Effects of Watering Regime and Mycorrhizal Inoculation on the Growth and Drought Tolerant Traits of Seedlings of Cocoa (*Theobroma cacao* L.) Varieties

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Abstract: Two experiments were conducted to examine the effects of watering regime and mycorrhizal inoculation on growth, development and drought tolerant traits of seedlings of five cocoa varieties (*Theobroma cacao*) obtained from the Cocoa research Institute Nigeria (CRIN), Ibadan, Nigeria. For each experiment, treatments were 5 x 3 x 2 factorial scheme consisting of cocoa hybrids/varieties, watering regimes in addition to mycorrhizal inoculation or without. Treatments were arranged using Completely Randomized Design with five replications. In experiment one, cocoa hybrids: CRIN: TC1, TC2, TC3, TC4 and TC5) were subjected to watering regimes (100, 60 and 40% field capacity (FC) equivalent to 1.5 0.9 and 0.6 litres of water per pot and were applied twice per week. Treatment effects were significant ($P \leq 0.05$) for the measured growth variables of cocoa such as plant height, stem girth, root length, root and shoot weight were enhanced by 100 and 60 % FC watering regime. Variety TC2 that were watered at 100 and 60 % FC were taller in height and produced higher number of leaves and stem girth compared to those watered at 40 % FC. Among varieties, increasing orders of water use by seedlings were TC1, TC2, TC5, TC3 and TC4. The 100 and 60 % FC TC1 and TC2 had taller plants and produced higher number of leaves and stem girth compared to those watered at 40 % FC. The response of cocoa varieties to mycorrhizal inoculation varied, inoculated seedlings of TC1, TC2 and TC3 had enhance vigor than the non-inoculated. Across varieties, both inoculated and non-inoculated seedlings that were subjected to 100 % FC and 60 % FC watering regimes enhanced growth and development. Cocoa variety TC3 showed best response to 100 % FC and 60 % FC watering and AMF inoculation. Watering regime and AMF Inoculation affected the concentration of chlorophyll and water soluble carbohydrate and water use efficiency of cocoa varieties. In experiment two, the elite varieties: T65/7 x N38, T65/7 x T57/22, T82/27 x T12/11, PA150 x T60/887 and T82/27 x T16/17 were subjected to watering regimes (4-, 8- and 14-day watering intervals). Cocoa seedling water use, plant height, stem girth, plant biomass and number of leaves and branches were enhanced by the 4- and 8- day watering intervals and with or without mycorrhizal inoculation compared with 14-day watering. Varieties T82/27xT12/11 and PA150xT60/887 watered at 4- and 8-day intervals was consistently taller in height, had enhanced biomass and produced higher number of leaves and branches compared with those watered at 14-day intervals. The variety PA150xT60/887 consumed more water and had wider stem girth. The inoculated seedlings of varieties T82/27xT12/11 and T65/7xN38 were taller than the non-inoculated seedlings. The interaction effects of watering regime and variety enhanced the growth parameters of varieties T82/27xT12/11 and PA150xT60/887 that were watered at 4- and 8-day intervals. Mycorrhizal inoculation enhanced vigour of growth and survival of seedlings of tested cacao varieties that were subjected to the evaluated watering regimes in the nursery in the dry season.

Keywords: Theobroma cacao, varieties, AMF, soil moisture, growth, development.

INTRODUCTION

Cacao (*Theobroma cacao* L.) belongs to the family of Sterculiaceae and the genus *Theobroma*. Recently, with the application of molecular marker, cacao was reclassified to belong to the family Malvaceae [1]. Its natural habitat is the lower storey of the evergreen rainforest. There are over twenty species in the genus but *Theobroma cacao* is the only one cultivated widely. Since its discovery in the 18th century at the Amazon basin, its cultivation has spread to other tropical areas of South and Central America, and indeed West Africa, which became the major producer from the mid-1960s [2]. Cocoa is one of the most important perennial type cultivation in the world, with an estimate world production of 3.6 Mt [3]. Cocoa is a major cash crop in many tropical countries and it is produced within 10 ^{o}N and 10 ^{o}S of the equator where the climate is suitable for its growth. West Africa has been the center of cocoa cultivation for many decades, as two-thirds of the world's cocoa is produced in West Africa [4]. Globally, the six main world cocoa producers are Ivory Coast, Ghana, Indonesia, Nigeria, Brazil, and Cameroon in descending order [3]

Cacao plant is highly sensitive to changes in climate from hours of sunshine to rainfall and application of water, soil condition and particularly to temperature due to effects on evapotranspiration [5, 6, 7]. Water account for large proportion of living weight of plants, for example in some herbaceous species, water forms 70-90% of the fresh organic weight in many woody species, is essential for many physiological functions and it is most critical at certain periods in the life of cacao. Legavre *et al*. [8] reported that varietal improvement for tolerance to abiotic and biotic stress

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factors has been identified as a priority of research programs of cocoa producing countries. The Nigerian National Cocoa Breeding Programme through selection resulted in “Established Ability Elites” which had better yields and adapted to marginal and drought prone areas [9]. The Cocoa Research Institute of Nigeria (CRIN) has developed sets of improved cocoa varieties. Improvement implies development of varieties that are high yielding, pest and disease tolerant, tolerant to environmental stress. Cocoa varieties were registered by the National Centre for Genetic Resources and Biotechnology (NACGRAB) and presented at CRIN headquarters to the Nigerian public and farmers on September, 2011. These varieties were CRIN TC-1(Kasimawo), CRIN TC-2 (Olowo), CRIN TC-3 (Peta-1), CRIN TC-4 (Eskes), CRIN TC-5 (Esan), CRINTC-6 (Iremiren), CRIN TC-7 (Semobita), CRIN TC-8 (Efeh.) [9].

Most plants form symbiotic relationship with a group of fungi called mycorrhizal, which function as a bridge for the flow of energy and matter between plants and soils [10]. Mycorrhizal fungi have a long history in having symbiosis relation with most plants families and they exist in most ecosystems [11]. The symbiotic association involves most plant species and certain fungal species which has great relevance to soil ecosystem functions, especially nutrient dynamics, microbial processes, plant ecology and agriculture. The mycorrhizal performs beneficial functions for crops, like other microorganisms such as; phosphate-solubilizing bacteria [12] and N_2-fixing bacteria [13]. The fungus colonizes the host plant's roots inside the cortical tissues, the association may be either intracellular like Arbuscular Mycorrhizal Fungi (AMF), or extracellularly as in Ectomycorrhizal fungi. The root infection by the mycorrhizal increases active absorptive surface area and stimulate nutrient and water uptake even in water stress condition. Increasing attention is paid to the potential role of mycorrhizae fungi in agriculture due to their ability to increase water and nutrient uptake by agricultural plants [14]. Improvement in plant performance may be due to the increase in the absorption of the roots as a result of the wide extension of fungus mycelium in the soil around the root system that allows the agricultural plant to have access to higher volume of soil [15, 16]. Mycorrhizal-plant root association may be either intracellular such as AMF or extracellular by action of ectomycorrhizal fungi. The root infection by the mycorrhizal increases active absorptive surface area and stimulate nutrient and water up take even in water stressed condition [16, 17]. Presently, there is paucity of information on the effect of cocoa variety and mycorrhizal inoculation on water use, growth and development of cocoa seedlings, Hence, this study evaluate the effect of variety and mycorrhizal inoculation on water use, growth and development of cocoa seedlings as affected by wet-dry cycle.

