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Effects of Carbon Dioxide Concentration on the Growth and Physiology of *Albizia saman* (Jacq.) Merr

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ABSTRACT

This study was conducted to determine the effects of CO_2 concentration on the growth and physiology of rain tree (*Albizia saman* (Jacq.) Merr), by increasing the CO_2 concentration in a greenhouse automated system. The objective of this study was to evaluate the response of rain tree to CO_2 in terms of growth and physiology. CO_2 at an average concentration of 800 µmol mol^{-1} was injected daily for 2 h from 9 am to 11 am. The seedlings were placed in a greenhouse during the control trial with a mean ambient CO_2 concentration of 400 µmol mol^{-1}. In this study, the entire randomised block design has been applied, and growth was observed every 30 days for 120 days. Almost all seedling growth parameters were significant under elevated and ambient concentrations. The leaf area in the control samples (400 ppm of CO_2) was 243.37 cm^2, and this value increased to 277.30 cm^2 in the sample treated with 800 ppm of CO_2. The biomass increased, and the original wet weight ratio and root dry weight of the canopy and the principal (9.06 and 10.12 g, respectively) increased to 9.7 and 16.06 g, respectively, after treatment. Physiology was analysed in terms of relative levels of photosynthesis, stomatal conductance and water use efficiency (WUE). Such parameters increased in the principal treatment of CO_2 (800 ppm), whilst the CO_2 content and transpiration levels declined. As the CO_2 concentration increased, the value of the levels of photosynthesis and stomatal conductance in both samples increased. As the photosynthesis levels increased, the WUE activity increased. However, as photosynthesis levels decreased, the WUE activity also decreased. Transpiration levels but also rely on a certain age if the increased photosynthesis WUE has decreased.

Keywords: elevated CO_2, *Albizia saman,* growth, physiology.

INTRODUCTION

A range of changes, such as increasing atmospheric temperatures, melting ice caps in the Northern Hemisphere and unsustainably changing air and soil moisture levels, is caused by worldwide climate change [IPCC 2013a]. The growth of plants and crops can be affected by changes in soil moisture.The increased CO_2 concentration in the atmosphere is among the factors that affect global warming and climate change and cause greenhouse effect; the increase in CO_2 concentration stems from refinery industry activity and vehicles emissions [Fathurrahman et al. 2016]. The increase in CO_2 started in the Industrial Revolution; it resulted from increased activity from energy derived from coal, gas and crude oil [Keay 2007; Wright, Boorse 2011]. The primary cause of climate change is the increase in CO_2 concentrations in the atmosphere, which can have a negative impact on plant growth or production. Recent research has shown that higher CO_2 levels in a controlled environment, like greenhouses, can increase yields for C_3 plants by 17–29% and C4 plants by 6%–10% [Kimball 1983; Baker, Allen 1993]. In the case of a field where canopy size, structure and climatic conditions also affect water use, low CO_2 concentrations would not be enough to ensure that this area used less water [Meinzer et al.1997].

The addition of CO_2 causes a negligible increase in growth and production in some experiments, i.e C_3 soybean *(Glycine max*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) under the free-air CO_2 enrichment system [Ainsworth, Long 2005]. In order to increase the growth of many crops, it has been reported that the photosynthesis process has been improved with an increase in the concentration of CO_2 [Thongbai et al. 2010].

The Albizia saman is also referred to as a rain tree, given its canopy's ease of draining water from the soil. Either by increasing atmospheric CO_2 or reducing carbon supplies, such as forests, human activities have a direct influence on the carbon cycle. A strategy like forest management or non agriculture land protection that enables it to absorb CO_2 should be pursued in order to reduce greenhouse gas emissions, such as forest management and non-agricultural land protection that act as a net absorber of CO_2 as such, much CO_2 will be absorbed from the atmosphere and stored in plants, rather than being released. Investigations were carried out to understand its development and physiological of correlation with the ambient CO_2 and elevated CO_2 condition. In this study, rain tree was planted with two models, namely, ambient CO_2 model and elevated CO_2 model in a greenhouse. Phenotypical morphological traits, such as leaf area and biomass of roots, were studied. The purpose of this research was also to study the physiological characteristics of rain tree by determining its photosynthesis, stomata conductance, internal CO_2, respiration and water use efficiency (WUE).

