Leaf Gas Exchange and Stomata Properties of Oil Palm Seedlings (*Elaeis guineensis Jacq.*) Progenies Exposed to Elevated Carbon Dioxide

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HZEJ managed the analyses of the study. Author MHH managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: An experiment was carried out to examine the impact of increased CO_2 level on gas exchange characteristics and stomatal properties of three oil palm progenies (Deli URT, Deli Yangambi and Deli AVROS). The seedlings were exposed to three CO_2 enrichment treatments: ambient CO_2 (400 µL L^{-1}), twice (800 µL L^{-1}) and thrice ambient CO_2 (1200 µL L^{-1}) for six days a week for three months.
Study Design: The experiment used a 3x3 Randomized Complete Block Design (RCBD) in factorial split plot arrangement with the CO_2 levels as the main plot and progenies as the sub plot replicated three times. Each treatment contained 10 seedlings.
Place and Duration of Study: Malaysian Palm Oil Board, (MPOB) Headquarters, between January to March 2009.
Methodology: Carbon dioxide at 99.8% purity was provided from a high pressure CO_2 cylinder and

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injected through a pressure regulator into fully sealed growth compartments. The flow of CO_2 in the chamber was automatically controlled by a CO_2 P.P.M. Controller ™ (R and M Supply Inc. USA). Seedlings were exposed to elevated CO_2 for 2 hours per day from 0800 in the morning to 1000 continuously for six days every week. The leaf gas exchange was measured using a closed system, infra-red gas analyzer LICOR 6400 Portable Photosynthesis System (IRGA: LICOR Inc. Nebraska, USA). The net photosynthesis rate (A), transpiration rate (E), stomata conductance (gs) and intercellular CO_2 (Ci) were simultaneously recorded. Stomata densities were examined by means of peel surface imprints made using clear nail varnish (cellulose acetate). Before peeling off, the layers with the impression imprinted were then placed on 1 mm^2 graduated slide. All stomata were counted in six field to give a mean value for each sample.

Results: Significant interactions between CO_2 and progenies for gas exchange attributes were observed except for stomata density. Increased CO_2 significantly ($p \leq 0.05$) affected net photosynthesis (A), stomata conductance (g_s), transpiration rate (E), instantaneous water use efficiency (WUE), intercellular CO_2 concentration (C_i), adaxial, abaxial and total stomata density. As CO_2 concentration increased, A, and WUE increased. It was also observed that stomata conductance and transpiration rate reduced and could be explained by stomata closure and declining stomata densities (abaxial, adaxial, total) under elevated CO_2. The decreased stomata density (pores per mm^2) was likely due to greater leaf expansion as showed by increased in total leaf area per seedling.

Conclusion: Results showed that the increase in WUE was due more to an increase in net photosynthesis (A) than to a reduced transpiration rate (E). The experiment showed there was no significant difference ($p \leq 0.05$) between 800 and 1200 $\mu L\ L^{-1}$ CO_2 treatments in any of the variables measured.

1. INTRODUCTION

Since the industrial revolution, CO_2 concentration in the atmosphere has increased to the current level of 400 $\mu L\ L^{-1}$ and is expected to double by the year 2050 primarily as a result of burning fossil fuels. This change will have profound effects both on the climate system and on the earth's primary productivity because CO_2 is the main greenhouse gas as well as a substrate for the production of biomass [1]. Theoretically, a higher CO_2 concentration will increase plant photosynthesis and growth by increasing the availability of substrate for photosynthesis. In general, yield should increase by 33% with a doubling in ambient CO_2 concentration [2]. However, plant growth and physiological responses to elevated CO_2 may differ from agronomic responses due to differences in lifespan, water, nutrients and plant growth habit. Plants may be influenced by an elevated CO_2 depending on the physiological process studied, stage of development at exposure, species and the physiological conditions [3].

Leaves are the main plant organ to detect and respond to changes in the concentration of atmospheric CO_2 through rapid changes in photosynthetic rate and stomatal opening [4].

Stomatal density (stomata number per unit of leaf area) of a plant grown with high level of CO_2 is usually lower than normal [4]. Woodward [5] found that stomata densities for plane tree maple (*Acer pseudoplatanus* L.) decreased by 40% as the ambient CO_2 level doubled. As the stomatal densities reduced, g_s also reduced under elevated CO_2 levels [6]. Carbon dioxide directly affects plant water relations. Increasing CO_2 concentration in the atmosphere around the leaf causes the stomata to partially close, which reduces transpiration or rate of moisture loss from the leaf and stomata conductance. Stomatal conductance is a parameter that characterizes the ability of stomates to transmit water vapor from inside the leaf to the air surrounding it. The partial closing of stomata with elevated CO_2 has several consequences, which may or may not be important depending on circumstances. One immediate effect of partial closure of stomata is a reduction of transpirational cooling of the plant [2], which is greatest for plant under drought [7]. Idso [8] observed that water stressed plants of C_3, C_4 and CAM groups when exposed to 640 $\mu L/L^{-1}$ CO_2 concentrations were able to remain turgid and functional for a long period of drought condition compared to plants grown under ambient carbon dioxide. Morrison [9] analyzed data from previous studies and concluded that

doubling the ambient CO_2 concentration decreased stomatal conductance up to 60% with no significant difference between C_3 and C_4 species. It has been hypothesized that reduction in stomata conductance are due to increases in C_i which also has a direct effect on stomatal closure [10].

