SCREENING FOR DROUGHT TOLERANCE POTENTIAL OF NINE COCOA (*THEOBROMA CACAO* L.) GENOTYPES FROM COCOA RESEARCH INSTITUTE OF GHANA (CRIG)

This thesis/dissertation is submitted to the University of Ghana, Legon in partial fulfilment of the requirement for the award of MPHIL. Plant & Environmental Biology Degree

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JUNE 2016

Declaration

I hereby declare that except for references to other people's work which I have duly cited, this thesis is the result of an original research work and that the material has not been presented in either whole or in part elsewhere for another degree.

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Dedication

This study is dedicated to the memory of

Mr. Winfred Ellis Kojo Dzandu

1954-2011

As well as Felicia Mensah, Alma Dzandu and Benjamin Atieku-Dzandu

Acknowledgements

Many thanks also go to the various technicians of the Botany Department, Mr George Akwettey, Mr Kofi Baako and Mrs Bernice Asante.

I wish to also thank my supervisors, Prof Lewis Enu-Kwesi and Dr Ayeh, for their dynamic supervision, constructive criticisms, corrections and constant motivation during this project.

Abstract

Theobroma cacao L. (cocoa), a tropical forest understory plant, is very important for the chocolate industry and the economy of many developing countries in the tropics. However, cocoa production is under threat from climate change due to the general intolerance of the crop to drought. In Ghana, cocoa production is threatened by erratic weather patterns and the shifting of the cocoa belt southwards (particularly south of Brong-Ahafo region) due to the gradual extension of the dry savannah areas from the Northern regions of the country as a result of climate change. Thus, there is the need to develop drought-tolerant cocoa plants that can help not only to sustain but also boost cocoa production in the country. Consequently, two experiments were conducted in a shed constructed at the premises of the Department of Botany, University of Ghana, designed to keep out rain and simulate the shading required for establishment of cocoa seedlings. The two experiments were conducted between January – February 2016 and April – May 2016 in order to i) determine the drought-tolerance potential of nine (9) selected cocoa genotypes (T60 x Pound10, PA7 x 6035, T85 x PA7, T63/971 x Sca9, PA150 x 6020, AMAZ X 9006, T79 x 9006, PA150 x 6020 and PA7 x MAN) and ii) identify the relationship between their physiological and biochemical parameters under drought stress and their drought-tolerance ability so that these could be used as sources of traits for the development of drought-tolerant cocoa hybrids. In the First Experiment (Phase I), a Randomized Complete Block Design (RCBD) with three (3) replicates was used to rapidly assess the drought tolerance potentials of the 10 genotypes of cocoa at the seedling stage and determine the effect of small differences in age of seedlings on their drought-tolerance potential. The seedlings were slowly saturated with water to Full Saturation (FS) after which water was withheld from 27 out of 54 seedlings comprising of three (3) replicates of each of the nine (9) genotypes one (1) Day After Full Saturation (DAFS) (Water stressed); whereas the remaining 30 seedlings (Control) received water every other day till the end of the experiment. Data on leaf Relative Water Content (RWC) and Soil Moisture Content (SMC) were collected

in addition to the number of days for First Appearance of Drought Symptoms (FADS). Data on Leaf Relative Water Content (RWC) and Soil Moisture Content were taken at i) the beginning of the experiment i.e. 1DAFS and ii) First Appearance of Drought Symptoms (FADS). Based on the results of Experiment I, the genotypes were ranked using the number of days it took for FADS to appear in the selected cocoa genotypes. Further studies were conducted on the genotypes in Phase II (Experiment II). Experiment II was designed as a Randomised Complete Block Design (RCBD) with six replicates of each genotype. The free proline content of the leaves was used as a biochemical criterion for drought-tolerance potential. The selected genotypes differed in their drought-tolerance potential. Proline was observed to accumulate in water-stressed seedlings, and the differences in the mean proline accumulation in the genotypes was found to be statistically significant. There was no positive correlation between the accumulation of free proline of most of the genotypes and the relative water content of the leaves except for T63 x SCA9 and T60 x POUND10; T63 x SCA9 and T60 x POUND10 stood seemed to be the most drought-tolerant genotypes resulting from their relatively high leaf RWC and low water use. Free proline accumulation could be used broadly to distinguish between drought-tolerant and drought-sensitive varieties. The number of trichomes present on the leaves of the various genotypes could also broadly be used to distinguish between drought-tolerant and drought-sensitive varieties of the cocoa genotypes. Further studies should be carried out to investigate the role of proline accumulated in drought-stressed cocoa plants as well as the other factors e.g. presence of trichomes that can contribute to the low water use of the genotypes that showed higher drought-tolerance potentials.

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List of Abbreviations

DAFS – Days After Full Saturation

DW – Dry weight

CRIG – Cocoa Research Institute, Ghana

FADS – First Appearance of Drought Symptoms

FS – Full Saturation

FW – Fresh weight

OA – Osmotic adjustment

OSSD – Occurrence of Symptoms of Severe Drought

PEG – Polyethylene glycol

SMC – Soil Moisture Content

RWC – Relative Water Content

TW – Turgid weight

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 *Introduction*

Cocoa (*Theobroma cacao* L.) is an important humid crop plant grown in regions of high rainfall. It is also one of the most important tree cash crops in the world due to its use in the confectionary industry; specifically the exclusive use of its seeds in the manufacturing of chocolate and other cocoa products (Carr and Lockwood, 2011) as well as skin care products (Almeida and Valle, 2007; Bart-plange & Baryeh, 2003). It originates from South America (Carr & Lockwood, 2011; Mousa and Abdel-Aziz, 2008). It is specifically a native of the understorey species in the forests of the Amazon (Frimpong *et al.*, 2011). It belongs to the family Sterculiaceae and the genus Theobroma. With the application of molecular markers, it has been proposed that the cocoa species be reclassified to belong to the family Malvaceae (Alverson et al., 1999).

It is cultivated in most tropical regions, especially in West Africa accounting for 70% of the global annual production of the crop (ICCO, 2013). The cocoa crop was first introduced to West Africa in the mid-19th century and per official records, cocoa was introduced into Ghana by Dutch missionaries 1815 but it is widely credited to have been introduced in 1879 by Tetteh Quarshie (COCOBOD, 2016).

To date, known constraints to the growth, development and yield of cocoa include i) diseases such as the Cocoa Swollen Shoot Virus (CSSVD) and the black pod disease caused by *Phytophthora megakarya* and *P.* palmivora; and ii) water stress/drought which is the focus of this research work/study.

Water is a very essential requirement for the cultivation of cocoa which requires an optimal rainfall between 1500 and 2000 mm per annum though it can grow with an amount of between

1100 and 3000 mm per annum (Amoah, 1995). Therefore cocoa production is often adversely affected by periodic drought caused by changes in seasonal weather patterns that have prolonged dry cycles (Bae *et al.*, 2008). Thus in general cocoa cannot tolerate drought (Belsky and Siebert, 2003; Wood and Lass, 2001).

When natural water systems or managed water systems cannot provide sufficient water for established human and environmental needs, drought occurs (Robert *et al.*, 2000). Drought is a usual phenomenon in semi-arid and arid regions of the world (Jain *et al*., 2007) but the effects of climate change may cause regions that previously enjoyed high amounts of rainfall to experience drought conditions. This change in weather patterns will have a significant effect on agriculture (Alam *et al.*, 2011; Lobell *et al*., 2008).

Erratic weather patterns which are indicative of climate change have become more common in the world (Mary & Majule, 2009). These changes in the ambient abiotic environmental factors nationally and globally due to deforestation and general environmental degradation have resulted in the unpredictable nature of the world's climate (IPCC, 2007). Rainfall patterns have changed considerably both nationally and at the global level such that areas which previously experienced little rainfall are experiencing torrential rains whereas areas that had an abundance of rainfall are experiencing minimal precipitation (IPCC, 2007). Furthermore, both the onset of the rainy season and quantum of precipitation have become relatively difficult to predict at present (Alam *et al* 2011; Mary & Majule, 2009).

Agricultural production can be severely compromised by climate change and variability (Alam *et al* 2011; IPCC, 2007) and one of the main causes of crop loss in the world is drought which has been predicted to get worse in the years to come (Sharma and Lavanya, 2002); and as a result, hunger and poverty would become more common during those years.

Ghana previously experienced conditions of drought in the years 1977, 1983, 1992 and 1998 (Oduro-Afriyie and Adukpo, 2006) and may experience similar drought conditions in the near future, considering current state of deforestation and environmental degradation as well as the erratic and unpredictable rainfall patterns.

Cocoa is Ghana's major cash crop as well as its foreign exchange earner (Mull and Kirkhorn, 2005). In the year 2009, cocoa contributed approximately $1.87 billion to the revenue of the country (Anon, 2009); which constituted about 3.4% of its GDP (FAO, 2008); and also accounted for 70-100% of household incomes of cocoa farmers in the country (Ntiamoah and Afrane, 2008). Although Ghana is a major commercial producer of cocoa globally, it presently trails the Ivory Coast as the second-largest producer of the crop. However, Ghana's cocoa is still considered as the best cocoa in terms of quality (Laderach *et al.*, 2013).

The Cocoa Research Institute of Ghana (CRIG) at Akim Tafo is the mandated institution that carries out research on all aspects of the growth and production of cocoa as well as other mandated crops (e.g. coffee, shea and cashew). Appreciable amount of work has been done by CRIG which has resulted in the production of improved cocoa varieties (with respect to early maturity, disease resistance and higher yields) for distribution to Ghana's cocoa farmers (MMYE, 2006). There have been several efforts by researchers at CRIG to screen and breed for drought-tolerant cocoa hybrids which have resulted in the identification of some cocoa cultivars as having some drought-tolerance potential (Adu-Ampomah and Frimpong, 2002; Adu-Ampomah *et* al., 2001; and Amponsah, 1973). Furthermore, others e.g. Osei-Bonsu (2011) have also collected germplasm from CRIG to investigate their drought tolerance potential.

A considerable amount of research has been conducted on drought tolerance in other crop plants (e.g. rice, maize, cowpea etc.) but compared to these other crops, relatively very little

research has been done to screen for the potential drought-tolerance in different genotypes of cocoa even though as has been already been alluded to, the crop is very important to the confectionary industry and most importantly, to the economy of Ghana.

The cocoa growing belt in Ghana has gradually shifted southwards due to climate change with its associated global warming and it has been predicted to continue to move further towards the south of the Brong-Ahafo and Western regions of Ghana by 2030 (CIAT, 2011).

As afore-stated Ghana stands to lose a lot if nothing is done about the effects of drought and climate change on the production of cocoa. Consequently, this research work has been designed to add to efforts at the continued screening of additional cocoa germplasm for their drought-tolerance potential using of physiological, biochemical and morphological features as the criteria for selection. Ultimately, the results obtained from this study may be useful either in direct selection of drought-tolerant cocoa genotypes or in assisting to breed for drought-tolerant cultivars of the crop.

1.1.1 Problem statement

As alluded to earlier on, cocoa is a valuable crop globally in the confectionary industry. Most importantly, it contributes about $1.87 billion (3.4% of GDP) to the economy of Ghana where it is a major foreign exchange earner to the GDP (Anon, 2009). Unfortunately, climate change has resulted in the cocoa-growing belt within the country moving further south of the Brong-Ahafo and Western Regions, and efforts must be made to breed for drought-tolerant varieties of cocoa. In order to breed drought resistant varieties of *Theobroma cacao* L., there has to be a thorough understanding of the available germplasm that is currently cultivated in the country as well as collections from outside, with respect to their drought-tolerance potential.

Justification

As indicated cocoa is a major cash crop which contributes significantly to the foreign exchange of Ghana $1.87 billion (3.4% of GDP). This can be adversely affected by drought. Therefore, efforts must be made to breed for resistance of the crop to drought in order to maintain the livelihoods of people in the cocoa-growing areas of the countries as well as its contribution to the country's economy.

1.1.2 Hypotheses

- *Null Hypothesis (H_0)*: There are no variations in morphological, physiological, and biochemical responses/parameters of the nine cocoa genotypes obtained from CRIG in response to varying or different soil moisture content (i.e. drought).
- *Alternate Hypothesis (H_1)*: There are variations in morphological, physiological, and biochemical responses/parameters of the nine cocoa genotypes obtained from CRIG in response to varying soil moisture content (i.e. drought).

1.1.3 General objectives

To investigate the potential use of either morphological, physiological or biochemical parameters or their combination(s) in the rapid screening of the drought-tolerance potential of the nine cocoa genotypes obtained from CRIG.

1.1.4 Specific objectives

In order to achieve the general objective, the specific objective of this study was:

a. To assess nine (9) selected genotypes of cocoa obtained from CRIG for their drought-tolerance potential through:

(i) Determination and comparison of the actual water status of the seedlings of the nine (9) genotypes of cocoa as depicted in the values of their Leaf Relative Water

Content under normal irrigation/non-stress conditions and intermittent water stress as a basis for the determination of their drought-tolerance potential.

(ii) Investigating the possibility of the presence or absence of epidermal hairs (trichomes) on the surface of the leaves of each of the nine (9) genotypes and their probable relation to their drought-tolerance potential

(iii) Determination of the possible correlation of the following leaf anatomical features in transverse section to the possible drought-tolerance potential of the nine (9) cocoa genotypes by measurements of:

(a) Thickness of upper and lower epidermis

(b) Presence/absence of hypodermis (number of layers of cells, if present)

(c) Measure thickness of hypodermis (if present)

(d) Width of palisade and spongy mesophylls and their possible correlation with drought-tolerance potential

(iv) Obtain photomicrographs of the leaf of each of the nine (9) genotypes in transverse section

(v) Investigate the relationship between the leaf accumulation of free proline content in each of the nine (9) genotypes of cocoa and their drought-resistance potential

1.2 Literature review

1.2.1 The cocoa plant, origin and global distribution

As previously mentioned, cocoa (*Theobroma cacao* L.) is a tropical forest understory perennial tree species of the family Malvaceae (Alverson et al., 1999) originally classified under the family Sterculiaceae (Wicks, 2003; Cuatrecasa, 1964). The cocoa tree is native to the lowland tropical evergreen forests of South America (Argout et al., 2008; Borrone *et al*., 2004; Wicks, 2003 and Motamayor *et al*., 2002), but is now grown commercially in all tropical lowlands of the world, particularly in Central and South America, Asia and Africa, because of its seeds

(beans). Cocoa beans are used almost exclusively in the manufacture of chocolate and cocoa butter, a by-product, used in the cosmetics industry (Almeida and Valle, 2007).

Table 1.1: Top ten cocoa producing countries in 2015 *(source: www.perfectinsider.com)*

Rank	Country	Production (million metric tonnes)
1	Cote d'Ivoire	1.954
2	Indonesia	1.143
3	Ghana	0.879
4	Nigeria	0.583
5	Cameroon	0.356
6	Brazil	0.283
7	Ecuador	0.193
8	Mexico	0.083
9	Dominican Republic	0.072
10	Peru	0.057

Major constraints to the production of cocoa are the Blackpod Disease, the Cocoa Swollen Shoot disease and declining soil fertility (Mull and Kirkhorn, 2005). However, the most serious constraint now appears to be drought; particularly due to climate change which is said to be contributing to the gradual shifting of the cocoa growing-belt southwards of the Brong-Ahafo region (Vigneri, 2007).

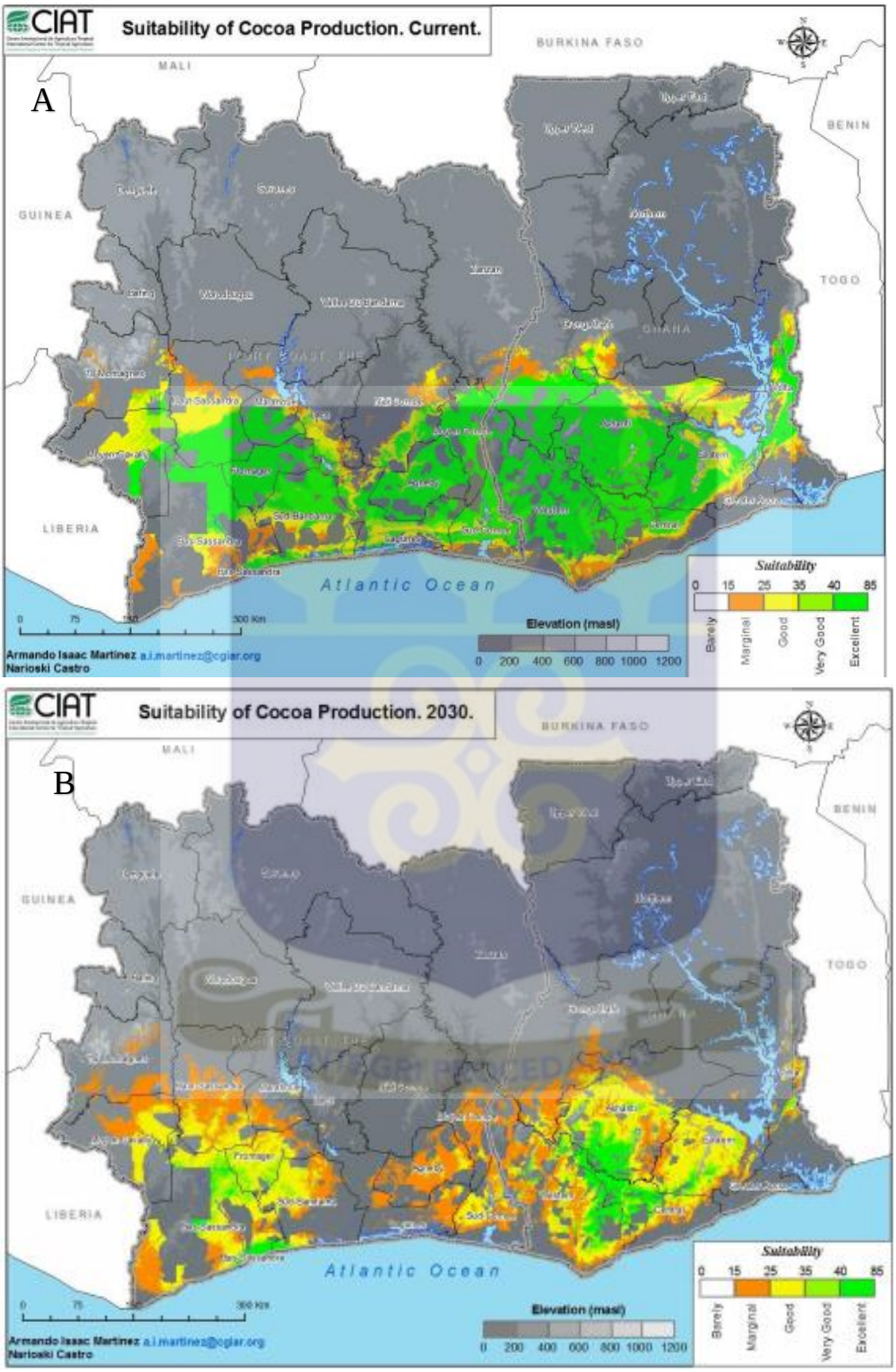

Plate 1.1: Geographical suitability of cocoa cultivation areas now (A) and projected suitability in 2030 (B) (CIAT, 2011)

1.2.2 Botany of cocoa

1.2.2.1 Habit and Morphology

Cocoa is a perennial woody species that grows up to heights of between 3 and 5m at maturity under cultivation (Almeida and Valle, 2007); but can reach heights of 10m or more (Wicks, 2003).

The shoots of cocoa seedlings exhibit orthotropic growth patterns with spiral leaf phyllotaxy (Amoah, 1995). Seedlings bear plagiotropic branches (i.e. 3 to 5 shoots coming out of the shoot apex) (Wicks, 2003) which develop at an angle to the horizontal (Amoah, 1995) and form the crown 8of the cocoa tree (Cuatrecas, 1964). The point of development of these fan branches is called the jorquette and the vertical stem is known as the chupon (Wicks, 2003; Amoah, 1995). The cocoa leaf has stomata occurring on only the lower side of the leaf (i.e. it is hypostomatous) and has hairs, most of which are thick and short with a multicellular rounded head (Abo-Hamed *et al*., 1983).

The root system of the cocoa tree comprises a tap root that functions both in structural support as well as in the absorption of water that lies relatively deeper underground and lateral roots that functions mainly in the uptake of water and nutrients (Amoah, 1995).

There are significant variabilities in the morphological and physiological traits that exist in cocoa (Daymond *et al*., 2002).

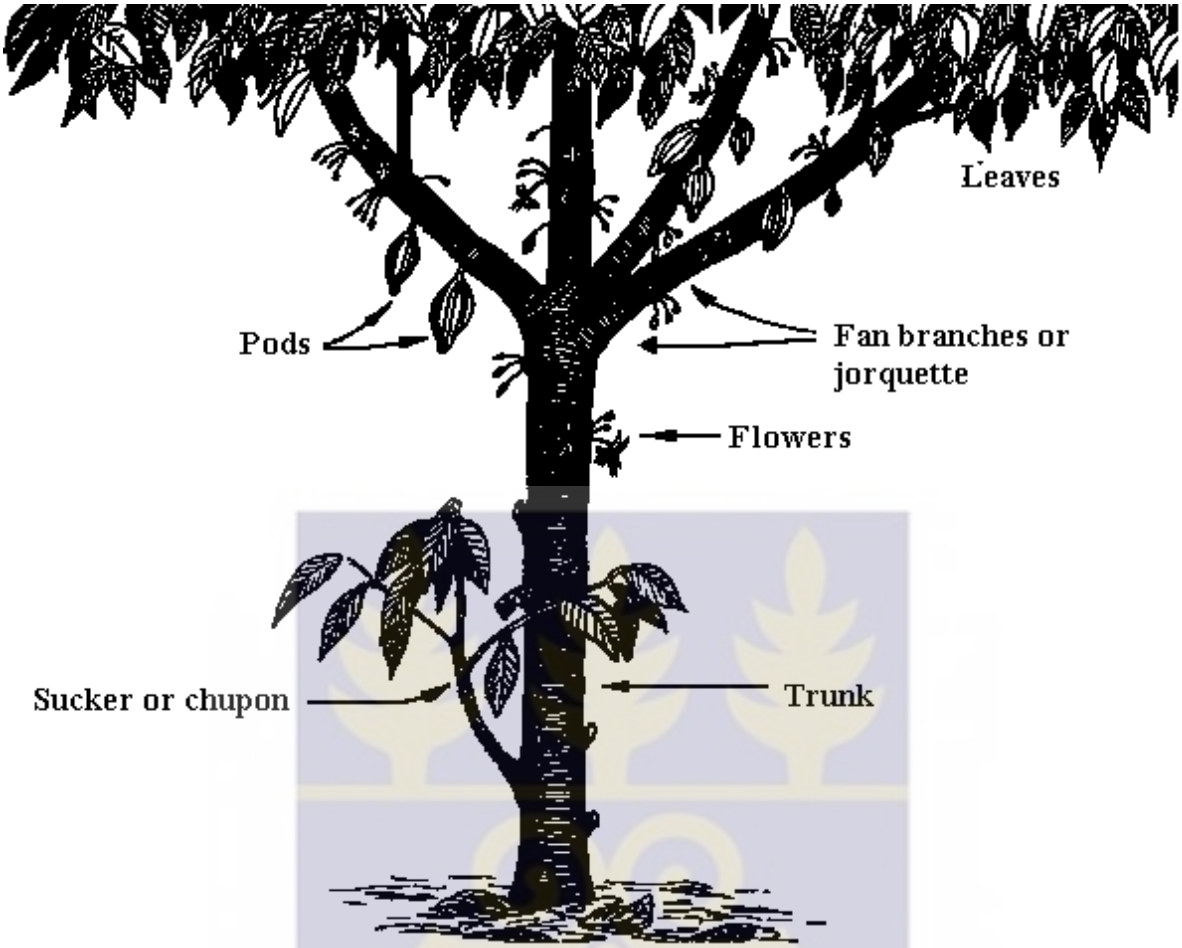

Plate 1.2: Diagram of habit of a mature cocoa plant *source: (University of Queensland, 2016)*

1.2.2.2 *Phenology*

The mature cocoa tree produces flowers and fruits throughout the year, though greater amounts of flowers are produced twice a year. The caulescent flowers are located on flower cushions (i.e. little swellings on the main trunk and main branches in positions previously occupied by leaves) (Wicks, 2003; Amoah, 1995). The flowers open in the morning or afternoon and usually fall off by the following day if they are not pollinated (Amoah, 1995). According to Almeida and Valle (1995), cocoa fruits (pods) are usually formed through cross-fertilisation. The pods vary in length and shape and are coloured differently depending on their stage of maturity; young pods are either light green or red (Wicks, 2003) and mature pods are yellow or orange (Wood and Lass, 2001). It takes approximately five to six months after pollination for the pods to ripen (Amoah, 1995).

1.2.3 Climatic requirements for the growth and development of cocoa

1.2.3.1 Light

Cocoa is a tropical understory plant and can therefore survive substantial shading. Shading is a requirement for seedling establishment and can be gradually reduced as the plant matures (Amoah, 1995). When subjected to full sunlight, the growth of a seedling is slowed; although nutrients may be more important for seedling establishment than shading (Okali and Owusu, 1975). Excessive shading in mature trees can reduce yield and increase the incidence of disease (Alvim, 1977). There is a positive correlation between cocoa yields and light, when nutrients are available (Ahenkorah *et al.*, 1987; Vernon, 1967). This higher yield under non-shaded conditions however results in a shorter productive lifespan and a higher requirement for fertilizers (Owusu, 1978)

1.2.3.2 Temperature

Cocoa, being a tropical plant, thrives in warm and humid conditions. It is intolerant to cold; and thus frosts are deadly to the cocoa plant (Wicks, 2003). The growth and development of the plant is highly temperature-dependent as temperatures below and above the range considered optimal lead to a reduction in rates of photosynthesis (Raja Harun and Hardwick, 1988). The maximum and minimum temperatures should therefore range between 30-32 ^{o}C and 18-21 ^{o}C respectively (ICCO, 2013); with an absolute minimum of 10^{o}C (Amoah, 1995).

