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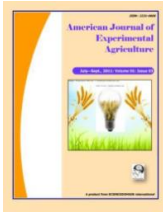
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The Influence of Shade and Organic Fertilizer Treatments on the Physiology and Establishment of *Theobroma cacao* Clones

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Authors' contributions

This work was carried out in collaboration between all authors. Author KA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AJD, PH reviewed the experimental design and all drafts of the manuscript. Authors KA and PAY managed the analyses of the study. Authors KA and AJD identified the plants. Authors KA and PH performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This experiment aimed to determine whether the soil application of organic fertilizers can help the establishment of cacao and whether shade alters its response to fertilizers.

Study Design: The 1.6 ha experiment was conducted over a period of one crop year (between April 2007 and March 2008) at the Cocoa Research Institute of Ghana. It involved four cacao genotypes (T 79/501, PA 150, P 30 [POS] and SCA 6), three shade levels ('light', 'medium' and 'heavy') and two fertilizer treatments ('no fertilizer', and 140 kg/ha of cacao pod husk ash (CPHA) plus poultry manure at 1,800 kg/ha). The experiment was designed as a split-plot with the cacao genotypes as the main plot factor and shade x fertilizer combinations as the sub-plots.

Methodology: *Gliricidia sepium* and plantains (*Musa sapientum*) were planted in different

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arrangements to create the three temporary shade regimes for the cacao. Data were collected on temperature and relative humidity of the shade environments, initial soil nutrients, soil moisture, leaf N, P and K⁺ contents, survival, photosynthesis and growth of test plants.

Results: The genotypes P 30 [POS] and SCA 6 showed lower stomatal conductance under non-limiting conditions. In the rainy seasons, plants under light shade had the highest CO₂ assimilation rates. However, in the dry season, plants under increased shade recorded greater photosynthetic rates ($P = .03$). A significant shade x fertilizer interaction ($P = .001$) on photosynthesis in the dry season showed that heavier shade increases the benefits that young cacao gets from fertilizer application in that season. Conversely, shade should be reduced during the wet seasons to minimize light limitation to assimilation.

Conclusion: Under ideal weather conditions young cacao exhibits genetic variability on stomatal conductance. Also, to optimize plant response to fertilizer application shade must be adjusted taking the prevailing weather condition into account.

Keywords: Cacao; shade; organic fertilizer; photosynthesis; establishment.

1. INTRODUCTION

Cacao is cultivated through the humid tropics with over 70% of cocoa being produced in West Africa [1]. In order to meet the increasing demand for cocoa [2], it will increasingly be necessary for farmers to selectively replant their cocoa farms thereby replacing aging stock with more productive varieties.

Re-planting of cacao farms in West Africa can be challenging particularly where there is scanty overhead shade [3,4]. The difficulty has often been ascribed to diminished soil fertility partly as a result of the transfer, each year, of large amounts of nutrients as economic yield from cacao ecosystems [5-7]. Therefore, there is a need to replenish the 'mined' soil nutrients in order to sustain cocoa production. Available data on mature cacao suggest that it is practicable to address the soil fertility problem through the use of fertilizers [8-11]. However, because of health and environmental concerns there has been a steady growth in the demand for organically produced cocoa in recent times [12,13]. Therefore, it is worthwhile to investigate whether organic fertilizers aid in re-establishing cacao on soils with a previous history of cacao cultivation. Since the use of overhead shade has been shown to aid seedling survival [14] the interaction between fertilizer addition and overhead shade also was studied.

Thus, the aims of this paper were to determine whether soil application of organic fertilizers can help the field establishment of young cacao and whether shade alters plant response to fertilizers under the prevailing conditions.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at the Cocoa Research Institute of Ghana, West Africa (latitude 6° 13' N, Longitude 0° 22' W), and sited on a 1.6 hectare plot with an ideal soil type classification but a poor soil phase for cacao establishment. Initial soil fertility of the experimental site was determined and compared with standard soil ratings for cacao [15].

2.2 Plant Material

Four cacao genotypes T 79/501, PA 150, SCA 6 (representing, respectively, a vigorous, an intermediate, and a low vigour genotype) and P 30 [POS] (a traditionally cultivated cacao genotype in Ghana, used in this work as control), were propagated by side grafting using a common seedling rootstock (T 60/887 x Amelonado). The scion was inserted below the point of attachment of the cotyledons of the rootstock to prevent chupons growing from below the graft union.

2.3 Experimental Design

The experiment was designed as a split-plot with the cacao genotype as the main plot factor and shade x fertilizer combinations as sub-plots. Each of its four blocks contained 24 sub-plots measuring 9 m x 12 m and having a guard row (shared by adjacent sub-plots) and 6 experimental cacao plants planted at 3 m x 3 m. The experiment was re-randomized in each block. The grafted cacao plants were transplanted in June 2007.

Three temporary shade regimes were created by planting *Gliricidia sepium* and plantains (*Musa sapientum*) in different arrangements:

1. The arrangement of the 'light' shade regime consisted of 1.5 m tall *Gliricidia sepium* stakes and plantains planted at 3 m x 3 m each.
2. The 'medium' shade regime consisted of the 'light' shade treatment plus three stands of 0.5 m tall *Gliricidia sepium* stakes planted at 0.6 m away from the young cacao in a triangle to enclose the cacao.
3. The 'heavy' shade treatment arrangement was the same in all respects as the 'light' shade except that, in relation to the position of the cacao, the plantain suckers were planted towards the west and at 0.6 m (instead of 1.5 m) away from the cacao plants.

2.4 Shade Measurement

Radiation intercepted by the shade was measured twice in each of the three main seasons of West Africa ("Major rainy season"- March to July, "Minor rainy season"- August to October, "Dry season"- November to February) by means of a 'SunScan' SystemSS1 (Manufacturers: Delta-T, UK). Measurements were taken around midday with the light sensor held below the canopy of the shade plant and just above the cacao plants.

2.5 Applied Fertilizer

140 kg per hectare of cacao pod husk ash (equivalent to 125 kg muriate of potash per hectare) plus poultry manure at 1,800 kg/ha was applied per year. Prior to its application the chemical properties of the poultry manure were determined at the Soil Science Laboratory of the Cocoa Research Institute of Ghana (results summarised in Table 1).

2.6 Soil Moisture Determination

Soil moisture content in each of the experimental plots was measured twice in the dry season on January 20 2008 and February 21 2008 using a 40 cm soil moisture probe (Delta-T, U.K; model PR2/4).