Increase in CO_2 generally enhances photosynthesis and increases water use efficiency (WUE). Jackson [11] defined WUE as the amount of CO_2 assimilated through photosynthesis relative to the water lost through transpiration. Numerous researchers have ascribed this increase to greater net photosynthesis associated with greater CO_2 availability, lower transpiration resulting from lower stomatal conductance (g_s), or the combination of the two. The increase in WUE, however is variable among different studies [12,13]. Nijs et al. [14] indicated that increased WUE in *Lolium perenne* at high CO_2 concentration was due to enhancement in net photosynthesis alone, without any concurrent decrease in transpiration; and by doubling the CO_2 concentration reduces WUE by 9%.

Studies regarding impact of elevated CO_2 in oil palm are still lacking in Malaysia. Previously, for oil palm seedlings under nursery condition, Henson [15] determined the CO_2 compensation point and RubP carboxylation efficiency but the information was insufficient because he only measured them at ambient level. Research by Awang and Furukawa [16] on oil palm seedlings just gives a brief detail of oil palm seedling photosynthetic responses to elevated CO_2 of 550 to 700 µL L^{-1} when grew under open top chamber. Information on CO_2 enrichment techniques and procedures is still lacking. The essential information is needed to develop strategies for seedling management especially in oil palm nurseries. This is explained by the fact that, oil palm seedlings took about 14 – 20 months in nurseries to establish before can be transplanted to field [17]. If the time under nurseries can be decreased by giving additional supplies of CO_2 to the seedlings, management and maintenance cost could be reduced to spur better income for oil palm nursery entrepreneurs. Lengthening the time in the nursery might produce vigorous palms that could ensure a successful field establishment, and that has the potential for high initial accumulated yield than plants coming from traditional nurseries. The use of carbon dioxide enrichment may substitute for the length of time in the nurseries. In the present study, we use two-hour carbon dioxide enrichment from 0800 to 1000 due to lower vapor pressure deficit (Vpd) during this time that enhanced CO_2 uptake that enhanced the benefit of CO_2 enrichment. The present study investigated the leaf gas exchange responses and stomatal density of three commercial *tenera* progenies of oil palm seedlings exposed to short duration of elevated CO_2, in order to determine the optimum CO_2 concentration for the palm carbon assimilation. The relationship between CO_2 concentration and progenies was also calculated to give important information on varietal gas exchange characteristics and stomata density to elevate levels of CO_2.

2. MATERIALS AND METHODS

2.1 Experimental Location and Treatments

The experiment described was carried out at the Malaysian Palm Oil Board (MPOB) headquarters in Bangi, Selangor Malaysia. The site is situated at longtitude 101° 44' and latitude 2° 58' and 68 m above sea level with a mean atmospheric pressure 1.013 kpa, mean daily temperature 30 °C, mean relative humidity 70% and highest irradiance level at 1600 µmol m^{-2}s^{-1} and whilst minimum at 40 µmol m^{-2}s^{-1}. Three month old oil palm seedlings were grown in 30 cm x 40 cm polyethyelene bags (polybags) containing a thoroughly mixed soil mixture of topsoil and sand in a ratio (6 kg / polybag) of 3:1 (v:v). Prior to seedling transplant, 50 g of rock phosphate was applied to each polybag.Three months old *tenera* (DxP) progenies of oil palm progenies of *Deli Urt*, *Deli Yangambi* and *Deli AVROS* were purchased and left to acclimatize in a nursery until ready for the experiment. The treatment started when seedlings reached 5 months, old where every progeny was exposed to three levels of CO_2 enrichment viz. ambient CO_2 (control; 400 µL L^{-1}), twice ambient CO_2 (800 µL L^{-1}) and thrice ambient CO_2 (1200 µL L^{-1}). The experiment used a 3x3 Randomized Complete Block Design (RCBD) in factorial split plot arrangement with the CO_2 levels as the main plot and progenies as the sub plot replicated three times. Each treatment contained 10 seedlings. The CO_2 level was selected as the main plot because higher differences were expected compared to different planting materials used, hence precision could be pooled for seedling effects to give a high degree of freedom for error.

