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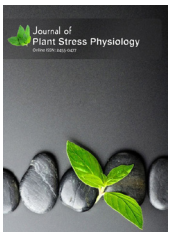

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Effects of watering regime on the morphological, physiological and functional traits of seedlings of cacao provenances under screen house conditions

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ABSTRACT

In the present study, morphological and physiological responses of cocoa provenances to watering regimes under screen house conditions and the implications of the measured variables as drought tolerance strategy in *Theobroma* was discussed. A 4 by 3 factorial scheme involving four cacao provenances and watering regimes (well watering at full field capacity, 60 and 40% field capacity: 1.5, 0.9 and 0.6 L/plant at each watering event) the *cocoa* genotypes evaluated are PA 150 Series (the elite varieties), F3 Amazon and Amelonado. Observations were made on the morphological and physiological traits of seedlings of the cacao genotypes affected by watering regimes. The measured variables were deployed to rank the drought performance of cacao genotypes following nursery desiccation studies. Data on root and shoot biomass, water use, stomatal conductance, proline, water soluble carbohydrate and leaf chlorophyll concentrations of cacao seedlings were collected. The results showed that root zone moisture status affected the morphological and physiological characteristics of cacao provenances. Differences were obtained in root and shoot biomass, water use, the densities of stomatal and its conductance of gases, and the concentrations of leaf chlorophyll, and shoot and leaf proline and water soluble carbohydrates among the watering regimes imposed. Cacao provenances evaluated also differed in their responses to watering regimes and in morphological and physiological characters. The imposed root zone moisture scenarios elicited differences in the responses of cacao provenances evaluated. Most of the measured morphological and physiological variables were driven by root zone moisture status among cacao provenances, the measured traits appeared to have played important roles as root zone moisture deficit stress tolerance mechanisms in cacao. Seedlings of cocoa provenances had better vigour of growth when grown under 100 and 60% field capacity watering compared with 40% FC. Adequacy of soil moisture promotes growth and physiological functions in the seedlings of cacao provenances tested. The measured morpho-physiological variables were statistically superior under well watered situations (100% FC) compared with the 40% FC. The results confirmed that cocoa seedlings cannot withstand soil moisture deficit stress as was obtained for seedlings that were watered with 40% FC. It is recommended that watering cacao seedlings at full field capacity (FC) and at 70% FC (mild root zone moisture stress) will ensure the production of vigorous seedlings of cacao in the nursery.

KEYWORDS: *Theobroma*, provenances, root zone, moisture, growth, physiology, biochemistry, tolerance

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INTRODUCTION

Cacao (*Theobroma cacao* L.), belongs to the family Sterculiaceae and the genus *Theobroma*. However, due to advances in molecular marker technique in recent times, cacao has been reclassified to the family Malvaceae. The natural habitat of cacao is the lower storey (understorey) of the evergreen rainforest of the tropics where it originated. The cultivation of cacao has spread within the tropics including South and Central America, Asia and West Africa, since its discovery in the 18th century in the Amazon basin (Alvim, 1977; Opeke, 2006). Cocoa is a major cash crop in many tropical countries and it is produced within 10° N and 10° S of the equator where the climate is suitable for its growth. Globally the six main world cocoa producers are Ivory Coast, Ghana, Indonesia, Nigeria, Brazil, and Cameroon. West Africa is an important center of cocoa cultivation for many decades, as two-thirds of the world's cocoa is produced in the region (Opeke, 2006; Agele *et al.*, 2018).

Cocoa seedlings are raised in nurseries by smallholder farmers for planting out on the field to meet the increasing demand for vigorous seedlings for the establishment of new plantations or rehabilitation of old/moribund fields. Therefore it is necessary to develop sustainable nursery management practices for the production of vigorous cocoa seedlings. Cocoa seeds are sown in pots in the nursery to raise seedlings which are subjected to variable watering regimes (soil moisture status and wet-dry cycles) while in the nursery. The watering regimes under which seedlings are raised in the nursery affect their vigour of growth (Famuwagun *et al.*, 2017; Agele *et al.*, 2018).

There is an increasing need to establish new cocoa fields, and or rehabilitate old and moribund plantations using more productive cocoa stock for increased productivity. The success of the establishment and rehabilitation of cocoa farms, aimed at replacing ageing and non-productive cocoa stocks in the field may be limited by the inadequacy of healthy cocoa seedlings (CRIN, 2010; Agele *et al.*, 2016; Famuwagun *et al.*, 2017). The vigour of seedlings plays an important role in the establishment, survival percentage and growth following transplanting on the field.

Daymond and Hadley (2008) and Tezara *et al.* (2016) reported that varietal improvement for tolerance to abiotic and biotic stress factors has been identified as research priority programs for cocoa producing countries. Thus, cocoa varieties that are high yielding, and tolerant to pests and diseases and environmental stresses have been developed (CRIN, 2010).

Cocoa is highly sensitive to changes in climate from hours of sunshine to rainfall and application of water, soil condition and temperature (Almeida *et al.*, 2002; Acheampong *et al.*, 2013, Tezara *et al.*, 2020). In nature, water is usually the most limiting factor for plant growth. If plants do not receive adequate rainfall or irrigation, the resulting drought stress can reduce growth more than all other environmental stresses combined. Inadequate root zone moisture status halts plant growth and development resulting in reduced vigour and yield (Daymond & Hadley, 2008; Agele *et al.*, 2018). As water loss progresses, leaves of some species may change color, wilt and, if the plant is not irrigated, leaves will fall off followed by death (mortality) (Tezara *et al.*, 2016; Almeida *et al.*, 2018).

In plants, changes in the morphological and physiological traits of annual and perennial species under drought or soil moisture deficit have been evaluated severally (Glenn *et al.*, 2014; Tezara *et al.*, 2016). These reports affirmed that plants exhibit adaptive strategies to cope with root zone moisture deficit stress and recovery following stress alleviation via rewatering (Tyree *et al.*, 2003; Glenn *et al.*, 2014; Haeberle *et al.*, 2016). These strategies may involve complex, interacting mechanisms (e.g. desiccation tolerance and drought performance) (Tyree *et al.*, 2003; Li & Liu, 2016; Tombesia *et al.*, 2018).

Root-zone moisture deficits impinge on stomatal density, a parameter that is closely and inversely correlated with a starch concentration in roots and trunks of plants (Putra *et al.*, 2012). It has been suggested that the carbohydrate reserve status of plants may be an important endogenous determinant of stomatal density. Putra *et al.* (2012) found that the stomatal density of newly emerging leaves plants grown in dry and warm soil increased after the treatment had been removed. The differences in stomatal densities have consequences on stomatal gas exchange (conductance of the stomatal to gases, g) (Putra *et al.*, 2012; Glenn *et al.*, 2014). In addition, it has been reported that the low stomatal conductance characteristic of a dry root-zone environment may not be due to low stomatal density alone but also to the stomatal aperture (Putra *et al.*, 2012; Glenn *et al.*, 2014).