Cocoa seeds are sown in pots in the nursery to raise seedlings. In the nursery and on the field, cocoa seedlings are subjected to variable soil moisture status (wet-dry cycles) during its growth. Inoculation of crops with AMF is a common horticultural practice and variable responses of species to AMF inoculation had been reported. There is paucity of information on the effects of wet-dry cycles and AMF inoculation on water use, growth and development of seedlings of cocoa varieties. The establishment and rehabilitation of cocoa farms, aimed at replacing ageing and non-productive cocoa stocks in the field may be limited by inadequacy of healthy cocoa seedlings [7]. Efforts to increase cocoa seedlings by smallholder farmers and seed production unit for planting and or replanting through the raising of cocoa seedlings in the nursery is associated with poor growth as a result of inadequate water application rate [17]. Therefore it is necessary to investigate suitable water application rate and the imports of other nursery management practices required to raise vigorous hybrid cocoa seedlings in the nursery to replace the old and nonproductive stock in the field for optimum and quality production of cocoa in Nigeria. The objectives of this study are to evaluate the effects of watering regime and mycorrhizal inoculation on the growth, development and seedling survival among cocoa hybrids, water use leaf chlorophyll and water soluble carbohydrate content and to relate measured agronomic variables with soil moisture deficit stress tolerance in cocoa seedlings. Findings from this study is expected to enhance understanding of the responses of newly released cocoa varieties and hybrids to soil moisture deficit stress the information will be relevant to the development of management practices for the production of cocoa seedlings. The results will contribute to the development of improved nursery practices for the production of vigorous cocoa seedlings.

MATERIALS AND METHODS

Site of Experiment and Conditions

The experiment was conducted in the Screen House of the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure (Lat.7.16°N and Long. 5.12°E) in the rain forest zone of Nigeria.

Planting Materials

Seedlings of elite, high yielding and early maturing lines of cocoa (*Theobroma cacao L.*) were obtained from the Cocoa Research Institute of Nigeria (CRIN) Ibadan, Nigeria. The hybrids are: CRIN TC1, CRIN TC2, CRIN TC3, CRIN TC4 and CRIN TC5 and the varieties are T65/7 x N38, T65/7 x T57/22, T82/27 x T12/11, PA150 x T60/887 and T82/27 x T16/17 were obtained from the Cocoa Research Institute of Nigeria (CRIN) Ibadan, Nigeria. The seeds were planted into polybags perforated at the bottom to allow drainage and filled with 5 kg topsoil obtained from 10 years fallow vegetation. Arbuscular Mycorrhizal Fungus (AMF) was obtained from the International Institute of Tropical Agriculture IITA, Ibadan, Oyo State, Nigeria.

Experimental Design and Treatments

The treatments imposed on seedlings of cocoa varieties/hybrids and watered at two sets of watering regimes with or without mycorrhizal inoculation. In Experiment One, watering regimes consisted of well watered conditions at 100 % field capacity (FC) moisture content and at 60 and 40 % FC which are equivalent to 1.5, 0.9 and 0.6 litres of water/pot. These were separately applied twice per week. For experiment Two, there were three watering regimes which are 4-day, 8-day and 14-day watering intervals. The seedlings which were grown in perforated pots were arranged in rows of 3 consisting of 10 cocoa seedlings per row according to the watering regimes, with or without AMF inoculation. Each experiment was 5 x 3 x 2 factorial combinations arranged using Completely Randomized Design (CRD) with 3 replications.

Data Collection

Measurements of agronomic variables commenced 2 weeks after transplanting (WAT). The variables included seedling water use using weighing method (kg) to determine the water use by the seedlings, plant height (cm), stem girth (stem diameter) (cm), number of leaves and branches, leaf drop, crude leaf area (product of length and width in cm^2), weight of leaves per plant (g), root weight (g), tap root length (cm), sum total of root length (cm).

These parameters were determined in the following ways:

Seedling Water Use

Seedling water use was determined by weighing method sing a weighing balance, the weights of potted plants were measured before watering and a day after watering and the change in weight between measurement periods were recorded.

Plant height (cm): Plant height was done using a measuring tape calibrated in centimeters, measurements were taken form the base of the cocoa to the apical shoot level and recorded.

Stem girth (cm): The collar diameter of the seedling was assessed using digital Venier caliper and converted into girth calculating their circumference and recorded.

Number of leaves: Visual counting of the leaves on each plant in the pot was done and recorded.

Number of branches: Visual counting of the branches on each plant in the pot was done and recorded.

Plant Leaf Area (cm): The area of individual leaves and total leaf area/plant were measured using a Leaf Area meter (Delta, UK)

Leaf Fresh Weight (g): All the leaves on each plant were harvested and weighed using a weighing balance and were recorded.

Root Fresh Weight (g): The root of each plant was harvested and weighed using a weighing balance and was recorded.

Tap Root Length (cm): The tap root length of each plant was measured with a measuring tape and recorded.

Total Root Length (cm): This was carried out with a measuring tape and recorded.

Extraction and Determination of Leaf Chlorophyll

Chlorophyll extraction and its determination were done at the laboratory of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The 2 uppermost leaves of legumes species from each treatment were harvested. One gramme of the fresh plant samples were cut into pieces and smashed in a mortar. The samples were put in a test tube and its chlorophyll content was repeatedly extracted with successive volume of 100ml acetone/water (80:20v/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 hot water bath. The total volume of the extract was also recorded at end of the extraction. Three millimeter (3ml) of the extract was taken and the absorbance was determined with a spectrophotometer (Spectronic 20) at two wave length of 663nm and 645nm that corresponds to maximum absorption of chlorophyll "a" and "b" respectively. The total chlorophyll content was calculated as follows:

Total chlorophyll content (mg/100g tissue) = (20.2A645 + 8.02A663) (V/10w)

where, A645=absorbance at 645nm wavelength; 663=absorbance at 663nm wavelength,

V is the final volume (cm^3) of chlorophyll extract in 80% acetone and W is fresh weight (g) of tissue extracted

Determination of Leaf Water Soluble Carbohydrate

About 2ml of extracts were pipetted into a test tube.10ml of anthrone reagent was rapidly added and mixed by shaking and placed in a boiling water bath. The absorbance of the extract was determined on a spectrophotometer device (using a 10mm diameter cuvette). About 0.5g of plant samples were ground and transferred into 250ml test tube and 220ml of water was added. The bottles was capped and shaken on a shaker for about an hour and filtered. The first few ml was ejected and the filtrate was retained for the determination of soluble carbohydrate using Antrone reagents.770ml of concentrated H_2SO_4 was added to 330ml of distilled water, in addition to 1g of thiourea, 1g of antrone, stired until dissolved and was stored in a refrigerator. Glucose stock solution, 1.0g of anhydrous D (+) glucose in water and diluted to one litre prepared immediately before use. From the glucose working standard solutions, 10ml of stock to 100ml was diluted to produce100ppm. From these, 0, 5, 10, 20, 40, 80ml was pipetted and made up to 100ml and these produced 0, 5, 10, 20, 40, 80ppm.Samples of 2ml of each glucose working standard solutions were pipetted into the glass test tube and rapidly,10ml 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 20minutes.The absorbance was measured using spectrophotometer device in a 10mm optical cell at 620mm.The graph of absorbance was plotted against glucose concentration in ppm and prepare standard graph with each batch of extracts examined. The glucose standard becomes 0, 0.8, 1.7, 3.3, 6.7, 13.3ppm respectively.

Statistical Analysis

The data collected were subjected to Analysis of Variance (ANOVA) to test the significance of the treatment means using the Statistical Analysis System (SAS) software package version 9.2 [2007]. The means were separated using the Duncan's Multiple Range Test (DMRT).

RESULTS

Experiment One

Effects of Variety, Watering Regime and Mycorrhizal Inoculation on Growth and Water Use of Cocoa Seedlings

There were consistence pattern in the amount of water use among cocoa varieties (Table **1**). Varieties T65/7x T57/22 appeared to have consumed the highest volume of water while PA150 x T60/887 was the least. Consistently, variety T82/27 x T12/11 produced tallest plants while variety PA150 x T60/887 that was among the tallest in the earlier months declined in height. Although, there seems to be no consistent trend in leaf production, variety T82/27 x T12/11 significantly had the highest number of leaves while T65/7 x N38 had the least number of leaves. Cocoa variety T65/7 x N38 had the least number of branches per plant. The effects of variety on plant biomass components (leaf and root

Table 1: Effect of Variety on Root and Shoot Biomass of Cocoa Seedlings (Measurement Taken at 11 MAT)

Cocoa varieties	Leaf weight (g)	Root weight (g)	Total root length (cm)	Top root length (cm)	Water use efficiency (g/l)
T65/7 x N38	41.67b	63.00a	265.43a	56.57ab	0.82
T65/7x T57/22	43.22ab	61.61a	227.34ab	68.59a	0.69
T82/27 x T12/11	53.61a	50.83b	233.79ab	45.68b	0.96
PA150 x T60/887	48.22ab	63.22a	230.49ab	56.37ab	1.05
T82/27 x T16/17	44.28ab	60.06a	207.87b	53.37ab	0.73

Means that do not share a letter are significantly different from each other at 5% level of probability by Tukey method.

