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Research

Enhanced Growth Performance and Steviol Glycosides Content in *Stevia Rebaudiana* Under Elevated Carbon Dioxide

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ABSTRACT

In the current climate-changing scenario with a steadily rising CO_2 concentration, there is a chance that crop performance will be affected in terms of growth, yield, and quality. Therefore, an experiment was conducted in a glasshouse using a randomized complete block design with four replications to investigate the effect of short and long-term elevated CO_2 on growth performance and chemical markers of *Stevia rebaudiana* Bertoni. The CO_2 in the glasshouse was gradually elevated from 400 ppm to 1800 ppm weekly. The plants were exposed to elevated CO_2 for four months (T1), two months (T2), and one month (T3), while the control plants (T4) were grown under ambient CO_2 (aCO_2) levels to assess the effect of elevated atmospheric carbon dioxide (eCO_2) on stevia crop growth performance and steviol glycosides content. The number of branches per plant, plant height, number of leaves per branch, and plant biomass were found to be significantly increased under eCO_2 treatment over aCO_2 treatment. The eCO_2 increased photosynthetic rate by 46% for T1, 45% for T2, and 29% for T3 over control plants (T4) at 3rd month of planting. The enhancement in photosynthesis is attributed to an increase in stevioside; with a 33% increase for T1 28.83% for T2 and 11% for T3 over aCO_2. Similarly, the rebaudiosides A were also significantly increased by 32.8% for T1, 25% for T2, and 15% for T3 compared to the control under aCO_2. Based on our findings, we concluded that eCO_2 levels positively influenced the growth, biomass, and glycoside content by enhancing the physiological performance of *Stevia rebaudiana* Bertoni.

Key words: Climate change, rebaudioside, root fresh weight, stevioside, stomatal conductance, transpiration

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INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herbaceous species of plant that belongs to the family Asteraceae and it is from the genus stevia (Ahmad *et al*., 2020). Stevia originated in South America, but nowadays it is cultivated in several parts of the world including Europe, Asia, and even North America (Lemus-Mondaca *et al*., 2012; Ahmad *et al*., 2020). There are more than 200 species of stevia present around the world but *Stevia rebaudiana* is the only one of those 200 species which produces sweet taste. Due to its sweet taste, it is also known as honey leaf, sweet leaf, or candy leaf (Lemus-Mondaca *et al*., 2012; Shivanna *et al*., 2013). Steviol glycosides, which are non-caloric sweeteners in nature are the chemical compounds found in *Stevia rebaudiana* which is responsible for the sweetness (Madan *et al*., 2010). It has been showed by several studies that it may have beneficial effects on type II diabetes (Misra *et al*., 2011; Vazquez-Baxcajay *et al*., 2014; Ahmad *et al*., 2020). The sweetening power of stevia powder is 30 times greater while its extract is found to be 200-300 times sweeter than table sugar (Cruz, 2015). The main content found in stevia as sweeteners are stevioside and rebaudioside A, which are its focal active ingredients, and it mainly found in leaves in higher amounts than other parts of the plant (Vazquez-Baxcajay *et al*., 2014; Cruz, 2015). The leaves of stevia also contain a high amount

of phenolic compounds, chlorophylls, carotenoids, and vitamin C. It is also a good source of crude fiber, carbohydrates, protein, and minerals that promote wellness and reduce the risk of certain diseases (Khiraoui *et al*., 2017).

It is well understood that the greenhouse gas concentration has been increasing in the atmosphere since the pre-industrial revolution because of human activities. The concentration of carbon dioxide is dramatically increasing among other greenhouse gasses, due to the consumption of fossil fuel and the change in the use of land resulting in the 19th-century industrial revolution (Abzar *et al*., 2017; Dusenge *et al*., 2019; Zheng *et al*., 2019). It was reported by the Intergovernmental Panel on Climate Change (IPPC), in 2013, that the amount of CO_2 in the atmosphere has grown dramatically from 280 parts per million(ppm) (Before the industrial revolution) to over 400 ppm (Current level), growing at a rate of about 1.5 to 2.0 parts per year. The concentration of CO_2 may even surpass 1000 ppm by the end of this century (Sivaramanan, 2015; Abzar *et al*., 2018). The higher atmospheric CO_2 concentration may drastically impact the structure and function of natural and managed ecosystems (Wand *et al*., 1999; Zhang *et al*., 2011).

