameters can be measured as heritable traits. For cowpea these include the traits mentioned earlier like stomatal conductance, chlorophyll fluorescence, abscisic acid (ABA) levels, free proline levels, wooden box screening for drought tolerance at the seedling stage, and delayed leaf senescence (DLS).

The second approach is to make an 'educated guess' from published data, i.e. select candidate genes (CG) that are known to be functionally relevant for drought tolerance and test in cowpea plants whether these genes can be linked to drought tolerance. Candidate genes refer either to cloned genes presumed to affect a given trait ('functional CGs') or to genes suggested by their close proximity on linkage maps to loci controlling the trait ('positional CGs') (Pflieger, 2001). The final validation of a CG will be provided through physiological analyses, and genetic transformation. The most detailed studies relating candidate genes to drought QTLs have looked at genes that determine ABA levels, at genes involved in dehydrin production, at invertase activity and transcription factors (Pflieger, 2001). However, there has also been interest in mapping a wide range of regulatory and structural candidate genes to determine QTLs with effects on drought tolerance and this approach has been particularly effective in the case of rice (Nguyen *et al.*, 2004). Recently, genes involved in ABA biosynthesis, ascorbate eroxidase, glutathione reductase and transferase, and putative phosphatidate phosphatases have been cloned from cowpea under water stress conditions. However, clear evidence that these genes affect drought tolerance for instance through transgenic analyses has not been reported so far. Other CGs can be inferred from studies in related crops and model crops. Cowpea orthologues of these genes that have been characterized in other species and crops as being involved in drought tolerance will be increasingly easy to discover, as the number of cowpea EST sequences as well as genespace sequences is increasing rapidly.

The third approach is comparative genomics. Earlier studies indicated that members of Papilionoideae subfamily to which cowpea belongs exhibit extensive genome conservation, based on comparative genome analysis between mungbean and cowpea (Menancio-Hautea *et al.*, 1993), between pea and lentil and orthologous seed weight genes in cowpea and mungbean (Fatokun *et al.*, 1992). Recent advances in comparative mapping among the legumes has clarified the genetic relationship of model and crop legumes and enabled linking of the genomes of the tropical and temperate legumes that represent the major clades of the legume family (Choi *et al.*, 2004a; 2004b).

Drought tolerance is a highly appropriate target for comparative plant genomics because this information-rich approach has the potential to unveil the key genetic contributors to the complex physiological processes involved (Bennetzen, 2000). With the already extensive and rapidly increasing publicly available genomic data for cowpea, comparative genomics of cowpea with other legumes such as common bean (Blair *et al.*, 2002; Schneider *et al.*, 1997) and soybean (Mian *et al.*, 1996; 1998; Specht *et al.*, 2001) could be applied. This will allow aligning of drought QTLs between legume species including cowpea and determine the most important regions for saturated mapping. Moreover, the micro and macrosyntenic relationships detected between cowpea and other cultivated and model legumes (Timko *et al.*, 2008) would simplify the identification of informative markers for marker-assisted trait selection and map-based gene isolation necessary for cowpea improvement.

2.9. Transgenic cowpea

Until recently cowpea remained one of the last major grain legume species for which an efficient genetic transformation/regeneration system had not been developed (Van Le *et al.*, 2002; Avenido *et al.*, 2004; Popelka *et al.*, 2004), despite substantial efforts for more than ten years by several groups of researchers (Machuka, 2002a; Machuka *et al.*, 2002b). Ikea *et al.* (2003) reported the successful genetic transformation of cowpea using the particle-gun bombardment of shoot meristems. They were able to isolate several plants in the T3 generation that showed strong expression of the transgene “bar” that confers resistance to the herbicide Basta, but these studies were inconclusive. An efficient and stable cowpea transformation/regeneration system has been developed recently (Popelka *et al.*, 2006), so that transgenic cowpea is now a reality.

Transgenic approaches should be undertaken to develop varieties of cowpeas with strong resistance to insect pests. Insect-resistant cowpeas would dramatically increase cowpea productivity in many developing countries and reduce costs, safety hazards, and environmental risks in virtually all cowpea producing countries. Traditional plant breeding has made only limited progress in breeding for resistance to the major insect pests of cowpea and “new genes” are apparently needed to protect cowpea.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study site

The potted experiment was conducted from August to October 2011, in a planthouse at Soil Research Institute (CSRI/SRI) at Kwadaso, which is about 8 km away from the city of Kumasi. Geographically, the site lies between latitudes 06º.39' and 06º.43' North, and 01º.39' and 01º.42' West of Greenwich meridian. The area is located in the Semi – Deciduous Forest Zone of Ghana (Taylor, 1952) and is characterized by a bimodal rainfall distribution. The major rainy season starts from March – July while the minor season starts from September – November. Generally, the area receives a mean annual rainfall of 1500 mm with an average monthly temperature range of 24 – 28 ºC. The soil is a sandy loam classified as Ferric Acrisol according to (FAO, 1990) equivalent to Typic Haplustult in the USDA (1998) soil classification system. The soil properties are shown on Table 4.1.

3.2. Experimental materials and sources

Plastic pots, each measuring 7857 cm^3, were filled with 7 kg each of top soil. Six cowpea varieties Asontem and Nhyira supplied by the Crops Research Institute (CRI)/Fumesua, Dan illa, IT96D-610, TN5-78 and TN88-63 from the National Agricultural Research Institute of Niger (INRAN) were used in the study. The characteristics of these varieties are shown on Table 3.1. The SSP (Single Super Phosphate) (18 % P_2O_5) fertilizer was applied at the rate of 100 kg/ha at planting by incorporating into the soil. This is equivalent to 5 g per pot.

3.3. Cowpea genotypes

Table 3.1 Characteristics of cowpea varieties used for the study

Variety	Source	Seed size	testa texture	Growth habit	Maturity	Yield (t/ha)
1.Asontem	Ghana	Small	Smooth	Erect	Early	3.00
2.Dan Illa	IITA	Small	Rough	Semi-erect	Medium	2.50
3.IT96D-610	IITA	Medium	Smooth to rough	Erect	Early	3.50
4.Nhyira	Ghana	Small	Smooth	Semi-erect	Medium	2.50
5.TN5-78	Niger	Small	Rough	Semi-erect	Medium	1.00
6.TN88-63	Niger	Small	Smooth	Semi-erect	Medium	3.00

3.4. Experimental design

A 2 x 6 Completely Randomized Design (CRD) with three replications was used in this study. A total of thirty six (36) pots were used out of which eighteen (18) were irrigated at four days interval until ten days after planting (10 DAP) after which water was withdrawn. The remaining eighteen (18) pots received water throughout the experiment (up to 35 DAP) and this served as the control. Experimental pots were arranged to obtain a planting distance of 50 cm x 25 cm.

3.5. Soil sampling and analyses

Soil samples were collected in a forest around the Soil Research Institute from 0 - 20 cm depth in July, 2011. The samples were bulked, air-dried, ground and passed through an 8 mm and 2 mm sieves for filling pots and analysis, respectively. The 2 mm bulk samples were stored in polythene bags for analysis later. The bulk soil sample was analyzed by standard laboratory procedures.

3.5.1. Soil pH

Soil pH was determined using the H1 9017 Microprocessor pH meter in a 1:1 suspension of soil and water. A 25 g soil sample was weighed into plastic pH tube to which 25 ml water was added from a measuring cylinder. The suspension was stirred frequently for 30 minutes. After calibrating the pH meter with buffer solutions at pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.5.2. Soil organic carbon

A modified Walkley and Black procedure as described by Nelson and Sommers (1982) was used in the determination of organic carbon. One gram of soil sample was weighed into an Erlenmeyer flask. A reference sample and a blank were included. Ten milliliters of 1.0 *N* (0.1667 *M*) potassium dichromate was added to the sample and the blank flasks. Concentrated sulphuric acid (20 ml) was carefully added to the soil from a measuring cylinder, swirled and allowed to stand for 30 minutes in a fume cupboard. Distilled water (250 ml) and 10 ml concentrated orthophosphoric acid were added and allowed to cool. A diphenylamine indicator (1 ml) was then added and titrated with 1.0 *M* ferrous sulphate solution.

Calculation:

The organic carbon content of soil was calculated as:

$$\%C = \frac{M \times 0.39 \times mcf\ (V1-V2)}{w}$$

Where:

M = molarity of ferrous sulphate

V1 = ml ferrous sulphate solution required for blank

V2 = ml ferrous sulphate solution required for sample

w = weight of air - dry sample in gram

mcf = moisture correcting factor (100 + % moisture) / 100)

0.39 = 3 × 0.001 × 100 % × 1.3 (3 = equivalent weight of carbon,

1.3 =compensation factor for incomplete oxidation of the organic carbon).

3.5.3. Percent organic matter (O.M)

This was obtained by multiplying the organic carbon value by a correlation factor (1.72) to convert it to percent organic matter as described by Landen (1991).

3.5.4. Total Nitrogen

This was determined by the Kjeldahl digestion and distillation procedure as described in Soils Laboratory Staff (1984). A 0.5 g soil sample was weighed into a Kjeldahl digestion flask. To this 5 ml distilled water was added. After 30 minutes, concentrated sulphuric acid (5 ml) and selenium mixture were added and mixed carefully. The sample was then digested for 3 hours until a clear digest was obtained. The digest was diluted with 50 ml distilled water and mixed well until no more sediment dissolved and allowed to cool. The volume of the solution was made to 100 ml with distilled water and mixed thoroughly. A 25 ml aliquot of the solution was transferred to the reaction chamber and 10 ml of 40 % NaOH solution added followed by distillation. The distillate was collected in 2.0 % boric acid and was titrated with 0.02 *N* HCl using bromocresol green as indicator. A blank distillation and titration was also carried out to take care of the traces of nitrogen in the reagents as well as the water used.

Calculation:

The % N in the sample was expressed as:

$$\% \text{ N} = \frac{\text{N x (a-b) x 1.4 x mcf}}{\text{w}}$$

Where:

N = concentration of HCl used in titration

a = ml HCl used in sample titration

b = ml HCl used in blank titration

w = weight of air-dry soil sample

mcf = moisture correcting factor (100 % + % moisture) /100)

1.4 = 14 × 0.001 × 100 % (14 = atomic weight of N).

3.5.5. Available Phosphorus (Bray's No.1 phosphorus)

The available phosphorus was extracted with Bray's Nº 1 extracting solution (0.03 *M*NH4F and 0.025 *M* HCl) as described by Bray and Kurtz (1945). Phosphorus in the extract was determined by the blue ammonium molybdate method with ascorbic acid as the reducing agent using a spectrophotometer.

A 5 g soil sample was weighed into a shaking bottle (50 ml) and 35 ml of extracting solution of Bray's Nº 1 added. The mixture was shaken for 10 minutes on a reciprocating shaker and filtered through a Whatman No. 42 filter paper. An aliquot of 5 ml of the blank, the extract, and 10 ml of the colouring reagent (ammonium molybdate and tartarate solution) were pipetted into a test tube and uniformly mixed. The solution was allowed to stand for 15 minutes for the blue colour to develop to its maximum. The absorbance was measured on a spectronic 21D spectrophotometer at a wavelength of 660 nm at medium sensitivity.

A standard series of 0, 1, 2, 3, 4 and 5 mgP/L was prepared from 20 mg/L phosphorus stock solution.

Calculation:

$$P\ (\text{mg/kg}) = \frac{(a-b) \times 35 \times 15 \times \text{mcf}}{w}$$

Where

a = mg/L P in sample extract

b = mg/L P in blank

mcf = moisture correcting factor

35 = ml extracting solution

15 = ml final sample solution

w = sample weight in gram

3.5.6. Determination of exchangeable bases (K, Ca, Mg, and Na)

Exchangeable bases (calcium, magnesium, potassium and sodium) in the soil were determined in 1.0 *M* ammonium acetate extract (Black, 1986) and the exchangeable acidity (hydrogen and aluminium) was determined in 1.0 *M* KCl extract (Page *et al.*, 1982).

