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Evaluation of river sand as a medium for raising cocoa (*Theobroma cacao* L.) seedlings

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Abstract: A nursery experiment was conducted between November, 2011 and May, 2012 at the Cocoa Research Institute of Ghana to investigate the effect of using river sand and river sand-topsoil mixture on cocoa seedlings. Treatments tested were sole topsoil, sole river sand, river sand + foliar fertilizer (Sidalco liquid fertilizer, NPK-10:10:10), river sand + topsoil (1:1) and river sand + topsoil mixture (1:1) + foliar fertilizer. The results showed that sole topsoil retained more moisture and supported significantly taller seedlings. The river sand-topsoil mixture + foliar fertilizer seedlings had higher chlorophyll content and was more photosynthetically efficient thus resulting in significantly ($P<0.05$) larger stem diameter than all other treatments. Stem volume of seedlings in the river sand + topsoil +foliar fertilizer were also not different from those found in the sole topsoil medium. There were positive correlations between moisture retention and chlorophyll content of the seedlings. Chlorophyll content was found to be positively correlated to stem diameter and height gain by the seedlings. It was therefore concluded that cocoa seedlings can be raised in polybags filled with a mixture of river sand and topsoil (1:1) for a period of six months provided foliar fertilizer is applied at the rate of 10 mls NPK (10:10:10) in 15 litres of water at bi-weekly intervals.

Keywords: *Theobroma Cacao*, River Sand, Chlorophyll Fluorescence, Photosynthesis, Stem Volume, Foliar Fertilizer

1. Introduction

Raising of cocoa seedlings in nurseries using polybags is the recommended practice because the management of individual seedlings result in much viguor and improved capability of successful establishment [3, 13, 14]. This is attributed to minimized transplanting shock caused by the absence or reduced damage to the roots as compared to bare-root seedlings [12, 18]. The polybags also allow the nursery period to be prolonged until the environment is conducive for transplanting to the field [12]. These polybags are filled currently using topsoil. The major problem associated with the use of topsoil as seedling medium is scarcity [2]. This scarcity has led to use of various alternatives and mixtures of other media with topsoil for nursing seedlings of other crops such as coconut [7, 15]. In raising coconut seedlings, topsoil mixed with river sand in the ratio of 3:1 is a satisfactory mixture for filling the polybags [7]. Even if such a ratio works very well in the raising of cocoa seedlings, the requirement of topsoil could still be large. Related nursery studies [10] concluded that cocoa pod husk-based compost was suitable in combination with topsoil at ratios of 1:2 and 1:3. Earlier work [15] indicated that river sand did not adversely affect germination when substituted for topsoil. However, the rate of germination was slightly delayed and early seedling viguor was reduced in the sand substituted mixtures. Whereas potting media has not be shown to affect percent germination [8], nutrient composition and availability potting media significantly affects growth parameters such as leaf number, plant height, root and stem diameters, and subsequently dry matter accumulation [1, 5, 8, 9]. The addition of mineral nitrogen is also reported to [8] to improve chlorophyll concentration in the leaves. It is therefore possible to overcome these problems experienced with low nutrient media which also have poor water holding capacity by increasing the moisture and nutrient supply to the seedlings established in such media through frequent watering and application of fertilizers [8, 13].

The recommended polybag size for raising cocoa seedlings (17.5 x 25 cm) usually takes in an average of 6 kg of top soil per bag. The current directive from Ghana COCOBOD for the Seed Production Unit (SPU) to raise 20 million seedlings each year would amount to a topsoil requirement of 120,000 MT per annum, excluding that of private nurseries. Excessive scraping of the topsoil to meet this demand will eventually result in serious degradation of productive farmlands. It is therefore necessary to investigate possible options to replace or reduce the topsoil requirement as the sole medium for filling polybags, with particular interest in the use of river sand found abundantly in close proximity to most farm settlements. The sole objective of this work therefore was to determine the suitability of river sand in appropriate proportions with topsoil as alternatives to sole topsoil in raising cocoa seedlings.

