Effect of Water Deficit Imposed during the Early Developmental Phase on Photosynthesis of Cocoa (*Theobroma cacao* L.)

Kayode Olufemi Ayegboyin¹, Ezekiel Akinkunmi Akinrinde²

¹Agronomy and Soils Division, Cocoa Research Institute of Nigeria, Ibadan, Nigeria
²Department of Agronomy, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria

Received 11 November 2015; accepted 25 January 2016; published 28 January 2016

Abstract

A greenhouse study was carried out at Cocoa Research Institute of Nigeria, Ibadan to study the effect of water stress on the four popular cocoa genotypes at the institute. F₃ Amazon, T₁, T₇ and Amelonado were raised under different water regimes (daily, 3-day interval, 5-day interval and 7-day interval) at 100%, 50% and 25% field capacities. Data were collected on the height, leaf area, root length, stomata conductance, photosynthetic rate and water use efficiency of the plants. Results showed that plant performances showed genotypic variation in their response to water stress. Generally, there were linear and positive relationships between water level and values in both physiological and morphological responses of cocoa genotypes.

Keywords

Cocoa, Water Stress, Photosynthesis, Growth

1. Introduction

For highest level of successful establishment of most tree crop plantations to be guaranteed, a lot of work must be put in place before the field operation is actually set in motion. One of such activities is the selection of appropriate materials which will be raised for a specific period of time in the nursery. Response of cocoa to drought and/or other stress conditions varies considerably [1]. Such variations are mostly due to adjustments in their morphological parameters like leaf area, number and thickness of leaves as well as other features that are

How to cite this paper: Ayegboyin, K.O. and Akinrinde, E.A. (2016) Effect of Water Deficit Imposed during the Early Developmental Phase on Photosynthesis of Cocoa (*Theobroma cacao* L.). *Agricultural Sciences*, 7, 11-19.

http://dx.doi.org/10.4236/as.2016.71002
generated by phylogeny and adaptations [2]. Aside its morphological adjustment, several studies have shown that plant tolerance or sensitivity to water stress depends on their physiological characteristics [3]. Many physiological mechanisms displayed by plants across different agro-ecological zones are not only related to environmental parameters but also genetic dependant [4]. Consequently, the genetic, morphological and environmental factors that influence plants survival and growth during stress are inter-dependent.

Plant species or genotypes with better tolerance abilities to drought are different morphologically and/or physiologically from other species of the same crop due to variability in their mechanisms for survival and growth under limited water supply. When compared with other tree crops, cocoa is less efficient in the control of water loss [5] and does not tolerate periods of long water stress [6] while ability for osmotic adjustment during water stress varies in cocoa [7]. According to [8], ability to identify the genotypes that combine the traits for good growth and high yield with efficient WUE is an essential mechanism for breeding crops for drought-prone areas. Also, [9] positioned that traits which favour drought tolerance in plants include greater allocation of biomass to root than above ground parts, lower evaporative surface (leaf area) and thicker leaves.

The limited drought tolerance ability of the crop is a growing concern in the cocoa production areas mainly due to the inconsistent rainfall patterns [10]. While [11] explained that many research findings had already reported evidences of decline annual rainfall in the cocoa grown areas of Ghana. In Nigeria, a lot of the newly transplanted cocoa seedlings and/or cuttings die during the “longer-than-usual” dry spell in the cocoa production area of the country.

Increase in the length of dry seasons and high potential of expansion of cocoa production to many marginal environments in Nigeria demand the identification and selection of drought tolerance cocoa genotypes. According to [12], it is now paramount to study the water requirement of cocoa and breed for new genotypes that are more tolerant to environmental stress which is currently being experienced in the crop’s production areas. This work, therefore, presents a greenhouse experiment that studied the effect of water stress conditions on some morphological and physiological parameters in the growth and early development of four genotypes of cocoa in Nigeria.