1.2.3.3 Rainfall (water)

The limits of altitudes and latitudes in which cocoa can be grown profitable are determined by the temperature and rainfall requirements of the plant (Amoah, 1995). Cocoa thrives in regions with annual rainfall between 1,100 and 3,000 mm, with a dry season of not more than three months and minimum monthly rainfall of about 100 mm per month. Nonetheless, the optimal rainfall range is between 1,500 and 2,000 mm per annum (Amoah, 1995). In Ghana, the total

annual rainfall in the cocoa growing regions is less than 2000 mm (Anim-Kwapong and Frimpong, 2005). Cocoa trees appear to be very sensitive to water logging (Wicks, 2003; Amoah, 1995) so the distribution of rainfall is more important than the total level of rainfall (Amoah, 1995).

1.2.3.4 Soil

Cocoa can be cultivated in a wide range of soils, however, the soil should be able to retain moisture throughout the year and have good drainage because as already indicated cocoa trees are very sensitive to water logging (Wicks, 2003; Amoah, 1995). The suitability of a particular type of soil for cocoa production varies with climate since the moisture holding capacity of the soil becomes a limiting factor only when rainfall is not well distributed (Amoah, 1995). Generally, a soil that is high in nutrients, has a moderate water retention capacity, is permeable, with a pH range of 5.5-7.5 (Wicks, 2003) and has the ability to support a firm root-hold is desirable (Amoah, 1995).

1.2.4 Importance and world production of cocoa

Cocoa is the third most important internationally traded raw material, being ranked only third behind sugar and coffee. Globally, the cocoa industry is a US$73 billion industry (Ploetz, 2007). It provides income for an estimated 14 million people around the world and is a major cash crop for many tropical countries at present (Argout *et al.*, 2008).

The top-three cocoa-producing countries world-wide between 2009-2014 are Cote d'Ivoire, Ghana and Indonesia. These countries produced an average of 1.5 million tonnes, 542 thousand tonnes and 447 thousand tonnes of cocoa a year. Cote d'Ivoire commands 37% of global supply of cocoa, Ghana supplies 21% and Indonesia supplies 11% (Fairtrade, 2016).

Cocoa production is however plagued by a number of abiotic (nutrient deficiency, occasional drought) and biotic stresses (diseases and pests) that drastically affect yield (Wood and Lass, 2001; Gotsch, 1997).

1.2.5 Cocoa production in Ghana

Cocoa has gradually become the nation's major cash crop after its introduction in the 19th century. Currently, Ghana is the second largest producer of cocoa after the Ivory Coast (ECOWAS-SWAC/OCED, 2007).

The crop is grown in six (6) out of the ten (10) regions of Ghana, namely; Ashanti, Brong-Ahafo, Central, Eastern, Western and Volta regions (COCOBOD, 2016). Ghana has one of the lowest yields per hectare in the world (CSAE, 2009) despite it having a relatively large area under cultivation [1.8 million hectares in 2004, (ECOWAS-SWAC/OECD, 2007)].

Due to the importance of the crop to the country and the then British West African Sub-region, the West African Cocoa Research Institute (WACRI) was set up in Tafo in 1944 (initially as the Tafo Cocoa Station in 1938) to conduct research into all aspects of growth and productivity of cocoa (ECOWAS-SWAC/OECD, 2007). However, after independence in 1957, the Institute was taken up by the Government of Ghana and converted into the Cocoa Research Institute of Ghana (CRIG).

1.2.6 Drought and effects of drought stress on crops

Drought occurs when natural or managed water systems can no longer provide sufficient water to meet established human and environmental needs due to natural short-falls in precipitation and/or of stream flow (Robert *et al*., 2000). This phenomenon is a common place in rain-fed areas because of infrequent rains and poor irrigation (Wang *et al*., 2005).

Drought stress has a profound effect on many aspects of a plant's growth and development. The loss of water under drought stress leads to growth inhibition as well as induces changes in the metabolism and physiology of a plant (Vajrabhaya *et al.*, 2001). The vegetative as well as reproductive growth of plants are also affected by drought stress as a result of the decreased production of assimilates due to the reduction in stomatal conductance that impairs gaseous exchange. The effect of drought stress on the yield of crops depends on the point in the life cycle of the crop in which it occurs (Salter and Goode, 1967). The yield of a crop is not greatly affected if drought stress occurs during the vegetative phase (Boote, 1982) but when it occurs during the flowering stage, there is a dramatic reduction in yield (Ahmad *et al.*, 2003; Specht *et al.*, 1999).

1.2.6.1 Drought Stress and Cocoa

Generally cocoa is sensitive to drought (Anim-Kwapong and Frimpong, 2005; Wicks, 2003) and this may possibly be attributed to its fine root system (Moser *et al.*, 2010). Seedlings subjected to drought rapidly reach the permanent wilting point from which they cannot recover (Carr and Lockwood, 2011). Drought also has a number of other effects on the development of the cocoa crop; decreasing the following; (i) leaf area (Orchard and Saltos, 1988) (ii) net carbon assimilation (Hutcheon, 1977), (iii) flowering (Sale, 1970) and ultimately (iv) causes a reduction in yield (Moser *et al*., 2010).

Significant reduction in shoot growth, transpiration, the rate of photosynthesis and stomatal conductance occurs when the crop is exposed to drought stress (Antwi, 1994). It has been documented by Alvim (1959) that stomatal openings reduced appreciably when soil moisture of cocoa seedling decreased by 50-60%.

In cocoa, visual symptoms of drought stress include yellowing of basal leaves, wilting, premature leaf fall (abscission of progressively younger leaves), small leaves and slow trunk

growth (Carr and Lockwood, 2011). Some biochemical changes that occur in cocoa plants subjected to drought stress include the accumulation of the amino acid proline (Balasimha, 1988) and polyamines (Bae *et al.*, 2008).

1.2.7 *Mechanisms Used by Plants to Cope With Drought Stress*

It has been observed that there are four adaptive mechanisms that plants employ in response to dehydration stress (Fang and Xiong, 2015; Mitra, 2001). These are drought avoidance, drought-tolerance, drought-escape and drought-recovery.

Drought avoidance is the capability of plants to maintain 'normal' physiological processes under mild or moderate drought stress conditions by effecting changes in particular morphological structures or varying growth rates to avoid the negative effects caused by drought stress. It is characterized by the maintenance of high plant water potentials in the presence of a water shortage (Luo, 2010; Mitra, 2001). Plants generally adopt three strategies in order to avoid drought (or achieve drought avoidance); (i) reducing water loss via rapid stomatal closure, leaf rolling (Tardieu, 2013), and increasing wax accumulation on the leaf surface in many plant species such as alfalfa, tobacco, and rice (Islam *et* al., 2009; Cameron et al,. 2006; Zhang *et al*., 2005); (ii) enhancing the water uptake ability through a well-developed root system (especially increased rooting depth, rooting density or root/shoot ratio) and enhancing the water storage abilities in specific organs (such as fleshy water-storing tissues of cacti, the truck of candlenut, earthnuts or tubers of some plants, etc.) (Tardieu, 20013; Ogburn, 2010; Sawidis *et al*., 2005); (iii) accelerating or decelerating the conversion from vegetative growth to reproductive growth to avoid complete abortion at the severe drought stress stage (Luo, 2010; Mitra, 2001).

With drought tolerance or "true xerophytism" (Maximov, 1929), plants exposed to dehydration stress are able to continue their normal physiological/metabolic activities uninhibited even at high levels of dehydration stress (Scott, 1979). Drought tolerance refers to the ability of plants to sustain a certain level of physiological activities under severe drought stress conditions through the regulation of thousands of genes and series of metabolic pathways to reduce or repair the resulting stress damage (Luo, 2010; Mitra, 2001; Passioura, 1997). Plants commonly exert protoplasmic tolerance by increasing osmoregulatory molecules in the cells to maintain the cell turgor pressure, and adjusting the activities of cell defence enzymes to reduce the accumulation of hazardous substances. Crops that are drought tolerant produce higher yields than other genotypes under similar conditions of drought stress (Naveed, 2008).

Drought Escape refers to natural or artificial adjustment of the growth period, life cycle, or planting time of plants to prevent the growing season from encountering local seasonal or climatic drought (Mohamed *et* al,. 2002; Mitra, 2001).

Drought recovery allows the plants to recover and resume normal growth from the devastating effects of drought stress when it occurs during plant development (Luo, 2010; Kholodova *et al.*, 2007).

1.2.8 Morphological and physiological characters as indicators of drought stress

1.2.8.1 Morphological characteristics

Most plants that are drought-tolerant often have xeromorphic structures such as smaller and thicker leaves, more epidermal trichomes, smaller and denser stomata, a thicker cuticle epidermis, thicker epidermis (Fang & Xiong, 2015; Ashton & Berlyn, 1994), thicker palisade tissue (Fang & Xiong, 2015; Karaba *et al.*, 2007), a higher ratio of palisade to spongy parenchyma thickness and a more developed vascular tissue (Fang & Xiong, 2015; Abdulrahaman & Oladele, 2011; Sack & Holbrook, 2006). These plants also protect themselves

from drought by protecting themselves from sunlight either by rolling their leaves or by possessing mechanical tissues like sclerenchyma. In other drought tolerant species lipids accumulate in the epidermis to form wax that increases the reflectivity of sunlight to prevent them from losing water through excessive transpiration (Mohammadian and Watling, 2007)

Plate 1.3: Examples of drought tolerant plant species i) *Opuntia sp.* and ii) *Sisal sp.*

The stomata of plants play an important role in photosynthesis, transpiration and respiration. They function in the control transpiration and CO_2 exchange between the plant and its environment. It has been discovered that the characteristics/architecture of a plant's stomata may be useful as an indicator of its drought-tolerance potential (Shearman & Beard, 1973; Olyslaegers *et al*., 2002); e.g. small stomatal pore sizes has been linked to increased drought tolerance (Schaller & Paschold, 2009). Stomatal density and aperture has also been linked to drought tolerance in plants (Hetherington & Woodward, 2003).

1.2.8.2 Physiological characteristics

Leaf Relative Water Content (RWC)

Generally, drought-tolerant species maintain high water status irrespective of soil moisture level (Naveed, 2008). The Relative Water Content (RWC) of the leaves of a plant is a measure that gives an indication of the internal water status the plant. Relative Water Content was first

described by Weatherley (1950 and 1951), and has been widely accepted as a reproducible and meaningful measure of plant water status (Barrs, 1968; Yamasaki, 1999). RWC has been used by many researchers to successfully measure water stress in leaf tissues (Obeng-Bio, 2010; Naveed, 2008; Balasimha, 1988). The RWC expresses the water content of a leaf (in percent) at a given time as related to the water content in the fully turgid leaf. A dichotomy between drought-tolerant and drought-sensitive plants can easily be created by the use of leaf RWC (Naveed, 2008) by demonstrating that drought-tolerant plants generally have higher Leaf RWCs compared to drought-sensitive plants under similar water stress conditions.

1.2.9 Biochemical (metabolic) changes as indicators of drought stress

Plants undergo certain changes in their metabolic reactions when exposed to drought stress. Some of these changes enable the plants to withstand some degree of tissue degradation by increasing the stability of cell membranes, increasing cell wall elasticity and osmotic adjustment (Fang and Xiong, 2015; Gebre *et al.*, 1994; Turner, 1986; Morgan 1984). Increased cell wall elasticity and osmotic adjustment (OA) allow for greater cell turgor at low tissue water potential, which in turn may help plants to maintain gas exchange and growth during drought (Fang and Xiong, 2015; Seiler and Johnson, 1988; Morgan 1984). Soluble carbohydrates (fructose, trehalose, fructans), inorganic ions (K^+, Ca^{2+}) and amino acids (proline and citrulline) are largely accumulated in most species to contribute to the Osmotic Adjustment (OA), though other compounds may also be accumulated (Gebre *et al.*, 1994).

Accumulation of these compounds, (osmolytes or compatible solutes), results in a more negative osmotic potential of the cytosol, which in turn attracts water passively (osmotically) into the cell thereby maintaining turgor (Babu *et al.*, 1999). Osmotic adjustment has been considered as an effective component of drought tolerance in many crop plants (Fang and Xiong, 2015; Morgan, 1984). Aside the functions stated, compatible solutes also protect specific cellular structures and their functions such as enzymes, liposomes, nucleic acids and

membranes irrespective of turgor by acting as active oxygen scavengers or replacing water molecules around them (Akashi *et al.*, 2001; Hoekstra *et al.*, 2001; Hincha *et al.*, 2000; Shen *et al.*, 1997).

Abscisic acid (ABA), a phytohormone, has also been reported to accumulate in plants under water stress conditions (Fang and Xiong, 2015; Sauter *et al*., 2001). The accumulation of ABA is thought to trigger responses such as reduction of stomatal aperture that enable drought-stressed plants to cope with the stress (Fang and Xiong, 2015; Yokota *et al.*, 2006; Mustilli *et al.*, 2002).

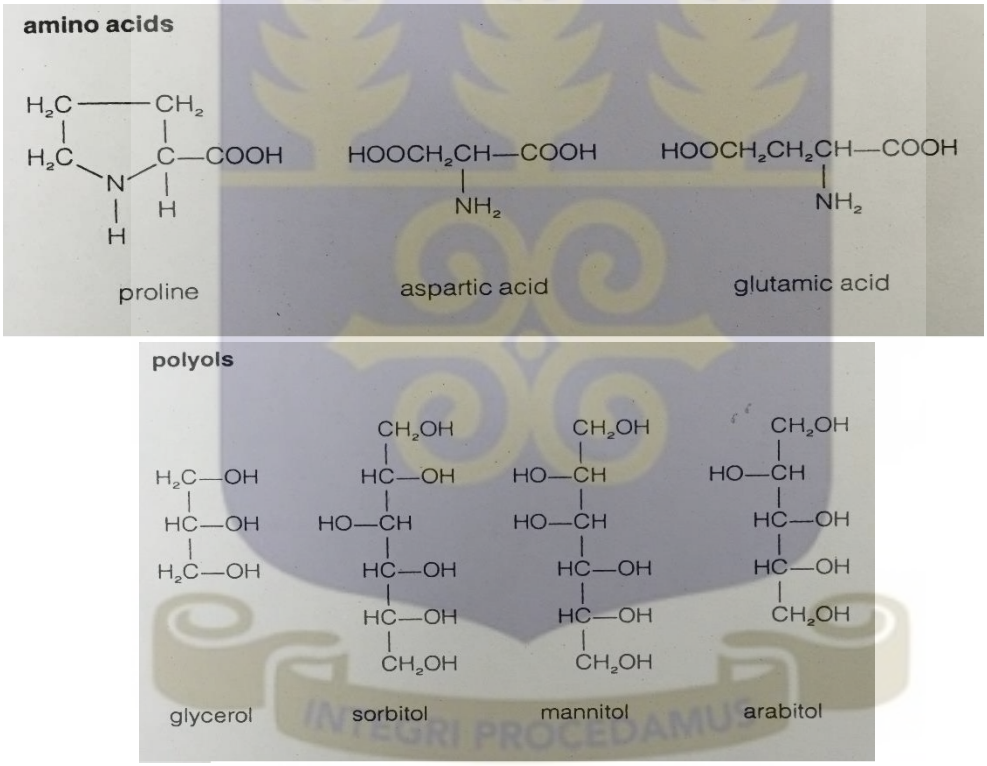

Plate 3.4: Molecular structures of some compatible solutes found in stressed plants i) amino acids (proline, aspartic acid and glutamic acid ii) polyols (glycerol, sorbitol, mannitol and arabitol

1.2.9.1 Free proline accumulation as an indicator of drought stress

Proline is a compatible solute which is accumulated by plants under water stress in order to contribute to the negativity of the osmotic potential of the cytosol and thereby maintain turgor. It is the most readily accumulated substance in plants subjected to water stress (Hayat *et al.*,

2012; *Karamanos*, 1995). Drought-induced accumulation of proline has been found in many plants including canola (Din *et al.*, 2011), coconut (Gomes *et al.*, 2010), maize (Mohammadkhani and Heidari, 2008; Ibarra-Caballero *et al.*, 1988), wheat (Dib *et al.*, 1994; Karamanos *et al.*, 1983), soybean and sorghum (Waldren and Teare, 1974), barley (Riazi *et al.*, 1985) and cocoa (Bae *et al.*, 2009; Balasimha, 1988). However, the specific function of proline in each of the species is unclear. For example, Gomes *et al.* (2010) found out that proline accumulation did not correspond to osmotic adjustment and that it may be functioning to protect cellular membranes. On the other hand, Blum and Ebercon (1976) have proposed that proline serves as a source of carbon for energy release, and nitrogen which enhance recovery of tissues following release from stress. Waldren and Teare (1974) reported that proline accumulation was not a sensitive indicator of drought stress in sorghum and soybean since the plants did not accumulate proline until they were severely stressed. They proposed the use of proline as an indicator of drought tolerance or sensitivity due to the observation that soybean which is less drought-tolerant than sorghum accumulated proline under less severe stress compared to sorghum.

In maize seedlings, Ibarra-Caballero *et al.* (1988) found that proline accumulated only in green leaves under drought stress. These same authors have also reported that exogenous application of ABA to green leaves did not cause accumulation of proline. Subsequently, they are of the view that accumulation of proline under drought stress may probably not be an indication of drought stress tolerance, but rather a symptom of drought stress. However Moussa and Abdel-Aziz (2008) found that in 21 d old maize seedlings under polyethylene glycol (PEG) induced water stress a known drought-tolerant variety accumulated more proline than its drought sensitive counterpart did. Similarly, in wheat, Karamanos *et al.* (1983) observed a drought-tolerant cultivar to accumulate more proline than a less tolerant one and subsequently proposed a positive relationship between proline accumulation and drought tolerance.

Table 1.2: Generalized sensitivity to water stress of plant processes or parameters [a]

Process or Parameter Affected	Sensitivity to Stress: Very Sensitive / Relatively Insensitive — Tissue Ψ Required to Affect Process [b]: 0 MPA	-1.0 MPA	-2.0 MPA	Remarks
Cell growth	_____			Fast-growing tissue
Wall synthesis	_____			Fast-growing tissue
Protein synthesis	_____			Etiolated leaves
Protochlorophyll formation	_____			
Nitrate reductactase level	_____			
ABA accumulation	_____			
Cytokinin level	_____			
Stomatal opening	_ _ _ _ _____ _ _ _ _ _			Depends on species
CO_2 assimilation	_ _ _ _ _____ _ _ _ _ _			Depends on species
Respiration	_ _ _ _____			
Proline accumulation	_ _ _ _____			
Sugar accumulation	_____			

[a] Length of the horizontal lines represents the range of stress levels within which a process first becomes affected. Dashed lines signify deductions based on more tenuous data.
[b] With Ψ of well-watered plants under mild evaporative demand as the reference point
Source: Hsiao, 1973

1.2.10 Efforts Made by CRIG to breed for Drought-Tolerance in Cocoa

Several researchers have studied and reported the responses of plants to drought stress using a variety of methods; either under drought conditions only or under partial drought conditions where some form of irrigation is used. Three drought-tolerant cocoa genotypes were selected by Frimpong *et al.* (1999) under shade conditions under "drought alone" evaluation. Padi *et al.* (2013) also identified some drought-tolerant genotypes of cocoa not grown under shade.

Other experiments were conducted to identify the physiological basis of observed drought tolerance in some cocoa genotypes. The specific leaf weight (SLW – Frimpong and Boafo,

1994) and total non-structural carbohydrate (TNC) accumulation in roots of seedlings under non-stress and drought stress conditions were measured (Frimpong *et al.*, 2001; Frimpong *et al.*, 1997 (a) and (b)). Frimpong and Boafo (1994) found that the cocoa genotypes with high drought tolerance potential had higher SLW and thus tended to have thicker leaves or thicker epidermal cells; although these same authors admitted that the difficulty in selecting leaves of similar morphological characteristics even from the same canopy makes the use of SLW very limiting in screening for drought resistance.

Over years, very few of the material different germplasm have been evaluated for drought tolerance (Frimpong et al. 1999; Padi *et al.*, 2013). Besides, the cocoa clones that have been evaluated so far are mostly the early and Posnette introductions and do not include the recent introductions. The number of identified drought-tolerant cocoa genotypes available in Ghana is not large enough and with recent changes in the prevailing climatic factors in Ghana, efforts are urgently needed to increase the availability of drought-tolerant cocoa genotypes for cultivation in plantations, deforested and drier areas. It is required that a large number of clones from the various germplasm collections have to be evaluated for their physiological and biochemical responses to drought and hence their drought tolerance potential. To be able to achieve this, relatively simple, inexpensive, fast and accurate screening methods have to be developed to screen germplasm for their drought tolerance potential.

CHAPTER TWO

MATERIALS AND METHODS

The study was carried out in two phases; the first phase (Phase I - Experiment I) involved a rapid screening of the nine (9) cocoa genotypes obtained from CRIG for their drought tolerance potential using Leaf Relative Water Content (RWC), and Soil Moisture Content. Following the completion of the first phase (Phase I), the second phase (Phase II - Experiment II) proceeded; and in this experiment, the nine (9) selected cocoa genotypes obtained from CRIG were studied in more detail using the following criteria: Leaf RWC, Soil Moisture Content, accumulated free proline content of uniformly-aged leaves, and also the morphological characteristics of uniformly-aged leaves.

2.1 Phase I (Experiment I): Rapid screening of nine (9) genotypes of cocoa obtained from CRIG for their drought tolerance potential

Experiment 1 was carried out between the months of January and February 2016.

2.1.1 Location of study

The study was undertaken under a shed constructed for that purpose at the premises of the Department of Botany, University of Ghana, Legon. The shed was designed to keep out rain and simulate shading conditions required for establishment of cocoa seedlings. The shed was made of galvanized steel frames with bamboo trusses overlaid with transparent plastic sheeting and black shading nets to reduce the light intensity and provide the shade required for growth of cocoa seedlings.

i)  ii) 

Plate 2.1: i) Arrangement of seedlings in screenhouse during Phase I- Experiment I (ii) Part of the roof of the screenhouse showing the plastic sheeting covered with black shading nets used in the study *Source: Osei- Bonsu, (2011)*

2.1.1.3. Source of Plant Materials

The plant materials used in this study included six (6) genotypes of cocoa each consisting of nine (9) plants/seedlings (Table 2.1) on the basis of viz T60 x Pound10, PA7 x 6035, T85 x PA7, T63/971 x Sca9, PA150 x 6020, AMAZ X 9006, T79 x 9006, PA150 x 6020 and PA7 x MAN (Table 2.1) developed at the Cocoa Research Institute, Ghana (CRIG) that were obtained from the Plant House of the Institute at Tafo and transported to the study site at the Department of Botany, University of Ghana, Legon. The seedlings, each in a 7 x 10 cm black plastic planting bag, were acclimatized under the shade of a tree.

There were variations in the age and size (height and total leaf surface area) of the seedlings, which could be due to genotypic effects. Within each genotype, seedlings were of approximately uniform size. The genotypes were randomly assigned single letter codes (a – i) for easier identification (Table 2.1).

Table 2.2: Genotypes of cocoa seedlings used and their ages (in weeks)

Genotype`	Code	Age (weeks)[1]
T60 x Pound10	A	40-42
PA7 x 6035	B	40-42
T85 x PA7	C	40-42
T63/971 x Sca9	D	40-42
PA150 x 6020	E	40-42
AMAZ X 9006	F	40-42
T79 x 9006	G	40-42
PA7 x MAN	H	40-42
PA150 x 9006	I	40-42

Source: CRIG nursery *[1]Estimated age as of 15/09/2015.*

2.1.2 Preparation of soil and repotting of seedlings

Prior to initiation of the treatments, the seedlings were transferred into bigger plastic buckets (height = 17.5 cm; upper diameter = 21 cm, base diameter = 20 cm; made by Decorplast, Accra, Ghana; Plate 2.2i) in order to increase the water holding capacity of the root environment and provide greater volume of rooting medium for increased provision of nutrients for root extension and normal seedling growth.

Before seedlings were transferred into the plastic buckets, five (5) holes were carefully punched at the bottom of each bucket.

2.1.3.1 Methods

The study was carried out in two (2) Phases (Experiment I and II). The first phase i.e. Experiment I involved the rapid screening of the nine (9) cocoa varieties for their drought tolerance potential using Leaf RWC and Soil Moisture Content (SMC) as the parameters.

2.1.3 Experimental Design and set-up

The experiment was a Randomized Complete Block Design (RCBD) with two (2) treatments (Stressed – S and Control – C), nine (9) blocks comprising of the ten (10) genotypes with three (3) replicates each making twenty-seven (27) seedlings for each of the two (2) treatments; and a total of fifty-four (54) seedlings for the whole experimental set up.

2.1.3.1 Experimental Set-up

Following repotting, the seedlings were randomly assigned to rows on all three benches with spacing of 18 cm across and 21 cm along the length of the bench. The soil surface in each bucket was covered with four scoops of finely chopped Styrofoam (using a 180 ml cup) to minimize the rate of evaporation of water from the soil surface and ensure that all the water lost was due to uptake by each seedling. The Styrofoam was held in place using black polyethene netting tied to the opening of the bucket with a nylon string. Styrofoam was used because it is an inert substance and would not add any nutrients to the soil. To check the effectiveness of the Styrofoam in reducing evaporation, six (6) other buckets of similar size were filled with soil to the same level (i.e. 19 cm) as that in buckets with seedlings. Three (3) of these pots were covered with the same amount of Styrofoam and the remaining three (3) left without any styrofoam cover. No seedling was planted in any of these six (6) pots and these were spread randomly in the intervening spaces between adjacent seedlings on the central bench.

