Growth Improvement of Rain Tree (*Albizia saman* Jacq. Merr) Seedlings under Elevated Concentration of Carbon Dioxide (CO_2)

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A study was conducted to determine the growth effects of rain tree (*Albizia saman* Jacq. Merr) seedlings under elevated concentration of carbon dioxide (CO_2) in an automated greenhouse system. The CO_2 was injected for two hours daily at 9 am– 11 am with mean concentration of 800 µmol mol^{-1}. For control trial, seedlings were placed in another greenhouse with mean ambient CO_2 concentration of 400 ± 50 µmol mol^{-1}. A completely randomized block design was used in this study, and growth observations were made in every 30 days for duration of 120 days. The results showed that almost all parameters observed for the seedling growth under elevated and ambient concentrations were significant. Concentrations of chlorophyll b in leaves of seedlings exposed for 30 days and 60 days at elevated CO_2 were 941.28 ppm and 993.56 ppm, compared to 492.06 ppm and 635 ppm at ambient CO_2. The chlorophyll b content of the leaves at 90 days of ambient CO_2 was significantly higher (688.51 ppm) compared to the leaves exposed to the elevated CO_2 with chlorophyll b content of 276.23 ppm. Moreover, the roots dry weight at ambient CO_2 indicated an average of 9.70 gram, while at elevated CO_2, the dry weight of plant roots is higher up to 38.65% of 13.45 gram. Root volume at ambient CO_2 showed an average volume of 40.36 ml^3, while at elevated CO_2 the volume increased to 90.33 ml^3 (up 123.81%). The results of this study indicate that the rain tree seedlings growth conditions were improved under the elevated CO_2 concentration, which reflect the efficiency in the use of CO_2 and water for photosynthesis process.

Keywords: Elevated CO_2, rain tree, growth, chlorophyll.

Global climate change shows various changes on climate parameters that include rising of atmospheric temperatures, melting ice caps in the poles, irregular seasonal changes of air and soil moisture (IPCC, 2013a). Changes of moisture in soil might affect the growth of plants and crops. The increase of CO_2 concentration in the atmosphere which is the main cause of climate change, may inhibit or stimulate the growth and production of plants. Previous research indicated that increased CO_2 concentrations in a controlled environment such as in greenhouses, had increased the yield on C_3 plants (17% -29%) and C_4 plants (6% - 10%) (Kimball, 1983; Baker and Allen, 1993). However, the low CO_2 concentration would not ensure lower water usage in the field, where the canopy size, structure, and microclimate would also regulate the use of water (Meinzer *et al.*, 1997).

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The relatively low usage of water is not necessarily led to increased growth and production, as the soils are in a moist state. In some experiments, the addition of CO_2 caused a relatively small increase in growth and production of C_3 soybean (*Glycine max*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) under the free-air CO_2 enrichment (FACE) system (Ainsworth and Long, 2005). Improved process of photosynthesis with increasing concentrations of CO_2 to enhance the growth of many crops has been reported (Calver, 1972; Madsen, 1976; Jacob *et al.*, 1995; Ohyama, *et al.*, 2005; Sanchez *et al.*, 2005; Thongbai *et al.*, 2010). Chlorophyll is a photosynthetic pigment and plays an important role in the absorption of light energy during the process of photosynthesis. Several studies have demonstrated that chlorophyll content is positively correlated with the rate of photosynthesis (Thomas *et al.*, 2005; Peng *et al*., 2008)

Albizia saman Jacq.Merr also called as "rain tree" because of its canopy that easily releases the rain water to the soil. Human activities directly the carbon cycleeither by adding more CO_2 into the atmosphere or to eliminate sources of carbon stocks, such as forests. Efforts should be done to reduce greenhouse gas emissions including forest management and non-agricultural land protection that has acted as a net absorber of CO_2, which means that much more CO_2 absorbed from atmospher, and stored in a plant, rather than being released (Dahlan, 2004).The investigations will be carried out to understand the mechanisms of rain tree with respect to its physiological and development of correlation with the ambient CO_2 and elevated CO_2 condition. The methods used in this study involves planting plants rain tree with two models namely ambient CO_2 planting in the greenhouse and elevated CO_2 in the greenhouse method. The study will run phenotipe morphological traits such as plant height, number of leaves, leaf area, root volume, stem loop and biomass.This research also aimed to study the physiology of rain tree by determining the chlorophyll content from leaves by ages.