2. Materials and Methods

2.1. Experimental Design and Treatments

The experiment was conducted within the nursery premises of the Cocoa Research Institute of Ghana (lat. 6^o 13' N, long. 0^o 22' W, altitude 222 m above sea level), from November 2011 to May 2012. The experiment was laid out in a randomized complete block design with four replicates and five treatments (Table 1). Nursery bags were first perforated at the bottom with a perforator to create a 4 cm^2 hole to facilitate drainage of excess water before filling with the respective media.

Table 1. *Treatment combinations.*

Treatment	Description
i	Topsoil only (control)
ii	River sand only
iii	River sand + foliar fertilizer
iv	River sand (50 %) + topsoil (50 %)
v	River sand (50 %) + topsoil (50 %) + foliar fertilizer

For each treatment forty polybags were filled with topsoil and 2 seeds sown per polybag. The polybags were then arranged on thick polythene sheet to prevent the growth of the roots into the ground, to avoid possible uptake of nutrients. The experiment was carried out under a shade net allowing 40 % sunlight through. Two weeks after germination an approved liquid foliar fertilizer (Sidalco NPK - 10:10:10) was applied to the seedlings at a rate of 10 ml per 15 litres of water at bi-weekly intervals using a knapsack sprayer. One insecticide treatment (60 ml Confidor 200 SL in 4.5 l of water) was carried out once each month using a motorized sprayer to prevent seedling damage by insect pests.

2.2. Data Collected

The number of seedlings that emerged per treatment was counted two weeks after sowing and expressed as a percentage of the 80 seeds initially sown. Data on seedling survival was obtained through monthly census of living plants which was then expressed as percentage of the number of seedlings that emerged. Moisture contents of the polybag media were recorded monthly using soil moisture meter (HH2 by Delta Devices Ltd) after 48 hrs of no watering. Twenty core plants were randomly selected and tagged for physiological and morphological data measurements. A leaf count was conducted once every month on individual plants. Data on leaf chlorophyll content was measured with a chlorophyll content meter (CL-01 by Hansatech Instruments Ltd) while leaf chlorophyll fluorescence was measured with a leaf chlorophyll fluorometer (FP 100 by Photon System Instruments Ltd). The rates of stomatal conductance, transpiration and photosynthesis were measured at the leaf level on ten of the twenty test plants at monthly intervals with a portable infra-red gas analyzer (LcPro, ADC Bioscientific, UK). To provide estimates for growth, stem diameter and plant height measurements were taken at monthly intervals from the twenty test plants using digital calipers and a standard metre rule respectively. The data on stem diameter and height was then used to derive their respective stem volumes for treatments and months, following the procedure described in earlier report [11]

$$Vol_{st} = \frac{\pi}{2}(d^2 * h)$$

Where:
Vol_{st} - stem volume (cm^3)
d - stem diameter
h - seedling height

Total leaf area per plant (cm^{-2}), root length, below and above ground plant dry matter were taken in the sixth month after sowing by destructively harvesting all the twenty test plants from each treatment. The harvested plant materials were first washed with tap water and oven dried at 80 ^oC for three days until a consistent weight was obtained. The weights of the dried samples were then taken as the dry matter accumulated by the seedlings after 6 months of growth.

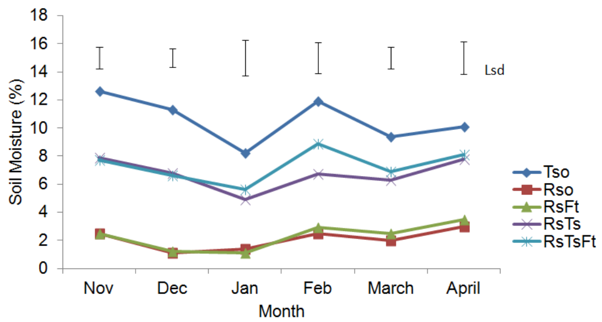
2.3. Data Analysis

Data on seedling growth and physiology were analysed using ANOVA and treatments separated by least significant difference. The data on final emergence, plant survival and bag moisture content were subjected to arcsine transformations before analysis.