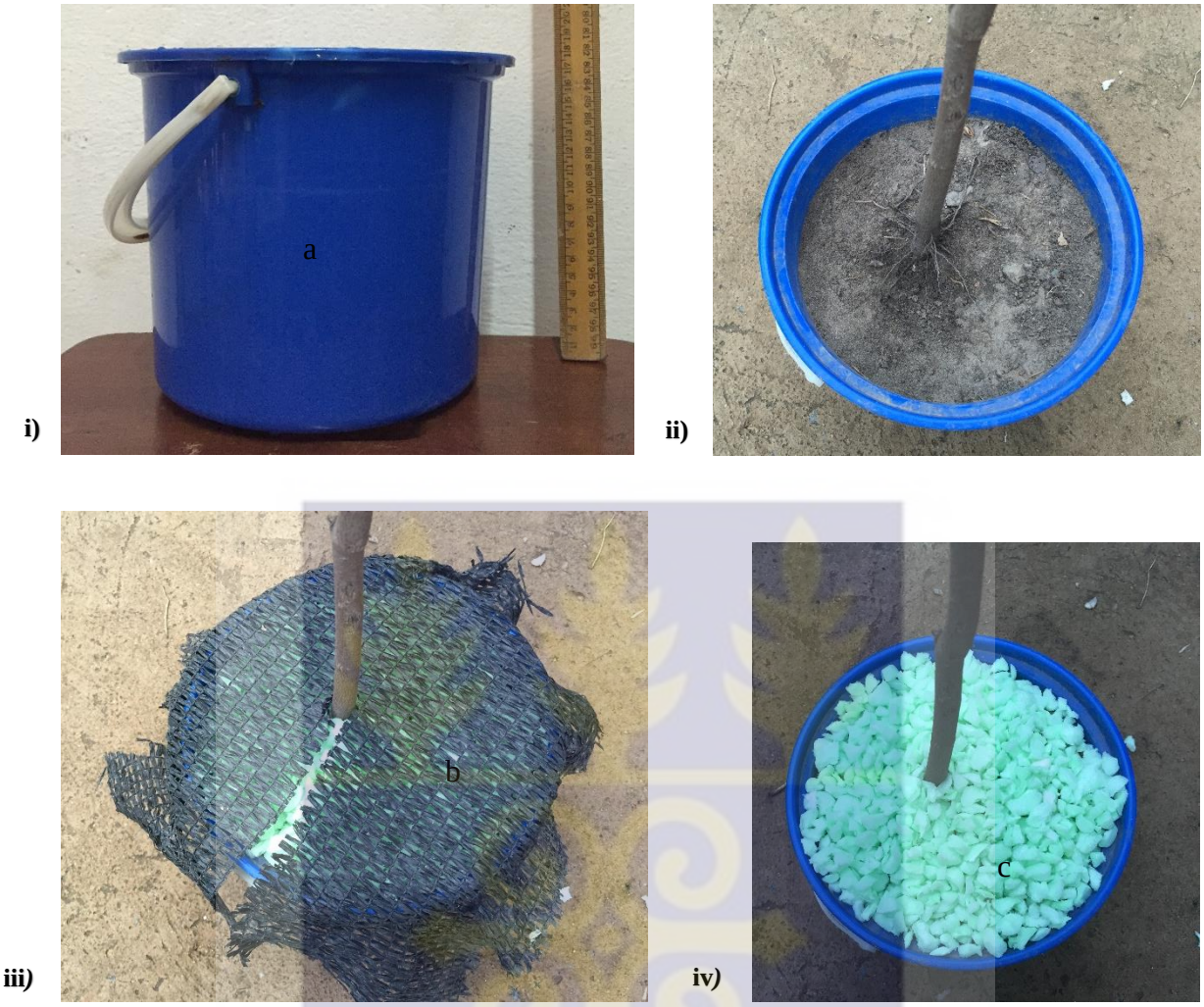

Plate 2.2: i) Plastic bucket used for repotting ii) soil level in bucket iii) soil-filled bucket with plant and Styrofoam iv) Styrofoam covered soil surface held in place with black plastic netting

2.1.4 Stress treatments

At the start of the experiment, all seedlings were slowly saturated with water until water was seen dripping from the bottom of the bucket (assumed indicative of Full Saturation – FS) and allowed to drain overnight to field capacity before initial Leaf RWC and Soil Moisture Content measurements were made. There were two treatments namely: *Stressed (S)* – which comprised a set of twenty-seven (27) seedlings (3 seedlings per genotype) from which water was withheld 1 Day After Full Saturation (i.e. 1 DAFS) until the First Appearance of Drought Symptoms (FADS), and *Control (C)* – which comprised a set of thirty seedlings (3 seedlings per genotype)

which received water every other day until the end of the experiment (i.e. when all seedlings in Stress group had shown first symptoms of drought).

2.1.4.1 Visual Symptoms of Drought Stress

For this study, the first observable symptoms of drought stress in the cocoa seedlings included: rapid yellowing of leaves starting from the leaf margin and/ or lamina, necrosis of leaf lamina, death of tender leaves, wilting, paling of leaves, drooping (especially of tender leaves)

2.1.5.2 Irrigation Schedule

The seedlings used as controls were watered every other day to ensure a constant supply of water. The idea was to maintain a moisture level greater than or equal to field capacity (FC).

2.1.5 Data collection for Experiment I

The number of days it took for each seedling to exhibit signs of First Appearance of Drought Symptoms – FADS (i.e. when seedlings first showed visual symptoms of drought) was recorded. The following parameters were also measured during the experiment: mid-day measurements of Leaf Relative Water Content (RWC), Soil Moisture Content, leaf area, plant height (H), stem diameter of seedlings at 10 cm from the soil surface.

2.1.6 Measurement of Leaf Relative Water Content (RWC)

2.1.7.1. Standardization of procedure for determination of Leaf RWC from Barrs and Weatherley (1962)

To determine the maximum number of hours for floating of leaf discs before measuring their turgid weight, a preliminary experiment was set up in which six (6) leaves were detached from different cocoa seedlings. Subsequently, three (3) discs were bored from each excised leaf with a 1 cm inner diameter cork-borer. Each set of three (3) one-cm inner diameter leaf discs was weighed together in order to determine their fresh weight using a fine balance (AL 104; Mettler

Toledo, Columbus, OH, USA). Each of these sets of three (3) one-cm inner diameter leaf discs was then floated separately on 10 ml distilled water in a covered Petri dish under diffuse light at room temperature and the turgid weight of each set of 3 floated discs was determined at 30 min intervals for 5 h. Prior to the determination of the turgid weight of each set of three (3) one-cm inner diameter leaf discs after each 30 min floatation on distilled water, each set of 3 leaf discs was surface blotted between two (2) layers of tissue paper (3 folds of a 3-ply tissue paper on each side) pressed with a 1.15 kg flat bottom weight precisely for 1 min.

Plate 2.3: i) The cork borer (a), pair of forceps (b), flat tile and glass vial (d) used in sampling of leaf discs for determination of Leaf RWC, ii) Mature green leaves of a hardened flush (red arrow) and a leaf of the most recent flush (white arrow), iii) Floatation of leaf discs and iv) The 2 layers of tissue paper and the constant flat bottom weight (e) used in blotting leaf discs *source: Osei-Bonsu (2011)*

2.1.7.2 Determination of Leaf RWC of experimental seedlings

To assess the water status of the seedlings, the mid-day Leaf Relative Water Content (RWC) of selected leaves of each seedling was measured. The Relative Turgidity Method of Barrs and Weatherley (1962) was standardized (afore-described in 2.1.7.1.1) and followed or used for the determination of the Leaf RWC. The Leaf RWC was measured on four sampling occasions

namely: at i) 1 DAFS (i.e. RWC at field capacity viz initial RWC) ii) at First Appearance of Drought Symptoms – FADS (i.e. when the cocoa seedlings subjected to water-stress first showed drought symptoms). Samples were taken between 11:30 am and 2:00 pm on each sampling occasion or day. Three mature green leaves of each seedling were selected and thoroughly cleaned with cheese cloth (These leaves were usually part of the most recently hardened flush or were found below the most recent flush). Avoiding the mid-rib and major veins, discs were quickly bored with a sharp 1 cm inner diameter cork-borer from each selected leaf, placed in a clean glass vial, and transferred immediately to the laboratory where the fresh weight (FW) of each set of three (3) leaf discs from each replicate of each genotype of cocoa was determined together using a fine balance (AL 104; Mettler Toledo, Columbus, OH, USA). Subsequently, each set of 3 leaf discs of each selected leaf was floated on 10 ml distilled water in a covered 2.5 cm diameter Petri dish under diffuse light at room temperature (25 °C) for four hours (as predetermined in the standardization procedure for leaf RWC as described in paragrapgh 2.1.7.1.1; after which they were removed with a clean pair of forceps, surface blotted between 2 layers of tissue paper and weighed in order to obtain their fully turgid weight (TW). The leaf discs were then dried in an oven at 60 °C for 24 h (in order to remove only the moisture content), and cooled in a desiccator before determining their dry weight (DW). The Leaf RWC for each set of three (3) leaf discs for each genotype was calculated as follows:

$$RWC = \frac{Fresh\ Weight\ (FW) - Dry\ Weight\ (DW)}{Turgid\ Weight\ (TW) - Dry\ Weight\ (DW)} \text{ x } 100$$

Where,

FW: Fresh weight of the three (3) 1cm inner diameter leaf discs

DW: Dry weight of the three (3) 1cm diameter leaf discs after oven drying at 60^0C for 24 hours (hr)

TW: Turgid weight of the three (3) 1cm inner diameter leaf discs of each seedling/replicate after floating in 10 ml distilled water and surface blotting for 1 min between tissue paper under a constant weight.

2.1.7 Measurement of Soil Moisture Content (SMC)

Soil Moisture Content was determined by the Gravimetric Method and expressed as a percentage of the initial weight. Soil samples were taken on from each potting bucket (at 5 cm) on each RWC sampling occasion along the sides of the bucket (in order to avoid breaking any roots) using a 1cm inner diameter stainless steel tube, quickly emptied into a glass Petri dish, covered and transferred to the laboratory to determine the initial weight (IW) with a fine balance (AL 104; Mettler Toledo, Columbus, OH, USA). The soil was then dried in a hot-air oven at 60 °C for 24 h (to remove only the moisture), cooled in a desiccator before measuring the dry weight (DW). The Soil Moisture Content was then calculated and expressed as a percentage of the initial weight of soil using the following equation:

$$SMC = \frac{Initial\ Weight\ (IW) - Dry\ Weight\ (DW)}{Initial\ Weight\ (IW)} \text{ x } 100$$

2.2 Phase II (Experiment II)

Experiment II was carried out between the months of April and May 2016.

2.2.1 Location of Study

The study was undertaken under a shed constructed at the premises of the Department of Botany, University of Ghana, Legon. Location of the study had to be changed because the shed that was used collapsed after a heavy downpour. The shed was designed to keep out rain and simulate shading conditions required for establishment of cocoa seedlings. It was constructed with galvanized steel frames, and transparent plastic roofing materials and the insides lined

with black netting to reduce light intensity as well as provide the shade required for the normal growth of cocoa seedlings.

Plate 2.4: Shed under which Phase II was carried out

2.2.2 Experimental design and set-up

The experiment was a Randomized Complete Block Design (RCBD) with two (2) treatments (Stressed – S and Control – C), nine (9) blocks comprising the ten (10) genotypes with three (3) replicates each making twenty-seven (27) seedlings for each of the two (2) Treatments and a total of fifty-four (54) seedlings for the entire Experimental set up.

2.2.2.1 Experimental Set-up

The experimental set-up in Phase II was similar to that of Phase 1; with the only difference this time was the location of the experiment and that the seedlings were placed on a concrete slab on the floor of the shed (Plate 2.5).

Plate 2.5: Arrangement of potted seedlings under shed used in Phase II of the study

2.2.3 Irrigation Schedule

The irrigation schedule for Phase II was the same as in Phase 1

2.2.4 Data Collection for Phase II

The number of days it took for each seedling to exhibit signs of First Appearance of Drought Symptoms – FADS (i.e. when seedlings first showed visual symptoms of drought) was recorded. The following parameters were also measured during the experiment: mid-day Leaf Relative Water Content (RWC), Soil Moisture Content, leaf area, plant height (H), stem diameter of seedlings at 10 cm from the soil surface.

2.2.4.1 Measurement of Leaf Relative Water Content

Procedure for measuring the Leaf Relative Water Content for the seedlings in Phase II was the same as that in Phase I.

2.2.4.2 Measurement of Soil Moisture Content

The method described earlier in Phase 1 was followed. Soil samples were taken on each RWC sampling occasion.

2.2.4.3 Leaf Anatomical Study

Three (3) leaves of relatively uniform size, chronological age, physiological and metabolic activity from each of the nine (9) varieties were randomly selected, placed in an airtight container and quickly taken to the Physiology Laboratory of the Botany Department, University of Ghana.

Transverse sections (TS) of each leaf was made and observed under the low power (LP) and high power (HP) magnifications of a Leica binocular light microscope. Due to the unavailability of a working microtome, transverse sections of the leaves were obtained manually using carrots to hold the leaves firmly in place and sharp razor blades for sectioning and mounted temporarily in water for microscopic examination. Prior to making any observations and measurements of the various features of interest of the leaves of the nine (9) selected cocoa genotypes, the microscope was calibrated.

For each section of each of selected leaf of each genotype the following features were studied or examined for;

a) *Presence or absence and number of epidermal hairs or trichomes if any*
b) *Presence or absence of hypodermis*

Followed by measurements/estimation of the under listed:

i. *Thickness of various layers in/of the leaf:*

- *Upper epidermis*

- *Hypodermis, if any*

- *Number of layers of cells constituting the hypodermis if any*

- *Entire mesophyll*

- *Lower epidermis*

Photomicrographs were taken (using a Leica ICC50 camera attached to a Leica microscope) of each temporary mounted section for each selected genotype studied and measurements of the selected features (upper epidermis, lower epidermis and the mesophyll layer) were taken at a magnification of x400 calibrated automatically using Leica LAS Z and the values obtained were recorded.

2.2.4.4 Cleaning of Glassware for Proline Study

All glassware used in this study for collection of samples, extraction and determination of proline content were thoroughly cleaned by first washing them with a detergent and rinsing three times with tap water followed by 24 h immersion in a potassium dichromate (K_2CrO_4) and concentrated sulphuric acid (H_2SO_4) solution (highly corrosive); after which they were rinsed three times with distilled water and dried in an oven. This was necessary because of the relatively low concentrations of the amino acid and the high potential of contamination which would produce artificial and unusually high values.

2.2.4.5 Determination of free proline content of leaves

The free proline content of the selected leaf of seedlings of each of the nine (9) selected genotypes was determined using leaf discs from the same leaves from which discs were taken for the determination of Leaf RWC. Samples of leaf discs were taken at 1DAFS, and FADS for each seedling. The procedures of Troll and Lindsley (1955) as modified by Mukherjee (1974) and that of Bates et al. (1973) were modified and adapted for this study (Fig. 2.1).

On each sampling occasion, three (3) leaf discs were obtained from the lamina of each selected leaf (of approximately uniform size, chronological and physiological age) using a sharp 1cm inner diameter cork-borer and a clean flat tile, avoiding the mid-rib and major veins. Each set of three (3) leaf discs bored from each selected leaf was placed in a clean 10 ml glass vial, into which 5 ml of 80% methanol was poured and subsequently boiled in a water bath for 5 minutes before storage in a refrigerator at 5 °C for further extraction and analysis.

2.2.4.4.1 Proline extraction

Extraction of the free leaf proline content of each set of three (3) leaf discs boiled in 80% methanol was carried out following the flow diagram (Fig 2.1) for extraction of free proline from the leaf discs that had been boiled in 80% methanol in a water bath and stored at low temperature (i.e. 5^{o}C) in a refrigerator.

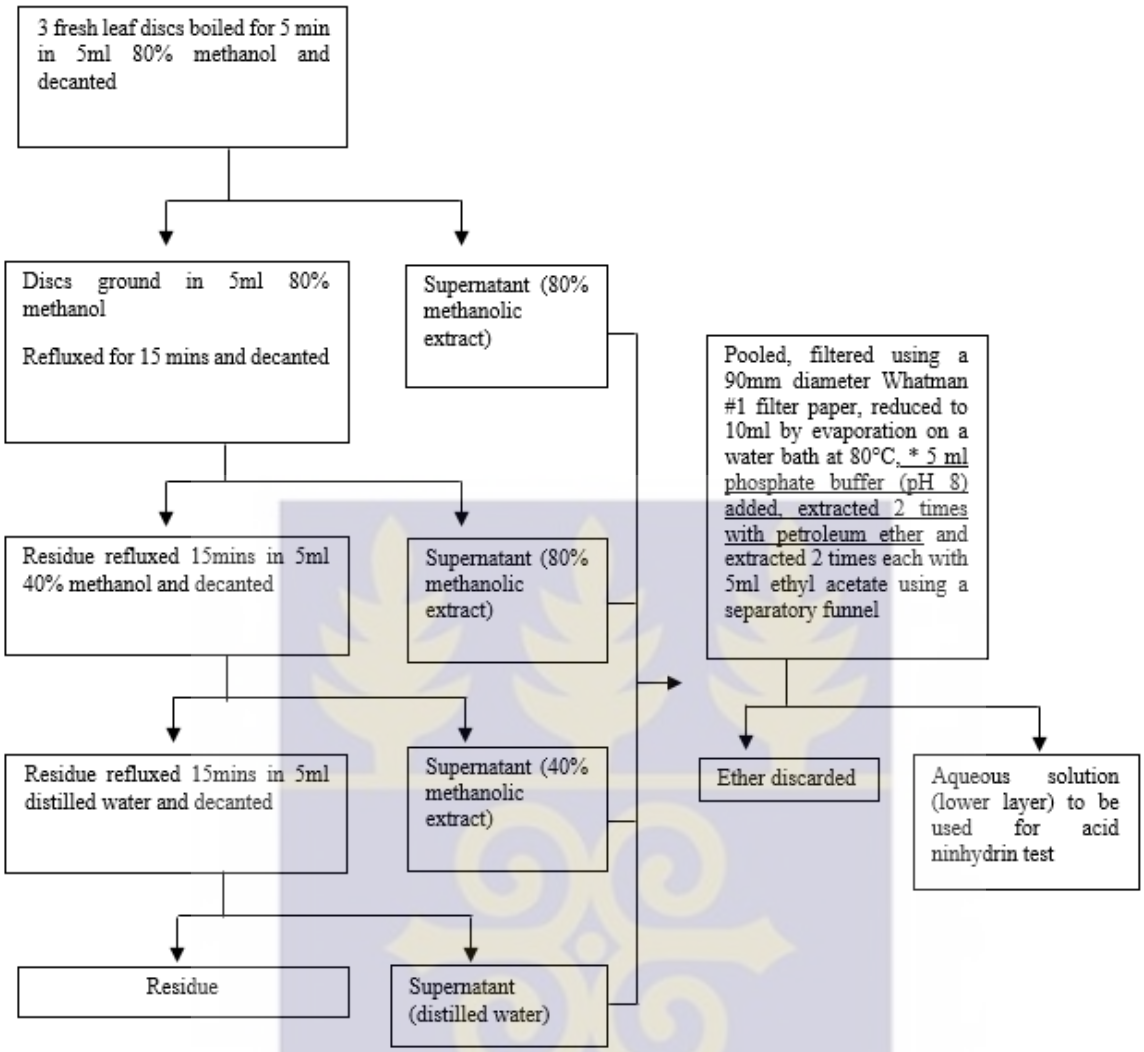

Fig. 2.1: Proline Extraction Flowsheet *(source: Troll and Lindsey (1955) as modified as modified by Mukherjee (1974) and that of Bates et al. (1973)*

2.2.4.4.2 Modifications to the extraction procedure

The steps in the Flow Sheet of the original procedure denoted by an asterisk and underlined in Fig 2.1 (i.e. the addition of the 5 ml phosphate buffer of pH 8 and the extraction with petroleum ether) were eliminated in this study because the leaf tissue of cocoa used for the study did not contain neutral to alkaline lipids.

2.2.4.4.3 Determination of free proline content of leaves

An acid-ninhydrin reagent was prepared by warming 1.25 g of ninhydrin with 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid over a water-bath with agitation. Two (2) ml of the aqueous solution obtained from the extraction procedure was reacted with 2 ml of acid-ninhydrin reagent and 2 ml glacial acetic acid in a capped test tube in a water-bath at 100 °C for 1 h. The reaction was terminated in an ice bath for 5 min. The colour thus developed was extracted by shaking vigorously with 4 ml benzene (in lieu of toluene) in a separatory funnel for 15 sec. The colour-containing benzene phase (upper layer) was then allowed to warm to room temperature (25°C) in a test tube, transferred into a glass cuvette and its absorbance was determined at 540 nm with a spectrophotometer (Jenway 6320D Spectrophotometer) using benzene as the blank. The actual proline content was expressed as µg proline per gram leaf dry weight using the following formula:

$$Proline\ (\mu g/g) = \frac{Proline\ \left(\frac{\mu g}{ml}\right) x\ ml\ benzene}{weight\ of\ discs\ (g)}$$

2.2.4.6 Development of Standard Curve of Authentic Analytical Grade D-Proline

In the development of the proline standard curve a stock solution was prepared by dissolving 0.03g of proline in of distilled water. Serial dilutions were made with the following concentrations: 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml. A standard curve of authentic analytical grade *D*-proline (Sigma-Aldrich Inc., USA) was prepared using the aforementioned procedure described previously and the concentration of proline in the leaf samples was estimated from the standard-curve.

2.2.4.7 Correcting for losses due to extraction procedure

To correct for losses in the concentration of proline due to the extraction procedure, 1 ml of a known concentration of authentic analytical grade *D*-proline (Sigma-Aldrich Inc., USA) was taken through the extraction procedure in Fig 2.1 and paragraph 2.2.4.4.3. The concentration of the resultant aqueous solution (about 10 ml) was determined by taking it through the steps outlined in paragraph 2.2.7.4.2 – concentration Y. Subsequently another 1 ml of the known concentration of authentic analytical grade *D*-proline was diluted to 10 ml, taken only through the steps in paragraph 2.2.7.4.2 and its concentration determined as concentration Z. Any differences observed between concentrations Z and Y would be due to losses in the extraction procedure.

2.2.6 Measurement of abiotic parameters

The amount of moisture/water that the seedlings received was the only abiotic factor that was regulated. A whirling psychrometer was used to measure the relative humidity of the study site; a thermometer was used to measure the ambient air temperature of the study sites on a tri-daily basis (at 0800GMT, 1200 GMT and 1600GMT) throughout the study period.

2.3 Data Analysis

All data obtained in this study were analysed using the statistical software "Statgraphics Centurion XVI" version 16.1.11.

The average values of water-stressed seedlings were compared using analysis of variance (ANOVA) and LSD was used to determine differences in block (genotype) average value (means) at 5% level of probability.

CHAPTER THREE

RESULTS

3.0 Phase I (Experiment I): Rapid screening of the nine selected (9) cocoa genotypes for their drought tolerance potential at seedling stage

3.1 Physical properties of soil used for the study

The soil used for the study was identified to be sandy loam with a pH of 7.3, indicating that it was neutral to alkaline. It had a moisture content of 13.3% and 13.8% at field capacity at depths of 5 cm and 10 cm respectively.

3.2 Climatic conditions at the study site during the period of the experiments

Results of the ambient abiotic climatic conditions during the study period are presented in Appendices 1 and 2 and summarised in Tables 3.1 and 3.2 respectively.

During Experiment I, the mean weekly minimum and maximum temperatures ranged between 18.57-22.14°C and 32.86-36.00°C respectively; whereas the mean minimum relative humidity (RH) ranged between 26.24-42.59% and 48.50-84.29% respectively (Table 3.1).

In Phase II (Experiment II), carried out in the months of March and May, 2016, the mean minimum and maximum weekly ambient air temperatures were 25-27°C and 32.86-33.43°C respectively,; whereas the mean minimum relative humidity (% RH) ranged between 60.90-64.3% and 69.6-84.03% respectively (Table 3.2).

Table 3.1: Mean weekly ambient air temperatures and relative humidity at the study site of Experiment I

Parameter / Week	Ambient Air Temperature (°C		Relative Humidity (%)	
	Minimum	Maximum	Minimum	Maximum
1	18.57	32.86	26.24	48.50
2	22.14	32.86	42.59	84.29
3	19.71	36.00	35.93	74.89
4	21.25	33.00	38.85	69.53

Table 3.2: Mean weekly ambient air temperatures and relative humidity at the study site of Experiment II

Parameter / Week	Ambient Air Temperature (°C		Relative Humidity (%)	
	Minimum	Maximum	Minimum	Maximum
1	25.00	32.86	63.21	69.60
2	27.00	33.43	60.90	82.46
3	26.00	33.29	64.30	84.03

3.1.3. First Appearance of Drought Symptoms (FADS)

The seedlings used for the study exhibited varied symptoms of drought stress even within the same genotype. The visual symptoms indicative of FADS in this study included: rapid yellowing of leaves starting from the leaf margin and/ or lamina, necrosis of leaf lamina, drooping and death of young tender leaves, wilting and paling starting from the leaf margin and lamina similar to the observations made by Osei-Bonsu (2011: Plate 3.1)

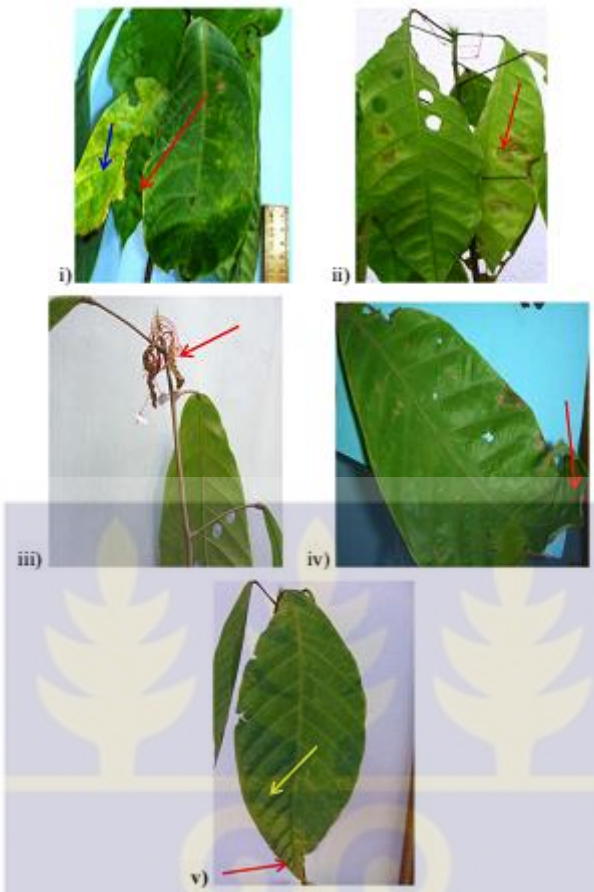

Plate 3.1: Various visual symptoms of drought stress observed in seedlings of the different genotypes of cocoa, indicative of First Appearance of Drought Symptoms (FADS). i) Yellowing of leaves starting from the leaf margin (red arrow) and/ or lamina (blue arrow) ii) Drooping and necrosis (on leaf lamina – arrowed) of immature green leaves, iii) Drooping and death of young tender leaves (arrowed), iv) Wilting of old dark green leaves (arrowed) and v) Paling starting from the leaf margin (red arrow) and lamina (yellow arrow) (source: Osei-Bonsu, 2011)

3.1.4. Number of days for First Appearance of Drought Symptoms (FADS) as a measure of drought tolerance potential

The mean number of days for First Appearance of Drought Symptoms (FADS) for all the genotypes is presented in Fig 3.1. Genotypes *'a'*, and *d*' took the longest number of days to show FADS with 16.3, and 14.7 DAFS respectively. The genotypes that showed FADS earliest were *'f'*, *'i'* and *'h'*, with 11.3, 12.7.3 and 13.3 DAFS respectively (Fig 3.1). However, the differences observed in number of days for FADS between the genotypes were not statistically significant ($p > 0.05$).