2.2 CO_2 Exposure Methods

Carbon dioxide at 99.8% purity was supplied from a high pressure CO_2 cylinder and injected through a pressure regulator into fully sealed growth compartments that located outside MPOB headquarters. The flow of CO_2 in the chamber was automatically controlled by a CO_2 P.P.M. Controller ™ (R and M Supply Inc. USA). Seedlings were exposed to elevated CO_2 for 2 hours per day from 0800 in the morning to 1000 continuously for six days every week. At the time of CO_2 exposition, two fans with low revolution per minute placed on the left and right of the chamber were operated simultaneously to produce mean wind velocity inside the sunlit growth compartments between 0.5 and 1 ms^{-1} to distribute CO_2 evenly inside the growth compartment. The low wind velocity would create small wind turbulence that removed leaf. mesophyll boundary layer hence, increased CO_2 uptake by the leaf. The wind velocity was measured using a portable anemometer Windscribe™ (Davis Instrument, Haywood, USA). After CO_2 exposure, the temperature inside the growth compartment was regulated with mounted exhausted fan installed at the left and right side of growth compartments.

2.3 Leaf Gas Exchange Analysis

The measurements were carried out in March 2007 for on 29 weeks old *tenera* oil palm seedlings grown in 2 m by 3 m sunlit growth compartments after 9 weeks of exposure. Ten seedlings with uniform growth were selected. Measurements were taken using a closed system, infra-red gas analyzer LICOR 6400 Portable Photosynthesis System (IRGA :LICOR Inc. Nebraska, USA) by placing the lamina of fully expanded leaves of the second frond on the terminal bifid lobes in a leaf cuvette set with optimal growth conditions. Prior to use, the instrument was warmed up for at least an hour and calibrated by taking two steps; first the initial zeroing process for the built-in flow meter, and second, zeroing of the infra-red gas analyzer. Measurements used standard optimal cuvette conditions for oil palm at 1000 µmol m^2 s^{-1} photosynthetically active radiation (PAR), 400 µL L^{-1} CO_2 concentration, 30°C leaf temperature and 60% relative humidity (Haniff, 2006). The leaf cuvette was supported by a tripod. Measurements of gas exchange were carried out between 1000 to 1100 after the CO_2 enrichment procedure. Net photosynthesis rate, transpiration rate, stomata conductance and intercellular CO_2 were simultaneously recorded. Data were stored in the LICOR computer within the console, and analyzed by "Photosyn Assistant " software that calculated several parameters. Several precautions were applied to avoid errors during measurement, e.g. cleaning and drying of leaf surfaces before placing them in leaf cuvette.

2.4 Stomatal Density (pores mm^{-2})

Stomata have important roles both in CO_2 / O_2 gas interchange and in transpiration. Stomatal density and its variations have been used as an important eco-physiological parameter that reflect variations in the environment. Since 1700, stomata densities have been the parameter that was widely used to show changes in the environment. Two samples per subplot were taken randomly from fully expanded leaves of the second frond. Stomata were examined by means of peel surface imprints made using clear nail varnish (cellulose acetate). The thin layer of nail varnish was applied on both leaf surfaces and dried for 10 minutes. Before peeling off, the layers with the impression imprinted were then placed on 1 mm^2 graduated slide. All stomata were counted in six field of view to give a mean value for each sample. The slide was then observed under a digital microscope (Model Ken A Vision T 1952, USA) with a magnification of 400x, and the stomata numbers per mm square were counted.

2.5 Data Analyses

SPSS software version 13 was used to run an analysis of variance (ANOVA) between treatments at $p \leq 0.05$. Mean separation tests between treatments were performed using Duncan Multiple Range Test (DMRT) with the assumption that data were normally distributed and equally replicated.

3. RESULTS AND DISCUSSION

3.1 Net Photosynthesis

Photosynthesis measurements, conducted on second fully expanded leaf, showed increased leaf photosynthesis solely due to elevated CO_2 ($p \leq 0.05$; Table 1) regardless of the progenies used. As CO_2 concentration increased from 400 µL L^{-1} to 800 or 1200 µL L^{-1}, net photosynthesis of oil palm seedling also increased (Fig. 1; $p \leq 0.05$). Doubling CO_2 levels, increased net photosynthesis by 60% over the ambient, whilst