Physiological variables such as water relations and stomatal gas exchange are important to plant survival under unfavourable environmental conditions (Agele *et al.*, 2016; Haeberle *et al.*, 2016; Tezara *et al.*, 2020). Limitation of the water supply has an impact on photosynthesis, plant growth, and yield production in plants, and drought is an important environmental stress which affects various levels of plant metabolism (Sheffield *et al.*, 2012; Witt *et al.*, 2012; Glenn *et al.*, 2014; Li & Liu, 2016; Zhang *et al.*, 2017). The effects of limitation in soil moisture status on phytochemistry, especially parameters such as total soluble solids, soluble sugar, organic acids and vitamin C has been reported (Khan *et al.*, 2015).

It is reported that soluble carbohydrates (sugars), other metabolites and osmolytes increase under drought (Keller & Ludlow, 1993; Hoekstra *et al.*, 1994; Bray, 1997; Watanabe *et al.*, 2000; Garcia-Sanchez *et al.*, 2007; LiXin *et al.*, 2009; Khan *et al.*, 2015). In other studies, Mafakheri *et al.* (2010), Budak *et al.* (2015) and Soni *et al.* (2015) asserted that fluctuations in metabolic pools of carbohydrates and amino acids have implications for drought tolerance in plants via the activation of osmotic adjustment (Boyer *et al.*, 2008; Scalabrin *et al.*, 2015). Increases in the quantities of total soluble sugars occur in proportion to the intensity of moisture deficits (Scalabrin *et al.*, 2015). Soluble carbohydrates (sugars) protect plants against water shortage induced damage to proteins and cell membranes (Sawhney & Singh, 2002) maintain leaf turgidity (Khan *et al.*, 2015) and serves to activate protective enzymes (Li & Liu, 2016).

The accumulation of osmolytes exerts protection against drought stress (Yancy *et al.*, 1982; Herbinger *et al.*, 2002; Verbruggen & Hermans, 2008). For example, proline accumulation in plant tissue serves as a marker and an important part of the stress signal influencing adaptive responses to environmental stress, particularly in plants under drought stress (Routley, 1966; Sanchez *et al.*, 1998; Maggio *et al.*, 2002; Mafakheri *et al.*, 2010). Osmolytes accumulation offers protective functions to plants under environmental stress (Tokihiko *et al.*, 2003). Studies have shown that proline content increased under drought stress in peas (Sanchez *et al.*, 1998; Verbruggen & Hermans, 2008) while proline accumulation has also been obtained for plants under high temperature and poor soil fertility status. Severe wilting under soil drought has been reported to stimulate proline synthesis and accumulation of carbohydrates (Routley, 1966; Mafakheri *et al.*, 2010).

In addition to ecophysiology, various fields of omics including molecular biology, transcriptomics and metabolomics have been deployed to clarify mechanisms of abiotic stress tolerance in plants (Cramer *et al*., 2007; Kantar *et al*., 2011; Wang *et al*., 2015). Results affirmed the potential of metabolic pathways and their manipulation for ameliorating adverse effects of root zone moisture deficits on plant performance (Budak *et al*., 2015; Soni *et al*., 2015; Zhang *et al*., 2017). Drought-induced accumulated solutes offer plant protective functions in response to environmental stresses, as was reported in studies with Arabidopsis (Tokihiko *et al*., 2003) and grapevine (Kantar *et al*., 2011; Soni *et al*., 2015; Chmielewska *et al*., 2016).

Changes in chlorophyll and carotenoid contents have served as an index for the evaluation of plant response to drought or soil moisture deficit stress (Pastori & Trippi, 1992; Kpyoarissis *et al*., 1995; Zobayed *et al*., 2005; Khayatnezha & Gholamin, 2012). Decreases in chlorophyll concentration are referred to as a non-stomatal limiting factor for plants under drought stress (Pastori & Trippi, 1992; Kpyoarissis *et al*., 1995). Ommen *et al.* (1999) and Agele *et al.* (2018) reported that drought stress caused a large decline in leaf chlorophyll a, b and total chlorophyll content in cacao. The decrease in chlorophyll under drought stress has been ascribed to damage to chloroplasts caused by active oxygen species (Smirnoff, 1995; Khayatnezha & Gholamin, 2012).

The implications of soil moisture deficit stress on stomatal gas exchange in plants have been variously reported (Berninger *et al*., 1996; Daymond & Hadley, 2008; Miranda *et al*., 2013; Martin-StPaul *et al*., 2017). Reports of gas exchange variable measurements (net CO_2 assimilation rate (A), stomatal conductance (g_s) and transpiration (E) of leaves) spanning daytime hours (early morning, midday and late afternoon periods) have confirmed the effects of various root zone moisture status (Cuevas *et al*., 2006; Glenn *et al*., 2014; Agele *et al*., 2016). Stomatal conductance increases with sunlight intensity from sunrise, attaining a maximum at mid-morning followed by midday depression (Tyree *et al*., 2003; Agele *et al*., 2016; Haeberle *et al*., 2016). The decline in stomatal conductance (gs) just after sunrise toward midday had been attributed to increases in leaf temperature and vapor pressure deficit and incident photon flux density (PPFD) which increased after sunrise and reached a maximum around noon (Cuevas *et al*., 2006; Agele *et al*., 2016; Haeberle *et al*., 2016). Changes in stomatal conductance under root-zone moisture deficits appear as a regulatory signal for transpirational water loss (Tyree *et al*., 2003; Agele *et al*., 2016; Li & Liu, 2016). Changes in the daytine course of gas exchange have been reported for plant species including forest trees (Cuevas *et al*., 2006; Haeberle *et al*., 2016). The response of gs and leaf transpiration characterized by midday depression is known to increase daily water-use efficiency (Cuevas *et al*., 2006; Agele *et al*., 2016) while the midday depression of plant water status (water potential), stomatal conductance and photosynthesis have important consequences for ecosystem water and carbon exchange (Agele *et al*., 2016; Haeberle *et al*., 2016). Several studies have reported the midday and afternoon reductions in stomatal conductance as a common phenomenon across species, and that this phenomenon be incorporated leaf-level stomatal regulation into process-based models of ecosystem gas exchanges of forest trees (Tyree *et al*., 2003; Glenn *et al*., 2014; Trenberth *et al*., 2014; Li & Liu, 2016).

Drought affects the establishment, growth and yield of cocoa (Tezara *et al*., 2016), especially during the juvenile stage (Almeida *et al*., 2016; Famuwagun *et al*., 2017). Inadequate (sub-optimal) water application may profoundly affect the vigour of cacao seedlings (growth, development and survival rate) Improved insight is required for the adjustment of physiological processes for enhanced tolerance of drought including photochemical compounds of cacao (Daymond *et al*., 2011; Tezara *et al*., 2020).

Can the expression of physiological attributes of cacao provenances, varieties and clones to drought suggest differences in drought tolerance? Are the morphological (growth, development) and physiological traits (water use, leaf proline, chlorophyll and water soluble carbohydrate contents) measured indicators of the sensitivity of cacao provenances to variables of soil moisture status (adequacy and deficits)? It is hypothesized that during episodes of drought (root zone moisture deficit stress), changes in physiology and functional attributes would cause physiological plasticity with implications for drought tolerance in cacao provenances.