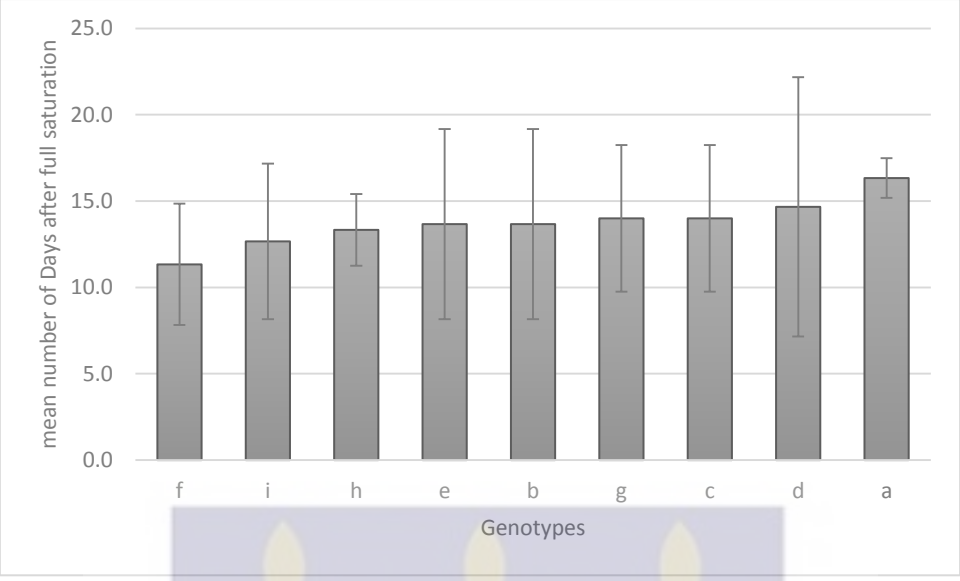

Fig 3.1: Mean number of Days After Full Saturation (DAFS) for First Appearance of Drought Symptoms for the nine (9) different genotypes of Cocoa (*Bars are means of 3 replicates with the standard error)*

3.1.5. Leaf RWC

3.1.5.1. Standardization of the procedure for determination of leaf RWC

During the first 30 minutes of immersion of the three (3) 1cm inner diameter leaf discs of each of the nine (9) cocoa genotypes in the distilled water, there was an initial rapid uptake of water followed by a gradual uptake after 1 h up to about 4 h. Uptake of water then levelled-off from 4 h to 5 h, indicated by the minimal to no change in weight of leaf discs after 3 h (Fig. 3.2a and 3.2b).

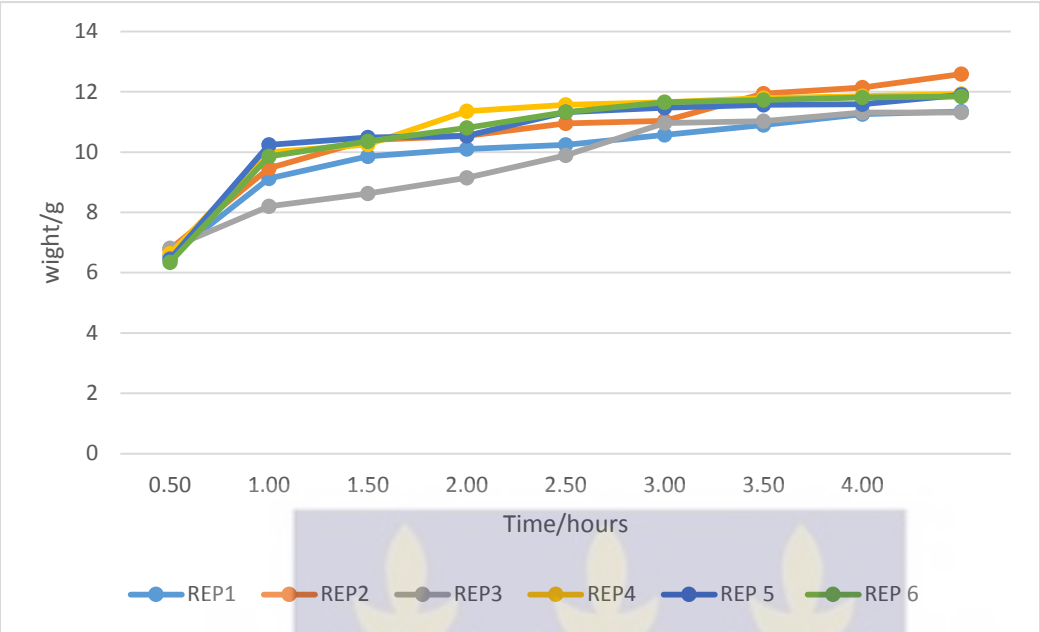

Fig. 3.2a: Means of time taken for leaf discs of selected cocoa genotypes to reach constant weight

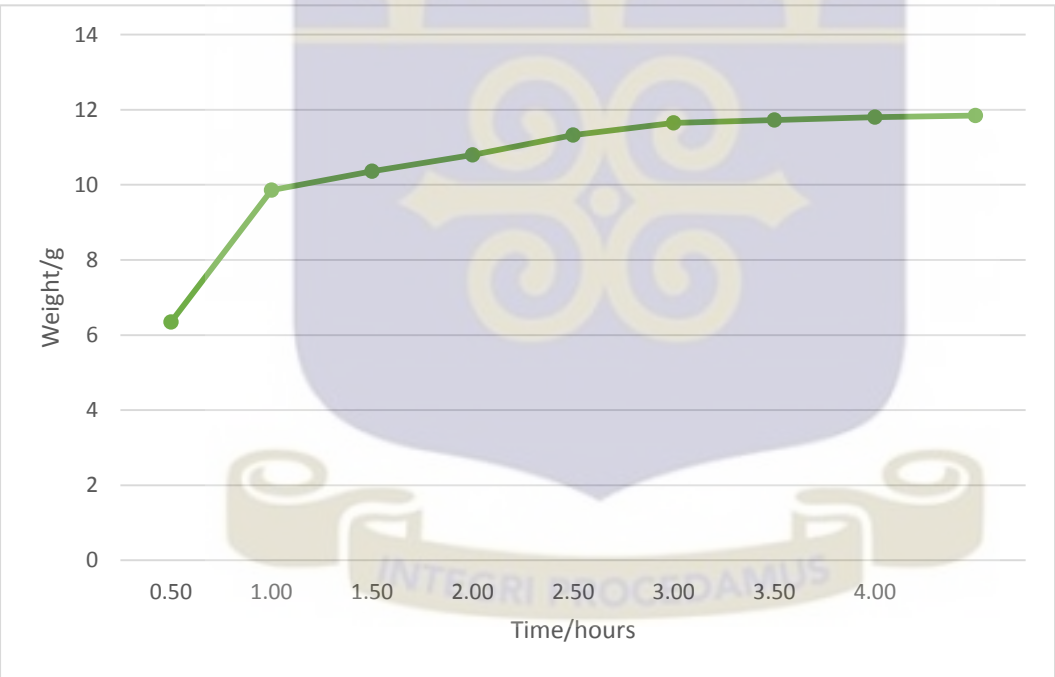

Fig. 3.2b: Mean of the mean duration (in minutes) times taken for leaf discs of selected cocoa genotypes to reach constant weight

3.1.5.2. Leaf RWC and Soil Moisture Content of water-stressed seedlings of the nine (9) selected cocoa genotypes

Results for the Leaf RWC and Moisture Content of the soil in which seedlings of all the nine (9) selected genotypes were grown are presented in Table 3.3 and Appendix 3a.

The initial mean Leaf RWC and Moisture Content of the soil (at Field Capacity one day after full saturation) in which the seedlings of all the nine (9) selected genotypes of cocoa were grown prior to exposing them to water stress were within the range of 77.49 – 91.77% (for percentage leaf RWC) and 10.88 – 13.74% (for percentage SMC) respectively. However, the differences in mean Leaf RWC and mean Soil Moisture Content in which all the different cocoa genotypes were grown were similar and not statistically significant/different as expected/intended ($p \leq 0.05$).

3.1.5.3. Mean Leaf RWC and Mean Soil Moisture Content at FADS

Data of mean leaf RWC at FADS (Table 3.3) were also not statistically significant ($p \leq 0.05$); although mean values for Leaf RWC of genotypes 'd', 'a', 'b' and 'i' (66.7%, 64.59%, 63.57% and 61.23%) were the highest when compared to the values of the other genotypes ('e': 59.70%; 'g': 55.47%; 'f': 52.63%; 'h': 51.09% and 'c': 42.77%).

The values of moisture content of the soils in which the selected genotypes were grown are shown in Table 3.3. and their means were not statistically significant ($p \leq 0.05$). Similarly the mean moisture content of the soil in which genotypes 'c', 'e' and 'g' were grown were the highest (1.50%, 2.78% and 1.48%) when compared to the mean moisture values of the soils in which the other genotypes were grown ('a': 0.66%; 'b': 1.29%; 'd': 1.23%; 'f': 1.31%; 'h': 0.99%; 'i':0.98%).

The soil in which genotype 'a' was grown had the lowest mean Soil Moisture Content (0.66%) though it had one of the highest leaf RWCs (64.59%). Replicates in genotype 'i', 'e' and 'f'

had the largest variation in leaf RWC (40.5%-85.9%, 35.2%-83.3% and 19.4%-74.3% respectively) while replicates of genotype 'g' had the lowest variation (54.6%-56.2%) followed by genotypes 'a' and 'b' (61.7%-67.5% and 59.7-65.7%) (Table 3.3)

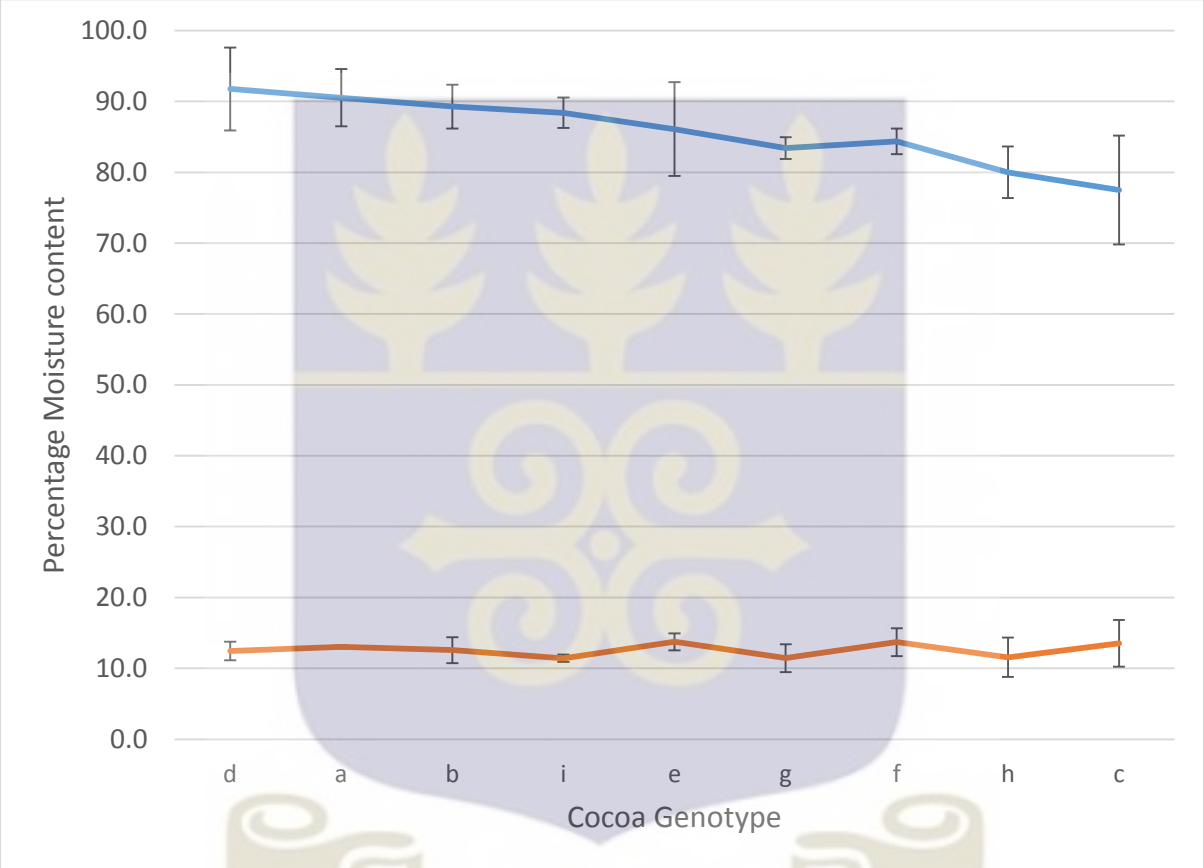

Fig. 3.3: Mean RWC and moisture content of the soil in which the nine (9) cocoa genotypes were growing at 1DAFS

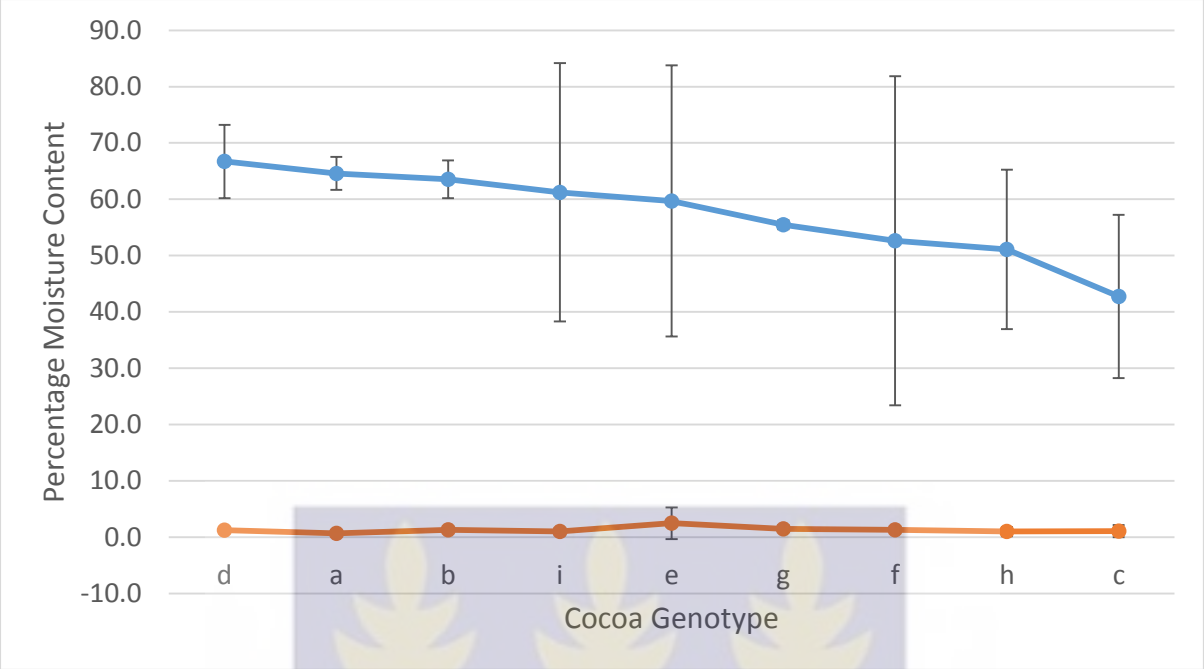

Fig. 3.4.: Mean RWC and moisture content of the soil in which the seedlings of the nine (9) cocoa genotypes were growing at FADS in Experiment I

3.2 Results for Phase II (Experiment II)

3.2.1 Apparent drought tolerance potential of the nine (9) selected genotypes based on number of days for FADS

In Experiment II, it took all the genotypes less number of days to show FADS than they did in Experiment I.

Based on the number of days for FADS, the genotypes could roughly be ranked into four (4) Groups – I, II, III, IV in increasing order of drought tolerance potential as follows:

f (11.3) < i (12.7) < h (13.7) < e (13.7) < b (13.7) < g (14.0) < c (14.0) < d (14.7) < a (16.3)

Group I *Group II* *Group III* *Group IV*

3.2.2 Leaf RWC and moisture content of the soil of water stressed seedlings of genotypes

Initial mean leaf RWC and Moisture Content of the soil (at Field Capacity one day after full saturation) in which seedlings of all the nine (9) selected genotypes of cocoa were grown prior to exposing them to water stress were in the range of 79.17 – 91.84% and 6.35 – 10.26% respectively (Appendix 5b).

Data of the parameters for Leaf RWC and Soil Moisture Content measured in Experiment II are summarized in Fig. 3.5 and Appendix 5b.

At FADS, the mean leaf RWC of the genotypes were also no statistically significant but the mean SMCs of the genotypes were statistically significant ($p < 0.05$). Genotypes *'e'*, *'h'*, *'g'* had the highest mean leaf RWC (65.55%, 58.16% and 56.16%) whereas the moisture content of the soil in which genotypes 'e' was grown was the highest (1.52%); being almost twice as much as the genotype with the second highest SMC, 'a' (0.81%). The soil moisture content of the soil in which genotype 'g' was grown. The moisture content of the soil in which genotype 'g' was grown was the lowest (0.37%).

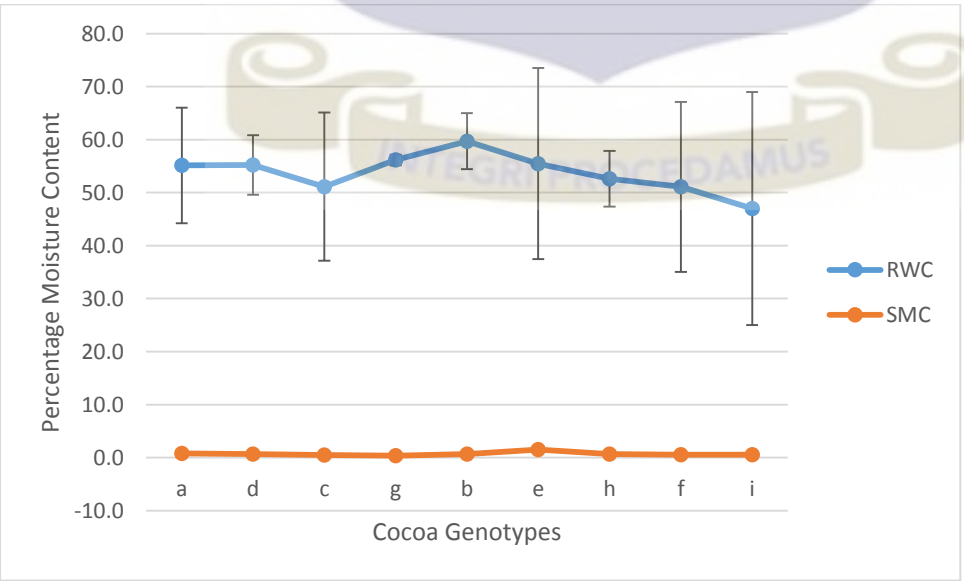

Fig. 3.5: Mean RWC and moisture content of the soil in which the nine (9) cocoa genotypes at FADS in Experiment II

3.2.2.1 Ranking and Possible Trends among mean Leaf RWC, mean Soil Moisture values and Drought-tolernace potential of nine (9) selected cocoa genotypes in Phase I and II

Ranking of the mean values of Leaf RWC and SMC of Phases I and II (i.e. Experiments I and II) and the possible trends in relationship amongst the nine (9) selected cocoa genotypes in terms of their drought-tolerance potentials is presented in Table 3.5.

Table 3.3: Rankings of Values of mean leaf RWC (%), Soil Moisture Content – SMC (%) and standard errors (se) of the nine (9) selected cocoa genotypes and possible trends in their drought-tolerance potential (Phases I and II)

Phase	RWC value of genotype (%) and rank								
I	d	a	b	i	e	g	f	h	c
	66.70	64.59	63.57	61.23	59.70	55.47	52.63	51.09	42.76
se	± 1.33	± 0.23	± 1.82	± 1.98	± 2.78	± 1.95	± 0.53	± 1.19	± 3.30
SMC (%)	1.23	0.66	1.29	0.98	2.47	1.48	1.31	0.99	1.50
II	e	h	g	d	a	i	c	f	b
	65.55	58.16	56.17	55.2	55.13	51.87	51.12	47.00	42.80
se	± 18.02	± 5.24	± 0.99	± 5.62	± 10.92	± 16.06	13.98	± 21.98	± 5.28
SMC (%)	1.520	0.667	0.371	0.673	0.810	0.570	0.497	0.540	0.663

During Phase I genotypes ‘d’ and ‘a’ had the highest Leaf RWC values (66.70% and 64.59% respectively) with genotypes ‘e’, ‘g’, ‘f’ and ‘h’ having values in the medium to high-low range (59.70%, 55.47%, 51.09% and 51.09% respectively) and genotype ‘c’ having the lowest value (42.76%). For Phase II (Experiment II) however the RWC values of ‘e’, ‘h’ and ‘g’ had the highest values (65.5%, 58.16% and 56.17% respectively) with genotype ‘c’ still showing one of the lowest values (51.12%).

3.2.3 Leaf morphology and anatomical features

Results of the leaf morphological and anatomical features are presented in Tables 3.4, 3.7a, 3.8a, 3.9a, 3.10and Plates 3.1-3.6.

Table 3.4: Summary of the mean number of trichomes, mean width of the upper epidermis, mesophyll and lower epidermis of the nine (9) cocoa genotypes used in this study

Genotype	Mean Width of			Mean Number of Trichomes
	Upper Epidermis (µm)	Mesophyll (µm)	Lower Epidermis (µm)	
a	15.97 ± 4.11	71.15 ± 17.09	9.52 ± 0.53	2.67 ± 1.25
b	22.71 ± 1.36	83.86 ± 5.35	15.02 ± 1.54	2.00 ± 1.63
c	22.15 ± 1.99	84.96 ± 10.68	11.82 ± 0.93	2.06 ± 0.82
d	23.87 ± 3.63	83.05 ± 10.65	11.33 ± 1.58	3.67 ± 2.06
e	19.303 ± 2.76	82.88 ± 16.36	12.35 ± 0.68	2.00 ± 0.82
f	18.19 ± 3.67	73.98 ± 2.91	12.87 ± 1.60	1.33 ± 1.25
g	16.39 ± 1.27	46.60 ± 5.03	11.44 ± 0.57	0.50 ± 0.5
h	18.51 ± 2.47	57.25 ± 14.77	12.46 ± 1.22	3.30 ± 0.47
i	18.17 ± 2.7	91.10 ± 9.91	11.01 ± 0.39	0.33 ± 0.47

3.2.3.1 Upper Epidermis

Results of the mean widths of the leaves of the upper epidermis of the nine (9) selected genotypes are summarized in Table 3.5a. Genotypes 'a' and 'g' had the lowest mean upper epidermal width (15.97 microns and 16.39 microns respectively); whereas genotypes 'b', 'c' and 'd' had the largest mean upper epidermal width (22.71 microns, 22.15 microns and 23.87 microns respectively) (Table 3.5a). However the sizes of the sections (TS) also appeared variable.

Analysis of variance conducted (ANOVA) indicated that there were no statistically significant differences ($p \leq 0.05$) among the mean width values of the mean upper epidermal widths of the various genotypes (Table 3.5b). The genotypes that showed the least variation in width of the upper epidermal widths amongst their replicates were genotypes 'b' and 'c' (values ranged between 21.35 microns – 24.07 microns for genotype 'b' and 20.16 microns – 24.14 microns for genotype 'c'). The widest variation in the width of the upper epidermis were observed in

genotypes ‘a’ (11.86 – 20.08 microns), genotype ‘e’ (16.54 – 22.06 microns), genotype ‘f’ (14.52 – 18.19 microns), genotype ‘g’ (15.12 – 16.39 microns), genotype ‘d’ (20.24 – 27.5 microns) and ‘i’ (15.47 – 20.87 microns).