2. Materials and Methods

The study was carried out in the greenhouse of Cocoa Research Institute of Nigeria, Ibadan, with the daylight and temperature during the growth season varied from 14 h to 17 h and 22 °C to 30 °C, respectively. Four cocoa varieties (F3 Amazon, T1, T7 and Amelonado) were sown at the rate of 4 seeds per pot which were previously filled with about 3 kg soil and thinned to 2 seedlings per pot at 4 weeks after sown (WAS). T1 and T7 were selected randomly from the CRIN’s newest release (T1, T2, T3, T4, T5, T6, T7 and T8 cocoa genotypes) which were also known as CRIN Seed Garden or 18-month Cocoa Varieties. While F3 Amazon is described as the 3rd filial generation of the Upper Amazonian variety released by CRIN, Ibadan, Tsimply means Theobroma. Both F3 Amazon and Seed Garden are high yielding and early bearing cocoa varieties. However, the latter was the newest release of CRIN and has an advantage of earlier fruit production, about 18-month after planting. F3 Amazon starts fruit bearing around 24 months old. Unlike the first two varieties, Amelonado cocoa, also known as West African Amelonado, is less efficient in terms of time and quantity of fruits production.

The experimental soil was a top-soil of a Sandy loam Alfisol, Olorunda Series (USDA Soil Classification). The soil was moderately rich in organic content and collected within 0 - 15 cm depths with a soil auger from a virgin forest in the nearby Onigbambari Forest Reserve. The soil was air-dried before and passed through 10mm sieve to remove stones, roots and any other debris. All pots were circular and of equal size while weather parameters like light intensity and duration, temperature and relative humidity were same for all treatments.

All pots were watered at 2-day interval till 4WAS when different water regimes were imposed on the plants. After the determination of the rate of water loss from cocoa seedlings, appropriate quantities of water were applied at 4 different frequencies (daily, 3-day, 5-day and 7-day intervals) till 28WAS. Field capacity of the soil was determined using the modified [13] and applied to the plants at 100%, 50% and 25%. There were 4 cocoa genotypes, 3 field capacities (100%, 50% and 25%), 4 watering frequencies (daily, 3-day, 5-day and 7-day intervals), 3 replications and 2 stands per genotype per replicate of cocoa plants totalling 288 experimental pots arranged in Completely Randomised Design (CRD). All cocoa plants were sampled for data collection.

Data was collected at 5WAS, 12WAS, 16WAS, 20WAS, 24WAS and 28WAS on some of the morphological parameters (plant height, leaf area and root length) and gas exchange characteristics (stomatal conductance, photosynthetic rate and water use efficiency) of the cocoa plants. Plant height was measured non-destructively in-situ while the leaf area was measured destructively with portable Leaf Area Meter AM 300 (ADC Bio Scien-
K. O. Ayegboyin, E. A. Akinrinde

The plant total harvest was conducted twice at 14 WAS and 28 WAS to study the effect of variation in water regimes on the root length of the plants while a portable infra-red gas analyser (ADC Bio Scientific, United Kingdom) was used to measure stomatal conductance as well as the transpiration and photosynthetic rates. Water use efficiency was a ratio between the photosynthetic rate and transpiration or the photosynthetic rate and stomatal conductance, as the case may be.

Measurements on gas exchange characteristics were collected between 12:00 pm and 3:30 pm during each sampling day in order to obtain an accurate indication of cocoa response to environmental stress [14]. However, the plants were sometimes moved out of the greenhouse to the open place in order to get ≥300 µmol m⁻² s⁻¹ of sunshine needed for optimum performance of the cocoa. Genstat (VSN International Limited) 13th edition was used to analyse the data by means of analysis of variance with significant means were determined by least significant difference (LSD) at P = 0.05 value.