It has been illustrated by several scientific reports that higher CO_2 levels generally increase plant net photosynthetic rate, referred to as the "CO_2 fertilization effect" in particular for C_3 species (Lee *et al*., 2001; Morgan *et al*., 2007; Yu *et al*., 2012; Singh & Reddy, 2016). However, in contrast to the above findings some other reports have shown that the photosynthetic rate was not marginally enhanced and even dropped when the plants were exposed to long-term high CO_2 concentrations (Salvucci *et al*., 2004; Robredo & Perez-Lopez, 2010). Elevated atmospheric CO_2 concentrations typically have two immediate and transient physiological impacts on plants. First, it will increase the rate of photosynthesis in the leaves because enzymes responsible for fixing CO_2 operate more efficiently at higher CO_2 concentrations. Second, it will reduce transpiration water loss by causing partial stomatal closure. Additionally, if plants are exposed to high CO_2 concentrations for an extended period, secondary consequences can occur. These include changes in the plant's chemical content, leaf morphology, and overall architecture, as well as alterations in respiration rates (Poorter & Perez-Soba, 2002). Excessive electrons can combine with O_2 under ambient CO_2 levels to create hazardous reactive oxygen species (ROS), which can then cause photo-inhibition, particularly in plants like stevia that follow the C_3 photosynthetic pathway (Foyer & Shigeoka, 2011; Sanoubar *et al*., 2016). Extreme ROS in plants can seriously damage protein, lipids, and nucleic acids, leading to oxidative stress if photo-protective mechanisms are not present or if the decline in CO_2 assimilation is accompanied by an increase in the potency of another sink for absorbed radiation (Garcia-Plazaola *et al*., 2003; Gill & Tuteja, 2010). Several terrestrial C_3 plant species have increased ribulose biphosphate (RuBP) carboxylation by enhancing rubisco's affinity for CO_2 at the expenditure of the oxygenation process under short-term elevated CO_2 (Ibrahim & Jaafar, 2017; Walker *et al*., 2021). This increase in carboxylation will decrease photorespiration which is the cause of a decrease in photosynthesis by 20-30% ultimately increasing the net photosynthetic rate (Moroney *et al*., 2013). In addition to increased carboxylation, higher CO_2 levels often result in decreased stomatal conductance, which declines consumptive water demand to support plant development. Increased CO_2-induced decreases in photorespiration may also lessen the build-up of water, which would moderate oxidative stress and potentially shield the photosynthetic machinery (Zinta *et al*., 2014).

By increasing the photosynthetic carbon uptake, CO_2 elevation could also enhance the amounts of secondary metabolites, ultimately enhancing the plant's antioxidant activities and lowering ROS levels (Zinta *et al*., 2014). Generally, the increase in plant growth happens due to enhanced photosynthesis under elevated CO_2. Elevated levels of CO_2 can change carbon partitioning/allocation in addition to promoting photosynthesis and aboveground growth. The distribution of photosynthate belowground can be preferentially induced by increased atmospheric CO_2 and carbon supply. (Prior *et al*., 2011; Rai *et al*., 2016). Mostly the extra biomass produced by plants under elevated CO_2 is for belowground (Rogers *et al*., 1994; Prior *et al*., 2010), frequently leading to a higher ratio of roots to shoots (Rogers *et al*., 1996). This makes sense because, according to Prior *et al*. (2011), plants typically allocate photosynthate to the tissues that require it to obtain the most limiting resource water or nutrients when CO_2 levels are raised. Previous studies mainly focused on cereals crops such as wheat, rice, and barley, but comparatively less work has been carried out on stevia growth and its productivity under elevated CO_2, specifically under short-term elevated CO_2. Therefore, the growth and biomass of stevia under elevated CO_2 are the focus of this study. It has been observed that various C_3 crops promote plant growth under elevated CO_2 and, on the other hand, increase biomass by favorably impacting the plant's physiological processes. The concept of growing stevia plants under short and long-term elevated CO_2 is the new aspect of this research. In this context, a greenhouse study was conducted to investigate the effect of short and long-term elevated CO_2 on the growth performance, biomass, and chemical markers of *Stevia rebaudiana* Bertoni.

MATERIALS AND METHODS

Experimental site and planting materials

The current experiment was conducted under glasshouse conditions. The glasshouse was in Putra Agriculture Center (PAC), Universiti Putra Malaysia (UPM), Serdang, Selangor and Tenaga National Berhad Research (GHTNBR), Kawasan Institusi Penyelidikan, Jalan Ayer Itam, Kajang, Selangor. The glasshouse with elevated CO_2 was constructed in such a way that the plant can receive a 12 hr photoperiod and an average photosynthetic photon flux density of 330 μmoL m^{-2} s^{-1}. High-pressure CO_2 cylinders were used which provided 99.8% pure CO_2 and were continuously applied from 8:00 to 10:00 a.m. at two hr a day through a pressure regulator, into the fully sealed 5 m × 3.67 m greenhouses. Air sense CO_2 sensors designated to each chamber were used to measure the CO_2 concentrations during the CO_2 exposition period. The CO_2 level increased weekly from 400 ppm to a maximum of 1800 ppm with 400 ppm of increment per week. The greenhouse was equipped with the dripped fertigation for irrigation purposes. The seedlings of S*tevia rebaudiana* Bertoni were prepared from stem cutting. The seedlings of *Stevia rebaudiana* Bertoni with a height of 7-8 cm were transferred to medium containing coco-peat without soil in 16 cm × 16 cm size (16 × 16) polyethylene bags. Day and night temperatures were maintained from 27-35°C and 18-21°C, respectively. The relative humidity was maintained from 50% to 60%. To allow the plant to grow under their natural environment stevia plants were grown under 50-60% shade (light intensity 225±50 μmoL m^{-2} s^{-1}) using black netting.