MATERIALS AND METHODS

Seedlings preparation and growth measurement

Sixty rain tree (*Albizia saman* Jacq.Merr) seedlings of one week old were grown in polybags (25 cm diameter x 30 cm height) with growing medium of topsoil podzolic type that was mixed with organic fertilizer at a ratio of 4:1. The NPK fertilizer (15:15:15) was applied at a low dose of 5 g/polybag/month and seedlings were sufficiently watered for its growth. Thirty seedlings were exposed to elevated CO_2concentration of 800 µmol mol^{-1}in an automated CO_2 glasshouse system. These seedlings were exposed for two hours at 9-11 am daily where photosynthesis is anticipated to be optimum for control trial experiment, similar numbers of 30 seedlings of the same age were exposed to ambient CO_2outside of the glasshouse with ambient concentration of 400±50 mol mol^{-1}. The first observation was made after 14 days of treatment, and subsequent observations were recorded weekly for a period of 16 weeks (4 months). Several plant growth parameters that were measured and counted include plant height (cm), number of branches, and number of leaves, whilst the diameters of stems were measured at the base of the stems using a caliper.

Chlorophyll Concentration, Fresh and Dry Weight, and Root Volume

Determination of chlorophyll concentration was conducted using standard procedure by the International Rice Research Institute IRRI (Cock and Gomez, 1976), with modifications by Nurdin *et al*. (2009) on reduction of the acetone volume.0.1 g of rain tree leaves were chopped into small pieces(about 2 mm), and the leaves were put into a test tube, after which 20 ml80% acetone was added into the test tube. The mixture was homogenized by a shaker, and it was then incubated in the dark for 48 hours. Concentrations of chlorophyll *a* and chlorophyll *b* were analyzed using a spectrophotometer at the wavelength » of 663 nm and 645 nm, respectively. The chlorophyll concentrations were calculated using (Arnon, 1949); Mac Kinney, 1941) equation as follows:

$$C_{chl\text{-}a} = 12.7A_{663} - 2.69B_{645}$$
$$C_{chl\text{-}b} = 22.9\,A_{645} - 4.68\,B_{663}$$
$$\text{Total chlorophyll} = C_{chl\text{-}a} + C_{chl\text{-}b}$$

Fresh and dry weights of the seedlings were measured using a digital scale, of which the dry weight was obtained after the samples were dried in the oven at 65°C for seven days.Root volume was measured to determine the root growths of the seedlings. Seedling roots were put into a measuring cup with half-filled of water, and the volume difference before and after the roots were put into the cup was recorded.

Statistical analysis

All data were statistically analyzed using one way Analysis of Variance (ANOVA). Mean Separation was carried out to determine significance using the Duncan's Multiple Range Test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

Seedlings preparation and growth measurement

Seedlings growth parameters (plant height, number of branches and leaves, stem diameter between treatments of elevated and ambient CO_2 displayed various responses depending on number of days of treatments. Observations on plant height and number of branches showed no significant difference between the treatments after 30-60 days of exposure. Subsequent observation after 90-120 days of treatments revealed that the mean plant height exposed to high CO_2 differed significantly compared to the mean height of ambient concentration (Table 1).At 120 days of exposure, the mean height of plants under elevated CO_2 concentration was 196.02±15.8 cm, whereas the plants under ambient CO_2 concentration showed mean height of 173.16±10.90 cm. Likewise, after 120 days of exposure to elevated CO_2, the number of branches of seedlings was significantly different compared to ambient, of which the former indicated mean number of 36.82±2.50 whilst the latter of 35.22±2.10. Leaves number of the seedlings exhibited significant differences between treatments at 30 days and 120 days of exposure, whereby 1360.90±171.16 leaf sheets/plants were produced at 120 days under the elevated CO_2, whilst 1078.80±119.16 sheets/plants were of ambient CO_2. As for the stem diameter, the stems of plant sunder