A 5 g soil sample was weighed into a leaching tube and leached with 100 ml buffered 1.0 *M* ammonium acetate solution at pH 7.

To analyze calcium and magnesium, a 25 ml aliquot of the extract was transferred into an Erlenmeyer flask. To this were added 1 ml portion of hydroxylamine hydrochloride, 1 ml of 2.0 % potassium cyanide, 1 ml of 2.0 % potassium ferrocyanide, 10 ml ethanolamine buffer and 0.2 ml Eriochrome Black T solution. The solution was titrated with 0.01 *M* EDTA (ethylene diamine tetraacetic acid) to a pure turquoise blue colour.

A 25 ml aliquot of the extract was transferred into a 250 ml Erlenmeyer flask and the volume made up to 50 ml with distilled water. Following this, were added 1 ml hydroxylamine, 1 ml of 2.0 % potassium cyanide and 1 ml of 2.0 % potassium ferrocyanide solution. After a few minutes, 5 ml of 8.0 *M* potassium hydroxide solution and a spatula of murexide indicator were added. The resultant solution was titrated with 0.01 *M* EDTA solution to a pure blue colour.

Calculation:

The concentrations of calcium + magnesium or calcium were calculated using the equation:

$$\text{Ca + Mg (or Ca) (cmol/kg soil)} = \frac{0.01 \times (Va - Vb) \times 1000}{w}$$

Where

w = weight (g) of air – dried soil used

Va = ml of 0.01 *M* EDTA used in sample titration

Vb = ml of 0.01 *M* EDTA used in blank titration

0.01 = concentration of EDTA

Potassium (K) and sodium (Na) in the leachate were determined by flame photometry. A standard series of potassium and sodium were prepared by diluting both 1000 mg/l K and Na solutions to 100 mg/l. In doing this, 25 ml portion of each solution was taken into 250 ml volumetric flask and made up to the volume with distilled water. Portions of 0, 5, 10, 15, 20 ml of the 100 mg/l standard solution were put into 200 ml volumetric flasks. One hundred milliliters of 1.0 *M* NH4OAc solution was added to each flask and made to the volume with distilled water. This resulted in standard series of 0, 2.5, 5.0, 7.5, 10 mg/l for K and Na. Potassium and sodium were measured directly in the leachate by flame photometry at wavelengths of 766.5 and 589.0 nm respectively

Calculation:

$$\text{Exchangeable K (cmol/kg soil)} = \frac{(a - b) \times 250 \times mcf}{10 \times 39.1 \times w}$$

$$\text{Exchangeable Na (cmol/kg soil)} = \frac{(a - b) \times 250 \times mcf}{10 \times 23 \times w}$$

Where

a = mg/l K or Na in the diluted sample percolate

b = mg/l K or Na in the diluted blank percolate

w = weight (g) of air- dried sample

mcf = moisture correcting factor

The soil sample was extracted with unbuffered 1.0 *M* KCl solution for the determination of exchangeable acidity (Al^{3+} and H^+). Ten grams of soil sample was weighed into a 200 ml plastic bottle and 50 ml of 1.0 *M* KCl solution added. The mixture was shaken on a reciprocating shaker for 2 hours and filtered. An aliquot of 25 ml of the extract was pipetted into a 250 ml Erlenmeyer flask and 4 - 5 drops of phenolphthalein indicator solution added. The solution was titrated with 0.025 *N* NaOH until the colour just turned permanently pink. A blank was also included in the titration.

Calculation:

$$\text{Exchangeable acidity (cmol/kg soil)} = \frac{(a - b) \times M \times 2 \times 100 \times \text{mcf}}{w}$$

Where

a = ml NaOH used to titrate with sample

b = ml NaOH used to titrate with blank

M = molarity of NaOH solution

w = weight (g) of air-dried sample

2 = 50/25 (filtrate/ pipetted volume)

mcf = moisture correcting factor (100 + % moisture)/100

The effective cation exchange capacity (ECEC) was calculated by summation of exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+) and exchangeable acidity ($Al3^+$ and H^+).

The Percentage base saturation was obtained by dividing the total exchangeable bases (T.E.B) by the ECEC, and multiplied by 100.

3.6. Particle size analysis

Soil texture was determined by the hydrometer method (Boyoucos, 1962). A 50 g of air-dried soil was weighed into a measuring cylinder and 50 ml of calgon (sodium hexamethaphosphate) added. The suspension was shaken and allowed to stand. Corrected hydrometer readings at 40 seconds and 3 hours were taken.

Calculation:

$$\% \text{ sand} = 100 - [(A / W) \times 100]$$

$$\% \text{ clay} = 100 \times (B / W)$$

$$\% \text{ silt} = 100 - (\% \text{ sand} + \% \text{ clay})$$

Where

A= corrected hydrometer reading at 40 seconds

B = corrected hydrometer reading at 3 hours

W = weight of dry soil

The textural class was then determined from the textural triangle.

3.7. Soil bulk density

The mass (Mo) of an empty cylindrical core sampler of inner radius 2.5 cm and of height 5.0 cm, was determined on an electronic balance. The core sampler was used to take moist soil sample at a depth of 0 - 15 cm. The mass of the moist soil (Mt) was derived by subtracting the mass of empty core sampler (Mo) from the mass of empty core sampler (Mo) + mass of moist soil (Mt). The dry mass (Ms) of soil sample was determined (after drying the moist soil sample to equilibrium in an oven at 105 ºC) by subtracting mass of water (Mw) from Mt.

The volume (Vt) of soil sample taken was derived from the relation:

$$Vt = \pi r^2 h$$

Where

$\pi = 22/7$

r = inner radius (cm) of the cylindrical core sampler

h = height (cm) of the cylindrical core sampler

Dry bulk density (*Pb*) was then determined from the equation:

$$Pb\ (\text{g/cm3}) = \frac{\text{Mass of dry soil sample (Ms)}}{\text{Volume of soil (Vt)}}$$

3.8. Calibration of required volume of water to apply

Based on the optimum mean annual rain of 1,500 mm in the Deciduous Forest Agro-ecological zone, and the minimum rainy days of 150 days in the major season (Table 3.2), the moisture availability for cowpea [volume of water (cm^3) to apply] per day and per plant was calculated as follows:

Table 3.2: Rainfall distribution by agro-ecological zones in Ghana

		Growing Period (Days)	
Agro-ecological Zone	Mean annual Rain (mm)	Major season	Minor season
Rain Forest	2200	150-160	100
Deciduous Forest	1500	150-160	90
Transitional	1300	200-220	60
Coastal	800	100-110	50
Guinea savana	1100	180-200	**
Sudan savana	1000	150-160	**

Source: Meteorological Services Department, Accra Ghana. **= Records not available

$$\text{Available moisture per day} = \frac{1500\text{ mm}}{150\text{ days}} = 10\text{ mm/day} = 1\text{ cm/day}$$

Depth of water (θz) = Volumetric water content (θv) x depth of soil

$$\text{Depth of water } (\theta z) = \frac{\text{Volume of water x depth of soil}}{\text{Volume of soil (Volume of container)}}$$

$$\text{Volume of water per day} = \frac{\text{Depth of water } (\theta z) \text{ x volume of soil (volume of container)}}{\text{Depth of soil}}$$

But:

Depth of water (θz) = 1 cm

Volume of soil (volume of container) = Л r² h

$$= \frac{22}{7} \times (12.5)^2 \times 16$$

$$= 7857 \text{ cm}^3$$

Depth of soil (depth of container) = 16 cm

Therefore:

$$\text{Volume of water per day} = \frac{1 \text{ cm} \times 7857 \text{ cm}^3}{16 \text{ cm}}$$

$$= 491.06 \text{ cm}^3 \approx 491 \text{ cm}^3$$

But, to initially saturate the air dried soil to field capacity, a four day volume of water was considered. Thus:

Volume of water applied initially = 491 cm^3 x 4 = 1964 cm^3

A volume of 1964 cm^3 of water was applied initially to the soil in each pot and their individual masses were recorded. The pots were left for two days for the soil to settle before planting was done.

3.9. Planting and cultural practices

Three seeds each of the six cowpea genotypes were planted in 36 plastic pots (three seeds/pot) and seedlings were later thinned 7 days after planting (DAP) to one plant per pot. A total of 36 plants were therefore, obtained. Sowing was done on August 26th, 2011.

Plants were watered at four days interval until 10 days after planting (DAP) and 35 DAP respectively for water-stressed pots and the control. Prior to every irrigation, each pot was weighed and the weight differences (kg) were converted to volume (ml). The values obtained for each pot represented the volume of water applied to that particular pot at that period. The idea was to regain the initial soil moisture content at 4 days interval. Five grams of SSP (18 % P_2O_5) was applied per pot by incorporating into the soil at planting. In order to ensure a clean pot, hand weeding was done first at one week after planting and subsequently when necessary.

3.10. Data collection

Water was withdrawn ten days after planting (10 DAP) in the water-stress treatment but the control treatment continued to receive water until 35 DAP when the experiment was terminated. Data collection was then initiated. All data were determined on plants from which leaves had not been sampled for relative water content measurements

Relative water content (RWC)

During the period of moisture stress, 10 discs of leaf tissue of each genotype were taken using a cork borer with a diameter of 1.5 cm. The fresh weight was quickly measured, followed by flotation on distilled water for up to 4 hours. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 50 °C. RWC was then calculated according to Barrs (1968) as follows:

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$$

Where: FW (fresh weight), TW (turgid weight) and DW (dry weight).

Plant height

This was measured at four days interval until 35 DAP in order to assess plant growth. The first measurement was taken 17 DAP and at each sampling date, the height of each genotype was taken. Plant heights were measured from the base of the plant to the tip using a metallic measuring tape. Then, the average for each cowpea genotype was determined.

Number of leaves per plant

The number of leaves was recorded for each genotypes one week (17 DAP) after imposing the water stress. Then the average number was determined for each cowpea varieties.

Stem diameter

The stem diameter of each genotype was measured with a digital caliper at 1.5 cm above soil surface to the nearest millimeter. The first measurement was taken 17 DAP and at each sampling date, the diameter of the plants of each genotype was taken. After, then the average diameter of each genotype was calculated.

Leaf senescence

Plants were scored for leaf senescence on a scale of 0 - 10, dividing the percentage of estimated total leaf area that is dead by 10 as described by Bänziger *et al.* (2000), as follows:

1 = 10 % dead leaf area	6 = 60 % dead leaf area
2 = 20 % dead leaf area	7 = 70 % dead leaf area
3 = 30 % dead leaf area	8 = 80 % dead leaf area
4 = 40 % dead leaf area	9 = 90 % dead leaf area
5 = 50 % dead leaf area	10 = 100 % dead leaf area

The first scoring was done at 17 DAP when some of the leaves had initiated senescing till end of the experiment. This date was also appropriate for taking leaf senescence scores because an array of different leaf senescing levels could be observed. Scoring was done at four days interval on sunny days between 13:00 and 14:00 GMT on three occasions.

NB: The scoring was done only on droughted cowpea plants.

Biomass

At harvest, the biomass of one plant of each genotype in a replication excluding the roots was oven-dried at 72 °C to a constant mass and their masses were taken with an electronic balance.

Root dry mass

At harvest, roots were separated from the shoots and were gently removed from the soil mass. The roots were gently washed to remove all soil, and then dried at 72 °C to constant mass in order to get the dry mass.