2.7 Photosynthesis and Leaf Chlorophyll Fluorescence Measurements

Starting from September 2007 an infrared gas analyser (LC pro+, ADC Bioscience, U. K.) was used to measure stomatal conductance and photosynthesis on single attached leaves twice in each of the three seasons. To minimize differences caused by diurnal fluctuations in photosynthesis, measurements were taken on selected plants between 9:00 am and 11:00 am on three out of the six core plants of each treatment per replication.

2.8 Temperature and Relative Humidity Data Collection

Three data loggers (Tinytag, Gemini data loggers, U.K.) put in a miniature Stevenson screen which was placed below the cacao canopy were used to record the temperature and relative humidity at 15-minute intervals in samples of the three shade environments.

2.9 Plant Growth Measurements

Growth (stem diameter at 20 cm from ground level and height) measurements of experimental plants were carried out once every two months from September 2007. Values were transformed into stem volume indices using the formula developed by Oliet [16]:

$$\text{Stem volume} = \left(\frac{1}{2}\right)\pi\left(\frac{1}{4}\right)(\text{stem diameter})^2 \times \text{height} \quad (1)$$

2.10 Determination of Leaf Chlorophyll, N, P and K⁺ Concentrations

In March 2008, data on leaf chlorophyll were taken. Also N, P and K⁺ concentrations were determined at the Soil Science Division of the Cocoa Research Institute of Ghana on leaves that were approximately of uniform size and physiological age. The data were taken once on a leaf on each of the three plants which were used for photosynthetic data collection in each treatment.

Table 1. Chemical properties of applied poultry manure

Total N	Organic C	Organic matter	Total P	C/N ratio	Moisture content
2.45%	35.10%	59.67%	1.19%	14.3	12.30%

2.10.1 Leaf chlorophyll content determination

Leaf chlorophyll concentration was measured on intact leaves by means of an automatic-calibrated and temperature-compensated leaf chlorophyll meter (CL-01 chlorophyll content meter, Hansatech, UK) with a capability range of 0 – 2000 chlorophyll units.

2.10.2 Determination of leaf N

Samples of leaves were dried overnight at 80°C. The samples from different treatments were ground separately and then, using copper as a catalyst, 0.5 g of the samples were digested for two hours with 12 ml of nitrogen-free concentrated H₂SO₄. The digestate was then distilled by the Kjeldahl method for 3 minutes according to the method of Stuart [17] using methyl red as an indicator. A back titration was done to determine the end point. % Nitrogen (N) was calculated as:

$$(\% N) = [\text{Titre value} \times N (= 0.02) \times 1.401] / 0.5] \% \quad (2)$$

2.10.3 Determination of leaf total phosphorus (P)

Samples of leaves (1 g each) were placed in digestion tubes. 15 ml of nitric acid (HNO₃) was added to each sample and then digested for 30 minutes at 150°C in a digester. 10 ml of perchloric acid (HClO₄) was then added to each digest which was re-digested at 200°C for one hour. Total phosphorus (P) in each sample solution was then determined via the ascorbic acid method using spectrophotometry [18].

2.10.4 Determination of leaf K

15 ml of nitric acid (HNO₃) was added to each of 1 g of ground, oven-dried leaf samples and digested for 30 minutes at 150°C in a digester in a fume cupboard. 10 ml of 1 molar perchloric acid was then added to each of the digested samples and re-digested for another one hour at

200°C. The digests were allowed to cool in a fume cupboard and were then filtered through Whatman No. 42 filter papers into 250 ml volumetric flasks. The filtrates were topped up with distilled water to the 250 ml mark and mixed well. The sample filtrates in the 250 ml volumetric flasks were used to determine the leaf potassium content on an atomic absorption spectrometer [19].

3. RESULTS

3.1 Attained Shade Levels

The 'light' 'medium', and 'heavy' shade regimes attained between 50% and 52.5% shade, between 52% and 57% shade and between 58% and 61% shade, respectively. The lower shade levels for the shade regimes were obtained in the dry season as the shade plants experienced more rapid leaf senescence and leaf shedding.

3.2 Soil Fertility of Experimental Site

Table 2 shows the initial soil nutrient levels of the experimental site. Compared with Smyth's suggested standard [15], soils at the experimental site were low on % N, % O.M. and % C and the C/N ratio was below standard.

3.3 Soil Moisture

Soil moisture content was not statistically different between fertilized and non-fertilized plots. However, soil moisture increased ($P < .001$) further down the soil profile (Fig.1).

3.4 Microclimatic Data

Table 3 shows the air temperature and relative humidity under the different shade treatments across the seasons. Under increasing shade slightly higher relative humidity and lower temperature values were recorded particularly in the dry season.

Table 2. Initial soil fertility of experimental site compared with Smith's suggested standard [15]

Soil fertility parameters	Soil depth(cm)	pH	% N	% O.M.	Avail. P ($\mu\text{g g}^{-1}$)	% C	C/N
Experimental site	0-15	5.7	0.13	1.67	20.55	0.97	7.46
Experimental site	15-30	5.5	0.08	1.02	16.69	0.59	7.35
Medium rating (standard range)	-	5.1-5.5	0.2-0.5	2.0-4.2	6.5-13.0	1.16-2.44	13.0- 20.0
Low rating (standard range)	-	-	0.1-0.2	1.0-2.0	3.0- 6.5	0.58-0.16	> 20.0

The dry season had the highest daytime temperature and the lowest relative humidity values whereas the minor rainy season had the lowest daytime temperature and the highest relative humidity values. Therefore, daytime water vapour pressure deficit was lowest in the minor rainy season and highest in the dry season.

3.5 Gas Exchange

3.5.1 Stomatal conductance

The mean stomatal conductance for both the major and minor rainy seasons was 7.5 times higher ($P < .001$) than that of the dry season (Fig. 2). In the major rainy season cacao plants under light shade had the highest stomatal conductance ($P = .006$). Furthermore, fertilized plants had higher stomatal conductance than non-fertilized ones ($0.534 \text{ mol m}^{-2} \text{ s}^{-1}$ compared with $0.326 \text{ mol m}^{-2} \text{ s}^{-1}$, $P < .001$) and P 30 [POS] had the highest rates while PA 150 had the lowest rates ($P = .02$) in the dry season. None of the interactions was significant.

Unlike the major rainy season, a significant genotype x shade interaction ($P = .005$) was observed during the minor rainy season.