Table 1. Growth parameters subjected to different CO_2 concentrations of rain tree (*Albizia saman* Jacq. Merr)

Measurement	CO2 concentration (ppm)							
	30 Days		60 Days		90 Days		120 Days	
	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2
Plant height	45.41± 13.9	45.63± 13.03	92.89± 12.7	95.62± 13.17	175.25± 34.1[a]	142.5± 17.4 [b]	196.02± 15.8 [a]	173.16± 10.9 [b]
Number of branches	12.66± 2.0	12.21± 2.6	19.94± 2.2	20.39± 1.6	28.13± 2.8 [a]	27.78± 2.6 [b]	36.82± 2.5 [a]	35.22± 2.1 [b]
Number of leaves	182.38± 63.8 [a]	160.28± 59.8 [b]	438.7± 115.8	408.55± 80.0	862.45± 149.5	703.19± 115.5	1360.9± 171.16 [a]	1078.8± 119.16 [b]
Diameter of stems	5.38± 1.3	4.68± 0.7	11.37± 2.1 [a]	8.72± 1.7 [b]	16.33± 1.3	13.24± 1.0	20.49± 1.5 [a]	16.17± 0.8 [b]

Note: Mean ± standard error (SE) followed by different letter of the same days of treatment is significant tested using Duncan multiple range test at $p<0.05$

Table 2. Responses of fresh weigh, dry weigh, fresh weigh roots, dry weigh roots and roots volume of rain tree (*Albizia saman* Jacq. Merr) to elevated CO_2 and ambient CO_2 conditions

Treatment	Fresh weigh (gram)	Dry weigh (gram)	Fresh weigh roots (gram)	Dry weigh roots (gram)	Roots volume (ml^3)
Elevated CO_2	647.02 ±32.31 [a]	191.66±9.13 [a]	69.10±1.42 [a]	13.45±0.49 [a]	90.33±1.05 [a]
Ambient CO_2	368.60±73.66 [b]	130.40±6.23 [b]	40.03±1.01 [b]	9.70±0.51 [b]	40. 36±1.10 [b]

Note: Mean ± standard error (SE) followed by different letter of the same column of treatment is significant tested using Duncan multiple range test at $p<0.05$

Table 3. Comparison concentration (ppm) of Chlorophyll a and b rain tree (*Albizia saman* Jacq.Merr) age leaves of 30, 60 and 90 days respectively

Measurements	Ages					
	30 Days		60 Days		90 Days	
	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a*	Chlorophyll *b*
Elevate CO_2	2166.7±54.11 [a]	941.3±16.50 [a]	2256.7±17.55 [a]	983.6±19.97 [a]	917.1±0.66 [b]	275.5 ±1.69 [b]
Ambient CO_2	1507.8±2.78 [b]	492.1±8.80 [b]	1611.3±11.30 [b]	635.3±33.10 [b]	982.3±11.23 [a]	493.9±4.19 [a]

Note: Mean ± standard error (SE) followed by different letter of the same days of treatment is significant tested using Duncan multiple range test at $p<0.05$

elevated CO_2 indicated larger in size compared to the plants at ambient CO_2. The measured there significant diameter stems where at elevated CO_2 produced an average age of 60 days of exposure have diameter 11.37±0.92 mm, while ambient CO_2 averaged of only 8.72±0.77 mm. However at age at 90 days of exposure, there was no difference. While age of 120 days of exposure stems diameter growth at elevated $C0_2$ and ambient $C0_2$ significant with an averaged at 20.49±1.5 mm and 16.17±0.8 mm respectively.