Water Use (WU) and Water Use Efficiency (WUE)

Prior to every irrigation, each pot was weighed and the weight differences (kg) were converted to volume (ml). The values obtained for each pot represented the volume of water applied to that particular pot at that period. The average volume of the water used rate was determined for each genotype. The water use efficiency based on biomass was calculated according to Larcher (2003) as follow:

$$\text{WUE (g/kg)} = \frac{\text{Biomass (g/plant)}}{\text{Water used rate (kg/plant)}}$$

Drought susceptibility index (S)

Drought intensity based on biomass, water use efficiency, relative water content, plant height, number of leaves per plant, stem diameter and root dry mass was first determined using both water stressed and well-watered conditions. This was done for the six cowpea varieties separately. Subsequently, drought susceptibility index (*S*) based on relative biomass; water use efficiency (WUE) and relative water content (RWC) of water stressed to well-watered conditions were estimated. The following relations proposed by Fischer and Maurer (1978) were used:

$$D = 1 – X_s/Xw \qquad \text{[Equation 3.1]}$$

Then the drought susceptibility index (*S*) of individual varieties was calculated:

$$Y_s = Y_w\,(1 - SD) \qquad \text{[Equation 3.2]}$$

$$S = \frac{X_w\,(Y_w - Y_s)}{(X_w - X_s)Y_w} \qquad \text{[Equation 3.3]}$$

Where D = drought intensity

X_s = respective average yield under water stress condition

X_w = respective average yield under well-watered condition

Y_s = Individual yield under water stress condition

Y_w = Individual yield under well-watered condition

Varieties with average susceptibility to drought have an S value of 1.0. Values of S less than 1.0 indicate less susceptibility and greater tolerance to drought, with a value of S = 0.0 indicating maximum possible drought tolerance (no effect of drought on yield).

3.11. Data analysis

Data were subjected to ANOVA (Analysis of Variance) using Genstat statistical package 10th edition. Individual means of water-stressed genotypes were compared to their corresponding non-stressed in a pairwise comparison analyses (t-test) and LSD was used to determine differences in treatment means.

CHAPTER FOUR

4. RESULTS

4.1. Physical and chemical properties of the soil used

The physical properties of the soil used in the plant house at Kwadaso showed that the soil was silty loam (Table 4.2). The pH of the soil used was 5.59 which suggested a slightly acidic soil condition; also, a moderate value (2.21 %) was recorded for the percentage organic carbon and its corresponding organic matter (3.80 %). The soil used at the plant house contained again moderate values of total nitrogen (0.21 %) and potassium (2.77 cmol/kg), with low amount of phosphorus (8.94 cmol/kg).

4.2. Temperatures measured in the plant house

Temperature ranges in the plant house were from 24.5 °C to 30.1 °C and from 24.7 °C to 32.4 °C for the minimum and maximum temperatures, respectively. The highest minimum and maximum temperatures were recorded in the 4th week of October (Table 4.1).

Table 4.1: Weekly temperature measured in the plant house (September – October 2011)

	Temperature (°C)	
Week	Minimum	Maximum
1st week of September	24.5	24.7
2ndweek of September	27.8	28.3
3rd week of September	29.8	31.9
4th week of September	27.0	27.8
1st week of October	27.2	29.4
2nd week of October	29.5	31.5
3rd week of October	26.5	29.0
4th week of October	30.1	32.4

The largest difference between maximum and minimum over the period was 2.2 °C during the first week of October 2011.

Table 4.2: Physico-chemical properties of the soil used in the plant house experiment

Soil property	Kwadaso
Soil depth (cm)	0 – 20
pH (1:1 soil: H_20)	5.59
Organic carbon (%)	2.21
Organic matter (%)	3.80
Total N (%)	0.21
Available P (cmol/kg)	8.94
Exchangeable cations (cmol/kg)	
Ca^+	2.28
Mg^+	1.39
K^+	2.77
Na^+	0.55
Total exchangeable bases (cmol/kg)	7.19
Exchangeable acidity (Al + H) (cmol/kg)	0.75
Effective cation exchange capacity (cmol/kg)	7.93
Base saturation (%)	90.58
Bulk density (g/cm3)	1.64
Particle size (%)	
Sand	26.69
Silt	62.91
Clay	10.40

4.3. Effect of water stress on morphology and physiology of six cowpea varieties used in the plant house experiment

In the plant house experiment, the result from analysis of variance (ANOVA) on biomass, water use efficiency, relative water content, plant height, number of leaves, stem diameter, root dry mass, and leaf senescence suggested highly significant differences ($p < 0.01$) among cowpea genotypes, water regime and their interaction (Table 4.3) [Appendix 4.1- 4.8].

Table 4.3: Effects of cowpea genotypes, water treatment and their interaction on different morphological and physiological indicators used for the study.

				Effects		
Trait	Minimum	Maximum	Mean	G	WT	G x WT
Biomass (g)	0.56	10.12	4.47±3.60	*	*	*
Water use efficiency (g/kg)	0.97	35.25	13.08±12.30	*	*	*
Relative water content (%)	58.00	72.00	64.25±4.44	*	*	*
Plant height (cm)	57.00	115.0	84.25±14.70	*	*	*
Number of leaves	12.00	72.00	28.00±16.12	*	*	*
Stem diameter (cm)	0.28	0.62	0.43±0.12	*	*	*
Root dry mass (g)	0.21	2.20	0.79±0.70	*	*	*
Leaf senescence (%)	2.00	13.00	5.50±1.50	*	!!	!!

G= genotype, WT= water treatments, * = significance at 1%, G x WT= interaction, ± standard deviation, !!= measurement was made only on water-stressed cowpea genotypes

4.3.1. Effect of water stress on biomass, water use efficiency and relative water content

At the planthouse experiment, significant differences ($p < 0.01$) were observed for mean biomass of the six cowpea varieties subjected to water-stressed condition. Among the varieties, average biomass ranged from 9.99 g to 3.21 g for the control and form 2.20 g to 0.57 g for the water stressed varieties (Figure 4.1). Varieties TN88-63 and Asontem recorded the highest values of mean biomass under the control, while variety Dan illa was next to variety TN88-63 under water-stressed condition.

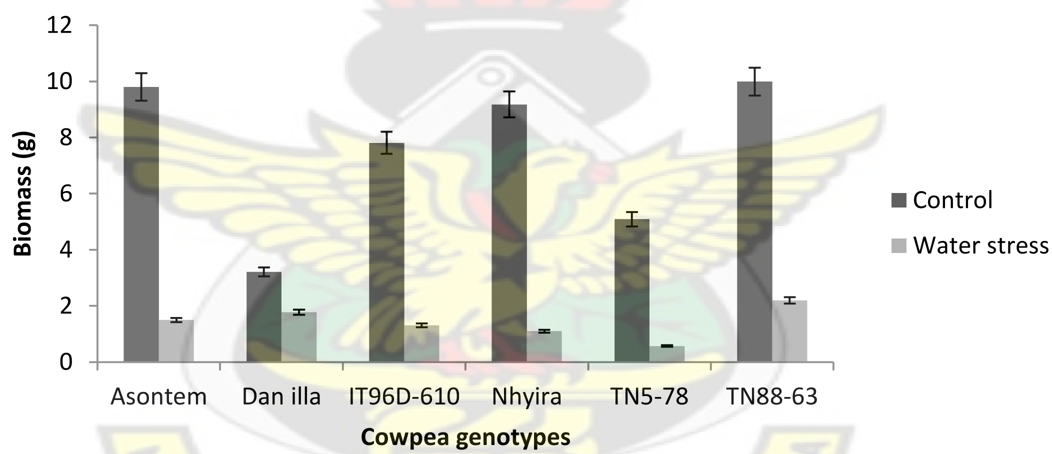

Figure 4.1: Effect of water stress on biomass of the six cowpea varieties used for the study

Highly significant differences ($p < 0.001$) were observed for water use efficiency. It was deduced from Figure 4.2 that the average water use efficiency ranged from 34 g/kg to 13 g/kg for the control and from 4 g/kg to 1 g/kg for the water-stressed varieties. The result indicated that variety TN88-63 relatively took the highest value of water use efficiency under water-stressed condition, while Nhyira had the highest value for the control. The variety TN5-78 consistently showed relatively least value of water use efficiency under water-stressed condition.

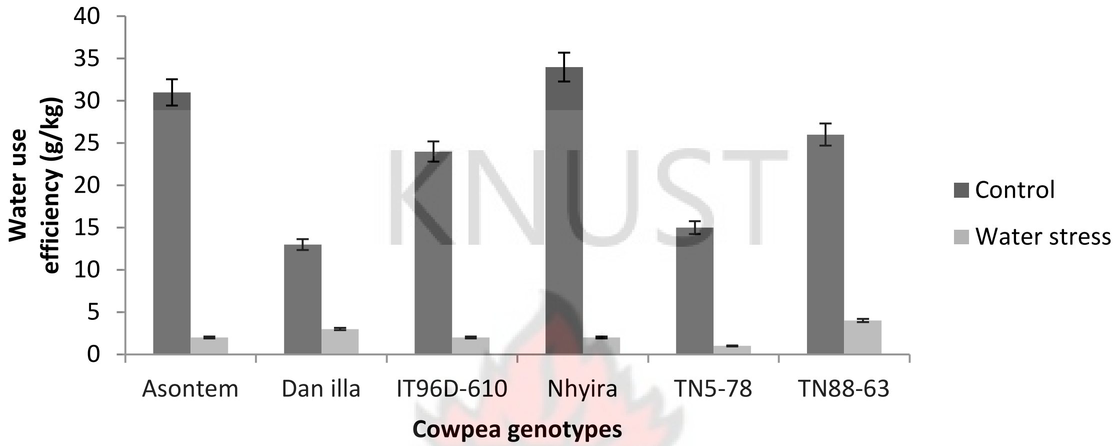

Figure 4.2: Effect of water stress on water use efficiency of the six cowpea varieties used for the study

For the relative water content, highly significant differences ($p < 0.01$) were observed among cowpea genotypes. Mean relative water content ranged from 71 % to 63 % for the control and from 65 % to 59 % for the water-stressed genotypes (Figure 4.3). The highest average values of relative water content were recorded by the three varieties TN88-63, TN5-78 and IT96D-610 under the control, while under the water-stressed condition variety Dan illa recorded the highest value.

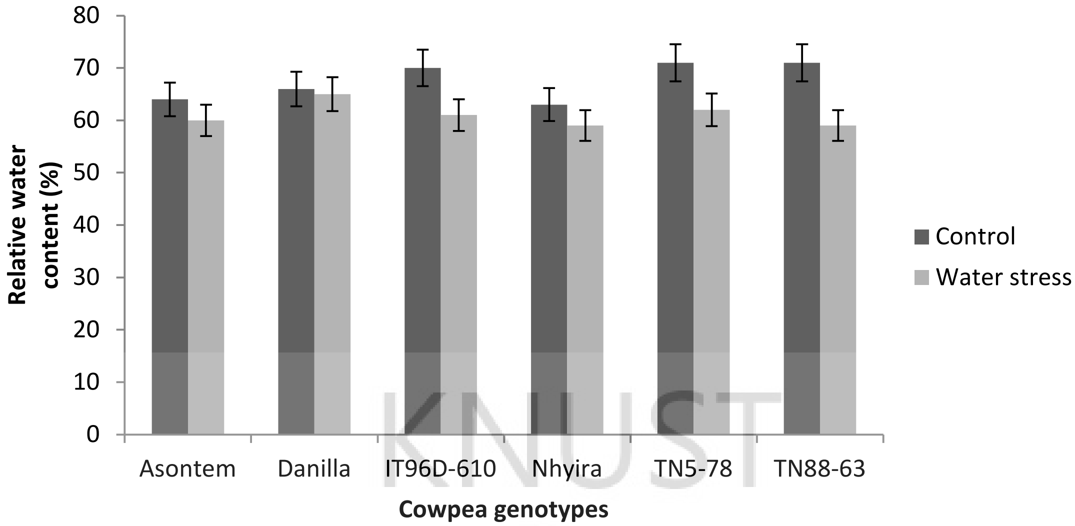

Figure 4.3: Effect of water stress on relative water content of the six cowpea varieties used for the study

4.3.2. Effect of water stress on plant height, number of leaves per plant and stem diameter

Average plant height ranged from 113 cm to 73 cm for the control and from 107 cm to 58 cm for the water stressed genotypes. The maximum value was recorded in the control which was the optimum condition (Figure 4.4). The results showed that variety TN88-63 had the highest plant height followed by Nhyira under the control while under the water-stressed condition, variety Dan illa was next to variety TN88-63.

