CHAPTER ONE

1.0 INTRODUCTION

Vigna unguiculata (L) Verdc commonly called cowpea is a member of the family Leguminaceae, and is characterized by possessing special kind of fruit pods that open along two sutures when ripe. Cowpea was formerly included in the genus *Phaseolus* but was separated to a new genus; *Vigna* because of the possession of stipules that are appendaged, and that the thickened part of the style is less twisted (Hill, 1994). Cowpea is the largest and most essential of the three main cultivated species of legumes; *Phaseolus, Pisum* and *Vigna.* There are nearly 11,000 known species of plants under the legume family and most of these are used as medicinal and industrial, and as food. They are said to be next in importance to cereals as sources of human food and contain more protein materials than any other vegetable product in Africa (FAO, 2000). Proteins occur as small granules in the same cells as the starch grains. The high protein content is correlated with the presence in the root of many legumes of tubercles containing nitrogen -fixing bacteria (www.en.wikipedia.org/wiki/vigna, 2007).

The cowpea is scientifically classified as follows:

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledonae

Super-order: Magnoliidae

Order: Fabales

Family: Leguminaceae

Sub Family: Papillionatae (Lotoideae)

Tribe: Phaseoleae

Genus: *Vigna*

Species*: unguiculata* (www.en.wikipedia.org/wiki/vigna, 2007)

Cowpeas have different growing habits namely: erect, semi-erect and creeping. The plants mostly show treading at certain stages of their growth and they possess fruit pods of varying sizes and shapes. The leaves are variable large, and show dark to dark green colours. The plants exhibit both self and cross pollinations, and may be determinate or indeterminate in growth. The rate of leaf appearance is dependent on temperature and the plants have stems that branch at most nodes except the cotyledonary and the first leaf node (Stephen, 2006).

The pattern of tissues in the plant is a typical example of that seen in most dicotyledons. There is an outer epidermal strip layer that encloses the delicate inner tissues consisting of cortical cells; collenchyma and parenchyma, and the vascular systems made up of the phloem, cambium, xylem and their associated cells. The vascular system of the stem consists of a network of interconnected leaf traces and the vascular supply to the leaf was described as emerging around tree gaps of leaf traces and dividing into five main bundles and several minor ones before extending through the petiole to the leaf. There is a unique layer of paraveinal mesophyll that extends horizontally between the vascular bundles in the lower layer of the palisade parenchyma (Stephen, 2006).

Cowpeas grow over a wide range of soil types provided the soils are moist or well drained. The plants do not tolerate high salinity and temperatures. They are however, moderately tolerant to acidity, pH 5.5-6.5. The availability of soil moisture has great impact on the growing pattern of the plants and this includes the girth and height of plants, flowering and the fruiting yields (Ahedor, 1996).

Drought stress is among the important environmental factors that affect plant growth in the tropics as other climatic factors have little variation. Drought occurs frequently in the arid and semi-arid regions and may be due to erratic start or early cessation of rainfall regimes. Plants may develop adaptive mechanisms to forestall inhibitions to their developmental and reproductive growth stages that may be posed by the limiting water factor. The mechanisms may be morphologic, physiologic or anatomic, and may be expressed innately by the inherent genes or as a temporal modification due to the presence of unfavorable environmental condition. Plants do also survive drought by avoidance as in ephemerals.

Cowpeas have great recoverability against water stress and have no critical phase in development, thus resulting in the overlapping nature of their ability to branch profusely even through late vegetative development and reproductive phases (Souza, 2000). Fruit yield in cowpeas under water stress was investigated by Ahedor (1996) and he concluded that the number and sizes of the flowers and fruits reduced under increasing severity of drought. Though works have been done on the effect of water stress on morphology, physiology and fruit yield in cowpeas, little literature can be found on the effect of water stress on the anatomical features of the plant.

1.1 OBJECTIVES

The objective of this work is to determine the effects of different water treatments on the growth of six accessions of cowpea from three agro-ecological zones in Ghana, and to find out which of the accessions will be suitable for cultivation under water deficit conditions.

1.1.1 SPECIFIC OBJECTIVES

(i) To assess the variations in the morphological organs of the different accessions under normal irrigation and water stressed conditions.

(ii) To assess the variations in the anatomical structures of the different accessions normal irrigation and water stressed conditions.

(iii) To assess the maturation (in terms of flowering) of the different accessions under water stressed conditions.

(iv) To assess the amount of fruits (bean pods) produced by the different accessions under normal irrigation and water stressed conditions.

1.2 JUSTIFICATION

Drought is a problem to agriculture in Sub-Saharan Africa. Desertification is taking over most of the region as a result of climate change and the inadequate supply of water to plants. Though most plants thrive well in areas where there is adequate water, some accessions of the plants may also, thrive well under water deficit conditions. The introduction of water deficit irrigation farming (DI) to Africa by the Food and Agriculture Organization (www.en.wikipedia,org/wiki/Deficit irrigation, 2009) buttress the need for studies into plants that have the ability to grow well under water deficit conditions.

Cowpeas are arable crop being grown widely by peasant farmers of Africa, especially in the Sub-Saharan region. There are many accessions of cowpeas but the ordinary farmers do not make effort to find out about the water stress adaptability of the type of cowpea seeds they acquire before cultivating them. Some accessions may tolerate water deficit conditions while many of them will not grow well in such condition. There is thus, heavy loss in both manpower and harvests during the faming periods. Hence, there is the need to investigate the response of the accessions of cowpeas that are available to farmers to enable them make proper decisions before selecting the cowpea seeds for planting; especially in the drier ecological regions.

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN AND DISTRIBUTION

Cowpeas are diverse and well distributed over the globe. It was suggested that the centers where great diversity of different species of the cowpea were found could be the primary source or origin. Places like India, Africa and Persia were primarily cited though it was later observed that cowpeas were known during the sanskritic times (Hill, 1994). Cowpeas were also thought to have originated from Arabia and were disseminated to the Nile and Niger basins by the Mohammedan caravans. Cowpea is presently accepted to have originated from the West African region, the Niger basin where a lot of wild and weedy species abound in the savannah and forest zones. It is distributed among the wild, weedy and cultivated forms, and ethno-botanical evidence supports such claim (Stephens, 2006). The importance of cowpeas as food and animal forage has made it one of the most cultivated crops by peasant farmers in the Sub- Saharan region of Africa (www.en.wikipedia.org/wiki/longyard, 2009).

2.2 PRODUCTION AND IMPORTANCE

Official statistics placed global cowpea production at 1.4 million metric tones of dry seeds per year in Africa (FAO, 2000). However, unreported yields from kitchen gardens and inaccurate reporting as [dry bean] may have under-estimated real production by as much as fifty percent (50%) or more. This suggests an equivalent of more than two million tones annually. Cowpeas contribute about 13-17% of available food pulses in the world. Africa however, produces 75% of the world's cowpea; Nigeria 58.6%, Burkina

Faso 5.6%, Uganda 3.6%, Niger 2.5% and Senegal 2.2%. Seed yields are however, low at only 0.35 t/ha with many countries reporting between 0.15 – 0.2 t/ha (FAO, 2000).

Cowpeas have many uses as a vegetable and as a pulse. It provides food for million of people and also serve as feed for a vast array of livestock. The tender green leaves and the whole seedling are used as potherbs. Cowpeas have relatively high level of protein while the pulses (dry seeds) form a major storage of protein. It has a nutrient value of 24% protein, 62% soluble carbohydrate and small amount of other nutrients. The true digestible cowpea protein was found to be 85% as compared to the 95% for albumen. The cowpea protein was however, free from metabolites and other toxic principles (Ackroyd and Walker, 1995).

2.3 SOIL TYPES AND GROWING CONDITIONS

Cowpeas are grown over a wide range of soil types. The plants prefer well drained or moist soils but not waterlogged soils. Loamy soils are noted for the optimum seed yields. Heavy clays and soils of high fertility usually result in high yields of hay, but poor seed yield. Cowpea does not tolerate high salinity though it is reasonably tolerant to acidity, 5.5 – 6.5 (Stephen, 2006). The plants grow best in semi-arid and low humid regions of tropical Africa, South-East Asia and Central America. They prefer a temperature range of 25-38^{o}C, and may survive up to 45^{o}C if adequate soil moisture level is maintained. The plants are known to thrive better in warm and moist soil moisture conditions. It was found to be sensitive to very cold conditions and exhibit optimum growth characteristics at day temperature of 37^{o}C (www.en.wikipedia.org/wiki/vigna , 2007).

2.4 WATER STRESS AND PLANT GROWTH

Water stress affects many processes that are involved in plant growth. The processes are mostly anatomical, physiological and morphological. The processes affect cell turgor, stomata movements and leaf expansions which have direct influence on the growth and fruiting of plants (Singh and Rachie, 1985) established that water availability is the major factor in determining yield and variations in yield of temperate pastures.

Reduction in plant sizes is associated with soil moisture stress though legumes are said to have high recoverable growth rate. The roots of legumes contribute to the survival of the plants during drought conditions through a greater water capturing potential offered by their greater root mass (Davies and Zhang, 1991). However, the advantages of such strategy to deal with shot term soil moisture deficit may not be readily apparent.

Experiments by Souza (2004) indicated a significant reduction in seedling biomass, stem diameters and root to shoot ratio in relation to water stress in legumes whereas the roots of coniferous species remain dormant in response to the stress. Tardieu *et, al.* (2000) noted from his study on cowpea in relation to water stress that there was a general decrease in the average leaf area, shoot dry mass, and the number of leaves. More branching, tallness, larger stems and dry root matter were observed in normal irrigated pigeon peas than in stressed ones by Petrie and Hall (1992). Belford *et al.* (2001) made similar observations while working on *Pachyrrhizus* species.

Water stress on cowpea production is known to have reduced the fresh and dry shoot weigh by 56% and 45% respectively; fresh root and leaf weight by 44% and 25% respectively, and nodule weight and number by 44% to 25% respectively (National Research Council, 2006). Prolonged water stress led to a reduction in leaf area and subsequent shedding of leaves in semi-deciduous plants. Working with a whole plant, Nonami and Boyer (1988) established that reduction in the sizes of leaves is the first response of legumes to water stress. It was also found that changes in leaf thickness of herbaceous plants were related directly to turgor pressure in the cells though, the amount of leaf shrinkage varied among species. Leaves that were relatively rigid and well supported by veins exhibited modest shrinkage under water stress conditions while soft leaves showed considerable changes (reductions) in thickness. Munns *et al.* (2000) however, attributed the changes in the thickness of leaves to the rate of transpiration and stomata openings that influenced the rapid development of water deficits in the tissues of the plants.

Tardieus *et al.* (2000) observed reductions in the morphological features of maize plants grown under water deficit conditions and attributed the trend to changes in the internal structures of the plants as a response to the water stress treatments. The relative rate of water absorbed into the plants and the rate of transpiration may have created a deficit in the internal pressures of the tissues. The reduction in the cell turgor and the tissues would then reflect on the morphology of the plants, and thereby causing the decrease in the sizes of the various organs of the plants.

Reproductive tissues increase in size during development as a result of cell divisions expansion. Superimposition of stress conditions to plants during reproductive stages result in reversible changes in size (reductions) because of hydration changes in the reproductive tissues and leaves. Under such stress conditions, shrinkage in the flowers and fruits of plants would then prevail (Westgate and Boyer, 1985). The effects of water deficit on fruits and seed yields were studied extensively through the analysis of precipitation and yield records, and by irrigation studies that was based on soil moisture measurements (Steudle *et al.*, 2000). Under soil moisture stress of 0.3 - 0.5 bar, he observed severe decreases in the fresh weights of flowers and pods in the plants under stress when compared to those of the plants under 0 bar treatment. Reductions in fruit yields of corn (*Zea mays*) were also observed under water stressed conditions, especially after the time of pollination (Chapin, 1987).

2.5 WATER STRESS ON PLANT TISSUES

The anatomical structure of the cowpea plant is that of a typical example of a dicotyledonous plant, and consists of a middle cortical tissue enclosed outwardly by an epidermal strip and by the stele on the inside. The cortex is made up of loosely arranged cells with thinner walls that are parenchymatous and cells that may have partially thickened cell walls (collenchyma) or fully thickened walls (sclerenchyma). The stele is also made up of an outer phloem, middle cambium and inner xylem elements. The center is mostly occupied by loosely arranged parenchyma cells known as the pith (Esau *et al.*, 2007, Souza, 2000).

Xylem elements conduct the water and mineral salts absorbed by the roots from the soil to the leaves for the synthesis of complex food products while the phloem and their companion cells translocate prepared food from the leaves to the other parts of the plants body. The cambium consists of cells that differentiate perpetually to give rise to other specialized cells. These specialized cells become the permanent vascular tissues. They are therefore, responsible for secondary growth in dicotyledonous plants (Souza, 2004). The primary vascular system of the stem consists of a network of interconnected leaf traces, and the vascular supply to the leaf was described by Esau *et al*. (2007) as emerging from the three leaf gaps and fusing into a ring at the pulvinus. The leaf has a reticulate venation pattern with large interveinal dimensions that average 157-236 µm. It also, has a paraveinal mesophyll that consists of a unique layer of parenchyma cells that extends horizontally between the vascular bundles and the plane of the phloem. The cells are large and completely in contact with the bundle sheath (Esau *et al*., 2007).

Water stress was found to have a negative influence on all the organs of temperate pastures (Proseus *et al*., 2000). There were reductions in the tissues of the various organs of the stressed plants. The decreases in the sizes of the tissues were however, more significant in the roots of the affected plants. The thinner stems of stressed dicotyledon plants were observed to contain more vessels in the vascular tissues as a result of stimulated cell divisions in the anticlinal plane. This was in contrast to an earlier assertion that water stress inhibits transverse cell divisions (Hsiao and Zing, 1987). Petrie and Hall (1992) however, recorded increases in the number of xylem vessels of the stele in plants they grew under different water stress conditions and attributed the occurrence to the rate of transverse cell proliferation of meristematic tissues in the plants under the different water deficit condition.

Working with maize seedlings at two different water treatment levels, Laing and Shaw (1987) observed reductions in the tissues of the stressed plants. They attributed the reductions to reduced cell expansion, and that the rate of tangential and radial expansions decreased in both the stele and the cortical tissues while the total numbers of cells in them remained unchanged. Tardieus (2000) found significant reductions in the tissues of varieties of plants he worked with as a result of water stress. He attributed the reductions to changes in the energy status of the water imposed as well as cell turgor. The turgor changes were found to be controlled by relative absorption water and transpiration, and the internal distribution of absorbed water in the tissues of the plants (Tardieus, 2000).

2.6 WATER STRESS ON STOMATA

Stomata are pores on the leaves of vascular plants through which water is lost, mainly through the process of transpiration. These pores are surrounded by special bean-shaped cells called the guard cells. The stomata form conduits for about 90% of the total water lost in plants (Munns *et al*,. 2000). They are responsible for gas exchanges in plants and occur evenly on both surfaces of monocotydonous leaves. They are however found more on the lower surface of dicotyledonous leaves than the upper surfaces (Esau *et al*., 2006). Stomata tend to close under water deficit conditions. The extent of closure however, may depend on other environmental factors and the developmental stage of the plant. It was noted by Souza (2000), that stomata closure in response to water stress is a classical feedback mechanism; when leaf water potential is lowered below a critical level, the stomata began to close to reduce the rate of water loss and to allow the water potential to recover. Experiments have established that cowpeas are excellent drought tolerating plants and show minimal changes in water potential with increasing water deficit conditions (Hsiao and Zing, 1987). Maize was said not to have osmotic adjustment but responds to water stress by closing their stomata (Tardieus *et al.*, 2000), though Chapin (1987) had observed earlier during diurnal measurements that the stomata of stressed plants close throughout the day. Belford *et al.* (2001) worked on *Pachyrhizus* species and observed a decreasing trend in the features of the plants under stressed conditions.

CHAPTER THREE

MATERIALS AND METHOD

3.1 SEED ACQUISITION AND VIABILITY TEST

Seeds of six accession s of cowpea (*Vigna unguiculata*) from three agro-ecological zones in Ghana; Semi deciduous, Coastal savanna and Sudan savanna were acquired from the Plant Genetic Resource and Research Centre at Bunso and used for the investigation. Each of the agro-ecological zones was represented by two accessions. The accessions were given treatment codes for the purpose of this investigation; Semi-deciduous (SD1, SD2), Coastal savanna (CS1, CS2) and Sudan savanna (SS1, SS2). The accessions from the Semi-deciduous zone had the accession numbers GH2332 and GH2334. The accessions from the Coastal savanna had the numbers GH3673 and GH3667, with those from the Sudan savanna zone having the numbers GH4770 and GH4771.

Seed viability was tested through laboratory germination. The seeds were soaked in water for 30 minutes before being laid on trays filled with vermiculite media. Germination was determined by physical observation of the emergence of radicle and plumule of the seeds. The seeds of the six different accessions under investigation germinated within five days of observation.

3.2 SITE PREPARATION

The experiment was carried out on the benches in the plant house of the Department of Theoretical and Applied Biology of KNUST. Three hundred and sixty black poly-bags of sizes 10 inches by 5 inches were obtained and each was filled with 2 kilograms of well mixed loamy soil. Holes were bored under the bags to improve drainage. Before the start of the experiment, the water holding capacity of a sample of the soil was determine by the '0' pressure method (Agvise laboratories, 2009). In this method, no external pressure was needed or applied. The fresh loamy soil of mass 2 kilograms was put into a beaker and dried in an oven at 70^{o}C for 24hr. It was transferred into a beaker with a hole at the bottom. Water was poured gradually into it till no bubble was seen to escape. The saturated soil was allowed to stand for 6 hours to allow the excess water to be drawn away under gravity into a high density tissue paper. The soil was then, weighed again to get the mass of water retained in the soil. This was calculated to be 300 ml of water per the 2 kg of soil used.

3.2.1 PLANTING METHODS AND WATER STRESS TREATMENTS

The soil in each poly-bag was initially watered with 300 ml of water to saturate the soils. Twenty (20) seeds of each of the six accessions of the cowpeas were sown in the poly-bags; with each seed in a poly-bag. The seeds were allowed to germinate and the plants were given 300ml of water at three days interval till the end of the 3rd week of growth when they were grouped into three water treatment regimes. The watering regimes were normal irrigation (WO) at which the plants were not stressed and therefore given 300ml of water every 3 days, medium water stress treatment (W1) at which the plants were water stressed at 50% and therefore given 150ml of water at every 3 days, and the severe water stress treatment (W2) at which the plants were water stressed at 85% and therefore, given 50ml of water at 3 days interval.

The total number of plants in each treatment protocol (plot) was 20 X 6 = 120 plants. The total sample size used for the investigation in the three treatment protocols was 360 plants.

The water stress treatment thus, started at the end of the 3rd week of growth of the plants in the poly-bags. Each plant in the normal (W0) treatment regime was given 300ml of water at three days interval from the beginning of the treatment till the end of the experimental period. The plants in the medium stress (W1) treatment regime were each given 150 ml of water at three days interval till the end of the experimental period. The plants in the severe stress treatment (W2) were given 50 ml of water each at three day intervals from the day the treatment started till the end of the experimental period. Watering was done between 8 and 9 am in the mornings of the scheduled days. The experimental period stretched over nine weeks from the day the treatments were started.

Another experimental plot was set up where accessions were grouped into the three treatment protocols but in this case, the water watering applications depended on differences between watering periods. Thus, the normal irrigation regime was given 300ml of water every 3 days, the medium water stress was given 300ml of water every seven days while the severe water stress was given 300ml of water every 12 days. This method of stress treatments was adopted from the works of Boyer (1985) and Ahedor (1996).

3.2.2 SAMPLING OF TREATMENT PLANTS

Sampling of the plants was performed three times during the treatment period. These were at the end of the 3rd, 5th and 7th weeks. From each treatment, five plants were randomly selected on each sampling occasion and their morphological features, and possible flowering and fruiting patterns noted. The sampling periods were adopted from earlier works by Signet and kramer (1977), and Petrie and Hall (1992). The roots of the plants were cut at a distance of 2 cm from the base of the stem. This length was taken because the root branches profusely after the 2 cm length in all the plants. Samples of the stems of the plants were taken at points of 1 cm distance above the first, second and the third nodes of the harvested plants. This was to prevent the effects of branching on the vascular arrangements in the stems from affecting the investigation. In the leaves, the middle leaflets of the 2nd, 3rd and 4th leaves from the base of the stem were chosen. A cut was made transversely across the leaflets at 1 cm from the trifoliate joint and 2 cm piece of the leaflets that included the mid vein were taken.

After each harvest, the organs of interest (roots, stems and leaves) were washed, cut and fixed in small bottles containing formal-acteto-alcohol (FAA). These were labeled accordingly and stored for further treatment. Exception to this was found in the study of the leaf strips. Due to the sensitivity of the stomata guard cells to environmental changes, leaf strip examinations were performed immediately after each harvest. These preparations were made for each plant of the accessions under all the treatment protocols after the harvests. The methods of preparation were used in earlier works by Hsiao and

Zing (1987), Nonami and Boyer (1988), and Souza (2004). Photographs of the plants were taken at the end of the 5th week of the treatment period.

3.3 MONITORING OF SOIL MOISTURE CONTENTS

The water stress treatments were monitored by measuring the relative soil moisture contents for the different treatment protocols. From the determination of the water holding capacity of the soil used from the laboratory analysis, ie. 300 ml per 2 kg of the soil, the water stress treatments were determined at 50 % for the medium treatment and 15 % for the severe water stress.

Three soil samples from each treatment protocol were collected randomly with a medium sized cork borer, having a volume of 10 ml on the 3^{rd}, 5^{th} and 7^{th} weeks of treatment. The soils were weighed immediately after being collected and the values recorded. The soils were subsequently dried in an oven with a temperature of $70^{o}C$ for 24 hours. The soils were then removed and placed in desiccators to cool before being reweighed. The changes in the weights represent the soil moisture that was contained in each sampled soil. The mean values and the percentages were computed and recorded. The methods used to monitor the relative soil moisture contents in this investigation were adopted from the works of Ahedor (1996), and Clark and Bell (1996).

3.4 MONITORING OF CLIMATIC FACTORS

Two hydro-thermograph machines were obtained from the Botany Department of the University of Ghana and set up in the plant house of the Theoretical and Applied Biology

Department. A calibrated graph sheet to indicate the humidity and thermometer readings were wrapped around the drum of the machine and the markers set on them. The settings were standardized with ordinary hygrometer and mercury thermometer. The hygrometer was measured in percentages (%) and the temperature in degrees Celsius (^oC). The light intensity was measured with a light meter in Lux. The mean values for the minimum and maximum readings for the experimental period were computed for all the measured climatic factors. Ahedor (1996) used this environmental measuring technique for his earlier work.

3.5 MEASUREMENTS OF MORPHOLOGICAL FEATURES

The morphological measurements of plants height, plant girth, root lengths, amount of leaves per plant, and twinning were determined. The heights of the stems (plant heights) were measured with meter rules from the base of the stem to the tips of the terminal buds. The girth of the plants was measured at three points; after the 1^{st}, 2^{nd} and 3^{rd} nodes with a Vernier caliper. The lengths of the roots were also taken with a measuring tape (mm) from the base of the root to the tip. These were done after the roots have been uprooted and cleaned with tap water.

The width and the length of the leaves were measured with a meter rule. The length of the leaves was measured from the petiole to the tip of each leaf and the width was measured for the 2^{nd} middle leaflets at their largest stretch. The amount of leaves per plant was performed by counting the entire leaves on each plant. The methods used in the measurements were adapted from that used by Ahedor (1996) and Hsiao and Zing (1987) and Steudle (2000) in earlier work. The means of the measurements were computed and recorded.

3.6 MEASUREMENTS OF REPRODUCTIVE STRUCTURES

The effects of soil moisture stress on the reproductive organs (flowering and fruiting) in the cowpeas were studied. The days on which flower buds appear on the plants after the start of the treatment were noted. The total number of flowers and the flowers that were aborted because of the stress were counted and recorded. The total number and mass of fruits per plant was computed for each accession at the end of the experimental period. The width of each bean pod was measured with Vernier calipers and the length with a standard meter rule.

The mass of each fruit (bean pod) on the plants was weighed and the means recorded. This was carried out for all the plants in the different treatment protocols. The dried mass of the fruits produced by each accession was weighed. The total amount of bean pods produced per each plant was put into a furnace at 70 ^{o}C for 48 hours. The dried mass was then taken out from the furnace and weighed to obtain the dried mass of the fruits produced per each accession. The method used has been employed in earlier works by Tan and Blake (1997), and Singh and Rachie (1985).

3.7 PREPARATION OF ANATOMICAL TISSUES

The organs of the plants that were fixed in the formol-aceto-alcohol were subjected to the processes of permanent slide preparation which includes dehydration, infiltration, embedding, microtomy, staining and mounting. Permanent slides of roots, stems and leaves were thus, prepared before their examination. The methods used for the preparation of the permanent slides were adopted from works by Arkoh (1991) and Tardieu (2000).

3.7.1 DEHYDRATION

The organs of the plants that were stored in the specimen bottles were passed through series of diluted alcohol and formalin in a process referred to as dehydration. The procedure for dehydration practice is as follows:

i. Dehydrated tissues in 70% alcohol …………………………………….. 1 hr

ii. Transferance of tissues to absolute alcohol ………..……………….. 1 hr

iii. Transferance of tissues into 75% alcohol and 25% chloroform …….. 1 hr

iv. Transferance of tissues into 50% alcohol and 50% chloroform ……..... 1 hr

v. Transferance of tissues into 25% alcohol and 75% chloroform …...… 1 hr

vi. Tissues were sent into absolute (100%) alcohol ……………………… 1 hr

vii. Tissues were transferred into another absolute alcohol ..…………….. 1 hr

3.7.2 INFILTRATION AND EMBEDDING

The organs were then infiltrated or impregnated with wax to enable them withstand subsequent treatments. A medium temperature melting wax ($60^{o}C$) was crumbled into the bottles containing the last absolute alcohol. The wax melted and penetrated the tissues. The bottles and their contents were left to stand for 24 hours before being heated on a water bath to evaporate the alcohol solvent.

The infiltrated organs were picked with hair brush into small boxes filled with the embedding wax. The heated wax was used to fill the embedding moulds. The organs of the plants were arranged with their cutting faces downwards. The embedded organs were left to stand for 12 hours to cool and harden into blocks. The wax blocks were shaped to fit onto the stage holder of the hand microtome.

3.7.3 MICROTOMY

A hand microtome was obtained from the laboratory of the Theoretical and Applied Biology Department and used to section the embedded plant specimens. The hand microtome was selected because of its lightness and easy operation. The knife was sharpened on a stropping leader and fixed onto the knife holder of the microtome. The wax block was also fixed onto the specimen holder of the microtome. The cutting width was set at 5µm and the machine was rotated by the hand roller to move the stage against the cutting edge of the knife at a predetermined pace of 5µm. Thin sections of the organs were obtained from the cuttings. Continuous cutting results in the production of ribbons or series of sections.

3.7.4 STAINING

The sectioned tissues were detached from one another and sorted out for good ones. The good sections were heated gently on water baths to evaporate the solvents used for the dehydration. The sections were then, stained by the retrogressive method. This is the best staining method as more than one staining reagent was used. The double staining helped to give contrasting features to the different components of the tissues being prepared.

