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Response of *in vitro* Cultured Palm Oil Seedling Under Saline Condition to Elevated Carbon Dioxide and Photosynthetic Photon Flux Density

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ABSTRACT

Salt stress elicits various physiological and growth responses of oil palm. A laboratory experiment was conducted to determine the responses of oil palms cultured in vitro under varying salinity levels (0, 85.5, 171.11, 342.21 and 684.43 mM NaCl) to elevated CO_2 (1000 µmol CO_2/mol) and PPFD (100±5 µmol m^{-2} s^{-1}) in terms of growth characteristics, pigment contents and photosynthetic abilities. After 14 days of culture, net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of oil palms across varying salinity levels was 5.33 times higher than those cultured under ambient CO_2 (380±100 µmol CO_2/mol) and PPFD (50±5 µmol m^{-2} s^{-1}). At increased net photosynthetic rate (elevated CO_2 and PPFD), despite having no significant difference in pigment contents (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) between different CO_2 and PPFD levels, dry weight and percent dry matter were 0.26 and 0.11 times higher, respectively, as compared to those cultured under ambient CO_2 and PPFD. In the same elevated CO_2 and PPFD level, across all salinity levels, stomatal conductance was 0.30 times lower than those cultured under ambient CO_2 and PPFD. At reduced stomatal conductance (elevated CO_2 and PPFD), transpiration rate was also reduced by 0.30 times. Thus with increased net photosynthetic rate and reduced transpiration rate, water use efficiency was increased by 7.22 times, across all salinity levels, than those cultured at ambient CO_2 and PPFD. These were considered essential for NaCl produces iso-osmotic stress.

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INTRODUCTION

It is projected that the demand for oil will increase as a result of future increase in the world per capita consumption of oils and fats (Oil World, 2004). Palm oil can contribute significantly to meet this demand in view of its high yield (4-5t/ha/year) (Barison and Ma, 2000); almost three times the yield of coconut and more than ten times that of soybean (Rajanaidu and Jalani, 1994). Expansion of oil palm plantations will mean encroachment of forested lands which has an adverse effect on the environment. Hence, areas other than forested land such as saline areas should be considered for oil palm production. However, since oil palms are known to be susceptible to salinity, breeding for salt tolerance in oil palm is needed to be able to fully utilize the vast saline areas for expansion of oil palm plantations.

Salinity is one of the major factors affecting agricultural productivity worldwide. In Asia alone, with an estimated area of 21.5 million hectares, 12 million hectares of which are considered saline. Salt negatively affects plants by inhibiting water absorption due to increased osmotic pressure around the roots and death of cells or tissues due to abundance of Na^+ (Munns and Tester, 2008). Moreover, physiological parameters have been developed as effective indices for tolerance screening in plant breeding programs (Parida and Das, 2005; Ashraf and Foolad, 2007). Jalani *et al.* (1997) considered tolerance of oil palm to salinity along with other abiotic stresses such as water-deficit, extreme temperature, mineral deficiency, heavy metal toxicity and ultraviolet irradiation as a fruitful topic in its improvement.

Thus, this study aimed to determine the responses of oil palm seedlings cultured in vitro under varying salinity levels to increased CO_2 and PPFD level in terms of growth characteristics, pigment contents and photosynthetic abilities. These issues were considered as a profitable venture for the purpose of salt tolerance screening using physiological parameters as quicker and simpler index in oil palm breeding programs such as high net photosynthesis in plant translating to better performance under saline condition.

MATERIALS AND METHODS

Plant materials and culture condition

Two-month-old oil palm plantlets of the variety Tenera derived from zygotic embryos of approximately 5 cm height with 1 to 2 opened leaves cultured in ½ strength Murashige and Skoog (MS) medium supplemented with 3% sucrose and 0.25% Phytagel® (Pronadisa, Hispanlab, S.A. Madrid) were selected and grown with roots removed in glass vessel under photoautotrophic condition supplemented with 50 ml ½ strength MS liquid medium (without agar and sugar) supported by vermiculite (Fig. 1 a and b)

The pH of the media was adjusted to 5.7 to 5.8 before autoclaving. Except for the differences in treatments (CO_2 concentration and PPFD), oil palm seedlings cultured *in vitro* in plant growth chamber (Eyela) were subjected to the same 28±2^{0}C/25±2^{0}C temperature shift(light/dark), 60±5% relative humidity (RH) and 16 h d^{-1} photoperiod as provided by 40W white fluorescent tube (Fig. 1 c). Air exchanges between the chamber environment and the glass vessels were facilitated with two air perforations on its cover and placed with gas-permeable microporous polyethylene film (0.22 µm pore size) over the holes.