Within the varieties, number of leaves per plant ranged from 71 to 20 for the control and from 31 to 13 for the water stressed genotypes (Figure 4.5). As expected, more leaves were observed in the control. The highest number was recorded by TN88-63, while the lowest number was recorded by IT96D-610 under water-stressed condition.

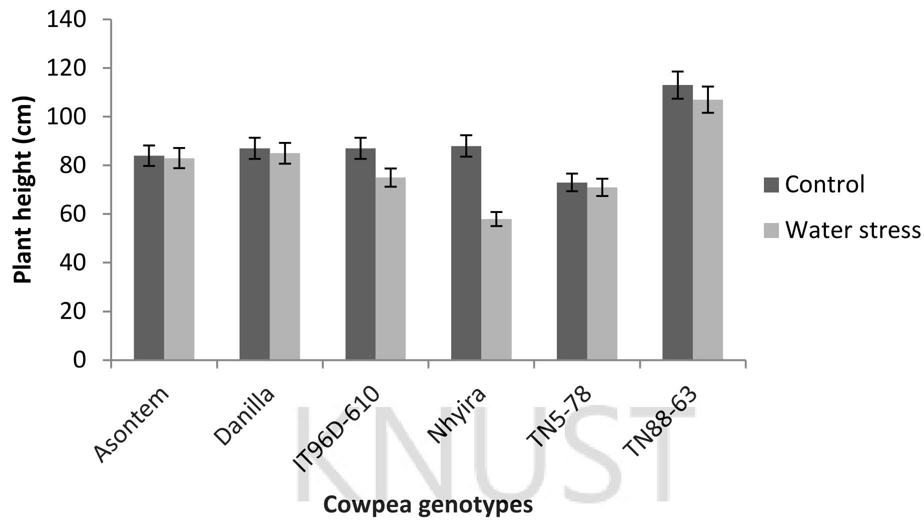

Figure 4.4: Effect of water stress on plant height of the six cowpea varieties used for the study

Mean stem diameter ranged from 0.60 cm to 0.41 cm for the control which formed the optimum condition and from 0.40 cm to 0.30 cm for the water-stressed cowpea genotypes. Variety TN88-63 relatively, recorded the highest value of stem diameter. Among the water-stressed genotypes TN5-78, Nhyira and Asontem gave the lowest values of stem diameter (Figure 4.6).

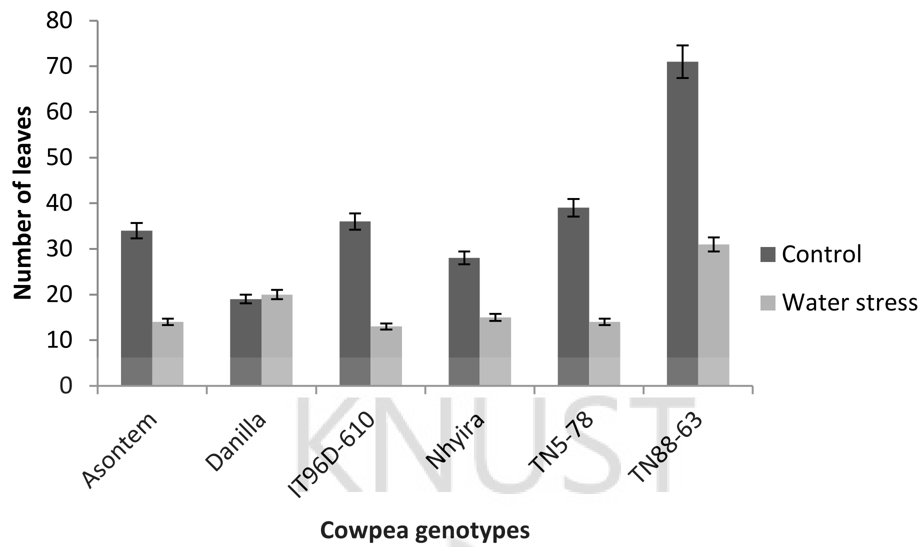

Figure 4.5: Effect of water stress on number of leaves per plant of the six cowpea varieties used for the study

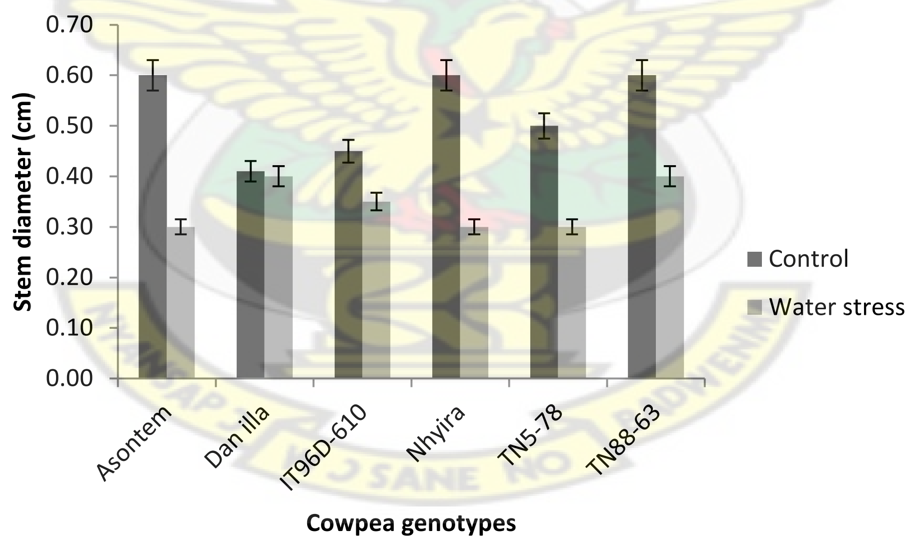

Figure 4.6: Effect of water stress on stem diameter per plant of the six cowpea varieties used for the study

4.3.3. Effect of water stress on root dry mass

Figure 4.7 shows that mean root dry mass ranged from 2.08 g to 0.52 g for the control and from 0.59 g to 0.22 g for the water-stressed cowpea genotypes. Variety TN88-63 proved the most outstanding for root dry mass both under control and water-stressed condition. On the other hand, varieties TN5-78 and Nhyira recorded the lowest value for root dry mass under water-stressed condition.

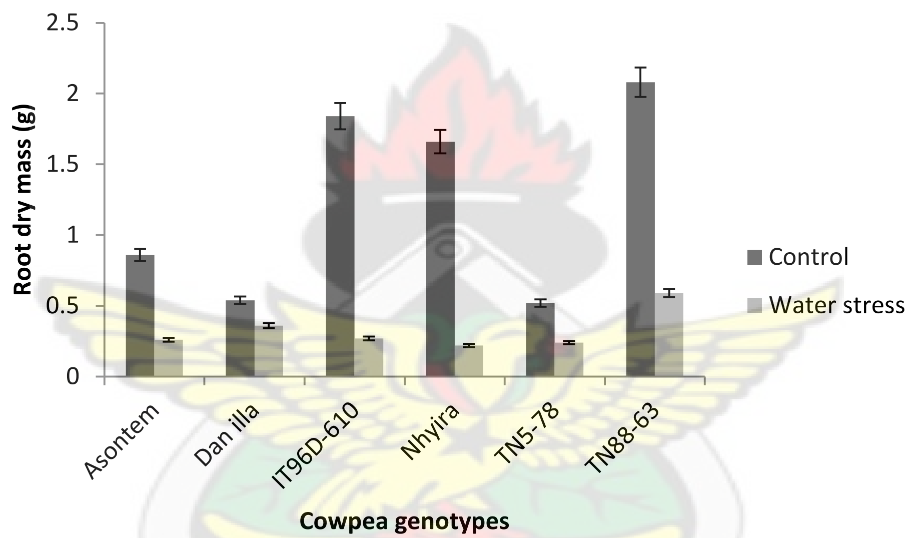

Figure 4.7: Effect of water stress on root dry mass of the six cowpea varieties used for the study

4.3.4. Leaf senescence of water-stressed cowpea genotypes

Highly significant differences ($p < 0.01$) exist among cowpea varieties for leaf senescence. Mean leaf senescence ranged from 6 % to 2 %. The highest value of 6 % was recorded for TN5-78, while the least value of 2 % was recorded equally for Asontem and IT96D-610 (Figure 4.8).

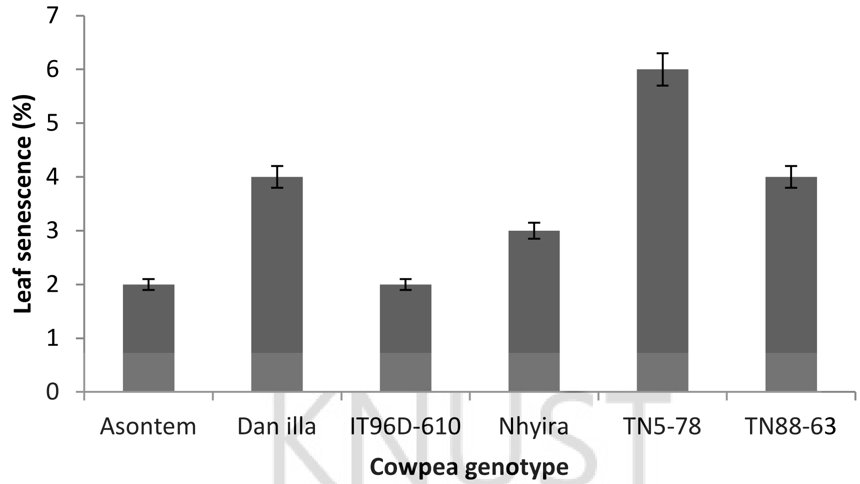

Figure 4.8: Leaf senescence of water-stressed cowpea genotypes

Furthermore and more importantly, the mean values of the water-stressed cowpea genotypes were compared to their corresponding non-stressed plants using pairwise comparison analysis. Cowpea genotypes that showed no significant difference between the control and the corresponding water-stressed genotypes may have the potential of drought tolerance. Those that showed significant differences when both conditions were compared individually may be apparently susceptible to drought. Plate 4.1 shows cowpea varieties under the stressed (left) and non-stressed (right) conditions at the plant house.

Cowpea varieties under water stress | Cowpea varieties under optimum condition

Plate 4.1: Cowpea varieties under the stressed (left) and non-stressed (right) conditions at the plant house

4.4. Pairwise comparison of means for biomass, water use efficiency and relative water content of the water-stressed and non-stressed cowpea varieties

Within the varieties, all genotypes (Asontem, Dan illa, IT96D-610, Nhyira, TN5-78 and TN88-63) showed highly significant differences ($p < 0.01$) in biomass production after twenty-five (25) days of water stress (Table 4.4). Compared to the control, the water-stressed cowpea genotypes showed significant differences. Only variety Dan illa under water-stressed condition showed continuous growth very much close to that of the control for the whole period of water stress. Under the water deficit TN88-63 and Dan illa recorded the highest values of 2.20 g and 1.78 g respectively, while the least value of 0.57 g was recorded by TN5-78. The same trend was observed under the control where Asontem was next to TN88-63 with biomass production of 9.80 g and 9.99 g, respectively.