The retrogressive staining process was as follows;

i. Dewaxed in xylene I …………………………………………….. 5 min

ii. Dewaxed in xylene II …………………………………………….. 5 min

iii. Dehydrated in absolute alcohol ………………………………….. 2 min

iv. Washed in 95% alcohol …………………………………. 2 min

v. Transferred to 75% alcohol …………………………………… 2 min

vi Then to 50% alcohol …………………………………… 2 min

vii. Stained in Safranin ………………………………….. 10-15 min

viii. Transferred to 50% alcohol ……………………………………… 2 min

ix. Dehydrated in 70% alcohol ……………………………………….. 2 min

x. Then to 95% alcohol ……………………………………………… 2 min

xi. Counter stained in eosin …………………………………………… 1 min

xii. Differentiated in 95% alcohol …………………………………… 2 min

xiii. Transferred to absolute alcohol …………………………………….. 1 min.

3.7.5 CLEARING AND MOUNTING

The stained specimens were transferred into two bottles containing xylene reagent to clear off excess stains. The clearing process was as follows:

i. Clearing in Xylene I ………………………………………… 5 min

ii. Clearing in Xylene II ………………………………………. 5 min

At this stage, the specimens were rigid and devoid of water. Glass slides were obtained from the Stores and cleaned with cotton wool. A drop of Canada Balsam was put on each slide and allowed to spread. The stained sections were picked with hair brush laid on the mountants with care. Cover slips were laid on the specimen on each slide gently and the slides were allowed to dry on a hot plate at a temperature of 60^oC. The slides were then labeled and stored for examination.

3.8 STUDY OF THE TISSUES

The anatomical study of the tissues from the plants of the different accessions was performed quantitatively. It involved the use of an Olympus Research Microscope fitted with an eye-piece graticule. The microscope was obtained from the Theoretical and Applied Biology laboratory of the Kwame Nkrumah University of Science and Technology. The graticule was calibrated with a stage micrometer before use. Photomicrographs of the slides under study were taken with an Olympus camera fitted to the Olympus microscope.

The variables measured from the stems and roots slides were: sizes of epidermal and of cortical cells, width of cortex, width of vascular band/bundles, sizes of xylem vessels, frequency of xylem vessels, width of pith region and the sizes of pith cells. The variables measure in the leaves of the plants of the accessions were: length and width of leaves, sizes of epidermal cells, width of cuticle layers, width of vascular bundle, sizes of palisade and spongy cells, frequency of stomata, sizes of stomata guard cells and the pores of the stomata.

The phloem tissues of the accessions were not able to be measured because phloem and its associated cells are covered immediately that the plants are cut with cutinized substances that hinder their study.

3.8.1 MEASUREMENTS OF THE SIZES OF CELLS

The slide examinations were performed under medium magnification of X40. The stage of the microscope was manipulated to shift the slides forwards and backwards during the observation. The pointer on the eye-piece was rotated randomly under each field of view and the diameters of cells of the tissues that fell under the pointer were measured in microns. Ten counts were made for each slide and the means recorded.

3.8.2 MEASUREMENTS OF THE SIZES OF CORTICAL TISSUES

The stage and the eye-piece of the microscope were manipulated to present many parts of the specimen onto the field of view. The pointer of the eye-piece was rotated randomly across the cortical region of the specimen and the width of the cortex was found at five different positions. A magnification of X40 was used for this study. The means of the values were computed and recorded.

3.8.3 MEASUREMENTS OF THE SIZES OF VASCULAR TISSUES

The vascular tissue measurements were taken in a similar manner as was performed for the width of the cortical tissues. A magnification of X40 was used. The eye-piece and the stage of the microscope were operated to enable the pointer of the eye-piece move randomly across the vascular regions of the specimens on the slides. The distance from one end of the vascular band to the other in the roots, and that of the vascular bundles in the stems were measured.

A transect was made on the eye-piece to dissects the slides into four sections. One sector was chosen randomly and studied for the frequency of xylem vessels and their diameters. The diameters of the xylem vessels were measured by finding the distance across the individual xylem vessels that fell within the demarcated sector.

The frequency of the cells was determined by counting the xylem vessels that were found within the sector on the field of view. Five different readings were taken for each slide and the means recorded.

3.8.4 MEASUREMENTS OF THE SIZES OF LEAF TISSUES

In the leaves, the variables measured were; diameters of the main vein of the leaves and their xylem vessels, epidermal cells, width of palisade and spongy tissues, width and frequency of stomata, and the width of cuticles. The sizes of the cells and the vessels were taken by measuring the diameters of the epidermal cells on both the adaxial and abaxial leaf surfaces and the xylem vessels in the main vein respectively. These were measured from five different positions of the slide by rotating the pointer of the eye-piece randomly across the slide. Five cells in each tissue were selected and their diameters measurement.

The examination of the stomata was facilitated by taking leaf strips of the various plants of the accessions and mounting them with glycerine immediately after the harvests/ samplings. A magnification of X60 was used for the stomata study. Samples from both the upper and lower leaf surfaces were prepared and examined. The total number (frequency) of stomata that appeared under the field of view of the microscope was counted and their means recorded. The diameter of the guard cell was found by taking the reading for the widest part of the guard cell under the field of view of the eye-piece.

The width of the pores of the stomata was found by taking the reading for the widest part of the stomata aperture under the field of view of the microscope for five different stomata. The width of the cuticles was measured for both the upper and lower surfaces of the leaves. The mean values for all the measure variables were computed and recorded.

The anatomical measurement procedures used in this investigation were adopted from earlier works by Hsiao and Zing (1987), Nonami and Boyer (1988), Ahedor (1996), Proseus (2000) and Steudle (2000) on plant anatomy and growth analysis.

3.9 STATISTICAL ANALYSIS

The data was subjected to analysis of variance (ANOVA) by using the Microsoft Excel software. The means were separated where necessary by using the least significant difference at P=0.05.

CHAPTER FOUR

RESULTS

4.1 ENVIRONMENTAL FACTORS

The environmental parameters measured were that of the soil moisture content, ambient light intensity, ambient temperature and relative humidity.

4.1.1 RELATIVE SOIL MOISTURE CONTENT

The mean soil moisture content measured during the period of the experiment is shown in Figure 1. The water holding capacity of the soil used was found to be 300 ml of water per 2 kg of the soil. The water stress for the medium was 50% and that for the severe stress 85%. As was expected from the different water treatments, there was a gradual decrease in the percentage of the soil moisture contents of the soil across the stressed protocols. The normal irrigation protocols showed more soil moisture content than the stressed protocols. The water stressed treatments reduced around 33% for medium stress (W1) and 52% for the severe stress treatment (W2) on all sampling occasions (Fig. I, Appendix 1). The decreases in the soil moisture contents on the 2^{nd} and 3^{rd} samplings could be attributed to the increase absorption of the water through the growing plants.

4.1.2 CLIMATIC FACTORS

The parameters measured under climatic factors were the ambient light intensity, ambient temperature and the relative humidity. There were no wide variations in the measured parameters during the study period in the plant house (Appendix II). The mean values of the ambient temperature and the relative humidity however, showed opposite tendencies. A decrease in the mean values of the temperature resulted in an increase in the mean values of the relative humidity (Appendix II).

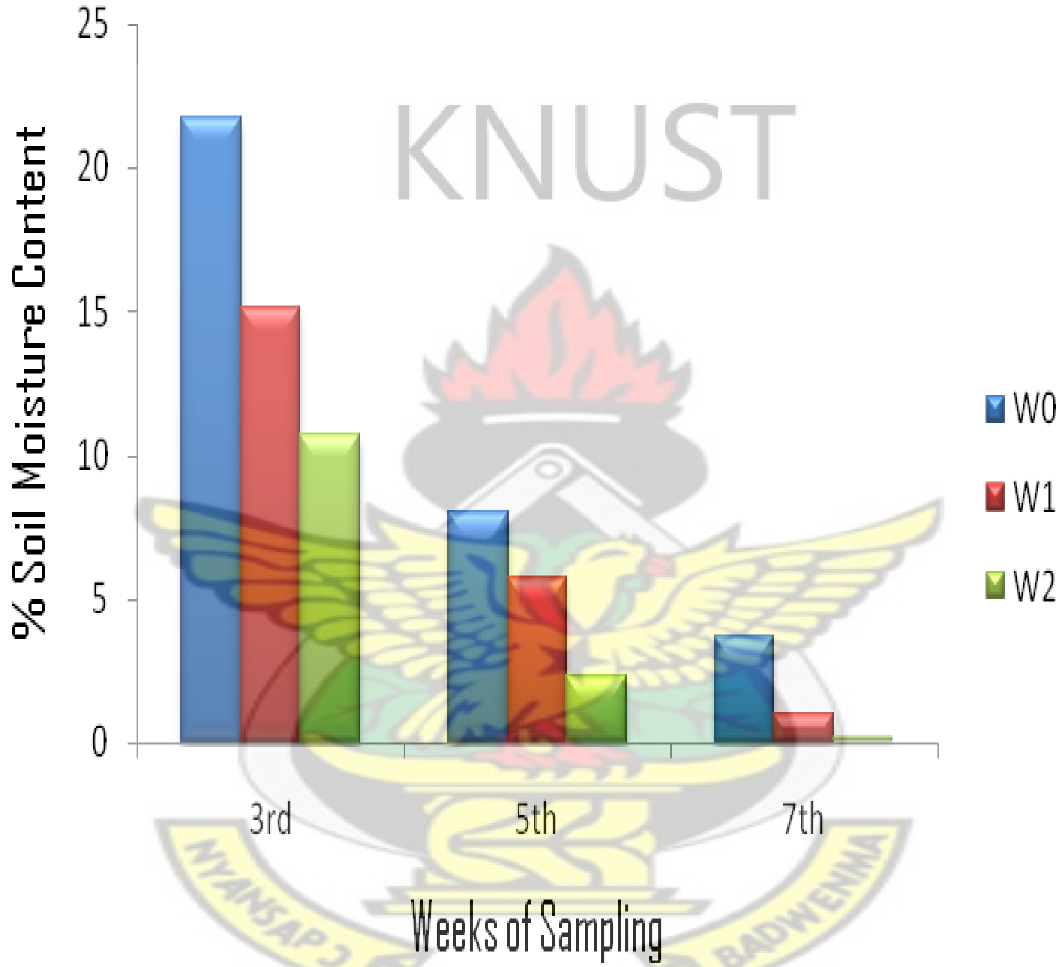

Fig.1. Variations in the percentage soil moisture contents on three sampling occasions

Where;
W0: Normal irrigation (Control)
W1: Medium water stress irrigation
W2: Severe water stress irrigation

4.2 MORPHOLOGY OF THE ACCESSIONS

The growth forms of the accessions are shown in Plates 1-4. Those from the Semi-deciduous zone were semi-erect, while the accessions from the Coastal savanna were creepers and those from the Sudan savannah were erect. All the plants of the accessions showed twinning at some stages in their growing period, except those from the Sudan savanna zone that did not showed twinning of the stems (Table I). The accessions SS1 however, showed twining in few replicates under the normal irrigation by the 3rd sampling period.

TABLE 1. EFFECTS OF WATER STRESS ON THE TWINING OF STEMS OF THE SIX ACCESSIONS ON THREE SAMPLING OCCASIONS

ACCE-SSIONS	TREAT-MENTS	HARVEST PERIODS		
		1ST (3rd week)	2ND (5th week)	3RD (7th week)
SD1	W0	+	+	+
	W1	+	+	+
	W2	--	+	+
SD2	W0	--	+	+
	W1	+	+	+
	W2	+	+	+
CS1	W0	+	+	+
	W1	+	+	+
	W2	+	+	+
CS2	W0	+	+	+
	W1	+	+	+
	W2	+	+	+
SS1	W0	–	–	-
	W1	–	–	–
	W2	–	–	–
SS2	W0	–	–	–
	W1	–	–	–
	W2	–	–	–

SD1 and SD2 : accessions from the Semi-deciduous zone, CS1 and CS2 : accessions from the Coastal savanna zone, SS1 and SS2 : accessions from the Sudan savanna zone. W0, W1 & W2 for normal, medium and severe water stresses respectively.

4.2.1 MORPHOLOGY OF STEMS

4.2.1.1 HEIGHTS OF STEMS

The morphological features showed a general decrease in most of the mean values of the measured parameters toward increasing soil moisture stress treatments on the three harvests (Figs. 2-6). The height of the accessions showed increasing growth under the normal irrigation on all sampling occasions. There was however, a decrease in the plant heights along the water stress treatments; W0>W1>W2. Exception was observed in the 1st harvest where the accessions from the Semi-deciduous zone (SD1 and SD2) and those from the Coastal savanna (CS1 and CS2) showed an initial increase in the medium water stress (W1) before reducing in heights under the severe water stress treatment (W2) (Fig.2, Appendix IIIa). It was observed that the reductions in plant heights were greater in the accessions from Semi-deciduous zone; 75% reduction in the SD1 between the W0 and W2 treatments on the 3rd harvest as compared to that of the savanna zones that reduced between 20-50% within the same treatments. The accession SS2 reduced by 50% while the CS1 reduced by 20% (Fig. 2). The accessions from the Coastal savanna had the longest lengths with those from the Sudan savanna having the shortest (Plate 1).

With the accessions from the coastal savanna, the lengths were measured as they twinned along the supporting stick. The plants may have been termed creepers if they had remained on the ground without being supported. They exhibited the longest length at the end of the investigation under all the treatments and on the tree harvest times (Fig.2).

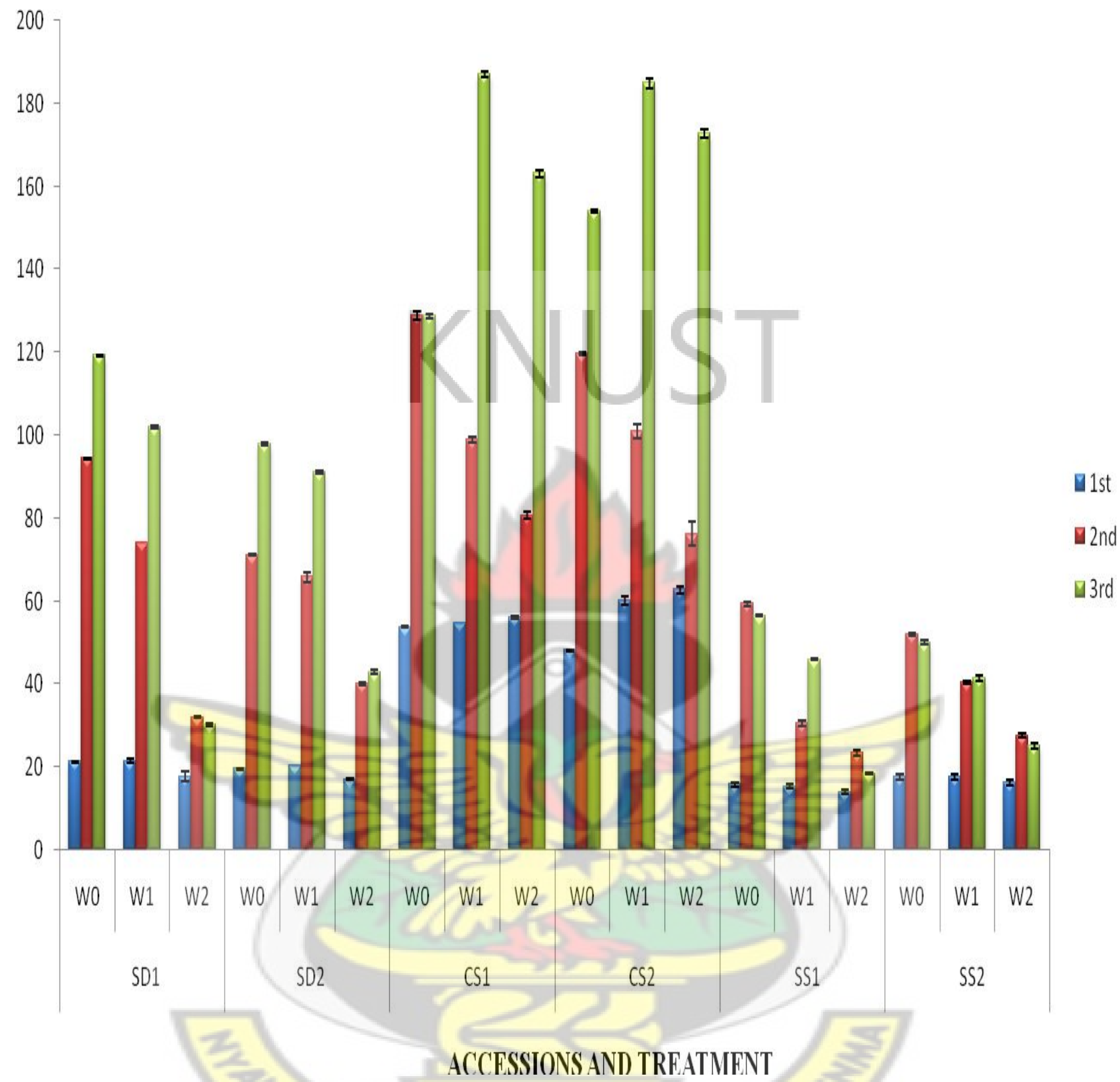

Figure 2: Effects of water stress on the plant heights of the six accessions on three sampling occasions

Where;
SD1 & SD2: accessions from the semi-deciduous zone;
CSI & CS2: accessions from the coastal savanna zone;
SS1 & SS2: accessions from the Sudan savanna zone;
W0: Normal water treatment (control);
W1: Medium water stress treatment;
W2: Severe water stress treatment.

4.2.1.2 WIDTH OF STEMS

The width of the stems of the accessions increased in sizes in the normal irrigation (W0) along the three harvesting occasions. However, the plants reduced in sizes under the water stress treatments (Fig 3). Exception was observed during the 1st harvest where all three accessions showed an initial increase in the width of stems under the medium water stress treatment (W1) before reducing in sizes under the severe water stress treatment (W2). Thus, only the accessions from the Sudan savanna (SS1 and SS2) maintained their reduction in stem width from the normal irrigation to the severe stress protocols (W0>W1>W2) (Fig 3). The Coastal savanna accessions CS1 and CS2 showed increases in their stem width in the medium water stress treatment during the 2nd and 3rd harvests though, the others reduced in sizes with age under the water stress treatments (Appendix IIIb, Fig 3). The accessions from the Coastal savanna were thin while those from the Sudan savanna were thicker in stem width (Plate 2-4).

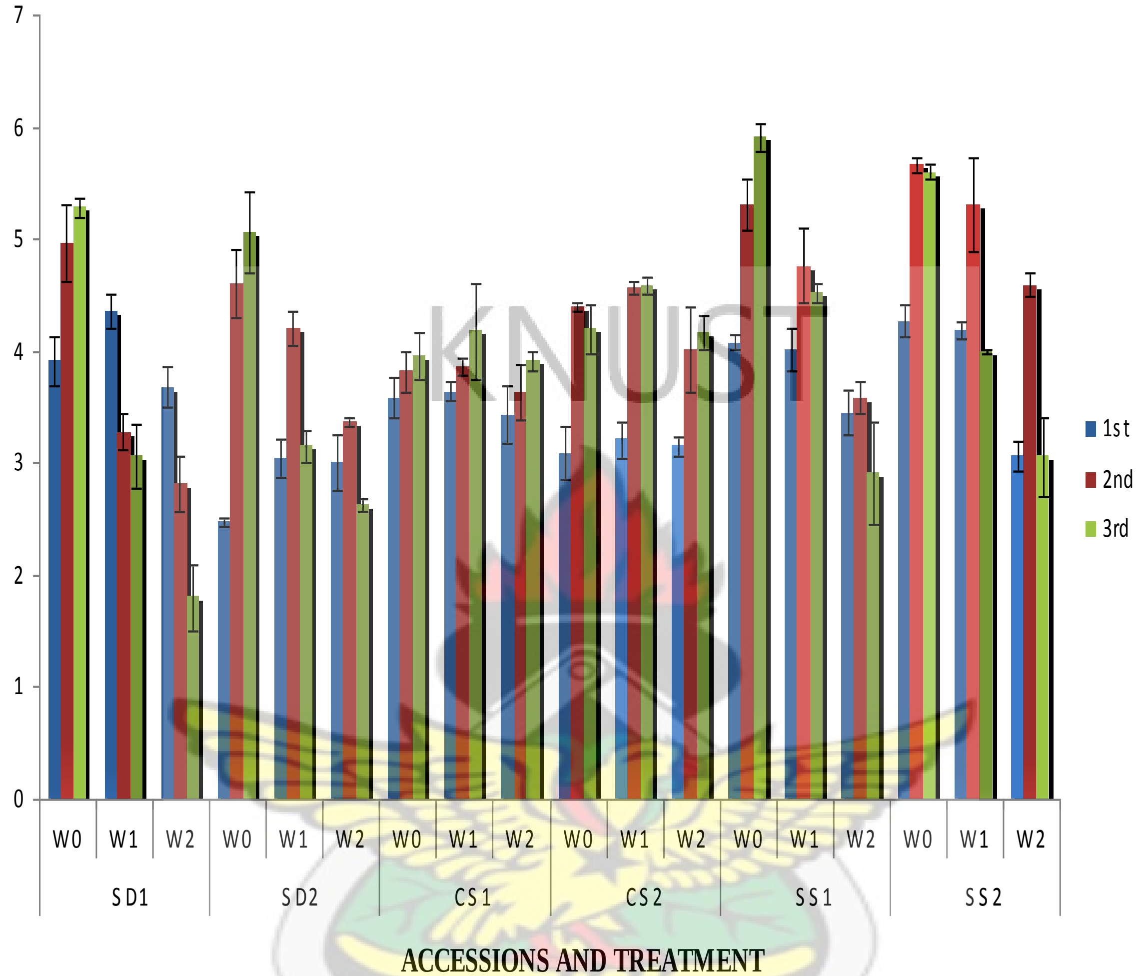

Figure 3: Effects of water stress on the width of stems of the six accessions on three sampling occasions

Where;

SD1 & SD2: accessions from the semi-deciduous zone;

CSI & CS2: accessions from the coastal savanna zone;

SS1 & SS2: accessions from the Sudan savanna zone;

W0: Normal water treatment (control);

W1: Medium water stress treatment;

W2: Severe water stress treatment.

4.2.2 MORPHOLOGY OF ROOTS

The accessions showed increase in the length of their roots along the treatments. Exception was found in the CS1 and CS2 accessions which reduced in root lengths across the treatments. This was prominent in the 2nd and 3rd harvests where the accessions SD1 and SD2 reduced by 20% under the W2 treatments. Accessions from the Sudan savanna zone were observed to possess the longest roots under all the treatment protocols and on the three harvesting occasions (Fig 4). Though the accessions Sd1 and Sd2 possessed the shortest root system, the increase in their root lengths was high under the W1 before reducing in the W2 treatment. There was a continuous growth in the roots of the accessions along the three harvesting periods except CS1 and CS2 which reduced in root length in the W1 before increasing again under the W2 treatment. The variations in the roots lengths of the accessions, except the CS1 and CS2 on the 3rd harvest were found to be significantly different at $P<0.05$ (Appendix IIIc).

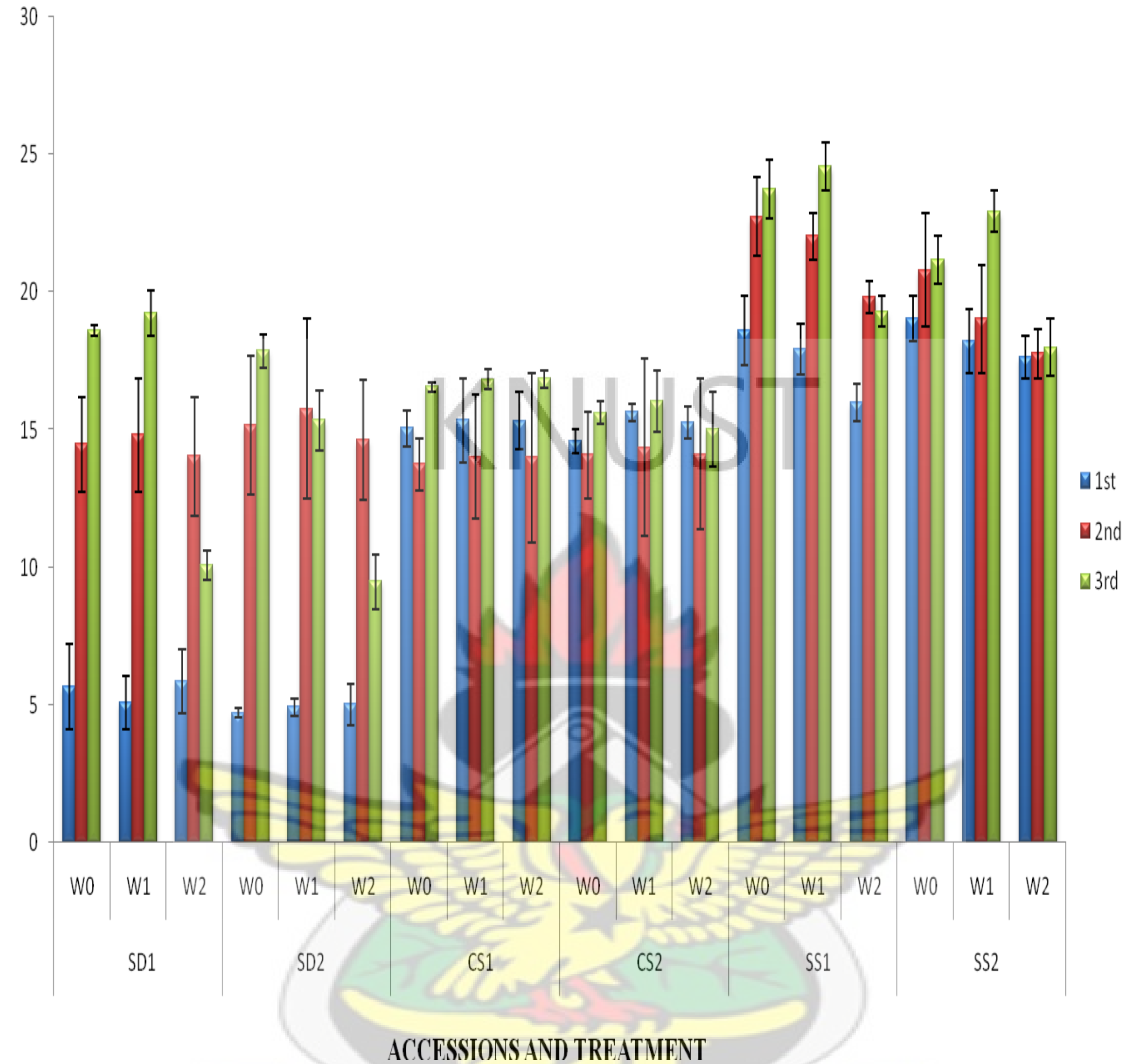

Figure 4: Effects of water stress on the root length of the six accessions on three sampling occasions

Where;
SD1 & SD2: accessions from the semi-deciduous zone;
CSI & CS2: accessions from the coastal savanna zone;
SS1 & SS2: accessions from the Sudan savanna zone;
W0: Normal water treatment (control);
W1: Medium water stress treatment;
W2: Severe water stress treatment).

4.2.3 MORPHOLOGY OF LEAVES

4.2.3.1 SIZES OF LEAVES

The sizes of the leaves and their frequency in the different accessions generally decreased under the water stress applications. Accessions in the normal irrigation showed increment in the sizes of leaves on all harvesting occasions (Fig 5). The leaves of the accessions from the Semi-deciduous zone (SD1 and SD2) were smaller in sizes than those of the Savanna zones. The accessions SS1 and SS2 possessed the largest leaves. The reduction in leaf sizes along the treatment protocols was however, more prominent in the Semi-deciduous accessions as compared to the other accessions; the SD2 reduced by 46% on the 1st harvest between the W0 and W2 treatments, while the CS2 reduced by 11% and SS2 reduced by 7.2% . On the 3rd harvest, the SD2 reduced by 60%, CS2 had 38% while SS2 reduced by 44%. Only the variation in the size of the leaves of accessions SS2 between the W1 and W2 treatments during the 1st and 3rd harvest showed significant differences at 0.05 confidence limit (Appendix IIId).