3. Results

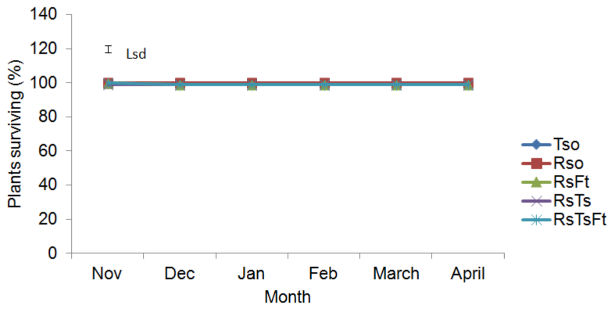
3.1. Percent Soil Moisture, Final Emergence and Number of Plants Surviving

The sole river sand and river sand + fertilizer recorded significantly ($P<0.05$) lower percent moisture content compared to other treatments throughout the study period (Fig. 1). Also, river sand-topsoil mixtures improved moisture retention compared to the sole river sand even though its moisture retention capacity was significantly lower (P<0.05) than that of the sole topsoil. The higher moisture content of the sole topsoil medium did not translate into significant differences (P<0.05) in percent final emergence and subsequently, survival of cocoa seedlings afterwards (Fig. 2).



Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 1. *Effects of polybag medium and foliar fertilizer on available soil moisture at various months after sowing.*

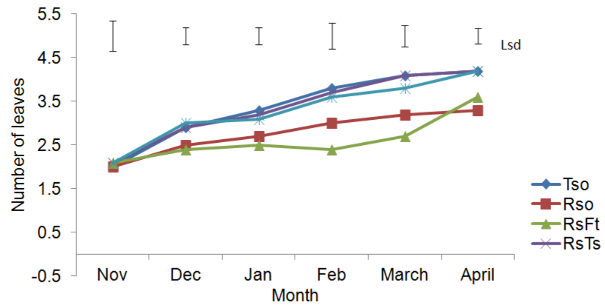


Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 2. *Effects of polybag medium and foliar fertilizer on the proportion of plants surviving in the nursery at various months after sowing.*

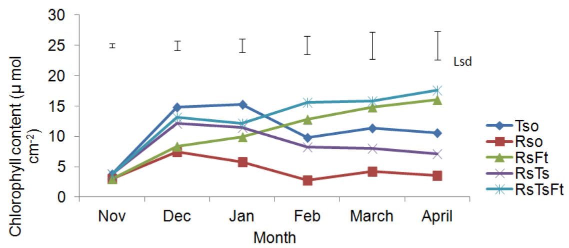
3.2 Number of Leaves Per Seedling and Leaf Chlorophyll Content

The use of river sand as polybag medium with or without fertilizer significantly (P<0.05) reduced the number of leaves per cocoa seedling (Fig. 3). Leaf numbers of seedlings in the river sand + topsoil, river sand + topsoil + foliar fertilizer and sole topsoil treatments were similar throughout the six months of growth. That of the river sand + foliar fertilizer and sole river sand treatments were also similar during the first three months of growth, and in the 6th month, but significantly different (P<0.05) in the 4th and 5th months. Both treatments however, recorded leaf numbers that were significantly lower (P<0.05) than those of the remaining treatments throughout the six months (Fig. 3). The leaves of seedlings in the sole river sand and the river sand + foliar fertilizer treatments recorded similar chlorophyll values during the first two months of growth but was significantly different (P<0.05) from each other from the 3rd to the 6th month (Fig. 4). Leaf chlorophyll values of the sole topsoil and river sand + topsoil treatments which started very high showed a steady decline throughout the six months of seedling growth. From the 3rd month, leaf chlorophyll content of seedlings in the river sand + foliar fertilizer and river sand + topsoil + foliar fertilizer treatments continued to increase, recording significantly higher values from 4 - 6 months (Fig. 4). Leaves of seedlings grown in the sole river sand medium recorded the least chlorophyll content during the 6 months of monitoring.