The result indicated that there were significant differences ($p < 0.01$) among the varieties for water use efficiency. All genotypes failed to show no significant differences between the water-stressed and the control conditions. Under water-stressed condition Dan illa and TN88-63 did not show any significant difference, while under the control significant differences were observed among the varieties for water use efficiency. Under the control, Nhyira recorded the highest value of 34 g/kg followed by Asontem 31 g/kg for water use efficiency, while under the water-stressed condition TN88-63 and Dan illa recorded the highest values of 4 g/kg and 3 g/kg, respectively. The least value of 1 g/kg for water use efficiency was recorded by the variety TN5-78 after 25 days of water stress (Table 4.4).

With reference to relative water content, it was observed that after twenty-five (25) days of water-stressed condition, only Dan illa within the cowpea varieties did not show any significant difference from its control. This variety may have the potential of drought tolerance for relative water content. The remaining varieties did not show any significant differences under water- stressed condition. TN88-63 and TN5-78 recorded equally the same value of 71 % which was the highest under the control (Table 4.4), while under the water-stressed condition, TN88-63 and Nhyira equally recorded lower values of 59 %.

4.5. Pairwise comparison of means for plant height and number of leaves of the water-stressed and non-stressed cowpea varieties

The result indicated that, after twenty-five (25) days of water stress, varieties Asontem, Dan illa and TN5-78 did not show any significant differences compared with the control. These varieties may probably have the potential of drought tolerance for plant height. Significant genetic variations were observed among the cowpea varieties under the control and water-

Table 4.4: Effect of water stress on biomass, water use efficiency, relative water content and plant height

	Biomass (g)		Water use efficiency (g/kg)		Relative water content (%)		Plant height (cm)	
Variety	WS	Ctrl	WS	Ctrl	WS	Ctrl	WS	Ctrl
Asontem	1.50±0.01	9.80±5.0	2.00±0.03	31.00±16	60.00±2.0	64.00±2.0	83.00±1.0	84.00±2.0
Dan illa	1.78±0.03	3.21±1.0	3.00±0.04	13.00±5	65.00±0.5	66.00±1.0	85.00±2.0	87.00±2.0
IT96D-610	1.31±0.01	7.81±4.0	2.00±0.02	24.00±12	61.00±0.3	70.00±5.0	75.00±3.0	87.00±7.0
Nhyira	1.10±0.01	9.18±4.0	2.00±0.01	34.00±18	59.00±0.0	63.00±3.0	58.00±1.0	88.00±17
TN5-78	0.57±0.01	5.09±3.0	1.00±0.04	15.00±8	62.00±1.0	71.00±5.0	71.00±2.0	73.00±1.0
TN88-63	2.20±0.01	9.99±4.0	4.00±0.06	26.00±12	59.00±2.0	71.00±6.0	107.0±1.0	113.00±4.0
LSD (0.05)	0.07		1.94		2.07		2.84	
CV (%)	16		14		21		19	

CV= co-efficient of variation, ± standard deviation, WS= water stress, Ctrl= control,

stressed conditions. TN88-63 recorded the highest value of 107 cm which was significantly different from Dan illa and Asontem which were, also different from IT96D-610, TN5-78 and Nhyira, with 85 cm and 83 cm, 75 cm, 71 cm and 58 cm, respectively under water-stressed condition. The same trend was observed under the control where TN88-63 recorded the highest value of 113 cm, while the least value of 73 cm was recorded by TN5-78 (Table 4.4).

With this indicator, significant genetic differences exist among cowpea varieties for number of leaves under the control and water-stressed conditions. Variety Dan illa did not show any significant variation from its control after twenty-five (25) days of water stress. This may probably be due to its potential to withstand water stress, an important feature for drought adaptation. Under water-stressed condition (Table 4.5), TN88-63 recorded the highest number of 31, which was different from Dan illa (19), also significant different from Nhyira (15), Asontem (14), TN5-78 (14) and IT96D-610 (13), while under the control TN88-63 again recorded the highest value of 71, which was significantly different from TN5-78 (39), also different from Asontem (34), IT96D-610 (36), Nihyira (28) and Dan illa (20).

Table 4.5: Effect of water stress on number of leaves per plant, stem diameter, root dry mass and leaf senescence

	Number of leaves		Stem diameter (cm)		Root dry mass (g)		Leaf senescence (%)
Variety	WS	Ctrl	WS	Ctrl	WS	Ctrl	Water stress
Asontem	14.00±1.0	34.00±11	0.30±0.03	0.60±0.2	0.26±0.01	0.86±0.3	2.00±1.0
Dan illa	19.00±2.0	20.00±1.0	0.40±0.02	0.41±0.02	0.36±0.02	0.54±0.1	4.00±2.0
IT96D-610	13.00±1.0	36.00±13	0.35±0.02	0.45±0.05	0.27±0.04	1.84±1.0	2.00±1.0
Nhyira	15.00±1.0	28.00±7.0	0.30±0.02	0.60±0.2	0.22±0.01	1.66±1.0	3.00±0.0
TN5-78	14.00±1.0	39.00±14	0.30±0.0	0.50±0.1	0.24±0.02	0.52±0.2	6.00±1.0
TN88-63	31.00±0.0	71.00±22	0.40±0.01	0.60±0.1	0.59±0.02	2.08±1.0	4.00±0.0
LSD (0.05)	2.43		0.03		0.07		0.18
CV (%)	5.2		7.50		4.90		2.9

CV= co-efficient of variation, ± standard deviation, WS= water stress, Ctrl= control.

4.6. Pairwise comparison of means for stem diameter and root dry mass of the water-stressed and non-stressed cowpea varieties

After twenty-five (25) days of water stress, variety Dan illa did not show any significant variation for stem diameter compared with its corresponding treatment under the control. Varieties Dan illa and TN88-63 recorded equally the highest value of stem diameter of 0.40 cm which was significantly different from IT96D-610 (35 cm), also different from Asontem, Nhyira and TN5-78 with 0.30 cm both under water-stressed condition, while under the control the highest value of 0.60 cm was equally recorded by Asontem, Nhyira and TN88-63, which was significantly different from TN5-78 (0.50 cm), also different from IT96D-610 and Dan illa which recorded 0.45 cm and 0.40 cm, respectively (Table 4.5).

Within the varieties, all genotypes (Asontem, Dan illa, IT96D-610, Nhyira, TN5-78 and TN88-63) showed significant variation in root dry mass under the control compared to their corresponding treatment under water-stressed condition. Variety TN88-63 recorded the highest value of 0.59 g, which was significantly different from Dan illa (0.36 g), and also different from Asontem (0.26 g), IT96D-610 (0.27 g), TN5-78 (0.24 g) and Nhyira (0.22 g) under water-stressed condition, while the same variety TN88-63 recorded the highest value of 2.08 g which was significantly different from the other counterparts under the control (Table 4.5).

4.7. Relationship between biomass and water use efficiency

In the planthouse experiment, there was highly significant ($p < 0.01$) correlation between biomass and water use efficiency. Within the varieties, a correlation co-efficient of 0.96 was obtained which indicated a strong positive relationship between biomass and water use efficiency. Increased water use efficiency resulted in increased biomass. A co-efficient of determination (r^2) of 0.92 was observed among the varieties implying that about 92 % of the variation in biomass was explained by its association with water use efficiency (Fig. 4.10). Variety with high water use efficiency may therefore have high biomass production and vice-versa.

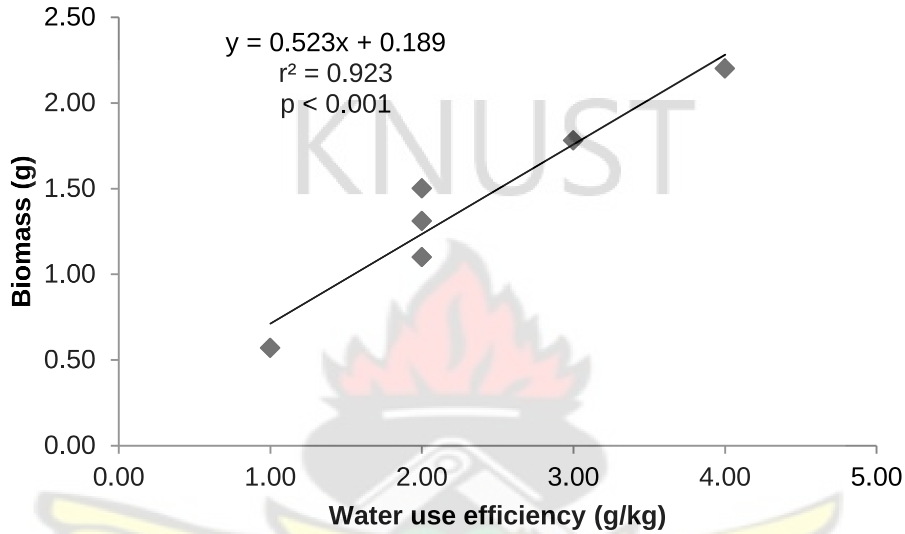

Figure 4.9: Relationship between biomass and water use efficiency of water-stressed cowpea genotypes

4.8. Relationship between biomass and root dry mass

A highly significant correlation ($p < 0.01$) with a strong positive relationship ($r = 0.82$) was observed between biomass and root dry mass (Figure 4.11). A co-efficient of determination (r^2) of 0.703 was obtained for the varieties under water stress. This suggests that about 70.3 % of the variation in biomass could be attributed to root dry mass. Biomass of the varieties increased with increasing root dry mass and vice-versa.

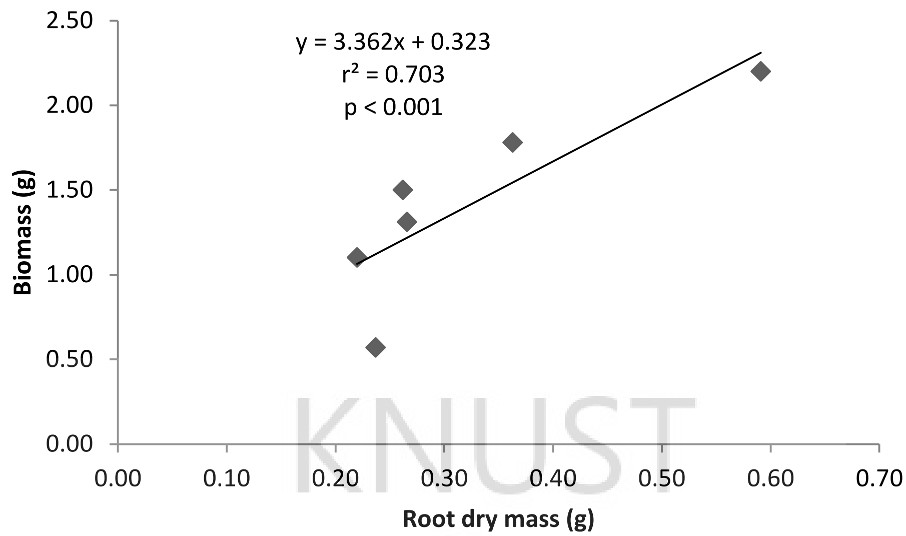

Figure 4.10: Relationship between biomass and root dry mass of water stressed cowpea genotypes

4.9. Relationship between water use efficiency and number of leaves per plant

Figure 4.12 shows that, number of leaves per plant had significant correlation ($p < 0.01$) with water use efficiency. Cowpea varieties had a strong positive relationship between water use efficiency and number of leaves per plant with a correlation co-efficient (r) of 0.90. A corresponding co-efficient of determination (r^2) of 0.82 was found implying that about 82 % of the variation in water use efficiency may be attributed to its association with number of leaves per plant. Again, increased water use efficiency was found to be associated with increased number of leaves per plant and vice-versa.