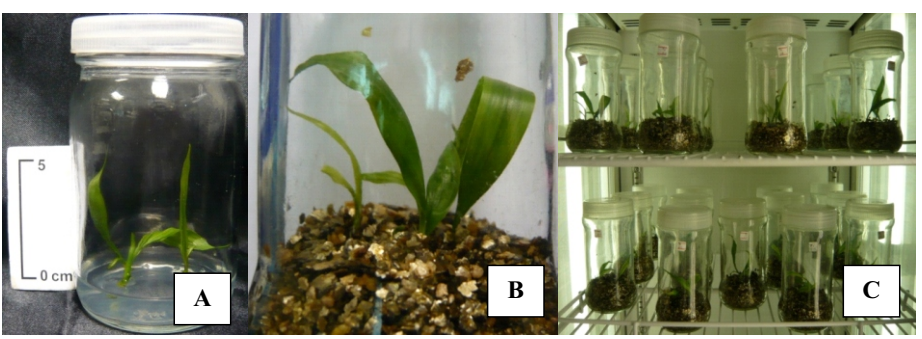

Figure 1. Two-months-old tenera plantlets derived from zygotic embryos cultured in ½ strength MS medium (A) sub-cultured with the roots removed in glass vessel under photoautotrophic condition supported by vermiculite (B) and placed under plant growth chamber (C).

Experimental design and treatments

A 2 × 5 factorial experiment arranged in Completely Randomized Design (CRD) with five replications at three plantlets per replication was used in this study. Different carbon dioxide (380±100 and 1000±100 µmol CO_2/mol) concentrations along with light intensities (50±5 and 100±5 µmol m^{-2} s^{-1}), and with varying levels of salinity (0, 85.5, 171.11, 342.21 and 684.43 mM NaCl) were tested (Table 1). The zygotic embryo-derived plantlets of oil palm were exposed to the different treatment conditions for a period of two (2) weeks before data were gathered. The following were the treatments of the study

Factor A – CO_2 and PPFD Level

A - Ambient CO_2 and PPFD Level (380±100 µmol CO_2/mol and 50±5 µmol m^{-2} s^{-1})
E - Elevated CO_2 and PPFD Level (1000±100 µmol CO_2/mol and 100±5 µmol m^{-2} s^{-1})

Factor B – Salinity Level

T1 - 0.00 mM NaCl
T2 - 85.50 mM NaCl
T3 - 171.11 mM NaCl
T4 - 342.21 mM NaCl
T5 - 684.43 mM NaCl

Data gathered

Physiological responses

Chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TC) and carotenoid (C_{x+C}) were measured following the methods of Shabala *et al*. (1998) and Lichtenthaler (1987), respectively. One hundred milligram of leaf tissue were collected and placed into 25 ml glass vial, added with 10 ml of 95.5% acetone and blended with homogenizer. The Chla, Chlb, and C_{x+C} were measured with UV – visible spectrometer. Solution of 95.5% acetone was used as blank.

The net photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (gs) of oil palm plantlets were measured using an Infra-Red Gas Analyser (IRGA). The E and gs were measured by continuously monitoring the H_2O of the air entering and exiting in the IRGA headspace chamber. Water use efficiency (WUE) of plantlets was calculated by ratio of P_n and E (Cha-um *et al.*, 2007).

Growth characteristics

Shoot fresh and dry weights of oil palm seedlings were measured at the end of the experiment. For dry weight, oil palm seedlings were dried at 80^0C in a hot-air oven for 2 days, and were incubated in desiccators for 1 day before the measurement of dry weight. Dry matter data are essential as dry matter production generally increases as the plant grows and develops making it a very reliable index of plant development compared to other parameters which are greatly dependent on moisture content. Percent dry matter was calculated by ratio of dry weight and fresh weight multiplied by 100. This is also important since weight of plantlets varies even before treatment application. On the other hand, root parameters were not gathered anymore because the study focused only on determining the potential of using Pn as index for salinity tolerance.

Data Analysis

Data were statistically analyzed through ANOVA for CRD. Comparisons among means were done using DMRT to determine the specific significant differences among treatment means.