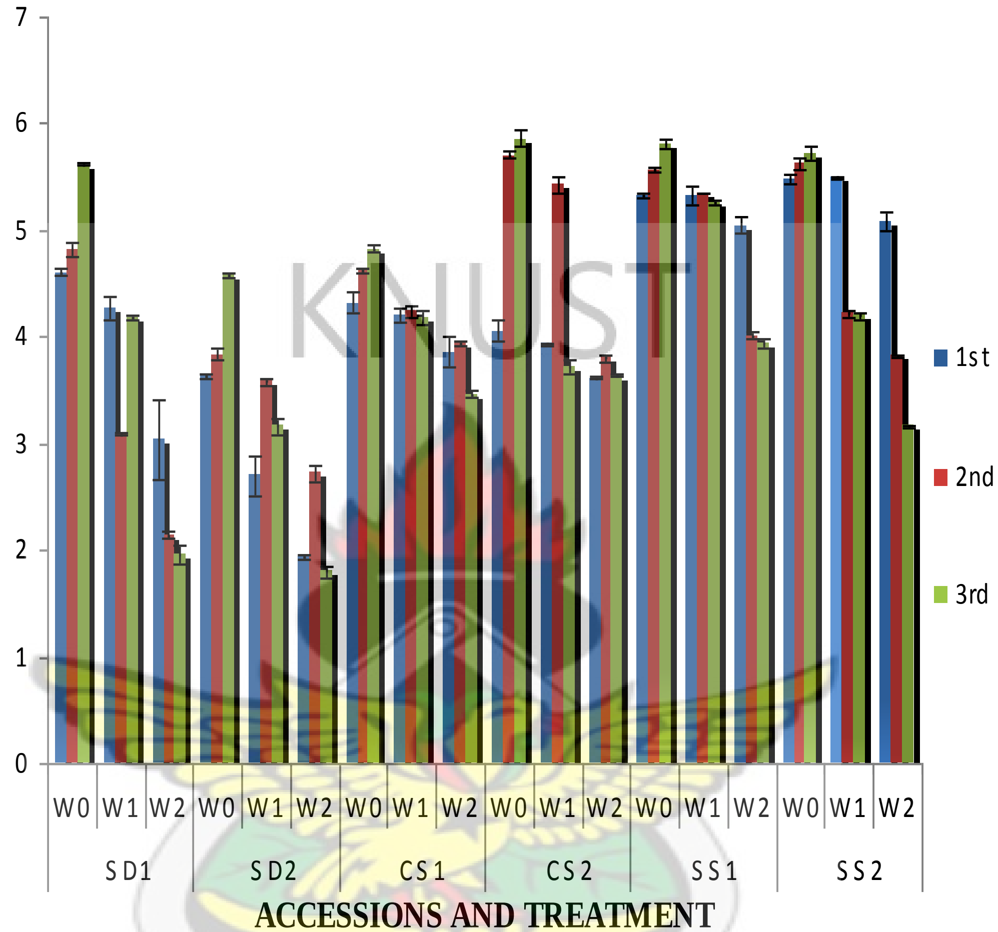

Figure 5: Effects of water stress on the sizes of leaves of the six accessions on three sampling occasions

Where;
SD1 & SD2: accessions from the semi-deciduous zone;
CSI & CS2: accessions from the coastal savanna zone;
SS1 & SS2: accessions from the Sudan savanna zone;
W0: Normal water treatment (control);
W1: Medium water stress treatment;
W2: Severe water stress treatment.

4.2.3.2 TOTAL NUMBER OF LEAVES

The amount of leaves on the accessions varied along the sampling occasions. The leaves of the different accessions increased with growth under the normal irrigation protocol. However, there was a general decrease in the amount of leaves on the accessions under the water stress treatments (Fig. 6). On all the three harvests, the reduction in the amount of leaves in the Semi-deciduous accessions was significantly different at $P<0.05$. The other accessions showed no significant difference on the 1st harvest between the W0 and W1 treatments except CS2 (Appendix IIe). The accessions SS1 and SS2 from the Sudan savanna showed no significant variation in the amount of leaves under the treatment protocols on the 2nd and 1st harvests respectively. The accessions SS1 and SS2 possessed the lowest amount of leaves under the normal irrigation treatment though, the SD1 and SD2 from the semi-deciduous zone experienced the lost of leaves more under the water stress treatments. It was observed that the accessions SD1 and CS1 from the Semi-deciduous and Coastal savanna zones respectively lost 50% of their leaves on the 1st harvest between the W0 and W2 treatments while the SS1 from the Sudan savanna lost 20% of its leaves. However, the CS1 reduced by 33%, SS1 reduced by 40% while the Sd1 reduced by 80% between the W0 and W2 treatments.

The trends whereby the morphological features of the plants of the accessions reduced in sizes with respect to increasing water stress treatments were noted in earlier works by Boyer (1985), Hsiao and Zing (1987), Ahedor (1996) and Steudle (2000).

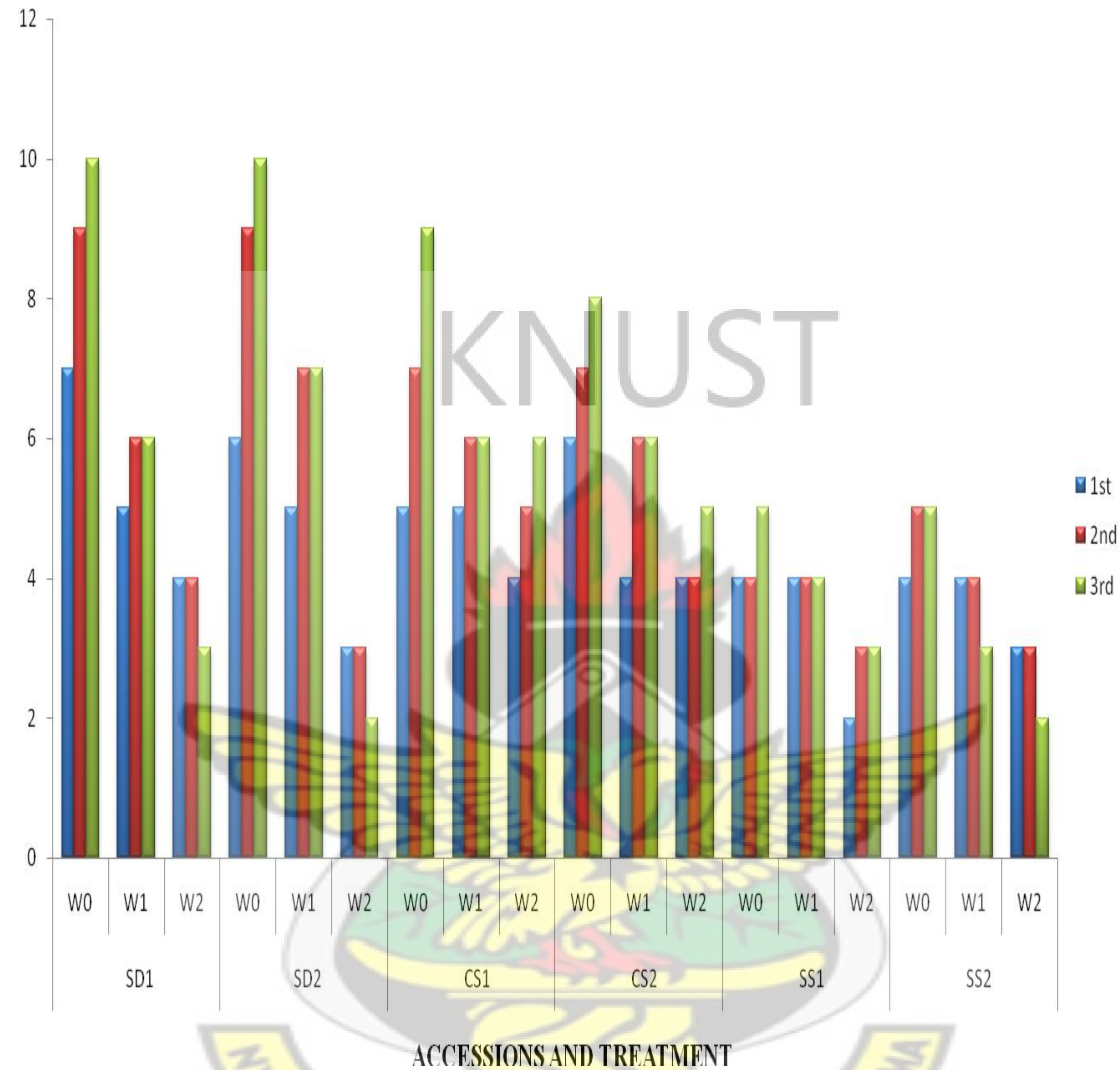

Figure 6: Effects of water stress on the total number of leaves of the six accessions on three sampling occasions

Where;

SD1 & SD2: accessions from the semi-deciduous zone;

CSI & CS2: accessions from the coastal savanna zone;

SS1 & SS2: accessions from the Sudan savanna zone;

W0: Normal water treatment (control);

W1: Medium water stress treatment;

W2: Severe water stress treatment

4.3 REPRODUCTIVE STRUCTURES OF THE ACCESSIONS

Generally, the plants of the accessions showed varied patterns in the onset of their flowers and subsequent fruiting among themselves and within the water stress treatment protocols. The mean values of the lengths and masses of their bean pods varied as well along the water stress treatments (Appendix V).

The plants of the accessions CS1, CS2 and SS1, SS2 from the savannah zones showed early flowering in both the normal watering and stressed protocols as compared to that of the Semi-deciduous. After the experimental period, the accessions from the Semi-deciduous zone were observed to produce more fruits under the normal irrigation regime while that in the water stressed protocols and the other accessions ceased fruit production. The imposed water stress may have inhibited the production of the hormones responsible for flowering in the accessions under the stressed protocols.

4.3.1 MORPHOLOGY OF THE FRUITS

4.3.1.1 LENGTHS OF BEAN PODS

The sizes of the individual bean pods of the accessions from the Sudan savanna were bigger while those from the Coastal savanna recorded the smallest sizes. The lengths of the bean pods were longer in the accessions (SD1 and SD2) from the Semi-deciduous zone with SD2 recording 10.05cm under the control (W0). This was followed by accessions SS1 and SS2 from the Sudan savanna with the SS1 recording 9.00cm. This was followed by the Coastal savanna accessions (CS1 and CS2) with the CS1 recording 7.65cm under the W0 treatment. The accessions from the Semi-deciduous zone however, recorded much reduction in the lengths of bean pods between the W0 and W2 treatments; 50% reduction in size in SD1. This was followed by those from the Sudan savanna with 38% reduction. The accessions from the Coastal savanna recorded little reduction of 29% between the same treatment protocols (Fig.7).

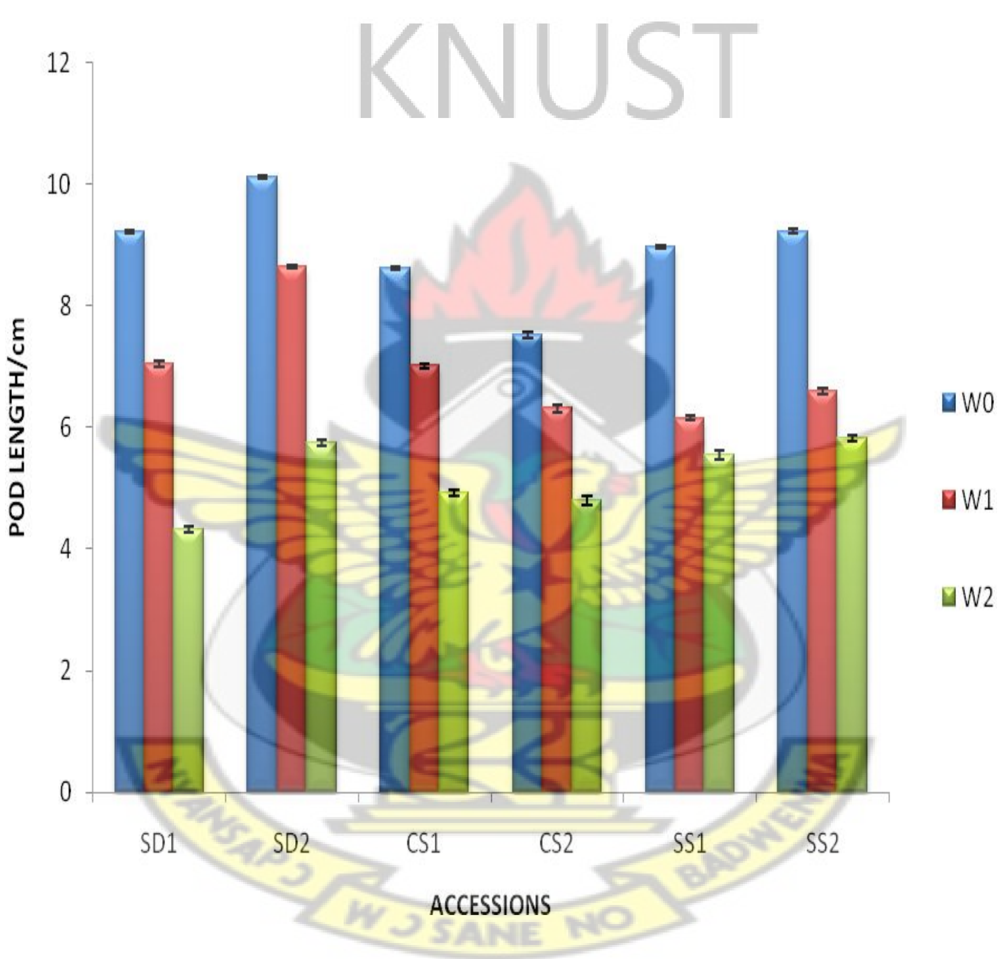

Fig. 7 Effects of different water treatments on the length of bean pods of the six accessions.

Where;

SD1 & SD2: accessions from the semi-deciduous zone;

CSI & CS2: accessions from the coastal savanna zone;

SS1 & SS2: accessions from the Sudan savanna zone;

W0: Normal water treatment (control);

W1: Medium water stress treatment;

W2: Severe water stress treatment

4.3.1.2 WIDTH OF BEAN PODS

The bean pods from the accessions SS1 and SS2 from the Sudan savanna recorded the largest width with the SS1 having 1.23cm in size under the normal irrigation protocol. It was followed by the Semi-deciduous accessions with SD1 recording 0.91cm, then the Coastal savanna accessions with CS2 recording the lowest size of 0.71cm. However, the effects of the water stress were observed to be higher in the SD1 and SD2 from the Semi-deciduous zone between the W0 and W2 treatments with the SD1 recording a reduction of 50% in size. It was followed by the accessions SS1 with 44% and CS1 with 32% reductions between the same treatment protocols.

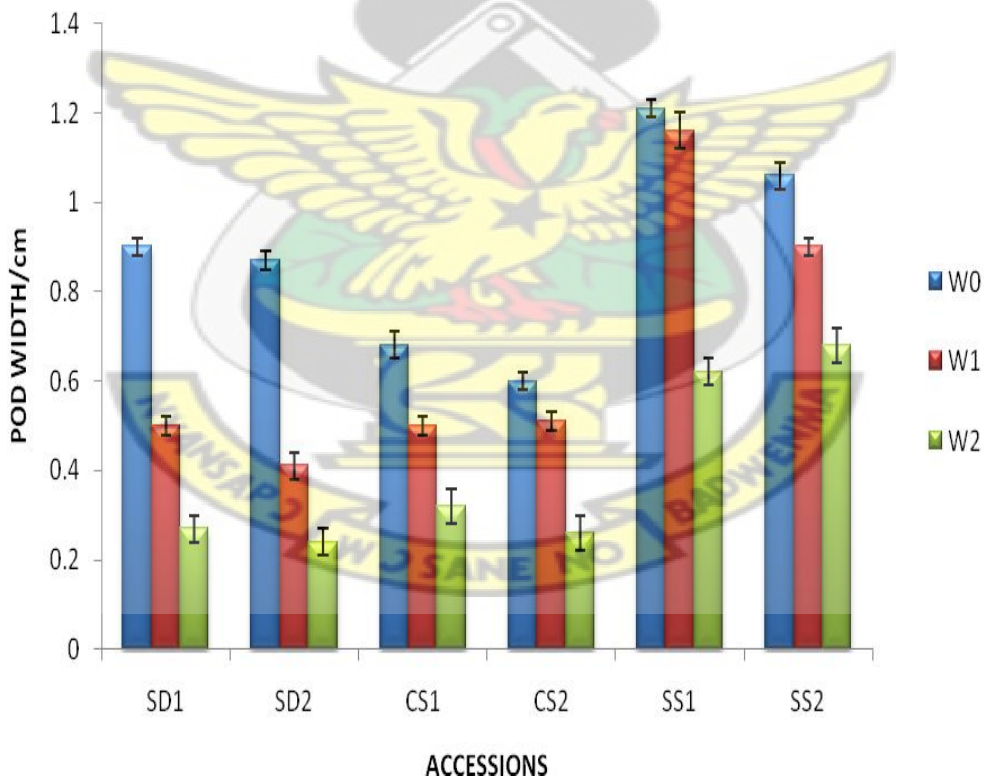

Fig. 8 Effects of different water treatments on the width of bean pods of the six accessions.

Where; SD1 & SD2 (Semi-deciduous accessions), CS1 & CS2 (Coastal savanna accessions) and SS1 and SS2 (Sudan savanna accessions). W0 (control), W1 (medium stress) and W2 (severe water stress)

4.3.2 WEIGHTS OF FRUITS

The six different accessions produced fruits under all the treatment protocols. There were more fruiting in the normal and medium water stress treatments as compared to that of the severe water stress in all the accessions (Figs 9&10).

4.3.2.1 MASS OF INDIVIDUAL BEAN PODS

The accessions produced bean pods with different weights under the normal irrigation (Fig. 9). The accessions SS1 and SS2 from the Sudan savanna produced fruits with individual mass average of 3.80g, followed by those of the Semi-deciduous zone with 2.75g. The accessions CS1 and CS2 from the Coastal savanna had the average least weight of individual bean pods of 1.90g (Fig 9). The weights of the individual bean pods decreased along the treatments as expected with the SS2 having the least reduction of 14% between the W0 and W1 treatments, followed by CS2 with 17% and SD2 with 29% reductions respectively. The accession from the Semi-deciduous zone great reduction of 88% in the mass of individual bean pods between the W0 and W2 treatments. It was followed by accessions from the Coastal savanna (CS2 with 28%) and SS2 of the Sudan savanna with 14% reduction.

It was observed that the accessions from the Sudan savanna reduced by an average of 14% across all the treatments while CS2 reduced by 17% under the W0 and W1, and 28% under the W2 treatment. The Semi-deciduous accession SD2 had reductions above 26% across the treatments (Fig 9). The trends whereby, the sizes and weights of fruits produced by leguminous plants reduced with respect to increasing water stress treatment were also observed in earlier works by Ahedor (1996), and Petrie and Hall (1992).

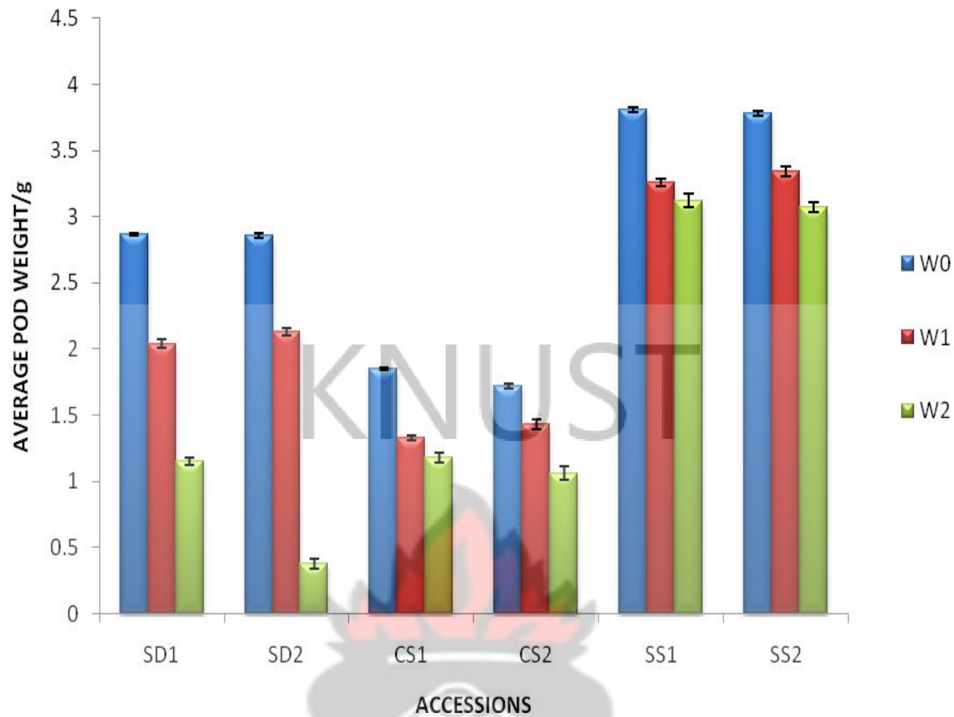

Fig. 9 Effects of different water treatments on the mass of individual bean pods of the six accessions.

Where; SD1 & SD2 (Semi-deciduous accessions), CS1 & CS2 (Coastal savanna accessions) and SS1 and SS2 (Sudan savanna accessions). W0 (control), W1 (medium stress) and W2 (severe water stress)

4.3.2.2 WEIGHTS OF TOTAL FRUITS PRODUCED

The weight of total fruits produced by the different accessions varied between themselves under the normal irrigation (control). The accessions SD1 and SD2 from the semi-deciduous zone produced an average mass of 52g of fruits, followed by the CS1 and CS2 accessions with an average of 49g and the Sudan savanna accessions producing 32g. The accessions CS1 and CS2 of the Coastal savanna however, recorded the highest fruit production (18.00-20.00g) under the severe stress regime, followed by the Sudan savanna accessions (15.00g-12.00g). The accessions from the moist ecological zone recorded the lowest fruit production of 10.00g (Fig.10). The accession CS1 had the least reduction of 44% in the fruits produced between the W0 and W2 treatments while recording a reduction of 14% between the W0 and W1 treatments. The accessions SS1 of the Sudan savanna followed with a reduction of 50% between the W0 and W2 treatments while recording a reduction of 16% between the W0 and W1 treatments. The accession SD1 recorded the 88% reduction between W0 and W2.

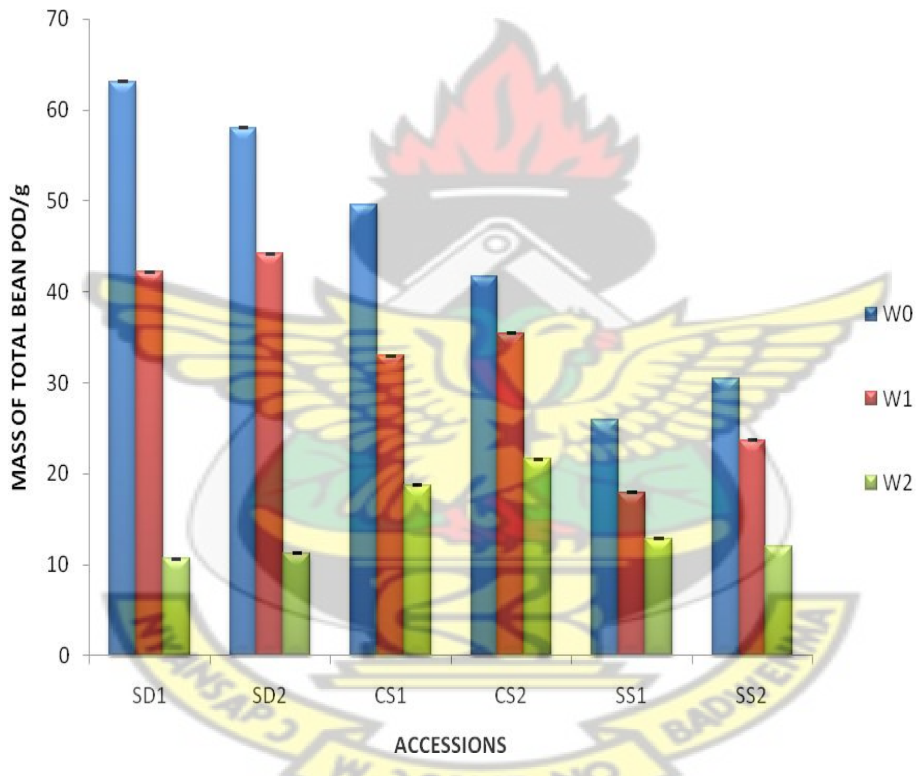

Fig. 10 Effects of different water treatments on the total fruits of the six accessions. Where; SD1 & SD2 (Semi-deciduous accessions), CS1 & CS2 (Coastal savanna accessions) and SS1 and SS2 (Sudan savanna accessions). W0 (control), W1 (medium stress) and W2 (severe water stress)

4.3.2.3 WEIGHTS OF DRIED FRUITS

The weights of the dried fruits produced by the six accessions at the end of the investigation varied between the accessions. The accessions from the Semi-deciduous zone had 10g under the normal irrigation while the accessions from the Sudan savanna had the least mass of 4.9g with the Coastal savanna accessions having 6g. Under the severe water stress treatment, the accessions SD1 recorded 3g with a reduction of 71%, followed by the SS2 with 40% (2.5g). The accession CS2 from the Coastal savanna recorded 5g with a reduction of 33% (Fig. 11). The reductions were found to be significantly between the accessions and the treatments (Appendix V).

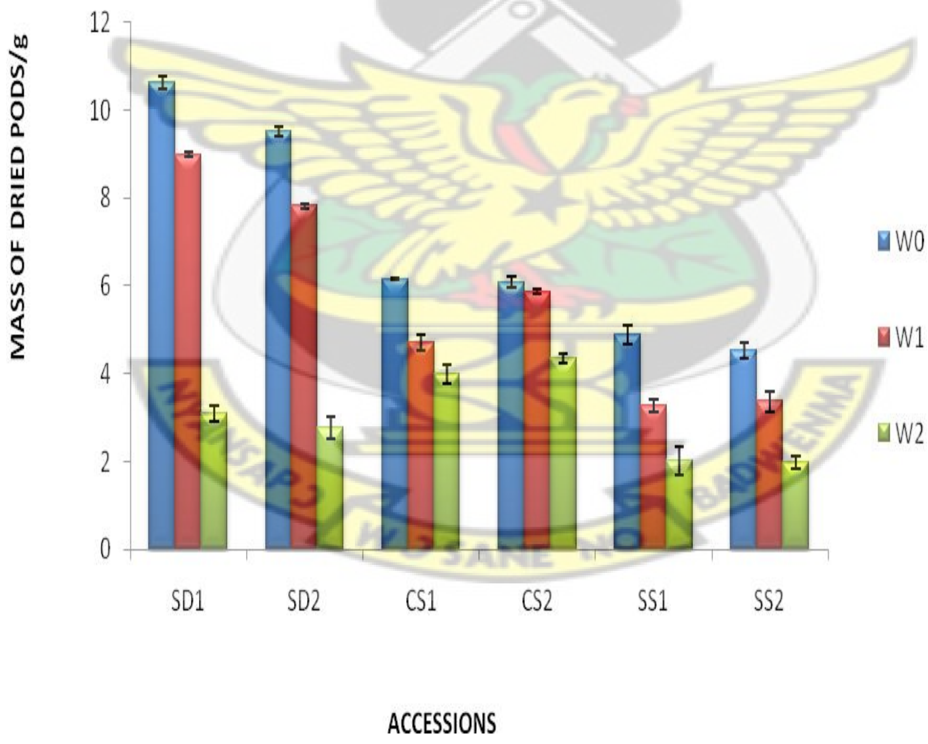

Fig. 11 Effects of different water treatments on the weights of dried fruits.