Table 2: Effects of Variety and Watering Regime on Growth Parameters of Cocoa Seedlings at 6 Months after Transplanting

Cocoa varieties	Watering regimes	Plant height (cm)	Stem girth (cm)	Number of leaves
T65/7 x N38	4-day	97.50a	5.78ab	27.67a-d
	8-day	91.98ab	5.64ab	30.00a-d
	14-day	81.83abc	5.35ab	15.67d
T65/7x T57/22	4-day	97.03a	6.46a	36.67a
	8-day	89.80ab	5.96ab	33.50ab
	14-day	70.77c	5.12b	19.50bcd
T82/27 x T12/11	4-day	87.32b	6.15ab	31.50ab
	8-day	96.57a	5.57ab	29.17a-d
	14-day	73.32bc	5.13b	22.83a-d
PA150 x T60/887	4-day	82.60abc	6.53a	31.16abc
	8-day	84.80abc	5.87ab	28.17a-d
	14-day	76.32bc	5.59ab	16.00cd
T82/27 x T16/17	4-day	84.77abc	5.79ab	36.67a
	8-day	81.42abc	5.41ab	32.83ab
	14-day	76.32bc	5.58ab	16.00cd

Means that do not share a letter are significantly different from each other at 5% level of probability by Tukey method.

Table 3: Effects of Mycorrhizal Inoculation and Watering Regime on Growth Parameters of Cocoa Seedlings

Mycorrhizal inoculation	Watering regimes	Plant height (cm)	Stem girth (cm)	Number of leaves
Inoculated	4-day	85.29ab	6.14a	34.53a
	8-day	89.99a	5.70ab	31.40a
	14-day	77.65bc	5.17b	18.80b
Non inoculated	4-day	94.40a	6.14a	30.93a
	8-day	87.83a	5.68ab	30.07a
	14-day	73.77c	5.54ab	17.20b

weight and total root length) taken at 11 months after transplanting (MAT) are shown in Table **2**. Among the varieties, leaf weight was highest in T82/27 x T12/11 and least in T65/7 x N38 and treatment differences between the two varieties were significant. The reverse was the case for root weight where the highest value occurred in T65/7 x N38 and the least value was in T82/27 x T12/11, which was significantly lower than values obtained in each of the other four cocoa varieties. T65/7 x N38 variety also had the longest total root length while T38/27 x T16/17 had the least and treatment differences between the two were significant. However, water use was highest in variety PA150 x T60/887 and least in T65/7 x T57/22. The effects of watering intervals were significant on water use of cocoa seedlings across most of the sampling dates (Table **2**). Seedlings that were watered at 4-day intervals consistently used more water than those watered at 8-day intervals while seedlings watered at 14-day intervals used least amount of water. Table **2** shows the effects of watering regimes on plant height of cocoa seedlings. Seedlings that were watered at 4-day intervals were consistently taller than those watered at 8- and 14-day intervals across the different periods of observation. Significant interactions ($P \leq 0.05$) were obtained between watering regimes and varieties for plant height, stem girth, number of leaves and branches per plant. At 6 MAT, seedlings watered at 4-day intervals were the tallest for all the varieties, apart from T82/27 x T12/11 and PA150 x T60/887 where those watered at 8-day intervals were tallest (Table **2**). Effects of cocoa varieties and watering regimes on leaf development showed that watering at 8-day intervals led to more leaf development than watering at 4-day intervals for varieties T65/7 x N38 (Table **8**), while watering at 4-day intervals surpassed in all other varieties.

The effect of mycorrhizal inoculation was significant on plant water use measured at 8 WAT (Table **3**). Mycorrhizal inoculation enhanced water use for varieties T65/7x T57/22, T82/27 x T12/11 and PA150 x T60/887 than the non-inoculated. The effect of mycorrhizal inoculation was not significant on plant height, stem girth, number of leaves and branches as shown in Table **3**. Plant height and stem girth were generally higher throughout in inoculated cocoa seedlings. Significant interactions ($P \leq 0.05$) were obtained between mycorrhizal inoculation and watering regime for plant height, stem girth, number of leaves and branches per plant (Table **4**). At 6 WAT,both inoculated and non-inoculated seedlings subjected to 4- and 8- day watering intervals were consistently taller than the inoculated. At 6 MAT, the varieties T65/7 x N38 and T82/27 x T12/11, that were inoculated were taller than those not inoculated. Cocoa seedlings not inoculated were taller than inoculated ones. Interaction effects of mycorrhizal inoculation and watering regime on number of leaves showed that, more leaves developed for the inoculated seedlings than the non-inoculated seedlings except for variety PA150 x T60/887 which have the same leaves development (Table **4**). Significant interactions ($P \leq 0.05$) were obtained between mycorrhizal inoculations and watering regime for plant height, stem girth, number of leaves and branches per plant (Table **5**). At 6 WAT, results showed that both inoculated and non-inoculated seedlings subjected to 4- and 8- day watering intervals were consistently taller than the inoculated and non-inoculated seedlings subjected to 14-day watering intervals. Interaction effects for stem girth and number of leaves followed similar trends as observed for plant height.

Effects of Mycorrhizal Inoculation, Variety and Watering Regime on Growth Characters and Water Use of Cocoa Seedlings at 6 MAT

The interaction effect of mycorrhizal inoculation, variety and watering regime for plant height of cocoa

Table 4: Effect of Mycorrhizal Inoculation and Variety on Growth of Cocoa Seedlings at 6 MAT

Varieties	Mycorrhizal inoculation	Plant height (cm)	Stem girth (cm)	Number of leaves
T65/7 x N38	Inoculated	91.84a	5.50a	25. 11a
	Non inoculated	89.03a	5.69a	23.78a
T65/7x T57/22	Inoculated	85.57a	5.67a	30.67a
	Non inoculated	86.17a	6.02a	29.11a
T82/27 x T12/11	Inoculated	86.46a	5.59a	30.00a
	Non inoculated	85.01a	5.64a	25.67a
PA150 x T60/887	Inoculated	79.7a	5.59a	25.11a
	Non inoculated	82.78a	6.04a	25.11a
T82/27 x T16/17	Inoculated	77.98a	5.66a	30.33a
	Non inoculated	83.69a	5.53a	26.67a

Table 5: Interaction Effect of Mycorrhizal Inoculation and Watering Regime on Growth Parameters of Cocoa Seedlings

Mycorrhizal inoculation	Watering regimes	Plant height (cm)	Stem girth (cm)	Number of leaves
Inoculated	4-day	85.29ab	6.14a	34.53a
	8-day	89.99a	5.70ab	31.40a
	14-day	77.65bc	5.17b	18.80b
Non inoculated	4-day	94.40a	6.14a	30.93a
	8-day	87.83a	5.68ab	30.07a
	14-day	73.77c	5.54ab	17.20b

Table 6a: Interaction Effect of Variety, Mycorrhizal Inoculation and Watering Regime on Growth Parameters of Cocoa Seedlings