3. Results

3.1. Soil Analysis

The physico-chemical analyses of the soil (Table 1) showed that it was slightly acidic (pH (H₂O) = 6.66) and quite suitable for optimum cocoa production [15]. The values of nitrogen (5.7 g·kg⁻¹) and phosphorus (4.77 mg·kg⁻¹) of the soil were above their critical levels of 1.8 g·kg⁻¹ and 0.13 mg·kg⁻¹ respectively, while potassium value (0.25 cmol·kg⁻¹) was below its critical level of 1.2 cmol·kg⁻¹ for raising cocoa [16]. The soil can be described as sandy loam while both calcium and magnesium values (0.41 cmol·kg⁻¹ and 0.24 cmol·kg⁻¹) were also above their critical levels of 0.3 cmol·kg⁻¹ and 0.2 cmol·kg⁻¹, respectively [16].

3.2. Morphological Parameters

Most of the morphological traits measured varied between clones. Plant heights ranged from 69.9 cm for F₃ Amason watered 3-day interval at 50% field capacity to 10.2 cm for Amelonado watered 7-day interval at 25% field capacity (Table 2). Differences in the leaf area ranged from 252.3 cm² for T₁ watered daily at 50% field capacity to 99.0 cm² for Amelonado watered 7-day interval at 25% field capacity (Table 3). The root length ranged from 39.4 cm for T₁ watered 3-day interval at 50% field capacity to 22.3 cm for Amelonado watered 7-day interval at 50% field capacity with most plants watered 3-day interval at 100% and 50% FC having longer root length.

### Table 1. The physico-chemical property of the experimental soil before planting.

| Property                        | Value  |
|---------------------------------|--------|
| pH (H₂O)                        | 6.66   |
| Organic Carbon (g·kg⁻¹)         | 25.4   |
| Total Nitrogen (g·kg⁻¹)         | 5.7    |
| Available Phosphorus (mg·kg⁻¹)  | 4.77   |
| Calcium (cmol·kg⁻¹)             | 0.41   |
| Magnesium (cmol·kg⁻¹)           | 0.24   |
| Potassium (cmol·kg⁻¹)           | 0.25   |
| Manganese (mg·kg⁻¹)             | 70.5   |
| Iron (mg·kg⁻¹)                  | 2.88   |
| Zinc (mg·kg⁻¹)                  | 3.7    |
| Boron (mg·kg⁻¹)                 | 6.9    |
| Sand (g·kg⁻¹)                   | 809    |
| Silt (g·kg⁻¹)                   | 164    |
| Clay (g·kg⁻¹)                   | 117    |
Table 2. Plant height (cm) of cocoa under different field capacities and water frequencies.

| Cocoa genotype | Field capacity (%) | Daily (cm) | 3-d interval (cm) | 5-d interval (cm) | 7-d interval (cm) |
|----------------|--------------------|------------|-------------------|-------------------|------------------|
| F₃ Amazon      | 100                | 67.1       | 69.8              | 68.6              | 69.5             |
|                | 50                 | 66.9       | 69.9              | 59.8              | 56.0             |
|                | 25                 | 65.0       | 55.0              | 45.0              | 40.6             |
| T₁             | 100                | 68.9       | 66.7              | 61.4              | 66.3             |
|                | 50                 | 66.5       | 66.5              | 46.5              | 56.0             |
|                | 25                 | 64.2       | 53.8              | 45.7              | 42.2             |
| T₇             | 100                | 68.3       | 66.1              | 67.6              | 68.0             |
|                | 50                 | 69.2       | 69.3              | 63.2              | 63.1             |
|                | 25                 | 61.6       | 58.4              | 48.6              | 38.6             |
| Amelonado      | 100                | 69.2       | 70.1              | 64.2              | 64.2             |
|                | 50                 | 67.5       | 67.1              | 53.5              | 47.5             |
|                | 25                 | 68.9       | 44.0              | 28.9              | 10.2             |
| LSD genotype   | NS                 | 8.8        | 7.8               | 20.6              |                  |
| LSD genotype * field capacity | NS | 9.2 | 7.3 | 11.6 | |

Values represent the means of 3 replicates measured 6 times at 8, 12, 16, 20, 24 and 28 weeks after sowing.