Whereas higher stomatal conductance was recorded in PA 150 and P 30 [POS] under increasing shade, no clear trends were observed for T 79/501 and SCA 6.

In that season fertilized cacao had 14.5% higher stomatal conductance ($P = .05$).

In the dry season, a significant ($P = .004$) cacao genotype x shade interaction was observed in which T 79/501 had its lowest rates under the medium shade whereas the other genotypes had their lowest stomatal conductance under heavy shade. Also fertilized cacao plants had 25.4% higher stomatal conductance than non-fertilized plants ($P = .007$) in the dry season.

3.5.2 Photosynthesis

Mean photosynthetic rates were higher in the major rainy season than in the minor rainy and dry seasons by 19.26% and 71.86%, respectively (Fig. 3). Also reduced shade was associated with higher ($P = .03$) photosynthetic rates in the rainy seasons. There were no statistical differences between the cacao genotypes in any of the seasons.

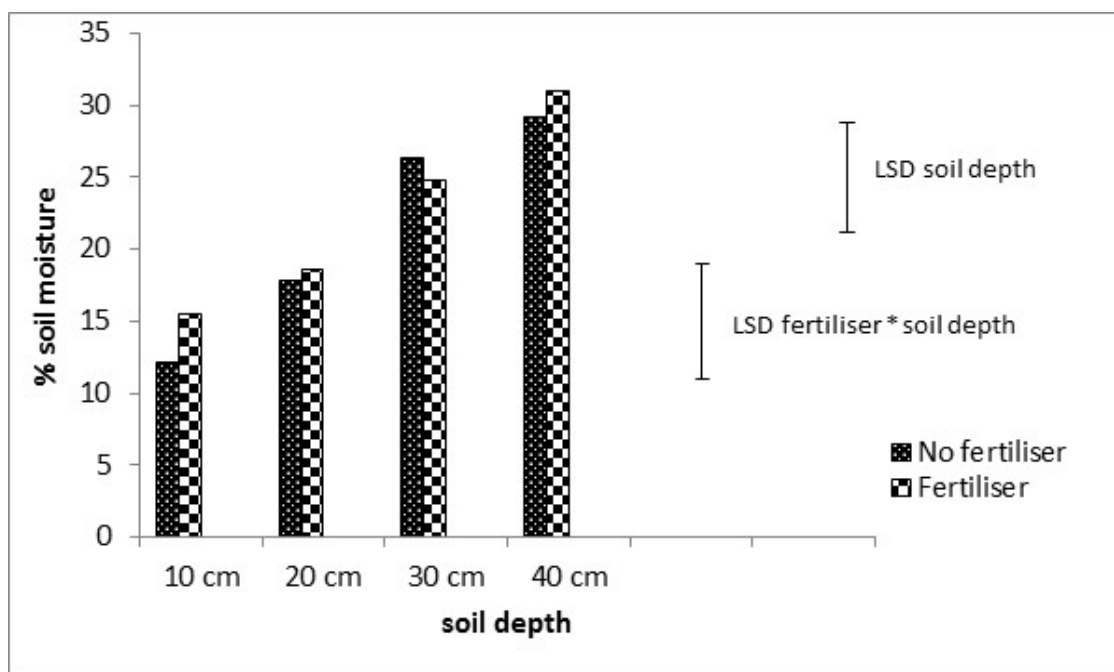


Fig. 1. Soil moisture measured under different fertilizer treatments and soil depths in the dry season

Table 3. Mean relative humidity (R.H.), daytime temperature (T °C) and vapour pressure deficit (VPD) under the shade regimes during the seasons. Each value represents the mean of data recorded every 15 minutes from 3 data loggers

Shade type	Major rainy season			Minor rainy season			Dry season		
	R.H. (%)	T °C	VPD kPa	R.H. (%)	T °C	VPD kPa	R.H. (%)	T °C	VPD kPa
Light	64.25	30.50	1.56	79.25	28.65	0.82	52.25	34.70	2.64
Medium	74.50	29.25	1.04	82.50	27.00	0.63	54.50	33.50	2.36
Heavy	78.50	30.00	0.91	83.25	26.80	0.59	56.00	33.00	2.22