Where; SD1 & SD2 (Semi-deciduous accessions), CS1 & CS2 (Coastal savanna accessions) and SS1 and SS2 (Sudan savanna accessions). W0 (control), W1 (medium stress) and W2 (severe stress)

4.4 ANATOMY OF THE ACCESSIONS

The anatomical parameters were studied in the organs of the roots, stems and leaves. These include the diameter of cells, width of cortex, width of vascular tissues, diameter of xylem vessels, frequency of xylem vessels in the stele of the plants, frequency and distribution of stomata, width of guard cells as well as the width of the cuticles.

4.4.1 CELLS OF THE EPIDERMIS

The epidermal cells of the organs of the accessions were squamous and flattened in shape (Plates 5-9). The cells had diameters that ranged from 1.53X10µm to 2.22X10µm. Though the epidermal cells of the various organs did not differ much in sizes within the accessions, there was a little decrease in the sizes under the stress treatments.

The epidermal cells in the roots of the SD1 and SD2 accessions from the Semi-deciduous zone showed greater variation between the normal irrigation and the severe water stress protocol (from 2.05X10µm to 1.53X10µm). Though the others showed smaller decreases in sizes (from 2.22X10µm to 2.21X0µm) across the water stress protocols (Table 2). The accessions from the Semi-deciduous thus, reduced in the sizes of their epidermal cells by 2X of that shown by the accessions from the drier ecological zones. The sizes of the epidermal cells of the roots of the six accessions showed no significant differences between themselves (P=3.1911; df.5) and within the treatment protocols (P=0.5322; df.2) (Appendix VI).

The sizes of the epidermal cells of the stems of the accessions were not different from each other under the normal irrigation treatment. There was a general decrease within the treatments protocols with the reductions ranging between 1.92X10µm -1.89X10µm in the accessions from the savanna zones (Table 2). Those from the moist ecological zone had a relatively greater reduction; from 2.04X10µm to 1.96X10µm in the SD2 accession.

The epidermal cells of the leaves of the six accessions showed decreases in sizes along the water stress treatments. There was greater reduction in the plants; esp. the SD1 and SD2 from the W1 treatment to the W2 treatment where the sizes reduced from 1.50X10µm to 0.14X10µm while the savanna accessions reduced from 2.01x10µm to1.88X10µm. The variations were found not to be significant (P> 0.05) within the treatment regimes and within the accessions (Appendix VI). Reductions in the sizes of epidermal cells were observed by Palmer and Davies (1996) and Steudle (2000) in an earlier works.

TABLE 2: Effects of different water treatments on the sizes of epidermal cells of the six different accessions of cowpeas.

ACCESSION CODES	TREATMENTS	EPIDERMAL CELLS DIAMETERS / x10 µm		
		ROOTS	STEMS	LEAVES
SD1	W0 W1 W2	2.05± 0.02f 1.94± 0.02e 1.53± 0.03a	2.22± 0.02ef 2.21± 0.04ef 2.08± 0.03c	1.53± 0.01de 1.50± 0.01d 0.14± 0.03a
SD2	W0 W1 W2	1.85± 0.03c 1.83± 0.04c 1.73± 0.02b	2.04± 0.02c 1.98± 0.02b 1.96± 0.03b	2.03± 0.01g 1.97± 0.02g 1.08± 0.03b
CS1	W0 W1 W2	2.22± 0.02g 2.21± 0.01g 2.21± 0.03g	1.92± 0.02ab 1.91± 0.02a 1.89 ±0.03a	1.51± 0.02d 1.37± 0.02c 1.08± 0.03b
CS2	W0 W1 W2	1.90± 0.01d 1.84± 0.01c 1.83± 0.02c	2.05± 0.01cd 2.04± 0.02c 1.87 ±0.03a	2.00 ± 0.01g 1.97 ± 0.01g 1.48 ± 0.02d
SS1	W0 W1 W2	2.53± 0.01h 2.51± 0.02h 2.48± 0.03h	2.02± 0.02f 2.01± 0.03f 2.00± 0.03e	2.02± 0.02g 2.01± 0.03g 1.88± 0.03f
SS2	W0 W1 W2	2.06 ±0.02f 2.00± 0.03f 1.94± 0.03e	2.22± 0.01c 2.21± 0.02c 2.15± 0.03c	1.98± 0.02fg 1.96± 0.03fg 1.52± 0.03de

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

NOTE:
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone
W0: Normal water treatment
W1: Medium stress treatment
W2: Severe treatment

4.4.2 CORTICAL TISSUES

The size of cortical tissue in the roots of all the accessions reduced across the water stress treatments. In the SD1, the cortex reduced from 25.58X10μm to 14.76X10μm. while the accessions CS1 showed small reduction (23.43X10μm to 18.83X10μm). There was variation in the cortical tissues of the stems of the accessions under the water stress protocols with the accessions SD1 and SD2 exhibiting little reduction between the W1 and W2 treatments; 20.45X10μm to 18.32X10μm while the savanna plants rather showed greater decrease in sizes within the same treatments; e.g. from 25.07X10μm to 22.50X10μm for CS2 (Table 3). The size of the cortex in both the roots and stems of the accessions varied significantly ($P<0.05$) within the treatment protocols.

4.4.2.1 SIZES OF CORTICAL CELLS

The diameters of the cells of the cortex of both the roots and the stems of the plants of all the accessions showed significant differences at ($P<0.05$) within themselves at df.5 and the water treatment protocols at df.2. The variation was great between the W0 and W1 treatments in the accessions from the moist ecological zones (SD1 and SD2) while those from the drier zones had a greater reduction between the W1 and the W2 treatments. In the accessions SD2, the reduction was 0.62μm from the W0 to the W1 while the SS1 accessions had 0.15μm within the same treatments (Table 3). Between the W1 and the W2 treatments, the SD2 reduced further by 0.41μm while the SS1 reduced more by 1.11μm. The trend of decreases in the sizes of the cortex and its cells with respect to different water stress treatments were observed in earlier works by Signit and Kramer (1977), Kramer (1983) and Souza (2004).

TABLE 3: Effects of the different water treatments on the sizes of the cortex of six different accessions of cowpeas

ACCESSION CODES	TREAT-MENTS	SIZE OF THE CORTEX / x10 µm		DIAMETER OF CELLS OF THE CORTEX / x10 µm	
		ROOTS	STEMS	ROOTS	STEMS
SD1	W0 W1 W2	25.58±0. 02k 22.11±0.03g 14.76± 0.05b	22.50±0.04g 20.45±0.06d 18.32±0.06a	4.04± 0.03j 3.42 ±0.02h 3.01± 0.03e	3.35±0.03e 3.14±0.04d 3.06±0.06cd
SD2	W0 W1 W2	28.91± 0.01l 24.43± 0.02i 17.37± 0.02c	23.48±0.04i 21.15±0.05f 19.01±0.05b	3.81± 0.06i 3.36± 0.03g 2.53± 0.02c	3.95±0.02g 3.51±0.04f 2.94±0.04b
CS1	W0 W1 W2	23.43± 0.02h 21.00± 0.04f 18.83± 0.04d	26.90±0.02m 23.15±0.04h 20.04±0.05c	4.53± 0.02l 4.00± 0.04j 3.03± 0.03e	4.02±0.03h 3.51±0.03f 3.08±0.06c
CS2	W0 W1 W2	19.62± 0.01e 14.69± 0.03b 11.01± 0.03a	28.88±0.05n 25.07±0.06j 22.50±0.06g	3.78± 0.01i 2.81± 0.02d 1.93± 0.04a	4.13±0.03i 3.56±0.02f 2.72±0.04a
SS1	W0 W1 W2	32.76± 0.03n 30.42± 0.03m 24.91± 0.05j	32.78±0.03p 26.40±0.04l 20.66±0.05e	3.36± 0.02g 3.21± 0.05f 2.10± 0.03b	4.68±0.03k 3.36±0.02e 3.05±0.04c
SS2	W0 W1 W2	42.70± 0.02q 40.05± 0.04p 33.76± 0.05o	36.88±0.04q 30.53±0.05o 25.82±0.05k	4.41± 0.02k 4.02± 0.04j 3.45± 0.05h	6.57±0.02l 4.52±0.03j 3.30±0.05de

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

NOTE:
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone
W0: Normal water treatment
W1: Medium stress treatment
W2: Severe treatment

4.4.3 VASCULAR TISSUES

Vascular tissues appeared in both the roots and stems of the accessions. The vascular tissues of the roots appeared as bands. The vascular tissues of the stems were however, of bundles and connected by a cambial ring (Plates 6-13).

4.4.3.1 SIZES OF VASCULAR TISSUES

The main vascular tissues examined were that of the xylem and the cambium. The sizes of the vascular tissues of the roots of the accessions reduced under the water stress treatments while that of the stems increased in dimensions (Table 4). The reductions in the vascular tissue of the accessions were not uniform as the roots of the accessions from the Semi-deciduous zone reduced more drastic than those from the drier zones. The SD1 reduced from 55.30X10µm in the W0 to 32.03µm under the W2. The decrease in size across the treatments was 23.27X10µm. The accession SS1 from the Sudan savannah had a variation of only 2.98X10µm across the treatments. The reduction in the size of the vascular tissues of the roots did not vary much in the accessions SS1 and SS2. The variations were found to be significant ($P<0.05$) under the treatment protocols and within the accessions (Appendix VI).

4.4.3.2 SIZES OF VASCULAR BUNDLES

The xylem vessels were numerous and made up of the protoxylems, metaxylems and fiber cells (Plates 6-13). The xylem vessels in all the organs of the plants of the accessions were measured. The plants of the accessions SD1 and SD2 showed greater decrease in the sizes of the xylem vessels of their roots across the treatments; accession

SD1 reduced in the size of the vascular tissue of the roots from 55.30X10µm in the W0 to 44.06X10µm in the W1 treatment and 32.03X10µm in the W2 treatment. The accessions in the savanna zone reduced marginally by a difference of 3.00X10µm across the treatment protocols (Table 4). Correspondingly, there was a greater increase in sizes of the vascular tissue in the stems of the six accessions as the water stress got severer. The differences in the sizes of the vascular tissues of the stems of accessions SD1 and SD2 were higher than that of the savanna accessions by 3 folds; 16.91X10µm increase for SD1 while those of CS1 and SS1 had an increment of 6.51x10µm in sizes within the same treatments. The leaves showed reductions in the sizes of their vascular tissues. The reductions were greater in the SD1 and SD2 accessions along the stress treatments. It was wider within the W1 and W2 treatments than those of the savanna zones which showed gradual decreases along the stress treatments (Table 4). The variations were found to be significant between the treatments ($p<0.05$) but within the accessions, the variation was not significant ($p<0.58$) for the roots and ($p<0.22$) for the stems.

This trend of decreasing in sizes of vascular tissues with respect to water stress treatments was noted in earlier works by Boyer (1985), and Hsiao and Zing (1987).

TABLE 4: <u>Effects of different water treatments on the vascular band / bundles of the six different accessions of cowpeas</u>.

ACCESSION CODES	TREAT-MENTS	WIDTH OF VASCULAR BANDS / x10µm		
		ROOTS	STEMS	LEAVES
SD1	W0	55.30±0.02m	25.50±0.05a	33.48±0.07d
	W1	44.06±0.03c	37.43±0.02e	32.81±0.05c
	W2	32.03±0.03a	42.82±0.02g	31.92±0.04a
SD2	W0	57.12±0.02o	42.60±0.03f	37.16±0.05f
	W1	45.81±0.04f	44.21±0.09h	36.06±0.07e
	W2	40.06±0.05e	46.36±0.01j	32.64±0.08b
CS1	W0	35.74±0.03d	34.58±0.02b	52.62±0.02o
	W1	34.92±0.06c	35.97±0.11c	50.32±0.03l
	W2	33.51±0.04b	37.16±0.02d	48.99±0.05k
CS2	W0	59.56±0.06p	44.40±0.03i	49.93±0.04k
	W1	56.06±0.09n	46.37±0.05j	47.36±0.03j
	W2	51.28±0.03i	49.22±0.03k	46.08±0.07i
SS1	W0	53.36±0.05l	50.05±0.03l	44.74±0.07h
	W1	52.40±0.07k	52.83±0.04m	42.94±0.05g
	W2	50.38±0.06h	53.54±0.02n	41.83±0.06g
SS2	W0	52.03±0.03j	58.66±0.02o	54.18±0.02p
	W1	50.44±0.06h	59.71±0.03p	52.02±0.04n
	W2	47.55±0.02g	61.29±0.04q	50.76±0.04m

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

<u>NOTE</u>:
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone
W0: Normal water treatment
W1: Medium stress treatment
W2: Severe treatment

4.4.3.3 SIZES OF XYLEM VESSELS

The sizes of the xylem vessels of the six accessions decreased along the stress treatments. The reductions were uniform in the vessels as the decreases ranged between 2.00X10µm to 3.00X10µm within the different water treatments. The accessions from the savanna zones showed lower reduction of 0.15X10µm in the xylem vessels between the W0 and W1 treatments as compared to the reduction between the W1 and the W2 treatments that was 2.43 X10µm (Table 5). The variations that occurred in the xylem vessels were found to be significant ($p<0.05$) within the treatments but not significant ($p>0.05$) within the accessions.

TABLE 5: Effects of the different water treatments on the sizes of xylem vessels of the six different accessions of cowpeas

ACCESSION CODES	TREAT-MENTS	DIAMETERS OF XYLEM VESSELS / x10µm		
		ROOTS	STEMS	LEAVES
SD1	W0 W1 W2	7.05± 0.03m 5.04± 0.03f 3.74± 0.04a	8.24± 0.01k 6.27± 0.03e 5.06± 0.03c	4.26± 0.02i 3.90± 0.04f 2.86± 0.05b
SD2	W0 W1 W2	6.88± 0.03l 6.84± 0.04l 4.72± 0.02e	7.03± 0.03f 5.22± 0.03d 3.62± 0.05a	4.13± 0.03h 3.72± 0.03e 3.05± 0.04c
CS1	W0 W1 W2	7.02± 0.03m 6.24± 0.04j 3.92± 0.04b	8.05± 0.03j 7.00± 0.02f 6.21± 0.04e	5.12± 0.03n 3.88± 0.02f 2.94± 0.03b
CS2	W0 W1 W2	6.71± 0.02k 5.43± 0.04h 3.80± 0.04a	9.71± 0.01m 8.05± 0.03j 7.24± 0.02g	4.09± 0.02h 3.36± 0.02d 2.66± 0.03a
SS1	W0 W1 W2	5.57± 0.02i 5.23± 0.03g 4.01± 0.03c	8.43± 0.04l 7.84± 0.02i 5.00± 0.04c	5.71± 0.02o 5.30± 0.03m 4.93± 0.02l
SS2	W0 W1 W2	6.93± 0.01l 6.92± 0.02l 4.32± 0.03d	7.45± 0.02h 5.26± 0.01d 3.81± 0.03b	4.75± 0.03k 4.48± 0.04j 4.03± 0.03g

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

4.4.3.4 FREQUENCY OF XYLEM VESSELS

There was a general decrease in the frequency of the xylem vessels in the roots, while that of the stems increased in the accessions under increasing severity in water stress treatment (Table 6, Plates 5-9). The accessions SD1 and SD2 from the Semi-deciduous were found to decrease in the frequency slightly from the W0 to the W1 treatments but experienced more impact of the water stress under the severe water stress. The difference was about 2.00X10µm reduction between the W0 and W1, and about 4.02X10µm between the W0 and W1 protocols. The accessions from the savanna zones decreased in size by 1.50X10µm between the W0 and W1 treatments, and about 2.00X10µm between the W1 and the W2 stress treatments. It could be seen that the savanna accessions were able to resist the reduction in sizes under the water stress treatment protocols (7). ANOVA analysis showed the variation to be not significant (P>0.05) within the treatment protocols at df.2 and within the accessions at df.5 (Appendix VI).

4.4.4 LAYERS OF CAMBIAL CELLS

The layers of the cambium cells decreased in the accessions under water stressed treatments. The accessions CS1 and CS2 showed no reduction in the cambial layers of the roots within the normal watering (W0) and the medium water stress (W1) treatments, while that of the stems showed no variation between the W1 and W2 water stressed treatments. The variations were found to be significant (P<0.05) within the accessions at df.5 and the treatment protocols at df.2.

TABLE 6: Effects of the different water treatments on the frequency of xylem vessels and the layers of cambium cells of the six accessions

ACCESSION CODES	TREAT-MENTS	FREQUENCY OF XYLEM VESSELS		LAYERS OF CAMBIUM CELLS	
		ROOTS	STEMS	ROOTS	STEMS
SD1	W0	19.67± 0.06e	10.33± 0.07a	3	5
	W1	17.08± 0.02c	12.43± 0.03c	2	3
	W2	13.42± 0.04a	16.55± 0.04h	0	0
SD2	W0	18.00± 0.07d	11.44± 0.05b	2	5
	W1	17.07± 0.04c	15.54± 0.09g	2	3
	W2	14.94± 0.03b	17.10± 0.13i	0	1
CS1	W0	28.54± 0.04n	12.45± 0.06c	3	4
	W1	26.13± 0.08l	14.04± 0.05e	3	2
	W2	23.74± 0.04i	15.57± 0.03g	2	2
CS2	W0	27.00± 0.10m	13.71± 0.02d	3	5
	W1	24.48± 0.04j	15.06± 0.04f	3	2
	W2	22.09± 0.03f	16.54± 0.03h	2	2
SS1	W0	26.01± 0.11l	14.10± 0.09e	3	5
	W1	24.36± 0.08k	19.60± 0.05j	2	4
	W2	22.30± 0.05g	22.10± 0.02k	1	2
SS2	W0	28.84± 0.06o	11.36± 0.04b	3	5
	W1	25.61± 0.03k	17.09± 0.08i	3	4
	W2	23.27± 0.04h	22.50± 0.03l	2	2

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

NOTE:
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone
W0: Normal water treatment
W1: Medium stress treatment
W2: Severe treatment

4.4.5 PITH TISSUES

The pith region of the different accessions was relatively lager in sizes than that of the other anatomical structures studied. The average size ranged between 13.34-20.61X10µm in the pith cells. The pith showed large parenchyma cells that were loosely arranged in the center of the roots and stems of the accessions under the microscope. The pith region of the roots of most of the accessions however, had open spaces within the parenchyma cells of the pith (Plates 6-11).

4.4.5.1 SIZES OF PITH REGION

The mean values of the size of the pith region in the roots of the accessions varied little under the normal irrigation that also served as a control. There was a mean value of 19.91 X10µm for the accessions from the moist ecological zone (SD1 and SD2), followed by savanna accessions; CS1 and CS2 with 21.00 X10µm, and 23.04 X10µm for the SS1 and SS2 (Table 7). The different accessions however, differed in their trends of reductions in the pith region of their roots and stems. There was a smaller decrease in the pith size of the roots in the accessions CS1 and CS2; ie. 2.00X10µm from the W0-W1 and between W1-W2 water stress treatments. The accessions from the moist ecological zone showed greater reductions in their pith; with an average of 7.00X10µm within the W0 to W1, and between the W1 to the W2 water stress treatments (Table 7). These variations were found to be significant within the treatments at df.2 but not significant within the accessions at df.5. (Appendix VI).

The pith of the stems of the different accessions was found to be larger than that of their roots by about 25% (Table 7). The difference was about 5.00X10µm in the SD1 and SD2 accessions, 2.50-3.00X10µm in the Coastal savanna accessions (CS1 and CS2) and 6.00X10µm in the SS1 and SS2 accessions. The stems of the Sudan savanna accessions were found to be morphologically bigger than the other accessions in the control treatment (Plate 1). There was a decrease in the sizes of the pith in the stems of the different accessions under increasing water stress application (Table 7). The decrease in the sizes of the pith of the stems was smaller in the accessions CS1 and CS2 under the stress applications; 1.53X10µm between the W0 and W1 treatments, and the W1-W2 water stress treatments. This was followed by the accessions SS1 and SS2 with a reduction of 1.65X10µm between the W0 and W1 treatments and 2.30X10µm – 3.21X10µm between the W1 and W2 water stress treatments. The accessions SD1 and SD2 exhibited the greatest reduction with a decrease of 3.00X10µm – 5.80X10µm between the W0 and W1 treatments, and 2.50X10µm – 4.20X10µm between the W1 and the W2 water stress treatments.

4.4.5.2 SIZES OF PITH CELLS

The cells of the pith decreased in their sizes along the water stress treatments. The reductions were prominent in the pith cells than the cells of the other tissues. The water stress imposition may have had a higher impact as a result of plasmolysis in the soft-walled parenchyma cells. The sizes of the pith cells of the accessions SD1 and SD2 showed wider reduction as compared to the others with a difference of 2.00X10µm between the W0 and W1 treatments and 4.15X10µm from the W1 to the W2 water stress treatments. This was followed by the accessions CS1 and CS2 with a difference of 1.50X10µm while the accessions SS1 and S2 had the least reduction with a mean of 1.20X10µm. The variations were found to be significant at P<0.05 (Appendix VI).

TABLE 7. Effects of different water treatments on the sizes of the pith region and the pith cells of the six accessions of cowpeas

ACCESSION CODES	TREAT-MENTS	SIZE OF PITH REGION X10µm		DIAMETER OF PITH CELLS/ x10µm	
		ROOTS	STEMS	ROOTS	STEMS
SD1	W0	20.61± 0.03k	26.18± 0.02l	8.31± 0.03n	10.46± 0.02l
	W1	17.50± 0.02e	23.54± 0.01h	5.50± 0.04e	9.26± 0.03j
	W2	14.14± 0.02b	19.73± 0.04b	2.71± 0.05b	5.15± 0.05a
SD2	W0	19.72± 0.02j	25.99± 0.04k	7.43± 0.04l	9.43± 0.01j
	W1	17.02± 0.01d	20.01± 0.04c	5.01± 0.03d	8.36± 0.04h
	W2	13.37± 0.03a	18.25± 0.03a	2.25± 0.04a	5.06± 0.06a
CS1	W0	21.05± 0.05m	23.45± 0.05g	7.25± 0.02k	7.11± 0.03d
	W1	20.67± 0.04k	22.73± 0.06f	6.92± 0.06i	6.68± 0.05c
	W2	17.95± 0.06f	20.92± 0.03d	4.18± 0.04c	6.05± 0.04b
CS2	W0	20.88± 0.02l	23.91± 0.03i	7.08± 0.03o	8.12± 0.01g
	W1	19.05± 0.03h	21.35± 0.02e	5.96± 0.04g	8.01± 0.03f
	W2	16.72± 0.04c	19.75± 0.05b	4.24± 0.06c	7.32± 0.05e
SS1	W0	22.52± 0.05o	28.53± 0.04n	8.94± 0.02p	12.16± 0.02o
	W1	22.13± 0.08n	27.92± 0.07m	6.26± 0.05h	11.20± 0.02m
	W2	18.61± 0.02g	24.68± 0.04j	5.78± 0.05f	9.42± 0.03j
SS2	W0	23.30± 0.04p	29.01± 0.08o	8.20± 0.03m	11.74± 0.03n
	W1	21.09± 0.03m	28.03± 0.07m	7.43± 0.04l	10.18± 0.05k
	W2	19.25± 0.06i	26.18± 0.03l	6.01± 0.03g	9.42± 0.04j

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

4.4.6 TISSUES OF THE LEAVES

Transverse section of the leaves indicated that the leaf blades were made up of mesophyll tissues bounded by two epidermal layers. The spongy mesophyll was made up of cells that were loosely arranged and have intercellular spaces within while the palisade mesophyll formed a single layer of square-shaped cells under the upper epidermis (Plates 11-13).

4.4.6.1 VARIATIONS IN THE MESOPHYLL TISSUES

Generally, there was a reduction in the sizes of both the palisade and the spongy cells as a response to increasing severity in the water stress treatment (Table 8). The width of the mesophyll tissues thus, reduced in the accessions as the water stress became severer. However, there was a greater decrease in the sizes between the medium water stress (W1) and the severe water stress (W2) treatment protocols in the accessions. The variation was found to be significant within the treatment protocols ($p<0.05$) though not significant between the different accessions ($p>0.05$) (Appendix VI).

The palisade tissues reduced slightly under the treatment protocols though, the reduction was more prominent within the accessions from the Semi-deciduous zone. The accessions SD1 and SD2 had a reduction of 0.87X10µm from the normal irrigation to the medium water stress treatment while those from the Savanna zone had a mean reduction of 0.30X10µm. This showed that the water stress had greater impact on the Semi-deciduous accessions than those of the savanna zone. There was a greater reduction in the sizes of the palisade cells from the medium water stress to the severe water stress regimes. The accessions from the Semi-deciduous zone reduced by a mean value of 0.54X10µm while that of the drier ecological zones reduced by a mean value of 0.25X10µm.

The spongy tissues decreased greatly in sizes along the water stress treatments. Similarly, the spongy cells of the accessions from the Semi-deciduous zone decreased prominently under each of the treatment protocols than the others from the Savanna zones. The accessions SD1 and SD2 reduced by a mean value 1.07X10µm from the normal irrigation to the medium water stress treatment while the accessions from the drier ecological zones decreased by a mean value of 0.30X10µm. The reduction in the spongy cells from the medium water stress to the severe water stress treatments was higher in the accessions SD1 and SD2 by a mean value of 0.64X10µm while those from the drier zones; CS1, CS2, SS1 and SS2 reduced by a mean value of 0.31X10µm within the same treatment protocols.

4.4.6.2 VARIATIONS IN THE CUTICLES

The cuticles showed slight increase in sizes in the accessions under the water stress treatment. There was a general increase in the sizes of the cuticles from the normal irrigation (W0) to the medium water stress (W1) protocols in all the accessions. There was however, no difference in the sizes of the cuticles between the medium stress (W1) and the severe stress (W2) treatments except that in the accessions from the moist ecological zone; SD1 and SD2. The variation was not found to be significant ($P>0.05$) between the accessions though, it was significant between the treatments ($p<0.05$) at df.5 and df.2 respectively.

The water stress imposition may have caused a hormonal action in the plants that might have prevented the epidermal cells of the leaves to produce additional cutin under the severe water stress protocol though there was an initial increase in the cuticles of the accessions from the normal irrigation (W0) to the medium water stress (W1) treatment. The accessions from the moist ecological zones showed a reduction after the initial increase in the width of the cuticle from the medium water stress (W1) to the severe water stress (W2) treatments (Table 8).

The other accessions from the drier ecological zones were observed to maintain the sizes of their cuticles between the medium water stress (W1) treatment to the severe water stress (W2) treatment after the initial increase in size from the normal irrigation (W0) to the medium water stress treatment (W1) (Table 8). Reductions in the tissues of leaves with respect to water stress treatments were noted in earlier works by Tardieu (2000) and Souza (2004).