MATERIALS AND METHODS

Seedling preparation and growth measurement

Sixty 1-week old rain tree seedlings were grown in polybags (25 cm × 30 cm) with a growth medium of topsoil podzolic type mixed with organic fertiliser at a ratio of 4:1. The NPK fertiliser (15:15:15) was applied at a low dose of 5 g/polybag/month, and the seedlings were sufficiently watered. In the automated CO_2 greenhouse system, thirty seedlings were exposed to an increased level of 800 μmol mol^{-1}. These seedlings have been exposed daily for two hours from 9:00 am to 11:00 am o'clock, when photosynthesis is expected to be optimum in a controlled trial. Outside the glasshouse with ambient CO_2 concentrations of 400 mol mol^{-1}, similar number of seedlings aged between 1 and 2 years were exposed. After 14 days of treatment, the plants were first observed and weekly observations were made for 16 weeks. The plant growth parameters that were measured and counted included leaf area and root biomass. Leaf area was measured by collecting the fresh leaves of *A. saman*. These leaves were subjected to the principal sample maintainability of CO_2 (800 ppm) and principal escort CO_2 (400 ppm). A total of 30 leaves were obtained at random from each treatment. Leaf sampling was done on the basis of leaf age. The number of whole leaves was 630 strands, whereas that of the sample treatment and control was 630. The leaf area was observed for seven times and used as a data retrieval end. Image-J software was used for digital image processing by investigators at the Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA. Image-J is extensively used in digital image analysis in the field of health and biology. ImageJ has been also used for a variety of specific purposes, such as measuring the leaf area and height of a subject in agricultural studies.

Photosynthesis, stomata conductance, internal CO_2, respiration and WUE were analysed using Licor 6400 XT. For each data, a statistical analysis using SAS version 9.1 and min separation has been carried out in order to test the different parameters with T-Test at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of increase in carbon dioxide concentration to leaf area

The increased carbon dioxide concentrations had a positive effect on leaf area as shown in Figure 1. The leaf of the treated samples (800 ppm) was 13.94% higher than that of the control samples (400 ppm). Thus, high CO_2 concentrations result in a large leaf area at 2, 4, 6, 8, 10, 12 and 14 weeks after treatment.

The tree reaction to the outside CO_2 can be controlled by a steady increase of carbon dioxide concentration from the environment [Farquhar, Caemmerer, 1982; Franks 2013]. Refund of excess nitrogen in the Rubisco enzyme can improve the efficiency of N use at high CO_2 concentrations [Parry et al. 2003; Ainsworth, Rogers 2007]. The increase in N content can affect plant photosynthesis through chlorophyll content and photosynthetic enzymes. As the N content of leaves increases, photosynthates will rise, and vice versa. The increase in N compounds increase the green colour of the leaves and promote stem and leaf

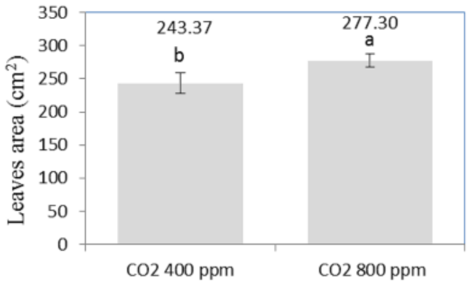

Figure 1. Leaf area index (cm^2) *A. saman* control (400 ppm) and treatment (800 ppm). The lowercase a and b are significant (*t*-test p <0.05)

growth [Fathurrahman et al. 2015]. N plays a role in protein and enzyme syntheses. Rubisco acts as a catalyst in setting the CO_2 needed for photosynthesis [Schaffer et al. 1999].