This study aims to investigate morphological and physiological responses of cocoa provenances to watering regimes under screen house conditions and the implications of the measured variables as drought tolerance strategy in *Theobroma*.

MATERIALS AND METHODS

Experiments were conducted at the Nursery and Experiment Station of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. Two months old potted cacao seedlings were subjected to three watering regimes in the screen house. The cacao provenances are Amelonado and F3 Amazon and two elite lines (PA 150/34 and PA 150/36).

Planting Materials, Experimental Design and Treatments

Seedlings of the cacao genotypes were obtained from the Cocoa Research Institute of Nigeria (CRIN) in Ibadan, Nigeria. The seeds of the cacao genotypes were planted in polythene pots (10cm diameter x 30cm length) filled with topsoil obtained from fallow vegetation. After 16 weeks at the nursery, the seedlings were later transplanted into standard plastic pots (25 x 15cm) with drainage holes at the bottom and transferred to the screen house. The seedlings of cacao genotypes were subjected to wet-dry cycles. The seedlings were well watered before treatments were imposed.

Treatments consisting of 4 x 3 factorial combinations of 4 cacao genotypes by 3 watering regimes were assigned using a complete randomized design with five replications. Cacao seedlings were subjected to three (3) field capacity percentages; 100, 60 and 40%. The full field capacity (100% FC: 1.5 L/plant), 60% field capacity (0.9 L/plant) and 40% field capacity (0.6 L/plant) were applied once a week throughout the experiment. To maintain the

preferred percentage FC in the soil, water was added whenever the water level is lower than the required field capacity conditions.

Data Collection

Measurement of agronomic variables started 4 weeks after transplanting (WAT) and lasted for 10 months before they were transplanted to field plots.

Water use characters of cacao

Seedling water use was by weighing method, the weights of the potted seedlings were measured using a weighing balance before watering and a day after watering. The differences between measurement periods were carefully recorded. Plant water use (kg) was determined using a weighing balance before and 24 hours after watering.

Growth parameters

Cacao height, number of leaves, leaf area and number of branches were measured weekly. Plant height was measured from the base to the crown of the plant using a meter tape graduated in millimetres. The total number of leaves was obtained by counting. Leaf area was measured on a Leaf area Meter (Delta T, UK). The average leaf area measured was multiplied by the total number of leaves on the plant to obtain the total leaf area available for photosynthesis. The total number of branches was also determined by counting. For root and shoot biomass determination, cacao seedlings were gently uprooted and separated into roots and shoots, then oven-dried separately at 60 °C for 48 hours and re-weighed to obtain the dry weight of samples. The mass of each fraction was then averaged to obtain the corresponding final dry mass per plant. The final biomass was a combination of root and shoot dry masses:

$$Biomass\,of\ plant(g) = root\,DM(g) + shoot\,DM(g) + fruit\,DM(g) \quad (1)$$

where DM = dry mass.

Stomata characters of cacao

Stomatal densities

The Impression Method uses clear nail polish to make an impression or cast of the leaf surface placed on a microscope slide. Leaf samples were harvested from each treatment replication and were placed into plastic envelopes and transported immediately to the laboratory, where leaf surface impressions were taken using clear fingernail polish. The upper and lower surfaces of leaves were identified as they are under normal conditions. Clear nail polish was spread as a thin layer on each surface, upper side and lower side, the leaf surface and left to dry. The casts of fully expanded mature leaves were made by pressing leaf sections onto a microscope. The number of stomata of the sampled leaves was counted using a light microscope, and stomatal density was averaged per ring and viewed under the microscope (100 or 400x magnification). The number of stomata of the sampled leaves was counted using a light microscope, and stomatal density was averaged per ring.

Stomatal conductance

Stomatal conductance was measured using a steady state porometer device (Delta T, UK). Seedling water use was estimated by a weighing method in which the weights of the potted seedlings were measured using a weighing balance before watering and 24 hours day after watering. The differences between measurements were recorded. Soil moisture content of each watering treatment was determined using soil moisture sensor (Decagon Device, USA), at a three weeks interval. The ambient air temperature during the period of analysis was measured using ordinary mercury in a glass thermometer, suspended at 1.5 m above ground level.

Proline content

The proline content was determined following the free proline accumulation method. Plant samples were harvested, and approximately 100 mg of fresh samples were deployed for analysis. Plant samples can be snap frozen in liquid nitrogen and when necessary stored below 80 °C. The plant materials are ground and kept in tubes and stored on ice. The samples are centrifuged for 5 min at room temperature using a benchtop centrifuge with maximum speed. Afterwards, exactly 100 µL of 3% sulfosalicylic acid and 200 µLglacial acetic acid is added in addition to 200 µL acidic ninhydrin to 100 µl to the supernatant of the plant extract and properly mixed while the tubes are incubated at 96 °C for 60 mins. Plant samples are extracted with toluene (1 mL toluene which was added to the mixture in the tube). Samples are taken and vortexed for 20 seconds, and left on the bench for 5 min to allow the separation of the organic and water phases. The chromophore containing toluene is removed into a fresh tube while the absorbance of the extract is measured using a Spectrophotometer at 520 nm using toluene as a reference. The proline concentration was determined using a standard concentration curve and calculated on a fresh weight basis (usually expressed as microgram per gram FW or micromole per gram FW: µmoles g^{-1}).

Relative water content

After harvesting, the samples were immediately weighed (Wf). Plant samples were then oven dried at 70°C for 2 days and dry weight was calculated (Wd). Then their average was calculated.

$$\text{Relative Water Content} = (Wf - Wd)/Wt \times 100 \quad (2)$$

Where Wf is fresh weight and wd is dry weight of samples.

Determination of chlorophyll

The leaf chlorophyll concentration was determined using the acetone method. Leaf samples were collected from intact leaves still attached, and placed in vials with 10 mL 95% ethanol in the cold room for 24 hours for chlorophyll extraction and chlorophyll determination. Leaf chlorophyll was extracted and determined using leaf samples from the uppermost leaves of the

cacao genotypes from each treatment. One gram of the fresh plant samples was cut into pieces and smashed in a mortar. The samples were put in a test tube and their chlorophyll content was repeatedly extracted with successive volumes of 100 mL acetone/water (80:20 v/v) until no traces of green colour were noticed (residue became white). While adding the solvent (acetone), the test tubes containing the samples were kept boiling in a hot water bath. The total volume of the extract was also recorded at the end of the extraction. Three millimeters (3 mL) of the extract was taken and the absorbance of chlorophyll was determined with a spectrophotometer at two wavelengths of 663 nm and 645 nm that corresponds to the maximum absorption of chlorophyll "a" and "b" respectively. The total chlorophyll content was calculated as follows:

$$\text{Total chlorophyll content (mg/100 g tissue)} = (20.2A_{645} + 8.02A_{663})\ (V/10\ w) \quad 3$$

Where, A645 = absorbance at 645 nm wavelength; A663 = absorbance at 663 nm wavelength; A = absorbance, C_a = chlorophyll a, C_b = chlorophyll b, C_{a+b} = total chlorophyll, V is the final volume (cm^3) of chlorophyll extract in 80% acetone and W is fresh weight (g) of tissue extracted.