Note: Values are square root transformations. Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 3. *Effects of polybag medium and foliar fertilizer on leaf number per plant of cocoa seedlings at various months after sowing in the nursery.*

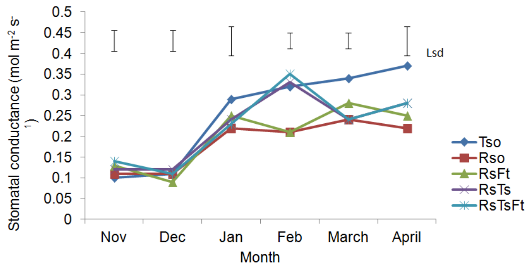


Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 4. *Effects of polybag medium and foliar fertilizer on leaf chlorophyll content of cocoa seedlings in the nursery at various months after sowing.*

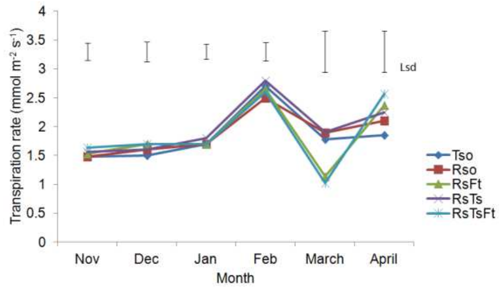
3.3 Stomatal Conductance, Transpiration Rates, Leaf Chlorophyll Fluorescence and Photosynthetic Rate

There were no significant differences (P<0.05) in stomatal conductance during the first three months of seedling growth (Fig. 5). Four months after sowing, seedlings in the sole river sand and river sand + foliar fertilizer media recorded significantly lower (P<0.05) stomatal conductance compared to those of seedlings in the remaining treatments. In the 5th and 6th months after sowing, stomatal conductance of seedlings grown in the sole topsoil medium was significantly higher (P<0.05) than that of the remaining treatments (Fig. 5).



Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 5. *Effects of polybag medium and foliar fertilizer on stomatal conductance of cocoa seedlings at various months after sowing in the nursery.*

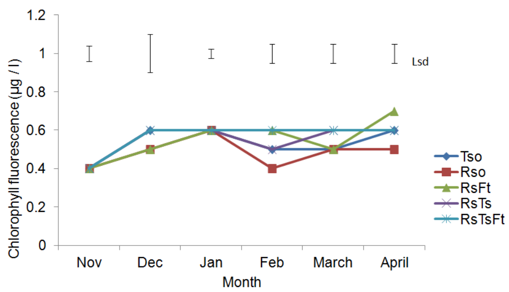


Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 6. *Effects of polybag medium and foliar ferilizer on transpiration rates of cocoa seedlings at various months after sowing in the nursery.*

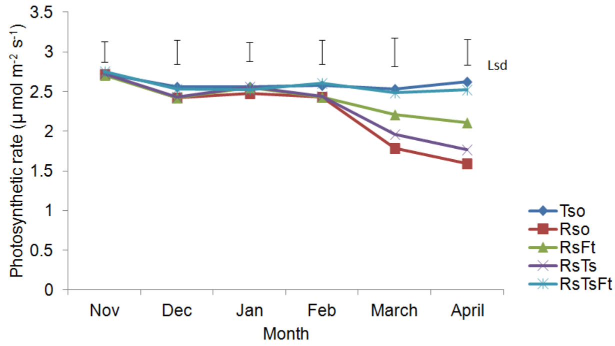
There were no significant treatment influences (P<0.05) on the transpiration rates of cocoa seedlings during the first four months, and at six months after sowing (Fig. 6). At five months, the rate of transpiration recorded by the river sand + foliar fertilizer and the river sand + topsoil + foliar fertilizer were similar and significantly lower (P<0.05) than that recorded by the remaining treatments (Fig. 6).