RESULTS

The combined effect of CO_2, PPFD and salinity levels on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) is shown in Table 1. Oil Palms cultured under elevated CO_2 (1000±100 μmol CO_2/mol) and PPFD (100±5 μmol m^{-2} s^{-1}) and exposed to a salinity level of 85.50 mM NaCl (T1E) exhibited the highest net phosynthetic rate, but was not significantly different to those exposed to the same elevated CO_2 and PPFD level that were untreated with NaCl (T0E). Net phosynthetic rate in T1E was seven times higher than those seedlings cultured under ambient CO_2 (380±100

µmol CO_2/mol) and PPFD (50±5 µmol m^{-2} s^{-1}) level without NaCl (T0A). At salinity level above 85.50 mM NaCl, however, Pn of oil palm exposed to elevated CO_2 and PPFD level was not anymore statistically different to those grown under ambient CO_2 and PPFD level and exposed to saline condition up to 342.21 mM NaCl. Several authors have reported that an increase in PPFD enhanced net photosynthetic rate of *in vitro* plantlets in plant species such as *Eucalyptus* (Kirdmanee *et al.*, 1995), melon (*Cucumis melo* L.) (Adelberge *et al.*, 1999) and coffee (*Coffea arabusta)* (Nguyen *et al.*, 2000). However, none has been reported on plants exposed to saline condition.

Table 1. Net photosynthetic rate of oil palms after 14 days of culture under controlled environments as affected by different CO_2, PPFD and salinity levels. Different letters indicate significant differences between the treatments at $P \leq 0.05$ determined by Duncan's Multiple Range

CO_2 and PPFD Levels	Salinity Level (mM NaCl)					Mean
	T0	T1	T2	T3	T4	
A	0.00027^{cd}	0.00084^{cd}	- 0.00386^{cd}	- 0.00440^{cd}	- 0.00998^{d}	-0.00343b
E	0.02191^{ab}	0.02699^{a}	0.00927^{bc}	0.00811^{bc}	0.00005^{cd}	-0.00343b
Mean	0.01109^{a}	0.01391^{a}	0.00270^{ab}	0.00185^{ab}	-0.00049^{b}	

The increase in net photosynthetic rate, despite having no significant difference in pigment contents (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in µg g^{-1}FW) between ambient and elevated carbon dioxide and PPFD level (Table 2), mean dry weight (mg/plant) and percent dry matter were increased by 0.26 and 0.11 times at elevated CO_2 (1000±100 µmol CO_2/mol) and PPFD (100±5 µmol m^{-2} s^{-1}), respectively (Table 3). Plantlets grown under elevated CO_2 (1000±100 µmol CO_2/mol) and PPFD (100±5 µmol m^{-2} s^{-1}) levels that were untreated with NaCl (T0E) had the heaviest dry weight and percent dry matter which was 0.76 and 0.37 times higher, respectively, than those oil palms gown under ambient condition that were untreated with NaCl (T0A). Generally, photosynthetic photon flux (PPF) and CO_2 concentration are key environmental factors determining plant growth. In St. Johns wort, increasing PPF from 100 to 300 µmol m^{-2} s^{-1} and increasing CO_2 concentration from 500 to 1500 µmol mol^{-1} significantly increased net photosynthetic rate that resulted to increase in fresh and dry mass (Mosaleeyanon *et al.*, 2005). However, there were no reports on plants subjected to salinity stress.

Table 2. Pigment contents (chlorophyll a, chlorophyll b, total chlorophyll content and carotenoid) of oil palms after 14 days of culture under controlled environments as affected by different CO_2, PPFD and salinity levels. Different letters indicate significant differences between the treatments at $P \leq 0.05$ determined by Duncan's Multiple Range Test.

Treatment	Chla ($\mu g\ g^{-1}FW$)	Chlb ($\mu g\ g^{-1}FW$)	TC ($\mu g\ g^{-1}FW$)	C_{x+c} ($\mu g\ g^{-1}FW$)
Ambient CO_2 and PPFD				
0.00 mM NaCl	11.85^{ab}	4.71^{ab}	16.56^{ab}	3.71^{ab}
85.50 mM NaCl	13.67^{a}	5.52^{a}	19.20^{a}	4.43^{a}
171.11 mM NaCl	10.76^{ab}	4.57^{ab}	15.34^{ab}	3.36^{ab}
342.21 mM NaCl	8.34^{bc}	3.60^{bcd}	11.94^{bc}	2.85^{bc}
684.43 mM NaCl	5.35^{cd}	2.59^{cd}	7.95^{cd}	1.81^{cd}
Mean	9.99	4.19	14.19	3.23
Elevated CO_2 and PPFD				
0.00 mM NaCl	10.95^{ab}	4.27^{abc}	15.22^{ab}	3.85^{ab}
85.50 mM NaCl	10.43^{ab}	4.29^{abc}	14.72^{ab}	3.67^{ab}
171.11 mM NaCl	10.43^{ab}	4.39^{ab}	14.82^{ab}	3.92^{ab}
342.21 mM NaCl	7.85^{bc}	3.53^{bcd}	11.39^{abc}	2.97^{abc}
684.43 mM NaCl	3.79^{d}	2.03^{d}	5.82^{d}	1.35^{d}
Mean	8.69	3.70	12.39	3.15