Water soluble carbohydrate

Water soluble carbohydrate was determined using the Anthrone extraction method. About 1.0 g of plant samples were ground and transferred into a 250 mL test tube and 220 mL of water was added. The bottles were capped and shaken on a shaker for about an hour and filtered. The first few ml were ejected and the filtrate was retained for the determination of soluble carbohydrates using Anthrone reagents. 770 mL of concentrated H_2SO_4 was added to 330 mL of distilled water, in addition to 1 g of thiourea, and 1 g of anthrone, stirred until dissolved and stored in a refrigerator. Glucose stock solution, 1.0 g of anhydrous D (+) glucose in water and diluted to one litre prepared immediately before use. From the glucose working standard solutions, 10 mL of stock to 100 mL was diluted to produce 100 ppm. From these, 0, 5, 10, 20, 40, and 80 mL were pipetted and made up to 100 ml and these produced 0, 5, 10, 20, 40, and 80 ppm. Samples of 2 mL of each glucose working standard solution were pipetted into the glass test tube and rapidly, 10 mL of anthrone reagent was added and mixed by shaking. The test tube was loosely covered with a glass bulb stopper and placed immediately in boiling water for 20 minutes. The absorbance was measured using a spectrophotometer device in a 10 mm optical cell at 620 mm. The graph of absorbance was plotted against glucose concentration in ppm and prepared a standard graph with each batch of extracts examined. The glucose standard becomes 0, 0.8, 1.7, 3.3, 6.7, and 13.3 ppm respectively.

Data analysis was carried out using the Minitab Version 17 statistical package (Minitab Inc., PA, USA) and where necessary, significance was determined at the 95% level ($\alpha = 0.05$) using Tukey HSD test at $\alpha = .05$, after tests for normality and homogeneity of variance

RESULTS

The watering regimes and cocoa provenances evaluated affected root zone moisture. The 40% Fc had consistently lower root zone moisture followed by 60% Fc and highest under full field capacity watering. The trends in root zone moisture were similar among the provenances. While root zone moisture for 40 and 560 5 Fc among provenances, differences were found for root zone moisture among the provenances under field capacity conditions (Figure 1).

There was a gradual decline in root and shoot biomass as the quantity of water applied increased (40, 60 and 100% FC) (Table 1). Root biomass decreased as the quantity of water applied increased (12, 23 and 27 g). Plant biomass was similar for PA150/36 and PA150/34 at 40% FC at 60% and 100 FC. Amelonado produced the heaviest plant biomass compared with F3 Amazon at 40, 60 and 100% FC. The significantly heavier root (tap and lateral roots) biomass was produced by Amelonado followed by F3 Amazon and the least by the PA 150 Series at 60% FC. Leaf biomass was heaviest at 60% FC (26.25 g) and least at 100% FC (20 g). Stem biomass was heaviest at 100%FC (30.3 g) and lowest at 60% FC (27.5 g). Heaviest plant biomass was obtained for 60% FC (69 g) compared with 100% FC (63.8 g) and 40% FC (64.5g) respectively. Leaf biomass was heaviest at 60% FC (26.25 g) and least at 100% FC (20 g). Stem biomass was heaviest at 100% FC (30.3 g) and lowest at 60% FC (27.5 g). Heaviest plant biomass was obtained for 60% FC (69 g) compared with 100% FC (63.8 g) and 40% FC (64.5g) respectively. The effects of differential watering

Table 1: Effects of watering regime and provenance on root and shoot biomass of cocoa

Watering regimes	Provenances	Fresh Leaf Weight (g)	Fresh Stem Weight (g)	Fresh Root Weight (g)	Fresh Plant Weight (g)	Dry Stem Weight (g)	Dry Root Weight (g)	Dry Plant Weight (g)	Total Root Length (cm)	Root Volume (cm^3)
40% FC	PA/150/36	35.28i	45.0i	25.0g	85.24g	19.0h	17.0h	26.0h	25.20de	28.8cd
	PA/150/34	38.76h	50.0h	37.18f	105.80g	17.0i	15.0i	24.0i	21.86f	31.0c
	F3 AMAZON	45.30e	106.0f	62.6e	233.94e	27.0f	21.0f	70.0f	34.04ac	35.0c
	AMELONADO	37.3d	135.7a	112.6b	460.72b	36.0a	24.0a	138.0a	33.94ac	43.0b
60% FC	PA/150/36	42.40k	72.50j	30.0i	25.36h	21.0l	18.3	5.3	23.00ef	22.5d
	PA/150/34	45.25f	87.56g	34.0g	167.61f	24.0g	20.0g	48.0g	27.74cd	25.0d
	F3 AMAZON	79.3b	150.0e	75.0d	334.29d	40.0e	24.0d	105.0e	41.40a	40.0b
	AMELONADO	84.3c	212.56c	100.0c	406.87c	51.0d	27.0c	118.0c	31.78bc	50.0a
100% FC	PA/150/36	52.8l	112.52j	58.0i	29.38h	27.5k	18.0k	4.00k	27.60cd	30.2c
	PA/150/34	57.47j	115.0i	62.56h	47.93h	30.4j	16.2j	11.0j	31.80bc	33.5c
	F3 AMAZON	83.31a	200.0d	132.38a	505.69a	44.0b	20.0b	134.0b	36.42ab	44.0b
	AMELONADO	78.5g	250.0b	100.0c	398.95c	52.0b	24.0e	106.0d	39.10ab	55.0a

were profound on the root characteristics of cocoa. Root biomass was highest at 60% FC (15.3 g) and lowest at 40% FC (12.8 g). There were increases in the tap root length and the number of root hairs as the quantity of water applied increased. Taproot length was longest at 100% FC (33.7 cm), and the number of root hairs was significantly higher at 100% FC compared to other watering regimes. The number of lateral roots was significantly higher at 60% FC (71.1) and least at FC (47.1).

Cacao seedlings that were watered at 40% field capacity consistently consumed the highest amount of water while the least was obtained for full field capacity watering while the provenances differed in their water use (Table 2). Significantly higher water consumption was obtained for PA 150/34, F3 Amazon and Amelonado under 40% FC compared to PA 150/36 across measurement dates. Cacao consumed more water 100 and 60% FC watering and at least at 40% FC, water use values were similar for 60 and 100% FC. Significantly higher water use was obtained for PA 501/34 followed by Amelonado and F3 Amazon and lowest by PA 150/34. For the well watered treatment (field capacity moisture content), PA 150 Series had significantly higher water use followed by F3 Amazon and Amelonado. Cacao consumed more water 100 and 60% FC watering and at least at 40% FC, water use values were similar for 60 and 100% FC.

The effects of differential watering were significant on the relative water content and stomatal density of cacao (Table 3). At the various watering levels, relative water content was highest at 60% FC and least at 40% Fc for Amelonado while PA150/36 at 100% FC had the highest leaf water content and least for F3 Amazon at 100% FC. There were significant ($P<0.05$) differences in stomatal densities for both the upper (adaxial) and lower (abaxial) leaf surfaces among varieties and watering regimes. Stomatal densities (adaxial) were highest at 100 FC for the PA 150 series (the elite varieties) and least for F3 Amazon at this level of watering level (100% FC) compared to Amalonado. PA150/36 had the highest abaxial stomatal density at 60% FC and F3 Amazon at 100% FC and the least values for Amelonado at 40% FC.

