Growth performance of ICCRI 06 H cocoa seedling in response to different microclimate and soil moisture conditions

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Abstract. Climate and environmental factors play an important role for cocoa (Theobroma cacao L.) optimal growth and production. This study aims to evaluate growth performance and stomatal characteristics of cocoa seedling in response to microclimate, i.e. temperature, relative humidity, light intensity and soil moisture condition. The cacao variety of ICCRI 06 H was used for greenhouse experiment arranged by a completely randomized design with three replications. The first factor was evapotranspiration (ET) i.e. ET<sub>0</sub> 100% (control), ET<sub>ε</sub>-2 50% (moderate stress), and ET<sub>ε</sub>-3 25% (severe stress). The second factor was microclimate condition defined by its location in the greenhouse, i.e. inside the glass box (IB) and outside the glass box (OB). IB microclimate conditions were modified through the installation of 50-Watt LED artificial lamps. Our seven weeks observations demonstrated the specific microclimate condition created both in IB and OB. An insignificant effect of different soil moisture level to cocoa seedling growth was evidenced. Conversely, microclimate condition shows a significant effects especially the plant height and stem diameter. The multiple regression analysis suggests the presence of connection between microclimate and crop growth, i.e. plant height, stem diameter, and number of young cocoa leaves in IB. The stomatal characteristics indicate the more stressed condition created outside the box.

1. Introduction
Cocoa (Theobroma cacao L.) growth and yield are highly influenced by the climatic and environmental condition. Climate provides a great challenge for cocoa to support the optimal growth and yield, because it is sensitive to drought [1]. The on-going climate change now has resulted in the obvious and consistent increase in the global temperatures and subsequent evapotranspiration with high level of anomalies with respect to precipitation patterns [2]. This condition may not be preferential in terms of long-term cocoa sustainability. Cacao is particularly sensitive to changes of soil moisture and will probably not get any further recovery above the critical level. Soil moisture deficit, will affect morphological changes and performance growth of cocoa seedlings [3]. The nursery stage is considered to pose the most sensitive part of cocoa growth to environmental stress. This stage is characterized by a high requirement of water used to support the increasing in plant height, diameter enlargement, leaf propagation, and root growth [4]. A soil moisture content down to 25–50% from field capacity observed to limit the number of leaf and height of crops [5]. Additionally, with 25% soil
moisture status, leaves were subjected to early biological aging and drop. It indicates the important of soil moisture among microclimate determinants [6].

Temperature stresses could inhibit plant growth and diminish the yields down to 41% [7]. It also reduces the potential of germination seed, produces poor germination, and accelerates the cell aging [8]. Additionally, it is suggested that its combination with drought stresses may provide the greatest effect in decreasing of growth and productivity of cocoa, compared to effect of an individual factor. Cocoa with subject to combination of both temperature and drought stresses demonstrate rapid growth and easy-to-fall leaf characters [9]. Their combination are also suggested to produce the high temperature of leaf and an incapability to stomata opening [10]. This way the cell turgor is maintained by adjusting the water level so that the cells remain stable. The response of stomata to combination of drought and temperature stresses in a way of crops to adapt with new environmental condition may provide an approach to develop new accession of stress-tolerant cocoa [11]. Therefore, different variety of cocoa may be expected to give a various response to different stresses.

This research evaluated cocoa variety ICCRI 06 H to different soil moisture level in combination with different microclimate conditions, i.e. temperature, relative humidity, and light intensity during the nursery stage. This variety was derived from superior hybrid clone (TSH 858 x KW 162) with high yield potential, and also reported as resistant to Vascular Streak Dieback (VSD) [12]. We take into account the temperature effect as an interplay with different climate variables, as stated before, to improve the understanding of microenvironment effect to growth of cocoa seedlings. The vegetative growth, i.e. plant height, stem diameter, number of leaves, and stomata measurement will also be discussed in the present paper.

2. Materials and Methods
A greenhouse research was carried out in MIPA center building, University of Brawijaya. To improve the understanding dealing with enviromental background, in relation to microclimate variation, the temperature, light intensity, and relative humidity were recorded during experiments. The 3-months-old of cacao seedlings of ICCRI 06 H was subjected to simulation of soil moisture treatments using an evapotranspiration (ETa) approach. This way, the ETa value is used to indicate the crop water requirement by eliminating the evaporation (ET) value obtained from blank pots (without crops). The experiment was arranged using a completely randomized design (CRD) with three replications. The first factor was three level of soil moisture condition as indicated by ETa, i.e. ETa=1 100% (control), ETa=2 50% (moderate stress), and ETa=3 25% (severe stresss). The second factor was microclimate condition where the separated location, i.e. inside the glass box (IB) and outside the glass box (OB), assumed to provide a different microclimate background. Therefore, a totally 18 pots using a < Φ 2 mm sized soil media were prepared for this study.