Chlorophyll fluorescence of cocoa seedlings was not significantly influenced (P<0.05) by treatments from 1 to 3, and at 5 months after sowing (Fig. 7). At four and six months however, leaf chlorophyll fluorescence of the river sand + foliar fertilizer was significantly higher (P<0.05) than that of the sole river sand treatment (Fig. 7).



Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 7. *Effects of polybag medium and foliar fertilizer on leaf chlorophyll fluorescence of cocoa seedlings at various months after sowing in the nursery.*



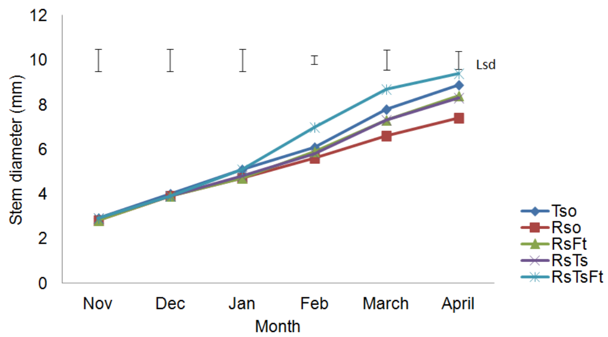
Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 8. *Effects of polybag medium and foliar fertilizer on the rate of photosynthesis of cocoa seedlings at the leaf level at various months after sowing.*

Initially, all treatments recorded identical rate of photosynthesis at the leaf level up to the fourth month after sowing (Fig. 8). At five and six months however, the rate of photosynthesis of the sole topsoil and river sand + topsoil + foliar fertilizer were significantly higher (P<0.05) than the rates in the leaves of seedlings in the sole river sand and the river sand + topsoil media (Fig. 8).

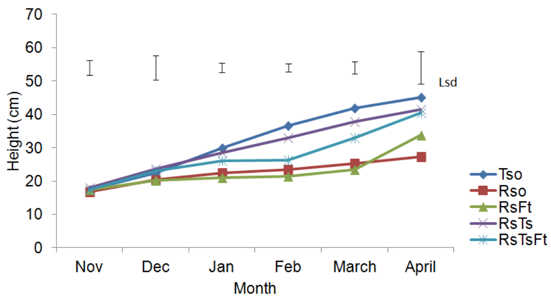
3.4. Seedling Growth and Dry Matter Accumulation

There were no significant differences (P<0.05) in stem diameter of cocoa seedlings up to three months after sowing (Fig. 9). From four-six months after sowing, seedlings grown in the river sand + topsoil media and supplied with foliar fertilizer recorded significantly bigger (P<0.05) stems compared to the remaining treatments. Also, the effect of the sole topsoil treatment on stem diameter was significantly higher (P<0.05) than the effects of sole river sand, and river sand + topsoil treatments at five and six months after sowing.



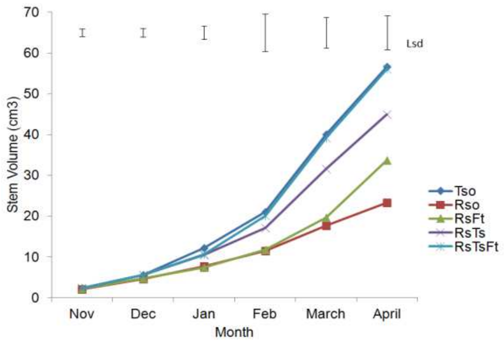
Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 9. *Effects of polybag medium and foliar fertilizer on stem diameter increase in cocoa seedlings at various months after sowing.*



Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 10. *Effects of polybag medium and foliar fertilizer on height gain in cocoa seedlings at various months after sowing.*



Note: Tso-topsoil only, Rso-river sand only, RsFt-river sand + fertilizer, RsTs-river sand + topsoil, RsTsFt-river sand + topsoil + fertilizer.