TABLE 8: Effects of the different water treatments on the sizes of the mesophyll tissues and the cuticles in the leaves of six accessions of cowpeas

ACCESSION CODES	TREAT-MENTS	SIZE OF MESOPHYLL TISSUES / X10µm		SIZE OF CUTICLES / X10µm	
		SPONGY CELLS	PALISADE CELLS	UPPER SIDE	LOWER SIDE
SD1	W0 W1 W2	4.92± 0.02j 4.16± 0.04f 3.38± 0.06a	3.50± 0.02jk 2.63± 0.03f 2.00± 0.03b	0.20± 0.01b 0.21± 0.01b 0.17± 0.01a	0.18± 0.01a 0.19± 0.02a 0.16± 0.02a
SD2	W0 W1 W2	5.02± 0.03k 4.36± 0.04g 3.67± 0.04c	3.65± 0.02l 3.08± 0.02i 2.77± 0.03g	0.20± 0.01b 0.21± 0.02b 0.17± 0.01a	0.18± 0.02a 0.19± 0.02a 0.15± 0.03a
CS1	W0 W1 W2	4.62± 0.03h 3.91± 0.05de 3.58± 0.06bc	2.51± 0.01e 2.13± 0.03c 2.00± 0.03b	0.25± 0.02bc 0.27± 0.01c 0.27± 0.02c	0.24± 0.03ab 0.25± 0.02b 0.25± 0.03b
CS2	W0 W1 W2	3.87± 0.03d 3.59± 0.03b 3.34± 0.05a	2.45± 0.02d 2.07± 0.02c 1.68± 0.04a	0.26±0.01c 0.28±0.02c 0.28±0.02c	0.26± 0.01b 0.27± 0.02b 0.27± 0.03b
SS1	W0 W1 W2	5.80± 0.03m 5.41± 0.04l 4.78± 0.06i	4.23± 0.01o 4.07± 0.02n 3.86± 0.03m	0.33± 0.01d 0.35± 0.02d 0.35± 0.03d	0.32± 0.01c 0.33± 0.03c 0.33± 0.02c
SS2	W0 W1 W2	4.86± 0.04j 4.25± 0.05f 3.99± 0.03c	3.45± 0.03i 2.89± 0.05h 2.05± 0.05bc	0.33± 0.02d 0.36± 0.02d 0.36± 0.01d	0.33± 0.03c 0.34± 0.02c 0.34± 0.03c

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

NOTE:
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone
W0: Normal water treatment
W1: Medium stress treatment
W2: Severe treatment

4.4.6.3 VARIATION IN THE STOMATA

The stomata pores of the accessions from the Semi-deciduous zone were larger than that of the Savanna zone accessions under the normal irrigation (control) (Plates 11-13, Table 9). The accessions SD1 and SD2 had a mean value of 0.43X10µm in their stomata pores, while those of the Savanna zones had a mean value of 0.32X10µm for the CS1 and CS2, and 0.30X10µm for the SS1 and SS2. The sizes of the stomata openings in the different accessions thus, varied little between the accessions from the Savanna zones under the normal irrigation treatment by 0.01X10µm while they differed from that of the Semi-deciduous zone by 0.10X10µm.

All the accessions responded to the stress treatments by showing reductions or closure of the pores of their stomata (Table 9). The stomata of the Savanna accessions showed little reduction between the treatment protocols as compared to that of the moist ecological zone accessions. The Savanna accessions reduced their stomata pores by a mean value of 0.11X10µm (0.35%) from the normal irrigation to the medium water stress treatment while the Semi-deciduous accessions reduced by a wider margin of 0.23X10µm (54%) within the same treatments. The accessions from the moist ecological zone (SD1,SD2) however, reduced little in their stomata opening from the medium stress to the severe water stress protocol by 0.04X10µm as compared to the accessions from the Savanna zone that exhibited a mean reduction of 0.09X10µm. The accessions SD1 and SD2 might have already closed their stomata between the normal watering (W0) and the medium water stress treatment (W1) so there was no need for them to continue narrowing their stomata pores.

The sizes of the guard cells in accessions showed slight variation within the different accessions under increasing water stress treatments. Though the guard cells of the SD1 and SD2 were smaller than that of the Savanna zones, the reduction in the sizes of the guard cells was generally uniform between all of them (Table 9). The differences were between 0.08X10µm in the SD1 and SD2 accessions from the normal to the medium water stress protocol while the Savanna accessions showed a reduction of 0.06X10µm in the CS1 and CS2, and 0.04X10µm in the SS1 and SS2 accessions. The accessions from the Savanna zones thus, showed slight response to the stress treatments in their guard cells. The variations were found to be significant ($p<0.05$) between the treatments while the variation was not significant within the accessions ($p>0.05$) (Appendix VI).

The lower epidermal surface of the leaves of the different accessions possessed more stomata than the upper epidermal surfaces, and that of the SD1 and SD2 had more than the other accessions by a difference of 3 (Table 9). There were reductions in the number or frequency of the stomata on the leaves surfaces of the accessions SD1 and SD2 along the treatments protocols (W0-W1-W2), while there was only a little decrease in the frequency of the stomata in the accessions from the Savanna zones from the normal irrigation (W0) to the medium water stress (W1) treatments. There was no difference in the frequency of the stomata from the medium water stress (W1) to the severe water stress (W2) treatments in the savanna accessions.

TABLE 9: Effects of the different water treatments on the frequency of stomata, sizes of guard cells and stomata pores of the six accessions

ACCESSION CODES	TREAT-MENTS	SIZE OF PORES OF STOMATA	SIZE OF GUARD CELLS	FREQUENCY OF STOMATA	
				UPPER SURFACE	LOWER SURFACE
SD1	W0	0.44± 0.02e	1.94± 0.02c	20	26
	W1	0.21± 0.02b	1.82± 0.05b	19	24
	W2	0.18± 0.01ab	1.73± 0.03a	18	24
SD2	W0	0.42± 0.01e	2.09± 0.03de	19	24
	W1	0.25± 0.02bc	2.01± 0.06d	17	21
	W2	0.21± 0.02b	1.93± 0.03c	16	20
CS1	W0	0.31± 0.02d	2.13± 0.01f	16	21
	W1	0.24± 0.01bc	2.05± 0.02d	15	19
	W2	0.15± 0.03a	2.01± 0.02d	15	18
CS2	W0	0.33± 0.01d	2.12± 0.02f	17	22
	W1	0.25± 0.02bc	2.06± 0.02d	16	21
	W2	0.20± 0.01b	2.02± 0.03d	16	21
SS1	W0	0.31± 0.02d	2.23± 0.04gh	14	21
	W1	0.23± 0.01bc	2.17± 0.02g	13	19
	W2	0.12± 0.02a	2.08± 0.04d	13	19
SS2	W0	0.30± 0.01d	2.01± 0.02d	13	20
	W1	0.24± 0.01bc	1.96± 0.03cd	12	18
	W2	0.14± 0.03a	1.91± 0.01c	12	18

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

NOTE:
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone
W0: Normal water treatment
W1: Medium stress treatment
W2: Severe treatment

PLATE 1: GROWTH FORM OF THE DIFFERENT COWPEA ACCESSIONS UNDER NORMAL IRRIGATION AT THE END OF WEEK 5

NOTE:

C1 : Accessions from the Semi-deciduous zone. Accessions are semi-erect and showed twining of stems.

C2 : Accessions from the Coastal savannah zone. Accessions are creepers / stranglers and showed twining of stems.

C3 : Accessions from the Sudan savannah zone. Accessions are erect and do do not show twining of stems.

PLATE 2: THE GROWTH RESPONSE OF THE ACCESSIONS FROM THE SEMI-DECIDUOUS ZONE **(SD**) TO DIFFERENT WATER TREATMENTS AT THE END OF WEEK 7

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NOTE:
W0 : Normal water treatment
W1 : Medium stress treatment
W2 : Severe stress treatment

Plant heights and leaf sizes were observed to decrease along the water stress treatments; W0>W1>W2.

PLATE 3: THE GROWTH RESPONSE OF THE ACCESSIONS FROM THE COASTAL SAVANNA ZONE **(CS)** TO DIFFERENT WATER TREATMENTS AT THE END OF WEEK 7

NOTE:

W0 : Normal water treatment
W1 : Medium stress treatment
W2 : Severe stress treatment

Plant heights and leaf sizes were observed to decrease along the water stress treatments; W0>W1>W2.

PLATE 4: THE GROWTH RESPONSE OF THE ACCESSIONS FROM THE SUDAN SAVANNA ZONE (**SS**) TO DIFFERENT WATER TREATMENTS AT THE END OF WEEK 7

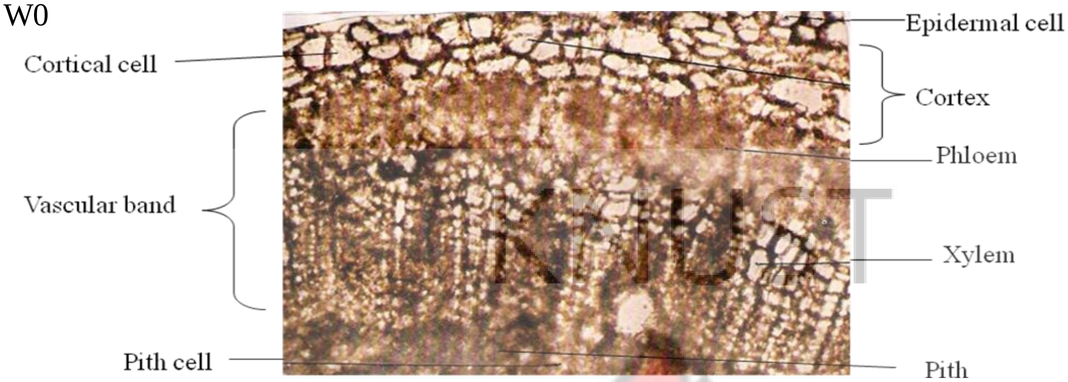
NOTE:
W0 : Normal water treatment
W1 : Medium stress treatment
W2 : Severe stress treatment

Plant heights and leaf sizes were observed to decrease along the water stress treatments; W0>W1>W2.

PLATE 5: PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION (T/S) OF THE ROOT OF 7 WEEKS OLD ACCESSIONS **(SD)** UNDER DIFFERENT WATER TREATMENTS

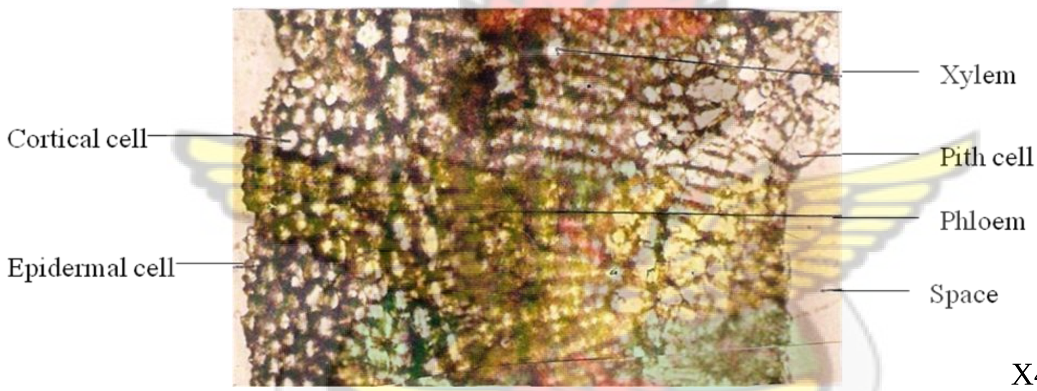
TREATMENTS

W0



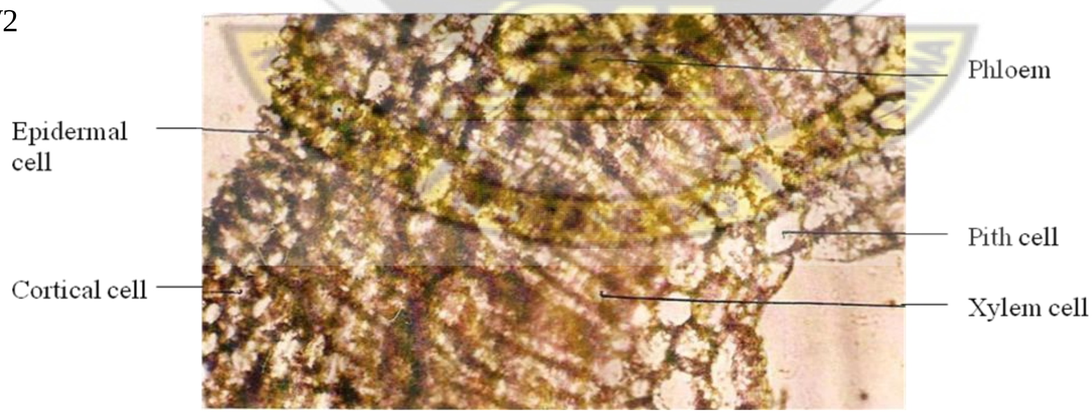
X40

W1



X40

W2



X40

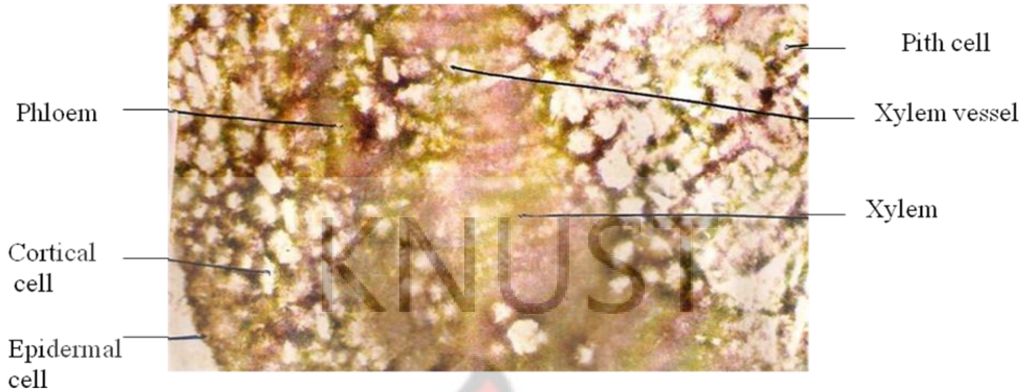
NOTE:

Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 6: PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION (T/S) OF THE ROOT OF 7 WEEKS OLD PLANT OF ACCESSIONS (**CS)** UNDER DIFFERENT WATER TREATMENTS

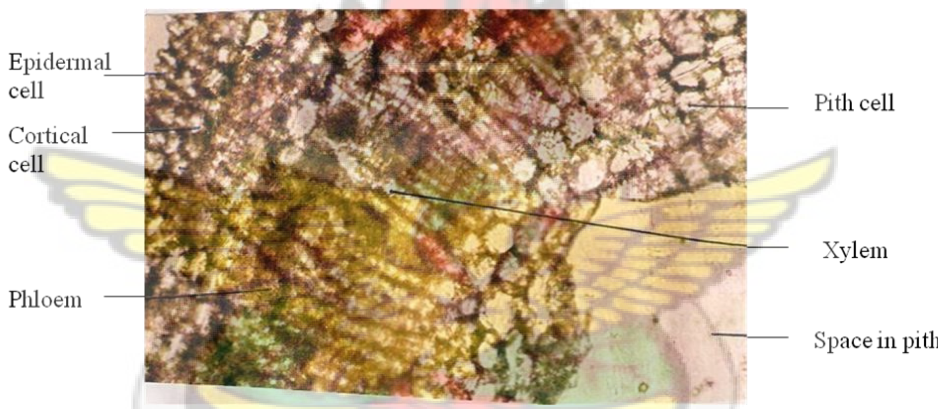
TREATMENTS

W0



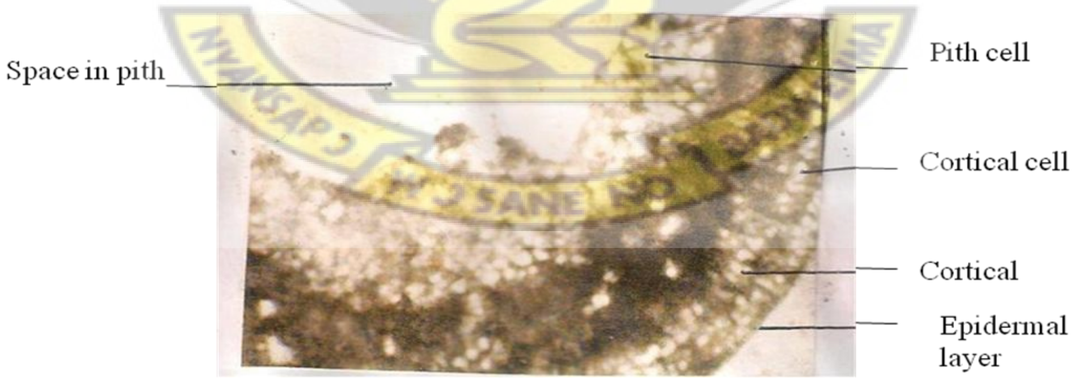
X40

W1



X40

W2



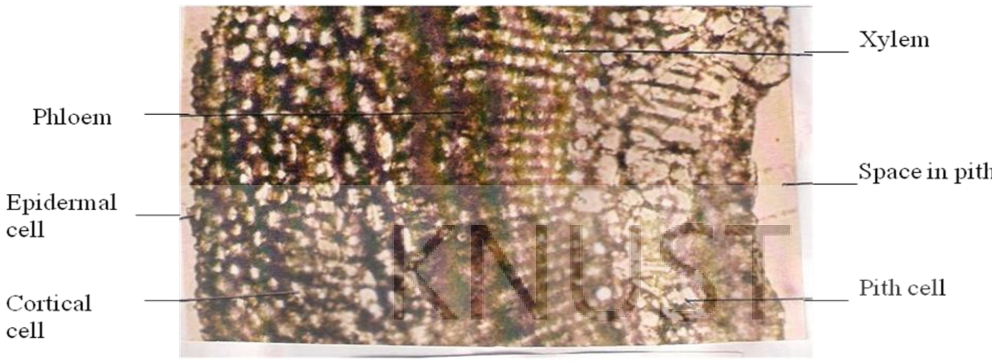
X40

NOTE:
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 7: PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION (T/S) OF THE ROOT OF 7 WEEKS OLD ACCESSIONS **(SS)** UNDER DIFFERENT WATER TREATMENTS

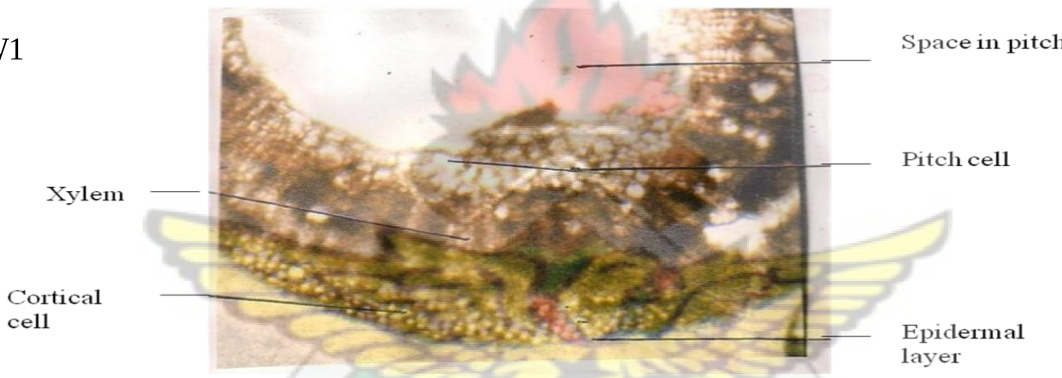
TREATMENTS

W0



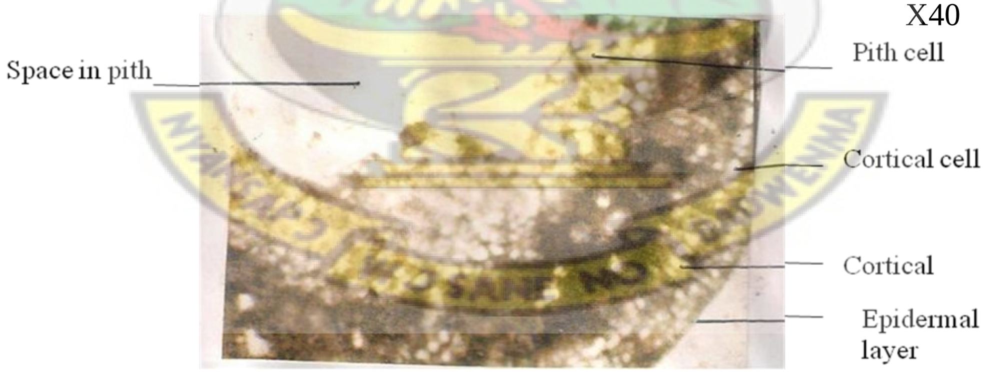
X40

W1



X40

W2



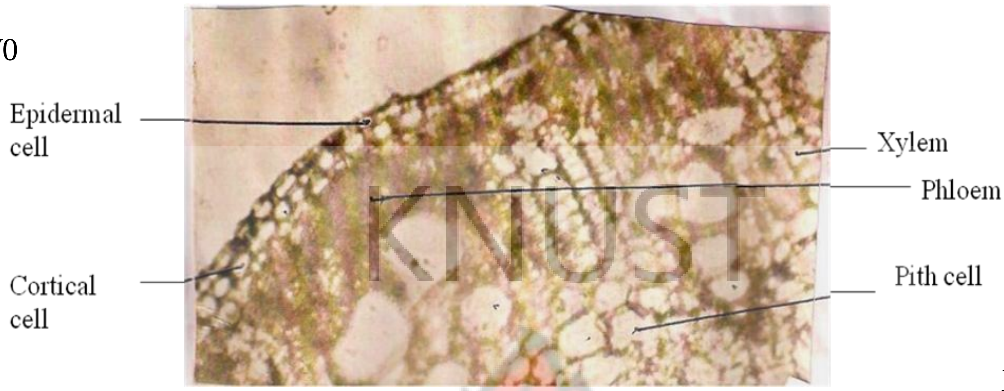
X40

NOTE:
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 8: PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION (T/S) OF THE STEMS OF 7 WEEKS OLD ACCESSIONS (SD) UNDER DIFFERENT WATER TREATMENTS

TREATMENTS

W0

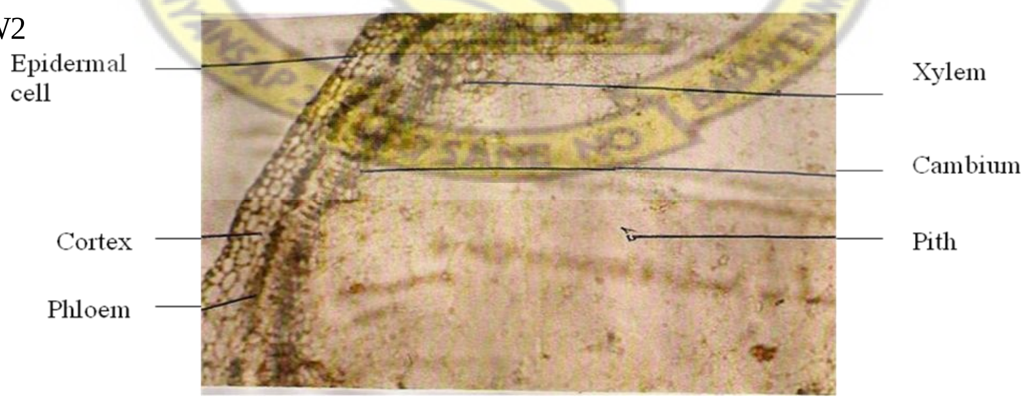


X40

W1

X40

W2

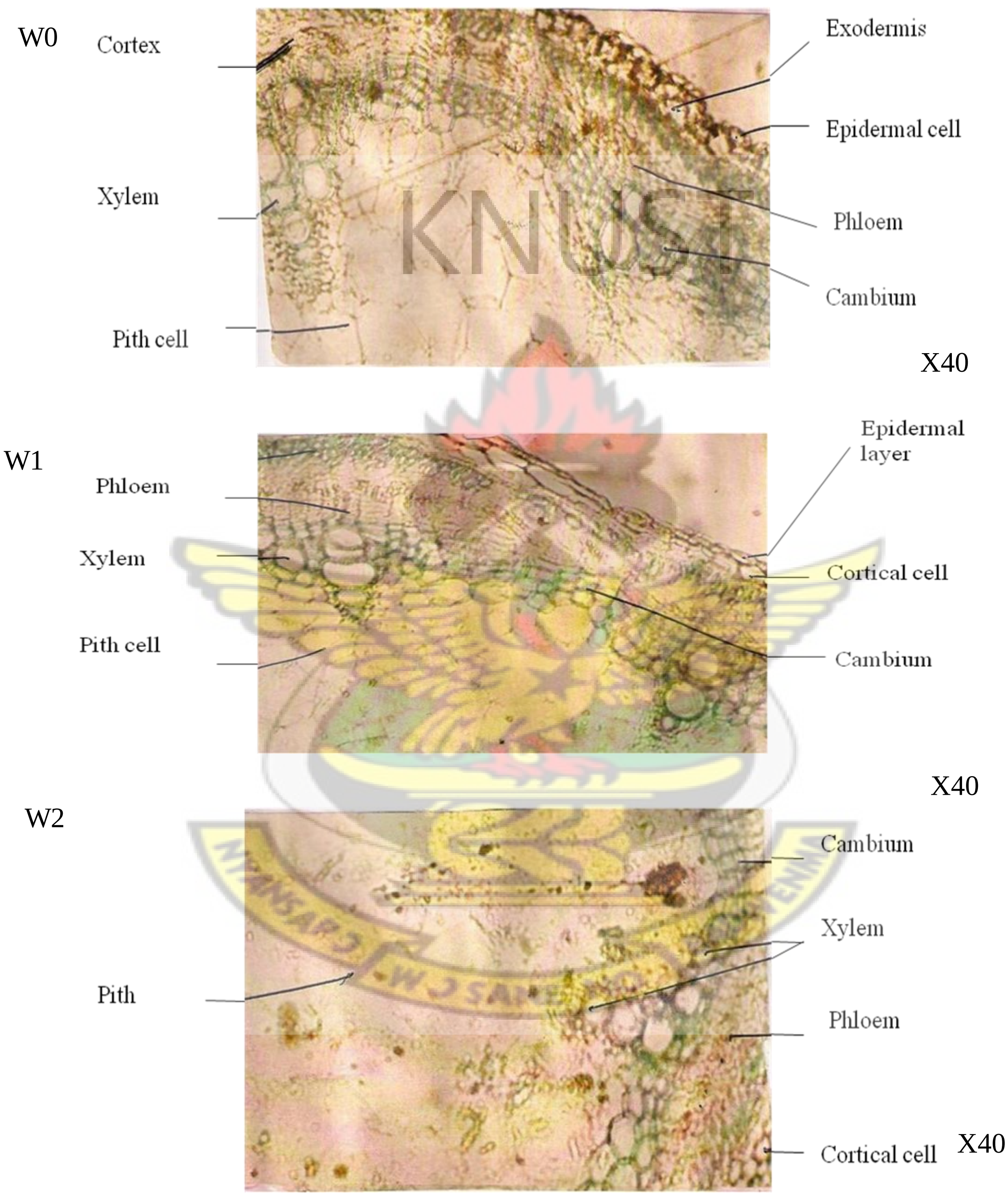


X40

NOTE:
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 9: <u>PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION (T/S) OF THE STEMS OF 7 WEEKS OLD ACCESSIONS **(CS)** UNDER DIFFERENT WATER TREATMENTS</u>