Cocoa varieties	Mycorrhizal inoculation	Watering regime	Plant height (cm)	Stem girth (cm)	Number of leaves	Number of branches	Water use (l/week)
T65/7 x N38	Inoculated	4-day	95.87ab	5.76ab	28.67ab	2.67a	0.73ab
		8-day	95.73ab	5.52ab	28.67ab	2.00a	0.73ab
		14-day	83.93ab	5.21ab	18.00ab	1.67a	0.37ab
	Non inoculated	4-day	99.13ab	5.81ab	26.67ab	1.00a	0.60ab
		8-day	88.23ab	5.78ab	31.33ab	1.33a	0.43ab
		14-day	79.73ab	5.50ab	13.33b	2.00a	0.93ab
T65/7x T57/22	Inoculated	4-day	93.20ab	6.00ab	36.67ab	3.00a	0.73ab
		8-day	92.33ab	6.05ab	33.67ab	2.67a	0.33b
		14-day	71.17b	4.97ab	21.67ab	2.33a	0.73ab
	Non inoculated	4-day	100.87a	6.93a	36.67ab	1.67a	0.37ab
		8-day	87.27ab	5.86ab	33.33ab	4.33a	0.67ab
		14-day	70.37b	5.28ab	17.33ab	4.00a	1.10a

Table 6b: Interaction Effect of Variety, Mycorrhizal Inoculation and Watering Regime on Growth Parameters of Cocoa Seedlings

Cocoa varieties	Mycorrhizal inoculation	Watering regime	Plant height (cm)	Stem girth (cm)	Number of leaves	Number of branches	Water use (l/week)
T82/27 x T12/11	Inoculated	4-day	85.33ab	6.29ab	36.00ab	1.33a	0.33b
		8-day	96.60ab	5.63ab	29.00ab	1.67a	0.83ab
		14-day	77.13ab	4.86b	25.00ab	2.33a	0.27b
	Non inoculated	4-day	89.00ab	6.01ab	27.00ab	0.67a	0.47ab
		8-day	96.53ab	5.52ab	29.33ab	4.00a	0.87ab
		14-day	69.50b	5.40ab	20.67ab	2.00a	1.00ab
PA150 x T60/887	Inoculated	4-day	76.30ab	6.62ab	31.00ab	2.00a	0.47ab
		8-day	84.80ab	5.89ab	29.67ab	1.33a	0.87ab
		14-day	78.00ab	5.41ab	14.67b	2.00a	0.73ab
	Non inoculated	4-day	88.90ab	6.44ab	31.33ab	1.33a	0.90ab
		8-day	84.80ab	5.95ab	26.67ab	3.33a	0.43ab
		14-day	74.63ab	5.77ab	17.33ab	1.67a	0.43ab
T82/27 x T16/17	Inoculated	4-day	75.53ab	6.05ab	40.33a	2.67a	0.90ab
		8-day	80.50ab	5.53ab	36.00ab	1.33a	1.10a
		14-day	78.00ab	5.41ab	14.67b	2.67a	0.40ab
	Non inoculated	4-day	94.10ab	5.53ab	33.00ab	0.67a	0.80ab
		8-day	82.33ab	5.29ab	29.67ab	0.67a	0.40ab
		14-day	74.63ab	5.77ab	17.33ab	1.67a	0.40ab

seedlings at 6 WAT were virtually not significant. However, seedlings of variety T65/7xT57/22 that were not inoculated and were watered at 4-day intervals were the tallest while those watered at 14-day intervals were the shortest (Table **6** and **b**). All varieties both inoculated and non-inoculated watered at 4- and 8-day intervals were taller than those watered at 14-day intervals. Treatment differences for plant height were significant between 4- and 14-day watering regimes of T65/7 x T57/22 inoculated seedlings and, also, the

Table 7a: Interaction Effect of Variety, Mycorrhizal Inoculation and Watering Regime on Root and Shoot Biomass of Cocoa

Cocoa variety	Mycorrhizal inoculation	Watering regime	Leaf weight (g)	Root weight (g)	Tap root length (cm)	Total root length (cm)
T65/7 x N38	Inoculated	4-day	22.33a	73.67a-d	67.33a	282.47a
		8-day	46.33a	63.00a-e	52.90a	325.90a
		14-day	40.00a	40.00cde	49.80a	200.43a
	Non inoculated	4-day	28.33a	83.00ab	65.80a	293.73a
		8-day	63.33a	71.33a-e	66.40a	274.80a
		14-day	49.67a	47.00b-e	37.17a	215.23a
T65/7x T57/22	Inoculated	4-day	22.00a	67.33a-e	61.63a	216.33a
		8-day	44.00a	64.00a-e	78.73a	230.83a
		14-day	60.67a	38.00cde	51.87a	167.57a
	Non inoculated	4-day	32.33a	102.33a	80.07a	339.07a
		8-day	44.67a	58.67b-e	67.13a	221.57a
		14-day	55.67a	39.33cde	72.12a	188.70a

Table 7b: Interaction Effect of Variety, Mycorrhizal Inoculation and Watering Regime on Root and Shoot Biomass of Cocoa

T82/27 x T12/11	Inoculated	4-day	22.00a	63.00a-e	57.93a	318.90a
		8-day	55.67a	44.00b-e	44.23a	226.20a
		14-day	63.33a	31.00e	33.50a	220.47a
	Non inoculated	4-day	40.00a	67.00a-e	49.37a	244.43a
		8-day	69.67a	54.33b-e	43.63a	183.43a
		14-day	71.00a	45.67b-e	45.43a	209.30a
PA150 x T60/887	Inoculated	4-day	50.00a	73.00a-d	69.90a	266.47a
		8-day	53.00a	71.67a-e	55.00a	202.60a
		14-day	44.67a	50.33b-e	43.00a	201.23a
	Non inoculated	4-day	51.33a	77.67abc	52.10a	236.33a
		8-day	49.33a	56.33b-e	44.17a	233.20a
		14-day	41.00a	50.33b-e	74.07a	243.13a
T82/27 x T16/17	Inoculated	4-day	37.00a	68.00a-e	49.73a	198.20a
		8-day	61.00a	84.00ab	71.07a	241.40a
		14-day	46.67a	43.00b-e	46.70a	169.00a
	Non inoculated	4-day	23.67a	54.67b-e	57.77a	232.77a
		8-day	54.33a	78.00abc	58.87a	229.53a
		14-day	43.00a	32.67de	36.10a	176.30a

non-inoculated seedling of T82/27 x T12/11. The interaction effect on number of leaves also showed consistent higher values for both inoculated and non-inoculated at 4- and 8-day intervals respectively Mycorrhizal inoculation, watering interval and variety were profound for T82/27xT16/17 on leaf formation for the inoculated and at 4-day watering intervals. Significant differences were also obtained for water use among the varieties tested. Leaf weight, tap root length and total root length showed no significant interactions ($P \leq 0.05$) for effects of variety, mycorrhizal inoculation and watering regime (Table **7a** and **b**). The tap root length was also least in T82/27 x T12/11 and highest in T65/7 x T58/22 and treatment differences were

Table 8: Effect of Variety and Watering Regime on Growth Parameters of Cocoa Varieties at 6 Months after Transplanting

Cocoa varieties	Watering regimes	Plant height (cm)	Stem girth (cm)	Number of leaves
T65/7 x N38	4-day	97.50a	5.78ab	27.67a-d
	8-day	91.98ab	5.64ab	30.00a-d
	14-day	81.83abc	5.35ab	15.67d
T65/7x T57/22	4-day	97.03a	6.46a	36.67a
	8-day	89.80ab	5.96ab	33.50ab
	14-day	70.77c	5.12b	19.50bcd
T82/27 x T12/11	4-day	87.32b	6.15ab	31.50ab
	8-day	96.57a	5.57ab	29.17a-d
	14-day	73.32bc	5.13b	22.83a-d
PA150 x T60/887	4-day	82.60abc	6.53a	31.16abc
	8-day	84.80abc	5.87ab	28.17a-d
	14-day	76.32bc	5.59ab	16.00cd
T82/27 x T16/17	4-day	84.77abc	5.79ab	36.67a
	8-day	81.42abc	5.41ab	32.83ab
	14-day	76.32bc	5.58ab	16.00cd

Means that do not share a letter are significantly different from each other at 5% level of probability by Tukey method.

significant (Table **6a** and **b**). However, variety T82/27xT12/11 non-inoculated at 14-day watering intervals had the highest leaf weight while variety T65/7xT57/22 non-inoculated at 4-day watering intervals had the highest value for both tap root length and highest total root length. Significant interactions occurred for root weight of the tested varieties, and T65/7xT57/22 non-inoculated at 4-day watering intervals had the highest root weight while T82/27xT12/11 inoculated at 14-day watering interval had the least value for root weight (Table **7**). Significant differences were also obtained in the level of water used among the varieties tested. Cocoa varieties T65/7xT57/22 watered at 14- day interval and non-inoculated and T82/27xT16/17 8-day inoculated watering intervals had the highest values for water use respectively (Table **8**). However, varieties T65/7xT57/22 that were watered at 8-day intervals but inoculated and T82/27xT12/11 and watered at 14- days intervals but inoculated had the least values for water use among the varieties (Table **9**).