Table 3.5a: Mean values for the width of upper epidermis of the leaves of the nine (9) selected cocoa genotypes

Genotype	Mean width of upper epidermis (µm)
a	15.97 ± 4.11
b	22.71 ± 1.36
c	22.15 ± 1.99
d	23.87 ± 3.63
e	19.30 ± 2.76
f	18.19 ± 3.67
g	16.39 ± 1.27
h	18.51 ± 2.47
i	18.17 ± 2.70

Table 3.5b: ANOVA of the width of the Upper Epidermis of the leaves of the nine (9) selected cocoa genotypes

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	189.157	8	23.6447	1.97	0.1106
Within groups	215.952	18	11.9973		
Total (Corr.)	405.109	26			

3.2.3.2 Mesophyll

Observations on the mean width of the mesophyll of the different genotypes are summarized in Table 3.6a. The genotype with the smallest mean mesophyll width was genotype 'c' (11.80 microns) whereas genotype 'i' had the largest mean mesophyll width (91.10 microns) closely followed by genotypes 'b', 'd' and 'e' (with 83.02 microns, 83.05 microns and 82.88 microns respectively). The mean mesophyll width of the rest of the genotypes are 'a' (71.15 microns), 'f' (73.98 microns), 'g' (47.60 microns) and 'h' (57.23 microns).

An analysis of variance conducted (ANOVA – Table 3.6b) indicated that there were statistically significant differences ($P \leq 0.05$) among the mean widths of the mesophyll of the various genotypes following subsequent orthogonal comparisons using LSD, DMR and SNK (Table 3.8c). The genotype that showed the least variance in mesophyll width amongst its replicates were genotypes 'f' with values ranging between 71.07 microns – 76.89 microns. The largest variation in the width of the upper epidermis were observed in genotypes 'e' (66.52 microns – 99.24 microns) and 'h' (42.48 microns – 72.02 microns).

Table 3.6a: Mean values of the width of the leaf mesophyll of each of the nine (9) selected genotypes

Genotype	Mean width of mesophyll (µm)
a	71.15 ± 17.09
b	83.86 ± 5.35
c	84.96 ± 10.68
d	83.05 ± 10.65
e	82.88 ± 16.36
f	73.98 ± 2.91
g	46.60 ± 5.03
h	57.25 ± 14.77
i	91.10 ± 9.91

Table 3.6b: ANOVA of the width of the leaf mesophyll of the nine (9) selected cocoa genotypes

Source	*Sum Squares*	*of Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4933.07	8	616.634	3.17	0.0199
Within groups	3498.38	18	194.354		
Total (Corr.)	8431.45	26			

Table 3.6c: Orthogonal analysis of the mean values for the width of the leaf mesophyll of the nine (9) selected cocoa genotypes

Genotype	*Count*	*Mean*	*Orthogonal Comparisons*		
			LSD	***DMR***	***SNK***
a	3	71.15	***	***	**
b	3	83.86	*	**	**
c	3	84.92	*	*	**
d	3	83.05	*	**	**
e	3	82.88	*	**	**
f	3	73.98	**	**	**
g	3	47.60	*	*	*
h	3	57.25	**	**	**
i	3	91.10	*	*	*

3.2.3.3 Lower Epidermis

Data of the mean widths of the lower epidermis of leaves of the nine (9) different genotypes are presented in Table 3.9a. The genotype with the smallest lower epidermal width was 'a' (9.52 microns), whereas genotype 'b' had the largest lower epidermal width (15.02 microns). The upper epidermal widths of the rest of the genotypes are 'c' (12.35 microns),'d' (11.82 microns), 'e' (12.35 microns) 'f' (12.87 microns), 'g' (11.44 microns), 'h' (12.46 microns) and 'i' (11.01 microns).

Analysis of variance conducted (ANOVA – Table 3.9b) indicated that there were statistically significant differences ($P \leq 0.05$) amongst the mean widths of the lower epidermal layers of the various genotypes (Table 3.9c). Subsequent orthogonal comparisons using LSD, DMR and SNK indicated the differences amongst the data values. The genotypes that showed the least

variance in lower epidermal widths were genotypes 'i' with values ranging between 10.62 microns – 11.40 microns. The widest variation in the width of lower epidermal widths were observed in genotypes 'b' (13.48 microns – 16.56 microns), 'd' (9.75 microns – 12.91 microns) and 'f' (11.27 microns – 14.47 microns).

Table 3.7a: Mean vales of the widths of the lower epidermis of each of the nine (9) selected cocoa genotypes

Genotype	Lower Epidermis (µm)
a	9.52 ± 0.53
b	15.02 ± 1.54
c	11.82 ± 0.93
d	11.33 ± 1.58
e	12.35 ± 0.68
f	12.87 ± 1.60
g	11.44 ± 0.57
h	12.46 ± 1.22
i	11.01 ± 0.39

Table 3.7b: ANOVA of the lower epidermal widths of the nine (9) selected cocoa genotypes

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	54.3242	8	6.79053	3.70	0.0100
Within groups	32.9988	18	1.83327		
Total (Corr.)	87.323	26			

Table 3.7c: Orthogonal analysis of the mean values of the width of the lower epidermis of the leaf of each of the nine (9) selected cocoa genotypes

Genotype	*Count*	*Mean*	*Orthogonal Comparisons*		
			LSD	***DMR***	***SNK***
a	3	9.52	*	*	*
b	3	15.02	*	*	*
c	3	11.82	**	**	**
d	3	11.33	**	**	*
e	3	12.35	*	*	**
f	3	12.87	**	**	**
g	3	11.44	**	**	*
h	3	12.46	*	*	**
i	3	11.01	**	**	*

3.2.3.4 Presence/Absence of Trichomes

The presence/absence and number of trichomes are seen in Table 3.8 and Plates 3.2-3.7 for the different cocoa studied.

It was observed that most of the replicates of the nine (9) genotypes selected cocoa genotypes possessed trichomes although a few of them did not have any (e.g. genotype 'b' - Plate 3.3 left; 'f' – Plate 3.5 right).

The ranking of the mean number of trichomes among replicates of the nine (9) genotypes may be presented in four (4) categories I, II, III, IV as follows (Table 3.8):

'd' (4) > 'a' (3) = 'h' (3) > 'b' (2) = 'c' (2) = 'e' (2) > 'f' (1) = 'g' (1)
Category (I) Category (II) Category (III) Category (IV)

However, these categories were not statistically significant.

Table 3.8: Mean number of trichomes on the selected leaf of each of the nine (9) selected genotypes

Genotype	Mean number of trichomes	Alternate Rationale*
a	2.67 ± 1.25	3
b	2.00 ± 1.63	2
c	2.06 ± 0.82	3
d	3.67 ± 2.06	4
e	2.00 ± 0.82	2
f	1.33 ± 1.25	1
g	0.50 ± 0.5	1
h	3.30 ± 0.47	3
i	0.33 ± 0.47	1

*the fact that is a fraction of the value of the trichome means it is present; it does not matter the value/quantum of that fraction

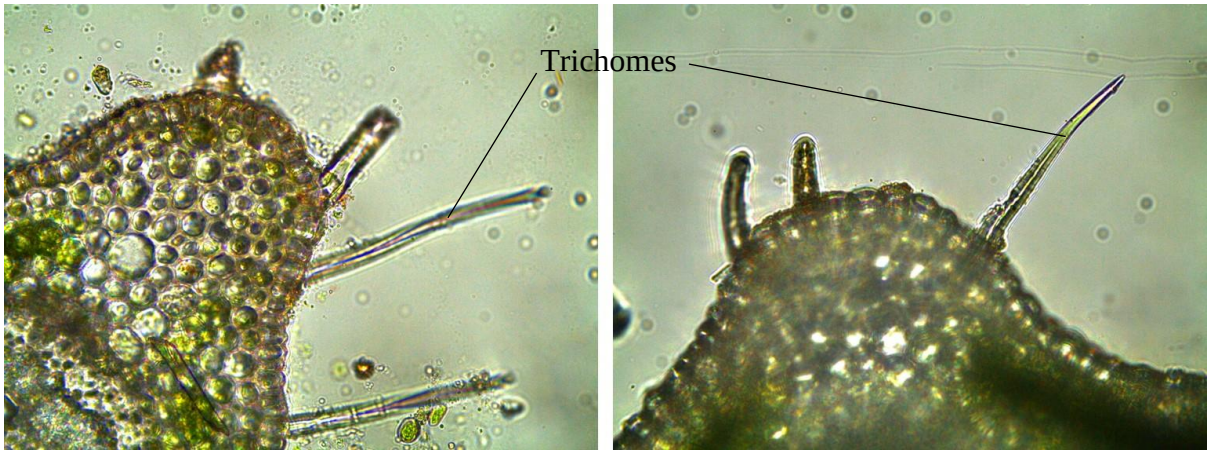

Plate 3.2: Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (a) through the mid-rib under the high power (HP) magnification (x400) showing the presence of many epidermal hairs on the mid-rib

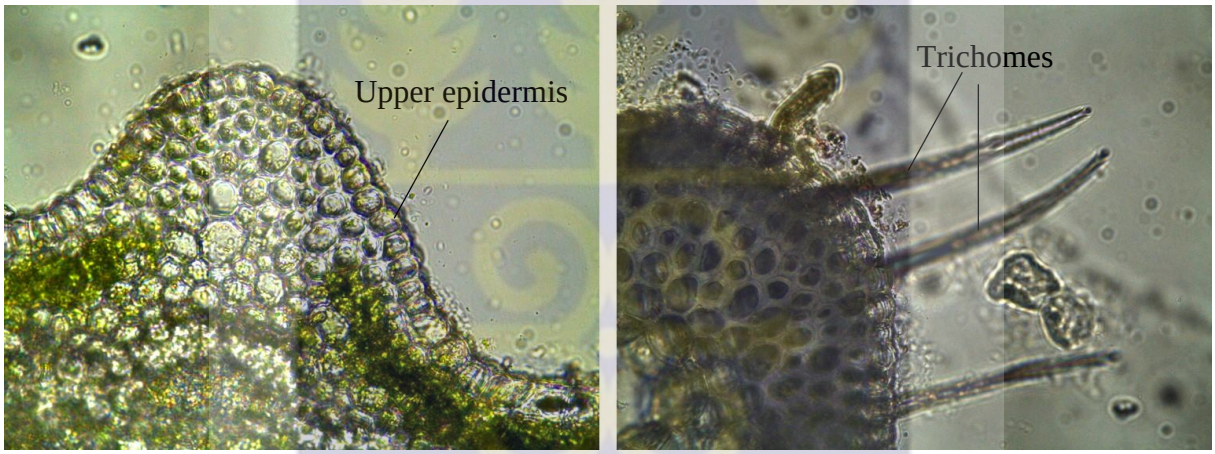

Plate 3.3: Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (b) through the mid-rib under the high power (HP) magnification (x400) showing the presence of many epidermal hairs (right) and lack of epidermal hairs (left) on the mid-rib

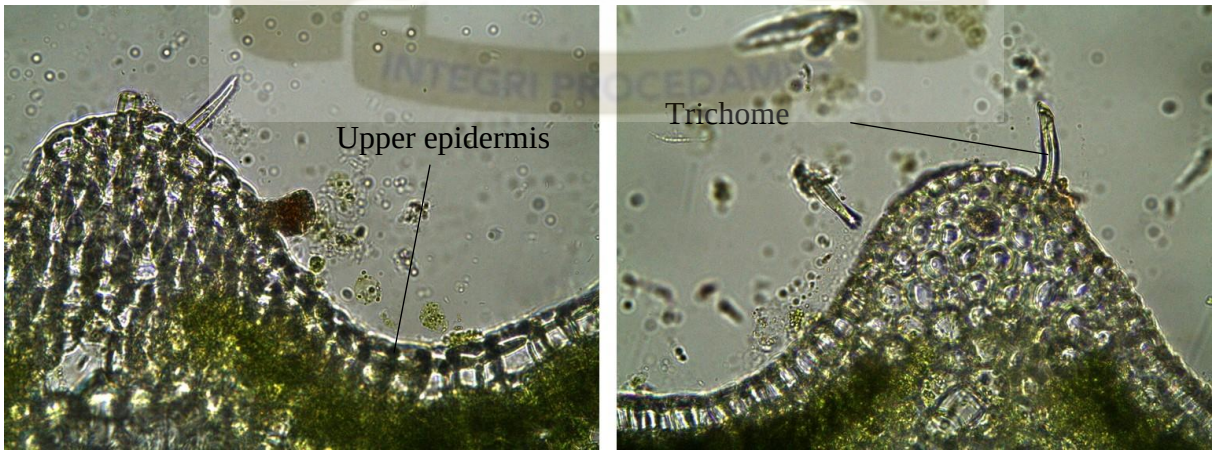

Plate 3.4.: Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (c) through the mid-rib under the high power (HP) magnification (x400) showing the presence of many epidermal hairs (left) and lack of epidermal hairs (right) on the mid-rib

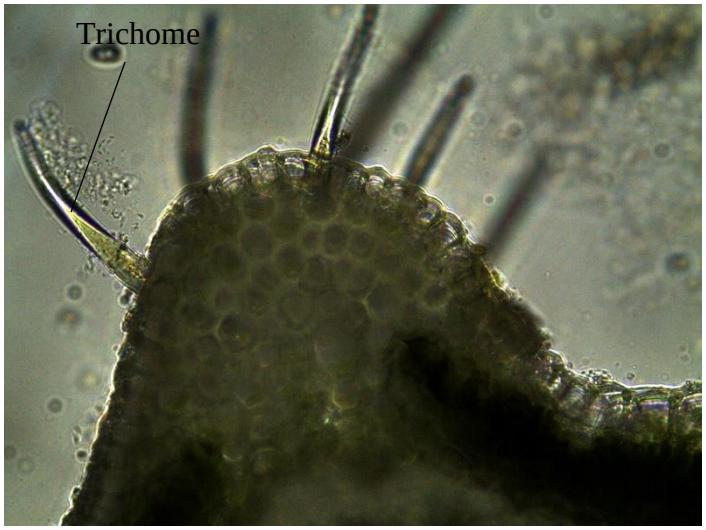

Plate 3.5: Photomicrograph of transverse section of selected leaf of cocoa genotype (d) through the mid-rib under the high power (HP) magnification (x400) showing the presence of many epidermal

Plate 3.6.: Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (e) through the mid-rib under the high power (HP) magnification (x400) showing a single epidermal hair (left) and presence many epidermal hairs (right) on the mid-rib

Plate 3.7.: Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (f) through the mid-rib under the high power (HP) magnification (x400) showing the presence of a single epidermal hairs (left) and lack of epidermal hairs (right) on the mid-rib

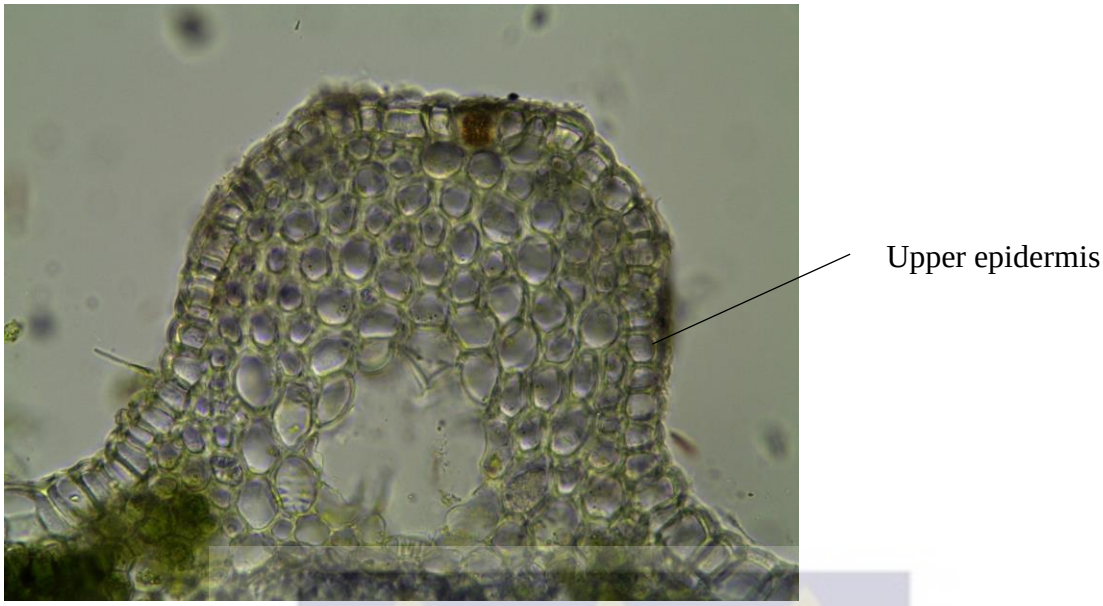

Plate 3.8 Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (e) through the mid-rib under the high power (HP) magnification (x400) showing a lack of epidermal hairs on the mid-rib

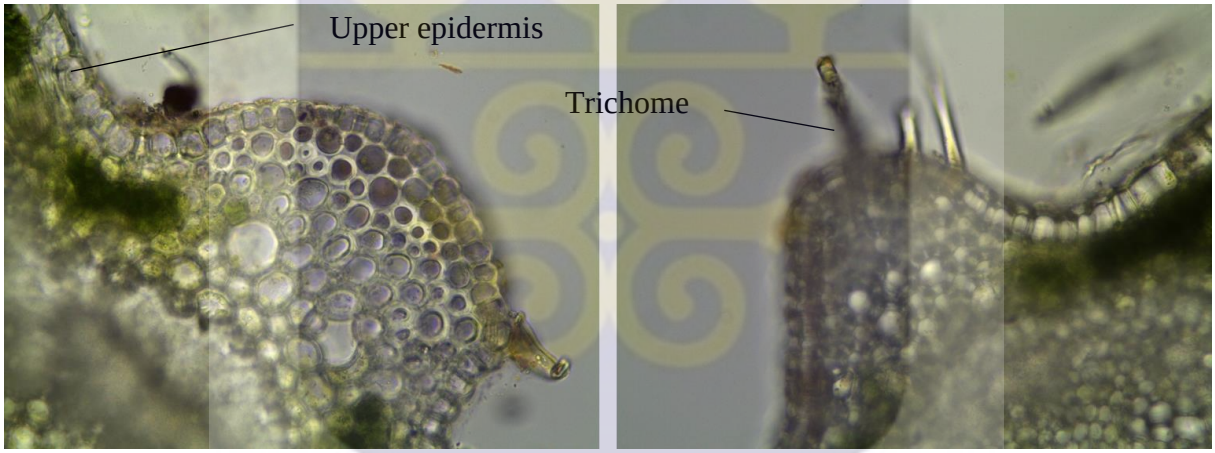

Plate 3.9.: Photomicrograph of transverse section (TS) of selected leaf of cocoa genotype (h) through the mid-rib under the high power (HP) magnification (x400) showing the presence of many epidermal hairs (left) and lack of epidermal hairs (right) on the mid-rib

3.2.4 Free Proline Accumulation in the leaves of the selected cocoa genotypes

The standard curve of authentic analytical grade *D*-proline (Sigma-Aldrich Inc., USA) used in estimation of the free proline content of leaves of seedlings is shown in Fig 3.6. The lower exponential range of the standard curve (Fig. 3.6) suitable for the estimation of the concentration of accumulated free proline in the leaves was within the range of about 0.001 µg/ml and 0.005 µg/ml.

Colour development obtained by the treatment of different dilutions or concentrations of free proline with acid-ninhydrin reagent is shown in Plate 3.7 and the standard curve for this is presented in Fig. 3.6.

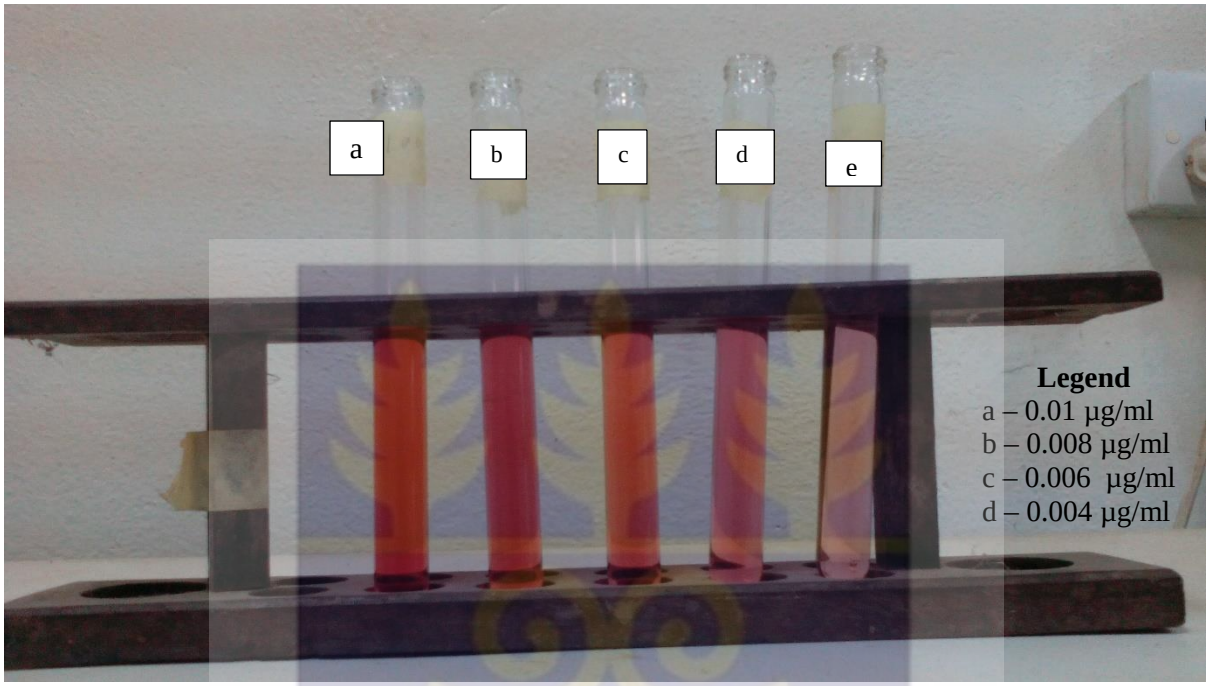

Plate 3.10: Colour development after the treatment of different concentrations of authentic D-proline stock solution with acid-ninhydrin reagent

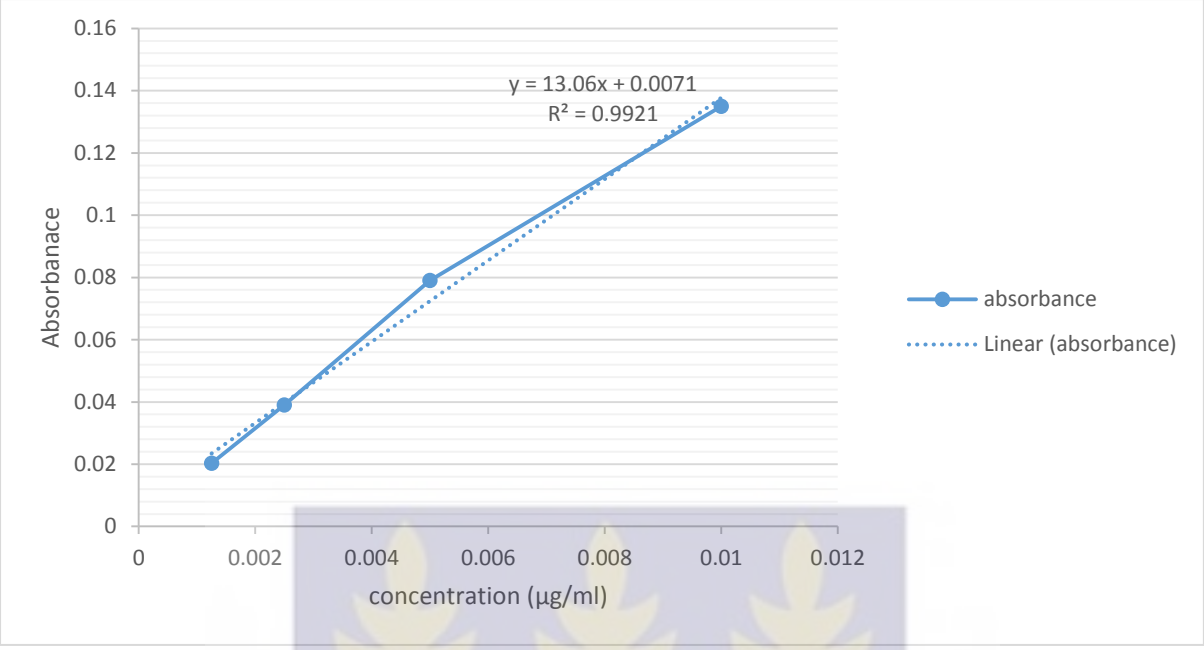

Fig 3.6: Standard Curve for authentic analytical grade D-proline

The linear/exponential range of the standard curve (Fig. 3.5) suitable for the estimation of the concentration of the accumulation of free proline in the leaves of the cocoa genotypes was between approximately 0.001 – 0.005 µg/ml.

The mean free proline content of leaves of the nine (9) selected genotypes of cocoa is shown in Table 3.11a. It was observed that the free proline content in the leaves of all water-stressed seedlings of the selected cocoa genotypes at FADS were significantly higher when compared to those their controls. However, the analysis of variance (ANOVA) indicated that the differences in the mean accumulation of proline of the replicates of the various genotypes that were subjected to drought-stress were statistically significant ($P \leq 0.05$) (Table 3.11b).

Genotype 'd' had the highest concentration of free proline at FADS (3.69 ± 0.05 µg/g DW) followed by genotype 'a' (3.49 ± 0.09 µg/g DW) whereas genotype 'i' had the lowest amount of accumulated proline in its leaves (0.56 µg/g DW). Plate 3.8 shows the colour development of the control and water-stressed at FADS.