Based on the measurement of raw data, it can be assumed that after 30 days of treatments plants at elevated CO_2 concentrations of 800 µmol mol^{-1} at two hours per day on morning (9.00-11.00 am) indicated higher CO_2 absorption before and after those hours. At that time the photosynthesis

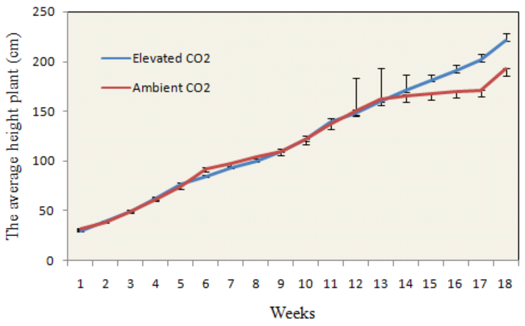

Fig. 1. Comparative responses fromelevated CO_2 and Ambient CO_2 of average height (cm) rain tree (*Albizia saman* Jacq.Merr)

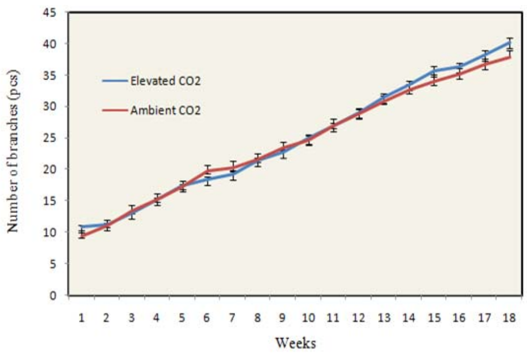

Fig. 2. Comparative responses fromelevated CO_2 and Ambient CO_2 of the number of branches (pcs) rain tree (*Albizia saman* Jacq.Merr).

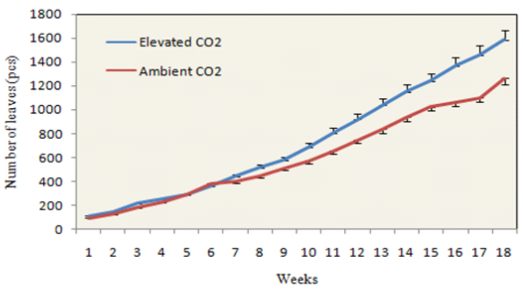

Fig. 3. Comparative responses fromelevated CO_2 and Ambient CO_2 of the number of leaves (pcs) rain tree (*Albizia saman* Jacq.Merr)

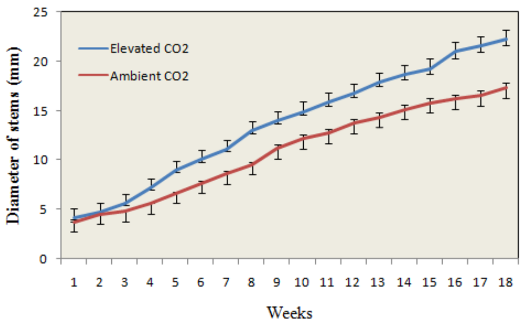

Fig. 4. Comparative responses fromelevated CO_2 and ambient CO_2 of the diameter of leaves (mm) rain tree (*Albizia saman* Jacq.Merr)

process went very well and effectively. Photosynthesis process will be gradually slow down until the sunset. The opening and closing of stomata on the leaf surface cells gradually adapted to high concentrations of CO_2 and the water content available within the plant tissue.The age of 120 days of exposure showed four readings in Table 1, and all produce significant observations. It is proven that the longer the plants were exposed to high concentrations of CO_2, the better the growth rate due to high photosynthetic process thus proving rain tree grow faster at increased CO_2 concentration than at ambient CO_2 conditions.

The growth of plant height at elevatedCO_2 and ambient CO_2 data were collected every weeks, starting the second week until week 14, and it shows no significant results. Significant plant height difference can be seen from week 14 and further are shown in Figure 1. Furthermore, significant differences in plant height observed between the treatment of elevated CO_2 and ambient CO_2 observations obtained at weeks 16, 17 and 18. The observations of the weeks was the best compared to observations in the previous weeks.