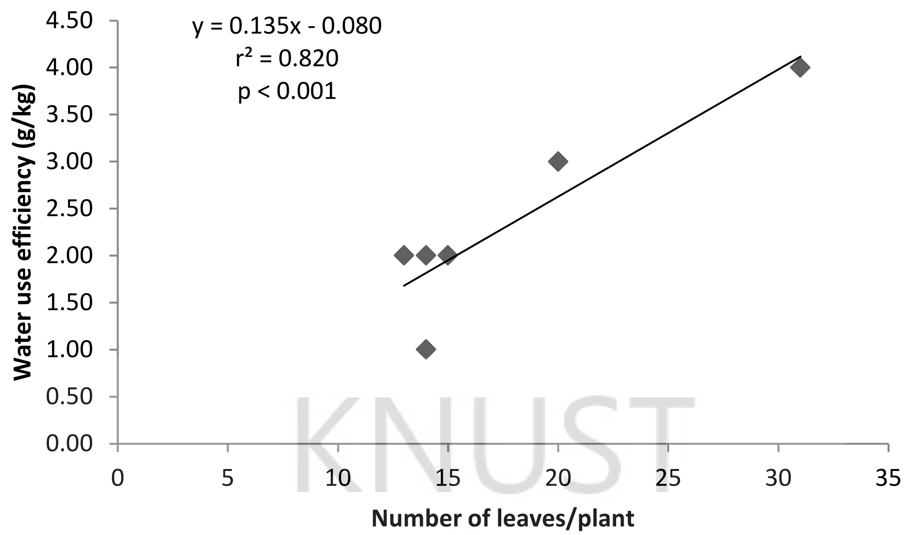

Figure 4.11: Relationship between water use efficiency and number of leaves per plant of water stressed cowpea genotypes

4.10. Drought intensity and drought susceptibility index (*S*)

The drought intensity (D) was calculated for biomass, water use efficiency, relative water content, plant height, number of leaves per plant, stem diameter and root dry mass (Table 4.5) using equation 4.1. Then the drought intensities for these indicators were subsequently used for the calculation of drought susceptibility index (*S*) (equation 4.3) for the six cowpea varieties evaluated for their drought tolerance in the planthouse (Table 4.6).

Table 4.6: Drought intensity of the six cowpea varieties used for this study

	Drought intensity						
Traits	BM	WUE	RWC	NL	PHT	SD	RDM
D	0.80	0.90	0.10	0.50	0.10	0.30	0.70

D: drought intensity, BM: biomass, WUE: water use efficiency, RWC: relative water content, PHT: plant height, NL: number of leaves, SD: stem diameter, RDM: root dry mass.

Among the varieties, water use efficiency followed by biomass recorded higher values of drought intensity, while relative water content and plant height recorded the lowest value. The drought susceptibility index of these indicators was used for the selection and ranking the cowpea genotypes for their drought tolerance. The drought index based on these parameters ranged from 0.10 to 1.63 (Table 4.6).

Table 4.7: Drought susceptibility index for the six cowpea varieties used for the study.

Variety	BM	WUE	RWC	NL	PHT	SD	RDM
Asontem	1.04	1.04	0.79	1.11	0.12	1.43	0.94
Dan illa	0.55	0.85	0.19	0.10	0.24	0.10	0.45
IT96D-610	1.02	1.02	1.63	1.21	1.44	0.64	1.15
Nhyira	1.08	1.04	0.80	0.88	3.56	1.43	1.17
TN5-78	1.09	1.03	1.60	1.21	0.29	1.15	0.73
TN88-63	0.96	0.94	2.14	1.07	0.55	0.95	0.97

BM: biomass, WUE: water use efficiency, RWC: relative water content, PHT: plant height, NL: number of leaves, SD: stem diameter, RDM: root dry mass.

Selection and ranking of the six cowpea varieties were done based on a correlation between their relative performance with respect to these parameters (biomass, water use efficiency, relative water content, plant height, number of leaves, stem diameter and root dry mass) under water-stressed condition and their respective drought indices obtained. The result indicated negative relationship between parameters and drought indices for the six cowpea varieties under water-stressed condition (Appendix 4.9), implying that the drought susceptibility indices for these parameters could be a good tool for selecting drought tolerant cowpea varieties.

Table 4.8: Scoring and ranking of cowpea genotypes based on drought susceptibility indices of the seven morpho-physiological parameters under water-stressed condition.

Variety	BM	WUE	RWC	PHT	NL	SD	RDM	Total score	Ranking
Asontem	3	2	5	3	6	2	4	25	2
Dan illa	6	6	6	6	5	6	6	41	1
IT96D-610	4	4	2	2	2	5	2	21	4
Nhyira	2	2	4	5	1	2	1	17	6
TN5-78	1	3	3	2	4	3	5	21	4
TN88-63	5	5	1	4	3	4	3	25	2

BM: biomass, WUE: water use efficiency, RWC: relative water content, PHT: plant height, NL: number of leaves, SD: stem diameter, RDM: root dry mass.

The scale made was as follows:

42 - 35: drought tolerance

35 – 25: Moderatly tolerant

< 25: Susecptible

The six cowpea genotypes were ranked according to their tolerance level to water stress. The scoring was done in such a way that genotype with lowest value of drought susceptibility index was scored number six (6), the following genotype was scored five (5), till to the highest index which was scored 1. The rule subjectively adopted for the ranking in the plant house was that any genotype that recorded drought index value from 42 to 35 may be considered to be tolerant to drought, from 35 to 25 may be considered moderately tolerant and lastly, less than 25 may be considered susceptible to drought stress. The following ranking was therefore obtained for the six cowpea varieties in decreasing order of drought tolerance; Dan illa > Asontem = TN88-63 > IT96D-610 = TN5-78 > Nhyira. Dan illa was relatively the most tolerant variety, while Asontem and TN88-63 showed relatively moderate drought tolerance. Varieties TN5-78 and Nhyira showed apparent susceptibility to drought. Therefore, from the planthouse studies, varieties Dan illa, Asontem and TN88-63 could be used as sources for drought tolerance in a cowpea breeding programme in the future.

Similar trend was observed, while ranking the varieties based on the relative percentage of each parameter determined by the ratio of individual performance of a variety under water stress to that of the control (Table 4.8). The rule adopted for ranking was the same, but on the contrary here the most tolerant genotype was that one which recorded high value of relative percentage, while the most susceptible was the one which recorded the lowest value (Table 4.9). Therefore, with respect to the relative percentage, the following ranking in decreasing order was obtained; Dan illa > TN88-63 > Asontem > IT96D-610 > TN5-78 > Nhyira. Here again, Nhyira and TN5-78 showed their apparent susceptibility to drought.

Table 4.9: Relative percentage of the seven morpho-physiological parameters used for study

	Relative percentage (%)						
Variety	BM	WUE	RWC	PHT	NL	SD	RDM
Asontem	15	6	94	99	41	50	30
Dan illa	55	23	98	98	95	98	67
IT96D-610	17	8	87	86	36	78	15
Nhyira	12	6	94	66	54	50	13
TN5-78	11	7	87	97	36	60	46
TN88-63	22	15	83	95	44	67	28

BM: biomass, WUE: water use efficiency, RWC: relative water content, PHT: plant height, NL: number of leaves, SD: stem diameter, RDM: root dry mass.

Table 4.10: Scoring and ranking of cowpea genotypes based on the relative percentage of the seven morpho-physiological parameters used for the study.

Variety	BM	WUE	RWC	PHT	NL	SD	RDM	Total score	Ranking
Asontem	3	2	5	6	3	2	4	25	3
Dan illa	6	6	6	5	6	6	6	41	1
IT96D-610	4	4	4	2	2	5	2	23	4
Nhyira	2	2	5	1	5	2	1	18	6
TN5-78	1	3	4	4	2	3	5	22	5
TN88-63	5	5	3	3	4	4	3	27	2

BM: biomass, WUE: water use efficiency, RWC: relative water content, PHT: plant height, NL: number of leaves, SD: stem diameter, RDM: root dry mass.

CHAPTER FIVE

5.0. DISCUSSION

5.1. Soil physico-chemical properties

The pH of the soil used at the plant house was 5.59 which suggested a slightly acidic soil condition; although the soil was silty loam, the chemical properties of the soil used in the plant house was relatively good according to the soil manual given by Landen (1991). Moderate level of organic carbon (2.21 %) and its corresponding organic matter (3.82 %) were recorded. Also, moderate level of total nitrogen (0.21 %) and potassium (2.77), whereas low amount of phosphorus (8.94 cmol/kg) [8 < 8.94 < 20 cm0l/kg] were obtained from the soil used for the plant house study.

The inherent capacity of the soil used in the plant house as regards cowpea production can be said to be better, and this might have a strong bearing on the conditions to which this soil was subjected to prior to its use for the experiment. The soil was obtained from woodland savanna vegetation where the area has not been cultivated. The dark color of the soil implied that there was adequate time for organic matter decomposition and hence availability of other important nutrients such as N, P and K.

5.2. Temperature in the plant house

The maximum temperature recorded from 24.73 to 32.4 °C (Table 4.2) in the plant house could have caused yield reductions if plants were allowed to grow to maturity as reported by Hall (2004) that high night temperatures that commonly occur in the tropics can cause male sterility and substantially reduce grain yield of cowpea by increasing floral abscission and decreasing the number of pods/plant. Male sterility, as induced by high night temperature, is mainly due to lack of anther dehiscence which results from incomplete pollen development. Also, Ismail *et al.* (1997) stated that if sowing is too early, however, and the soil is cooler than 19 °C, chilling damage can cause slow and incomplete emergence in cowpea. On the other hand, some studies have reported that drought greatly exacerbates the effects of heat stress on plant growth and photosynthesis (Xu and Zhou, 2005; 2006).

5.3. Biomass production

Water deficit occurs when water potentials in the rhizosphere are sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development. On a global basis, it is a major cause limiting productivity of agricultural systems and food production (Boyer, 1982). In this study, water stress significantly reduced above ground biomass resulting in low biomass in severe water-stressed genotypes (25 days of water deficit). Relative reduction in biomass was more significantly ($p < 0.01$) pronounced in TN5-78 and Nhyira, with 89 % and 88 % respectively, while the least relative reduction was recorded by Dan illa (45 %) (Figure 4.1), as compared with the control plants. The result confirms the findings of Lu *et al.* (1999) while identifying the specific physiological mechanisms at the whole-plant and cellular levels responsible for drought resistance in barley. The authors reported that when subjected to -0.4 MPa root water deficit, the shoot growth in cv. Mona (on the basis of dry weight) decreased by 85.2 %, as compared with the control plants; while the shoot growth in Wadi Qilt 23-39 was significantly less inhibited (74.8 %) by the same root water deficit. The results of this study suggested that the effect of drought was severe to reduce leaf area and stem growth reducing ability of the crops to intercept solar radiation. This observation agrees with the findings of Prabhu and Shivaji (2000) who reported that the main effect of drought in the vegetative period was to reduce leaf, so that the crop intercepts less sunlight. It also, supports a report by Vianello and Sobrado (1991) who also reported that drought stress during the vegetative stage caused diminution of growth in maize crop leaves and stems. The mechanisms underlying drought-tolerance strategy in Dan illa, appeared to be related to the higher ability of osmotic adjustment because when plants are subjected to drought stress, a number of physiological responses are expressed (Ludlow and Muchow, 1990; Fukai and Cooper, 1995). In some cultivated cereals, osmotic adjustment has been found to be one of the most effective physiological mechanisms underlying plant tolerance to water deficit (Turner and Jones, 1980; Morgan, 1984; Blum, 1988; Zhu *et al.*, 1997). Osmotic adjustment, as a process of active accumulation of compatible osmolytes in plant cells exposed to water deficit, may enable a continuation of leaf elongation, though at reduced rates (Turner, 1986).