Table 3.9a: Mean free leaf proline accumulation values for the nine (9) selected cocoa genotypes

Genotype	Proline Concentration (µg/g DW)	
	1DAFS	FADS
a	0.24 ± 0.01	3.49 ± 0.90
b	0.24 ± 0.04	2.47 ± 0.39
c	0.22 ± 0.02	2.27 ± 0.15
d	0.22 ± 0.02	3.69 ± 0.50
e	0.25 ± 0.06	2.37 ± 0.05
f	0.24 ± 0.04	1.17 ± 0.36
g	0.24 ± 0.05	2.06 ± 0.22
h	0.22 ± 0.01	1.03 ± 0.49
i	0.24 ± 0.04	0.56 ± 0.27

Table 3.9b: ANOVA of the mean free proline accumulation in the leaf of each of the nine (9) selected cocoa genotypes

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	27.26	8	3.407	17.65	0.0000
Within groups	3.47	18	0.193		
Total (Corr.)	30.73	26			

Table 3.9c: Orthogonal comparison of the free proline accumulation in the each of the selected leaves of each of the nine (9) selected genotypes

Genotype	*Count*	*Mean*	*Orthogonal Analysis*		
			LSD	*DMR*	*SNK*
i	3	0.56	X	X	X
h	3	1.03	X	X	X
f	3	1.17	X	X	X
g	3	2.06	X	X	X
c	3	2.27	X	X	X
e	3	2.37	X	X	X
b	3	2.47	X	X	X
a	3	3.49	X	X	X
d	3	3.69	X	X	X

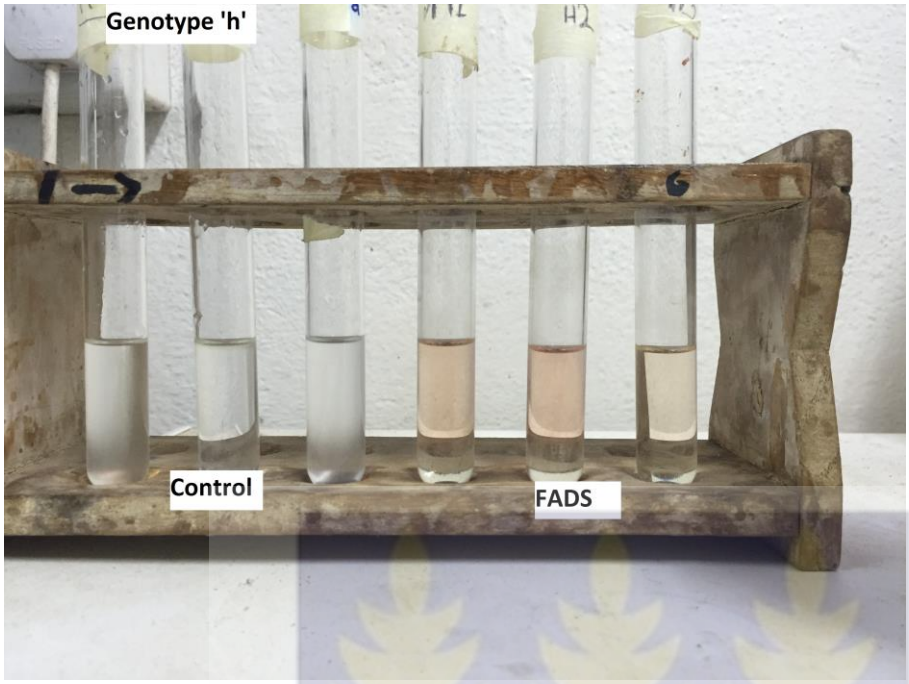

Plate 3.11: Difference in colour intensity and hence free proline concentration in one of the water stressed genotypes 'h' (PA150 x 6020) at First Appearance of Drought Symptoms (FADS), right, as compared to its control, left.

3.2.5 Possible interrelations amongst the various parameters (Leaf RWC, SMC, free proline accumulation in the leaves and mean number of leaf trichomes on the upper epidermis) of the nine (9) different genotypes of cocoa used in this study

The possible interrelations and trends amongst the different parameters used for the investigation of the drought resistance potential of the nine (9) selected cocoa genotypes are presented Fig. 3.7. The moisture content of the soils used to simulate drought in Phase II (Fig. 3.7 and Appendix 5d) ranged between 0.37% for the soil in which genotype 'g' was grown through 0.67% (for the soils in which genotype 'd' was grown) to a maximum of 1.5% (for the soils in which genotype 'e' were grown). These soil moisture levels mimicked water stress as had been planned as an objective for this study.

Under the drought-induced soil moisture levels, the values of the mean leaf RWCs ranged between 42.8% for genotype 'b' through to 55.20% and 55.13% (for genotypes 'd' and 'a') to 65.55% for genotype 'e' (Fig. 3.7 and Appendix 5d).

There appeared to be a general tendency of correlation of the mean values of all the four (4) parameters (i.e. Leaf RWCs, accumulation of free leaf proline the number of trichomes present on the mid-rib of the upper epidermis of the leaves of most of the nine (9) selected cocoa genotypes and the moisture content of the soil (SMC) in which the genotypes were grown (Fig. 3.7 and Appendix 5); except for genotype 'h' where the value of its accumulated free leaf proline did not follow the trend i.e. was lower (1.03 µg/g DW) whereas the value of the mean number of upper epidermal leaf trichomes was the second highest value 3.30 and also for genotype 'g' where its mean value for free leaf proline was 2.06µg/g DW and the sixth (6^{th}) in the ranking whereas the mean value for the number of upper epidermal trichomes of 0.5 was the eighth (8^{th}) out of the nine genotypes.

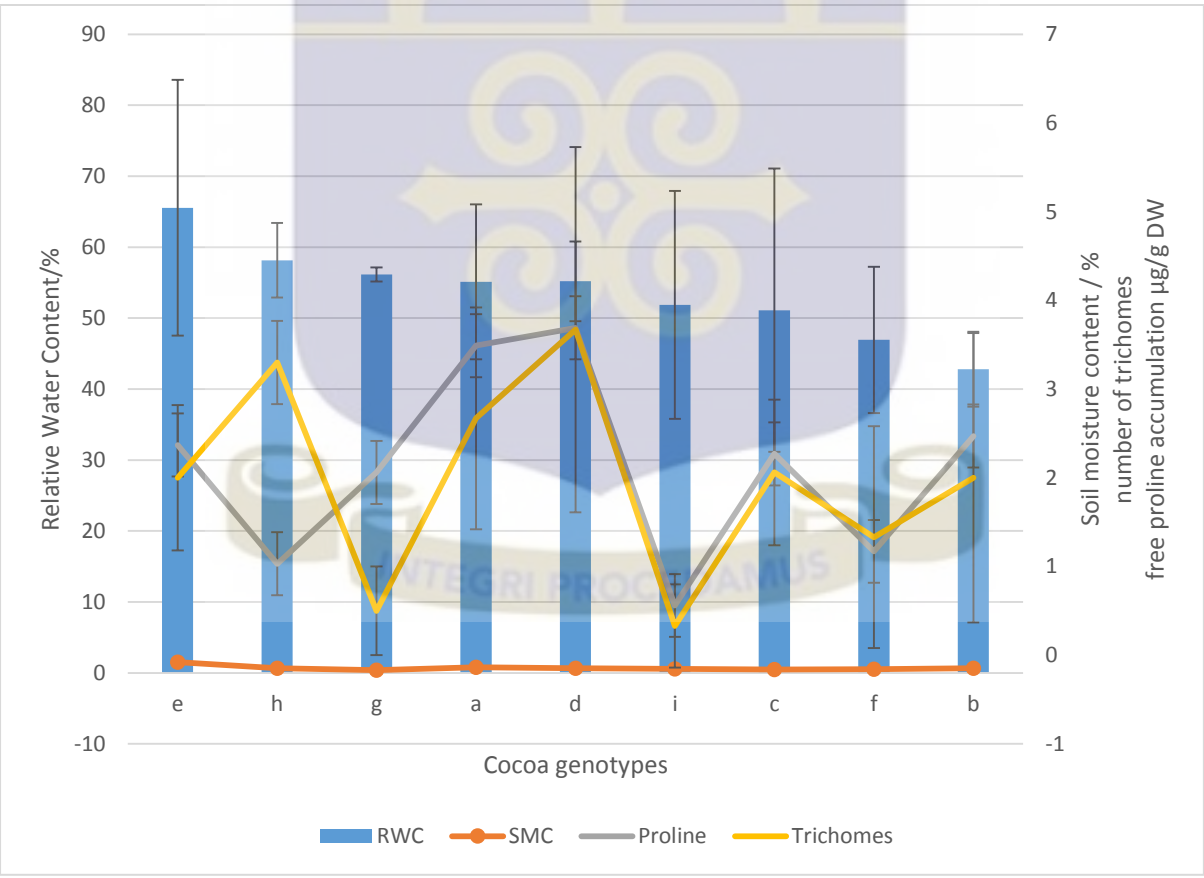

Fig. 3.7: Possible interrelations amongst the various parameters (Leaf RWC, SMC, and proline) used in the study of the nine (9) selected genotypes obtained in Phase II

The mean Leaf RWCs values of genotypes ‘d’ and ‘a’ were 55.20% and 55.13% i.e. the fourth (4^{th}) and fifth (5^{th}) respectively in the ranking of RWC values whereas the mean leaf proline accumulation in their leaves were 3.69 µg/g DW and 3.49 µg/g DW which were first (1^{st}) and second (2^{nd}) respectively (Fig. 3.7 and Appendix 5).

CHAPTER FOUR

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1. Discussion

4.1.1. *Identification and physical properties of the soil used for the study*

The soil used in the potting of the seedlings in this study was identified to be a sandy loamy soil which is different from the recommended soil required for the growth of cocoa as described by Amoah (1995) although it was also found to be free-draining and not easily water logged. The mixing of the top soil (sandy loam soil) used in this study with leaf compost ensured a good and adequate supply of minerals and nutrients to the growing cocoa seedlings which was similar to recommended soil types for cocoa cultivation.

The pH of the soil which was 7.3 was also within the range 5.0-7.5 recommended by the ICCO (2013) and Wicks (2003) for the cultivation of cocoa.

4.1.2. Observed climatic conditions (ambient temperature and relative humidity) of the study sites during the study period

The mean minimum weekly ambient air temperature of 18.57– 22.14 °C and 25.00 – 27.00 °C recorded in Experiments I (Table 3.1) and II (Table 3.2) of this study were slightly higher than the recommended minimum ambient air temperature for the cultivation of cocoa (18-21 °C) for optimum growth of cocoa as described by ICCO (2013) and Amoah (1995). The mean maximum weekly ambient air temperature of 32.86 – 36.00 °C (Table 3.1) and 32.86 – 33.43 °C (Table 3.2) recorded in Experiments I and II were also slightly higher than the recommended maximum ambient air temperature for the cultivation of cocoa (30 – 32 °C) for optimum growth of cocoa as described by ICCO (2013) and Amoah (1995).

In general, humidity conditions of Experiment II (Table 3.2) were higher (min: 60.90-64.30%; max: 69.60-84.03%) than that of Experiment 1 (Table 3.1; min: 26.24-42.59%; max: 48.50-

84.29), an indication that water loss through evapotranspiration would be lower under the conditions that prevailed in Experiment II compared to Experiment I. However, this was not the case. During Experiment II, the cocoa seedlings showed signs of drought-stress faster than those in Experiment I (Appendix 5a and Appendix 5b). This could have been due to differences in other abiotic factors that were not measured (i.e. wind speed and light intensity) during that period (January-February 2016 for Experiment I and April-May 2016 for Experiment II).

4.1.3. First Appearance of Drought Symptoms in Cocoa Seedlings

Some of the visible symptoms or signs of early drought-stress observed in the nine (9) cocoa genotypes used in both Phases I and II (Experiments I and II) of this study, for example the drooping of leaves (especially the young and tender leaves) have been reported earlier by Osei-Bonsu (2011) and Bae *et* al. (2009). The yellowing of basal leaves from the margin of the leaves of the cocoa genotypes studied has also been previously observed by Carr and Lockwood (2011) and Osei-Bonsu (2011).

Cocoa leaves are more rigid and have more wax compared to the leaves of other food crops that are usually screened for their drought tolerance potential (Osei-Bonsu, 2011). This makes the observation of the first symptoms of drought harder to notice. This is particularly so in the older, mature green cocoa leaves were symptoms of drought were usually only very conspicuous when the drought stress was very severe leading to their permanent wilting point and ultimate death i.e. lethal (Osei-Bonsu, 2011).

4.1.4 Number of days for FADS

The observation in this study that the nine (9) selected cocoa genotypes showed first symptoms of drought-stress (FADS) after different durations (the number of days) is similar to that made by Xu *et al*. (2008) in a number of plant species (rice, pepper, tomato, water melon and beet) where it served as a criterion for the screening for their drought-tolerance potential as can be

done in this study for the nine (9) genotypes of cocoa (i.e. FADS can be used potentially as a criterion for the screening of drought-tolerance potential in cocoa); although the values obtained during Phase I – Experiment I and Phase II – Experiment II of this study were different (most probably because of the differences in the range of the ambient air temperatures and relative humidifies that were observed (Tables 3.1 and 3.2) and the possible variations in prevailing wind speeds during Phases I and II of the study although they were not measured .

4.1.5 Leaf Relative Water Content (%)

The observations made in Phase I/Experiment I and Phase II/Experiment II of this study that the mean Leaf RWC values of genotypes 'd' and 'a' were the highest (66.70% and 64.59% respectively - Appendix 5d and Fig. 3.7) with genotypes 'e', 'g' and 'h' having values in the medium to high-low range (59.70%, 55.47% and 51.09% respectively – Appendix 5d) with genotype 'c' having the lowest value (42.76% - Appendix 5d and Fig. 3.7) whereas in Phase II the trend changed with genotypes 'e', 'h' and 'g' having the highest values (65.5%, 58.16% and 56.17% respectively – Appendix 5d and Fig. 3.7) with genotype 'c' still showing one of the lowest values (51.12% - Appendix 5d and Fig 3.7) indicates that RWC has the potential to be used as criterion to select for genotypes with low potential for drought stress and also high potential in selecting for high drought-tolerant varieties; with potential differences arising from the use of different replicates (seedlings) not clones with different gene combinations due to independence segregation of genes e.g. the shifting of different replicates of 'a' and 'd' from high drought-tolerance potential and genotypes 'e', 'h' and 'g' from medium drought tolerance-potential to high drought tolerance-potential from Phase I to Phase II. This is similar to the use of RWC to screen for drought tolerance potential in potatoes by Soltys-Kalina (2016) and sugar-cane (De A Silva, 2007). Also the Leaf RWC values obtained in Phase I were generally higher than those obtained in Phase II though the humidity levels generally had a smaller range in Phase II (26.24-74.89% in Phase I (Table 3.1) compared to 60.90-84.03% (Table 3.2) in

Phase II; it could be attributed to the different ambient conditions (wind, light intensity, temperature, and humidity) of the two different sites used in Phase I and Phase II as well as the varying genotypic differences within the cocoa genotypes.

4.1.6 Soil Moisture Content (%)

The observation that the moisture content of the soils used in Phases I and II did not vary significantly supports or justifies of the objective of this study in creating drought/water-stress conditions; and that the data obtained for all parameters (i.e. Leaf RWC and free proline leaf accumulation) were indeed under water stress conditions. However, soil moisture content alone cannot be used as a criterion in determining/selecting drought tolerant genotypes of cocoa unless it was studied concurrently with the other parameters such as the Leaf RWC and accumulation of free proline in the leaves of the seedlings grown in them (Osei-Bonsu, 2011). However, there is paucity of information on the combined use of Leaf RWC, proline accumulation and SMC in the screening of drought-tolerance potential in cocoa; although this has been done significantly for other species of crops e.g. wheat where it was established that they could be used to screen for drought-tolerant genotypes (De A Silva, 2007).

Consequently any of the cocoa genotypes that showed high drought-tolerance potential might probably due to the low water use and transpiration rate of those genotypes as a result of other characteristics. This supports the findings of Nunes (1967) that higher drought-tolerance potential in some genotypes of cocoa may be attributed to their stomatal behaviour and transpiration rate. Genotype ‘d’ (T63/971 x SCA9) had a relatively high mean Leaf RWC (55.2%) while the soil in which it was grown had one of the lowest mean soil moisture levels/content (SMC in Phase II/Experiment II of 0.67%). It was ranked as one of the genotypes with high drought-tolerance potential. On the other hand genotype ‘e’ (PA150 x 6020) had a high Leaf RWC (65.55% in Phase II) as well as the highest moisture content for the soil it was grown in (1.52%). Using the free proline accumulation in the leaves and the number of

trichomes present on the leaves, genotype 'e' was not ranked as a high drought-tolerant potential germplasm but one with medium drought-tolerance potential (Group II) (Table 4.2).

4.1.7 Free proline accumulation in the leaves and drought sensitivity of each of the selected genotypes

The observation in this study that free proline accumulation in the leaves of induced water-stressed genotypes was higher than that accumulated in their control counterparts (between 0.56 µg/g DW to 3.69 µg/g DW for the water-stressed replicates compared to between 0.22 µg/g DW to 0.24 µg/g DW for the controls – Table 3.11a and Appendix 5d) is similar to what was observed by Bae *et al*. (2009) and Balasimha (1988) in cocoa and by in other species. Apart from the function proline plays as an osmolyte for osmotic adjustment, proline also contributes to the stability of the cell membranes and cell proteins (Ashrad and Foolad, 2007), removes free radicals as well as buffering cellular redox potentials under drought-stress. From the results obtained, it was clear the free proline accumulation in leaves of the selected cocoa genotypes can be used as a criterion in the screening for drought-tolerance potential in cocoa. However, there was no positive correlation between the increasing trend of the accumulation of free proline of most of the genotypes and the relative water content of the leaves except for genotypes 'd' (which had a mean Leaf RWC of 55.20% and proline accumulation of 3.69 µg/g DW – Appendix 5d) and 'a' (which also had a mean Leaf RWC of 55.13% and proline accumulation of 3.49 µg/g DW – Appendix 5d). There also appeared to be some positive correlation between the mean number of trichomes observed in some of the genotypes studied and the leaf RWC e.g. the genotype – i.e. 'd' with the highest number of trichomes observed per transverse section (3.67) also had the second highest mean Leaf RWC value of 55.20% (genotype 'd'). The highest value for free leaf proline accumulation observed in the study (3.69µg/g DW) for genotype 'd' followed with the highest number of trichomes (3.67) with an appreciably high Leaf RWC (55.20% - Table and Appendix 5d). The ranking of the drought-

tolerance potential of some of the genotypes was not clear cut. This could be due to the high genetic variability in cocoa (Daymond *et al.*, 2002). In general, the observation that all the cocoa genotypes selected for this study appeared to have different drought-tolerance levels is similar to what was reported by Adu-Ampomah *et al*. (2001), Adu-Ampomah and Frimpong (2002, 1994, 1993) and as well as Balasimha *et al.* (1988).

4.1.8 Ranking of drought-tolerance potential of the nine (9) selected cocoa genotypes obtained from CRIG based on some of the criteria investigated i.e. FADS, mean Leaf Proline Accumulation, mean Leaf RWC values, presence/absence of trichomes and their mean numbers

Data obtained from FADS (Appendix 5a) indicates that generally, genotypes 'd' and 'a' show the highest number of days after saturation to full field capacity before showing first symptoms of flaccidity of the leaves i.e. 14.7 and 16.3 days respectively; whereas genotypes 'f', 'i' and 'h' showed leaf flaccidity within 11.3, 12.7 and 13.7 days after saturation to full field capacity (Table 4.1).

Table 4.1: Ranking of drought-tolerance potential of the nine (9) selected cocoa genotypes based on number of days for First Appearance of Drought Symptoms (FADS)

'f' (11.3) < 'i' (12.7) < 'h' (13.7) = 'e' (13.7) = 'b' (13.7)	< 'g'(14.0) < 'c' (14.0) < 'd' (14.7)	< 'a' (16.3)
Group I	Group II	Group III	Group IV
LOW	LOW MEDIUM	HIGH MEDIUM	HIGH

Similarly, based on the mean leaf proline accumulation (µg/ml) during Phase II of the study, genotypes 'a' and 'd' have the highest free proline accumulation in the leaves i.e. 3.49 µg/ml and 3.39 ±0.50 µg/ml whereas genotypes 'i', 'h' and 'f' had the lowest mean leaf proline content i.e. 0.56 ± 0.27 µg/ml, 1.03 ± 0.49 µg/ml, 1.17 ± 0.36 µg/ml respectively which had the lowest potential for drought-tolerance; with genotypes 'g', 'c', 'e' and 'b' showing medium drought-tolerance potential.

Table 4.2: Ranking of drought-tolerance potential of the nine (9) selected cocoa genotypes based on Mean Leaf Proline accumulation (µg/ml)

Group I	Group II	Group III	Group IV
'i' ($\frac{0.56}{\pm 0.27}$) < 'h' ($\frac{1.03}{\pm 0.49}$) < 'f' ($\frac{1.17}{\pm 0.36}$) = 'g' ($\frac{2.06}{\pm 0.22}$)	< 'c' ($\frac{2.27}{\pm 0.15}$)	< 'e' ($\frac{2.37}{\pm 0.05}$) < 'b' ($\frac{2.47}{\pm 0.39}$ (3)	< 'a' ($\frac{3.49}{\pm 0.90}$) < 'd' ($\frac{3.39}{\pm 0.50}$)
LOW	LOW MEDIUM	HIGH MEDIUM	HIGH

Based on mean leaf RWC values for Phases I and II, genotypes 'a' and 'd' also had the highest leaf RWC values i.e. 64.59 ± 0.23 and 66.70 1.33 respectively whereas, genotype 'c' had the lowest value of 42.76 ± 3.30%, 'h' had 51.09 ± 1.90% and 'f' had 52.63 ± 0.53% and thus showed the lowest drought-tolerance potential. With genotype 'e', 'i' and 'b' showing medium drought-tolerance potential.

Table 4.3: Ranking of drought-tolerance potential of the nine (9) selected cocoa genotypes based on Mean Leaf RWC values (%) – (Phases II & II – Appendix 5d)

Group I	Group II	Group III	Group IV
'c' ($\frac{42.76}{\pm 3.30}$) < 'h' ($\frac{51.09}{\pm 1.19}$) < 'f' ($\frac{52.47}{\pm 0.53}$) = 'g' ($\frac{55.47}{\pm 1.95}$)	< 'e' ($\frac{59.70}{\pm 2.81}$)	< 'i' ($\frac{61.23}{\pm 1.98}$) < 'b' $\frac{63.57}{\pm 1.82}$ (3)	< 'a' ($\frac{64.59}{\pm 0.23}$) < 'd' ($\frac{66.70}{\pm 1.33}$)
LOW	LOW MEDIUM	HIGH MEDIUM	HIGH

Based on the presence and absence of trichomes and their mean whole numbers, genotypes 'd' and 'h' had the highest drought-tolerance potential with four (4) trichomes each whereas genotypes 'i' and 'g' had one (1) each, genotypes 'f', 'e', 'b' 'c' 'a' had two trichomes each making them the genotypes with the least drought-tolerance potential.

Table 4.4: Ranking of drought-tolerance potential of the nine (9) selected cocoa genotypes based on both whole numbers and fractions of whole numbers of trichomes (rationale/logic)

Group I	Group II	Group III	Group IV
'c' (1) = 'g' (1)	< 'f' (2) = 'e' (2) = 'b' (2)	< 'c' (3) < 'a' (3)	< 'd' (4) < 'h' (4)
LOW	LOW MEDIUM	HIGH MEDIUM	HIGH

From all these criteria, genotype 'd' consistently presented with high drought-tolerance potential whereas genotype 'a' except for presence of trichomes also showed a high drought-

tolerance potential. Whereas genotypes 'i', 'g' and 'f' consistently exhibited a medium drought-tolerance potential.

4.2 Conclusions

i. Cocoa leaf discs floated on distilled water attained their maximum level of turgidity after 3 hours.

ii. Generally, genotypes 'd' (TA63/97 x SCA9) and 'a' (T60 x POUND10) were the most drought-tolerant amongst the nine (9) genotypes studied. They had the highest RWC in Phase though the moisture content of the soil that 'a' was grown in was relatively lower than the moisture content that 'd' was grown in ('a': 0.66% compared to 'd': 1.23%). Both genotypes also accumulated the most proline in their leaves at FADS ('a' 3.49 µg/g DW; 'd': 3.69 µg/g DW).

iii. Genotypes 'f' (AMAZ x 9006), 'h' (PA150 x 9006) and 'i' (PA7 x MAN) seemed to be the least drought-tolerant genotypes amongst the nine selectedgenotypes studied. Both genotypes 'f' and 'h' had some of the lowest mean Leaf RWCs in Phase I (52.63% and 51.09%); the only genotype that had lower mean leaf RWC values was genotype 'c' (42.76%).

iv. Mean Soil moisture Content (SMC) alone cannot be used as a criterion in the screening for drought-tolerance in the selected cocoa genotypes. It must be studied concurrently with other parameters such as the accumulation of free proline in the leaves of cocoa plants, Leaf Relative Water Content, presence/absence of leaf trichomes, width dimensions of leaf anatomical features e.g. thickness of upper and lower cuticle, width of epidermis, presence/absence of hypodermis, width of palisade and spongy mesophyll, width of lower epidermis, width of lower cuticle and presence/absence and number of stomata on the epidermis (particularly the lower epidermis).

v. The number of leaf epidermal trichomes varied amongst the selected nine (9) cocoa genotypes; and it appears that this anatomical/morphological parameter could be useful in the screening for drought-tolerance potential of the cocoa genotypes.

vi. The observation of the accumulation of free proline in the leaves of the nine (9) cocoa genotypes studied indicated that it could be used as a criterion to screen for potentially drought-tolerant genotypes of cocoa.

The null hypothesis that there are no variation in morphological, physiological, and biochemical parameters of the nine (9) selected cocoa genotypes obtained from CRIG in response to varying or different soil moisture content (SMC) - (i.e. drought) can be rejected based on the data obtained in this study. Instead the alternate hypothesis that there are variations in morphological, physiological, and biochemical responses/parameters of the nine (9) selected cocoa genotypes obtained from CRIG in response to varying soil moisture content (SMC) can be accepted.