Table 3. Mean leaf area (cm²) of cocoa under different field capacities and water frequencies.

| Cocoa genotype | Field capacity (%) | Daily (cm) | 3-d interval (cm) | 5-d interval (cm) | 7-d interval (cm) |
|----------------|--------------------|------------|-------------------|-------------------|------------------|
| F₃ Amazon      | 100                | 227.7      | 238.1             | 209.0             | 199.3            |
|                | 50                 | 216.0      | 230.0             | 199.4             | 186.4            |
|                | 25                 | 215.9      | 200.0             | 178.3             | 140.3            |
| T₁             | 100                | 219.9      | 238.9             | 203.1             | 198.1            |
|                | 50                 | 252.3      | 240.2             | 194.9             | 176.0            |
|                | 25                 | 217.0      | 196.1             | 173.8             | 134.6            |
| T₇             | 100                | 238.2      | 217.7             | 215.0             | 179.0            |
|                | 50                 | 219.2      | 210.5             | 197.9             | 178.1            |
|                | 25                 | 223.5      | 187.5             | 174.3             | 141.0            |
| Amelonado      | 100                | 252.0      | 231.6             | 102.9             | 174.9            |
|                | 50                 | 229.4      | 209.5             | 180.9             | 150.0            |
|                | 25                 | 202.7      | 170.5             | 158.5             | 99.0             |
| LSD genotype   | NS                 | 57.1       | 42.7              | 39.4              |                  |
| LSD genotype * field capacity | NS | 38.9 | 21.1 | 32.6 | |

Values represent the means of 3 replicates measured 6 times at 8, 12, 16, 20, 24 and 28 weeks after sowing.

roots than their counterparts which were watered daily (Table 4).

3.3. Variation in Stomatal Conductance, Photosynthesis and Water Use Efficiency (WUE) between Cocoa Clones

The stomatal conductance, photosynthetic rate and WUE varied significantly between cocoa clones (P < 0.01). The highest stomatal conductance of 0.099 mol m⁻² s⁻¹ was produced by T₃ Amazon watered 3-day interval at
100% field capacity while the lowest stomatal conductance value was 0.007 mol m$^{-2}$s$^{-1}$ from Amelonado watered 7-day interval at 25% field capacity (Table 5). Average photosynthetic rate ranged from 4.671 µmol (CO$_2$) m$^{-2}$s$^{-1}$ for F$_3$ Amazon watered daily at 50% field capacity and 1.021 µmol (CO$_2$) m$^{-2}$s$^{-1}$ for Amelonado watered 7-day interval of Amelonado at 25% field capacity (Table 6).

Instantaneous water use efficiency (defined as the ratio of photosynthetic rate to transpiration rate) varied between 4.9 mmol (CO$_2$) mmol$^{-1}$ (H$_2$O) and 3.0 mmol (CO$_2$) mmol$^{-1}$ (H$_2$O) while genotypic differences in the mean root length (cm) of cocoa under different field capacities and water frequencies.

| Cocoa genotype | Field capacity (%) | Daily (cm) | 3-d interval (cm) | 5-d interval (cm) | 7-d interval (cm) |
|----------------|-------------------|------------|------------------|------------------|------------------|
| F$_3$ Amazon   | 100               | 33.3       | 38.3             | 39.0             | 39.3             |
|                | 50                | 35.4       | 39.2             | 37.1             | 32.5             |
|                | 25                | 31.0       | 29.9             | 28.4             | 29.8             |
| T$_1$          | 100               | 31.5       | 38.8             | 35.5             | 39.1             |
|                | 50                | 32.3       | 39.4             | 34.5             | 32.6             |
|                | 25                | 27.0       | 28.0             | 25.2             | 27.4             |
| T$_7$          | 100               | 38.1       | 37.4             | 38.2             | 39.2             |
|                | 50                | 33.4       | 37.9             | 37.5             | 28.6             |
|                | 25                | 27.0       | 29.7             | 25.8             | 29.7             |
| Amelonado      | 100               | 35.0       | 35.6             | 37.6             | 34.7             |
|                | 50                | 34.4       | 34.5             | 27.2             | 22.3             |
|                | 25                | 26.5       | 25.3             | 24.0             | 22.5             |