CO_2 increment by three-fold only enhanced net photosynthesis by 40% more than when exposed to 400 µL L^{-1} (Table 2). However, net photosynthesis was not significantly different when seedlings were exposed to 800 µL L^{-1} or 1200 µL L^{-1} of CO_2 between progenies. Van and Megonigal [18] who applied elevated CO_2 and water stress treatment on *Taxidium distichum* seedlings (C_3), also observed significant increases in photosynthesis due to elevated CO_2. The increase in leaf photosynthesis obtained in the present work ranged between 58 to 128% from 18.6 µmol $m^{-2}s^{-1}$ to 29.8 and 26.0 µmol $m^{-2}s^{-1}$. Downton et al. [19] also obtained similar result with enrichment of *Garcinia mangostana*, i.e. as CO_2 increased from 400 and 800 and 1000 µL L^{-1}, net photosynthesis increased proportionally. The increase in photosynthesis is due to an increase in photosynthetic substrate, CO_2. Theoretically, a higher CO_2 concentration should increase plant photosynthesis by increasing the availability of the substrate. A high level of CO_2 also suppresses the rate of photorespiration, which is most beneficial for C_3 pathway plants [20]. The reduction of photosynthesis in high level of CO_2 has been observed by Bunce [21] in soybean. In their review of C_3 crops, it was documented that as CO_2 level increased above 1000 µL L^{-1} little responses in photosynthesis occurred. Similar result was observed in the present work, where increasing [CO_2] from 800 to 1200 µL L^{-1} did not cause any significant increase in photosynthesis. The reduced photosynthesis rate under ambient CO_2 might be attributed to lack of sink strength of the palms. If plants don't have the capacity to provide extra sinks for extra assimilates feedback inhibition will occur and these are manifested by a decreased rate of photosynthesis under normal CO_2 condition [22,23].
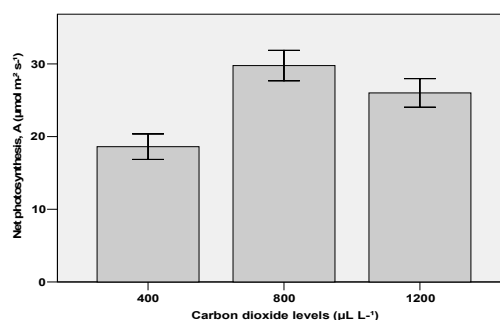

Fig. 1. Net photosynthesis (A) by different carbon dioxide levels. N = 18. Bars represent standard error differences between means (SED)

3.2 Stomata Conductance

Interaction between CO_2 and progenies contributed to effects of stomatal conductance (Table 1; p≤0.05). Seedlings of *Deli Yangambi* when exposed to 400 µL L^{-1} CO_2 recorded higher stomatal conductance than *Deli AVROS* (577 vs 549 mmol/m^2/s), but decreased its stomatal conductance markedly against *Deli AVROS* when exposed to higher CO_2 concentration of 800 and 1200 µL L^{-1} at respective values of 212 and 342 mmol/m^2/s compared to 399 and 441 mmol/m^2/s (Figure 2;). Under all CO_2 levels, progeny *Deli Urt* had displayed the highest stomatal conductance implying that there was a species difference between progenies. Meanwhile, levels of CO_2 concentrations were also observed to impose very significant impact (p≤0.01; Table 2) on the stomatal conductance of the progenies. Doubling and tripling the levels of CO_2 concentration did not enhance stomata conductance although rate of photosynthesis under both situation was improved than when

Table 1. Statistical significance of the effect of the factors carbon dioxide, progenies and the interaction of carbon dioxide x progenies on gas exchange and stomata properties of *Elaeis guineensis*, seedlings after 9 weeks. n.s = non significant, * p≤0.05, ** p≤0.01

	Treatments		
Parameters	**CO_2**	**progeny**	**CO_2 x progeny**
Net photosynthesis	*	ns	ns
Stomata conductance	**	*	*
Transpiration	**	**	ns
Water use efficiency	**	*	ns
Intercellular CO_2	*	ns	*
Abaxial stomata	*	ns	ns
Adaxial stomata	*	ns	ns
Total stomata	*	ns	ns

Table 2. Comparison between carbon dioxide enrichment treatments for all parameter. Plants were grown and measured in ambient (400 µL L^{-1}), double (800 µL L^{-1}) and triple (1200 µL L^{-1})

Parameters	400	800	1200	800/400	1200/400
Net photosynthesis (A)	18.614[a]	29.774[b]	26.014[b]	1.600	1.397
S. conductance (gs)	526.01[a]	322.21[b]	462.17[b]	0.630	0.880
Transpiration rate (E)	4.78[a]	3.244[b]	3.98[b]	0.670	0.823
WUE	3.967[a]	9.689[b]	6.978[c]	2.440	1.758
I. carbon (Ci)	232.55[a]	261.94[b]	285.07[c]	1.126	1.225
Abaxial stomata	44.59[a]	28.63[b]	27.96[b]	0.642	0.627
Adaxial stomata	182.96[a]	126.03[b]	130.22[b]	0.688	0.711
Total stomata	227.55[a]	154.67[b]	158.18[b]	0.670	0.695