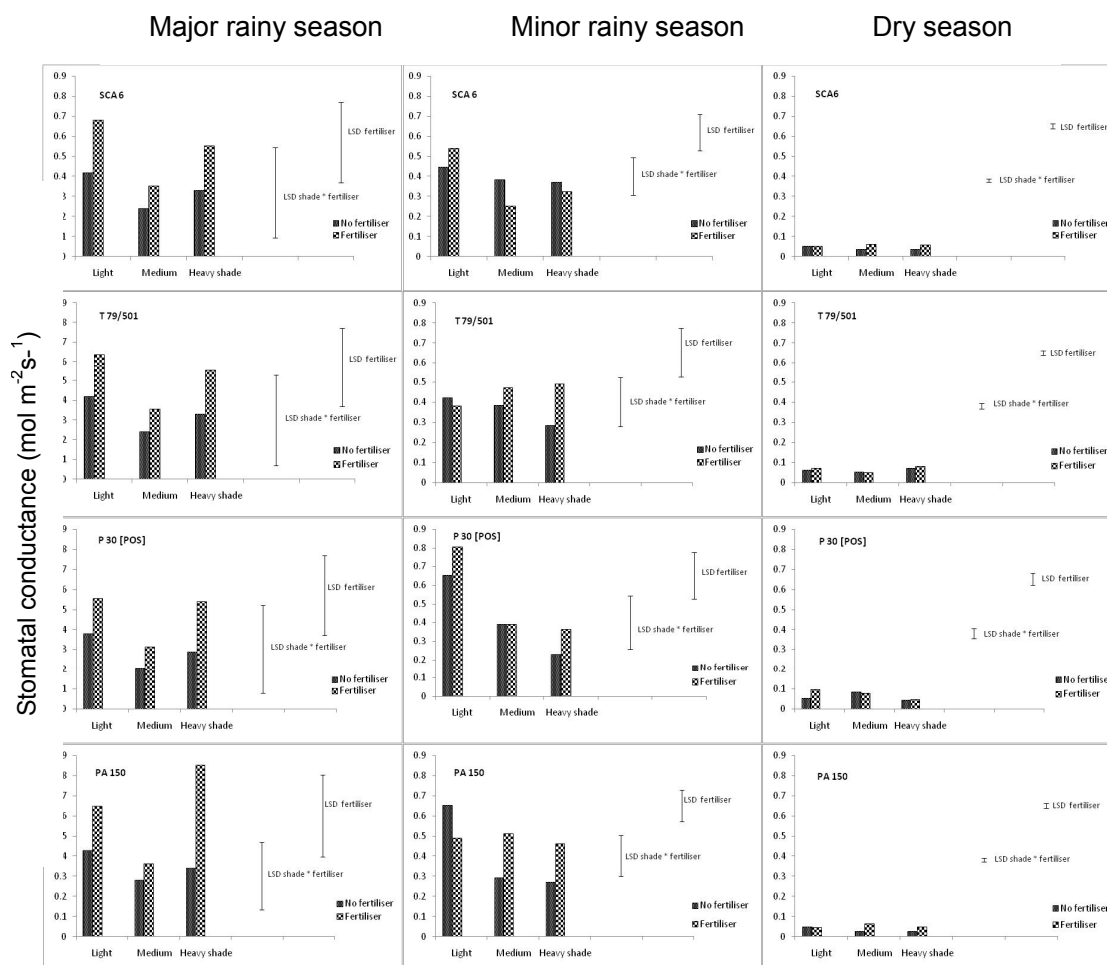


Fig. 2. Stomatal conductance of young cacao under the shade and fertilizer treatments and in different seasons. Each bar represents the mean value for 9 plants

A significant ($P = .001$) shade x fertilizer interaction was observed in the dry season with higher increases occurring in the fertilised plants under the medium (40%) and heavy (47%) shade than under the light shade (7%).

3.6 Water Use Efficiency

The mean water use efficiency of the cacao plants during the dry season was $3.2 \mu\text{mol} (\text{CO}_2)$

/ mmol (H_2O) being 12% and over 100% greater than in the minor and major rainy seasons, respectively.

In the major and minor rainy seasons no significant differences were observed between the cacao genotypes or shade or fertilizer treatments on water use efficiency (Fig. 4).

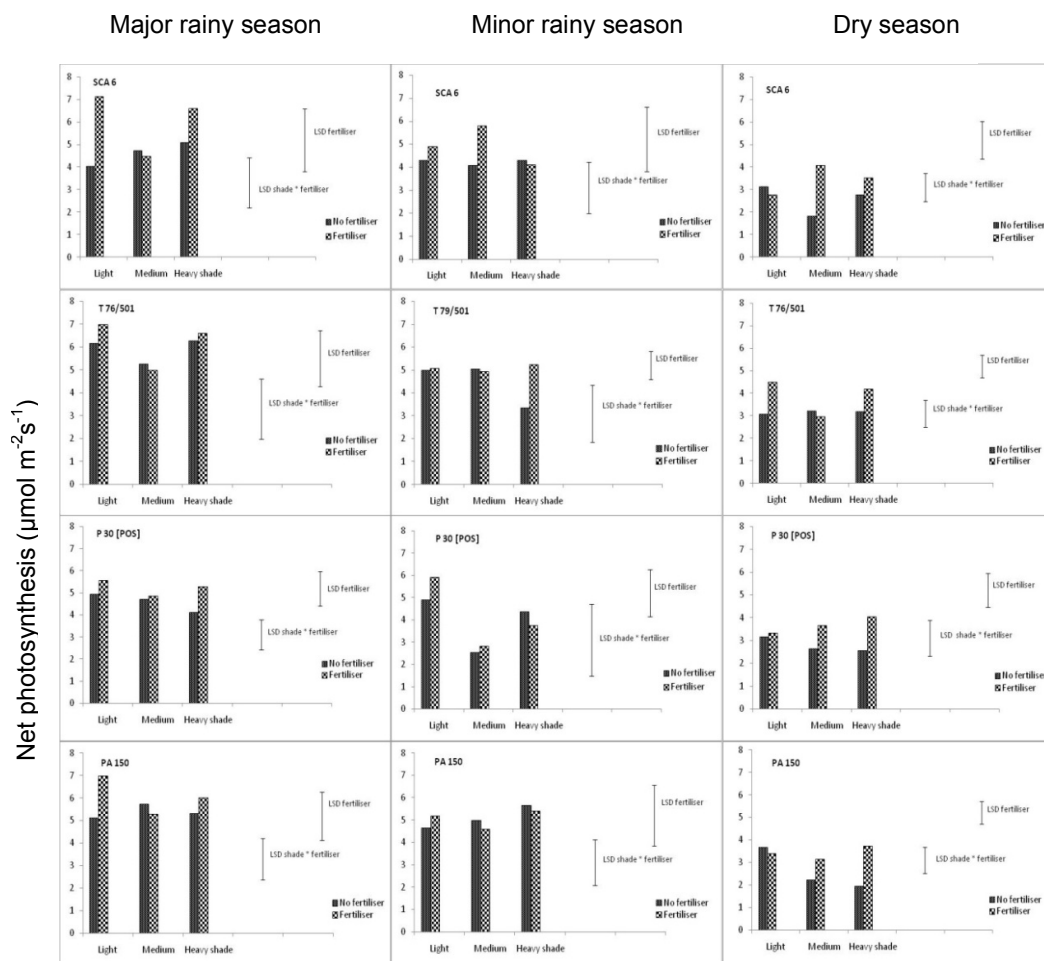


Fig. 3. Photosynthesis of young cacao under different shade and fertilizer treatments and in different seasons. Each bar represents the mean value for 9 plants

In the dry season higher ($P = .002$) water use efficiency was observed in plants under the light shade treatment than the other shade environments.

3.7 Leaf Traits

Only slight increases in leaf chlorophyll concentration were recorded under fertilizer application in the case of SCA 6 and T 79/501 and no clear trends were observed for P 30 [POS] and PA150. Similarly, no clear trends were observed in leaf chlorophyll content under the different shade regimes.

Differences ($P = .01$) were noted between cacao genotypes on leaf nitrogen concentration (Table 4a). Their values ranged from 1.78% in SCA 6 to 2.02% in PA 150. Whereas leaf nitrogen concentration increased ($P = .001$) with fertilizer

application, only slight differences were recorded for the shade treatments and none of the interactions was significant.

Total leaf phosphorus content did not differ substantially between the cacao genotypes or the shade treatments (Table 4b). Plants that grew under light shade had a 6.85% higher concentration than those that grew under heavy shade. Also fertilization was associated with a marginal increase ($P = .047$) in total leaf phosphorus. No significant interactions were observed.