LSD genotype: NS 3.1 7.2 10.1
LSD genotype * field capacity: 10.2 11.5 10.1 12.6

Values represent the means of 3 replicates measured 6 times at 8, 12, 16, 20, 24 and 28 weeks after sowing.

| Cocoa genotype | Field capacity (%) | Daily (mol m$^{-2}$s$^{-1}$) | 3-d interval (mol m$^{-2}$s$^{-1}$) | 5-d interval (mol m$^{-2}$s$^{-1}$) | 7-d interval (mol m$^{-2}$s$^{-1}$) |
|----------------|-------------------|-----------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| F$_3$ Amazon   | 100               | 0.081                       | 0.099                               | 0.067                               | 0.061                               |
|                | 50                | 0.098                       | 0.081                               | 0.056                               | 0.048                               |
|                | 25                | 0.051                       | 0.062                               | 0.043                               | 0.014                               |
| T$_1$          | 100               | 0.089                       | 0.091                               | 0.071                               | 0.067                               |
|                | 50                | 0.075                       | 0.092                               | 0.069                               | 0.051                               |
|                | 25                | 0.026                       | 0.067                               | 0.042                               | 0.017                               |
| T$_7$          | 100               | 0.078                       | 0.088                               | 0.097                               | 0.067                               |
|                | 50                | 0.077                       | 0.087                               | 0.073                               | 0.045                               |
|                | 25                | 0.067                       | 0.080                               | 0.055                               | 0.019                               |
| Amelonado      | 100               | 0.088                       | 0.087                               | 0.068                               | 0.059                               |
|                | 50                | 0.081                       | 0.082                               | 0.067                               | 0.060                               |
|                | 25                | 0.068                       | 0.077                               | 0.056                               | 0.048                               |

LSD genotype: 0.011 0.013 0.009 0.007
LSD genotype * field capacity: 0.034 0.025 0.041 0.021

Values represent the means of 3 replicates measured 6 times at 8, 12, 16, 20, 24 and 28 weeks after sowing.
Table 6. Mean photosynthetic rate ($\mu$mol m$^{-2}$ s$^{-1}$) of cocoa under different field capacities and water frequencies.

| Cocoa genotype | Field capacity (%) | Daily (µmol m$^{-2}$ s$^{-1}$) | 3-d interval (µmol m$^{-2}$ s$^{-1}$) | 5-d interval (µmol m$^{-2}$ s$^{-1}$) | 7-d interval (µmol m$^{-2}$ s$^{-1}$) |
|----------------|-------------------|-------------------------------|------------------------------------|------------------------------------|------------------------------------|
| F$_3$ Amazon   | 100               | 4.321                         | 4.331                              | 3.671                              | 3.045                              |
|                | 50                | 4.671                         | 3.491                              | 2.901                              | 2.962                              |
|                | 25                | 3.123                         | 2.011                              | 2.491                              | 1.050                              |
| T$_1$          | 100               | 4.034                         | 4.201                              | 3.718                              | 3.041                              |
|                | 50                | 3.982                         | 4.067                              | 3.541                              | 3.011                              |
|                | 25                | 3.171                         | 2.902                              | 2.703                              | 1.034                              |
| T$_7$          | 100               | 3.901                         | 4.067                              | 3.785                              | 2.991                              |
|                | 50                | 4.272                         | 3.552                              | 2.890                              | 2.091                              |
|                | 25                | 4.001                         | 3.078                              | 2.744                              | 1.072                              |
| Amelonado      | 100               | 4.098                         | 4.051                              | 2.901                              | 2.078                              |
|                | 50                | 4.321                         | 3.908                              | 2.090                              | 2.045                              |
|                | 25                | 4.671                         | 2.891                              | 1.997                              | 1.021                              |
| LSD genotype   |                   | 1.034                         | 1.012                              | 1.007                              | 1.008                              |
| LSD genotype*field capacity | NS          | 1.051                         | 1.257                              | 1.061                              |