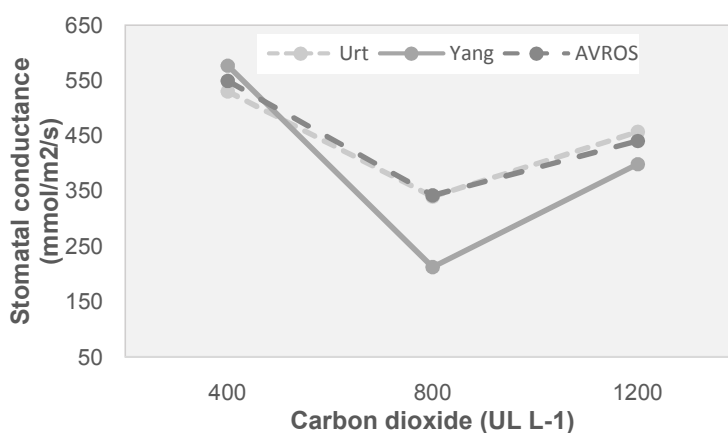*Mean in same rows followed by the same letter are not significant a p≤0.05*

Fig. 2. Interaction between carbon dioxide levels and progenies for stomatal conductance. (g$_s$) N = 9. Deli AVROS = (Av), Deli Urt = (Urt) and Deli Yangambi = (Yang)

under ambient CO_2 level. As CO_2 concentration increased, total stomata conductance was reduced, hence leaf resistance increased. The magnitude of stomata response to CO_2 enrichment varies between and within species. This has been observed by many researchers [24-27]. Morison and Jarvis [28] noted that stomata conductance decreased with increasing internal [CO_2] in the plant species they have observed. The difference in the latter can also be attributed to the difference in leaf structure. For instance, reduced stomata conductance is due to both decline in the aperture of stomata and lower stomata densities [29,30]. A reduction in stomatal conductance when growing plants at elevated CO_2 was also detected in *Quercus myrtifolia* [31] and in *Prunus avium* [32]. [33,34] demonstrated plants acclimated to change in ambient CO_2 concentration by changing the stomatal density. However, [35] suggested that changing in stomata aperture is more reliable in explaining reductions in stomatal conductances because stomatal density (pores mm^{-2}) varies between

species and growth condition. They found that stomatal density increased in *Solanum tuberasum* leaves when exposed to elevated CO_2 although g$_s$ reduced. The mechanism of stomatal response to CO_2 concentration is still unclear but there are suppositions that it is linked to malate synthesis, which is known to regulate anion channels in the guard cell plasma membrane [35].

3.3 Transpiration Rate

Similar to stomatal conductance, leaf transpiration rates are affected by interaction between CO_2 and oil palm progenies (P<0.05; Table 1). Both *Deli Yangambi* and *Deli Urt* recorded lower transpiration rate when exposed to 400 µL L^{-1} CO_2 concentrations (4.56 and 4.60 mmol/m^2/s) compared to *Deli AVROS* (5.12 mmol/m2/s). However, when exposed to 800 and 1200 µL L^{-1} CO_2 concentration transpiration rate of *Deli Yangambi* continued to decline than other progenies by 21% and 16% respectively (Fig. 3).

Transpiration proportionally reduced as CO_2 levels increased from ambient to 1200 µL L^{-1}. An increase in total plant transpiration rate might be due to a larger leaf area in both progenies [36]. As leaf area increased, transpiration rate also increased. Reductions in transpiration rates due to increased CO_2 concentration were caused by a decrease in stomatal conductance ($r^2 = 0.673*$; Table 3) probably through the partial closing of stomates which consequently increased leaf temperature at elevated CO_2 [37-39]. For instance, some of the researcher, observed that transpiration rate fell due to an increase in ambient CO_2 level. It can be concluded that as CO_2 increased, transpiration rate reduced in their studies.

3.4 Water Use Efficiency (WUE)

Increasing the concentration of CO_2 from 400 µL L^{-1} to 800 µL L^{-1}, increases the instantaneous water use efficiency (WUE) by 144% (9.6 vs 4.0; Fig. 4; p≤ 0.01). Further increase in CO_2 level to three fold, dropped WUE slightly to 76% of the reading at ambient carbon dioxide. As net photosynthesis increased, water use efficiency, increased ($r^2 = 0.772$; Fig. 5). This result suggests that, as photosynthesis increases, water use efficiency increases regardless of the transpiration rate. Instantaneous water use efficiency increased with increasing levels of CO_2. *Elaeis guineensis* seedlings exposed to where two and three-fold increase in CO_2 increased water use efficiency to 144% and 78% respectively (Table 2). The enhancements were due to higher photosynthesis and reduced transpiration rate. It was observed that WUE, was highest in 800 µL L^{-1} compared to 1200 µL L^{-1} treatments. Rosenberg [40] and Kramer [41] suggested that it is the combination of factors that produce an increase in WUE, especially lower g_s, which limits transpiration more than photosynthesis. Nijs et al. [14] supported this observation in their experiment with *Lolium perenne*. From that experiment, they inferred that increased water use efficiency was attributable to enhancement in net photosynthesis alone, without any concurrent increase in transpiration. In general, increases in water use efficiency of oil palm seedlings exposed to elevated CO_2 might mainly cause by increases in net photosynthesis and reduced transpiration rate [42-44].