Table 3. Growth characteristics (fresh weight, dry weight and percent dry matter) of oil palms after 14 days of culture under controlled environments as affected by different CO_2, PPFD and salinity levels. Different letters indicate significant differences between the treatments at $P \leq 0.05$ determined by Duncan's Multiple Range Test

Treatment	FW (mg/plant)	DW (mg/plant)	% DM (%/plant)
Ambient CO_2 and PPFD			
0.00 mM NaCl	117.64^{abc}	16.53^{bc}	13.98^{c}
85.50 mM NaCl	124.20^{abc}	21.34^{abc}	16.75^{abc}
171.11 mM NaCl	111.35^{bc}	16.13^{bc}	16.12^{abc}
342.21 mM NaCl	104.38^{c}	15.22^{bc}	15.27^{bc}
684.43 mM NaCl	91.66^{c}	13.53^{c}	14.48^{bc}
Mean	109.85	16.55^{b}	15.32^{b}
Elevated CO_2 and PPFD			
0.00 mM NaCl	149.09^{a}	29.15^{a}	19.27^{a}
85.50 mM NaCl	146.75^{ab}	25.57^{ab}	16.83^{abc}
171.11 mM NaCl	112.51^{abc}	20.37^{abc}	17.97^{ab}
342.21 mM NaCl	110.30^{bc}	18.36^{bc}	16.35^{abc}
684.43 mM NaCl	97.01^{d}	14.78^{c}	14.83^{bc}
Mean	123.13	21.65^{a}	17.05^{a}

In the same elevated CO_2 and PPFD level, across all salinity levels, stomatal conductance ($\mu mol\ m^{-2}\ s^{-}1$) of oil palm seedlings was 0.30 lower than those cultured under ambient carbon dioxide and PPFD (Fig. 2). Since

salinity induces iso-osmotic stress, a very common mechanism for plant to cope with further loss of water is through closing of stomata. With reduced stomatal conductance (elevated carbon dioxide and PPFD), transpiration rate (mmol H_2O m^{-2} s^{-1}) was also reduced by 0.30 times (Table 4).

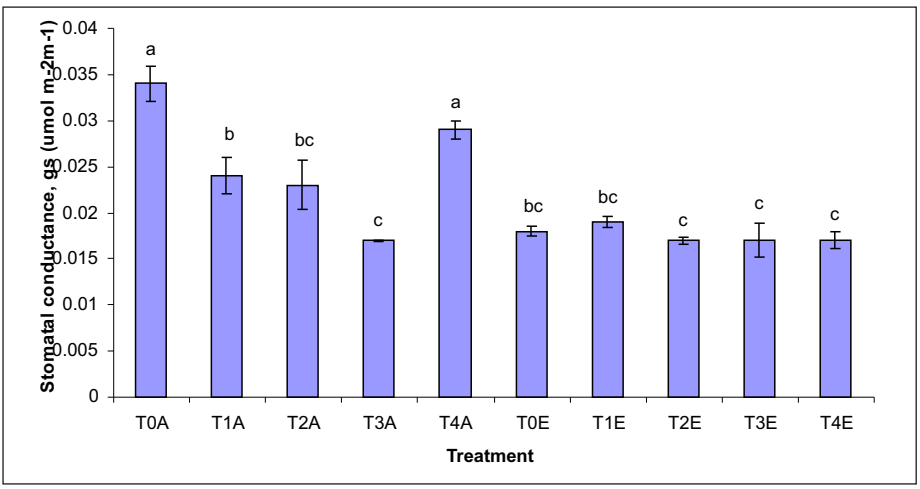

Figure 2. Stomatal conductance of oil palms after 14 days of culture under controlled environments as affected by different CO_2, PPFD and salinity levels. Different letters indicate significant differences between the treatments at $P \leq 0.05$ determined by Duncan's Multiple Range Test

Table 4. Transpiration rate of oil palms after 14 days of culture under controlled environments as affected by different CO_2, PPFD and salinity levels. Different letters indicate significant differences between the treatments at $P \leq 0.05$ determined by Duncan's Multiple Range Test