Global warming and climate change are mainly caused by an increase in concentrations of CO_2 in the atmosphere. This increase could inhibit or stimulate growth and tree production. CO_2 concentration increases in a controlled environment, such as in the greenhouse, and increases the yield of C3 and C4 plants by 17–29% and 6–10%, respectively [(Kimball 1983; Baker, Allen 1993]. The rapid growth of *A. saman* can have a positive effect on the environment, as it can reduce the temperature by 3–4 °C [Dahlan 2013]. In addition to micro temperature changes, it also has positive effects on microorganisms, which produce biomass. *A. saman* produces humus through decomposition and is used as media for the growth of soil microorganisms [Staples, Elevitch 2006]. Microorganisms in the soil have a good ability to absorb CO_2 and other harmful gases in the air. The tree is also reported to have a high ability to grow on barren land and poor soil. Through the symbiosis with rhizobium bacteria that play a role in absorbing nitrogen, *A. saman* can help bind N from the air and supply plants with nutrients.

Ratio of canopy and root on wet and dry samples

The data on the ratio of canopy and roots on wet and dry samples are shown in Figure 2. The ratio of the canopy and root in the wet sample of *A. saman* control sample (400 ppm of CO_2) was 9.06. The canopy and root ratio on the dried control sample (400 ppm of CO2) was 9.7. The proportion of the canopy and root on wet and dry samples was significant (p<0.05). The ratio of the canopy and root samples of in the wet treatment (800 ppm of CO_2) was 10:12. The ratio of the canopy and root dry sample treatment (800 ppm of CO_2) was 16:06, and the proportion of the canopy and root on wet and dry samples was significant (p <0.05). The ratio of the canopy and root wet and dry samples in the treatment (800 ppm of CO_2) is higher than the ratio of the canopy and root wet and dry samples in the control sample (400 ppm of CO_2).

The low root biomass from the canopy in the control and sample may be due to good root development that affects the root and canopy growth. Suitable root growth and nutrient absorption positively affect *A. saman* nutrition and stimulate the fast growth and development of the canopy. Bolinder et al. [2002] showed that the ratio of the canopy and roots can be affected by environmental and climatic conditions. Plants generally

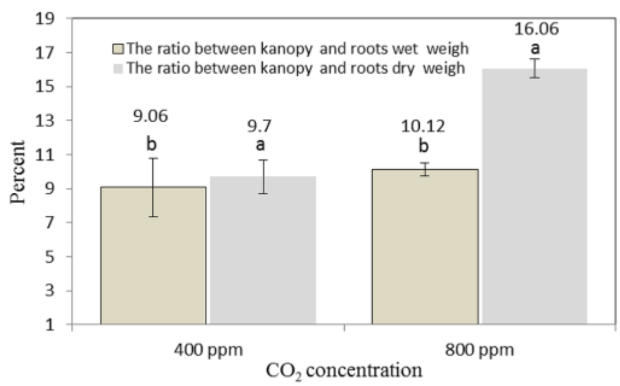

Figure 2. Ratio of canopy and root samples of wet and dry *A. saman* sample of the control (400 ppm of CO_2) and (800 ppm of CO_2). Lower case a and b indicate significance level (*t*-test, p <0.05)

store more food reserves in the trunk than in the roots. But in some plants, however, it is unlikely that an increase of CO_2 will have a significant effect on plant biomass, such as *Quercus acutissima* and *Rhynchophylla fraxinus*; instead, it can even decrease specific leaf area [Cha et al. 2017]. Other studies showed that elevated CO2 concentration stimulates photosynthesis, increases plant production and increases the amount of carbon stored in the canopy and roots [Reverchon, 2012].

The increase of CO_2 in plants, such as *Pubescens betula, Fraxinus excelsior* and *Platanoides acer*, results in low nitrogen levels in the tissues, resulting in the increase in leaf structural and non-structural carbon [Cotrufo et al. 1994]. Changes in plant tissues affect decomposition and mineral formation [Cornwell et al. 2008] by changing the parameters of quality compost, such as C/N ratio and lignin/N ratio [Taylor et al. 1989]. Changes in plant growth responses due to higher concentration of CO_2 depend on the variety's growth or ability to grow, as well as the development strategy of the species, such as the creation of sink current for additional carbon [Reich et al. 2014; Lopes et al. 2015]. In the long term, the growth of trees under high CO_2 conditions can help improve nitrogen content, carbohydrate transport and canopy or root alteration. However, whether the mechanism of the relationship between roots and canopy grown under low CO_2 conditions can optimise coordination and stimulate root growth after long-term exposure to high CO_2 remains unknown.