Experiment Two

Effect of Variety and Watering Regime on the Growth and Water Use of Cocoa

The effect of cocoa variety and watering regime was significant ($P < 0.05$) on water use, plant height, stem girth and number of leaves per plant (Table **8**). Seedlings grown at 100 % FC (1.5 litres of water per pot) were tallest for all varieties in addition to larger stem diameter (girth). Among the varieties, TC4 and TC5 showed the least response, compared with varieties TC1, TC2, and TC3 (Table **10**) varietal effects was significant on leaf development and water use, the varieties TC2, TC4 and TC5 100 % FC had similar water use pattern. However TC1 consumed the highest volume of water (1.5 litres per day). There were significant differences ($P<0.05$) in the growth responses of cocoa varieties evaluated. The result showed that variety TC1 was tallest while TC4 had tallest plants at 14 WAT. Although, there seems to be no consistence trend in leaf production, variety TC3 significantly had the highest number of leaves across the period of measurement while TC4 had the least.

Effects of Variety, Watering Regime, AMF Inoculation on Concentrations of Chlorophyll and Water Soluble Carbohydrate, Plant Biomass and Water Use of Cocoa

Significant interactions ($P<0.05$) were obtained for the effects of watering regime and variety on plant height, stem girth and numbers of leaves. Seedlings watered at 100% FC were the tallest for all the varieties except for varieties TC3 and TC4 that produce tallest plant at 60 % FC. Effect of cocoa varieties and watering regimes on leaf development showed that watering at 100 % FC enhanced leaf development than watering at 60 % FC except for variety TC3 (Table **8**). Watering at 40% produced least leaf development.

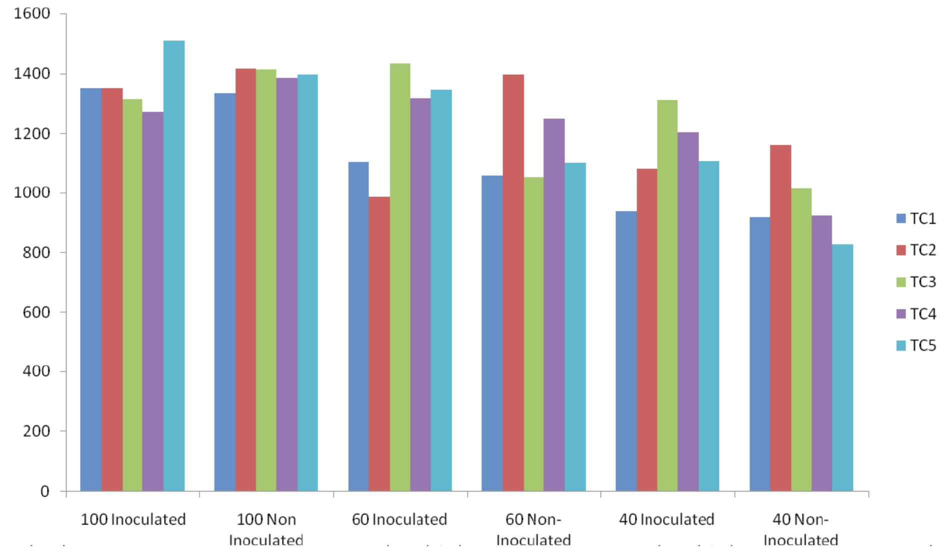

Figure 1: Interaction of watering regime and AMF inoculation leaf area of cocoa.

Table 9: Interaction Effect of Mycorrhizal Inoculation and Watering Regime on Growth Parameters of Cocoa Seedlings

Mycorrhizal inoculation	Watering regimes	Plant height (cm)	Stem girth (cm)	Number of leaves
Inoculated	4-day	85.29ab	6.14a	34.53a
	8-day	89.99a	5.70ab	31.40a
	14-day	77.65bc	5.17b	18.80b
Non inoculated	4-day	94.40a	6.14a	30.93a
	8-day	87.83a	5.68ab	30.07a
	14-day	73.77c	5.54ab	17.20b

Table 10: Effect of Variety and Mycorrhizal Inoculation on Growth of Cocoa Seedlings at 6 MAT

Varieties	Mycorrhizal inoculation	Plant height (cm)	Stem girth (cm)	Number of leaves
T65/7 x N38	Inoculated	91.84a	5.50a	25. 11a
	Non inoculated	89.03a	5.69a	23.78a
T65/7x T57/22	Inoculated	85.57a	5.67a	30.67a
	Non inoculated	86.17a	6.02a	29.11a
T82/27 x T12/11	Inoculated	86.46a	5.59a	30.00a
	Non inoculated	85.01a	5.64a	25.67a
PA150 x T60/887	Inoculated	79.7a	5.59a	25.11a
	Non inoculated	82.78a	6.04a	25.11a
T82/27 x T16/17	Inoculated	77.98a	5.66a	30.33a
	Non inoculated	83.69a	5.53a	26.67a

Means that do not share a letter are significantly different from each other at 5% level of probability Tukey method.

Across the varieties inoculated and non-inoculated both watered at 100 % FC and 60 % FC were taller than at 40 % FC. Treatment differences for plant height were significant between 100 % FC and 40 % FC watering for TC1 that was AMF inoculated, and similar trend obtains for variety TC3. Significant differences were also obtained for water use among the varieties tested. Varieties TC2 and TC5 at 40 % FC and non-inoculated had the highest values for water use while varieties TC2 at 60 % FC inoculated and TC3 at 40% FC

Table 11: Effects of Variety, Mycorrhizal Inoculation and Watering Regime on Growth Parameters of Cocoa

Cocoa variety	Watering regime	Mycorrhizal inoculation	Plant height (cm)	Stem girth (cm)	Number of leaves/plant
TC1	100FC	Inoculated	63.83a	1.30a	26. 11a
	60FC		61.12a	1.24a	25.11a
	40FC		69.42a	1.49a	23.78a
	100FC	Non inoculated	62.23a	1.65a	33.67a
	60FC		65.21a	1.23a	24.0b
	40FC		56.12a	1.03a	26.11a
TC2	100FC	Inoculated	66.66a	1.53a	32.00a
	60FC		61.43a	1.23a	23.0b
	40FC		65.45a	1.61a	22.67a
	100FC	Non inoculated	59.75a	1.29a	26.11a
	60FC		50.00a	1.32a	23.13a
	40FC		62.23a	1.14a	22.11a
TC3	100FC	Inoculated	57.34a	1.56a	31.33a
	60FC		72.12a	1.32a	1.21a
	40FC		63.69a	1.43a	33.67a
	100FC	Non inoculated	22.33a	1.67a	24.0ba
	60FC		46.33a	1.00b	26.11a
	40FC		40.00a	10.0b	32.00a
TC4	100FC	Inoculated	28.33a	1.00b	23.0ba
	60FC		63.33a	1.33a	22.67a
	40FC		49.67a	1.00a	26.11a
	100FC	Non inoculated	22.00a	1.23a	23.13a
	60FC		44.00a	1.20a	22.11a
	40FC		60.67a	1.80a	31.33a
TC5	100FC	Inoculated	32.33a	1.33a	1.21a
	60FC		44.67a	1.86	23.67a
	40FC		22.33a	1.67a	33.67a
	100FC	Non inoculated	46.33a	1.30a	24.0b
	60FC		40.00a	1.42a	33.67a
	40FC		28.33a	1.23a	24.0b