3.5 Intercellular CO_2

The effect of intercellular CO_2 C_i is contributed by the interaction effect between CO_2 and progenies (Table 1). Under ambient CO_2 condition, *Deli AVROS* registered significantly lowest C_i followed by *Deli Yangambi* (232 ppm) and *Deli Urt* (241 ppm) (Fig. 6). As CO_2 concentration doubled, C_i of *Deli AVROS* increased, although insignificant to other treatments. Ultimately, at triple CO_2, C_i of *Deli AVROS* recorded the highest value (293 ppm) followed by *Deli Urt* (283 ppm) and finally, *Deli Yangambi* (273 ppm; Fig. 5). Increasing CO_2 level from 400 to 800 and 1200 µL L^{-1} increased Ci of oil palm seedling by 13% and 22% respectively (Table 2). Intercellular CO_2, affects the temperature dependence of photosynthesis. The optimum temperature for photosynthesis is low when C_i
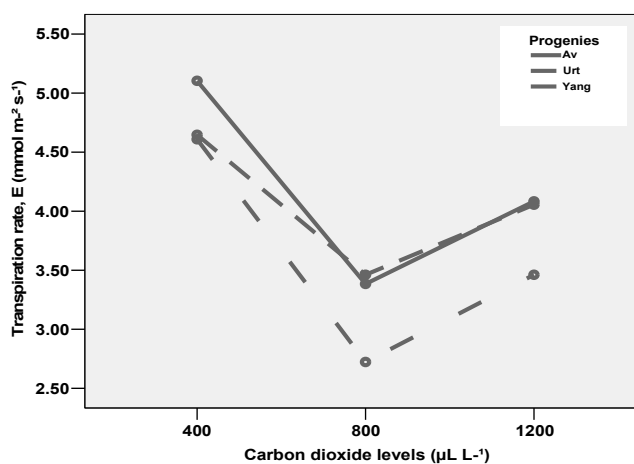

Fig. 3. Interaction between carbon dioxide levels and progenies for transpiration rate (E). N = 9. Deli AVROS = (Av), Deli Urt = (Urt) and Deli Yangambi = (Yang)
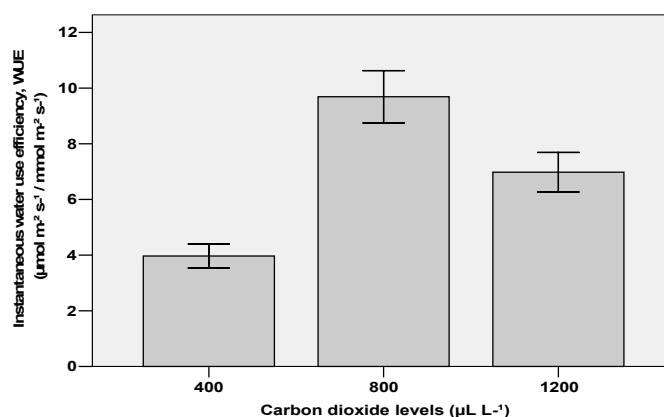
**Fig. 4. Instantaneous water use efficiency (WUE) by different carbon dioxide levels.
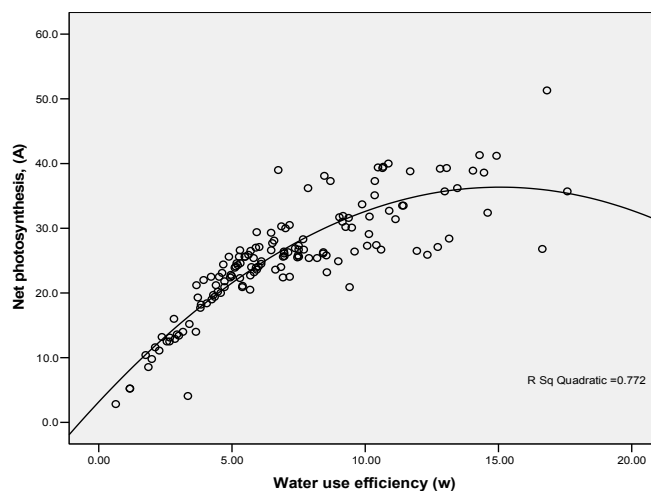N = 18. Bars represent standard error differences between means (SED)**