Photosynthesis rate

Photosynthetically active radiation flux density was in accordance with the method of Haniff [2006] and 1000 umol was used to measure the rate of photosynthesis in the leaves. The photosynthesis rates were analysed six times. Increasing CO_2 concentration led to different results for all ages between treatment (800 ppm of CO2) and control (400 ppm CO_2) as shown in Figure 3. The average reading initial observations of the leaf photosynthetic rate for 15 days was 3.03 and 1.77 μM CO2 $m^{-2}s^{-1}$ for the treatment and control, respectively. The increased photosynthesis rate is in line with the ageing of the leaves on either treatment or control samples. The maximum reading value increase was obtained in the 45-day-old leaves with a reading of 9.78–6.30 μM CO_2 $m^{-2}s^{-1}$. The highest rate of leaf photosynthesis was obtained in the 45-day-old leaves because of the physiology, quality and quantity of the cells that are directly related to the maximum photosynthetic activity. The reading decreased with increasing leaf age (60, 75 and 90 days). The leaf photosynthesis reading was higher at 90 days than at 15 days. Thus, the photosynthesis rate is expected to be a special feature of *A. saman* tree.

Photosynthetically active radiation flux density, such as 1000 umol, was used to measure the rate of photosynthesis in the leaves. The photosynthesis rates were analysed for six times, and increasing CO_2 concentration was different between treatment (800 ppm of CO_2) and control (400 ppm of CO_2) for all ages as shown in Figure 3. The average reading initial observations of leaf photosynthetic rate in 15 days was 3.03 and 1.77 μM CO_2 $m^{-2}s^{-1}$ for treatment and control, respectively. The rates of photosynthesis increased with the ageing of the leaves on the control (400 ppm of CO_2) and treatment (800 ppm of CO_2). The photosynthesis rate decreased after the optimum

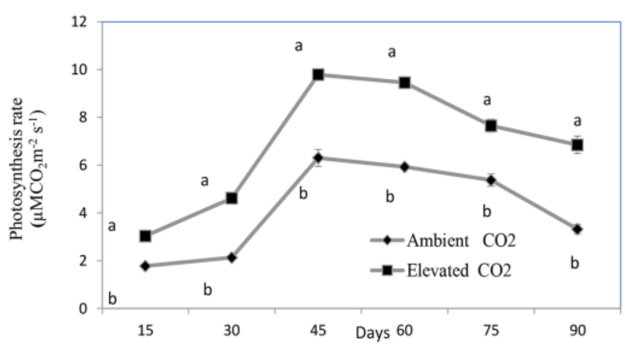

Figure 3. Relative effect on the rate of photosynthesis of *A. saman* control samples (400 ppm of CO_2) and treated samples (800 ppm of CO_2). Lower case a and b on the same day indicate significance level (*t*-test $p < 0.05$)

level with continuous increase in tree age. Photosynthetic activity may vary between plant species. However, the accumulation of carbon in plants, such as lettuce and spinach, does not increase at high CO_2 concentrations.

High CO_2 concentrations have adverse effects on the nutritional quality of vegetables (e.g. nitrogen, phosphorus, potassium, sulphur, magnesium, copper and zinc in lettuce) that are lower compared with ambient CO_2 [Giri, Rajasheka 2016]. Biomass and yield are expected to be increased due to higher levels of CO_2 [Prior et al. 2011; Azam et al. 2013], as it increases the photosynthetic rate in plants [Ainsworth, Long, 2005]. However, an increase in photosynthetic activity occurs within a short period and is not maintained under CO2 exposure. Plants have adapted to high levels of CO_2 and are less exposed to ambient concentrations. Being subjected to elevated CO2 for a long time leads to the accumulation of starch grains in the chloroplast thylakoid, leading to changes in the plant. A high rate of photosynthesis in plants grown under elevated CO2 conditions is accompanied by an increase in the number of mitochondria to meet the cellular energy demand [Sharma, Bhatnagar 2014].