While no statistical differences were noticed between the genotypes on leaf potassium ion concentration increasing shade was associated with higher leaf K^+ ($P = .01$). The genotype x shade interaction term was

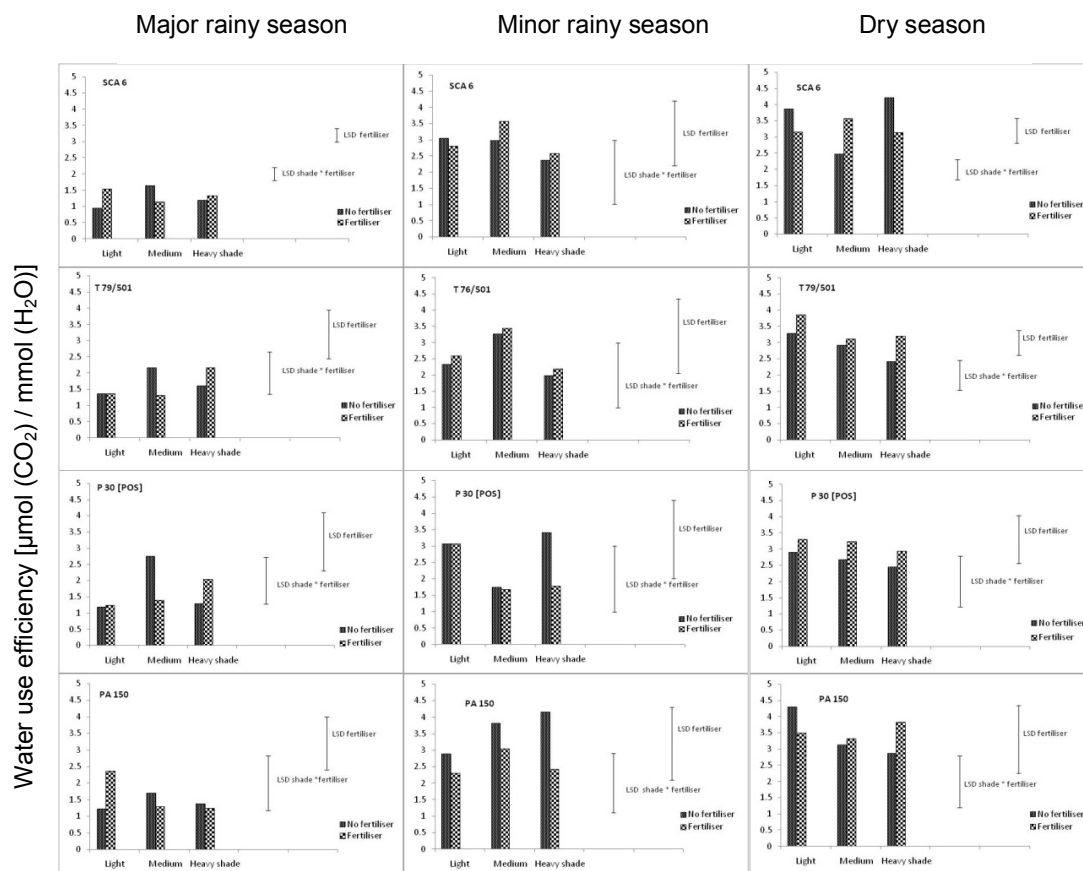


Fig. 4. Water use efficiency of young cacao under different shade and fertilizer treatments and in different seasons. Each bar represents the mean value for 9 plants

significant ($P = .02$) with lower leaf K^+ concentration occurring in SCA 6, T 79/501 and P 30 [POS] whereas PA 150 had higher leaf K^+ concentration under increasing shade. Also fertilized plants had a 13.7% higher leaf K^+ concentration (Table 4c) compared with the control ($P = .001$).

3.8 Plant Survival

Plant survival over the experimental period was different ($P = .03$) among the genotypes, the rates ranging from 79.9% for SCA 6 to 96.5% for PA 150 (Fig. 5). No substantial differences were recorded between the shade treatments but fertilized plants had a higher ($P = .02$) survival rate than the control. No significant interactions were observed.

3.9 Plant Growth

There were significant differences ($P = .02$) in stem volumes between the different cacao

genotypes. The genotype PA 150 which had the largest stem volume (mean = 287.6 cm^3) was 2.6%, 23.5% and 53.6% bigger than T 79/501, P 30 [POS] and SCA 6, respectively. No substantial differences were recorded between the shade or fertilizer treatments (Fig. 6).

4. DISCUSSION

4.1 Impact of Shade and Nutrition on Photosynthesis

The influence of overhead shade in this study on photosynthesis differed between seasons. The observation that in the rainy seasons on average, plants under light shade had the highest stomatal conductance and CO_2 assimilation rates was probably due to light limitation under shade during the prevailing cloudy condition.