There were significant ($P<0.05$) effects of watering regime and variety on the proline content of cocoa seedlings (Table 4). F3 Amazon had the highest leaf, stem and root proline contents. While F3 Amazon produced a significantly ($P<0.05$) higher proline content on the leaves and stem respectively at 40% FC and on the root at 60% FC, Amelonado on the other hand produced the least proline content on the leaves, stem and roots at 60% FC. The watering regime affected the proline content on the leaf, stem and root of cocoa seedlings. Proline content was significantly ($P<0.05$) higher in the leaves and stem at 40% FC and for root at 60% FC. Proline concentrations increased with decreases in water application (40<60<100 FC) and in plant tissue relative water content (moisture status of cell sap). Proline content was 4.4 μmoles/g of fresh weight in well watered treatment compared with 5.8 μmoles/g fresh weight under soil moisture deficit (40% FC watering). Decreases in proline contents were observed as tissue relative water content (water content of cell sap), decreased. The proline content was 4.4 μmoles/g for well watered treatment compared with 5.8 μmoles/g fresh weight under soil moisture deficit (40% FC watering).

Table 5 presents other functional traits of cocoa affected by the watering regime. The effects of watering and variety were significant leaf moisture content, water soluble carbohydrate and chlorophyll contents of seedlings of cocoa. Among the cacao provenances, the mean value of relative water content was 89.28% while that observed in drought conditions was 87.73%, PA150/36 at 100% FC and F3 Amazon at 40% FC had the highest leaf and stem water soluble carbohydrate contents (WSC), PA150/34 has least leaf WSC and stem WSC for F3 Amazon both at 100% FC. At 40% FC, the highest leaf and stem for PA 150/34 and F3 Amazon and the least for PA 150/36. At 60% FC, F3 Amazon had

Table 2: Effect of watering regime and cacao provenance on water use

Treatments	Varieties	Months after planting			
		8	12	16	20
40% FC	PA/150/36	0.40ab	3.22a	0.58bcde	0.30b
	PA/150/34	0.82a	0.66c	0.96a	0.14b
	F3 AMAZON	0.46ab	0.30c	0.56bcde	0.20b
	AMELONADO	0.70ab	0.66c	0.60bcd	0.30b
60% FC	PA/150/36	0.44ab	0.36c	0.26ef	0.58a
	PA/150/34	0.76ab	0.54c	0.56bcde	0.22b
	F3 AMAZON	0.46ab	0.64c	0.80ab	0.80a
	AMELONADO	0.26b	0.60c	0.56bcde	0.72a
100% FC	PA/150/36	0.32ab	0.60c	0.22f	0.22b
	PA/150/34	0.60ab	1.24b	0.34def	0.26b
	F3 AMAZON	0.56ab	0.64c	0.42cdef	0.14b
	AMELONADO	0.44ab	1.18b	0.74abc	0.30b

Table 3: Effect of Watering regime on stomatal densities of cocoa provenances

Treatments	Cocoa provenances	Relative Water Content %	Stomatal Density (adaxial:a)	Stomatal Density (abaxial:ab)	Stomatal Density (a/ab)
40%	PA/150/36	89.35bc	316.0cd	253.6cd	1.93a
	PA/150/34	84.26bc	440.8b	311.2ab	1.53bc
	F3 AMAZON	87.50bc	409.2b	326.0ab	1.62bc
	AMELONADO	96.64a	272.0e	151.2e	1.63bc
60%	PA/150/36	90.01ab	406.4b	344.8a	1.12e
	PA/150/34	87.18bc	289.2d	221.2d	1.97a
	F3 AMAZON	86.20bc	356.0c	279.6bc	1.30de
	AMELONADO	81.75cd	325.6cd	210.8d	1.33de
100%	PA/150/36	87.99bc	412.4b	248.4cd	1.61bc
	PA/150/34	83.65cd	552.8a	315.6ab	1.74ab
	F3 AMAZON	89.46ab	158.8f	124.8e	1.27de
	AMELONADO	91.29ab	346.0c	218.8d	1.45cd

Table 4: Effect of Watering regime on proline content of cocoa provenances

Watering regimes	Cocoa provenance	Proline contents (µg/g plant material)		
		Leaf	Stem	Root
40% FC	PA/150/36	265.28c	140.89i	19.40f
	PA/150/34	208.33g	181.78e	38.96b
	F3 AMAZON	329.17a	279.16a	36.15c
	AMELONADO	226.39e	188.93d	20.16g
60% FC	PA/150/36	204.317h	157.02h	11.30e
	PA/150/34	220.83f	166.50g	11.42e
	F3 AMAZON	289.61b	272.94b	37.78a
	AMELONADO	181.11l	159.71l	10.14g
100% FC	PA/150/36	195.83i	124.14j	26.44d
	PA/150/34	249.20d	176.17f	26.27d
	F3 AMAZON	184.72j	201.21c	18.14g
	AMELONADO	156.94k	51.26k	11.40g

the highest WSC in both leaf and stem followed by PA150/36 and Amelonado and the least for PA 150/34. At full field capacity, the highest leaf and stem WSC was obtained for PA 150/36 followed by Amelonado (leaf WSC) and lower but close values for PA 150/34 and F3 amazon. The highest chlorophyll a and b contents were recorded for PA 150/34 and PA150/36 at 60% FC while the least was for Amelonado at 40% FC and PA150/36 at 100% FC. The contents of chlorophyll a and b on cocoa leaves differed among varieties and watering levels. There were non-significant differences in total chlorophyll contents among cocoa varieties. Chlorophyll contents increased on average, from well watered (FC) to deficit watering (40 and 60% FC) showing decreases in total chlorophyll concentrations in leaves with increasing soil moisture deficit and values differed among cocoa varieties.

The trend among cocoa provenances and watering regimes of the time (900 to 1600 h) course of stomatal conductance (gs) is presented in Figures 2, 3 & 4). Root zone moisture affected stomatal conductance among cocoa provenances (Figure 2). Stomatal conductance was highest for F3 Amazon it was lowest for PA 150/36 series, however, at 40% FC, F3 Amazon had the lowest gs while the highest value was obtained for PA 150/34 and Amelonado (Figure 3). Among the varieties and across the daytime course of stomatal conductance, the highest stomatal conductance was observed for F3 Amazon and lowest for Amelonado for both morning and afternoon hours. The daytime pattern of stomatal conductance was similar between the PA 150 Series and F3 Amazon at 60% and 100 FC while differences were found for Amelonado for stomatal conductance under the various watering regimes. The responses of stomatal conductance to watering regimes (40 and 60% field capacity (FC) and full (100% FC) is presented in Figure 4. Increases in root zone moisture deficit consistently reduced stomatal conductance during the morning and afternoon hours of the day. Across the varieties and watering regimes, stomatal conductance was significantly higher during the morning hours (900 to 1100) and lowest in the late afternoon (1300 to 1600 hours) after which recovery of conductance occurred. The highest stomatal conductance values were obtained for the 60 and 100% field capacity moisture (0.9 and 1.5 L/plant) compared to 40% FC (0.6 L/plant). At the various measurement points during the day, 40% FC had the lowest stomatal conductance followed by 60% field capacity watering. Thus, the increasing trends in stomatal conductance were 40 < 60 < 100% field capacity (FC).