Values represent the means of 3 replicates measured 6 times at 8, 12, 16, 20, 24 and 28 weeks after sowing.

ratio of intrinsic water use efficiency (defined as the ratio of photosynthetic rate to stomatal conductance) were also significant ($P < 0.01$) with mean values ranged from 67.4 µmol (CO$_2$) mmol$^{-1}$ (H$_2$O) for F$_3$ Amazon to 42.3 µmol (CO$_2$) mmol$^{-1}$ (H$_2$O) for Amelonado (Table 7). A large proportion of the observed variation between cocoa genotypes in light-saturated photosynthetic rate across all water regimes and watering frequencies could be explained by variation in stomatal conductance (Figure 1).

4. Discussion

In the present work, most of the morphological parameters (especially, plant height and leaf area) had linear and positive relationships with the quantities of water supplied. In Ghana, [17] similarly found that cocoa plants that received smaller quantities of water, in terms of water volume and/or frequency, produced shorter and thinner plants. The study showed that water deficit also resulted in the reduction in leaf cell sizes which invariably led to declined leaf areas. Leaf growth is one of the first physiological processes affected by changes in plant water status under drought since a decrease in leaf expansion rate usually precedes any reduction in stomatal conductance or photosynthesis [18]. In cocoa, although growth and leaf expansion are controlled by endogenous mechanisms, the effects of environment override such influence [19].

Relationships between vegetative growth and photosynthesis are important in determining yield while availability of enough water to create non-water stress environment increase the proliferation of the plant root. Reduction in leaf area is considered as one of the earliest adaptation mechanisms to water deficits. The work of [20] discovered that in cocoa, leaf size is more sensitive to water stress than stomatal conductance and CO$_2$ assimilation. Persistence water stress leads to reduction in the leaf area and consequent lower net assimilation per unit leaf area and the harvestable yield of cocoa [21]. Ability to specifically diminish above-ground biomass and maintain good root system will afford cocoa the opportunity to exploit the limited water resources, to some extent, during drought. The significant differences ($P = 0.05$) that existed in the plant height, leaf area and root length of plants confirmed similar results of early genotypic variation in the morphological attributes of cocoa [22]. The results of the present work show that cocoa leaf area, stomatal conductance and photosynthetic rate had linear and positive relationships with the quantities of water supplied in agreement with [1].

The observed high correlation between plant photosynthetic rate and stomatal conductance showed that water potential has a serious impact on the growth and development of cocoa [23]. Decrease in photosynthetic rate
Table 7. Mean water use efficiency (WUE) traits of the four cocoa genotypes with standard error in brackets.

| Genotype     | WUE\textsubscript{INSTANTANEOUS} mmol (CO\textsubscript{2}) mmol\textsuperscript{−1} (H\textsubscript{2}O) | WUE\textsubscript{INTRINSIC} μmol (CO\textsubscript{2}) mmol\textsuperscript{−1} (H\textsubscript{2}O) |
|--------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| F\textsubscript{2} Amazon          | 4.9 (0.2)                                                                         | 67.4 (4.4)                                                                         |
| Theobroma\textsubscript{i}        | 4.1 (0.2)                                                                         | 67.0 (3.9)                                                                         |
| Theobroma\textsubscript{j}        | 4.8 (0.2)                                                                         | 66.9 (4.7)                                                                         |
| Amelonado    | 3.0 (0.1)                                                                         | 42.3 (3.2)                                                                         |

Figure 1. The relationships between stomatal conductance and light saturated photosynthesis of cocoa at different watering frequencies.