The result of number of branches of the plant during the first week of treatment was higher in elevated CO_2 compared to ambient CO_2 (Fig 2). Observations at week five to nine of the treatment of elevated CO_2 shows lower plant branches number. Plant branch growth at treatment of elevated CO_2 was higher at weeks 13 until weeks 18. Observations on the average number of leaves shows at the beginning of exposure are relatively similar between elevated CO_2 and ambient CO_2 (Fig. 3). However, from week 6 to week 18, the average number of leaves was higher in the treatment of elevated CO_2 compared to ambient CO_2. The diameter of each plants for the treatment of elevated CO_2 shows a quicker increased in size (mm) compared with ambient CO_2 treatment (Fig 4). Over all parameters investigated, stem diameter showed highest difference between elevated CO_2 and ambient CO_2 treatment. The difference increase vigorously starting from weeks 3 onwards. Meanwhile, other parameter show narrow difference throughout the weeks during experiment.

Chlorophyll Concentration, Fresh and Dry Weight, and Root Volume

The concentrations of chlorophyll *a*in the plant leaves at 30 days, 60 days and 90 days of elevated CO_2 were 2166.7 ppm, 2256.7 ppm, 917.1 ppm, respectively; with ambient CO_2 indicated concentrations of 1507.8 ppm, 1611.3 ppm and 982.3 ppm at the respective days of treatments (Table 3). It is apparent that the highest concentration of chlorophyll *a* was shown by plant leaves that were exposed to elevated CO_2 for 60 days, while the lowest concentration was in leaves of 90 days. Subsequently, concentrations of chlorophyll *b* were consistently lower than chlorophyll *a* in all plants of both treatments. Overall, the chlorophyll *a* and *b* contents for all plants were significantly higher in elevated CO_2 compared to the ambient condition (Figure 5).

Observation of the fresh weigh and dry weight , fresh and dry root weight and volume of rain tree plant after Duncant further test statistic indicates that there is significant difference for all parameters. The rain tree fresh weight is 75.53% higher in elevated CO_2 at 647.02 grams versus 368.60 grams at ambient CO_2 (Table 2). The weight of the plants are 46.97% higher in elevated CO_2 which is 191.66 grams versus 130.40 grams at ambient CO_2. The mean fresh weigh rain tree root at ambient CO_2 averaged at 40.03 grams, while the elevated CO_2 increased to 69.10 grams (up 72.62%). The dry root weigh at ambient CO_2 average 9.70 gram (dry weight) while at elevated CO_2 increased to 13.45 grams (up 38.65%). For root volume at ambient CO_2 averaged a volume of 40.36 ml^3 while at elevated CO_2 increased to 90.33 ml^3 rose to 123.81%. The highest percentage increase in growth is the root volume, followed by fresh weight, root fresh weight, dry weight and root dry weight respectively.

The results are in line with reports of Rogers *et al.* (1992) and Bowes (1993) who stated that elevated CO_2 might cause plants to produce more mesophyll cells and chloroplasts, leading to higher chlorophyll content. High chlorophyll content correlates with high nitrogen content, which would then reflect high protein contentin the plant cells. Bacerra *et al.* (1995) and Bui *et al.* (1995) reported duckweed (*Lemna* sp), has a high protein content, so many were used as cattle feed and poultry. The protein content in the leaves of ginger showed significant correlation with carbohydrates and chlorophyll *a* and *b* (Rai and Das 2010).

High carbon concentrations may increase

the length, diameter and number of roots (Lee-Ho *et al.* 2007) and stimulates lateral root production in plants grown under elevated CO_2 (Pritchard and Rogers 2000). It also changes the location of biomass from leaves to roots may occur under CO_2 enrichment (Stulen and Hertog 1993). Results of research Drake, *et al.* (1997) stated that elevated CO_2 increases photosynthesis carboxylation rate of Rubisco and competitively inhibiting the oxygenation of ribulose-1,5-bisphosphate (RuBP).

CONCLUSION

From this study, elevated CO_2 concentration affects the growth of rain tree seedlings which resulted in faster plant gowth, higher chlorophyll content and biomass content, increased root mass and volume, as compared to seedlings that were exposed at ambient CO_2. Optimization of acquisition and accumulation of CO_2 that is greater than the primary photosynthetic process will be beneficial to vegetative growth rain tree.

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