4.3 Recommendations

i. Tagging of leaves should be considered a potential measure to reduce or prevent the potential variations in the values of the parameters of the different genotypes studied (especially in the accumulation of free leaf proline in the leaves). This also ensures limitation of variations in chronological age of the leaf samples as well as metabolic/biochemical and physiological ages.

ii. It is also suggested that a microtome should be used in the study of leaf internal morphology/anatomy in order to provide uniform pressures, rather than the uneven pressures when using the razor blade and to reduce the potential variations in the number of slashes for sectioning when using the razor blade.

iii. Further studies should be carried out to on the transpiration rates and other morphological features like stomatal density, stomatal behaviour, and the thickness of

waxy cuticle of leaves of the different cocoa genotypes available in order to improve and better understand the reasons why relatively drought-tolerant genotypes of cocoa are efficient users of water.

iv. Studies should also be carried out to investigate the specific functions of proline in water-stressed cocoa plants.

v. Considering the wide variation in cocoa genotypes, future studies need to have large enough samples over a longer period of time to enable us be certain on the results of the experiment i.e. the drought-tolerance of the cocoa genotypes.

Summary

- This study involved the screening of nine (9) genotypes of cocoa (*T60 x Pound10, PA7 x 6035, T85 x PA7, T63/971 x Sca9, PA150 x 6020, AMAZ X 9006, T79 x 9006, PA150 x 6020 and PA7 x MAN*) for their drought-tolerance potential using Leaf Relative Water Content, free proline accumulation in the leaves, leaf morphological features (i.e. presence/absence of trichomes, width of upper epidermis, mesophyll and lower epidermis) as possible criteria for the screening.
- The study comprised of two (2) phases/experiments, Phases I and II - Experiments I and II. Experiment I was a rapid drought-tolerance screening involving counting the number of days from saturation of soils to field capacity till First Appearance of Drought Symptoms. Prior to the start of this phase (i.e. Phase I – Experiment I), all the replicates of the nine (9) genotypes were transplanted into larger blue plastic buckets containing the same quantity/volume of a thoroughly mixed sandy loam soil that contained 13.3% and 13.8% of moisture at field capacity at depths of 5 cm and 10 cm respectively.
- Experiment II involved the use of Leaf Relative Water Content (RWC), free proline accumulation in the leaves and leaf morphological features (i.e. presence/absence of trichomes, width of upper epidermis, mesophyll and lower epidermis) as criteria to screen for the drought-tolerance potential of the nine (9) cocoa genotypes.
- The experimental set-up was a Randomised Complete Block Design (RCBD) for both Phases using six (6) replicates for each of the nine (9) genotypes where three (3) replicates of each genotype served as controls.
- Phase I involved the rapid screening of the selected genotypes using the number of days it took to show symptoms of drought as a criterion for drought screening. Prior to the initiation of Phase I the seedlings were slowly saturated with water to Full Saturation

(FS) – Field Capacity after which water was withheld from 27 out of 54 seedlings comprising of three (3) replicates of each of the nine (9) selected genotypes one (1) Day After Full Saturation (DAFS) (Water stressed); whereas the remaining 27 seedlings (Control) received water every other day till the end of the experiment.

- Data on leaf Relative Water Content (RWC) and Soil Moisture Content (SMC) were collected in addition to the number of days for First Appearance of Drought Symptoms (FADS).
- Standardisation to determine the minimum number of hours to obtain full saturation of the leaf after floating on distilled water was found to be about two (2) hours.
- Mature leaves of approximately the similar chronological, physiological and biochemical ages were selected for the determination of free proline accumulation of the nine (9) selected cocoa genotypes.
- Mature leaves of similar chronological, physiological and biochemical ages were also selected for leaf morphological/anatomical studies (i.e. width of upper epidermis, mesophyll and lower epidermis, and the presence/absence of trichomes).
- At the end of the study period the data obtained indicated that the studied parameters measured varied in response to the induced drought stress and could prove useful in the screening of drought-tolerance in cocoa plants either in isolation or combination.

References

Abo-Hamed, S., Collin, H.A., Hardwick, K. (1983). Biochemical and physiological aspects of leaf development in cocoa (*Theobroma cacao* L.). VII. Growth, orientation, surface structure and water loss from developing flush leaves. *New Phytol.* 95:9-17.

Abdulrahaman, A. A., and Oladele, F. A. (2011). Response to Trichomes to water stress in two species of Jatropha. *Insight Botany*, 1, (2), 12-15

Adu-Ampomah, Y. and Frimpong, E.B. (1993). Drought resistance trials, p 97–102. In: *Rep. Cocoa Res. Inst. Ghana*, 1989/90. 154p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Adu-Ampomah, Y. and Frimpong, E.B. (1994a). Drought resistance trials, p 48–49. In: *Rep. Cocoa Res. Inst. Ghana*, 1990/91. 139p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Adu-Ampomah, Y. and Frimpong, E.B. (1994b). Breeding for drought resistance, p 51. In: *Rep. Cocoa Res. Inst. Ghana*, 1991/92. 188p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Adu-Ampomah, Y. and Frimpong, E.B. (2002).Breeding for easy establishment in degraded environment, p 65–69. In: *Rep. Cocoa Res. Inst. Ghana*, 1999/2000. 217p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Adu-Ampomah, Y., Frimpong, E.B. and Mante, S.O. (2001). Breeding for easy establishment in degraded environment, p 73–74. In: *Rep. Cocoa Res. Inst. Ghana*, 1998/99. 203p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Ahenkorah, Y., Halm, B.J., Appiah, M.R., Akrofi, G.S. and Yirenkyi, J.E.K. (1987). Twenty years' results from a shade and fertilizer trial on Amazon cocoa (*Theobroma cacao*) in Ghana. *Exp. Agric.* 23:31-39.

Ahmad, S., Mahmood, A., Malik, A.J., Karim, A. and Kumbhar, M.B. (2003). Response of okra to water stress. *Sarhad J. Agric.* 19(1):73-79.

Akashi, K., Miyake, C. and Yokota, A. (2001). Citrulline, a novel compatible solute in drought-tolerant wild water melon leaves, is an efficient hydroxyl radical scavenger. *FEBS Lett.* 508:438-442. 115

Alam, M. M., bin Toriman, M. E., Siwar, C., & Talib, B. (2011). Rainfall variation and changing pattern of agricultural cycle. *American Journal of Environmental Sciences*, *7*(1), 82–89. http://doi.org/10.3844/ajessp.2011.82.89

Almeida A-AF, Brito RCT, Aguilar MAG, Valle RR. 2002 Water relations' aspects of *Theobroma cacao* L. clones. *Agrotrópica* 14(2):35-44

Almeida, A.-A. F. de and Valle R.R. (2007). Ecophysiology of the cacao tree. *Braz. J. Plant Physiol.* 19(4):425-448.

Alverson, W.S., Whitlock, B.A., Nyffeler, R., Bayer, C., Baum, D.A. (1999). Phylogeny of the core Malvales: evidence from ndhF sequence data. *Am. J. Bot.* 86:1474- 1486.

Alvim, P. de T. (1959). Stomatal opening as a practical indicator of moisture deficiency in cacao, p 283-293. Proc. 7th International American Cacao Conference, Palmira, Columbia.

Alvim, P. de T. (1977). Cacao, p 279-313. In: Alvim P. de T., Kozlowski T.T. (eds.) *Ecophysiology of Tropical Crops*. Academic Press, London.

Amoah, J.E.K. (1995). Commercial production of cocoa, p 21-36. In: *Cocoa outline series 1. Development of consumption, commercial production and marketing*. Jemre, Accra, Ghana.

Amponsah, J.D. (1973). Akumadan drought tolerance trial p 154–155. In: *Rep. Cocoa Res. Inst. Ghana*, 1971/72. 233p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Anim-Kwapong, G. J. and Frimpong, E. B. (2005). Vulnerability of Agriculture to Climate – change impact of climate on Cocoa production. Cocoa Research Institute of Ghana. Retrieved May 15 2015 http://www.nlcap.net/fileadmin/NCAP/Countries/Ghana.2008

Anonymous. (2009). Ghana to revolutionarize cocoa industry - Fofie. Ghana Business News (GBN), Accra. 29 January 2009. Retrieved May 5, 2015 from the GBN website on the world wide web: http://www.ghanabusinessnews.com/2009/01/29/ghana-revolutionizes-cocoaindustry-fofie/

Antwi, A. (1994). The effects of water deficit on growth and development in young cocoa plants (*Theobroma cacao* L.). An Msc dissertation, University of West Indies, St. Augustine, Trinidad and Tobago.

Argout, X. Fouet, O., Wincker, P., Gramacho, P., Legavre, T., Sabau, X., Resterucci, A.M., Da Silva, C., Cascardo, J., Allegre, M., Kuhn, D., Verica, J., Courtois, B., Loor, G., Babin, R., Sounigo, O., Ducamp, M., Guiltinan, M.J., Ruiz, M., 116 Alemanno, L., Machado, R., Philips, W., Schnell, R., Gilmour, M., Rosenquist, E., Butler, D., Maximosa, S. and Lanaud, C. (2008). Towards the understanding of the cocoa transcriptome: Production and analysis of an exhaustive dataset of ESTs of *Theobroma cacao* L. generated from various tissues and under various conditions. *BMC*

Genomics 9:512-530. Retrieved April 4, 2016 from BioMed Central database on the world wide web: http://www.biomedcentral.com/1471- 2164/9/512.

Ashrad, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Environ. Exp. Bot., 59: 206-216.*

Babu, R.C., Pathan, M.S., Blum, A. and Nguyen, H.T. (1999). Comparison of measurement methods of osmotic adjustment in rice cultivars. *Crop Sci.* 39:150- 158.

Bae, H., Kim, S.-H., Kim, M.S., Sicher, R.C., Lary, D., Strem, M.D., Natarajan, S. and Bailey, B.A. (2008). The drought response of *Theobroma cacao* (cacao) and the regulation of genes involved in polyamine biosynthesis by drought and other stresses. *Plant Physiology and Biochemistry* 46:174-188.

Bae, H., Sicher, R.C., Kim, M.S., Kim, S.-H., Strem, M.D., Melnick, R.L. and Bailey, B.A. (2009). The beneficial endophyte *Trichoderma hamatum* isolate DIS 216d promotes growth and delays the onset of the drought response in *Theobroma cacao. J. Exp. Bot.* 60(11):3279-3295.

Balasimha, D. (1988). Water relations, growth and other indicators of plant water stress in cocoa under drought, p 215-217. In: *Proc. 10th Int. Cocoa Res. Conf.*, Santo Domingo, Dominican Republic.

Balasimha, D., Rajagopal, V., Daniel, E.V., Nair, R.V. and Bhagavan, S. (1988). Comparative drought tolerance of cocoa accessions. *Trop. Agric.* 65:271-274

Barrs, H.D. (1968). Determination of water deficits in plant tissues, p 235-368. In: T. T. Kozlowski, (ed.) *Water Deficits and Plant Growth, Vol. 1*. Academic Press.

Barrs, H. D. and Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* 24:519-570.

Barrs, H. D., Determination of water deficits in plant tissue. In: KOZLOWSKI, T.T. (Ed) Water deficits and plant growth. New York, Academic Press, 1968. v.1, p.235-368

Bart-plange, A., & Baryeh, E. A. (2003). The physical properties of Category B cocoa beans, *60*, 219–227. http://doi.org/10.1016/S0260-8774(02)00452-1

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-208. 117

Belsky, J.M and Siebert, S.F. (2003). Cultivating cacao: implications of sun-grown cacao on local food security and environmental sustainability. *Agric. Human Values* 20:277-285.

Blum, A. and Ebercon, A. (1976). Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. *Crop Sci.* 16:428-431

Blum, A., Sullivan C.Y. and Nguyen H.T. (1997). The effect of plant size on wheat response to agents of drought stress. II. Water deficit, heat and ABA. *Aust. J. Plant Physiol.* 24(1): 43-48.

Boote, K.J. (1982). Peanut. In: I.D. Teare and M.M. Peet (eds.) *Crop – water relations*. Wiley. p 256-280

Borrone, J.W., Kuhn, D.N. and Schnell R. J. (2004). Isolation, characterization and development WRKY genes as useful genetic markers in *Theobroma cacao*. *Theor. Appl. Genet.* 109: 495-507.

Cameron, K. D., Teece, MA, Smart LB (2006) Increased accumu- lation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol 140:176–183

Carr, M. K. V. (2014). *The water relations and irrigation requirements of mango* (Mangifera indica L.): A review. *Experimental Agriculture*, *50*(01), 1–23. http://doi.org/10.1017/S0014479713000288

Carr, M.K.V. and Lockwood, G. (2011). The water relations and irrigation requirements of cocoa (*Theobroma cacao* L.): a review. *Experimental Agriculture*, Available on CJO 2011 doi:10.1017/S0014479711000421.

CIAT. (2011). *Predicting the Impact of Climate Change on the Cocoa Growing Regions in Ghana and Cote d'Ivoire*. Retrieved May 13, 2016 from http://www.eenews.net/assets/2011/10/03/document_cw_01.pdf

CSAE. (2009). Fertile ground: Boosting cocoa production among Ghanaian smallholders. Centre for the Study of African Economies (CSAE) briefing paper 01, August 2009. 3p. Retrieved May 12, 2016 from the CSAE data base on the world wide web: http://www.csae.ox.ac.uk.

COCOBOD. (2007). Report on cocoa purchases for the year ending 2005/06. Research, Monitoring and Evaluation Department, Ghana Cocoa Board (COCOBOD), Accra. Ghana.

COCOBOD. (2016). The history of cocoa and its production in Ghana. Retrieved May 13, 2016 from the Ghana Cocoa Board (COCOBOD) website on the world wide web: http://www.cocobod.gh/history.php.

CSAE. (2009). Fertile ground: Boosting cocoa production among Ghanaian smallholders. Centre for the Study of African Economies (CSAE) briefing paper 01, August 2009. 3p. Retrieved May 13, 2016 from the CSAE data base on the world wide web: http://www.csae.ox.ac.uk.

Cuatrecasas, J. (1964). Cacao and its allies: a taxonomic revision of the genus Theobroma. *Bulletin of the United States National Museum*. Smithsonian Institution Press, Washington, 35: 379-614.

Da Matta, F.M., Loos, R.A., Rodrigues, R. and Barros, R.S. (2001). Actual and potential photosynthetic rates of tropical crop species. *Revista Brasileira de Fisiologia Vegetal* 13:24-32

Daymond, A.J., Hadley, P., Machado, R.C.R., and Ng, E. (2002). Canopy characteristics of contrasting clones of cacao (*Theobroma cacao*). *Exp. Agric.* 38:359-367.

Dib, T.A., Monneveux, Ph., Acevedo, E. and Nachit, M.M. (1994). Evaluation of proline analysis and chlorophyll fluorescence quenching measurements as Drought tolerance potential indicators in durum wheat (*Triticum turgidum* L. var. *durum)*. *Euphytica* 79(1-2):65-73

Din, J., Khan, S.U., Ali, I. and Gurmani, A.R. (2011). Physiological and agronomic response of canola varieties to drought stress. *Journal of Animal and Plant Sciences* 21(1):78-82.

ECOWAS-SWAC/OECD. (2007). Cocoa. Atlas on regional integration in West Africa: Economy series, 16p.

Ewusie, J.Y. (1980). The soil in tropical ecology, p 117-130. In: *Elements of tropicalecology. With reference to the African, Asian, Pacific and New World tropics.* Heinemann.

Fairtrade Foundation. (2016). Commodity Briefing: Cocoa. Obtained online at https://www.fairtrade.net/fileadmin/user_upload/content/2009/resources/2016-04_Cocoa_commodity_briefing_online7.pdf

FAO. 2008. *FAOSTAT online database*. Rome, Italy: The Food and Agriculture Organization `of the United Nations. [ONLINE] Available at: http://faostat.fao.org. Accessed 7/10/2011.

Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences*, *72*(4), 673–689

Frimpong, E.B. and Boafo, J.N. (1994). Specific leaf weight and response to drought, p 43. In: *Rep. Cocoa Res. Inst. Ghana*, 1992/93. 152p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Frimpong, E.B., Adu-Ampomah, Y. and Boafo, J.N. (1997a). Non-structural carbohydrate accumulation in roots of cocoa and its effect on drought tolerance, p 95–96. In: *Rep. Cocoa Res. Inst. Ghana*, 1995/96. 152p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Frimpong, E.B., Adu-Ampomah, Y., Dwapanyin, A.O. and Boafo, J.N. (1997b). Component analysis of yield responses to drought, p 96–97. In: *Rep. Cocoa Res. Inst. Ghana*, 1997/98. 236p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Frimpong, E. B., Y. Adu-Ampomah, and A. Abdul-Karimu. 1999. Effort to breed for drought resistant cocoa in Ghana. *In Proceedings of the 12thInternational Cocoa research conference*, 375–381. Lagos, Nigeria: Cocoa Producers' Alliance.

Frimpong, E.B., Adu-Ampomah, Y., Dwapanyin, A.O. and Boafo, J.N. (2001). Non-structural carbohydrate accumulation in roots of cocoa, p 69–70. In: *Rep. Cocoa Res. Inst. Ghana*, 1997/98. 187p. Cocoa Research Institute of Ghana (CRIG), Ghana.

Frimpong, E. B. (2011). Vulnerability and adaptation assessment under the Netherlands climate change studies assistance programme phase 2 (NCCSAP2) Vulnerability of agriculture to climate change- impact of climate change on cocoa production. *Journal of Pollination Ecology*, *5*(10), 74–80.

Galyuon, I. K. A, McDavid, F. B., Lopez, F. B. and Spence, J. A. (1996). The effect of irradiance level on cocoa (*Theobroma cacao* L.): 1. Growth and leaf adaptations. *Tropical Agriculture* (Trinidad) 73(1):23-28.

Gebre, G.M., Kuhns, M.R. and Brandle, J.R. (1994). Organic solute accumulation and dehydration tolerance in three water-stressed *Populus deltoides* clones. *Tree Physiol.* 14:575-587.

Gomes, F.P., Oliva, M.A., Mielke, M.S., Almeida A.-A. F. and Aquino, L.A. (2010). Osmotic adjustment, proline accumulation and cell membrane stability of leaves in *Cocos nucifera* submitted to drought stress. *Scientia Horticulturae* 126(3):379- 384.

Gotsch, N. (1997). Cacao crop protection: an expert forecast on future progress, research priorities and policy with help of the Delphi survey. *Crop Prot.* 16:227-233

Greathouse, D.C., Laetsch, W.M., and Phinney, B.O. (1971). The shoot-growth rhythm of a tropical tree, *Theobroma cacao*. *Am. J. Bot.* 58:281-286.

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant Signaling & Behavior*, *7*(11), 1456–66. http://doi.org/10.4161/psb.21949

Hetherington, A.M., and Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. Nature 424:901–908

Himcha, D. K., Hellwege, E.M., Heyer, A. G. and Crowe, J. H. (2000), Plant fructans stabilize phosphatidylcholine liposomes during freeze-drying. European Journal of Biochemistry, 267: 535-5540. Doi.10. 1046/j.1432-1327.2000.01028

Hoekstra, F.A., Golovina, E. A., Buitink J. (2001) Mechanisms of plant desiccation tolerance. *Trends Plant Science* 6:431–438

Hsiao, T.C. (1973). Plant responses to water stress. *Annu. Rev. Plant Physiol.* 24:519- 570. 120 Hutcheon, W.V. (1977a). Growth and photosynthesis of cocoa in relation to environmental and internal factors, p 222-232. In: *Proc. 5th Int. Cocoa Res. Conf.*, Ibadan, Nigeria.

Hutcheon, W. V. (1977b). Water relations and other factors regulating the seasonal periodicity and productivity of cocoa in Ghana, 233–244. In: *Proc. 5th Int. Cocoa Res. Conf.*, Ibadan, Nigeria.

ICCO. (2013). *Growing Cocoa*. Retrieved January 5, 2016, from www.icco.org/about-cocoa/growing-cocoa.html

IPCC (2007), '*Working Group II Fourth Assessment Report. Climate Change: Climate Change Impacts, Adaptation and Vulnerability*, http://www.ipcc.ch/SPM6avr07.pdf, retrieved on 5th March 2016

Ibarra-Caballero, J., Villanueva-Verduzco, C., Molina-Galan, J. and Sanchez-de-Jimenez, E. (1988). Proline accumulation as a symptom of drought stress in maize: a tissue differentiation requirement. *J. Exp. Bot.* 39: 889-897 (abs). Retrieved May 16, 2011 from the Oxford Journals data base on the World Wide Web: http://jxb.oxfordjournals.org/content/39/7/889.abstract.

Islam M. A., Du H., Ning J., Ye H,. Xiong L. (2009). Characterization of Glossy1-homologous genes in rice involved in leaf wax accumulation and drought resistance. Plant Molecular Biology 70:443–456

Jain, S. K., Agarwal, P. K., & Singh, V. P. (2007). *Hydrology and Water Resources of India* (1 ed). Springer Press. Doi:10.1007/1-4020-5180-8

Karaba, A., Dixit, S., Greco, R., Aharoni, A., Trijatmiko, K. R., Marsch-Martinez, N., Krishnan, A., Nataraja, K. N., Udayakumar, M. and Pereira, A. (2007). Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. Proc Natl Acad Sci 104(39): 15270-15275

Karamanos, A.K., Drossopoulos, J. B., Niavis, C. A. (1983). Free proline accumulation during development of two wheat cultivars with water stress. *Journal of Agricultural Science, Cambridge* 100: 429-439

Karamanos, A. J. (1995) The involvement of proline and some metabolites in water stress and their importance as drought resistance indicators. *Bulgarian Journal of Plant Physiology* 21(2-3): 98-110

Kholodova, V.P., Bormotova, T.S, Semenov, O.G, Dmitrieva, G.A. and Kuznetsov, VI.V. (2007). Physiological mechanisms of adaptation of alloplasmic wheat hybrids to soil drought. *Russian Journal of Plant Physiology* 54(4): 480-486.

Laderach, P., Martinez, A. I., Schroth, G., & Castro, N. (2013). Predicting the Future Climatic Suitability for Cocoa Farming of the World´s leading Producer Countries, Ghana and Côte d'Ivoire. *Climatic Change*, *119*(3), 841–854.

Luo, L.J. (2010) Breeding for water-saving and drought-resistance rice (WDR) in China. *Journal of Experimental Botany* 61:3509–3517

Lobell, D.B., Burke, M.B., Tebaldi, C., Mastrandrea, M.D., Falcom, W.P., Naylor,R.L. (2008), Prioritizing Climate Change Adaptation Needs for Food Security in 2030. Science 319: 607-610.

Mary, A. L., & Majule, A. E. (2009). Impacts of Climate Change, Variability and Adaptation Strategies on Agriculture in Semi Arid Areas of Tanzania: The Case of Manyoni District in Singida Region, Tanzania. *African Journal of Environmental Science and Technology*, 3(8), 206–218. http://doi.org/10.5897/AJEST09.099

Maximov, N. A. (1929). The plant in relation to water. George Allen and Unwin, Ltd., London

Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. *Curr. Sci.* 80(6):758-763. MMYE, (2006). Labour practices in cocoa production in Ghana (Pilot survey). Ministry of Manpower, Youth and Employment (MMYE), Accra, Ghana, 120p.

Mohammadkhani, N. and Heidari, R. (2008). Drought-induced accumulation of soluble sugars and proline in two maize varieties. *World Applied Sciences Journal* 3(3):448-453.

Mohammadian MA, Watling JR, Hill RS (2007). The impact of epicuticular wax on gas-exchange and photoinhibition in *Leucadendron lanigerum* (Proteaceae). Acta Oecol 31:93–101

Morgan, J. M., (1984) Osmoregulation and water stress in higher plants. Annu Rev Plant Physiol 35:299–319

Motamayor, J.C. (2005). Can we select for shorter cocoa plants? INGENIC Newsletter, St. Augustine, Trinidad & Tobago. September 2005 (10):16-19.

Morgan, J. M. (1984). Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiol.* 35:299-319.

Moser, G., Leuschner, C., Hertel, D., Holscher, D., Kohler, M., Leitner, D., Michalzik, B.,Prihastanti, E., Tjitrosemito S. and Schwendenmann, L. (2010). Response of cocoa trees (*Theobroma cacao*) to a 13-month desiccation period in Sulawesi, Indonesia. *Agroforestry Systems.* Retrieved September 23, 2010 from http://www.springerlink.com/content/q231330232348383/fulltext.html.

Motamayor, J.C., Risterucci, A.M., Lopez, P.A., Ortiz, C.F., Moreno, A. and Lanaud, C. (2002). Cacao domestication. I. The origin of the cacao cultivated by the Mayas. *Heredity* 89:380-386.

Moussa, H. R., and Abdel-Aziz, S. M. (2008). Comparative response of drought-tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Science* 1(1):31-36.

Mukherjee, I. (1974) Effect of Potassium on Proline Accumulation in Maize during Wilting. *Physiologia Plantarum* 31(4):288–291.

Mull, L. D. and Kirkhorn, S. R. (2005). Child labour in Ghana cocoa production: focus upon agricultural tasks, ergonomic exposures, and associated injuries and 122 illnesses. *Public Health Rep.* 120(6):649-656.

Mustilli, A.-C., Merlot, S., Vavasseur, A., Fenzi, F. and Giraudat, J. (2002). Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant cell* 14:3089- 3099.

Naveed, A. (2008). The potential of breeding okra (*Abelmoschus esculentus* L. Moench) for water stress tolerance. A PhD thesis, University of Agriculture, Faisalabad, Pakistan.

Ntiamoah, A. and Afrane, G. 2008. Environmental impacts of cocoa production and processing in Ghana: Life cycle assessment approach. Journal of Cleaner Production16:1735-1740

Nunes, A.M. (1967). A comparative study of drought resistance in cacao plants. *Ann. Bot.* 31(1): 189-193.