Increased CO_2 concentrations can significantly increase the cross sectional area of the mesophyll in leaves to increase photosynthesis [Pritchard et al. 1999]. In trees species, a marked increase of the thickness of fence parenchyma has been observed, such as birch and aspen, which grow and thrive in environments with high CO_2 concentrations [Wustman et al. 2001].

The results of other studies on C4 plants showed that their growth is impaired by elevated CO_2 levels, which may lead to changes in Rubisco enzyme, thereby interfering with photosynthesis efficiency. Mesophyll, which is a surface-active photosynthesis layer, is the most affected by high CO_2 concentrations. However, the magnitude of the reaction may vary depending on the species of plants; several reasons for these phenomenon, including the factors involved, have been put forward by scientists [Sharma, Bhatnagar 2014].

Stomatal conductance

Increasing CO_2 concentration varies at all leaf ages in the control (400 ppm of CO_2) and treatment (800 ppm of CO_2) as shown in Figure 4. The average readings of the stomatal conductance of leaves aged 15 days during the initial observation were 68.21 and 90.25 mM $m^{-2}s^{-1}$, respectively, for the control and treatment groups.

The increase in stomatal conductance is in line with the ageing of the leaves in the treatment and control samples. The maximum stomatal conductance value was on day 60 with a reading of 244.55 mM $m^{-2}s^{-1}$ in the treatment sample and 207.60 mM CO_2 $m^{-2}s^{-1}$ in the control sample. The maximum stomatal conductance reading was recorded on leaves aged 60 days. The stomatal conductance reading decreased at 75 and 90 days of observation. The decline in stomatal conductance between the two samples was the same.

The increase in stomatal conductance is directly proportional to the increase in the age of leaves on the treatment (800 ppm of CO_2) and control sample (400 ppm of CO_2). The control and patterns at the point of maximum image contrast with the pattern of photosynthesis rates. Monda et al. (2016) found that CO_2 enrichment increases stomatal activities. However, few studies have

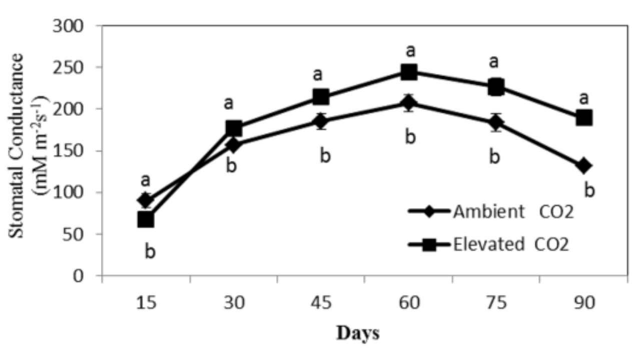

Figure 4. Effect of stomatal conductance on the control (400 ppm of CO_2) and treatment (800 ppm of CO_2). Lowercase a and b on the same day indicate significance levels (*t*-test, $p<0.05$)

reported the reduction in stomatal density [Lin et al. 2001; Teng et al. 2009] and stomatal conductance [Medlyn et al. 2001; Gao et al. 2015] when the CO_2 concentration is increased.

Increasing the size of the stomata can change the ability and structure of the stomata. Because of the low ratio between the surface area and the amount needed for solute transport to move CO_2 and O_2 the large stomata will open or close more slowly than the smaller ones. [Hetherington, Woodward 2003; Drake et al. 2013].

The size of the stomata in many Leguminosae species (e.g. *Canavalia ensiformis* or broad beans and *Arabidopsis thaliana*) is often not effective in achieving a significant change due to the slow solute transport. The movement of the solute is slow due to the low ratio of the membrane surface area and the amount of solute [Lawson, Blatt, 2014]. This type of cell stomata is large, and the cell wall thickness increases the size of the cell [John et al. 2013]. If the stomata's large cell walls are thick, the opening of stomatal aperture was smaller under the same turgor pressure.