watering regime and inoculated had the least values for water use. The effects of mycorrhizal inoculation and watering regime was not significant for tap root length (Table **9**). However, variety TC3 non-inoculated at 40 % FC watering had the highest leaf area (Figure **1**) while variety TC5 inoculated at 100 % FC watering regime had the highest value for both root length and highest shoot length. Significant interactions occurred for root weight of the tested varieties, and TC2 non-inoculated at 100 % FC watering had the highest root weight while TC1 had the least at 100% FC inoculated. Table **10** shows the effects of AMF inoculation and watering regime on growth and water use of seedlings of cocoa. The seedlings that were grown at 100 % FC were consistently taller than those watered with 60 and 40 % FC across the period of observation, likewise, had lager stem girth and more leaves. The results of the effects of AMF inoculation and watering regime on growth characters of cocoa varieties are presented in Table **11**. Cocoa seedlings grown at well watered condition (100 % FC) were consistently taller and had lager stem girth than those watered with 60 and 40 % FC, values were not different between the inoculated and non-inoculated treatments. However, differences

Table 12: Effects Variety, Watering Regime, AMF on Concentration of Chlorophyll, Carbohydrate and Plant Biomass and Water Use Efficiency of Cocoa Varieties

Variety	Watering Regime (%)	AMF Inoculation	Chlorophyll (mg/100g tissue)	Soluble Carbohydrate (mg/100)	Root length (cm)	Shoot weight (g)	Root weight (g)	Water use/litter	Water use efficiency gl/day
TC1	100FC	+	1.26a	13.19bc	60b	3.35c	3.35c	0.33b	0.72
		-	1.02a	11.49bc	61b	9.36b	9.36c	0.83ab	0.69
	60FC	+	1.09a	11.84bc	70ab	11.16a	11.16b	0.27b	0.96
		-	0.94b	11.94bc	50b	3.33c	3.33c	0.47ab	1.05
	40FC	+	1.08a	12.31ab	53b	8.34b	8.34c	0.87ab	0.73
		-	1.29a	14.327a	88a	10.11a	25.07a	1.00ab	0.82
TC2	100FC	+	1.82a	13.75a	60.00a	4.85c	4.85c	0.47ab	0.69
		-	1.33a	12.99a	70.00a	12.99b	12.99b	0.87ab	0.96
	60FC	+	1.32a	14.29a	65.00a	10.20b	10.20b	0.73ab	1.05
		-	1.17a	13.81a	60.00a	7.20b	7.20b	0.90ab	0.83
	40FC	+	1.17a	11.23a	68.00a	9.91b	9.91b	0.43ab	0.82
		-	1.23a	14.68a	80.00a	17.91a	38.07a	0.43ab	0.69
TC3	100FC	+	1.24a	14.03a	70.00a	5.16c	5.16c	0.90ab	0.96
		-	1.24a	13.86a	69.00a	16.79a	16.79b	1.10a	1.05
	60FC	+	1.09a	14.81a	78.00a	14.12a	14.12b	0.40ab	0.73
		-	1.12a	12.02a	73.00a	10.06b	10.06b	0.80ab	0.82
	40FC	+	1.13a	14.18a	60.00a	10.91b	10.91b	0.40ab	0.69
		-	1.32a	14.43a	60.00a	16.96a	41.39a	0.40ab	0.96
TC4	100FC	+	1.66a	14.32a	75.00a	5.14c	5.14b	0.23b	1.05
		-	1.14a	13.65a	70.00a	15.97a	15.97a	0.83ab	0.73
	60FC	+	0.94b	14.41a	76.00a	11.52b	11.52a	0.27b	0.82
		-	1.52a	13.80a	70.00a	11.01b	11.01a	0.47ab	0.69
	40FC	+	1.30a	14.12a	75.00a	12.74b	12.74a	0.87ab	0.96
		-	0.89b	14.88a	73.00a	16.06a	38.01a	1.00ab	1.05
TC5	100FC	+	1.01a	14.21a	93.00a	13.31b	16.74bc	0.47ab	0.63
		-	1.29a	13.41a	76.00a	20.86a	36.42b	0.77ab	0.82
	60FC	+	0.79b	14.96a	86.00a	18.78a	44.37a	0.73ab	0.89
		-	1.10a	14.32a	84.00a	16.27b	36.28b	0.90ab	0.86
	40FC	+	1.52a	14.09a	92.00a	14.72b	33.41b	0.43ab	1.05
		-	1.29a	13.87a	81.00a	13.50b	30.13b	0.43ab	0.73

Means along the column with the same superscript are not significantly different (DMRT) $P<0.05$.
+ ; Inoculated, - ; Non- Inoculated.

were obtained for number of leaves produced per plant for the variously watered plants under the inoculated and non-inoculated treatments. There were no significant differences in total chlorophyll and water soluble carbohydrate of the leaves of cocoa variety TC2, TC3, but for TC1, TC4 and TC5 (Table **12**). There were no significant different in the soluble carbohydrate content among the varieties except TC1. There were significant interactions for shoot weight and root weight among the varieties. The effect of watering regime and AMF were significant on water use of cocoa seedlings (Table **13**). Seedlings that were watered at 100 % FC watering regime consistently used more water than those watered at 60 % FC while seedlings watered at 40 % FC watering regime used least. The varieties TC2, and TC5 that were inoculated used more water than the non-inoculated. For varieties TC1 TC3 and TC4 water use in both the inoculated and non-inoculated seedlings were similar but differ significantly from TC1 TC3 and TC4 this imply that TC2 and TC5 cocoa varieties had inherent capacity for high water use. The effect of mycorrhizal inoculation for plant height and number of leaves was not significant ($P > 0.05$). While plant height and stem girth were generally higher in values for the inoculated cocoa seedlings, the trend was different for number of leaves per plant.

Table 13: Effect of Variety, Watering Regime, AMF on Growth and Waer Use on Cocoa

Cocoa varieties	Watering regimes	AMF Inoculation	Plant height (cm)	Stem girth (cm)	Number of leaves per plant	Water use
TC1	100FC	+	70.87ab	1.76a	28.67ab	0.30a
	60FC	+	70.73ab	1.52a	28.67ab	0.22b
	40FC	+	58.93ab	1.21a	18.00ab	0.13c
	100FC	-	74.13ab	1.81a	26.67ab	0.32a
	60FC	-	63.23ab	1.78a	31.33ab	0.22b
	40FC	-	54.73ab	1.50a	13.33b	0.11c
TC2	100FC	+	68.20ab	2.00a	36.67ab	0.29a
	60FC	+	67.33ab	2.05a	33.67ab	0.21c
	40FC	+	46.17ab	1.97a	21.67ab	0.13c
	100FC	-	75.87ab	2.93a	36.67ab	0.25b
	60FC	-	62.27ab	1.86a	33.33ab	0.21c
	40FC	-	45.37b	1.28a	17.33ab	0.12c
TC3	100FC	+	60.33ab	2.29a	36.00ab	0.26a
	60FC	+	71.60ab	1.63a	29.00ab	0.21c
	40FC	+	52.13ab	1.86a	25.00ab	0.12c
	100FC	-	64.00ab	2.01a	27.00ab	0.26a
	60FC	-	71.53ab	1.52a	29.33ab	0.21c
	40FC	-	44.50b	1.40a	20.67ab	0.11c
TC4	100FC	+	51.30ab	2.22a	31.00ab	0.24b
	60FC	+	59.80ab	1.89a	29.67ab	0.22b
	40FC	+	53.00ab	1.41a	14.67b	0.13c
	100FC	-	63.90ab	2.44a	31.33ab	0.25b
	60FC	-	59.80ab	1.95a	26.67ab	0.20c
	40FC	-	49.63ab	1.77a	17.33ab	0.13c
TC5	100FC	+	50.53ab	2.05a	40.33a	0.27a
	60FC	+	55.50ab	1.53a	36.00ab	0.22b
	40FC	+	53.00ab	1.41a	14.67b	0.13c
	100FC	-	69.10ab	1.53a	33.00ab	0.25b
	60FC	-	57.33ab	1.29a	29.67ab	0.21b
	40FC	-	49.63ab	1.77a	17.33a	0.11c

Means along the column with the same superscript are not significantly different using DMRT (P is 0.05).
FC is Field capacity moisture content, + (with AMF inoculation); - (without AMF inoculation).