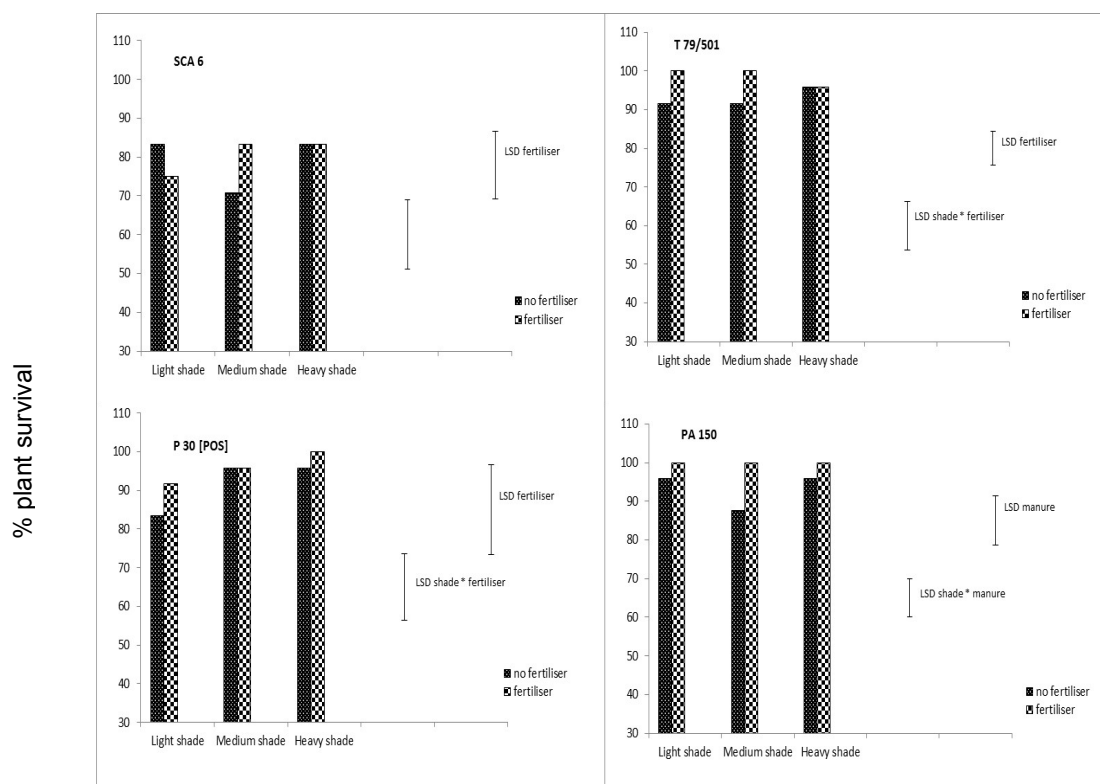


Fig. 5. % surviving cacao plants under varied shade and fertilizer treatments. Each bar represents the mean percentage value for 48 plants

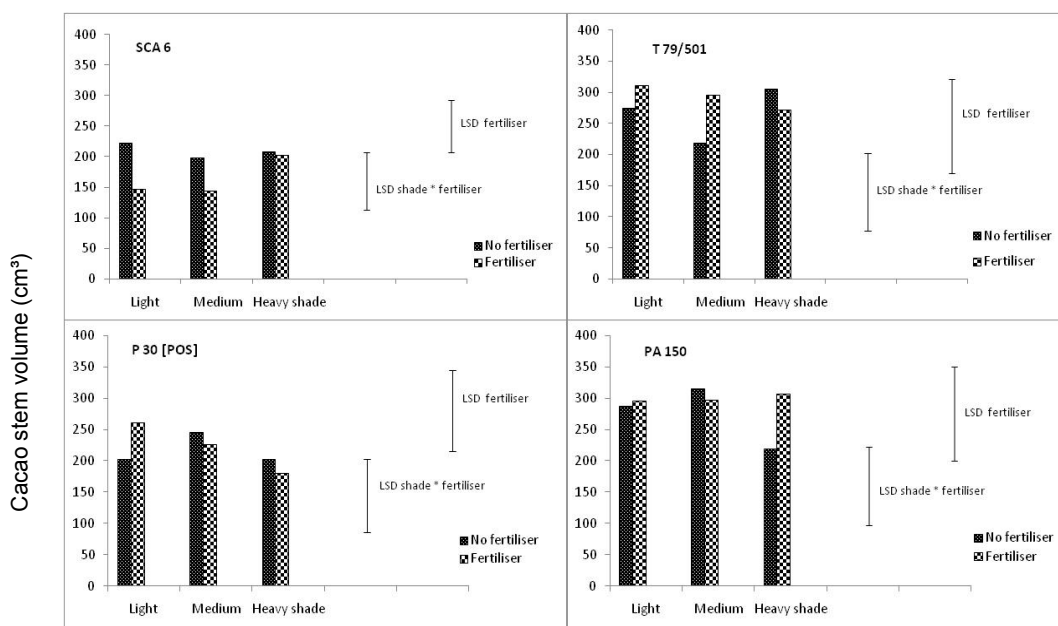


Fig. 6. Stem volume of young cacao under different shade and fertilizer treatments. Each bar represents the mean value of 48 plants

Table 4. Leaf traits of cacao under different shade and fertilizer treatments**Table 4a. % leaf nitrogen of cacao under varied shade and fertilizer treatments**

Genotype	Light shade		Medium shade		Heavy shade		Mean
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	
SCA 6	1.713	1.854	1.899	1.937	1.568	1.718	1.781
T 79/501	1.795	1.834	1.818	2.078	1.715	1.818	1.843
P 30 [POS]	1.815	1.903	1.769	1.927	1.881	1.986	1.880
PA 150	1.829	2.164	1.982	2.177	1.945	2.014	2.019
Mean	1.788	1.939	1.867	2.030	1.777	1.884	1.881

Shade treatments: ($P = .06$); Cacao genotypes: ($P = .01$); Fertilizer treatments: ($P = .001$)

Table 4b. Leaf total phosphorus ($\mu\text{g g}^{-1}$ of leaf tissue) of cacao under different shade and fertilizer treatments

Genotype	Light shade		Medium shade		Heavy shade		Mean
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	
SCA 6	1686	1836	1916	2135	1899	2073	1924
T 79/501	2103	2744	2267	2679	3030	2621	2574
P 30[POS]	1997	2066	2162	2442	2086	2248	2166
PA 150	2209	2272	2223	2201	1864	2253	2170
Mean	1998	2229	2142	2364	2219	2298	2208

Shade treatments: ($P = .31$); Cacao genotypes: ($P = .19$); Fertilizer treatments: ($P = .047$)

Table 4c. Leaf K^+ (meq 100 g^{-1}) of cacao under varied shade and fertilizer treatments

Genotype	Light shade		Medium shade		Heavy shade		Mean
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	
SCA 6	24.79	29.63	33.81	35.70	35.27	36.40	32.6
T 79/501	22.84	37.66	31.05	38.69	41.20	35.83	34.55
P 30 [POS]	33.96	35.20	31.49	39.54	37.90	45.19	37.21
PA 150	31.83	42.19	30.81	36.09	30.19	33.92	34.17
Mean	28.36	36.17	31.79	37.51	36.14	37.84	34.63

Cacao genotypes: ($P = .20$); Shade treatments: ($P = .01$); Genotype x shade ($P = .02$); Fertilizer treatments: ($P = .001$)

In contrast, in the dry season, increased shade, under which reduced water vapour pressure deficit was recorded, ameliorated the negative effect of atmospheric stress on stomatal conductance and hence photosynthetic rates were greater. A number of previous studies, including one that involved the four cacao genotypes, have shown that stomatal opening in cacao is sensitive to vapour pressure [14,20,21].

Differences in stomatal conductance were observed between cacao genotypes in the major rainy season (with PA 150 having the highest rates and P 30 [POS] the lowest). However, no such differences were observed in the dry season meaning that it was not possible to determine which of the cacao genotypes can better tolerate a condition of drought by means of a more effective stomatal regulation. Nevertheless, differences in the degree of plasticity among the genotypes were observed suggesting that P 30 [POS] and SCA 6 have an intrinsically lower stomatal conductance under non-limiting conditions.