With installed the 50-Watt LED artificial lamps, the microclimate variables observed in IB demonstrate a light difference in view of its daily fluctuations compared to OB. Given that the average efficiency energy lamps is 244.75 %, it is supposed the higher, temperature generated in IB than the same parameter in OB (Fig. 1).

The variable of growth was measured using some tools such as ruler, vernier caliper, and stomata printing. Prior to weekly observations, the cocoa seedlings were grown for about 3-months with a standard maintenance such as field capacity irrigation and fertilization to get an optimal condition. During this establishment stage, before treatment, all cocoa seedlings were arranged at the same location in OB. When they were ready for soil moisture level treatments a totally 9 pots were moved into a dimension about 100 x 60 x 80 cm² glass box (IB).

To see if there any relationship between microclimate data and morphological performance of cocoa seedlings, the regression analysis was done using a statistical software SPSS. Additionally, data were analyzed using the analysis of variance (ANOVA) followed by a Tukey post-hoc test at α = 5%, when a significant difference of treatment to observation variables occurred.
3. Results and Discussion

Our data show a clear difference between microclimate of IB and of OB (Fig. 1). For IB, it could be interpretable that the daily temperature ranging from 27.35 °C to 29.25 °C, relative humidity 72.48% to 79.83%, and light intensity 110 lux to 3120 lux. For OB, the microclimate is quite different with temperature’s range going from 26.77 °C to 27.56 °C, relative humidity 63.08% to 68.16%, and light intensity from 36 to 110 lux (Fig. 1). Since a temperature of 25 °C and 80% relative humidity have been reported to provide an optimal growth for cocoa seedlings [12, 13]. Therefore the modification of the microclimate conditions may potentially affect growth performance, stomatal characteristic and transpiration rates [14, 15]. The regression analysis revealed a strong connection of climate variables to cocoa growth components of IB (Fig. 4), with α = 0.02 (p<0.05), but not in OB with α = 0.66 (p<0.05). The effect of temperature may be related to the respiratory losses, though it depends on the CO2 concentration. At elevated CO2 concentrations, the respiratory losses may be reduced through higher carboxylation rates [16]. The level of air humidity may determine the photosynthesis rate of which the growth performance of cocoa seedlings are affected [17, 18]. Additionally, its combination with soil moisture condition in terms of crop water efficiency is most likely exacerbating stress [3], decreasing the effect of light. However, the stronger effect of relative humidity to growth of crop than light has been reported [19]. Light will actively stimulate the auxin for cell division and elongation [20]. The different light intensity requirement for crop under artificial and natural light have been confirmed [21].

The cocoa cultivars vary in their stress adaption to the different soil moisture regimes [2]. The present results show that a typical linear increase in the plant height, with higher growth response obtained for IB cocoa seedlings (Fig. 2a). Data suggest that microclimate condition may have played a more important role to support the cocoa seedlings growth than soil moisture condition. With larger plant height value at 25% level of soil moisture inside the glass box (IB-ETa 3) than 100% level of soil moisture outside the glass box (OB- ETa 1), it may be interpretable either the optimal condition is obtained inside the glass box or the level of soil moisture stress at IB- ETa 3 is not reached yet. However, the additional increase in terms of plant height is gradually to decrease from week to week. Observation demonstrated an average increase of IB (12%) and OB (4%) from 6th to 7th week. The consistent increase might be assumed the attained optimal condition for cacao seedlings growth [2]. However, this is different from previous research [22], that the plant improvement was occurred in response to drought condition.

Furthermore, data suggest that generally the stem diameter of cocoa seedling have a typically increased with observation periods (Fig. 2b). The biggest increase in stem diameter occurred at 3rd week in of IB. ETa-2. However, the percentage of stem diameter decreased from week to week through the
observation periods. Based on 7th week observations, it was found that the greatest increase in stem diameter observed in IB- ETa,3 (10%) and OB- ETa,3 (7%). However, cocoa under soil moisture stress condition pose a smaller stem diameter than control [23]. Similarly, a reduction in vegetative phase during this stress was also reported [24, 3].

A typical increase in the number of leaves at cocoa seedlings were observed (Fig. 2c). The pronounced growing in the number of leaves occurred in the 2nd week on OB- ETa,2 yet, with a gradual decrease with respect to their addition number from week to week. Observation at 7th week of trial shows an average increase in leaf number, i.e. 9.1% in OB and 8.9% in IB, compared to the 6th week. A reduced in the number of leaf of cacao seedlings was observed under drought and temperature stress [25]. Drought stress may be due to a decreased number of leaves, caused by a decrease in chlorophyll content for photosynthesis [23]. Additionally, during periods of drought flushing is suppressed [3]. A decrease in the number of cocoa leaves associated with changes in the size and cell division. Resulting in the leaf experiencing senescence and abscesses [26]. Senescence and abscesses on leaves were caused by the accumulation of abscisic acid which prevents the formation of flush [27, 28].