<u>TREATMENTS</u>

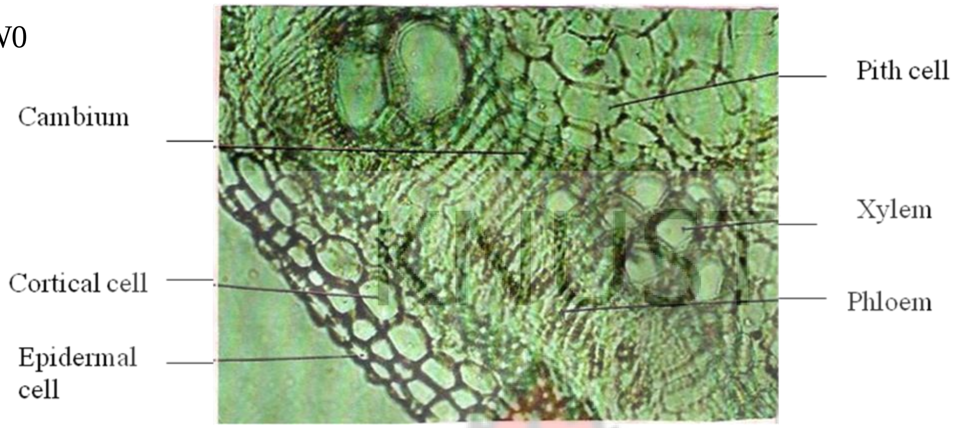


<u>NOTE:</u>
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 10: <u>PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION (T/S) OF THE STEMS OF 7 WEEKS OLD ACCESSIONS (**SS**) UNDER DIFFERENT WATER TREATMENTS</u>

TREATMENTS

W0



X40

W1

X40

W2

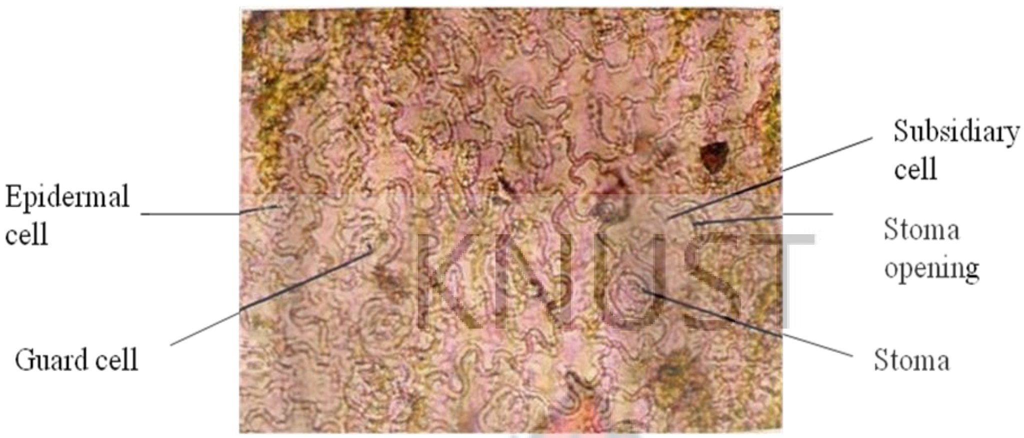
X40

NOTE:
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 11: <u>PHOTOMICROGRAPHS OF EPIDERMAL STRIP OF LEAVES OF ACCESSIONS (**SD)** IN RESPONSE TO DIFFERENT WATER TREATMENTS</u>

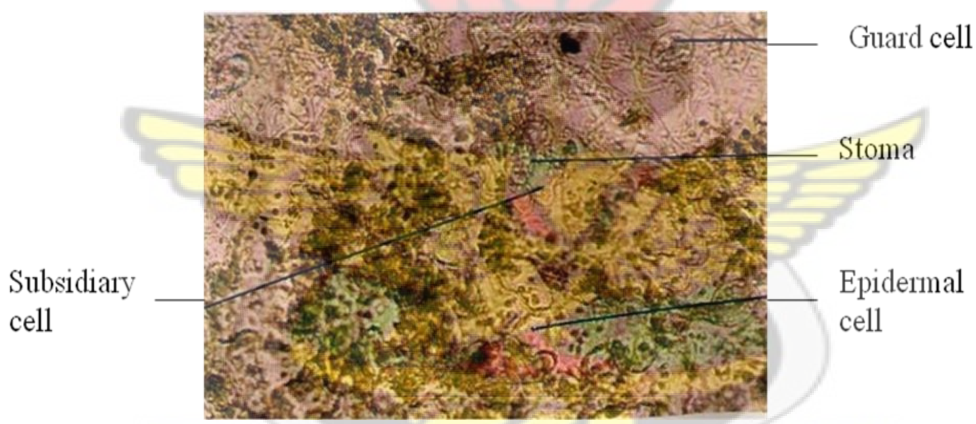
<u>TREATMENTS</u>

W0



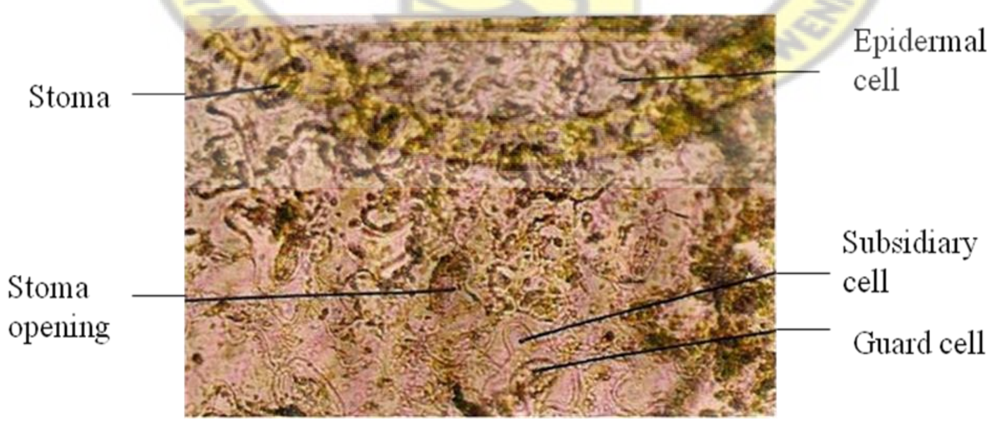
X100

W1



X100

W2



X100

<u>NOTE:</u>
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

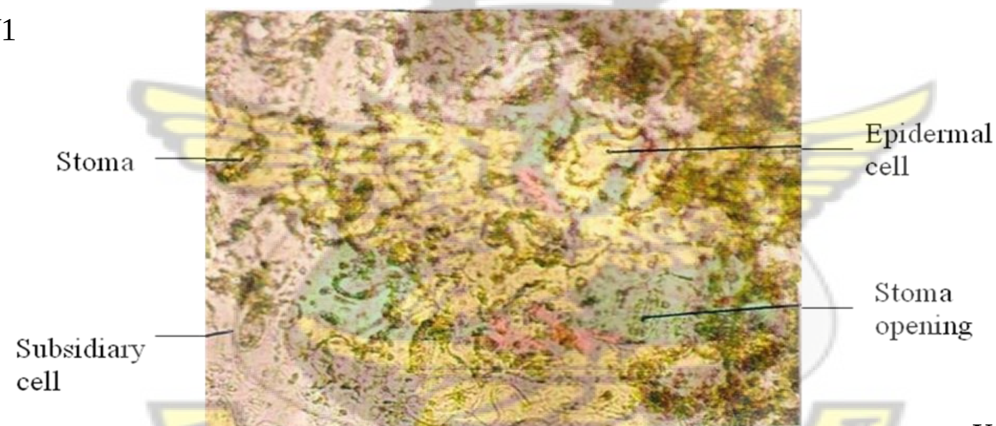
PLATE 12: <u>PHOTOMICROGRAPHS OF EPIDERMAL STRIP OF LEAVES OF ACCESSIONS **(CS)** IN RESPONSE TO DIFFERENT WATER TREATMENTS</u>

<u>TREATMENTS</u>

W0

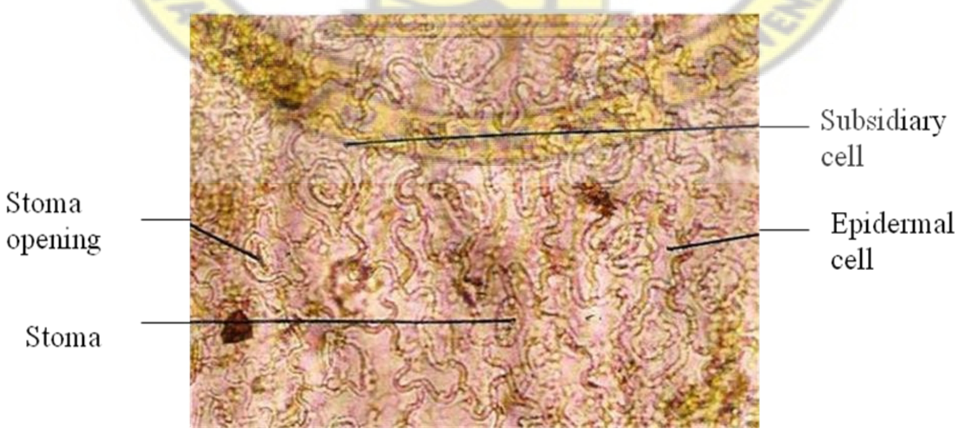
X100

W1



X100

W2



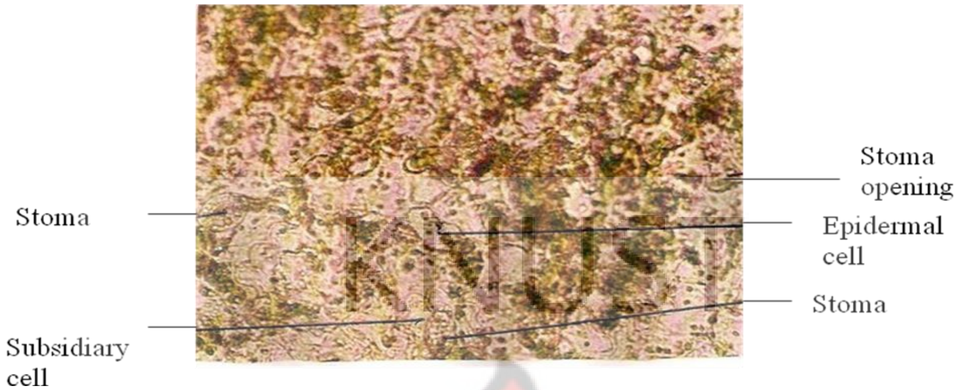
X100

<u>NOTE:</u>
Normal water (W0), medium stress (W1) and severe stress (W2) treatments.

PLATE 13: <u>PHOTOMICROGRAPHS OF EPIDERMAL STRIP OF LEAVES OF SUDAN SAVANNA (**SS**) ACCESSIONS IN RESPONSE TO DIFFERENT WATERTREATMENTS</u>

<u>TREATMENTS</u>

W0

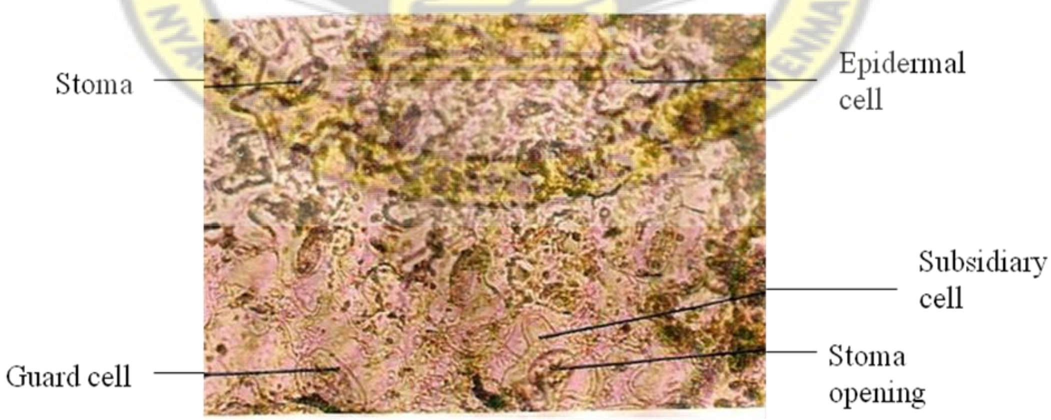


X100

W1

X100

W2



X100

<u>NOTE:</u>
Normal water (W0), medium stress (W1) and severe stress (W2) treatments

CHAPTER FIVE

DISCUSSION

5.1 ENVIRONMENTAL FACTORS

The water holding capacity was found to be 15 % of the 2 kg weight of the soil used as against the literature value of 25.9% (Agvise laboratories, 2007). The water stresses were monitored by the relative soil water content measurement. There were reductions in the soil moisture content with respect to the different water stress treatments on all sampling occasions (Appendix I). The reductions from the normal irrigation to the medium water stress treatment were found to be around 34% of the values of the control treatment on all the sampling occasions. The severe water stress also reduced by 52% of the control. The reductions could be attributed to water deficits created in the soil by the treatment applications and the increased uptake of water by the growing plants. The decreasing soil moisture contents observed in the different treatment protocols (W0>W1>W2) showed the effectiveness of the stress application.

The measured climatic factors showed relatively uniform trend with little variations in the means of the weekly values (Appendix II). The daylight periods were dry and hot while the nights had low temperatures with high humidity which are characteristic of the atmospheric conditions for the months of September to November. The high temperatures in the days reduced the moisture in the air during the day times whereas in the night, the air regained its humidity. Conducting the investigation in the plant-house of the Department however, provided a uniform ambient condition for the experiment.

5.2 VARIATIONS IN THE MORPHOLOGY OF THE ACCESSIONS

Plants of the accessions from the Sudan savanna (SS) zone were erect and did not show twining of stems throughout the experimental period. The accessions from both the Semi-deciduous (SD1, SD2) zone and the Coastal savanna (CS1, CS2) zones showed twining of their stems around the 4th and the 6th week of growth. The accessions from the Semi-deciduous zone were semi-erect in growth form while those from the Coastal savanna were vines / creepers in habit. Plants from both the Semi-deciduous and the Coastal savanna zones showed branching of their stems under both the normal watering and the water stressed protocols with that of the accessions CS1 and CS2 being more profuse (Plates 1-4). These characteristics exhibited by the different accessions are the growth forms of the cowpeas from the sampled ecological zones in Ghana.

5.2.1 VARIATIONS IN THE HEIGHTS OF PLANTS

Results on the morphological investigation revealed an increase in the heights of the accessions in the normal treatment (control) on all sampling occasions. This shows that the accessions in the normal irrigation protocol did not experience any inhibition to growth as they had enough soil water for continuous growth in heights.

The longest height in the six accessions was observed in the CS1 and CS2 under the medium water stress (W2) on the 2nd harvest (5th week) and the 3rd harvest (7th week). The shortest heights were seen in the accessions SS1 and SS2 on the 1st harvest period. The SS1 and SS2 accessions were erect and did not show any twinning of stems (Plate 4). The accessions therefore, showed lower heights on all the sampling occasions. The accessions CS1 and CS2 were creepers but because they were supported by sticks, they showed the greater length than all the other accessions. This was followed by the accessions SD1 and SD2 which showed a medium height; between that of the Coastal and the Sudan savanna accessions (Fig. 2, Plates 1-4).

The morphology showed a general trend of reductions in the plant heights of the six accessions under increasing water stress treatments; especially between the W1 and W2 treatments (Fig. 2). Though the accessions from the moist ecological zone (SD1, SD2) were not as tall as the CS1 and CS2 accessions on all the harvesting occasions, the reductions experienced in the height of the SD1 and SD2 between the normal watering and the severe water stress treatments were more drastic; 3X of what was observed in the CS1 and CS2 in the 2nd harvest. The SD1 and SD2 experienced a wide reduction in plant height between the normal watering (W0) and the medium water stress (W1) treatments (Fig. 2). The reductions in plant heights were greater in the accessions from Semi-deciduous zone; 75% reduction in the SD1 between the W0 and W2 treatments on the 3rd harvest as compared to that of the savanna zones that reduced between 20-50% within the same treatments. The accession SS2 reduced by 50% while the CS1 reduced by 20% (Fig. 2). This shows that the water stress application affected the heights of the accessions from the moist zones more than those of the Savanna zones. The accessions of the Coastal zone (CS1, CS2) were also, affected more than that of the Sudan savanna (SS1, SS2) within the accessions from the drier ecological zones. The heights reduced on the 3rd harvest in the stressed regimes as a result of the plasmolysis in the cells of the internal organs. This physiological phenomenon in the tissues resulted in the reduction of plant heights. Also growth hormones that may have incited by the water stress imposition in the W1 may have been inhibited under the W2 during the later part of plant growth. Growth hormones like auxin, cytokinine and giberellic acids (GA) may have caused the early differentiation of meristematic cells of the cambium into permanent tissues that in turn, increased the rate of growth and the subsequent increase in heights in the W1treatment. On the 3rd harvest (7th week of treatment), the effect of the water stress imposition; esp. the W2 treatment may have caused a decrease or total cessation of the production of growth hormone in the accessions. The plants thus started wilting and also dropped their leaves.

In the stress treatments, water loss from accessions through transpiration may have exceeded that absorbed by the root in the soil to replace the water deficit in the plants. However, the effects may be said to have been higher in the accessions in the severe stress protocol; esp. in the SD1 and SD2. The inadequate water supplied to the severe stress protocol provided very little soil water for root absorption to replace the amount of water lost through the leaves. There was thus, a gradual loss of turgor pressure in the tissues of the stressed plants that may have caused the reductions in the sizes of the accessions. Tan and Blake (1992) observed similar reductions in the morphology of black spruces they worked on under drought conditions and attributed the reductions to deficits in the internal tissues of the plants that were created by the inadequate water in the soil and low humidity.

5.2.3 VARIATIONS IN THE WIDTH OF STEMS

The width of the stems or girths of the accessions were higher in the accessions SS1 and SS2, followed by that of the SD1 and SD2 under the normal irrigation. The accessions CS1 and CS2 recorded the lowest girth (Fig. 3). The six accessions showed continuous increase in girth under the control treatment as the plants had adequate water for their physiological activities and growth.

The girth of the accessions reduced along the treatments with the plants in the severe water stress protocols recording their lowest values. The SD1 showed the lowest size under the W2 treatment. The effects of the water stress applications were more drastic on the accessions from the moist ecological zone, esp. the accession SD1than the others between the normal treatment (W0) and the medium water stress (W1) while the accessions CS2 reduced more drastic from the medium water stress (W1) to the severe water stress (W2) protocols in the 2nd harvest period (5th week of treatment). The SD1accession reduced in girth by 1.78X10µm from the normal irrigation as against 0.35X10µm for the SS2 accession. The accessions from the Savanna zone showed little but no significant reductions in their girth between the medium and the severe water stress regimes (Fig. 3).

The water stress applications thus, had wider effect on the accessions from the moist ecological zone than those of the drier ecological zones. The girth of the Sudan savanna accessions also, reduced much more than those of the Coastal savanna by 30% and 16% during the 2nd and 3rd harvests. It could be said that the accessions (SS1 and SS2) were also affected more by the severe stress application than the accessions from the Coastal savanna (CS1 and CS2). The reductions in the roots of the different accessions were found to be significant between the accessions and the treatment protocols ($P<0.05$).

Similar observations were noted in early works by Ahedor (1996) and Kirda (2002) on the effects of water stress on the growth of cowpeas and winter wheat respectively. They observed reductions in the morphology of the plants grown under different stressed regimes when compared to those grown under normal irrigation. Steudle (2000) attributed reductions in the morphology of the plants he worked with under water stress to imbalances in the tissues of the plants as a result of over-transpiration from the leaves of the plants.

The general reductions in the width of the stems under the stress treatments could be attributed to internal changes in the tissues of the plants which were brought about by physiological changes or imbalances in the stressed plants. The relative rate of absorption of water by the roots of the accessions, the internal distributions of the water, and the rate of transpiration could have created an osmotic deficit in the tissues of the accessions. These internal physiological conditions may have caused the width of the accessions to reduced, and thus resulting in wilting of the plant in the severe water stress protocols Signet and Kramer (1977) attested to this in their earlier work.

5.2.3 VARIATIONS IN THE ROOTS

The accessions showed increases in the length of their roots along the treatments. Exception was found in the CS1 and CS2 accessions which reduced in root lengths across the treatments during the 2nd harvest (5th week). Accessions from the Sudan savanna zone were observed to possess the longest roots under all the treatment protocols and on the three harvesting occasions (Fig 4). Though the accessions SD1 and SD2 possessed the shortest root system, the increase in their root lengths was high under the W1 before reducing by 20% under the W2 treatment. The short root system of the Semi-deciduous accessions is a growth feature of the accessions since they are endemic in moist ecological zones or habitats that have adequate soil water.

There was a continuous growth in the roots of the accessions along the three harvesting periods except CS1 and CS2 which reduced in root length in the W1 before increasing again under the W2 treatment. The variations in the roots lengths of the accessions except the CS1 and CS2 on the 3rd harvest was found to be significantly different at $P<0.05$. The variation of the roots of the accessions along the water stress treatments were thus, pronounced in the Semi-deciduous accessions; esp. SD2 though, the roots of these accessions differed slightly along the same stressed protocols (Fig.4). There was little variation in the roots of the accessions from the Savanna zones between the treatments on the three harvests occasions; esp. the CS1 and CS2 which had a minimal changes in their root lengths (Fig. 4).

The roots of the Sudan savanna accessions (SS1, SS2) were longer than the other accessions and this may have been an adaptation by the plants to reach water at lower depths in the drier ecological region. Pandey *et al* (2000) experimented with crop plants under Saharian conditions and observed the indigene breeds to possess longer root systems as compared to those collected from moist ecological regions.

5.2. VARIATION IN THE LEAVES

5.2.4.1 VARIATION IN THE SIZES OF LEAVES

The sizes of the leaves and their frequency in the different accessions generally decreased under the water stress applications. Accessions in the normal irrigation showed increment in the sizes of leaves on all harvesting occasions (Fig. 5). The leaves of the accessions from the Semi-deciduous zone (SD1 and SD2) were smaller in sizes than those of the Savanna zones. The accessions SS1 and SS2 possessed the largest leaves (Plate 1). The reduction in leaf sizes along the treatment protocols was however, more prominent in the Semi-deciduous accessions as compared to the other accessions; the SD2 reduced by 46% on the 1st harvest between the W0 and W2 treatments, while the CS2 reduced by 11% and SS2 reduced by 7.2% . The effects of the water stress application may not have been pronounced yet on the savanna accessions during the 1st harvest (3rd week).

On the 3rd harvest, the SD2 reduced by 60%, CS2 had 38% while SS2 reduced by 44%. The water stress application might have had pronounced effect on the accessions; esp. the SD which reduced by 60% in size as compared to the replicates in the W0 treatment (Fig 5).

The accessions shed some leaves in the water stressed regimes. The accessions (SD1, SD2) shed more leaves between the normal irrigation (W0) and the medium water stress (W1) treatment around the 5th week of stress application with that of the SD1 being more pronounced (Fig. 11). The Savanna accessions lost few leaves between the medium water stress (W1) and the severe water stress (W2) regimes around the 6th weeks of stress application with that of the Coastal savanna (CS1, CS2) being more pronounced. This shows that the SD1 and SD2 from the moist ecological zone were more sensitive to little deficit in soil moisture while the Savanna accessions became affected under severe water deficit condition. The changes in the sizes of leaves under water stressed conditions could be attributed to the thickness of cuticles, stomata openings and the degree of transpiration that influence the rapid development of water deficits in plants tissues (Souza, 2004).

Nonami and Boyer (1988) observed that prolonged dehydration led to reductions in leaf areas and the subsequent shedding of leaves in semi-deciduous plants. Working with a whole cowpea plant, Singh and Rachie (1985) established that reduction in the sizes of leaves or shrinkage was the first response of cowpea to water stresses. The amount of leaf shrinkage however, varied within the plant species that they worked on. Leaves that were relatively rigid and well supported by veins exhibited modest shrinkage under water stress conditions while soft leaves showed greater reduction (Singh and Rachie, 1985).

5.2.4.2 VARIATION IN THE NUMBER OF LEAVES

The accession had leaves that differed on the sampling occasions as a result of growth. This was mostly observed in the normal irrigation protocols. However, there was a general decrease in the amount of leaves on the accessions under the water stress treatments (Fig. 6). The accessions SS1 and SS2 from the Sudan savanna showed no significant variation in the amount of leaves under the treatment protocols on the 2nd and 1st harvests respectively. On all the three harvests, the reduction in the amount of leaves in the Semi-deciduous accessions (SD1 and SD2) was significantly different at P<0.05. The other accessions showed no significant differences on the 1st harvest between the W0 and W1 treatments except CS2 (Appendix IIe).

The accessions SS1 and SS2 possessed the lowest number of leaves under the normal irrigation treatment though, the SD1 and SD2 from the semi-deciduous zone experienced the lost of leaves more under the water stress treatments. As was observed from the results, the accessions SD1 and CS1 from the Semi-deciduous and Coastal savanna zones respectively lost 50% of their leaves on the 1st harvest between the W0 and W2 treatments while the SS1 from the Sudan savanna lost 20% of its leaves. However, the CS1 reduced by 33%, SS1 reduced by 40% while the SD1 reduced by 80% between the W0 and W2 treatments. This means that, the accessions from the Sudan savanna had fewer leaves but did not loss much leaves as the accessions SD1 and SD2 from the semi-deciduous ecological zone. The accessions SS1 and SS2 had longer roots that were able to absorb water from lower depth to sustain the plants from senescing their leaves as compared to the SD1 and SD2 which had smaller root system.

The Coastal savanna accessions; esp. CS1 had a lower reduction in leaf abortions under the W1 and W2 treatments on the 2nd and 3rd harvesting occasions. They were thus, able to tolerate the water stresses better than the other accessions. The Semi-deciduous accessions SD1 and SD2 are not genetically able to withstand drier conditions and thus, the accessions experienced wilting of their leaves during the 5^{th} week of stress treatment in the W1 and 3^{rd} week in the W2. The leaves however, senesced prominently from the 6^{th} week in the water stressed protocols.

Ahedor (1996) observed reductions in the amount of leaves produced by cowpeas under water stress conditions. He attributed the observation to the cessation of growth hormones in the plants under the severe water stress condition. Boyer (1985) and Nonami and Boyer (1988) recorded lower amount of leaves in maize plants under water stress conditions. They felt that the inadequate water supply might have initiated the production of ethylene hormone that caused the senescing of the leaves in the stressed plants.

5.3 VARIATIONS IN THE REPRODUCTIVE STRUCTURES

All the accessions under the treatment protocols flowered and produced fruits during the experimental period. The accessions from the Sudan savanna (SS1, SS2) showed early flowering within the accessions (around 6^{th} weeks) in the normal treatment and 4 weeks along the water stress treatments (Appendix V). This was followed by the accessions CS1 and CS2 from the Coastal savanna which also, produced the highest amount of flowers as a result of the numerous branching of their stems. The high lengths of the accessions and their abundant nodes provided numerous sites for the budding of flowers. The accessions from the Semi-deciduous zone (SD1, SD2) showed late flowering comparatively; around 8 weeks in the normal watering and 6 weeks in under the water stress treatments. The tendency to flower in the SD1 and SD2 accessions however, continued throughout the experimental period under the normal watering whilst flowering in the Savanna accessions ceased around the 8th week. The availability of water in the tissues of plants grown under normal irrigation enable to plants to perform better metabolic activities and provide enough food reserves for fruit production. The availability of adequate water to plants is therefore rated as being paramount to the proper growth and fruiting of plants (Signet and Kramer 1977; Francois *et al.*, 2000 and Munns *et al.*, 2000).