Plants have developed physiological responses as well as ecological strategies to cope with soil water shortage, either by stress avoidance or stress tolerance [25]. Mechanisms developed by cocoa plants to survive water deficits in the present study mainly involve avoidance of tissue water stress which includes stomatal closure and reduction in leaf area, among other factors.
5. Conclusion

Water deficit adversely affected development and growth of cocoa directly by dehydration of the photosynthetic apparatus or indirectly through stomatal closure. There was an evidence of genotypic variation in response of cocoa to water stress and considerable intra-specific variation in the photosynthetic performance and growth of cocoa. Therefore, ability for higher photosynthetic activity during water deficit may be an important step for enhancing cocoa cultivation and a factor for consideration in breeding of cocoa. The results of the experiment showed that F3 Amazon, T1 any T7 performed better than Amelonado during water stress.

References

[1] Ayegboyin, K.O. (2012) The Effect of Rootstock on the Genotypic Variability in Drought Tolerance of Theobroma cacao L. in Nigeria. Ph.D. Thesis, University of Reading, Reading, United Kingdom.

[2] Sack, L., Cowan, P.D., Jaikumar, N. and Holbrook, N.M. (2003) The “Hydrology” of Leaves: Co-Ordination of Structure and Function in Temperate Woody Species. Plant, Cell and Environment, 26, 1343-1356. http://dx.doi.org/10.1046/j.0016-8025.2003.01058.x

[3] Ahmed, C.B., Rouina, B.B. and Boukhris, M. (2007) Effects of Water Deficit on Olive Trees cv. Chemlali under Field Conditions in Arid Region in Tunisia. Scientia Horticulturae, 113, 267-277. http://dx.doi.org/10.1016/j.scienta.2007.03.020

[4] Villalobos, F.J., Morian, A. and Fereres, E. (2002) Stomatal and Photosynthesis Responses of Olive (Olea europaea L.) Leaves to Water Deficits. Plant Cell Environ, 25, 387-395.

[5] Raja-Harun, R.M. and Hardwick, K. (1988) The Effect of Different Temperatures and Water Vapour Pressure Deficits on Photosynthesis and Transpiration of Cocoa Leaves. Proceeding of the 10th International Cocoa Research Conference, Santo Domingo, Dominican Republic, 17-23 May 1987, 211-214.

[6] Bae, H., Kim, S., Kim, M S., Sicher, R.C., Lary, D., Strem, M.D., Natarajan, S. and Bailey, B.A. (2008) The Drought Response of Theobroma cacao (Cacao) and the Regulation of Genes Involved in Polyamine Biosynthesis by Drought and Other Stresses. Plant Physiology and Biochemistry, 46, 174-188. http://dx.doi.org/10.1016/j.plaphy.2007.10.014

[7] Balasimha, D. and Daniel, E.V. (1988) A Screening Method for Drought Tolerance in Cocoa. Current Science, India, 57, 395.

[8] Dias, P.C., Araujo, W.L., Moraes, G.A.B.K., Barros, R.S. and Damatta, F.M. (2007) Morphological and Physiological Responses of Two Coffee Progenies to Soil Water Availability. Journal of Plant Physiology, 164, 1639-1647. http://dx.doi.org/10.1016/j.jplph.2006.12.004

[9] Ludlow, M.M. (1989) Strategies of Response to Water Stress. In: Kreeb, K.H., Richter, H. and Hinckley, T.M., Eds., Structural and Functional Responses to Environmental Stresses, SPB Academic Publishing, The Hague.