Obeng-Bio, E. (2010). Selection and ranking of local and exotic maize (*Zea mays* L.) genotypes to drought stress in Ghana. An MSc Dissertation, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Oduro-Afriyie, K. and Adukpo, D.C. (2006). Spectral characteristics of the annual mean rainfall series in Ghana. *West African Journal of Applied Ecology* 9(15): 1-9

Okali, D.U.U. and Owusu, J.K. (1975). Growth analysis and photosynthetic rates of cocoa (*Theobroma cacao* L.) seedlings in relation to varying shade and nutrient regimes. *Ghana J Agric Sci* 8:51-67.

Ogburn, R., Edwards E. J. (2010). The ecological water-use strate- gies of succulent plants. Advances in Botanical Research 55:179–225

Olyslaegers, G., Nijs, I., Roebben, J. *et al*. (2002). Morphological and physiological indicators of tolerance to atmospheric stress in two sensitive and two tolerant tea clones in South Africa. *Experimental Agriculture* 38: 397-4110

Opoku, S.Y., Bhattacharjee, R., Kolesnikova-Allen, M., Motamayor, J.C., Schnell, R., Ingelbrecht, I., Enu-Kwesi, L. and Adu-Ampomah, Y. (2007). Genetic diversity in cocoa (*Theobroma cacao* L.) germplasm collection from Ghana. *J. Crop Imp.* 20(1/2) (39/40): 73-87.

Orchard, J.E. and Saltos, M.R. (1988). The growth and water status of cacao during its first year of establishment under different methods of soil water management p 193-198. In: *Proc. 10th Int. Cocoa Res. Conf.*, Santo Domingo, Dominican Republic.

Osei-Bonsu, I. (2011). Evaluation of the drought tolerance levels of selected *Theobroma cacao* l. genotypes developed at the cocoa research institute, Ghana (CRIG) (Unpublished master's thesis). University of Ghana, Accra, Ghana.

Owusu, J.K. (1978). Light requirements of cocoa: a review, p 112-121. In: *Proc. Int. Conf. Cocoa and Coconuts*, Kuala Lumpur, Malaysia.

Padi, F. K., Adu-Gyamfi, P., Akpertey, A., Arthur, A., and Ofori, A., (2013). Differential response of cocoa (*Theobroma* cacao) families to field establishment stress. *Plant Breeding* 132(2): 229-326. Doi: 10.1111/pbr.12039

Passioura, J. (1997) Drought and drought tolerance. Drought tolerance in higher plants: genetical, physiological and molecular biological analysis. *Springer*, Netherlands

Peprah, T., Kyeremeh, B., Owusu, K.A. and Adu-Bredu, S. (2009). Drought tolerance of *Garcinia kola* and *Garcinia afzelii* at the seedling stage. *Ghana J. Forestry* 25:13- 27.

Ploetz, R.C. (2007). Cacao diseases: important threats to chocolate production worldwide. *Phytopathology* 97:1634–1639. doi: 10.1094/PHYTO-97-12-1634

Raja Harun, R.M. and Hardwick, K. (1988). The effect of different temperatures and water vapour pressure deficits on photosynthesis and transpiration of cocoa leaves. In: *Proc. 10th Int. Cocoa Res. Conf*. 211-214, Santo Domingo, Dominican Republic.

Riazi, A., Matsuda, K. and Arslan, A. (1985). Water-stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. *J. Exp. Bot.* 36:1716-1725 (abs). Retrieved May 16, 2011 from http://jxb.oxfordjournals.org/content/36/11/1716.abstract.

Robert, L.S., Parris, N.G. and Kathleen, K.T. (2000). Final report of the Maryland statewide water conservation advisory committee, November 2000, 10p.

Sack, L., and Holbrook, N. M. (2006). Leaf hydraulics. *Annual Review of Plant Biology* 57: 361–381

Sale, P.J.M. (1968). Flushing and leaf growth of cacao under controlled temperature conditions. *J. Hort. Sci.* 43:475-489.

Sale, P.J.M. (1970). Growth, flowering, and fruiting of cacao under controlled soil moisture conditions. *J. Hort. Sci.* 45:99-118.

Salter, R.J. and J.B. Goode. (1967). Crop response to water at different stages of growth. *Common Wealth Agric*. Farham Royals Bucks, England. p 246.

Sauter A, Davies W. J., Hartung W. (2001). The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. *Journal of Experimental Botany* 52:1991–1997

Sawidis, T., Kalyva, S., Delivopoulos, S. (2005). The root-tuber anatomy of Asphodelus aestivus. *Flora - Morphology Distribution Functional Ecology of Plants* 200:332–338

Schaller, J., & Paschold, P. J. (2009). Stomata-characteristics and responses to soil drought indicating a cultivar specific drought stress susceptibility in asparagus. *European Journal of Horticultural Science, 74*(4), 145–151.

Scott, T. (1979). Plant Regulation and World Agriculture (1 ed.) New York: Springer. Doi: 10.1007/978-1-4684-3512-2

Seiler, J.R. and Johnson, J.D. (1988). Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. *For. Sci.* 34:487- 495.

Sharma, K.K. and Lavanya, M. (2002) Recent developments in transgenics for abiotic stress in legumes of the semi-arid tropics, p 61-73. In: *JIRCAS Working Report.*

Shearman, R. C., and Beard, J. B. (1972) Stomatal Density and distribution in Agrostis as influenced by species, cultivar, and leaf blade surface and position. *Crop Science* 12: 822-823

Shen, B., Jensen, R.G. and Bohnert, H.J. (1997). Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiol.* 115:527-532.

Silva, M. De A., Jifon, J. L., Silva, J. A. G. da, and Sharma, Vivek. (2007). Use of physiological parameters as fast tools to screen for drought tolerance in sugar cane. *Brazilian Journal of Plant Physiology*. 19(3): 193-201

Soltys-Kalina, D., Jaroslaw, P., Strzelczyk-Zyta, D., Sliwka, J. and Marczewski, W. (2016) The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-deprived potato cultivers. *Breeding Science* 66(2):t 328-331

Specht, J.E., Hume, D.J. and Kumudini, S.V. (1999) Soybean yield potential — a genetic and physiological perspective. *Crop Sci.* 39:1560-1570.

Tardieu, F. (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Front Physiol 4:17

Toxopeus, H. (1985). Botany, types and populations. In G. A. R. Wood & R. A. Lass (Eds) Cocoa (4th ed) p11-37. Oxford: Blackwell Science

Troll, W. and Lindsley, J. (1955). A photometric method for the determination of proline. *J. Biol. Chem.* 215:655-660.

Turner, N.C. (1986). Adaptation to water deficits: a changing perspective. *Aust. J. Plant Physiol.* 13:175-190.

University of Queensland (2016). Parts of a Cocoa Tree (Online Image). Retrieved 15th May 2016 from http://www.uq.edu.au/_School_Science_Lessons/CocoaProj.html

Vajrabhaya, M., Kumpun, W. and Chadchawan, S. (2001). The solute accumulation: The mechanism for drought tolerance potential in RD23 rice (*Oryza sativa* L) lines. *Science Asia* 27:93-97.

Vernon, A.J. (1967). Yield and light relationships in cocoa. *Trop. Agric.* 44:223-228.

Vigneri, M. (2007). Drivers of cocoa growth production in Ghana. Overseas Development Institute (ODI) Project Briefing No.4, December 2007, 4p.

Waldren, R.P. and Teare, I.D. (1974). Free proline accumulation in drought-stressed plants under laboratory conditions. *Plant and Soil* 40(3):689-692.

Wang, F.Z., Wang, Q.B., Kwon, S.Y., Kwak, S.S. and Su, W.A. (2005). Enhanced drought tolerance potential of transgenic rice plants expressing a pea manganese superoxide dismutase. *J. Plant Physiol.* 162:465-472.

Weatherley, P.E. (1950). Studies in the water relations of the cotton plant. I. The field measurements of water deficits in leaves. *New Phytol.* 49:81-97.

Weatherley, P.E. (1951). Studies of the water relations of the cotton plant. II. Diurnal and seasonal variations in relative turgidity and environmental factors. *New Phytol*. 50:36-51.

Wicks, C. (2003). Cocoa. Fact sheet FF3. Northern Territory Government, Darwin, Australia. Retrieved March 25, 2011 from the Northern Territory Government database on the world wide web: http://www.nt.gov.au/d/Content/File/p/Fruit/FF3_cocoa.pdf.

Wood, G.A.R. and Lass, R.A. (2001). Cocoa (4th ed). p620. Oxford: Blackwell Science. Wiley

Xu, P., Chen, F., Mannas, J.P., Feldman, T., Sumner, L.W. and Roossinck, M.J. (2008). Virus infection improves drought tolerance. *New Phytologist*, 11p. Retrieved May 23, 2016 from the New Phytologist database on the world wide web: www.newphytologist.org.

Yamasaki, S., & Dillenburg, L. R. (1999). Measurements of leaf relative water content in Araucaria angustifolia. *Revista Brasileira de Fisiologia Vegetal*, *11*(2), 69–75.

Yokota, A., Takahara, K. and Akashi, K. (2006). Water stress, p 15-39. In: K.V.M. Rao, A.S. Raghavendra and K.J. Reddy (eds.) *Physiology and molecular biology of stress tolerance in plants.* Springer

Zhang, J. Y., Broeckling, C. D., Blancaflor, E. B., Sledge, M. K., Sumner, L. W., Wang, Z. Y. (2005) Overexpression of WXP1, a putative Medicago truncatula AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (Medicago sativa). Plant Journal 42:689–707

Appendices

Appendix 1: Mean Daily ambient air temperature and relative humidity at the study site during Experiment 1

	Ambient Air Temperature (°C)		Relative Humidity (%)	
Day	**Min**	**Max**	**Min**	**Max**
1	19	31	40.1	56.4
2	20	32	31.9	53.1
3	18	33	20.7	64.5
4	17	33	16.8	52.2
5	19	34	22.0	52.2
6	19	33	37.6	51.1
7	18	34	14.6	62.2
8	22	33	42.2	84.4
9	22	33	42.2	92
10	22	33	42.2	92
11	21	31	40.1	84.7
12	22	34	42.2	85.0
13	22	33	42.2	85.3
14	24	33	47.0	66.6
15	20	33	37.6	84.7
16	17	33	16.8	84.4
17	21	33	33.1	66.6
18	20	33	33.1	66.6
19	20	33	33.1	71.7
20	20	30	49.4	65.2
21	20	29	48.4	85.0
22	21	33	33.1	78.3
23	22	33	37.6	66.6
24	22	33	37.6	66.6
25	20	33	47.0	66.6

Appendix 2: Mean daily ambient air temperature and relative humidity at the study site during Experiment 2

	Ambient Air Temperature (°C)		Relative Humidity (%)	
Day	**Min**	**Max**	**Min**	**Max**
1	25	33	63.3	85.0
2	26	32	62.6	85.6
3	26	32	62.7	82.5
4	25	33	63.3	82.5
5	24	33	62.6	79.5
6	24	34	66.0	91.9
7	25	33	62.0	62.7
8	27	34	52.8	85.6
9	27	32	67.7	79.2
10	27	33	62.7	79.2
11	27	32	63.3	85.6
12	27	35	58.6	85.6
13	27	34	58	82.5
14	27	34	63.3	79.5
15	27	34	67.7	82.5
16	27	34	62.7	84.4
17	27	32	63.3	84.4
18	26	33	63.3	82.5
19	26	34	67.7	85.6
20	25	34	62.7	84.4
21	24	32	62.7	84.4

Appendix 3a: Raw data for Phase I RWC

CATEGORY	VARIETIES	Control	mean	SE	Stress (initial)	mean	S.E.	Stress (FADS)	mean	S.E.
HDTP	(a)	91.880	85.807	6.12565371	92.250	90.533	4.03859299	64.500	64.567	2.900575
		79.630			93.430			61.700		
		85.910			85.920			67.500		
	(d)	86.340	82.350	3.56697351	85.400	91.765	5.83953551	71.300	66.700	6.505382
		79.470			93.020			62.100		
		81.240			96.875					
MDTP	(c)	78.260	78.993	1.13443084	82.738	77.491	7.66875238	58.800	42.767	14.49218
		80.300			81.045			38.900		
		78.420			68.690			30.600		
	(g)	86.270	80.603	4.97225636	81.990	86.773	5.8743028	56.200	55.467	0.80829
		78.570			85.000			55.600		
		76.970			93.330			54.600		
	(b)	92.590	91.640	3.25083066	90.710	89.273	3.10516237	65.700	63.567	3.354599
		88.020			85.710			65.300		
		94.310			91.400			59.700		
	(e)	90.900	88.523	8.1298606	78.260	79.990	3.63784277	83.300	59.700	24.06263
		79.470			84.170			60.600		
		95.200			77.540			35.200		
LDTP	(h)	78.620	81.690	4.08774999	88.570	86.100	6.62011329	55.800	51.087	14.17062
		86.330			78.600			62.300		
		80.120			91.130			35.160		
	(i)	84.210	86.447	4.65695537	83.890	84.377	1.81948711	85.900	61.233	22.95416
		83.330			82.850			57.300		
		91.800			86.390			40.500		
	(f)	87.500	88.233	3.99085622	89.510	88.397	2.1484956	64.2	52.633	29.2206
		84.660			85.920			74.3		
		92.540			89.760			19.4		

Appendix 3b: Raw data for Phase I SMC

CATEGORY	VARIETIES	Control	mean	S.E.	Stress (initial)	mean	S.E.	Stress (FADS)	mean	S.E.
HDTP	(a)	8.610	10.623	1.86081523	13.230	13.037	0.23158872	0.640	0.640	
		10.980			12.780					
		12.280			13.100					
	(d)	12.470	12.210	1.12280898	10.920	12.443	1.32039136	0.940	1.230	0.41012193
		13.180			13.150			1.520		
		10.980			13.260					
MDTP	(c)	16.680	13.513	3.30248896	11.070	11.917	1.02065339	0.240	1.080	1.08129552
		10.090			13.050			0.700		
		13.770			11.630			2.300		
	(g)	11.470	12.737	1.72401663	9.860	11.433	1.95359498	2.070	1.473	0.58071795
		12.040			10.820			0.910		
		14.700			13.620			1.440		
	(b)	13.75	12.083	1.73347435	12.480	12.567	1.82154696	1.380	1.287	0.43753095
		10.29			14.430			0.810		
		12.21			10.790			1.670		
	(e)	14.39	13.480	0.91	13.890	12.016	2.75885266	0.970	2.600	2.69438305
		12.57			13.310			1.120		
		13.48			8.848			5.710		
LDTP	(h)	14.69	13.370	1.23223374	12.490	13.743	1.19089602	1.620	1.200	0.45299007
		12.25			13.880			1.260		
		13.17			14.860			0.720		
	(i)	11.45	12.803	1.84892762	11.470	13.693	1.97849269	1.540	0.983	0.66289768
		12.05			14.350			1.160		
		14.91			15.260			0.250		
	(f)	9.33	11.460	1.9048097	11.990	11.418	0.52448673	1.890	1.307	0.51403632
		13			11.303			0.920		
		12.05			10.960			1.110		

Appendix 4a: Raw data for Phase II RWC

CATEGORY	VARIETIES	Control	mean	S.E.	Stress (initial)	mean	S.E.	Stress (FADS)	mean	S.E.
HDTP	(a)	88.460	91.263	6.09901905	85.710	85.927	1.19976387	66.929	55.133	10.91714
		87.070			87.220			45.385		
		98.260			84.850			53.086		
	(d)	78.510	85.343	5.92543951	88.000	79.817	12.9385406	59.460	55.220	5.616556
		89.060			86.550			57.350		
		88.460			64.900			48.850		
MDTP	(c)	80.000	84.870	7.16726587	78.110	87.877	8.5069932	49.650	51.118	13.97829
		93.100			93.670			37.931		
		81.510			91.850			65.772		
	(g)	88.300	90.400	6.37491961	91.280	91.837	3.86518219	56.875	56.173	0.993485
		97.560			95.950			55.470		
		85.340			88.280					
	(b)	94.390	95.433	0.92435563	85.080	86.533	10.5056667	48.052	42.803	5.276158
		95.760			97.690			42.857		
		96.150			76.830			37.500		
	(e)	86.730	87.673	1.79204725	90.680	89.597	1.38954429	78.290	65.545	18.02415
		86.550			90.080			52.800		
		89.740			88.030					
LDTP	(h)	80.670	86.800	9.69721094	90.100	86.737	4.40534145	58.900	58.160	5.239342
		97.980			88.360			52.590		
		81.750			81.750			62.990		
	(i)	89.660	87.990	4.80769175	86.090	86.057	0.9504385	40.510	51.865	16.0584
		91.740			85.090			63.220		
		82.570			86.990					
	(f)	84.430	84.157	0.24193663	84.780	87.660	4.51202837	39.840	47.000	21.98266
		84.070			92.860			29.490		
		83.970			85.340			71.670		

Appendix 4b: Raw data for Phase II SMC

CATEGORY	VARIETIES	Control	mean	S.E.	Stress (initial)	mean	S.E.	Stress (FADS)	mean	S.E.
HDTP	(a)	6.480	8.487	1.74712144	5.450	6.350	1.4309088	0.880	0.810	0.11269428
		9.310			5.600			0.680		
		9.670			8.000			0.870		
	(d)	9.630	8.727	1.37026761	5.790	7.107	1.351234	0.650	0.673	0.31564748
		7.150			7.040			1.000		
		9.400			8.490			0.370		
MDTP	(c)	8.470	9.060	3.98786911	6.820	7.660	2.52697448	0.520	0.497	0.0321455
		13.310			5.660			0.510		
		5.400			10.500			0.460		
	(g)	9.720	9.523	1.15759809	5.270	8.040	2.42802389	0.680	0.371	0.43699199
		8.280			9.800					
		10.570			9.050			0.062		
	(b)	8.170	7.683	1.4142607	9.350	9.507	0.90522557	0.660	0.663	0.07505553
		8.790			10.480			0.740		
		6.090			8.690			0.590		
	(e)	7.150	7.003	0.32578111	7.000	7.500	0.78102497	1.690	1.520	0.24041631
		7.230			8.400			1.350		
		6.630			7.100					
LDTP	(h)	11.130	9.103	1.85165692	7.570	9.513	2.40998617	0.540	0.667	0.14843629
		8.680			12.210			0.630		
		7.500			8.760			0.830		
	(i)	8.110	11.467	7.8993312	9.500	10.260	0.78619336	0.510	0.570	0.08485281
		5.800			10.210			0.630		
		20.490			11.070					
	(f)	6.650	7.660	1.9073804	10.220	8.303	1.67506219	0.480	0.540	0.21633308
		9.860			7.120			0.360		
		6.470			7.570			0.780		

Appendix 5a: Summary of results for Phase I (Experiment I)

	1 DAFS		FADS		
Genotype	**RWC**	**SMC**	**DAFS**	**RWC**	**SMC**
a	90.53 ± 4.04	13.03 ± 0.22	16.3 ± 1.15	64.59 ± 0.23	0.66 ± 0.02
b	89.27 ± 3.11	12.57 ±1.82	13.7 ± 5.50	63.57 ± 1.82	1.29 ± 0.44
c	77.49 ± 7.67	13.51 ± 3.30	14.0 ± 4.24	42.76 ± 3.30	1.50 ± 1.13
d	91.77 ± 5.84	12.44 ± 1.33	14.7 ± 7.50	66.70 ± 1.33	1.23 ± 0.41
e	79.99 ± 3.64	11.56 ± 2.78	13.7 ± 5.50	59.70 ± 2.78	2.47 ± 2.81
f	88.39 ± 2.15	11.42 ± 0.53	11.3 ± 3.5	52.63 ± 0.53	1.31 ± 0.51
g	83.43 ± 1.52	10.83 ± 1.95	14.0 ± 4.24	55.47 ± 1.95	1.48 ± 0.60
h	86.10 ± 6.60	13.74 ± 1.29	13.3 ± 2.01	51.09 ± 1.19	0.99 ± 0.60
i	84.38 ± 1.81	13.69 ± 1.98	12.7 ± 4.50	61.23 ± 1.98	0.98 ± 0.67

Appendix 5b: Leaf Relative Water Content and Soil Moisture Content at 1DAFS and FADS for Phase II

	1 DAFS		FADS	
Genotype	**RWC**	**SMC**	**RWC**	**SMC**
a	85.927 ± 1.21	8.487 ± 1.75	55.13 ± 10.92	0.810 ± 0.11
b	86.533 ± 10.50	7.683 ± 1.41	42.80 ± 5.28	0.663 ± 0.08
c	87.877 ± 8.50	9.060 ± 3.99	51.12 ± 13.98	0.497 ± 0.03
d	79.817 ± 12.94	8.727 ± 1.37	55.2 ± 5.62	0.673 ± 0.32
e	89.597 ± 1.34	7.003 ± 0.32	65.55 ± 18.02	1.520 ± 0.24
f	87.660 ± 4.51	7.660 ± 1.9	47.00 ± 21.98	0.540 ± 0.22
g	91.837 ± 3.87	9.523 ± 1.15	56.17 ± 0.99	0.371 ± 0.44
h	86.737 ± 4.41	9.103 ± 1.85	58.16 ± 5.24	0.667 ± 0.15
i	86.057 ± 0.90	11.467 ± 7.90	51.87 ± 16.06	0.570 ± 0.08

Appendix 5c: Leaf Anatomical Data (i.e. widths of the upper epidermis, mesophyll, lower epidermis and presence of trichomes and trichome number) for the selected genotypes

Category	VARIETIES	Upper Epidermal Width (µm)	Mesophyll Width (µm)	Lower Epidermal Width(µm)	Trichomes	Trichome number
High	(a)	20.380	84.810	8.800	present	1.000
		10.480	47.050	10.040	present	3.000
		17.040	81.600	9.720	present	4.000
	mean	15.967	71.153	9.520		2.667
	standard deviation	4.112	17.094	0.526		1.247
	(d)	18.790	67.990	9.100	present	6.000
		27.100	90.580	12.530	present	1.000
		25.710	90.590	12.370	present	4.000
	mean	23.867	83.053	11.333		3.667
	standard deviation	3.634	10.651	1.581		2.055
Medium	(c)	23.740	76.020	13.130	present	2.000
		23.360	99.930	11.240	present	1.000
		19.350	78.810	11.090	present	3.000
	mean	22.150	84.920	11.820		2.000
	standard deviation	1.986	10.675	0.928		0.816
	(g)	18.160	52.710	12.140	absent	
		15.740	49.340	10.740	absent	0.000
		15.270	40.760	11.450	present	1.000
	mean	16.390	47.603	11.443		0.500
	standard deviation	1.266	5.031	0.572		0.500
	(b)	23.66	76.52	14.06	absent	0
		23.69	85.94	13.8	present	4
		20.79	89.13	17.19	present	2

mean	22.713	83.863	15.017		2.000
standard deviation	1.360	5.353	1.540		1.633
(e)	15.45	59.82	11.44	present	2
	21.8	92.84	13.07	present	3
	20.66	95.99	12.55	present	1
mean	19.303	82.883	12.353		2.000
standard deviation	2.764	16.359	0.680		0.816
(h)	15.05	41.4	11.98	present	3
	20.62	53.4	11.27	present	3
	19.86	76.96	14.13	present	4
mean	18.510	57.253	12.460		3.333
standard deviation	2.466	14.771	1.216		0.471
(i)	16.72	103.48	10.69	absent	0
	21.96	79.21	10.78	present	1
	15.84	90.6	11.55	absent	0
mean	18.173	91.097	11.007		0.333
standard deviation	2.702	9.914	0.386		0.471
(f)	22.22	76.43	14.83	present	1
	19.01	69.89	12.87	present	3
	13.35	75.62	10.9	Absent	0
mean	18.193	73.980	12.867		1.333
standard deviation	3.667	2.911	1.604		1.247

Appendix 5d: Summary of data for parameters measured in both Phase I and II of this study

Genotype	Phase I		Phase II						
	RWC %	SMC %	RWC %	SMC %	Mean number of Trichomes	Upper Epidermis	Mesophyll	Lower Epidermis	Proline
a	64.59 ± 0.23	0.66 ± 0.02	55.13 ± 10.92	0.810 ± 0.11	2.67 ± 1.25	15.97 ± 4.11	71.15 ± 17.09	9.52 ± 0.53S	3.49 ± 0.90
b	63.57 ± 1.82	1.29 ± 0.44	42.80 ± 5.28	0.663 ± 0.08	2.00 ± 1.63	22.71 ± 1.36	83.86 ± 5.35	15.02 ± 1.54	2.47 ± 0.39
c	42.76 ± 3.30	1.50 ± 1.13	51.12 ± 13.98	0.497 ± 0.03	2.06 ± 0.82	22.15 ± 1.99	84.96 ± 10.68	11.82 ± 0.93	2.27 ± 0.15
d	66.70 ± 1.33	1.23 ± 0.41	55.2 ± 5.62	0.673 ± 0.32	3.67 ± 2.06	23.87 ± 3.63	83.05 ± 10.65	11.33 ± 1.58	3.69 ± 0.50
e	59.70 ± 2.78	2.47 ± 2.81	65.55 ± 18.02	1.520 ± 0.24	2.00 ± 0.82	19.303 ± 2.76	82.88 ± 16.36	12.35 ± 0.680	2.37 ± 0.05
f	52.63 ± 0.53	1.31 ± 0.51	46.94 ± 10.31	0.540 ± 0.22	1.33 ± 1.25	18.19 ± 3.67	73.98 ± 2.91	12.87 ± 1.60	1.17 ± 0.36
g	55.47 ± 1.95	1.48 ± 0.60	56.17 ± 0.99	0.371 ± 0.44	0.50 ± 0.5	16.39 ± 1.27	46.60 ± 5.03	11.44 ± 0.57	2.06 ± 0.22
h	51.09 ± 1.19	0.99 ± 0.60	58.16 ± 5.239	0.667 ± 0.15	3.30 ± 0.47	18.51 ± 2.47	57.25± 14.771	12.46 ± 1.22	1.03 ± 0.49
i	61.23 ± 1.98	0.98 ± 0.67	51.87 ± 16.058	0.570 ± 0.08	0.33 ± 0.47	18.17 ± 2.7	91.10 ± 9.91	11.01 ± 0.39	0.56 ± 0.27