Normally, reproductive tissues increase in sizes during development as a result of cell division and expansion (Esau, 2007). According to Ahedor (1996) and Pandey *et al.* (2000), water stressed conditions that are created by soil moisture deficit and excessive transpiration have deleterious effects on the growth of plants and their reproductive structures. In their investigations, they found that water deficits caused plants to abort their flowers that resulted in fewer fruit production.

In this investigation, there were observed reductions in the amount of flowers and the quantity of bean pods produced by the six accessions grown under the different water treatments (W0>W1>W2) (Figs. 9, 10). The reduction in the sizes of bean pods along the treatment protocols is shown in Figs.7-8 while the reduction in the individual fruit mass, the total fruits produced and their dried weights are shown in Figs. 9-11. The observed reductions in the fruits under the treatment protocols could be attributed to internal water deficit that were created in the water stressed plants. However, the six accessions reacted differently to the water stresses (Figs. 7-11).

Shrinkage in flowers and fruits of plants was observed in plants grown under the water deficit conditions by Nonami and Boyer (1988). Though the six accessions flowered under the normal irrigation treatment, the accessions SD1 and SD2 showed much shrinkage and abortion of their flowers under the water deficit protocols; esp. under the severe water stress treatment (W2). These accessions from the moist ecological zone showed much abortion of their flowers in the water deficit regimes and stopped producing flowers around the 4th week of treatment even under the medium water stress treatment. Cowpeas were found by Singh and Rachie (1985) to produce low fruit yield under water deficit and drought conditions.

As observed in the results (Fig. 10), the accessions from the Sudan savanna zone (SS1, SS2) produced the least amount of fruits (bean pods) produced at the end of the experimental period, though the mass of the individual pods were higher than those shown by the bean pods of the other accessions. This observation was so because the accessions from the Sudan savanna (SS1, SS2) produced bigger fruit pods, but the quantity was far less than those produced by the other accessions during the same experimental period. It was observed that the accessions from the Coastal savanna zone (CS1, CS2) produced slender fruit pods but in larger quantities as result of their profuse branching (Fig.7, 8). Working on cowpea and millets, Petrie and Hall (1992) observed that the branching of the stems of the cowpeas provided many sites for flowering as compared to the millet that is a non-branching plant. Fruiting was thus, pronounced in the cowpeas under both the normal irrigation and the stressed irrigations as compared to the millets grown under similar conditions.

The dried weights of the bean pods produced by the accessions CS1 and CS2 from the Coastal savanna zone were found to be the highest within the six accessions under the water stress regimes. This was followed by the accessions SD1 and SD2 from the Semi-deciduous zone with the Sudan savanna accessions (SS1, SS2) obtaining the least dried fruit weight (Fig 11). It could be assumed that the amount of bean pods produced by the different accessions may have affected the dried weights of the fruits obtained. The variations showed significance ($P<0.05$) within the treatments and the accessions under ANOVA analysis (Appendix V).

Similar observations have been made earlier by Ahedor (1996) while working on the growth and reproductive patterns of cowpeas under different water treatments. Some of the plants produced large-sized bean pods though in smaller quantities, while others produced small-sized bean pods but in greater quantities. These observed characteristics may have been due to hereditary influence. As mentioned earlier, the accessions from the Sudan savanna (SS1 and SS2) produced large but fewer fruits as compared to the other accessions (Figs. 10, 11). The accessions from the Coastal savanna produced smaller bean-pods but were in larger quantities.

According to Singh and Rachie (1985) and Stephen (2006), the cuticle covering of leaves and fruits contribute to the reaction of these organs to water stress conditions. The inherent thickness of the cuticles in the Savanna accessions; esp. SS1 and SS2 may have provided some resistance to the reductions in their fruits sizes under medium water stress treatment as was observed in this investigation (Figs. 7-8). The initial increase in the width of the cuticle on the leaves and the reproductive organs of the six accessions between the normal irrigation and the medium water stress treatment may have resulted from osmotic imbalances in the cells and the deactivation of some growth hormones in the plants.

The production of auxins and Gibbleric acids may have reduced while that of abscissic acid may have increased, and thereby causing the stagnation and reductions of growth under the water stressed regimes. The falling Westgate and Boyer (1985) observed reductions in some organs of maize plants that they investigated under water stressed conditions and attributed it to osmotic adjustments in the tissues of the plants to low water potential as a result of the water stress imposition. It was also, observed that the application of severe water stress on plants after their time of pollination hampered the ability of the plants to retain their flowers (Anyanaba and Wein, 1979).

5. 4 VARIATIONS IN THE ANATOMY OF THE ACCESSIONS

There was a reduction in the sizes of the cells and tissues in the different accessions under the normal watering and along the water stress treatments. The means of the measured variations were found to be significant ($P<0.05$) within both the accessions and the treatment protocols. The significant variations occurred mostly in the soft walled cells of the cortex and the pith. Exception to this trend of reductions in the sizes of the tissues was found in the vascular system. The xylem vessels of the accessions under the water stress treatments increased in number or frequency with those under the severe water stress (W2) possessing the highest number (Table 7).

The proliferation of numerous xylem vessels and the reductions observed in the organs could be attributed to hormonal influence on the growth of the plants, and the decreases in the sizes of the cells as a result of internal water deficit and plasmolysis. The increases in the xylem vessels of the accessions under the water stress regimes might have been due to early differentiation of the meristematic cambium cells into permanent xylem vessels in reaction to the imposed water stresses. The importance of growth hormones like auxin, Gibberellic acids and cytokinines cannot be overlooked in this phenomenon.

Nonami and Boyer (1988) and Kirda (2000) noted similar reductions in the tissues of plants grown under water deficit conditions, and attributed the trend to internal water deficits as a result of osmotic imbalance in the plant tissues. However, the reductions in the tissues of the plants may have also, been caused by reduced turgor in the cells as a result of over-transpiration as against water absorbed by the plants to replace the lost water. Soil moisture was monitored to be low in the water stressed regimes (Fig. 1) and thus, the reductions in the sizes of the tissues of the accessions along the water deficit protocols showed a reflection of the amount of water that was available to the growing plants under the different watering regimes (Table 1, 2). The reduction in tissue sizes along the water stress treatments may also, have resulted from the water deficits created in the plants as a result of excess loss of water through transpiration as compared to the volume of water absorbed by the roots.

The water absorbed by the roots of plants is conducted through the vascular tissues of the plants under concentration gradient to replace the water lost through the stomata and lenticels of the plants. According to Westgate and Boyer (1988) and Francois (2000), inadequate water supply as a result of an imposed water stress condition leads to low turgor pressure in the tissues of plants, and subsequently bring about reductions in the cell sizes. The hormone; abscisic acid is activated and this cause the tissues of the plants; esp. the leaves and flowers to fall or wither under the water stressed treatments. These physiological and anatomical processes might be speculated to have occurred in the tissues of the accessions under this water stress investigation.

The necessity of water in maintaining the internal water balance in plants to promote good growth was investigated by Nonami and Boyer (1988) and Steudle (2000). They concluded that the presence of inadequate water in soils and the difficulties the plants encounter in conducting the absorbed water in their tissues as a result of the apoplastic and symplastic barriers bring about osmotic imbalances in the cells. These then, lead to plasmolysis and reductions in the cells.

5.4.1 VARIATIONS IN THE EPIDERMAL TISSUES

The cells of the epidermis in the accessions were observed to be flattened and rigid as compared to the adjourning cells of the cortex (Plates 4-9). Epidermal cells of plants are normally flattened and thickened to serve as protective barrier to the delicate inner tissues (Souza, 2004). The epidermal cells of the roots, stems and leaves of the six accessions showed enlargement in the periclinal plane during growth under the normal watering though, the variation was found not to be significant ($P>0.05$). The cells however, showed reduction in sizes along the water stress treatments with the lowest sizes being recorded under the severe water stress regimes; W0>W1>W2 (Table 2).

According to Palmer and Davies (1996), the epidermal cells in young plants or seedlings show continuous enlargement during growth under normal watering treatment but reduced in sizes under water deficit conditions. Epidermal cells usually enlarged in the periclinal plane as a result of pressures from the inner tissues of the plants during growth. Esau *et al*. (2007) and Petrie and Hall (1992) observed similar orientation in the enlargement of epidermal cells of young dicotyledon and monocotyledon plants by using cowpea and millet respectively.

In this investigation, the sizes of the epidermal cells of the accessions in the control were observed to be similar; around an average size of 2.00X10µm with those of the Coastal savanna having theirs just a little below that. The reductions in the sizes of the epidermal cells of the six accessions as a result of the water stress treatments were minimal and statistically, not significant ($P>0.05$). The reductions ranged between 0.01X10µm to 0.15X10µm in the Savanna accessions from the medium water stress o the severe water stress protocols while the Semi-deciduous accessions showed a range of 0.03X10µm to 0.41X10µm within the same stress treatments.

The low reductions in the sizes of the epidermal cells under the water stress treatments could be attributed to the thickened nature of their cell walls that prevented the shrinking of the cells during plasmolysis. Epidermal cells are covered with other waxy substances like lignin and suberin during growth. These substances could give additional strength to epidermal cells to enable them withstand pressures from the inner tissues (Esau *et al*., 2007).

5.4.2 VARIATIONS IN THE CORTEX AND PITH TISSUES

The cortex of the six accessions was made up of parenchyma, collenchyma and sclerenchyma cells. The pith region was made up of only soft-walled parenchyma cells (Plates 4-9). The collenchyma cells were partially thickened at the angles while the sclerenchyma cells were heavily thickened around the cell. The parenchyma cells had no thickening and were found to be prominent in the pith region while the collenchyma cells dominated the cortex. The types of cells found in the cortex and the pith could explain the reactions of these tissues to the water stress treatments.

There were reductions in the sizes of the cells of the cortex and the cortex region in both the roots and the stems of the accessions within themselves and along the treatment protocols (Table 4). Under the control treatment, the accessions from the Savanna zones possessed the largest cells with an average size of 4.68X10µm, eg. SS1 and CS2 accessions and followed by that of the Semi-deciduous zone with an average size of 3.35X10µm as in the SD1 accession. The cells of the SD1 and SD2 reduced drastically under the different water treatments than those of the Savanna zones. From Table 4, the accessions from the Savanna reduced in sizes between the normal and the medium water stress treatment by an average of 0.05X10µm while that of the Semi-deciduous reduced by 0.35X10µm within the same treatment. There was a greater reduction in the SD1 and

SD2 from the medium water stress to the severe water stress treatments than that of the Savanna accessions though it is within these treatment regimes that the savanna accessions showed real reduction in cell sizes.

The sizes of the cortical region reduced pronouncedly in the roots and stems of the Semi-deciduous accessions as compared to the savanna accessions. Between the normal irrigation (W0) and the medium water stress (W1) treatment, the roots of SD1 reduced by 6.47X10µm, followed by that of the Coastal savanna (CS1) with 2.43X10µm while that of the Sudan savanna (SS1) had the least reduction in the roots with 2.28X10µm. However, the cortical region in the stems of the Coastal savanna accessions (CS1, CS2) proved to have the least reduction along the stress treatments (Table 4). The reduction in the sizes of the cortex was found to be significant within the accessions, and the treatment protocols ($P<0.05$).except the cortical width of the stems that was found to be non significant ($P>0.05$) (Appendix VI).

Meyer and Boyer (1972) observed reductions in the morphology of soy-beans under water stressed conditions and attributed the phenomenon to reductions in the radial expansion of the cortical cells under the influence of low water potential and pressures from adjourning cells. According to Steudle (2000) such reduction in the cortical tissue may have originated from changes in the energy status of the water stressed applications on cell turgor in the plants. Turgor changes were observed to be controlled in soy-bean plants by the relative absorption of water, transpiration, and the internal distribution of the absorbed water in the tissues (Meyer and Boyer, 1972).

The parenchyma cells of the pith region of the six accessions were comparatively, larger than the cells of the other tissues. The pith cells of the different accessions showed a least value of 7.08x10µm in the roots of the accessions CS2 under the control treatment while the same accession (CS2) showed a least mean value of 7.11X10µm under the same treatment. The accession SS1 showed the largest pith cells with average size of 12.16X10µm. This gives evidence to the fact that the pith cells were large in nature. The soft-walled parenchyma cells of the pith however, showed the greatest reduction in sizes as a result of the water stress applications. The nature of the soft wall may have allowed the cells to feel the effect of the inadequate water under the water stress protocols.

From Table 8, it could be observed that the pith cells of the SD1 and SD2 reduced drastically along the water stress treatments with an average of 2.79X10µm between the treatment protocols while the Savanna accessions showed a smaller average reduction 1.00X10µm. between the same protocols. The accessions CS1 and CS2 from the Coastal savanna showed the least reductions as a result of the water stress treatments. These variations were found to be significant ($P<0.05$) within the different accessions and within the treatment protocols.

Similar observation was made by Boyer (1985), and Tan and Blake (1992) while working on maize and black spruces respectively and according to them, the reductions in the pith and the general morphology of the plants were due to low water potential in the tissues and the plasmolysing of the cells as a result of inadequate soil moisture under the water stress applications. According to Steudle (2000), roots of plants present some barrier to the transport of absorbed water as a result of the differential passage of water and mineral salts through the symplastic and apoplastic pathways of the endodermal and exodermal rings in the cortex of roots of plants. The reduction in the cortical and pith tissues of the different accessions under this investigation could have been caused by low water potential and imbalances in osmotic pressures in the cells as a result of the water stressed conditions.

5.4.3 VARIATIONS IN THE VASCULAR TISSUES

The vascular tissues of the accessions consist of circular bundles in the early stages of growth but later joined to form circular bands in the roots of the matured plants, though the vascular tissues remained as bundles in the matured stems. The stems possessed interfascicular cambium cells that linked the bundles (Plates 5-10). The vascular bundles were observed to be made up of outer phloems and their companion cells, and inner xylem vessels with fibers. There were layers of fascicular cambium separating the two conducting tissues. This arrangement of the vascular tissues in the different accessions is peculiar to the arrangement of vascular systems of dicotyledon plants (Esau *et al.*, 2007).

A large number of the xylem vessels were found in each of the accessions from the Semi-deciduous ecological zone (SD1, SD2) in comparison to the accessions from the drier ecological zones in the control treatment (Table 7). The sizes of the xylem vessels in the accessions SD1 and SD2 reduced drastically from the normal treatment (W0) to the medium water stress (W1) protocol; ie. 2.01X10µm in the roots and 1.97X10µm in the stems of accessions SD1 (Table 6).

The accessions SS1 and SS2 from the Sudan savanna showed smaller reduction in the sizes of their xylem vessels across the treatment protocols. Between the normal water treatment (W0) and the medium water stress (W1), the reduction was 0.06X10µm and 0.59X10µm in the roots and stems of accession SS1 respectively while the reductions were higher from the medium (W1) to the severe water stress (W2) treatment; 1.62X10µm in the roots and 2.84X10µm in the stems. Analysis showed the reductions in the xylem vessel sizes to be non significant within the accessions ($P>0.05$) at df.5 but significant within the treatment protocols ($P<0.05$) at df.2 (Appendix VI).

It was observed in the investigation that there was the proliferation of many small-sized xylem vessels in the six different accessions along the water deficit treatments (W0<W1<W2) (Table 7). This phenomenon is found to be present in the anatomy of dicotyledon plants that experience seasonal changes in edaphic and climatic conditions. The environmental variation affects the amount of water contents of soils which in-turn induce hormonal actions in the water stressed plants to bring about the differentiation of the meristems into small-sized xylem vessels. This phenomenon is referred to as late growth in plant anatomy (Souza, 2004, Esau *et al*., 2007).

The six accessions were observed to grow continuously during the experimental period under the normal water irrigation (W0). The availability of water in this treatment protocol may have initiated and ensured the production of normal vascular tissues from the cells of the cambium unlike the situation observed in the plants under the water deficit regimes where the meristems differentiated into small-sized vascular tissues. The accessions under the severe water stress treatments thus became continuously thinner, and wilted at the later part of the experimental period (9th week); especially in the SD1 and SD2 accessions. The wilting resulted from the plasmolysis and the death of the cells in the permanent tissues.

As could be observed from the results; Table 9 and Plates 5-9, the cambium layers of the accessions were found to decrease under the water stress protocols. There were three layers of the cambium cells in the accessions under the normal irrigation regime but these reduced to mostly one layer under the severe water stress regimes. No cambium cell layer was observed in the roots of the accessions SD1 and SD2 from the Semi-deciduous zone under the severe water stress protocol on the 3rd sampling occasion. Differentiation of meristematic cells of the cambium might have ceased at this time, and resulted in the delayed growth and the subsequent wilting of the accessions. The accession from the drier ecological zones retained some of their cambium cells even, under the severe water stress treatment; especially in the accessions CS1 and CS2 from the coastal savanna.

It could be inferred that the accessions CS1 and CS2 from the Coastal savanna had the ability to produce new tissues from the cambium cells on watering occasions in the water stressed regimes as against the other accessions; especially the SD1 and SD2 that had lost their cambium cells. Cowpeas are known to possess fast recovery rate after drought (Anyanaba and Wein, 1979; www.en.wikipedia,org/wiki/longyard, 2009). This might be possible by means of the mass fibrous roots of the cowpea that provide a large surface area for faster absorption of water on watering occasions. This work revealed the cowpeas to possess mass fibrous roots but because there were no cambium cells in the roots of the SD1 and SD2 accessions under the severe water stress treatments, these accessions could not regain the ability to grow on watering occasions.

According to Proseus (2000), the differentiation of the cambium cells in the periclinal plane in the stele of plants increase the width of the plants while the apical meristems increase the heights. Thus, the surface area in roots for the absorption of water is not enhanced by the action of their cambium cells but rather, the differentiation of their apical meristems. Increment in the length of roots was observed in this investigation in the plants grown under the water deficit conditions (Table 1, Fig. 3).

5.4.4 VARIATIONS IN THE LEAF TISSUES

The leaves of the six accessions studied were net-veined with a large main vein running through the middle. The cross section revealed the leaves to be composed of inner mesophyll tissues that were enclosed by two outer epidermal tissues; the lower and upper epidermis. The epidermal layers were covered with waxy cuticle and interposed by a number of stomata. The features of the different accessions mentioned above showed varying degree of reduction in sizes under the water stress treatments. The variation was found to be not significant within the six accessions grown under the control treatment (W0).

5.4.4 1 VARIATIONS IN THE MESOPHYLL TISSUES

The mesophyll tissue of the leaves of the six accessions was made up of squamous palisade and round spongy cells. The mesophyll tissues of the accessions showed varying degrees of reduction in their sizes under the water stress treatments (W0<W1<W2) (Table 9). The palisade cells and their arrangement varied little within the leaves of the different accessions in the control treatment and within the treatment protocols. The reductions were 0.05X10µm to 1.24X10µm within the accessions and along the water stress treatments (Table 9). The variation was found to be non significant on analysis (Appendix IV).

The spongy cells in the leaves of the six accessions exhibited considerable reduction in sizes within the control (W0) and along the treatment protocols. The results of this investigation revealed greater reduction in sizes of the spongy cells of the accessions from the Semi-deciduous ecological zone (SD1, SD2) as compared to those from the drier ecological zones along the water deficit treatments (Table 9). The Semi-deciduous accessions (SD1) reduced in size by 0.76X10µm between the normal irrigation (W0) and the medium water stress (W1) treatments while the accessions (CS2) and (SS1) from the Coastal and the Sudan savanna reduced by 0.76X10µm and 0.19X10µm respectively. However, the accession from the Coastal savanna (CS2) showed a lower reduction in size from the medium (W1) to the severe water stressed (W2) treatment when compared to the other accessions. The accession (CS2) reduced by 0.55X10µm, followed by (SD1) with 0.68X10µm and (SS1) with 1.12X10µm. The results revealed that the accession from the Coastal savanna tolerated the severe water stress application better than the others.

The high degree in the reduction of the leaves of the accessions from the moist ecological zone (SD1, SD2) was caused by the plasmolysing cells of the spongy tissue as a result of the water stress treatments. Being genetically suited for moist environment condition than the other accessions, the cells of the mesophyll tissues of the SD1 and SD2 accessions might have had greater effect of the water deficit application than the Savanna accessions. Spongy cells are parenchymatous and soft-walled (Esau *et al*., 2007). The effect of imposed water stress was felt higher in the spongy tissue than the palisade which had thickened walls. This phenomenon was experienced in the six accessions under the investigation

Munns *et al*. (2000) observed reductions in the leaf sizes of maize and bailey plants that were subjected to different soil moisture treatments and attributed reductions to the effects of plasmolysis in the cells of their mesophyll tissues. It could be assumed that the presence of low turgor and high water potential in the leaves may have also, created an osmotic imbalance in the thin-walled spongy cells. The physiological conditions that occur in the cells cause their plasmallema to pull away from the walls and thus results in plasmolysis and the subsequent reduction in the sizes of leaves (Hsiao and Zing, 1987) and (Francois, 2000). Statistical analysis of the reductions in the spongy tissues showed significance ($P<0.05$) within the accessions and across the treatment protocols.

5.4.4.2 VARIATIONS IN THE STOMATA AND CUTICLES

The leaf lamina of the accessions was observed to be amphistomatous with the stomata occurring more on the lower surface than on the upper surface. The frequency of the stomata on the surfaces of the leaves varied pronouncedly within the accessions under the control treatment but varied slightly along the water stress treatments (Table 10). The accessions SD1 and SD2 had 20 and 19 stomata on their upper surface respectively, while having 26 and 24 stomata on their lower surface under the control treatment. The accessions CS1 and CS2 had 16 and 17 stomata on their upper leaf surface, but 21 on the lower surface of both plants. The accessions SS1 and SS2 had 14 and 13 stomata on their upper surface but 20 on the lower surface of both accessions under the normal irrigation regime. The accessions SD1 and SD2 thus, possessed more stomata than the Savanna accessions under normal watering condition.

The accessions from the Semi-deciduous zone (SD1, SD2) showed greater reduction in the frequency of their stomata between the normal irrigation and the medium water stress treatment as compared to the Savanna accessions (Table 10). However, the accessions from the savanna zones showed pronounced reductions in their stomata between the medium water stress (W1) and the severe water stress (W2) treatments. The results mean that there were differences between the distribution and frequency of stomata in the leaves of the six different accessions naturally (under the control). The Semi-deciduous accessions possessed more stomata on their leaves than the Savanna accessions, while the CS1 and CS2 possessed more stomata than the SS1 and SS2 accessions.

The Savanna accessions became sensitive to the water deficit applications in their stomata at a higher level; from the medium (W1) to the severe water stress (W2) treatments, while the Semi-deciduous accessions showed great reductions between the normal irrigation (W0) and the medium water stress (W1) treatments (Table 10).

The variations were found to be non significant (P>0.05) within the accessions in the control treatment and across the treatments (Appendix IV). The non significant variation observed in the frequency of the stomata could be attributed to the fact that the stomata were already formed before the water stress treatments were imposed. Thus, the impact of the water stress treatments was observed on the sizes of the stomata but not on the frequency. Munns, et al (2000) observed more stomata on the lower surface of the leaves of snap bean (*Phaseolus vulgaris*) than on their upper surface. The results of this investigation revealed similar observations. According to Munns et al (2000), the stomata on the upper and the lower leaf surfaces of the plants reacted differently to the imposed water deficit conditions. Similarly, this investigation showed that the stomata on the lower surface of the leaves reduced more under the water stress treatment than those on the upper surfaces (Table 10).

There were reductions in the pores and the guard cells of the stomata in the different accessions under the water stress protocols (Table 10). The reductions were found to be non significant within the accessions (P>0.05), though the reductions were significant within the water stress treatments. Stomata are the major exist through which water is lost from plants (Souza 2004, Esau 2007). According to Boyer (1985) and Kirda (2002) plants tend to close their stomata pores on the advent of harsh environmental conditions that includes drought, to enable the plants to tolerate the inadequate water supply and to conserve water. The six accessions in this investigation reduced the sizes of their stomata as an effort to reduce water lost by transpiration, and to conserve internal water.

The stomata reduction was observed to be more prevalent in the accessions from the moist ecological zone (SD1, SD2) between the normal irrigation and the medium stress treatments. The SD1 reduced its stomata by 0.23X10µm (almost ½ of the original size) between the normal irrigation (W0) and the medium water stress (W1) while the SD2 showed a reduction of 0.12X10µm. The Savanna accessions had lower reductions of 0.09X10µm in the CS1 (Coastal savanna) and 0.06X10µm in the SS2 (Sudan savanna). The closure of the stomata in the accessions from the moist ecological zone could be said to be more pronounced than that of the Savanna accessions under the medium water stress condition. There was a great reduction in the sizes of the stomata in all accessions between the medium water stress (W1) and the severe water stress (W2) treatments except the CS2 that showed a reduction of below 0.07X10µm. This means that the accessions CS2 was able to tolerate the water stresses more than the others under the severe water stress treatment. Plants have been observed to close their stomata on the advent of other adverse environmental conditions; like low humidity and high temperatures, in an effort to promote internal water conservation (Francois, et al 2000, and Pandey 2008)

Waxy cuticles were observed on both surfaces of the leaves of the six accessions. The thickness of the cuticle on the upper surfaces was higher as compared to that on the lower leaf surface. Comparatively, the accessions from the Sudan savanna (SS1, SS2) were found to possess thicker cuticle on the surfaces of their leaves under the control treatment than the other accessions. The accessions from the moist ecological zone (SD1, SD2) were found to possess thinner cuticle under the control irrigation (Table 9). The accessions SS1 and SS2 were observed to possess cuticle sizes between 0.32X10µm- 0.33X10µm, followed by accessions CS1 and CS2 with0.24X10µm- 0.26X10µm. The accessions SD1 and SD2 showed a least value of 0.20X10µm in the thickness of their cuticles under the normal irrigation. The variation in the cuticles within the accessions under the control treatment was found to be non-significant (P>0.05) at df.5.

The six accessions exhibited increases in the thickness of their cuticle between the normal irrigation (W0) and the medium water stress (W1) treatments (Table 9). The cuticles in the accessions SD1 and SD2 reduced in thickness between the medium water stress and the severe water stress treatments, from the initial increase that took place in the plants between the normal and the medium water stress treatments. The accessions from the Savanna ecological zone showed an initial increase in their cuticles from the normal irrigation to the medium water stress treatment but maintained the thickness of their cuticle between the medium and the severe water stress treatments (Table 9). The variations were found to be non-significant across the treatment protocols (P>0.05) at df.2.

The accessions became sensitive to changes in their internal water content when the first water stress level was initiated and the plants reacted by secreting additional cutinized substance on their leaf surfaces. Under the severe water stress application, the epidermal cells of the SD1 and SD2 accessions produced additional cutin to increase the thickness of the cuticle cover of their leaves in order the resist excessive transpiration as against the absorbed water by the roots. The savanna accessions showed no differences in their cuticle cover between the medium and the severe water stress treatments.

The results showed the cuticles of the Savanna accessions CS1, CS2, SS1 and SS2 to be physiognomically thicker than that of the SD1 and SD2 accessions (Table 9). The Savanna accessions did not show any change in their cuticles between the medium water stress (W1) and the severe water stress (W2) protocols after the initial increase between the control (W0) to the medium water stress treatment (W1). The differences in the thickness of the cuticles within the accessions from the different ecological zones under the control treatment were due to genetic dictates. Because the Savanna accessions are genetically suited for harsh environmental condition that includes drought, the cuticles are normally thicker.