The significant increase in stomatal conductance observed under fertilizer application in all the seasons seems to suggest that the amounts of nutrients supplied through fertilizer application were reasonable and not excessive considering the soil's water status. It has been noted in some plants that application of soil nutrients must be commensurate with available soil water. Also fertilization with N and K in their right amounts is associated with increases in transpiration rates when soil water is adequate but with a more effective stomatal control in a situation of soil water deficit [22-26]. For example, Turnbull [27] recorded a higher stomatal conductance rate in *Eucalyptus globules* plants after nitrogen fertilization. The results presented here are consistent with these earlier studies in that combining organic materials that were good sources of N and K was associated with improved water relations of the young cacao plants.

The observation that water use efficiency was highest in the dry season is consistent with that

of Rada [28] who observed, in young cacao, that under conditions of soil water stress stomatal closure reduced water loss through transpiration much more than it reduced the rate of photosynthesis resulting in an increase in water use efficiency. This also explains the higher water use efficiency recorded under the more stressful 'light shade' environment than under the heavier shade during the dry season.

Mineral nutrients are known to increase photosynthetic electron flow either as "constituents of the light-harvesting complex", or as "ions facilitating electron flow" [29]. Thus fertilizer application has been linked to increased photosynthetic rates in several plant species including cacao [9,27,29-35]. As the initial N and K levels in the soil at the site of the current experiment were below the critical values for successful establishment (see Table 2), fertilizer application was expected to stimulate photosynthesis.

The fact that increased photosynthesis was recorded in fertilized plants in the dry season more than in the rainy seasons may be attributed to the ability of organic fertilizers to improve plant water status. The benefits associated with such interventions become more manifest under harsher conditions such as were prevalent in the dry season. With increased stress in the dry season, plants that had not been provided with fertilizer would exhibit greater stomatal closure in order to conserve their tissue water inevitably causing reductions in CO₂ uptake and consequently, lower photosynthetic rates.

4.2 Leaf Chlorophyll, N, P and K⁺ Concentrations

Unlike leaf phosphorus and potassium contents which were not statistically different among the cacao genotypes, the significant difference in leaf nitrogen content among them indicates the presence of considerable genotypic variability as regards nitrogen uptake and/or translocation into leaf tissue. Changing the spatial arrangement of plantains or introducing additional *Gliricidia* shade plants was not found to be associated with a significant change in leaf N or P suggesting low inter-specific competition for the nutrients. A similar result was observed by Isaac [36].

The observed increase in leaf N and K in the plants following organic fertilizer application is consistent with an earlier finding [8]. The marginal increase in leaf P after fertilizer application suggests that the amount of P

supplied was below the threshold quantity that was needed for a substantial increase in leaf P. Initial soil analysis showed that the soil already contained more than the critical level of P for cacao establishment [37] (see Table 2).

4.3 Plant Survival

The different survival rates for the cacao genotypes was correlated with their different growth rates. With the annual dry spell in West Africa, varieties with greater vigour establish better as they often develop deeper root systems that have greater access to soil water before the dry season begins [38,39].

Although organic fertilization was not strongly associated with plant growth within the period of study it was clearly linked with higher plant survival. The recorded enhanced plant survival could have been caused not only by improved plant nutrition but also by indirect benefits such as reduced soil temperature and increased root and stem water content during the dry season. Furthermore, the higher N and K⁺ content in the plants' tissue (as evidenced by the higher leaf concentrations) likely helped plant osmoregulation [28,40,41] which contributes to plant survival under dry conditions.

Although fertilizer application failed to contribute significantly to faster cacao seedling growth, the recorded increases in assimilation rate and plant survival dispel any suggestion of nutrient antagonism [9,42,43]. Formation pruning was carried out in the experiment to remove basal chupons and unwanted developing branches and that may have confounded the results on growth to some extent. Also the time period within which the growth data were taken was, probably, too short to record any substantial difference in growth between the fertilizer treatments.

This experiment has shown a positive link between the application of organic fertilizers and the field establishment (survival) of cacao in a site that previously carried moribund cacao. Although differences in shade were not linked with plant survival, the significant shade x fertilizer interaction on photosynthetic activity in the dry season shows that during the dry season shade increases the benefits that young cacao plants get from fertilizer application. On the other hand, shade should be pruned back during the wet seasons to reduce light limitation to assimilation and growth.

5. CONCLUSION

It is concluded from this experiment that although soil application of organic fertilizers can improve cacao plant nutrition and help establishment, overhead shade must be adjusted in accordance with the prevailing weather conditions to optimize plant response to fertilizers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. ICCO. Annual forecasts of production and consumption and estimates of production levels to achieve equilibrium in the worldcocoa market, in annual report, ICCO: Berlin; 2008.
2. WCF. Introduction to cocoa market, in cocoa market update; 2012.
3. Ayanlaja SA. Rehabilitation of cocoa (*Theobroma cacao*) in Nigeria. Major problems and possible solutions. Causes of difficulty of seedling establishment. Plant and Soil, 1983. 73: p. 403-409.
4. Anim-Kwapong GJ, Osei-Bonsu K. Potential of natural and improved fallow using indigenous trees to facilitate cacao replanting in Ghana. Agroforest Syst. 2009;76:533-542.
5. Appiah MR, et al. The consequences of cocoa production on soil fertility in Ghana: A review. Ghana Jnl. agric. Sci. 1997;30:183-190.
6. Hartemink AE. Nutrient stocks, nutrient cycling, and soil changes in cocoa ecosystems: A review. Advances in Agronomy. 2005;86:227-253.
7. Omotoso TI. Amounts of nutrients removed from the soil in harvested Amelonado and F3 Amazon Cacao during a year. Turrialba, 1975;25:425-428.
8. Ahenkorah Y, Halm BJ, Amonoo RS. Cacao pod husk as source of potash fertilizer. Turrialba. 1981;31(4):287-291.
9. Ahenkorah Y, et al. Twenty years' results from a shade and fertilizer trial on amazon cocoa (*Theobroma cacao*) in Ghana. Experimental Agriculture, 1987;23(1):31.
10. Cunningham RK, Arnold PW. The shade, fertilizer requirements of cacao (*Theobroma cacao* L). Ghana J. Science, 1962;13(Food and Agric.):213-221.
11. Opoku IY, Ofori Frimpong K, Appiah MR. The use of fertilizer and fungicide to sustain cocoa production in severe black pod areas. Tropical Science. 2004;44(2):95-99.
12. ICCO. A study on the market for organic cocoa, London. 2006;1-16.
13. Pay E. Increasing incomes and food security of small farmers in West and Central Africa through exports of organic and fair-trade tropical products, FAO. 2009;1-14.
14. Acheampong K, Hadley P, Daymond AJ. Photosynthetic activity and early growth of four cacao genotypes as influenced by different shade regimes under West African dry and wet season conditions. Experimental Agriculture. 2013;49(1):31-42.
15. Smyth AJ. The selection of soils for cacao, in Soils Bulletin No. 51966. Food and Agriculture Organization of The United Nations: Rome. 52-53.
16. Oliet J, et al. Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-released fertilizer. Scientia Horticulturae: International Society for Horticultural Science. Elsevier; 2004.
17. Stuart NW. Adaptation of the Micro-Kjeldahl method for the determination of nitrogen in plant tissues. Plant Physiology. 1936;11(1):173-179.
18. Twine JR, Williams CH. The determination of phosphorus in Kjeldahl digests of plant material by automatic analysis. Communications in Soil Science and Plant Analysis, 1971;2(1532-2416)(6):485-489.
19. de Almeida M, et al. Interfacing multisyringe flow injection analysis to flame atomic emission spectrometry: An