Fig. 5. Quadratic relationship between net photosynthesis and water use efficiency

decreases with increasing leaf temperature [42]. From the data for oil palm seedlings, Ci intensified with increased levels of CO_2 from 800 to 1200 µL L^{-1}. The combination of Deli AVROS and 1200 µL L^{-1} gave the highest Ci among all treatment combination (CO_2 x progenies). The current result shows optimal temperature for photosynthesis might be increased with elevated CO_2. According to Hikosaka et al. [45], optimal temperature increases by c. 0.05ºC per 1 ppm increase on external CO_2 although the increment decreases with increasing CO_2 concentration. It is believed that stomatal conductance is regulated so as to maintain the ratio of C_i to C_a (ambient CO_2 concentration) constant, irrespective of temperature if the vapor pressure deficit (VPD) is constant. If leaf temperature is increased with constant water vapor pressure, then VPD increased with increasing leaf temperature, which decreased the value of C_i [46]. At low ambient CO_2 concentration, the carboxylation rate is less sensitive to temperature because an increase in kinetics of carboxylation (K_c) partly cancels the increase in maximum velocity of carboxylation (V_{cmax}). Furthermore, photorespiration rate increases with temperature due to increase compensation point increase [47,48]. These effects are smaller at high ambient CO_2, leading to increase in optimal temperature of photosynthesis carboxylation (P_c) [49]. It can be concluded, that the elevated Ci we observed on the current study, could be an indication of the increase in optimum temperature for the palms that treated with high levels of CO_2, but further measurements would be required to confirm this.

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Table 3. Pearson correlation coefficients for leaf gas exchange parameters

	Parameters	Net photosynthesis	Stomata conductance	Intercellular CO2	Transpiration rate	Water use efficiency
1	Net photosynthesis (A)	1.000				
2	Stomatal conductance (gs)	-0.395**	1.000			
3	Intercellular CO_2 (Ci)	-0.004^{ns}	-0.010^{ns}	1.000		
4	Transpiration rate (E)	-0.415**	0.673**	-0.199*	1.000	
5	Water use efficiency (WUE)	0.833*	-0.651**	0.023^{ns}	-0.790*	1.00

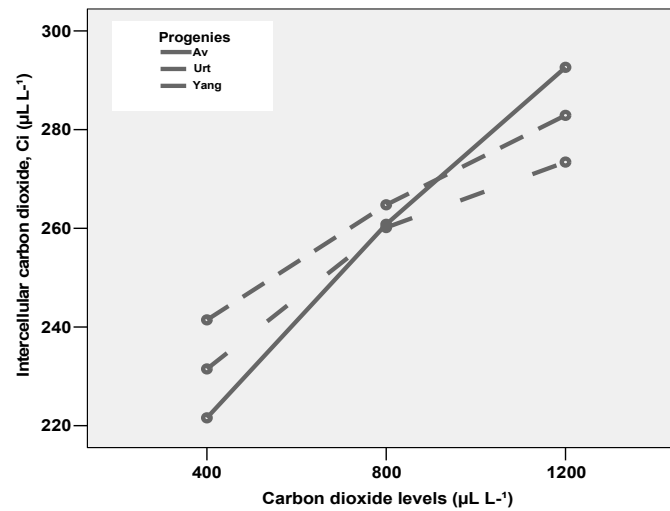***, * Significant at $p \leq 0.01$ and 0.05 respectively and ns = not significant*