DISCUSSION

Effects of Watering Regimes on Growth and Development of Cocoa Seedlings

The results of this study showed that the measured growth variables of cocoa seedlings responded to the watering regimes imposed and AMF mycorrhizal inoculation. The enhancement of plant height, stem girth, number of leaves and branches by more frequent watering may be attributed to higher moisture contents in the crop root zone. The measured growth variables of cocoa seedlings were statistically superior under 4- and 8-days watering regimes compared with 14-days watering interval. This implies that the cocoa seedlings require consistently moist root zone environment and favourable microclimate [18, 19, 20]. Khalil and El-Noemani [21] and Bahreininejad *et al.* [22] and Agele *et al.* [17] stated that, water stress reduces plant growth through inhibition of various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake and nutrient metabolism. The cocoa seedlings that were well watered were taller than those watered poorly. This result further confirms how essential water is to growth and development of cocoa seedlings, a crop that is known to be very sensitive to a soil water deficit [17, 23, 24]. Adequacy of soil moisture promotes leaf development as was obtained for seedlings that were well watered, possibly via enhance evapotranspiration. Adequate soil and plant water status are critical to the survival of cocoa seedlings during establishment especially in the premise of the unfavorable weather conditions of the dry season in Nigeria. The results of this study confirmed that cocoa seedlings cannot withstand soil moisture deficit stress as was obtained for seedlings that were watered with 40 % FC and 12- days watering intervals). In plants, moisture deficit stress reduces leaf area, branching and biomass development. These responses mean that plants experiencing water stress will end up smaller and poor in vigour [25]. In this respect, the cocoa varieties evaluated showed significant differences and values of measured parameters were statistically superior at well watered situation (4- days watering intervals and 100 % FC) compared with the 40 % FC (12- days watering intervals). Under serious water stress, the cocoa seedlings stopped growing, leaves turned yellow, wilted and later recovered or died in the 40% FC (12- days watering regimes). The terminal drought (dry spell) situation of the dry season is characterized by high intensity of soil and air moisture deficits and temperatures [7, 26]. These conditions have implications for survival and establishment of seedlings both in the nursery and on the field. Plants exposed to soil water stress elicit a number of physiological responses in an effort to conserve water, these include closing of stomatal and arresting cellular growth [20, 27]. If water stress is not alleviated, plants will close stomatal and shut down photosynthesis, carbon assimilation, and normal metabolism [20, 25].

Effects of Varieties on Growth and Development of Cocoa Seedlings

There were significant differences on the effects of varieties on growth characteristics, which include plant height, stem girth, number of leaves and branches, root and shoot biomass and water use efficiency. This is in agreement with the finding that growth characteristics in tree seedlings are affected by drought stress [15, 20, 27]. In this respect, all the varieties tested showed differences and were statistically superior under the 4- days watering and 100 % FC regimes compared with the 40 % Fc and 12- days watering regimes. Under these soil moisture deficit stress conditions, the seedlings stopped growing, leaves turned yellow, wilted and later recovered or died. This observation supported the findings of Haeberle *et al.* [20] and Agele *et al*. [17] reported that growth parameters were reduced under drought stressed condition in tree saplings and Shea butter seedlings. Our findings were also substantiated by the reports of DaMatta [28] and Carr [29] on the drought stress responses of coffee varieties.

Effects of Mycorrhizal Inoculation on Growth and Development of Cocoa Seedlings

Cocoa seedlings inoculated with mycorrhizal fungi and subjected to well watered conditions were more vigorous going by the measured growth parameters. Nevertheless, there were no significant differences for most of the measured parameters between weekly (4- and 8- days watering and fortnight watering for mycorrhizal inoculated seedlings. Across the varieties, cocoa seedling varieties that were subjected to well watered and mycorrhizal inoculation have significantly ($P < 0.05$) higher vigor than low watering regime. These findings support the observations of Levy and Kirkum [30], Read and Boyd [31] and Shinkafi [32] who reported that mycorrhizal inoculation increased soil water extraction and root activities of tree seedlings and Agele *et al*. [17] reported that mycorrhizal inoculation and mild water stress enhanced Shea butter seedling growth than the non-inoculated. In conformity with the findings of the present study, Osunubi [33] reported that mycorrhizal inoculation enhanced leaf number, collar diameter and shoot biomass in *Acacia* species. Inoculation of seedlings with AMF promoted the growth of cocoa seedlings in the nursery. Mycorrhizae fungi form symbiotic (mutualistic) association with plant roots. Govindarajulu *et al*. [34 reported that extra-radical hyphae take up inorganic nitrogen which is transported to intra-radical hyphae in the form of amino acids. In Shea butter, seedlings responses were greater for the inoculated plants [17]. The enhancement of growth characters under these treatment combinations may be due to the activity of the mycorrhizal fungi which enabled the cocoa seedling to explore a greater volume of soil for increased nutrient absorption. Ibiremo *et al.* [35] found that mycorrhizal inoculation increased the stem diameter of cashew. It is probable that mycorrhizal root colonization increased the ability of cocoa seedlings to take up nutrients and consequently increased vegetative growth.

CONCLUSIONS

The interaction among variety, watering regime, AMF Inoculation was profound on concentration of chlorophyll and water soluble carbohydrate, plant biomass and water use efficiency of cocoa variety. Mycorrhizal inoculation enhanced cocoa seedling growth and more frequent watering of seedlings at 4- and 8-day intervals (and 100 and 60 % FC) enhanced plant height compared compared with 14-day watering intervals (and with 40 % FC). Across varieties, both inoculated and non-inoculated seedlings that were subjected to 4- and 8-day watering regimes had enhanced growth and development. Seedlings that were mycorrhized and watered at 4- and 8-day intervals were taller, had wider stem girth and produced higher number of leaves compared with those watered at 14-day intervals. However, variety T82/27xT12/11showed greater response to 4- and 8-day watering regimes and AMF inoculation. In addition, cocoa hybrid TC3 showed greater response to 100 % FC and 60 % FC watering regimes and AMF inoculation compared with other varieties of cocoa. Across varieties, both inoculated and non-inoculated seedlings that were subjected to 100 % FC and 60 % FC watering regimes enhanced growth and development. Based on the measured growth parameters in this study, mycorrhizal inoculation and 8-day watering intervals (and 60 % FC) enhanced vigour of growth and development than the 14-day watering intervals and non mycorrhizal inoculation. Therefore, for a good vigor, growth and development of cocoa seedlings in the nursery and on the field, it is recommended that seedlings of variety T82/27xT12/11 subjected to weekly watering intervals. Cocoa hybrid TC3 showed greater response to 100 % FC and 60 % FC watering regimes and AMF inoculation compared with other varieties of cocoa. These treatments enhanced vigour of growth and development of cocoa seedlings in the nursery in the dry season.

REFERENCES

[1] Alvenson WS, Whitlock BA, Feller R, Bayer C, Baum DA. Phylogeny of the Core Malvales: Evidence from *NDHF* sequence Data. Ame J Bot 1999; 86:1474-1486. https://doi.org/10.2307/2656928

[2] Opeke LK. *Tropical Tree Crops*. John Willey and sons, Chichester: 1987. Pp 67-213.

[3] Food and Agriculture Organization FAOCLIM: Environment and natural resources service. Working Papers 2012; 5. FAO, Rome 2012.