The increase in the thickness of the cuticles of the six accessions between the normal and medium water stress was a reaction by the plants to the changes in the soil water supply. It was to help to plants conserve water by reducing water the rate of water loss. Tan and Blake (1992) observed cuticle increase in the progenies of black spruces that they worked on under water deficit conditions. Ahedor (1996) also, made similar observation in his investigations on cowpeas that were grown under water deficit field conditions. According to them, the increase in the thickness of cuticle was an attempt by plants under water deficit conditions to prevent over-transpiration and to tolerate the inadequate water supply.

Anyanaba and Wein (1979) observed an initial increase in the cuticles of the leaves in the plants they worked with and later, the folding of the leaves before abscission. They assumed the folding to be a further reaction of the plants to prevent over-transpiration and to conserve water. These observed phenomenon were also, seen in this investigation and that the accessions from the moist ecological zone (SD1, SD2) were the plants that showed early folding under the severe water stress; around the end of the 4th week of treatment and 7th week in the medium water stress treatments. The accessions SS1 and SS2 from the Sudan savanna showed folding under the severe water stress treatment only while the accessions CS1 and CS2 of the Coastal savanna never showed any folding of their leaves even under the severe water stress treatment. The accessions CS1 and CS2 could be said to have tolerated the water stress applications better than the other accessions.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

MORPHOLOGICAL

There was general reduction in the sizes of the morphological organs of the different accessions under increasing water stress treatments. The reductions were greater in the accessions from the Semi-deciduous ecological zone as compared to the accessions from the drier ecological zones. The accessions had different habits and this may have contributed to the response of the accessions to the water stress treatments. The accessions from the Semi-deciduous zone were semi-erect; those from the coastal savanna were creepers while those from the Sudan savanna were erect. Their exposure to atmospheric conditions thus causing evapo-transpiration may have differed. The accessions from the Semi-deciduous zone were severely affected, especially under the severe stress treatment as compared to those from the Savanna ecological zones. The accessions from the Coastal savanna (CS1, CS2) were the ones that had the minimal reductions in their morphological organs under the water stress treatments, with the accessions CS2 having the least reduction in plant size.

ANATOMICAL

The cells of the accessions from the Sudan savanna were larger than that of the other ecological zones under the normal irrigation protocol. However, the sizes of the cortical and pith tissues of the accessions from both the Sudan savanna and the Semi-deciduous ecological zones showed over 50% reductions under the water stress treatments; within the W0-W2, as compared to that from the Coastal savanna that reduced by about 15%. Plasmolysis of the thin-walled cells was less pronounced in the accessions from the Coastal savanna. The reductions in the tissues may have reflected on the over-all reduction in the sizes of the morphological organs of the accessions under the stress treatments. The sizes of the vascular tissues showed some reductions in all the accessions though the reductions were not that pronounced as compared to the parenchymatous and collenchymatous tissues of the pith and the cortex. The vascular system was made up of thick-walled cells of the sclereids, companion cells, fibers and xylem vessels. The layers of cambium cells reduced under increasing severity of water stress treatments with the accessions SD1 from the Semi-deciduous zone showing the greatest reductions under the severe water stress regime.

Exception to this trend of reduction in sizes of the tissues was found in the xylem vessels of the vascular system of the accessions. There was the proliferation of many xylem vessels with smaller lumen in the accessions under the stress regimes. The cambium may have differentiated most of its cells into smaller permanent xylem tissues as a result of hormonal action on the cambium cells which was initiated by the stress treatments. Thus, few cells of the cambium was found in the accessions from the Coastal savanna (CS1 and CS2) while none was found in the accessions from the Semi-deciduous ecological zone (SD1and SD2) under the severe water stress protocol at the latter part of the experimental period.

REPRODUCTIVE STRUCTURES

All the different accessions produced flowers and fruits in all the water treatment protocols. The accessions (SD1 and SD2) from the Semi-deciduous zone produced more fruits under the normal irrigation and the medium water stress treatments as compared to the accessions from the savanna ecological zones (CS1, CS2, SS1 and SS2). The accessions (SD1, SD2) however, produced the smallest amount of fruits under the severe water stress treatment as they aborted most of their flowers. The accessions from the Coastal savanna produced the largest amount of fruits under the severe water stress protocol; esp. the accession CS1. The ability of the accessions from the Coastal savanna to withstand or tolerate the imposed drought conditions by showing the least reductions in both their morphological and anatomical features may have contributed to their ability to produce larger amount of fruits under the severe stress regime.

The investigation showed that accessions from the Semi-deciduous zone perform well under well watered and semi dry conditions, while the accessions from the Coastal savanna thrived well under water deficit conditions. The accession (CS1) with accession number (GH3673) from the Coastal savanna ecological zone performed best under the water stressed treatments. This was followed by the other accession (CS2, GH3667) from the same ecological zone, and these could be concluded to be the best option for farmers in the dry ecological regions of Ghana. However, farmers in the moist ecological regions in Ghana could go in for accessions SD1 (GH2332) and SD2 (GH2334) as these proved to be the best accessions under the normal irrigation treatment.

6.2 RECOMMENDATION

This work revealed that the accessions from the Coastal savanna with accession number GH3673 and GH3667 were the best for cultivation in the drier ecological regions of Ghana. However, further investigation needs to be done in other countries across the tropical and sub-tropical regions of Africa to determine the accessions of cowpeas that can best maximize water use efficiency under drought or inadequate water conditions as being advocated by the Food and Agriculture Organization in their Water Deficit Irrigation (DI) program (www.en.wikipedia,org/wiki/Deficit irrigation, 2009).

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APPENDICES

APPENDIX I._Variation in the soil moisture content (%) on different sampling occasions.

WEEKS OF SAMPLING	PERCENTAGE SOIL MOISTURE CONTENT		
	W0	W1	W2
3^{td}	21.75 ± 0.05 a	15.13 ± 0.02 b	10.67 ± 0.03 c
5^{th}	8.0 ± 0.02 a	5.73 ± 0.03 b	2.30 ± 0.02 c
7^{th}	3.70 ± 0.02a	1.01 ± 0.01b	0.13 ± 0.01c

The means bearing the same letters along a row are not significantly different at the 5% confidence level.

Where;

W0 : Normal treatment

W1 : Medium stress treatment

W2 : Severe stress treatment

APPENDIX II. Details of the variations in some climatic factors (ambient light intensity, ambient temperature and relative humidity) during the period of study.

WEEKS OF STUDY	RANGE OF VALUES	TEMPERATURE / ^oC	LIGHT / x10 Lux	RELATIVE HUMIDITY / %
1	low	20.26 ± 0.04	10.86 ± 0.02	84.19 ± 0.03
	high	32.27 ± 0.06	14.32 ± 0.03	54.21 ± 0.03
2	Low	19.64 ± 0.05	9.89 ± 0.03	82.36 ± 0.04
	High	33.37 ± 0.03	13.94 ± 0.02	47.84 ± 0.03
3	Low	19.88 ± 0.02	10.34 ± 0.10	86.37 ± 0.02
	High	33.52 ± 0.03	14.22 ± 0.09	52.81 ± 0.04
4	Low	19.72 ± 0.03	10.11 ± 0.03	79.65 ± 0.03
	High	30.79 ± 0.02	13.42 ± 0.05	48.83 ± 0.05
5	Low	21.36 ± 0.04	9.14 ± 0.03	81.32 ± 0.03
	High	34.72 ± 0.03	13.72 ± 0.05	44.73 ± 0.04
6	Low	21.54 ± 0.03	10.03 ± 0.02	74. 52 ± 0.02
	High	33.72 ± 0.04	14.12 ± 0.03	46.57 ± 0.05
7	Low	23.71 ± 0.04	10.01± 0.04	69.53 ± 0.04
	High	35.33 ± 0.03	13.92 ± 0.03	52.06 ± 0.03
8	Low	23.68 ± 0.03	9.67 ± 0.03	71.21 ± 0.04
	High	37.21 ± 0.05	14.11± 0.02	53. 89 ± 0.05
9	Low	23. 22 ± 0.03	9.23 ± 0.03	68.80 ± 0.04
	High	36.72 ± 0.04	14.54 ± 0.04	50.21 ± 0.03

Means and S.E values.

APPENDIX IIIa. Effects of water stress on the plant heights of the six accessions on three sampling occasions

ACCESSIONS	TREAT-MENTS	HARVEST PERIODS		
		1ST	2ND	3RD
SD1	W0	21.30 ± 0.13 b	94.35 ± 0.04j	119.31 ± 0.18m
	W1	21.47 ± 0.45 b	74.06 ± 0.12g	102.02 ± 0.36k
	W2	17.67 ± 1.22a	32.03 ± 0.24c	30.26 ± 0.27bc
SD2	W0	19.62 ± 0.13a	71.15 ± 0.21g	97.84 ± 0.31j
	W1	20.37 ± 0.06ab	65.81 ± 1.13f	91.15 ± 0.28ij
	W2	17.00 ± 0.18a	40.06 ± 0.40cd	42.93 ± 0.61d
CS1	W0	53.80 ± 0.16e	128.95 ± 1.06n	128.71 ± 0.65n
	W1	54.71 ± 0.11e	98.92 ± 0.65j	187.06 ± 0.82t
	W2	56.16 ± 0.26f	80.65 ± 0.92i	163.12 ± 0.75r
CS2	W0	48.12 ± 0.08d	119.56 ± 0.3m3	154.03 ± 0.33q
	W1	60.05 ± 1.04ef	101.06 ± 1.64l	184.85 ± 1.27t
	W2	62.74 ± 0.93f	76.28 ± 3.02g	172.68 ± 0.94s
SS1	W0	15.82 ± 0.52a	59.31 ± 0.52e	56.54 ± 0.09e
	W1	15.32 ± 0.48a	30.60 ± 0.78bc	46.02 ± 0.23d
	W2	14.00 ± 0.52a	23.38 ± 0.63b	18.36 ± 0.17a
SS2	W0	17.54 ± 0.66a	52.03 ± 0.27c	50.03 ± 0.51de
	W1	17.06 ± 0.58a	40.44 ± 0.33de	41.44 ± 0.83c
	W2	16.15 ± 0.72a	27.55 ± O.56b	25.16 ± 0.67b

Where;
SD1 and SD2 : accessions from the Semi-deciduous zone
CS1 and CS2 : accessions from the Coastal savanna zone
SS1 and SS2 : accessions from the Sudan savanna zone
W0 : Normal water treatment (control).
W1 : Medium water stress treatment
W2 : Severe water stress treatment.

APPENDIX IIIb. Effects of water stress on the width of stems of the six accessions on three sampling occasions

ACCESSIONS	TREAT-MENTS	HARVEST PERIODS		
		1ST	2ND	3RD
SD1	W0	3.91 ± 0.22b	4.96 ± 0.34c	5.28 ± 0.09c
	W1	4.36 ± 0.15bc	3.28 ± 0.06b	3.06 ± 0.28b
	W2	3.68 ± 0.18b	2.82 ± 0.25b	1.80 ± 0.37d
SD2	W0	2.48 ± 0.04ab	4.60 ± 0.21bc	5.06 ± 0.66c
	W1	3.04 ± 0.17b	4.21 ± 1.15bc	3.15 ± 0.75b
	W2	3.00 ± 0.35b	3.36 ± 0.04b	2.63 ± 0.06a
CS1	W0	3.58 ± 0.08b	3.82 ± 0.18b	3.96 ± 0.21bc
	W1	3.64 ± 0.09b	3.96 ± 0.08b	4.18 ± 0.13bc
	W2	3.43 ± 0.66b	3.63 ± 0.25b	3.91 ± 0.08bc
CS2	W0	3.09 ± 0.23b	4.40 ± 0.04bc	4.20 ± 0.28bc
	W1	3.21 ± 0.16b	4.57 ± 0.06bc	4.58 ± 0.33bc
	W2	3.15 ± 0.08b	4.02 ± 0.38bc	4.16 ± 0.37b
SS1	W0	4.08 ± 0.07bc	5.30 ± 0.23c	5.91 ± 0.12c
	W1	4.01 ± 0.19bc	4.76 ± 0.33bc	4.52 ± 0.08bc
	W2	3.45 ± 0.20b	3.58 ± 0.14c	2.91 ± 0.15b
SS2	W0	4.27 ± 0.15bc	5.66 ± 0.07c	5.60 ± 0.07c
	W1	4.19 ± 0.08bc	5.31 ± 0.42c	3.99 ± 0.02b
	W2	3.06 ± 0.14b	4.59 ± 0.11bc	3.06 ± 0.35ab

Where;
SD1 and SD2 : accessions from the Semi-deciduous zone
CS1 and CS2 : accessions from the Coastal savanna zone
SS1 and SS2 : accessions from the Sudan savanna zone
W0 : Normal water treatment (control).
W1 : Medium water stress treatment
W2 : Severe water stress treatment.

APPENDIX IIIc. Effects of water stress on the root length of the six accessions on three sampling occasions

ACCE-SSIONS	TREAT-MENTS	HARVEST PERIODS		
		1ST	2ND	3RD
SD1	W0	5.67 ± 0.34ab	14.48 ± 0.52c	18.61 ± 0.21d
	W1	5.86 ± 0.96ab	14.81 ± 0.06c	19.22 ± 0.33d
	W2	5.89 ± 1.15ab	14.03 ± 0.15c	10.08 ± 0.54bc
SD2	W0	4.71 ± 0.16a	15.16 ± 0.34cd	17.86 ± 0.32d
	W1	4.92 ± 0.32a	15.76 ± 0.27cd	15.34 ± 0.09cd
	W2	5.02 ± 0.25a	14.64 ± 0.18c	9.47 ± 0.06b
CS1	W0	15.06 ± 0.06cd	13.75 ± 0.04c	16.55 ± 0.15d
	W1	15.34 ± 0.32cd	14.01 ± 0.25c	16.82 ± 0.36d
	W2	15.32 ± 0.05cd	13.99 ± 0.08c	16.85 ± 0.32d
CS2	W0	14.59 ± 0.45c	14.08 ± 0.38c	15.62 ± 0.41cd
	W1	15.63 ± 0.33cd	14.36 ± 0.22c	16.04 ± 0.10cd
	W2	15.24 ± 0.50cd	14.11 ± 0.33c	15.01 ± 0.36d
SS1	W0	18.61 ± 0.25d	22.75 ± 0.41e	23.73 ± 0.07e
	W1	17.94 ± 0.52d	22.03 ± 0.36e	24.56 ± 0.18e
	W2	16.00 ± 0.07d	19.83 ± 0.29d	19.30 ± 0.56d
SS2	W0	19.06 ± 0.43d	20.81 ± 0.07de	21.18 ± 0.39e
	W1	18.22 ± 0.15d	19.02 ± 0.05d	22.94 ± 0.54e
	W2	17.63 ± 0.09d	17.76 ± 0.08cd	18.00 ± 0.02d

Where;
SD1 and SD2 : accessions from the Semi-deciduous zone
CS1 and CS2 : accessions from the Coastal savanna zone
SS1 and SS2 : accessions from the Sudan savanna zone
W0 : Normal water treatment (control).
W1 : Medium water stress treatment
W2 : Severe water stress treatment.

APPENDIX IIId. Effects of water stress on the sizes of leaves of the six accessions on three sampling occasions

ACCE-SSIONS	TREAT-MENTS	HARVEST PERIODS		
		1ST	2ND	3RD
SD1	W0	4.61 ± 0.03bc	4.82 ± 0.06bd	5.62 ± 0.12c
	W1	4.26 ± 0.11b	3.09 ± 0.01c	4.18 ± 0.33bc
	W2	3.04 ± 0.38ab	2.14 ± 0.03ab	1.96 ± 0.08a
SD2	W0	3.64 ± 0.02b	3.84 ± 0.05b	4.58 ± 0.02bc
	W1	2.70 ± 0.18ab	3.58 ± 0.13b	3.16 ± 0.07b
	W2	1.94 ± 0.02a	2.72 ± 0.08ab	1.80 ± 0.06a
CS1	W0	4.32 ± 0.10bc	4.62 ± 0.05bc	4.83 ± 0.04bc
	W1	4.20 ± 0.07bc	4.24 ± 0.06c	4.18 ± 0.06b
	W2	3.86 ± 0.25b	3.94 ± 0.02b	3.46 ± 0.03b
CS2	W0	4.06 ± 0.09bc	5.71 ± 0.04cd	5.86 ± 0.08c
	W1	3.93 ± 0.02bc	5.43 ± 0.08c	3.72 ± 0.07b
	W2	3.62 ± 0.61b	3.80 ± 0.04b	3.64 ± 0.10b
SS1	W0	5.32 ± 0.04c	5.57 ± 0.02c	5.81 ± 0.09c
	W1	5.33 ± 0.09c	5.35 ± 0.11c	5.26 ± 0.03bc
	W2	5.05 ± 0.08bc	4.01 ± 0.03bc	3.94 ± 0.05b
SS2	W0	5.48 ± 0.05c	5.63 ± 0.05c	5.72 ± 0.06c
	W1	5.50 ± 0.11c	4.22 ± 0.03bc	4.20 ± 0.03bc
	W2	5.08 ± 0.09bc	3.82 ± 0.02b	3.16 ± 0.01b

Where;
SD1 and SD2 : accessions from the Semi-deciduous zone
CS1 and CS2 : accessions from the Coastal savanna zone
SS1 and SS2 : accessions from the Sudan savanna zone
W0 : Normal water treatment (control).
W1 : Medium water stress treatment
W2 : Severe water stress treatment.

APPENDIX IIIe. Effects of water stress on the number of leaves of the six accessions on three sampling occasions

ACCE-SSIONS	TREAT-MENTS	HARVEST PERIODS		
		1ST	2ND	3RD
SD1	W0	7	9	10
	W1	5	6	6
	W2	4	4	3
SD2	W0	6	9	10
	W1	5	7	7
	W2	3	3	2
CS1	W0	5	7	8
	W1	5	6	6
	W2	4	5	5
CS2	W0	6	7	8
	W1	4	6	6
	W2	4	4	5
SS1	W0	4	4	5
	W1	4	4	4
	W2	2	3	3
SS2	W0	4	5	5
	W1	4	4	3
	W2	3	3	2

Where;
SD1 and SD2 : accessions from the Semi-deciduous zone
CS1 and CS2 : accessions from the Coastal savanna zone
SS1 and SS2 : accessions from the Sudan savanna zone
W0 : Normal water treatment (control).
W1 : Medium water stress treatment
W2 : Severe water stress treatment.

APPENDIX IV. Effects of water stress on the reproductive structures of six different accessions of cowpeas.

ACCESSION CODES	TREATMENTS	FLOWERING AND FRUITING PATTERNS						
		Period before onset of 1st flower bud (weeks)	Length of bean pods /cm	Width of bean pods /cm	Mass of individual bean pods /g	Total bean pods produced /g	Mass of total dried bean pods	No. of bean pods
SD1	W0	8	9.21±0.03a	0.90±0.02f	2.87±0.01k	63.14±0.03n	10.63±0.13a	23
	W1	7	7.04±0.05b	0.50±0.02g	2.04±0.03l	42.16±0.05o	8.99±0.05a	10
	W2	5	4.32±0.05c	0.27±0.03h	1.15±0.03f	10..65±0.05a	3.08±0.18c	6
SD2	W0	8	10.1±0.02a	0.87±0.02f	2.86±0.02k	58.06±0.02p	9.51±0.11a	25
	W1	7	8.64±0.02a	0.41±0.03g	2.13±0.03l	44.18±0.04o	7.82±0.06b	9
	W2	5	5.75±0.05c	0.24±0.03j	0.38±0.04h	11.27±0.06a	2.76±0.25c	4
CS1	W0	7	8.62±0.03a	0.68±0.03i	1.85±0.01k	49.53±0.01o	6.15±0.01b	15
	W1	5	7.01±0.04b	0.50±0.02g	1.33±0.02l	32.94±0.03q	4.72±0.18c	10
	W2	4	4.92±0.06d	0.32±0.04h	1.18±0.04f	18.72±0.05r	4.00±0.22d	6
CS2	W0	7	7.52±0.04b	0.60±0.02i	1.72±0.02k	41.69±0.02o	6.08±0.13b	14
	W1	5	6.31±0.06f	0.51±0.02g	1.43±0.04l	35.47±0.02q	5.86±0.05c	11
	W2	4	4.80±0.07d	0.26±0.04j	1.06±0.05f	21.59±0.04r	4.35±0.11c	7
SS1	W0	6	8.96±0.02a	1.21±0.02f	3.81±0.02m	25.91±0.03r	4.89±0.22c	11
	W1	5	6.15±0.04e	1.16±0.04f	3.26±0.03m	17.94±0.05r	3.27±0.15d	9
	W2	4	5.55±0.07c	0.62±0.03i	3.12±0.05k	12.88±0.05a	2.01±0.31l	4
SS2	W0	6	9.22±0.04a	1.06±0.03f	3.78±0.02m	30.43±0.02q	4.52±0.17c	9
	W1	5	6.60±0.05e	0.90±0.02f	3.34±0.04m	23.67±0.04r	3.36±0.23d	7
	W2	4	5.82±0.06e	0.68±0.04i	3.07±0.04k	12.02±0.05a	1.98±0.14l	3

Mean and S.E values within the columns and followed by the same letters are not significantly different at 0.05 confidence level.

NOTES:
W0: Normal water treatment, W1: medium stress, W2: severe stress treatments
SD1&2 : accessions from Semi-deciduous zone
CS1&2 : accessions from Coastal savanna zone
SS1&2 : accessions from Sudan savanna zone

APPENDIX V. Summary of the p-values of ANOVA for the morphology and the fruit yields of the cowpea accessions

MEASURED PARAMETERS	P-VALUES	
	TREATMENTS	ACCESSIONS
HEIGHT OF PLANTS	0.012	0.035
WIDTH OF STEMS	0.028	0.041
LENGTH OF MAIN ROOTS	0.011	0.637
WIDTH OF LEAFLETS	0.046	1.062
TOTAL NUMBER OF LEAVES	0.002	3.066
ONSET OF FLOWERING(WEEKS)	0.001	0.042
LENGTH OF BEAN PODS	0.045.	0.009
WIDTH OF BEAN PODS	0.172	0.037
MASS OF INDIVIDUAL BEAN PODS	0.003	0.050
TOTAL BEAN PODS PRODUCED	0.021	0.048
MASS OF TOTAL DRIED FRUITS	0.003	0.002

*Degree of freedom (d.f) was 2 and 5 within the treatments and the accessions respectively

APPENDIX VI. Summary of the p-values of ANOVA for the anatomic parameters

MEASURED PARAMETERS	ORGAN / SITES IN PLANTS	P-VALUES	
		TREATMENTS	ACCESSIONS
Diameter of cells of epidermis	Roots	0.0615	3.1911
	Stems	0.0543	6.9512
	Leaves	0.1013	3.8214
Diameter of cells of cortex	Roots	0.0013	0.0173
	Stems	0.0046	0.0321
Width of cortex	Roots	0.0032	0.5784
	Stems	0.0024	0.2232
Diameter of pith cells	Roots	0.0172	0.0135
	Stems	0.0028	0.0206
Width of pith	Roots	0.0004	0.0083
	Stems	0.0447	0.0443
Width of vascular band	Roots	0.1348	0.5032
	Stems	0.0399	1.0244
	Leaves	0.0017	0.6407
Diameter of Xylem vessels	Roots	0.0014	5.1125
	Stems	0.0126	2.3170
	Leaves	0.0128	0.6027
Frequency of xylem vessels	Roots	0.0388	0.5721
	Stems	0.0112	0.4073
Number of cambium layers	Roots	0.0041	0.6815
	Stems	0.0306	0.4833
Diameter of mesophyll tissues	Palisade cells	0.4831	3.6312
	Spongy cells	0.0470	1.4844
Width of cuticles	Upper surface	0.0193	0.9318
	Lower surface	0.0468	0.6910
Width of stomata pores	Upper epidermis	0.0500	3.3418
	Lower epidermis	0.0572	1.5056
Width of stomata guard cells	Upper epidermis	0.0369	0.4679
	Lower epidermis	0.0473	0.1578
Frequency of stomata	Upper	0.5328	5.0409
	Lower	0.3085	5.6707

*Degree of freedom (d.f) was 2 and 5 within the treatments and the accessions respectively.

APPENDIX VII . ANOVA TABLES

ANOVA OF PLANT GIRTH/STEM WIDTH

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Accessions	7.698983	5	1.539797	10.60734	0.000953	3.325835
Treatments	4.647633	2	2.323817	16.00829	0.000764	4.102821
Error	1.451633	10	0.145163			
Total	13.79825	17				

ANOVA OF PLANT HEIGHTS/LENGTHS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Accessions	8378.727	5	1675.745	12.98151	0.000417	3.325835
Treatments	3552.73	2	1776.365	13.76098	0.001345	4.102821
Error	1290.871	10	129.0871			
Total	13222.33	17				

ANOVA OF LENGTH OF MAIN ROOT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Accessions	146.2114	5	29.24228	38.38666	3.28E-06	3.325835
Treatments	2.093644	2	1.046822	1.374175	0.296982	4.102821
Error	7.617822	10	0.761782			
Total	155.9228	17				

ANOVA OF SIZES OF EPIDERMAL CELLS

Source of Variation	SS	df	MS	F	P-value	F crit
Accessions	146.2114	5	29.24228	51.38815	2.21224	432835
Treatments	2.093644	2	1.046812	2.644123	0.339776	2.02411
Error	5.207562	10	0.331782			
Total	262.9215	17				

ANOVA OF WIDTH OF THE CORTEX

Source of Variation	SS	df	MS	F	P-value	F crit
Accessions	8378.727	5	1675.745	12.98151	0.000417	3.325835
Treatments	3552.73	2	1776.365	13.76098	0.001345	4.102821
Error	1290.871	10	129.0871			
Total	13222.33	17				

ANOVA OF SIZES OF CORTICAL CELLS

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	12.73633	5	2.547267	14.86009	0.000236	3.325835
Columns	4.0033	2	2.00165	11.6771	0.002422	4.102821
Error	1.714167	10	0.171417			
Total	18.4538	17				

ANOVA OF THE SIZES OF PITH CELLS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Accessions	7.862378	5	1.572476	8.585933	0.002185	3.325835
Treatments	6.922344	2	3.461172	18.89848	0.000401	4.102821
Error	1.831456	10	0.183146			
Total	16.61618	17				

ANOVA OF LENGTH OF BEAN PODS

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	6.243027778	5	1.248606	3.832678	0.033623	3.325835
Columns	42.15947778	2	21.07974	64.70566	1.9E-06	4.102821
Error	3.257788889	10	0.325779			
Total	51.66029444	17				

ANOVA OF WIDTH OF BEAN PODS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.786627778	5	0.157326	14.00663	0.000303	3.325835
Columns	0.717144444	2	0.358572	31.92353	4.55E-05	4.102821
Error	0.112322222	10	0.011232			
Total	1.616094444	17				

ANOVA OF MASS OF INDIVIDUAL FRESH PODS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	12.73633	5	2.547267	14.86009	0.000236	3.325835
Columns	4.0033	2	2.00165	11.6771	0.002422	4.102821
Error	1.714167	10	0.171417			
Total	18.4538	17				

ANOVA OF MASS OF FRESH BEAN PODS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	1026.679	5	205.3359	2.88641104	0.072275	3.325835
Columns	2786.801	2	1393.4	19.58705941	0.000348	4.102821
Error	711.3882	10	71.13882			
Total	4524.868	17				

ANOVA OF MASS OF DRIED BEAN PODS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	44.66251	5	8.932502	4.566947	0.019837	3.325835
Columns	48.22684	2	24.11342	12.32854	0.002	4.102821
Error	19.55902	10	1.955902			
Total	112.4484	17				