5.4. Water use efficiency

Drought stress significantly decreased water use efficiency. Cowpea under control recorded higher values of water use efficiency compared to their corresponding water-stressed genotypes. The variety Nhyira, recorded the highest value of 34 g/kg followed by Asontem and TN88-63 which recorded respectively 31 g/kg and 26 g/kg under the control treatment, while among the stressed-cowpea plants variety TN88-63 recorded the highest value of 4 g/kg, followed by Dan illa 3 g/kg. TN88-63 and Dan illa proved to be relatively drought tolerant variety. This may be probably due, according to Blum (2005), to their ability to reduce their water use, which is reflected in higher water use efficiency, and is generally achieved by plant traits (e.g. small plant size, small leaf area, reduced growth) and environmental responses that reduce yield potential. This result suggests that greater biomass production under water stress was associated with relatively low water use and greater water use efficiency as seen in TN88-63 and Dan illa. This observation agrees with the findings of Cordon *et al.* (2002), who compared the yield performance of two wheat genotypes differing in water use efficiency as defined by $\Delta^{13}C$, at two sites in Eastern Australia, differing in rainfall frequency, such that while one site was supplied with rain water the other site experienced prolonged drought for much of the season, relying only on stored soil water for crop production. Interestingly, under drier condition, the high water use efficient genotype realized relatively a higher yield than the corresponding genotype with low water use efficiency. But conversely, at the well-watered site the genotype experiencing higher water use efficiency realized a relatively poor yield, compared to the genotype with lower water use efficiency. The ability for crop plants to limit water use and transport, may be probably due to their osmotic adjustment within roots, because as soil water declines, it may provide an adaptive response to sustain root water uptake potentials to such an extent that the hydraulic driving force for water uptake and transport through the plant can be maintained (Turner and Jones, 1980). However, this value of osmotic adjustment has been challenged (Munns, 1998), particularly in relation to its suitability as a desirable trait in breeding programmes. He argues that genotypes expressing significant osmotic adjustment are likely to divert carbohydrates away from related processes, resulting in drought tolerance genotypes with low growth rates and poor biomass realization. In those cases, however where reduced water availability results in reduced rates of transpiration and sustained biomass accumulation, water use efficiency will be significantly increased. On the contrary, Munoz *et al.* (1998) documented that high yield potential and high yield under water-limited conditions are generally associated with reduced water use efficiency mainly because of high water use. Features linked to low yield potential, such as smaller plants (Martin *et al.*, 1999) or short growth duration (Lopezcastaneda and Richards, 1994); ascribe high water use efficiency because they reduce water use. Dehydration avoidance as achieved by enhanced capture of soil moisture by roots has been found to be associated with low water use efficiency in such diverse species as rice (Kobata *et al.*, 1996) and Ponderosa pine (Zhang *et al.*, 1997). On the other hand, reduced transpiration in rice (Kobata *et al.*, 1996) and reduced evapotranspiration in sorghum (Tolk and Howell, 2003) were associated with higher water use efficiency.

5.5. Relative water content

With this indicator, significant variations ($p < 0.01$) were observed among cowpea varieties. Higher relative water content of 71 % was recorded equally by TN5-78 and TN88-63 under the control, while Dan illa followed by TN5-78 and IT96D-610 conserved much more water in their leaves under water stress, with respective values of 65 %, 62 % and 61 %. High percentages recorded by these three genotypes under water stress give an indication that they were relatively able to maintain better plant water status within the water deficit period (osmotic adjustment), to extract deep soil moisture (root capacity) and to reduce transpiration via stomatal closure, as a water-saving mechanism. This shows that Dan illa, TN5-78 and IT96D-610 might not have only tolerated the drought but also might have avoided the drought as defined by Fisher and Sanchez (1979) and also Otoole and Chang (1979) that avoidance of drought is the ability of a plant to maintain relatively high water status despite the low moisture condition within the entire environment. The result agrees with the findings of Kumar *et al.* (2008) while screening and selecting cowpea genotypes for drought tolerance at early stages of breeding reported that the differences among the genotypes in leaf water potential (LWP) and relative water content at 1330 h were substantially large and significant. At 1330 h, genotypes CP6, CP4 and CP5 maintained highest (> 90 %) while genotypes CP12, CP14, CP16, and CP13 had the lowest relative water content (< 80 %). Higher relative water content may be maintained either by developing a leaf water potential gradient from the soil to plant as displayed by CP6, CP7, CP8, CP9, CP11 and CP19 or by reduced water loss from the plant organs as displayed by genotypes CP5, CP10 and CP4. The former genotypes had higher ability to extract moisture at low soil water content due to reduced leaf water potential which contributed to the maintenance of higher relative water content (Omae *et al.*, 2005). In cowpea also, osmotic adjustment had been found to be responsible in preventing the detrimental effects of drought in leaves (Sumithra *et al.*, 2007). On the other hand, the later genotypes maintained higher leaf water potential and relative water content perhaps due to reduced transpiration, as reported by Larbi and Mekliche (2004), that in situations of water stress, durum wheat lost much more water than bread wheat, whereas in the maximal evapotranspiration (MET) situation there were no differences.

5.6. Plant height

Plant height observed for the six cowpea varieties in this study was significantly higher in TN88-63 (113 cm), Nhyira (88 cm), IT96D-610 (87 cm) and Dan illa (87 cm) for the non-stressed plants, whereas TN88-63 (107 cm) followed by Dan illa (85 cm) and Asontem (83 cm) recorded the highest values under water-stressed condition. This result agrees with the findings of Onuh and Donald (2009) who reported that the highest mean plant height (117 cm) was observed from the cowpea plants that received 500 ml of water treatment, which was significantly different from the 47 cm; mean plant height observed from plants grown under rainfed condition. This was attributed to the physiological stress occasioned by the limiting water supply. According to the annual report of the Science Daily (2008), plants growing under water limiting condition tend to grow taller in an effort to scramble for nutrients around the growth environment.

On the other hand, mean plant height of Asontem, Dan illa and TN5-78 was not significantly different for the non-stressed compared to that of water-stressed conditions. Perhaps, this may be probably due to their relative tolerance to drought, and this group falls, as reported by Mai-Kodomi *et al.* (1999) into “Type 1” or “Type 2” drought tolerant lines. The “Type 1” reaction of drought tolerant lines like TVu11986 and TVu11979 which stopped growth after the onset of drought stress and maintained uniformity, but displayed a declining turgidity in all tissues of the plants including the unifoliates and the emerging tiny trifoliates for over two weeks, as seen in TN5-78. All plant parts such as the growing tip, unifoliates and epicotyl gradually died almost at the same time. But in contrast, Asontem and Dan illa fall into the ‘‘Type 2’’ drought tolerant lines like the same Dan illa and Kanannado which remained green for a longer time and continued slow growth of the trifoliates under drought stress. With continued moisture stress, the trifoliates of this variety started wilting as well and died about 4 weeks after drought stress started, as seen in Asontem and Dan illa. 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). Also, Mai-Kodomi *et al.* (1999) concluded 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.

5.7. Number of leaves per plant

Drought stress significantly decreased number of leaves per plant. There were significant differences among cowpea varieties. Variety TN88-63 recorded the highest value of 71 under the control while variety Dan illa recorded the least value of 20 for number of leaves per plant. Under the water-stressed condition, TN88-63 and Dan illa recorded the highest values of 31 and 19, respectively. These present results are consistent with previous study on cowpea, Hayatu and Mukhtar (2010) who reported that the results for specific leaf area at final harvest showed that, increases in specific leaf area (SLA) under both moderate and severe water stress were recorded in IT00K-835-45 and IT98K-819-118. The highest reduction was recorded in IT98K-555-1 under moderate and severe water stress. Similar findings were reported by Samson and Helmut (2007) in cowpea that water deficit reduced significantly the total leaf area and total dry matter. Variety Dan illa did not show any significant difference from the control compared to that of the water-stressed condition. This implies that this variety had the characteristics of plant adapted to water-limited environments, reduced plant size, leaf area and leaf area index (LAI) which are major mechanism for moderating water use and reducing injury under drought stress (Mitchell *et al.*, 1998). Also, reduced growth duration is associated with reduced leaf number (Blum, 2004). Blum and Arkin (1984), reported that in some drought adapted crop plants, typically sorghum, older leaves are selectively killed under stress while the remaining young leaves retain turgor, stomatal conductance, and assimilation, as a result of high osmotic adjustment in the younger leaves, while under favorable growth conditions upper leaves (above nodes 8 to 9) show a greater area at unfolding, attain a greater final area and exhibit higher expansion rate than the lower leaves, whereas, variation in the duration of expansion is less systematic (Karamanos, 1976; Dennett *et al.*, 1979). Many aspects of plant growth are affected by drought stress (Hsiao, 1973), including leaf expansion, which is reduced due the sensitivity of cell growth to water stress. Water stress also reduces leaf production and promotes senescence and abscission (Karamanos, 1980), resulting in decreased total leaf area per plant. Reduction in leaf area reduces crop growth and thus biomass production. Seed production, which is positively correlated with leaf area (Rawson and Turner, 1982), may also be reduced by leaf area reductions induced by drought stress.

5.8. Stem diameter

Stem diameter relatively significantly decreased by 50 % in Asontem and Nhyira, 40 % in TN5-78, 33 % in TN88-63, 22 % in IT96D-610 and lastly 2.43 % in Dan illa under the water-stressed condition, compared to the control. Variety Dan illa, which recorded the least reduction in stem diameter exhibits a relatively tolerance to drought. This perhaps may be due, to its initially ability to survive under extreme drought conditions and can respond against the latter drought, and this was mainly achieved by slowing growth and reducing transpiration, as stated by Vianello and Sobrado (1991) that drought stress during vegetative stage provides diminution of the growth in maize crop leaves and stems. This result matches with the findings of Omae *et al.* (2007) who reported that by the dry treatment, cowpea plants reduced their stem diameter and fresh plant weight by 32 % and 81 %, respectively. In terms of adaptation for cowpea varieties to drought stress, they also reported that all cultivar/strains changed their morphological features to adapt to the extreme drought, decreasing leaf areas, holding trifoliate leaves to gravitational direction, and turning leaf color to deep green like Dan illa. These changes indicated that the plants were exposed to severe drought. Results of the discriminate analysis, on the other hand, indicated that the drought-tolerant strain/cultivars might increase their stem size under non drought-stressed conditions (as seen in TN88-63) and, thus, could avoid from the drought effects such an assumption could be led by highly accurate classification in control, high and low temperature regimes, contrasted by a poor classification in dry treatment. Itani *et al.* (1992) also supported this assumption, mentioning that the values of transpiration rate differed in the two cowpea cultivars before drought but no difference in transpiration rate with decreased drought situations in xylem potential.

Chaves *et al.* (2002) argued that water deficit can cause reproductive failure. To avoid this some Mediterranean annuals exhibit phenological drought avoidance, meaning that they flower and produce seed before water supplies are exhausted. Others can resist drought-spells by accumulating reserves in different organs, normally stems and roots, prior to drought; the reserves are then remobilized during the reproductive phase. This is a well-known adaptive response to water deficit which has been documented in cereals (Austin, 1977; Palta, 1994; Gebbing, 1999), and was also observed in the Mediterranean native *Lupinus albus* (Rodrigues *et al.*, 1995).