Internal CO2 content

The increasing internal CO_2 concentration between the treatment (800 ppm of CO_2) and the control (400 ppm of CO_2) varies in terms of leaf age (Figure 5). The reading of the sample treatment for all observations is lower. The average initial observations of the internal CO_2 content of leaves aged 15 days are 253 and 310 ppm. The internal CO_2 content increased with increasing leaf age in the treatment and control. The maximum reading value showed increased internal CO_2 content in leaves older 45 days (404 and 479 ppm). The reading decreased at 60, 75 and 90 days. The increase in the internal CO2 content was amended in accordance with the age of the leaf. The readings between the maximum internal CO_2 content between the treatment and control differed. The reduction of the internal CO_2 in plant cells is correlated with photosynthesis. When a high rate of photosynthesis is reached, the concentration of CO_2 in leaf shall be reduced due to its use as an essential ingredient for photosynthesis. Concentrations of CO_2 in rice plants with *Trichoderma* spp. significantly decreases and the treatment shows higher growth than the control sample. Stomata are complex. Their opening and closing depend on light intensity and internal CO_2 concentration in the leaves [Ainsworth, Rogers 2007]. The time of maximal plant development when vegetation absorbed the most carbon dioxide, but this was already noticeable [Czubaszek 2019]. Plant growth was not only influenced by climatic factors, including CO_2 concentrations. Mutagens can also have an effect because there are changes in genotypic characteristics in plants early in the formation of vegetation [Fathurrahman 2023].

Transpiration rate

The increase in the rate of transpiration in CO_2 concentrations in the sample (800 ppm of CO_2) and control (400 ppm of CO_2) was different unless the leaf age was 30 days (Figure 6). The average initial reading of leaf transpiration rate at 15 days was 0.37 and 0.1 mM H_2O $m^{-2}s^{-1}$.

The further decline in the rate of transpiration occurred after 30 days in the treatment and control, and this reading is the minimum age compared with other leaves. Transpiration rate began

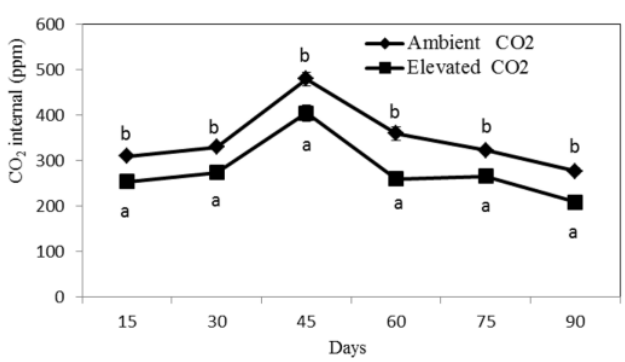

Figure 5. Effects of internal CO_2 concentration on the control (400 ppm of CO_2) and treatment (800 ppm of CO_2) based on the age of the leaf. Lowercase a and b on the same day indicate significant levels (*t*-test p <0.05)

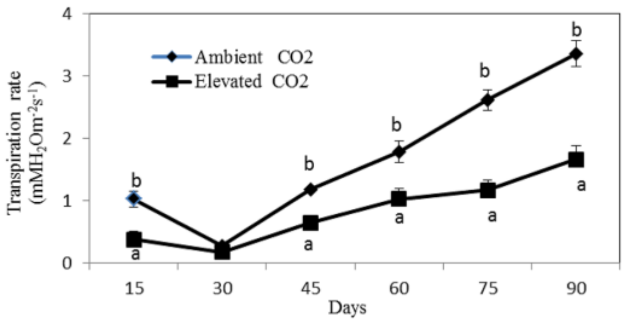

Figure 6. Effects of transpiration rates on the control sample (400 ppm of CO_2) and treatment (800 ppm of CO_2). Lowercase a and b on the same day indicate significance levels (*t*-test p <0.05)

to increase on days 45, 60, 75 and 90 for the two samples of leaves. The highest transpiration rate was 1.66 and 3.36 mM H_2O $m^{-2}s^{-1}$, and the reading was expected to increase with increasing leaf age. The high transpiration reading showed that water evaporation was high and water wastage occurred. Growth of cells is the reason for increased and reduced transpiration rates in leaves. If the reading is high, the transpiration process of catabolism in leaf mesophyll cells increases the amount of water used in the plant. The increased concentration of CO_2 is having a negative impact on the growth and development of *A. thaliana*, decreasing its transpiration rate.