**Figure 2.** Growth performance cacao seedlings a) plant height, b) stem diameter, c) number of leaf, IB = Inside of the glass box, OB= Outside of the glass box, ETa,1 = 100% (control), ETa,2 = 50% (moderate stress), and ETa,3 = 25% (severe stress).

The analysis of variance suggest an insignificant effect of soil moisture level to all growth performance characters, i.e. plant height, number of leaves, and stem diameter. This is an indication that the vegetative growth is not much reduced during the soil moisture treatment. Further analysis indicate a significant effects of microclimate to the plant height and stem diameter (Fig. 3a, Fig. 3c). As three microclimate variables were recorded simultaneously, therefore the variable of which play the important role for crop growth may be unable to confirm. But it is clear that IB condition is more optimal for cocoa seedling growth than OB (Fig. 3). While daily temperature may not significantly different, the intensity of light and relative humidity are obviously higher in IB than OB (Fig. 1). Though a temperature dependent of cocoa seedlings for growth performance has been reported [29].
however, its effect was not quite clear in the present research due to a similarity of daily temperature level between IB and OB. The microclimate effect to the performance of cocoa seedling vegetative growth and development has also been found earlier [30].

![Figure 3](image1)

**Figure 3.** Response growth performance in drought and temperature condition a) plant height, b) number of leaf, c) stem diameter. Bars with different letter on the top means a significant statistical difference at $\alpha = 5\%$.

Based on the regression analysis, all parameters growth (plant height, stem diameter and number of leaf) of IB show a strong relation to microclimate. Conversely, it did not clearly relate the crop growth of cocoa seedlings of OB. Based on the correlation analysis, light intensity of IB gives a positive effect to all parameters of crop growth. But, temperature and relative humidity of IB show a negative effect to all parameters. A positive correlation of humidity and light intensity to number of leaves and plant height were also observed in OB. Additionally, our finding suggested a negative effect to all parameters observed (Fig. 4).

![Figure 4](image2)

**Figure 4.** Scatter plot with regression line analysis plant growth performance vs microclimate a) plant height b) number of leaf c) stem diameter
Not only the growth of crops decreased under the stress conditions, but it will also produce stomata closure as a kind of an environmental adaptation [25]. Further strategies to reduce water loss are reported including the reduction in the number of stomata [30]. Stomata plays an important role as a tool to adapt to stress drought. Several types of plants adapt to stress drought by reducing the size and the number of stomata [31]. Result showed that stomata measurement was high in the IB environment, suggesting the presence of microclimate effect. The t-test between the number of stomata open (SA) and close (SO) in IB result in significantly different between the two as indicated by with $\alpha = 0.004$ ($p<0.05$) (Fig. 5). Our data shows a higher number of stomata open in IB than OB. Stomata open is higher in ETA3. Similar finding has been published pointing out the importance of stomata open in response to light condition [19, 32, 33]. Stomata opening as affected by the light intensity provide the same pattern in all species [33]. It may also be affected by elevated temperature regardless the CO$_2$ concentration [34]. In this case, as temperature levels are quite similar between IB and OB, therefore we suggest more to the light intensity and humidity related stomatal activities difference. It can be concluded from OB observation, that the response of stomata closure may be occurred when cacao seedling subjected to stress condition [35]. Stomatal closure in response to the decrease in relative humidity at the leaf surface has been observed in a wide variety species [36]. However, stomata regulation is a complex process as it depends on microclimate and soil water potential [37]. It may be concluded that the stomatal characteristics indicating the more stressed condition created as the case of OB treatment.

**Figure 5.** The difference in stomata measurement of OB and IB

### 4. Conclusion

Our study suggested a clear difference of microclimate condition between inside the glass box (IB) and outside the glass box (OB). During the observation periods, the plant height, stem diameter, and number of leaf are consistently increased with an indication of decreasing trends from week to weeks. Observation at 7th week demonstrate an average increase plant height of IB (12%) and of OB (4%), stem diameter of IB (10%) and of OB (7%), and the number of leaves of OB (9.1%) and of IB (8.9 %) from 6th week. Data suggest an insignificant effect of different soil moisture level to cocoa seedling growth. Conversely, microclimate condition shows a significant effects to crop growth especially the plant height and stem diameter. No interaction effect of soil moisture level and microclimate were observed. The analysis of multiple regression analysis suggest a relation between microclimate crop growth, i.e. plant height, stem diameter, and number of leaf of young cocoa in IB. stomata identifications may have indicated a more stressed condition created in OB.

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