5.9. Root dry mass

Significant variations were observed among cowpea varieties for root dry mass. Water stress relatively reduced root dry mass form 87 % to 33 % under drought stress, as compared to the control. The highest values of root dry mass were recorded by TN88-63 in both the control and water-stressed conditions with 2.08 g and 0.59 g respectively. The genotypic variation among cowpea varieties for root dry mass subjected to water deficit may be attributed to the differences in root morphology and growth. TN88-63 had the ability to develop deep and extensive rooting system, in order to enhance water and nutrient uptake under water-stressed condition. These results concur with that of Alyemeny (1998) in *Vigna ambacensis L.* that water stress results in significant reduction in stem dry weight and increased root length. Increase in root biomass in water-stressed genotypes may be due to ability of the cowpea to divert assimilates to enhance the growth of the roots so as to exploit deeper parts of the soil water. Also, it was later shown by White and Kirkegaard (2010) that root contact as driven by extensive root branching and long root hairs is a prime determinant of moisture extraction from dry soil. But in contrast, Matsui and Singh (2003), while investigating the root characteristics in cowpea related to drought tolerance suggested that drought tolerance of IT96D-604 is related neither to root dry matter nor root length, but due to the downward shift in root distribution. Consequently, IT96D-604 displayed survival under water-stressed conditions by maintaining a high water-absorption capacity indicated by root dry matter per unit leaf area.

Hall (1993) argued that assuming a cowpea genotype is developed with deeper roots than a local cultivar which would have greater grain yield potential in a water-limited environment, the deeper roots would enable the genotype to access more soil water and this would have the following benefits: the genotype could have a longer reproductive period, and through indirect effects on canopy conductance and canopy growth, greater daily transpiration and photosynthesis, and possibly greater nitrogen fixation than the local cultivar. However, according to Passioura (1982), roots of a particular depth would only be adaptive if they access adequate soil moisture at times when the water considerably influences grain yield. Since rainfall varies widely from year to year in semiarid climatic zones, any useful variation in trait expression would increase the extent of adaptation. For example, deeper roots would be more adaptive in years where adequate moisture is available deeper in the profile. In a dry year, when only the upper layers of the soil profile are moist, a more superficial system would be adaptive.

5.10. Leaf senescence

There was significant variation among cowpea varieties in the leaf duration under water-stressed condition. Variety TN5-78 scored the highest value of 6 %, which was significantly different from Dan illa and TN88-63 (4 %). The least value of 2 % was scored equally by Asontem and IT96D-610. This variation among cowpea varieties may be probably due to the ability to maintain green leaf duration and high relative water content in water-limited condition as seen in Asontem and IT96D-610, compared to the sensitive variety (TN5-78) with greater score for leaf senescence (6 %). This result confirms previous results of numerous studies conducted in rainfed and irrigated conditions between the durum wheat and bread wheat varieties (Deumier, 1987; Gate *et al.*, 1992; Mekliche, 1992). This was probably due to the osmotic adjustment by accumulation of solutes such as sugars, or by a good regulation of the stomatal conductance. Also, Di Fozon *et al.* (2000) reported that genetic variation exists for foliar senescence and genotypes and plants with leaves which remain green for longer periods than normal are defined as "stay-green". The result also, agrees with the findings of Belko *et al.* (2012) who reported that leaf senescence caused by drought stress varied across cowpea genotypes under both glasshouse and field conditions, and several cowpea genotypes preserved stem and leaf greenness more than others. Drought tolerant Mouride, Suvita 2, IT84S-2049, and IT97K-499-39 kept greener (lower scores) than sensitive Bambey 21, IT82E-18, IT97K-556-6, and UC-CB46 (higher scores).

According to Gwathmey *et al.* (1992) and Gwathmey and Hall (1992), another important morphological trait that may contribute to drought adaptation of cowpea is a delayed leaf senescence [DLS] under water stress, which would enhance plant survival after a mid-season drought and limit damages to the first flush of pods. Cultivars with delayed leaf senescence also have enhanced production of forage because their leaves remain green and attached to the plant until harvest.

5.11. Relationship between morphological and physiological indicators (biomass, water use efficiency, number of leaves per plant and root dry mass)

Since dehydration avoidance was achieved mainly, by enhanced capture of the soil moisture (deep rooting system) and reduced transpiration via stomatal closure, the question was then whether these observations could lead to high yield in cowpea plants under water-limited environment. Under water-stressed condition, biomass, water use efficiency, number of leaves per plant and root dry mass were indeed closely related with highly significant positive relationship. The interpretation is that greater biomass was relatively associated with low water use, and greater water use efficiency in one side and in the other side water deficit contributes to a significant reduction in leaf area, so as to reduce water loss through transpiration with immediate consequence of decreasing in photosynthesis because the crop plant intercepts less radiation. Also, the crop plant under water-limited condition tends to divert assimilates to root growth in order to capture deep soil moisture. For example, reduced plant size, leaf area, and leaf area index (LAI) are a major mechanism for moderating water use and reducing injury under drought stress (e.g. Mitchell *et al.*, 1998). Often, crop cultivars bred for water-limited environments by selection for yield under stress have a constitutively reduced leaf area. Pathways for constitutive reduction in plant size and leaf area are smaller leaves, reduced tillering, and early flowering. Reduced growth duration is associated with reduced leaf number (Blum, 2004). This observation agrees with the findings of Spollen *et al.* (1993) who reported that water stress led to stimulation of root growth and the suppression of shoot growth.

A reduction in transpiration can be achieved by reducing net radiation by way of reflection, namely increasing crop albedo. Various plant-surface structures allow an increase in albedo (e.g. Holmes and Keiller, 2002). Epicuticular wax or plant glaucousness reduces cuticular conductance and reflects incoming radiation at the ultra-violet (UV) and 400 – 700 nm wavelengths to the extent that leaf temperature and transpiration are reduced without a reduction in stomatal conductance. This is expressed in greater water use efficiency (WUE) for the glaucous genotype (Premachandra *et al.*, 1994). Also, Blum (2005) concluded that for conditions where high water use efficiency (WUE) is an advantage because it is a marker for low water use, selection for the preferred plant type can be done by directly selecting for small plant size, small leaf area, or reduced growth duration rather than by using the more expensive selection criterion of water use efficiency WUE by way of carbon isotope discrimination.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The three cowpea varieties Dan illa, TN88-63 and Asontem are recommended for use as sources for developing drought tolerance in cowpea breeding programmes based on their impressive performance under water-stressed condition in the plant house experiment.

Variety Asontem with its moderate tolerance to drought is recommended for use as source for developing delayed leaf senescence in cowpea breeding programme which is an important mechanism because it can enhance drought adaptation of early cowpea cultivars by enabling them to produce a greater second pod flush if the first flush is damaged by drought. Also, their genetic studies demonstrated that combining the delayed leaf senescence and heat tolerance traits could breed cowpea cultivars with enhanced yield stability.

Biomass, water use efficiency, relative water content, plant height, number of leaves, stem diameter and root dry mass are useful, reliable, cheaper and rapid indicators to identify and select drought tolerant cowpea genotypes using drought intensity and susceptibility index.

6.2. Recommendations

The six cowpea varieties should be tested in the field in order to evaluate their real performance,

Future studies should include biochemical analysis in addition to morpho-physiological evaluation and at molecular level, in order to get a better understanding of mechanisms responsible for drought tolerance in cowpea genotypes.

Future studies should also include large number of genotypes to increase genetic variation to the greatest extent possible.

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APPENDICES

APPENDIX 4.1: ANOVA for biomass

Variate: BM (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	66.479000	13.295800	8486.68	<.001
Water_regim	1	335.256100	335.256100	2.140E+05	<.001
Variety.Water_regim	5	54.122600	10.824520	6909.27	<.001
Residual	24	0.037600	0.001567		
Total	35	455.895300			

APPENDIX 4.2: ANOVA for water use efficiency

Variate: WUE (g/kg)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	547.250	109.450	82.36	<.001
Water_regim	1	4160.250	4160.250	3130.68	<.001
Variety.Water_regim	5	533.250	106.650	80.26	<.001
Residual	24	31.893	1.329		
Total	35	5272.643			

APPENDIX 4.3: ANOVA for relative water content

Variate: RWC (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	146.250	29.250	19.48	<.001
Water_regim	1	380.250	380.250	253.29	<.001
Variety.Water_regim	5	128.250	25.650	17.09	<.001
Residual	24	36.030	1.501		
Total	35	690.780			

APPENDIX 4.4: ANOVA for plant height

Variate: PLH (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	5723.250	1144.650	401.93	<.001
Water_regim	1	702.250	702.250	246.58	<.001
Variety.Water_regim	5	931.250	186.250	65.40	<.001
Residual	24	68.350	2.848		
Total	35	7425.100			

APPENDIX 4.5: ANOVA for number of leaves per plant

Variate: NL

Source of variation	d.f.	s.s.	m.s	v.r.	F pr.
Variety	5	4043.000	808.600	388.13	<.001
Water_regim	1	3600.000	3600.000	1728.00	<.001
Variety.Water_regim	5	1386.000	277.200	133.06	<.001
Residual	24	50.000	2.083		
Total	35	9079.000			

APPENDIX 4.6: ANOVA for stem diameter,

Variate: SD (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	0.0500000	0.0100000	28.57	<.001
Water_regim	1	0.3025000	0.3025000	864.29	<.001
Variety.Water_regim	5	0.1025000	0.0205000	58.57	<.001
Residual	24	0.0084000	0.0003500		
Total	35	0.4634000			

APPENDIX 4.7: ANOVA for root dry mass and

Variate: RDM (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	4.357700	0.871540	581.03	<.001
Water_regim	1	7.728400	7.728400	5152.27	<.001
Variety.Water_regim	5	3.115700	0.623140	415.43	<.001
Residual	24	0.036000	0.001500		
Total	35	15.237800			

APPENDIX 4.8: ANOVA for leaf senescence

Variate: Ls

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	37.82500	7.56500	756.50	<.001
Residual	12	0.12000	0.01000		
Total	17	37.94500			

APPENDIX 4.9: Correlation matrix (parameter under water-stressed condition vs. drought susceptibility index)

	BM	BMS	NL	NLS	PHT	PHTS	RDM	RDMS	RWC	RWCS	SD	SDS	WUE	WUES
BM	1													
BM*S*	**-0.52**^{ns}	1												
NL	0.781	-0.273	1											
NL*S*	-0.363	0.926	**-0.14***	1										
PHT	0.829	-0.352	0.834	-0.076	1									
PHT*S*	-0.255	0.356	-0.221	0.073	**-0.65**^{ns}	1								
RDM	0.833	-0.327	0.977	-0.127	0.915	-0.348	1							
RDM*S*	-0.096	0.762	-0.115	0.689	-0.262	0.66	**-0.14**^{ns}	1						
RWC	-0.086	-0.784	-0.205	-0.703	-0.011	-0.469	-0.126	-0.863	1					
RWC*S*	-0.792	0.56	0.418	0.754	0.375	-0.086	0.45	0.444	**-0.53**^{ns}	1				
SD	-0.148	-0.739	0.731	-0.527	0.74	-0.329	0.808	-0.386	0.357	0.094	1			
SD*S*	0.839	0.86	-0.196	0.676	-0.322	0.345	-0.311	0.598	-0.792	0.226	**-0.80***	1		
WUE	0.325	-0.505	0.894	-0.379	0.804	-0.148	0.909	-0.102	-0.085	0.131	0.853	-0.396	1	
WUE*S*	-0.322	0.945	-0.553	0.83	-0.556	0.389	-0.593	0.721	-0.65	0.3	-0.882	0.839	**-0.69**^{ns}	1

NS= non significant, *= significant at 5% level of probability, *S*= drought susceptibility index.
BM= biomass, WUE= water use efficiency, RWC= relative water content, PHT= plant height, NL= number of leaves per plant, SD= stem diameter, RDM= root dry mass.