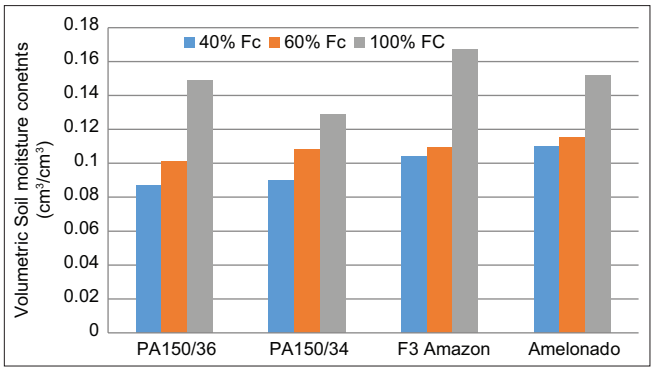

Figure 1: Effect of watering regime on mean root zone moisture under cocoa provenances

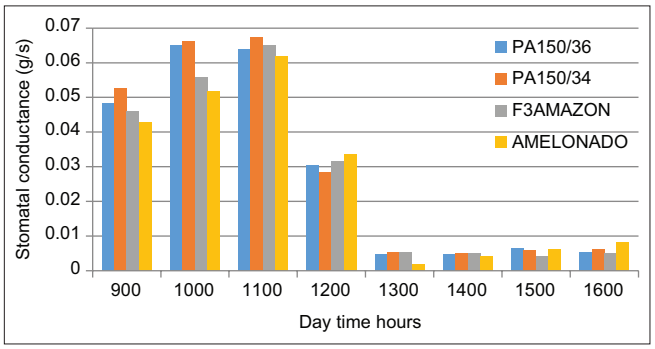

Figure 2: Daytime course of stomatal conductance among cocoa provenances

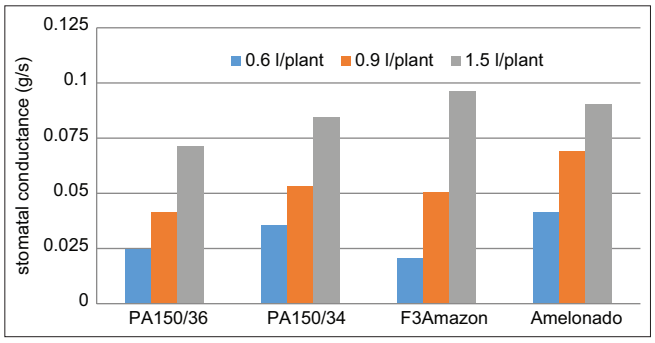

Figure 3: Responses of stomatal conductance to watering regimes among cocoa provenances

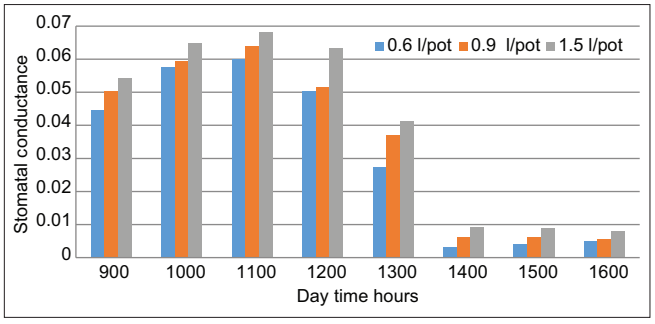

Figure 4: Effect of watering regime on stomatal conductance of cacao

Table 5: Effects of watering regime on functional traits of cocoa provenances

Watering regimes	Cocoa provenances	Relative Water Content (Leaf) (cm^3/m^3)	Leaf Soluble Carbohydrate Content (mg/g)	Stem Soluble Carbohydrate Content (mg/g)	Chlorophyll A (mg/l)	Chlorophyll B (mg/l)	Total Chlorophyll Content (mg/l)
40%	PA/150/36	0.23ab	18.88i	15.86g	9.15c	22.00c	31.13c
	PA/150/34	0.19bc	31.62c	22.78c	7.43h	14.48h	21.91g
	F3 AMAZON	0.22ab	33.59a	25.87a	8.23e	14.38i	22.61f
	AMELONADO	0.22ab	20.89g	19.06d	4.36k	5.97k	10.33j
60%	PA/150/36	0.22ab	27.81e	18.28e	9.27b	23.02a	32.28a
	PA/150/34	0.21bc	18.12j	15.52g	9.60a	20.26e	29.87d
	F3 AMAZON	0.26ab	29.23d	24.03b	7.35i	16.56f	23.90e
	AMELONADO	0.24ab	20.32h	18.18e	7.92g	7.48j	15.40h
100%	PA/150/36	0.30a	32.97b	26.16a	8.79d	6.07k	29.92d
	PA/150/34	0.21abc	18.05j	16.62f	9.63a	22.39b	32.02b
	F3 AMAZON	0.13c	18.70i	10.10i	8.06f	14.67g	22.71f
	AMELONADO	0.22ab	22.37f	12.86h	4.59j	21.14d	10.54i

DISCUSSION

The responses of cacao provenances to watering regimes were assessed through the measurement of morphological and physiological traits in screen house condition. The imposed root zone moisture scenarios elicited different responses in the evaluated provenances.

The status of root zone moisture and water use was affected by the watering regime and cocoa provenances. Across watering regimes, the highest soil moisture values were found for well watered plants while non-significant differences were found between 0.6 and 0.9 litres/plant (70 and 40% FC watering). Differences which were found among the provenances for status of soil moisture and cacao water use can be explained by genotypic differences among provenances. These observations, which were consistent with reports of Agele *et al.* (2018), imply that cocoa seedlings require a consistent moist root zone environment for optimum growth (Haeberle *et al.*, 2016; Agele *et al.*, 2018; Tezara *et al.*, 2020). The enhancement of crop water use (evapotranspiration) under adequacy of soil moisture would have promoted seedling growth compared with root zone moisture deficits.

Cacao biomass (root and shoot): Watering regime and provenances affected root and shoot biomass production in cocoa. The provenances exhibited a gradual decline in shoot root biomass as the quantity of water applied decreased (100 < 60 < 40% FC). The effects of differential watering were profound for other root parameters, tap root length was longest at 100% FC, and the number of root hairs was significantly higher at 100% FC compared to other watering regimes. There were increases in the tap root length and the number of root hairs as the quantity of water applied increased. The number of lateral roots was significantly higher at 60% FC and the least at FC. F3 Amazon had the highest dry leaf biomass at 100% FC, F3 Amazon and Amelonado had similar leaf biomass at 60% FC while PA150/36 and PA150/34 had similar leaf biomass at 40% FC. However, PA150/36 had the least leaf biomass at 100% FC (1g). Amelonado consistently had the heaviest root biomass at 40, 60 and 100% FC. Haeberle *et al.* (2016) stated that water stress reduces plant growth through inhibition of physiological and biochemical processes, including nutrient uptake and metabolism. Water stress reduces vigour and biomass (Agele *et al.*, 2018; Tezara *et al.*, 2020). Cocoa provenances subjected to mild and severe root zone moisture deficits had decreased root and stem biomass which had been described as survival (tolerance) strategic among cacao progenies under drought (Tezara *et al.*, 2020).