- intelligent system for automatic sample dilution and determination of potassium. *Journal of Analytical Atomic Spectrometry*. 2009;24(3):340-346.
20. Raja Harun RM, Hardwick K. The effect of different temperatures and water vapour pressure deficits on photosynthesis and transpiration of cacao leaves, in 10th International Cocoa Research conference: Santo Domingo. 1988;211-214.
 21. Sena Gomes AR, Kozlowski TT, Reich PB. Some physiological responses of *Theobroma cacao* var. catongo seedlings to air humidity. *New Phytol*. 1987;107:591-602.
 22. Boruah HPD, et al. Non-uniform, patchy stomatal closure of a plant is a strong determinant of plant growth under stressful situation. *Current Science*. 2008;94(10):1310-1314.
 23. Bosshart RP, von Uexkull HR. Some occasionally overlooked criteria for assessing fertilizer requirements of high yielding cacao in Seminar on palm kernel utilization and recent advances in cacao cultivation. *Sawan, Sabah Malaysia*; 1987.
 24. Cramer MD, Hoffmann V, Verboom GA. Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist*. 2008;179(4):1048-1057.
 25. Otoo E, Ishii R, Kumura A. Interaction of nitrogen supply and soil water stress on photosynthesis and transpiration in rice. *Japanese Journal of Crop Science*. 1989;58(3):424-429.
 26. de Melo AS, et al. Alteration of the physiologic characteristics in banana under fertirrigation conditions. *Ciencia Rural*. 2009;39(3):733-741.
 27. Turnbull TL, et al. Within-canopy nitrogen and photosynthetic gradients are unaffected by soil fertility in field-grown *Eucalyptus globulus*. *Tree Physiology*. 2007;27:1607-1617.
 28. Rada F, et al. water relations and gas exchange in *Theobroma cacao* var. Guasare under periods of water deficits. *Rev. Fac. Agric. (LUZ)*. 2005;22:105-112.
 29. Cakmak I, Engels C, eds. Role of mineral nutrition in photosynthesis and yield formation. *Mineral Nutrition of crops. Fundamental mechanisms and implications.*, ed. Z. Rengel. Published by Food Products Press, and imprinting of the Haworth Press Inc.: Binghamton; 1999,
 30. Lockard RG, Asomaning EJA. Composition of complete nutrient solutions, in *Rep. W. Afr. Cocoa Res. Inst. West African Cocoa Research Institute: Tafo, Ghana*. 1963;56-57.
 31. Efthimiadou A, et al. Effects of cultural system (organic and conventional) on growth, photosynthesis and yield components of sweet corn (*Zea mays* L.) under semi-arid environment. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 2009;37(2):104-111.
 32. Ahmed N, et al. Impact of zinc fertilization on gas exchange characteristics and water use efficiency of cotton crop under arid environment. *Pakistan Journal of Botany*. 2009;41(5):2189-2197.
 33. Rana NK, et al. A CsGS is regulated at transcriptional level during developmental stages and nitrogen utilization in *Camellia sinensis* (L.) O. Kuntze. *Mol Biol Rep*. 2009;37(2):703-10.
 34. Bown HE, et al. Chlorophyll fluorescence response of *Pinus radiata* clones to nitrogen and phosphorus supply. *Ciencia E Investigacion Agraria*, 2009;36(3):451-464.
 35. Liu HG, et al. Interactive effects of molybdenum and phosphorus fertilizers on photosynthetic characteristics of seedlings and grain yield of *Brassica napus*. *Plant and Soil*. 2010;326(1-2):345-353.
 36. Isaac ME, et al. Early growth and nutritional response to resource competition in cocoa-shade intercropped systems. *Plant and Soil*, 2007;298:243-254.
 37. Akirinde EA, Ayegboyin KO. Performance of *Theobroma cacao* seedlings irrigated with water from different sources. *Biotechnology*. 2006;5(3):330-336.
 38. Oppong FK, et al. The effect of time of planting at stake on cocoa seedling survival. *Ghana Jnl. Agric. Sci*, 1999;32(1):79-86.
 39. Oppong FK, Opoku-Ameyaw K. Comparison of methods of field planting on cocoa seedling survival and early growth in a marginal cocoa-growing area of Ghana. *Ghana Journal of Agricultural Science*, 2008;40(2):199-205.
 40. de Almeida, et al. Some water relations aspects of *Theobroma cacao* clones. in 13th International Cocoa Research Conference; 2002.

41. Orlova YV, et al. Contributions of inorganic ions, soluble carbohydrates, and multiatomic alcohols to water homeostasis in *Artemisia lerchiana* and *A-pauciflora*. Russian Journal of Plant Physiology. 2009;56(2):200-210.
42. Hardy F. Cacao ecology and physiology: Nutrient relations of cacao Cacao Manual (English Edition), ed. F. Hardy, Turrialba, Costa Rica: Inter-American Institute of agricultural Sciences; 1960.
43. Bonaparte EENA. Yield gradients in cacao (*Theobroma cacao* L.) shade and fertilizer experiments Acta Hort. 1975;49:251-257.

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