Treatment	E (mmol H_2O m^{-2} s^{-1})	WUE (Pn/E)
Ambient CO_2 and PPFD		
0.00 mM NaCl	0.88537^{a}	0.00030^{b}
85.50 mM NaCl	0.62821^{b}	0.00133^{b}
171.11 mM NaCl	0.60743^{bc}	-0.00635^{b}
342.21 mM NaCl	0.46688^{cd}	-0.00942^{b}
684.43 mM NaCl	0.76448^{a}	-0.01305^{b}
Mean	0.67047^{b}	-0.00544^{b}
Elevated CO_2 and PPFD		
0.00 mM NaCl	0.48423^{cd}	0.04524^{a}
85.50 mM NaCl	0.48735^{cd}	0.05538^{a}
171.11 mM NaCl	0.44871^{d}	0.02065^{ab}
342.21 mM NaCl	0.46000^{d}	0.01763^{ab}
684.43 mM NaCl	0.45623^{d}	0.00011^{b}
Mean	0.46730^{a}	0.02780^{a}

Stomatal conductance (T0A) and transpiration rate (T4A) peaks were significantly higher compared to the rest of the treatments. Thus with increased net photosynthetic rate and reduced transpiration rate, water use efficiency (Pn/E) was 7.22 times higher than those cultured at ambient carbon dioxide and PPFD, across all salinity levels.

Moreover, it is interesting to point out effect of elevated CO_2 and PPFD on stomatal conductance and transpiration rate as iso-osmotic stress derived from NaCl induced toxic damages especially in pigment degradation (de Herralde *et al.*, 1998; Lutts *et al.*, 2004; Neto *et al.*, 2004; Tonon *et al.*, 2004). In such case, minimal loss of water due to transpiration through the stomata is very important. With the increased net photosynthetic rate and reduced transpiration rate, water use efficiency (Pn/E) of seedlings was increased by 7.22 times higher than seedlings cultured at ambient carbon dioxide and PPFD. Moreover, the up-surge of stomatal conductance transpiration at 684.43 mM NaCl at ambient carbon dioxide and PPFD may have been attributed to epidermal cell death causing stomata to lock-open.

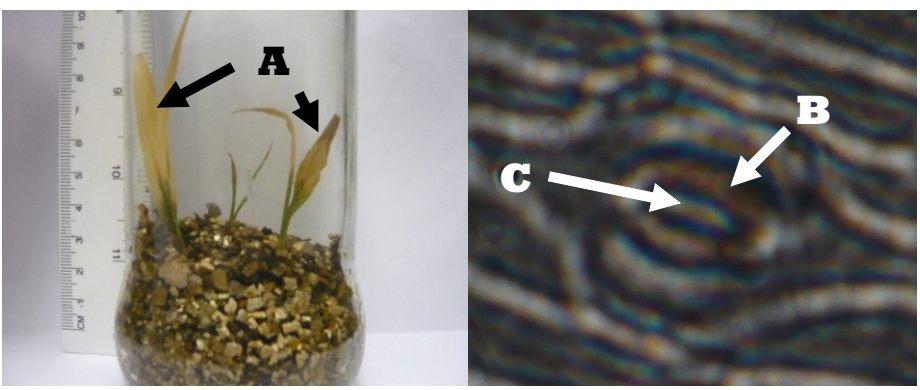

Figure 2. Severe chlorophyll degradation (A), due to high level of NaCl (684.43 mM), leading to damage or death of cells (B) thereby causing stomata to lock-open (C) in both ambient CO_2 and PPFD.

Prats *et al.* (2006) reported that in barley, stomata locked open as a consequence of epidermal cell death especially at the proximity of the dead cells. In general, the ionic toxicity of salt stress plays a major role in membrane injury, organelle damage and pigment degradation prior to cell death. This is well documented in many plant species such as *Centaurea rugusina* (Radic' *et al.*, 2005; Radic' *et al.*, 2006), *Fraxinus angustifolia*

(Tonon *et al.*, 2004), *Populus balsamifera* (Chen *et al.*, 2002), *Asteriscus maritimus* (Rodriguez *et al.*, 2005), *Lupinus consentinii* (Legocka and Kluk, 2005), *Vigna sinensis* (Costa *et al.*, 2007) and *Tamarindus indica* (El-Siddig *et al.*, 2004).

CONCLUSION

Elevated carbon dioxide (380±100 µmol CO_2/mol) and PPFD (50±5 µmol m^{-2} s^{-1}) increased the net phosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of oil palms under saline condition. With increased net photosynthetic rate, plant dry weight and percent dry matter were subsequently increased. The same increase in net photosynthetic rate is essential to affect an increase in water use efficiency. These findings show the possibility of using net photosynthetic rate as a physiological criterion for salinity tolerance screening in oil palm breeding programs e.g. higher Pn translating to better performance of oil palm under saline condition.

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