Differential watering affected relative water content and stomatal densities for both the upper (adaxial) and lower (abaxial) leaf surfaces. Stomatal densities also differed among the provenances which were highest at 100 FC for the PA 150 series (the elite varieties) and least for F3 Amazon at this level of watering level. PA150/36 had the highest abaxial stomatal density at 60% FC and the least values for Amelonado at 40% FC. Variable root-zone moisture impinged on stomatal density, this parameter has been closely and inversely correlated with a starch concentration in roots and trunks of plants. It has been suggested that the carbohydrate reserve status of plants may be an important endogenous determinant of stomatal density. Depleted starch reserves, elicited by long periods (several weeks) of high metabolism in dry root zones, would require replenishment. The stomatal density of newly emerging leaves plants grown in dry and warm soil increased after the treatment had been removed. The high stomatal densities obtained for well-watered treatment led to improved stomatal gas exchange (conductance of the stomatal to gases, g). Low stomatal conductance is characteristic of dry root-zone environments and may not be due to low stomatal density and stomatal aperture.

There were significant ($P<0.05$) effects of watering regime and variety on the proline content of cocoa seedlings. F3 Amazon had the highest leaf, stem and root proline contents compared with other provenances, especially for leaf and stem at 40% FC and on the root at 60% FC. Amelonado on the other hand produced the least proline content on the leaves, stem and roots across the watering regimes evaluated. Plants can protect themselves against mild drought stress by accumulating osmolytes (Yancy *et al.*, 1982; Herbinger *et al.*, 2002; Verbruggen & Hermans, 2008). Proline has been identified as an important compatible osmolyte in drought stressed plants (Sanchez *et al.*, 1998; Verbruggen & Hermans, 2008; Mafakheri *et al.*, 2010). For example, the proline content increased under drought stress in peas (Sanchez *et al.*, 1998). Proline accumulation in plant tissues has been described as a marker for environmental stress, and as an important part of the stress signal influencing adaptive

responses (Routley, 1966; Yancy *et al*., 1982; Herbinger *et al*., 2002; Maggio *et al*., 2002; Tokihiko *et al*., 2003; Verbruggen & Hermans, 2008, Mafakheri *et al*., 2010).

Water soluble carbohydrates (WSC) and chlorophyll concentration were affected by the watering regime and cacao provenance. PA150/36 at 100% FC and F3 Amazon at 40% FC had the highest leaf and stem water soluble carbohydrate contents (WSC), and PA150/34 has the least leaf WSC and stem WSC for F3 Amazon both at 100% FC. The results showed that root zone moisture status affected the quantities of total soluble sugars in plant parts with increases in the intensity of soil moisture deficit stress. Increased soil moisture deficits brought about increased accumulation of soluble sugars and proline. These observations confirmed other reports that soil moisture deficit stress increases the content of soluble sugars in plant tissues with increases in proportion to intensity of moisture deficits (LiXin *et al*., 2009).

Soluble carbohydrates (sugars) are among metabolites and osmolytes (Bray, 1997) which increased with increasing drought stress (reduced soil water content) (Hoekstra *et al*., 1994; Garcia-Sanchez *et al*., 2007; LiXin *et al*., 2009). Considerable changes in the accumulation of soluble sugars in response to drought stress have been observed both at intra-and inter-species levels in plants subjected to drought (dryness) (Ashraf & Harris, 2004; Verbruggen & Hermans, 2008; Mafakheri *et al*., 2010). The increase and accumulation of soluble sugars maintain leaf turgidity under soil moisture deficits and they prevent dehydration of proteins and cell membranes (Sawhney & Singh, 2002). Under drought stress or reduction of soil water content, soluble carbohydrates accumulate and would have served to activate protective enzymes in cacao.

Soil moisture status has been found to affect stomatal gas exchange and regulate stomatal conductance (g), transpiration (E) and carbon dioxide fixation (photosynthesis: A) in arables and perennial crops. The main effect of water stress is the reduction in carbon fixation associated with stomatal closure, reduction of photosynthesis and resultant decreases in carbohydrate synthesis and plant growth and yield. Cuevas *et al.* (2006) reported that approximately 60% of the variation in stomatal conductance was attributable to changes in soil water content (θ_v), and obtained a close correlation between g_s and θ_v) while net CO_2 assimilation rates were significantly correlated with g_s. Change in the daytime course of stomatal conductance (gs) has been reported in plants when measured between 09.00 h and 15:00 h with increases approximately by 20% from morning to afternoon and generally decreasing trends with decreasing soil water status (Cuevas *et al*., 2006; Agele *et al*., 2016; Haeberle *et al*., 2016).

Chlorophyll a and b contents of cocoa leaves differed among varieties and watering levels. Chlorophyll contents increased on average, from well watered (FC) to deficit watering (40 and 60% FC) showing decreases in total chlorophyll concentrations in leaves with increasing soil moisture deficit Among the provenances, the highest total chlorophyll contents were recorded for PA 150/34 and PA150/36 at 100 and 60% FC while the least was for Amelonado at 40%. Changes in chlorophyll and carotenoids have been associated with drought stress tolerance in plants (Pastori & Trippi, 1992). Zobayed *et al*. (2005) also reported that chlorophyll concentration is an important plant response to drought or soil moisture deficit stress. In crop species, changes in chlorophyll contents during drought stress have been reported depending on the duration and severity of drought (Kpyoarissis *et al*., 1995; Ommen *et al*., 1999; Mafakheri *et al*., 2010). Drought stress significantly decreased chlorophyll a, chlorophyll b and total chlorophyll and increase in proline content due to drought stress in Chickpeas (Verbruggen & Hermans, 2008; Mafakheri *et al*., 2010). The results were in agreement with earlier reports (Nyachiro *et al*., 2001; Agele *et al*., 2018) where a significant decrease of chlorophyll a and b was obtained under water deficits. It has been reported that the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, and this might be avoided by degrading the absorbing pigments (Yancy *et al*., 1982; Smirnoff, 1995; Herbinger *et al*., 2002). Therefore, a decrease in total chlorophyll with drought stress implies a lowered capacity for light harvesting and thus photosynthesis.