Given that high CO_2 concentrations can reduce transpiration, the situation may be beneficial in mitigating the effects of drought, and photosynthesis may continue. The transpiration rate discouraged the partial closure of the leaf stomata guard cells. Dugas et al. [1997] found that stem flow gauge use on the blade reduces the whole plant's transpiration when the CO_2 concentration increases for soybean crop C3 (and C4 millet). The decrease in transpiration, coupled with the increase in photosynthesis, can improve WUE [Baker et al. 1990a].

WUE

The analysis of WUE suggests that increased CO2 concentration varies between the treatment sample (800 ppm) and the control sample (400 ppm) (Fig. 7). After initial observation, the average readings of the WUE of leaf samples aged 15 days were 8.02 and 1.72 mol/mmol respectively.

Further increase in WUE occurred on leaves aged 30 days, in which the reading of the treatment sample CO2 was 35.96 mol/mmol compared

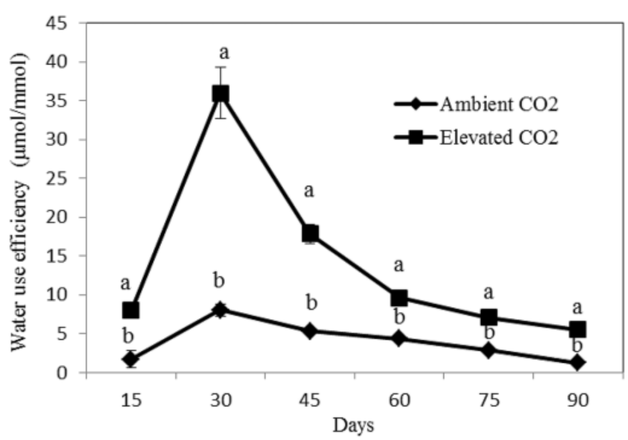

Figure 7. WUE of the control sample (400 ppm of CO_2) and treatment (800 ppm of CO_2). Lowercase a and b on the same day indicate significance levels (*t*-test p <0.05)

with the CO_2 reading of 8.04 mol/mmol in the control sample. Reading on the 30-day-old leaves is the highest. The WUE in the treatment after 45 days is more likely to decrease than in the control. The decline slowed down in the sample treatment from day 60 to day 90. In the sample CO_2 treatment, WUE decreased relatively slowly from 30 days to 90 days.

WUE in growing plants should be considered to prevent growth disorders. The WUE of treatment (800 ppm of CO_2) and control (400 ppm of CO2) varied. WUE increases at elevated CO_2 [Wu et al. 2004]. Various experiments have demonstrated through C3 species that increased CO_2 increased the rate of photosynthesis, plant growth and WUE [Pleijel et al. 2000; Loladze 2002].The study of Wu et al. [2004] showed that higher CO_2 contributes to plant growth, yields and WUE whereas grain quality has decreased with high CO_2, as shown by an increase in the crude starch content along with nutrient reduction and a decrease in lysine and protein concentrations. The WUE was increased by elevated CO_2 in the study with a view to increasing plant growth and reducing water consumption. This phenomenon will be beneficial in case of a long-term need for food production, especially in areas with limited water supply.

CO_2 results in increased root length and surface area. Water intake in the root becomes easy. High CO2 levels in bentgrass increases leaf photosynthesis and stomatal conductance but reduces the rate of transpiration, contributing to the efficient use of water. Efficient use of water is especially important for the survival of plants, such as turfgrass and rice, when irrigation is limited [Burgess, Huang 2014; Doni et al. 2014].

CONCLUSION

Increasing CO_2 in the treatment sample changed the plant's leaf area index. The ratio of root wet and dry samples within the treatment sample was higher than in control, as far as canopy profile is concerned. On dry samples, the ratio of canopy to roots was greater than it was on dried specimens. Compared to the control, the ratio of the canopy and root samples treated with CO_2 was higher. Increased CO_2 concentrations affected the physiology of *A. saman* and increased the flow rate of photosynthesis and stomatal conductance in the treatment sample compared with the control. WUE of treated samples, which had low levels of CO_2 in the leaves and underwent low respiratory treatments, was higher than in the control plants.

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