Fig. 6. Interaction between carbon dioxide levels and progenies for intercellular carbon dioxide (Ci). N = 9. Deli AVROS = (Av), Deli Urt = (Urt) and Deli Yangambi = (Yang)
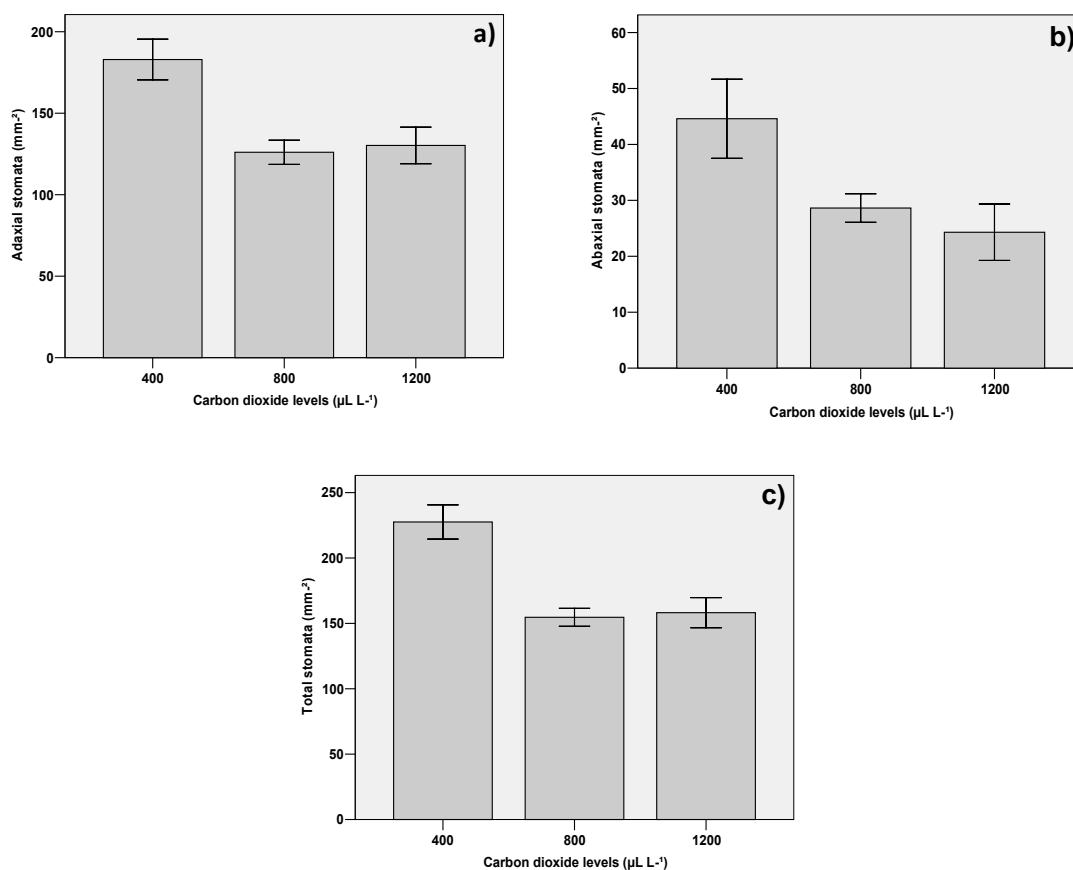
Fig. 7. Mean abaxial (a), adaxial (b) and total stomata density (c) by different carbon dioxide levels. N = 18. Bars represent standard error differences between means (SED)

3.6 Stomatal Density

Abaxial, adaxial and total stomatal density for oil palm after nine weeks treated with CO_2 are given in Fig. 7. There was no interaction between CO_2 and progenies nor progeny effects on stomatal density (Table 1). Carbon dioxide significantly reduced stomata density (mm^2) in the enriched plants (p≤0.01; Table 2). Both 800 and 1200 µL L^{-1} decreased adaxial, abaxial and total stomata by about 35%, 32% and 33%, respectively. These results demonstrate that, increased levels of CO_2 reduced stomata density but there is no significant difference between 800 and 1200 µL L^{-1} CO_2 levels. The data show that Oil palm seedlings leaves are *amphistomous* i.e. stomata occurs on both abaxial and adaxial leaf surfaces. This implies that they have the possibility to open and close their stomata on both sides independently, with transpiration rates being more sensitive to changes in stomatal aperture on the abaxial surfaces [48]. At the same time,

stomata density (pores/ mm^2) depends on levels of CO_2. As CO_2 increased, abaxial, adaxial and total stomata density declined (Table 2). This may be attributed to an increase in total leaf area per seedling when exposed to elevated CO_2. In the present study, total leaf area rose to 1.5 fold above ambient for plants that were enriched with CO_2 (data not provided). As total leaf area increased, stomata density per given unit area decreased because of enhanced leaf expansion. These factors contributed to decreased g_s per unit area with CO_2. According to Woodward [50], doubling of atmospheric CO_2 corresponds to a reduction of about 20 – 30% in stomata density. This observation is supported by Beryman et al. [51] who found tropical trees (*Maranthes corymbosa* and *Eucalyptus tetrodonta)* exhibited show an irreversible reduction in maximum stomata conductance, as a consequence of lower stomata densities. However, the effects of elevated CO_2 on leaf anatomy may differ depending on the stage of development, soil

fertility and seasonal variation. It is concluded that, reduced stomata density (pores mm^2) is caused by a stimulation of cell expansion (increased leaf area) which is a primary adaptive response of high levels of CO_2 [52,53].

4. CONCLUSION

Increase in Carbon dioxide have the potential to increase net photosynthesis (A) and instantaneous water use efficiency (WUE) of oil palm seedlings. Despite increase in net photosynthesis (A) and water use efficiency, stomatal conductance (g_s) and transpiration rate (E) was reduced. It can be concluded, that the reduction in stomatal conductance and transpiration rate of seedling enhanced with elevated carbon dioxide] probably due to reduced stomatal density on adaxial and abaxial parts of the leaves. On the present experiment, enhancement of instantaneous water use efficiency (WUE) in this experiment might be due to more of an increase in net photosynthesis (A) than to a reduced transpiration rate (E). This study also showed that oil palm would benefit with high levels of CO_2 that is expected to rise in the future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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