Daytime changes in magnitudes of stomatal conductance among cocoa provenances and watering regimes were obtained. Stomatal conductance (gs) was higher during the morning hours (900 to 1100) and decreased at midday under the various watering regimes. Gs was highest at FC and 60% field capacity moisture (1.5 and 0.9 L/plant/day). The depression of gs at noonday was followed by recovery of conductance late afternoon (1500 and 1600 h) hours. Across the varieties and watering regimes, values of gs were significantly higher during the morning hours (900 to 1100) and lowest in the late afternoon (1300 to 16 00 hours) during which recovery of conductance occurred. Among the varieties, the daytime course of stomatal conductance (gs) showed that higher values were observed at mid-morning hours (1000 and 1100 hours) and lowest in the afternoon. Environmental factors drive stomatal closure and result in leaf transpiration (Cuevas *et al*., 2006; Agele *et al*., 2016; Haeberle *et al*., 2016). The changes in the daytime course of stomatal conductance (gs) from after sunrise toward midday were attributed to increases in leaf temperature and vapor pressure deficit and incident photon flux density (PPFD) which increased after sunrise and reached a maximum around noon (Cuevas *et al*., 2006; Agele *et al*., 2016). It has been reported that midday depression can be considered to result from the combination of the effects of light intensity of stomatal opening. The daytime carbon and water exchanges of plant leaves reflect a balance between stimulation from high light exposure and depression from a high vapor pressure deficit. High light exposure during the day stimulates stomata opening, thereby driving gas exchange. Stomatal opening (aperture) negatively affects transpiration rate and stomata often close during the day when the humidity deficit is high (Cuevas *et al*., 2006; Agele *et al*., 2016; Haeberle *et al*., 2016).

Previous studies of forest trees had demonstrated a reduction of gs from sunrise followed by apparent midday depression (Cuevas *et al*., 2006; Agele *et al*., 2016; Haeberle *et al*., 2016). Cacao provenances differed in the daytime course of stomatal gas exchange, the highest gs was observed for PA 150/34 and the lowest for Amelonado for both morning and afternoon hours.

Across the watering regimes, the highest stomatal conductance was consistently obtained under field capacity watering closely followed by 60% FC (0.9 L/plant) and lowest for 40%FC (0.6 L/plant). The decrease in a daytime hour for stomatal conductance was substantially less in the water stressed treatment compared with the well watered. Reports of measurement net CO_2 assimilation rate (A), stomatal conductance (g_s) and transpiration (E) of leaves spanning daytime hours (early morning, midday and late afternoon periods) have shown these variables differed under various root zone moisture scenarios (Agele *et al.*, 2016; Haeberle *et al.*, 2016). Stomatal conductance was responsive to root zone moisture and appeared as a regulatory signal that is operative during the day for regulation of transpiration. Changes in the daytime course of gas exchange have been reported for plant species including forest trees (Cuevas *et al.*, 2006; Haeberle *et al.*, 2016). Cuevas *et al.* (2006) reported that approximately 60% of the variation in stomatal conductance was attributable to changes in soil water content (θ_v), and obtained a close correlation between g_s and θ_v) while net CO_2 assimilation rates were significantly correlated with g_s. The main effect of water stress is the reduction in carbon fixation associated with stomatal closure, reduction of photosynthesis and resultant decreases in carbohydrate synthesis and plant growth and yield (Tyree *et al.*, 2003; Agele *et al.*, 2016; Haeberle *et al.*, 2016). The consequences of leaf gas exchange and midday depression of gs on sunny days have been reported not only in dry regions but also in wet temperate and humid regions with implications for ecosystem-level carbon uptake (Tyree *et al.*, 2003; Agele *et al.*, 2016; Li & Liu, 2016). The stomatal gas exchange appears as an informative indicator of drought tolerance in cacao due to its high sensitivity to root zone moisture status (Daymond *et al.*, 2011; Almeida *et al.*, 2018; Tezara *et al.*, 2020). In this study, stomatal gas exchange was highly responsive to the root zone moisture environment. The stomatal gas exchange has been reported as an informative indicator of drought tolerance in plants because of its high sensitivity to environmental stress factors (Massacci *et al.*, 2008; Tezara *et al.*, 2020). The effect of water stress on plants to the reduction in carbon fixation associated with stomatal closure and concluded that the reduction of photosynthesis and resultant decreases in carbohydrate synthesis reduces plant growth and, therefore, impacts crop yield.

In this study, the growth and vigour of cocoa provenances tested were statistically superior under the full FC regimes compared with the 40% FC watering. This observation supported the findings of Agele *et al.* (2011) on the effects of soil moisture deficit on the of Shea butter seedlings. Our findings were also substantiated by the reports of DaMatta, Carr and Burkhardt on the drought stress responses of coffee varieties and cacao (Tezara *et al.*, 2020). Moisture deficit stress reduces leaf area and biomass accumulation which affirmed that plants which grow under water stress will end up smaller and poor in vigour. The results of this study showed that the measured morphological and physiological variables on the seedlings of cacao genotypes responded to watering regimes. This implies that cocoa seedlings require a consistently moist root zone environment for optimum growth and development (Haeberle *et al.*, 2016; Agele *et al.*, 2018).

CONCLUSIONS

The study investigated morphological and physiological responses of cocoa provenances to watering regimes under screen house conditions and the implications of the measured variables as drought tolerance strategy in Theobroma was discussed. The results showed that root zone moisture status affected the morphological and physiological characteristics of cacao provenances. The imposed root zone moisture scenarios elicited differences in the responses of cacao provenances evaluated. Differences were obtained in root and shoot biomass, water use, the densities of stomatal and its conductance of gases, and the concentrations of leaf chlorophyll, and shoot and leaf proline and water soluble carbohydrates among the watering regimes imposed. Cacao provenances evaluated also differed in their responses to watering regimes and in morphological and physiological characters. Most of the measured morphological and physiological variables were driven by root zone moisture status among cacao provenances, the measured traits appeared to have played important roles as root zone moisture deficit stress tolerance mechanisms in cacao. The measured morpho-physiological variables were statistically superior in well-watered situations (100% FC) compared with the 40% FC. Root zone moisture deficit stress reduced relative water content, chlorophyll and water soluble carbohydrate concentrations but reduced proline contents, stomatal densities and the conductance of the stomatal to gases. The best performance during drought was shown by the PA series while F3Amazon and Amelonado seemed to be less tolerant of drought. The latter provenance exhibited less change in chlorophyll, proline and total soluble carbohydrates concentration, and stomatal gas exchange with increasing soil moisture deficits. Adequacy of soil moisture promotes growth and physiological functions in the seedlings of cacao provenances tested. The results confirmed that cocoa seedlings cannot withstand soil moisture deficit stress as was obtained for seedlings that were watered with 40% FC. The responses of measured photochemical compounds under variable root zone moisture environments suggest inhibition of physiological functions for which the loss of phytochemical constituents can be implicated. It is concluded that root zone moisture stress-induced loss of physiological integrity is mediated by changes in phytochemistry. Seedlings of cocoa provenances had better vigour of growth when grown under 100 and 60% field capacity watering compared with 40% FC. Seedlings had greater growth when grown under 100 and 70% field capacity watering compared with the 40% FC in the nursery. The measured physiological variables varied among the provenances and root zone moisture appeared as important to cacao growth and survival, and thus, expression of cacao acclimation potential under current and future climate change scenarios of variable drought stress conditions. It is recommended that watering cacao seedlings at full field capacity (adequacy of watering/root zone moisture conditions) and at 70% FC (mild root zone moisture stress) will ensure the production of vigorous seedlings of cacao in the nursery.

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