Figure 11. *Effect of polybag medium and foliar fertilizer on stem volume of cocoa seedlings at various months after sowing.*

Plant height was not significantly influenced (P<0.05) by treatments during the first two months of growth (Fig. 10). From three-six months, height of seedlings grown in the sole topsoil medium was significantly greater (P<0.05) than that of seedlings in the sole river sand and river sand + foliar fertilizer treatments. The river sand + topsoil + foliar fertilizer treatment also supported seedlings that were significantly (P<0.05)) taller than seedlings found in the sole river sand medium. Total leaf area of the sole river sand medium seedlings was significantly reduced (P<0.05) when compared to those of the sole topsoil and river sand + topsoil + foliar fertilizer treatments (Table 2). Apart from the reduced leaf area, seedlings in the sole river sand medium also recorded significantly shorter (P<0.05) roots and lower leaf, stem and root dry matter weights (Table 2). The sole topsoil and river sand + topsoil + foliar fertilizer media produced similar but significantly larger (P<0.05) leaf area, longer roots and higher leaf, stem, root and total dry matter compared to those of the remaining treatments (Table 2). Consequently, stem volume values recorded by seedlings grown in sole topsoil and river sand + topsoil + foliar fertilizer were similar and significantly larger than that of seedlings grown in sole river with or without foliar fertilizer application at five and six months after sowing (Fig. 11).

Table 2. *Leaf area, root length and dry matter accumulation of cocoa seedlings as affected by polybag medium and foliar fertilizer six months after sowing.*

Treatment	Leaf area(cm^{-2})	Root length (cm)	Dry matter accumulation (g) at 6 months			
			Leaf (dm)	Stem (dm)	Root (dm)	Total (dm)
Topsoil only	45.97[a]	25.6[a]	4.21[a]	3.09[a]	4.78[a]	12.08[a]
River sand only	39.01[b]	20.6[b]	1.88[d]	1.58[c]	1.82[c]	5.38[c]
River sand + Foliar	41.10[ab]	22.8[ab]	2.59[c]	1.70[c]	2.26[c]	6.54[c]
Topsoil +River sand	42.56[ab]	26.1[a]	3.30[b]	2.45[b]	3.41[b]	9.16[b]
Topsoil +River sand + Foliar	46.56[a]	26.9[a]	4.51[a]	2.66[ab]	4.20[a]	11.37[a]
$Lsd_{0.05}$	5.84	3.6	0.61	0.56	0.73	1.75
CV (%)	8.8	21.1	26.1	34.6	31.2	12.8

Note: cm (centimeters, g (grams), dm (dry matter). Differences between means in the same column followed by the same superscripted letter are not significant at 5 % probability level.

3.5. Correlations between Some Physiological and Growth Parameters

Correlations between some soil and growth parameters are presented in Table 3. Soil moisture was positive and highly correlated to seedling stem diameter (r=0.69, P=0.01) and height (r=0.65, P=0.01). Leaf area was also found to be positively and highly correlated to dry matter of leaf (r=0.72, P=0.01), stem (r=0.73, p=0.01), root (r=0.74, P=0.01) and consequently total dry matter (r=0.77, P=0.01). There was also positive and high correlation between leaf dry matter and root length (r=0.66, P=0.01). Seedling stem diameter was found to be positive and highly correlated to stem dry matter (r=0.68, P=0.01) and total dry matter (r=0.63, P=0.05). There was also positive and significant correlation between root length and stem dry matter (r=0.55, P=0.05). Root dry matter correlated positively and strongly with stem dry matter (r=0.84, P=0.01) while stem dry matter correlated positively and strongly with total dry matter production (r=0.97, P=0.01).