[4] Famuwagun, IB, Agele SO. Effects of sowing methods and plant population densities on root development of cacao (Theobroma cacao L.) seedlings in the nursery. Int J Agric Res 2010; 5: 445-452 https://doi.org/10.3923/ijar.2010.445.452

[5] Almeida A-AF, Brito RCT, Agular MAG, Valle PR. Water relations aspects of *Theobroma cacao* L. clones. *Agrotropical* 2002; 14: 35-44

[6] Anim-Kwapong GJ, Frimpong EB. Vulnerability of Agriculture to Climate – change impact of climate on Cocoa production. Cocoa Research Institute, New Tafo Akim, Ghana 2005.

[7] Agele SO, Iremiren GO, Aiyelari OP, Famuwagun IB. Mainstreaming adaptation, resilience and disaster risk reduction into extension of frontiers of cacao to marginal soils and climate of the humid tropics in the wake of climate and weather variabilities. Proceeding of the 11th Annual International Symposium on Environment Athens, Greece 25–27 May 2016. Athens Institute for Education and Research 2016a.www.atiner.gr

[8] Legavre T, Gramacho K, Duchamp M, Sounigo O, Debert P, Fouet O, Sabau X, Argout X, Wincker P, Da Silva C, Lanaud C. Identification of *Theobroma cacao* genes differentially expressed during *Phytophthora* infection. Proceeding of the 15h International Cocoa Research Conference, Costa Rica. June 2006.

[9] Cocoa Research Institute of Nigeria (CRIN). Information Booklet. A Quarterly Magazine 2002; 3(4): 1-2. CRIN News, Ibadan, Nigeria 2002.

[10] Cardon ZG, Whitbeck JL. *The Rhizosphere*. Elsevier Academic Press 2007 235pp.

[11] Sheffield J, Eric F. W. Projected changes in drought occurrence under future global warming from multi-model, multi scenario, IPCC AR4 Simulations. Climate Dynamics 2008: 31 (1): 79-105. https://doi.org/10.1007/s00382-007-0340-z

[12] Silva EA, DaMatta FM, Ducatti C, Regazzi AJ, Barros RS. Seasonal changes in vegetative growth and photosynthesis of Arabica coffee trees. Field Crops Res 2004; 89: 349-357. https://doi.org/10.1016/j.fcr.2004.02.010

[13] Naher UA, Radziah O, Shamsuddin ZH, Halimi MS, MohdRazi I. Growth enhancement and root colonization of rice seedlings by *rhizobium* and *corynebacterium* spp. Int J Agric Biol 2009; 11: 586–590.

[14] Sardi P, Saracchi M, Quaroni S, Petrolini B, Borgonovi G, Meril S. Isolation of endophytic *Streptomyces* strains from surface sterilized roots. Appl Env Microbiol 1992; 58: 2691-2693.

[15] Agele SO, Osaigbovo AU, Ogedegbe SA, Nwawe AK. Effects of watering regime, organic manuring and mycorrhizal inoculation on the growth and development of Shea butter *(Vitellaria paradoxa* C.F.Gaertn) seedlings. Int J Agric Policy Res 2015; 4(3): 35-45. http://dx.doi.org/10.15739/IJAPR.016.006R

[16] Agele SO, Ajayi AJ, Olawanle FM. Effects of watering regime and rhizobium inoculation on growth, functional and yield traits of four legume species. Int J Plant Soil Sci 2017; 17 (4): 1-15. https://doi.org/10.9734/IJPSS/2017/32891

[17] Agele S, Famuwagun B, Ogunleye A. Effects of shade on microclimate, canopy characteristics and light integrals in dry season field-grown cocoa (*Theobroma cacao* L.) seedlings. J Hort 2016b; 11 (1): 47–56.

[18] Dias PC, Araujo WL, Moraes GABK, Barros RS, DaMatta FM. Morphological and Physiological responses of two coffee progenies to soil water availability. J Plant Physiol 2007; 164: 1639-1647 https://doi.org/10.1016/j.jplph.2006.12.004

[19] Henson IE, Harun MH, Chang K.C, Mohammed AT. Predicting soil water status, evapotranspiration growth and yield of young oil palm in a seasonally dry region of Malaysia. J Oil Palm Res 2007; 19: 398-415.

[20] Haeberle KH, Agele SO, Matyssek R, Hennlich M. Aspects of Water Relations and Gas Exchange of Katsura and Tilia Seedlings Subjected to Wet-Dry Cycles : Indication of Strategies for Whole Plant Drought Tolerance. Int J Plant Soil Sci 2016; 10(2): 1 -13 https://doi.org/10.9734/IJPSS/2016/17154

[21] Khalil SE, El-Noemani AA. Effect of irrigation intervals and exogenous proline application in improving tolerance of garden cress plant (*Lepidium sativum* L.) to water stress. J Applied Sci Res 2012; 8(1): 157-167.

[22] Bahreininejad B, Razmjoo J, Mirza M. Influence of water stress on morpho-physiological and phytochemical traits in *Thymus daenensis*. Int J Plant Prod 2013; 7(1): 151-166.

[23] Henson IE, Noor MRM, Harun MH, Yahya Z, Mustakim SNA. Stress development and its detection in young oil palms in north Kedah, Malaysia. J Oil Palm Res 2005; 17: 11 -26.

[24] Majumder AL, Sengupta S, Goswami L. Osmolyte Regulation in Abiotic Stress. 1st ed. New York: Oxford University Press 2010. Pp 349-370.

[25] Navarro Garcia A, Del PilarBanon Arias S, Morte A, Snachez- Blanco MJ (2011). Effects of nursery preconditioning through mycorrhizal inoculation and drought in Arbutus unedo L. Mycorrhiza 2011; 21: 53–64. https://doi.org/10.1007/s00572-010-0310-x

[26] Agele SO. Sunflower responses to weather variations in rainy and dry cropping seasons in a tropical rainforest zone. Int. J. Biotronics 2003; 32: 17-33.

[27] Kulac S, Nzokou P, Guney D, Cregg BM, Turna I. Growth and physiological response of Fraser Fir [*Abies fraseri* (Pursh) Poir.] seedlings to water stress: Seasonal and diurnal variations in photosynthetic pigments and carbohydrate concentration. HortSci 2012; 47(10): 1512-1519.

[28] DaMatta FM. Exploring drought tolerance in coffee: A physiological approach with some insights for plant breeding. Brazil J Plant Physiol 2004; 16: 1-6. https://doi.org/10.1590/S1677-04202004000100001

[29] Carr MKV. The water relations and irrigation requirements of coffee. Exp Agric 2001; 37: 1-36. https://doi.org/10.1017/S0014479701001090

[30] Levy Y, Krikum J. Effect of irrigation, water and salinity and root-stock on the vertical distribution of *Vesicular Arbuscular Mycorrhiza* on citrus roots. New PhytoPath 1983;15: 397-403. https://doi.org/10.1111/j.1469-8137.1983.tb03507.x

[31] Read DJ, Boyd RC. Water relations of mycorrhizal fungi and their host plant. *Water, Fungi and Plants.* Ayres PC, Boddy L (eds.). Cambridge University Press, Cambridge, UK. 1986.

[32] Shinkafi MA. Effects of macronutrients deficiency and mycorrhizal inoculation of *Faidherbla albida* (Del) A. chev. in a semiarid environment. Ph.D. Thesis. Usman DanFodiyo University Sokoto. 2000. 148pp.

[33] Osonubi O, Mulongony K. Interaction between drought stress and vesicular *Arbuscular mycorrhizal* on growth of *Faidherbia albida (Acacia albida)* and *Acacia nilotica* in sterile and non-sterile soils. Biol Fert Soils 1992; 3:159-165. https://doi.org/10.1007/BF00346056

[34] Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen J W, Bucking H, Lammers P J, Shacker-Hill Y. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 2005; 435: 819-823. https://doi.org/10.1038/nature03610

[35] Ibiremo OS, Daniel M A, Oloyede AA, Iremiren GO. Growth of coffee seedlings as influenced by *Arbuscular mycorrhizal* Inoculation and phosphate fertilizer in two soils in Nigeria. Int Res J Plant Sci 2011; 2(6): 160-165.

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