[10] Anim-Kwapong, G.J. and Frimpong, E.B. (2004) Vulnerability and Adaptation to Climate Change-Impact of Climate Change on Cocoa Production. Vulnerability and Adaptation Assessment under the Netherlands Climate Change Studies Assistance Programme Phase 2 (NCCSAP). Cocoa Research Institute of Ghana, New Tafo Akim. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.494.4508&rep=rep1&type=pdf

[11] Owusu, K. and Waylen, P. (2009) Trends in spatial-temporal variability in annual rainfall in Ghana (1951-2000). Weather, 64, 115-120. http://dx.doi.org/10.1002/wea.255

[12] Hadley, P. (2007) Delivering Research Outputs in Cocoa; a Physiological Roadmap. 3rd Meeting of Thames Valley Cocoa Club: How Can Cocoa Physiology Impact on Farmers’ Livelihoods? ICCO, London, 19 December 2007. http://www.thamesvalleycocoa.org/

[13] Craven, D., Gulambussein, S. and Berlyn, G.P. (2010) Physiological and Anatomical Responses of Acacia koa (Gray) Seedlings to Varying Light and Drought Conditions. Environmental and Experimental Botany, 69, 205-213. http://dx.doi.org/10.1016/j.envexpbot.2010.04.002

[14] Medrano, H., Escalona, J.M., Cifre, J., Bota, J. and Flexas, J. (2003) A Ten-Year Study on the Physiology of Two Spanish Grapvine Cultivars under Field Conditions: Effects of Water Availability from Leaf Photosynthesis to Grape Yield and Quality. Functional Plant Biology, 30, 607-619. http://dx.doi.org/10.1071/fp02110

[15] Mossu, G. (1992) Cocoa. The Tropical Agriculturalist, Maisonneuve et Larose. CTA/ Macmillan.

[16] Wessel, M. (1971) Fertilizer Requirements of Cacao (Theobroma cacao L.) in South-Western Nigeria. Royal Tropical Institute, Wageningen, The Netherlands.

[17] Acheampong, K. (2010) A Physiological Study on the Field Establishment of Cacao Clones through the Improvement of Agro-Ecological Conditions. Ph.D. Thesis, University of Reading, Reading, United Kingdom.

[18] Farooq, M., Kobayashi, N., Ito, O., Wahid, A. and Serraj, R. (2010) Broader Leaves Result in Better Performance of
Indica Rice under Drought Stress. *Journal of Plant Physiology*, 167, 1066-1075.  
http://dx.doi.org/10.1016/j.jplph.2010.03.003

[19] Abo-Hamed, S., Collin, H.A. and Hardwick, K. (1985) Biochemical and Physiological Aspects of Leaf Development in Cocoa (*Theobroma cacao* L.) IX. Water Relations and Abscisic Acid in the Control of Leaf Development. *Café, Cacao, Thea*, 29, 155-162.

[20] Rajagopal, V. and Balasimha, D. (1994) Drought Tolerance in Plantation Crops. In: Chalha, K. L. and Rethinam, P., Eds., *Advances in Horticulture, Vol. 10: Plantation and Spice Crops Part 2*, Malhotra Publishing House, New Delhi, India.

[21] Joly, R. J. and Hahn, D. T. (1989) An Empirical Model for Leaf Expansion in Cacao in Relation to Plant Water Deficit. *Annals of Botany*, 64, 1-8.

[22] Daymond, A.J., Hadley, P., Machado, R.C. R. and Ng, E. (2002) Canopy Characteristics of Contrasting Clones of Cacao (*Theobroma cacao* L.). *Experimental Agriculture*, 38, 359-367. http://dx.doi.org/10.1017/S0014479702003083

[23] Hsiao, T.C. (1973) Plant Responses to Water Stress. *Annual Review of Plant Physiology*, 24, 519-570.  
http://dx.doi.org/10.1146/annurev.pp.24.060173.002511

[24] Pezeshki, S.R. (1994) Responses of Baldcypress (*Taxodium distichum*) Seedlings to Hypoxia: Leaf Protein Content, Ribulose-1, 5-Bisphosphate Carboxylase/Oxygenase Activity and Photosynthesis. *Photosynthetica*, 30, 59-68.

[25] Kramer, J.K. and Boyer, J.S. (1995) Water Relations of Plants and Soils. Academic Press, California.