Table 3. *Correlations among some physiological and growth parameters of six month old cocoa seedlings grown in the nursery as affected by polybag medium and foliar fertilizer.*

1	%smc													
2	LA	0.45												
3	Ldm	0.68**	0.72**											
4	Lchlor	0.17	0.36	0.46										
5	Fv/Fm	0.06	0.17	0.30	0.62*									
6	Trans	-0.17	-0.09	0.11	0.14	0.36								
7	Stoma	-0.24	-0.26	-0.14	0.23	0.38	0.64**							
8	Prate	0.13	0.18	0.33	-0.17	0.01	-0.13	-0.18						
9	StemD	0.69**	0.38	0.57*	0.43	0.30	0.05	-0.08	0.06					
10	Height	0.65**	0.54*	0.60*	0.41	0.03	-0.36	-0.31	0.19	0.46				
11	Rlength	0.50	0.30	0.66**	0.23	0.28	0.38	0.11	0.43	0.55*	0.50			
12	Rdm	0.56*	0.74**	0.76**	0.19	-0.02	-0.19	-0.28	0.27	0.50	0.57*	0.37		
13	Sdm	0.82**	0.73**	0.88**	0.30	0.09	-0.11	-0.24	0.24	0.68**	0.74**	0.63*	0.84**	
14	Tdm	0.74**	0.77**	0.95**	0.35	0.14	-0.05	-0.23	0.30	0.63*	0.68**	0.60*	0.90**	0.97**
		1	2	3	4	5	6	7	8	9	10	11	12	13

Note:*significant at 0.05; **significant at 0.01. %smc – Percent soil moisture content; LA – Leaf area (cm^{-2}); Ldm – Leaf dry matter; Lchlor – Leaf chlorophyll content; Fv/Fm – Leaf chlorophyll fluorescence; Trans – Transpiration; Stoma – Stomata conductance; Prate – Photosynthetic rate; Rlength – Root length; Rdm – Root dry matter; StemD-Stem diameter; Sdm – Stem dry matter; Tdm – Total dry matter.

4. Discussion

The pattern exhibited by the polybag media contents with regard to moisture retention was expected as sand is known to have very low water holding capacity. The relatively lower moisture content of the sole sand and sand-topsoil mixtures primarily resulted from the presence of the sand component [16]. The wide variations in percent moisture content and potential availability of nutrients between the topsoil on one hand and the sole sand and sand-topsoil mixtures on the other hand did not translate into differences in percent final germination and plant survival afterwards. This was probably because the moisture and nutrient levels of the river sand treatments were sufficient for seed germination and continuous survival of the seedlings for up to six months. The relatively lower moisture and nutrient content of these treatments however, adversely affected leaf formation during the six months of growth in the nursery. With depleting nutrient levels in the polybags at the later stages, it became clear that leaf chlorophyll formation responded positively to the foliar fertilizer, with the highest leaf chlorophyll found in the river sand + topsoil + foliar fertilizer followed by the river sand + foliar fertilizer and the topsoil treatments. This higher leaf chlorophyll values did not lead to the expected improved photochemistry as seedlings recorded higher leaf chlorophyll fluorescence (fv/fm), indicating re-radiation of high amount of the photosynthetically active radiation (PAR) in long wave form [6]. This was probably due to low moisture levels and lack of complete complement of nutrients required for efficient photosynthesis since seedling growth was improved by the addition of foliar fertilizer to the river sand + topsoil medium over the sole topsoil medium [8]. The retardation of seedling growth by the sole river sand and river sand + foliar fertilizer treatments attributable to low moisture retention and nutrient availability support earlier findings [2] since the foliar fertilizer supplied only NPK and a few trace elements. The addition of the NPK to sole river sand only prevented the incidence of severe chlorosis compared to the sole river sand only treatment [8]. The increase in photosynthetic rate with increasing moisture and chlorophyll content was therefore translated into plant growth [2, 16]. This was probably the reason why the sole topsoil and river sand + topsoil + foliar fertilizer treatments with higher photosynthetic efficiency resulted in seedlings that had larger stem volumes, indicating better dry matter production and accumulation [1, 5, 8, 9]. Such seedlings will have a higher chance of establishment and survival in the field [4, 13, 14].

5. Conclusion

Based on the results obtained, It was concluded that the use of sole river sand with and without NPK (10:10:10) at this rate does not sufficiently support the growth of cocoa seedlings in the nursery. In situations of topsoil scarcity, cocoa seedlings can be raised in a mixture of sand and topsoil (1:1) and nutrient shortfalls provided for by application of foliar fertilizer.

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