Experimental setup

The experiment was carried out in a randomized complete block design (RCBD) with four replications for each treatment. The plants exposed to elevated levels of CO_2 for four months until the final harvest was considered as (T1). Plants exposed to elevated CO_2 levels for two months were named (T2), and after two months, the plants were transferred to a normal glasshouse and kept under ambient CO_2 levels for another two months until final harvest. For (T3) the plants were grown under elevated CO_2 for one month. The (T3) treated plants were transferred to a normal glass house under ambient CO_2 level after one month and were kept there until final harvest at the fourth month of transplanting. For control (T4), the plants were not exposed to elevated CO_2 levels but were grown under ambient CO_2 concentration for four months until the final harvest.

Growth components measurement

All the growth components for our stevia plants like plant height, number of branches per plant, and number of leaves per branch were measured monthly until four months after transplanting. To measure plant height, the plant was selected from ground level to the tip of the longest leaf while a number of branches and leaf number were visually counted for each treatment and control following the method from Wood and Roper, (2000).

Measurements of physiological components

The physiology-related components from our stevia plants were measured at 60 and 120 days of transplanting. The measurements of the physiological parameters namely, photosynthesis (µmoL CO_2 m^{-2} s^{-1}), leaf stomatal conductance (µmoL CO_2 m^{-2} s^{-1}), transpiration rate (µmoL H_2O m^{-2} s^{-1}), and intercellular CO_2 (µmoL CO_2 m^{-2} s^{-1}) were recorded in the morning (9:00 & 10:00 am) of a sunny day using a portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, NE, USA) adjusted at 400 µmoL moL^{-1} CO_2, 1000 µmoL m^{-2} s^{-1} irradiance and saturated light condition (solar radiation > 1200 µmoL. m^{-2} s^{-1}) on the abaxial surface of a fully expanded leaf from the top of the plant (Doni *et al*., 2018).

Biomass analysis

Biomass was evaluated at two harvesting frequencies. The plants grown in one m^2 area were cut manually for biomass analysis. The plants were carefully separated from the soil to measure plant fresh and root fresh weights. The plant shoot and root fresh weight was measured in kilograms (kg). For measuring dry weight for canopy and roots (biomass), the plants were dried in the oven at 70℃ for 48 hr, and canopy biomass and root biomass were measured in kilograms (kg). The total shoot and root fresh and dry weight were converted into ton/ha using the following formula suggested by (Morelli & Capurso, 2012).

(t/ha = (kg/m (obtained weight/1000) * 10000) - Equation 1

In the above formula, the biomass obtained in kg/m was divided by 1000 and the value was multiplied by 10000 to convert it into tons per hectare (t/ha).

Extraction and analysis of steviol glycosides

To extract steviol glycosides, healthy and fresh leaves were collected from the plants and washed and cleaned under running tap water. After washing, the leaves were dried in a hot air oven at 40°C till they reached a consistent weight. Afterward, a filtrate from the leaves was made, and the SGs (Steviol Glycosides) were measured using a Waters high-performance liquid chromatography system (996 Photodiode Array Detector). 10 mL of methanol was used to soak 100 mg of ground leaf sample for an overnight period to extract stevioside and Reb-A. After that, the mixture was then filtered through filter paper. N-hexane was used to help in the extraction of the fats from the sample after the filtrate was dried under low pressure. After the process of fat extraction, the extract was dissolved in 10 mL of HPLC-graded acetonitrile and water (8:2) mobile phase and afterward filtered through a microfilter with a pore diameter of 0.45 m. Stevioside and Reb-A were quantified using standard samples to create standard curves. To maintain the rest of the working conditions of the instrument, the methods from Pal *et al*. (2015b) were followed.

Statistical analysis

To statistically analyze the data one-way analysis of variance (ANOVA) of the SAS 9.4 was used. Means from all the treatments were separated using Fisher's protected Least Significance Difference (LSD) mean separation at a 5% probability level.

RESULTS

Growth attribute

Our results showed that plant height, number of branches, and number of leaves per plant were significantly ($P \leq 0.05$) enhanced with CO_2 elevation. Figure 1a illustrates that plant height was significantly increased by exposing the plants to elevated CO_2 by comparing to ambient CO_2 level. After one month of transplanting, no significant differences were seen among all the CO_2-treated plants, however, the results CO2 CO2-treated plants were significantly higher than those plants that were grown under ambient CO_2 as a control. At month four, the maximum plant height was produced by those plants that were exposed to elevated CO_2 for the entire growth cycle (T1) (87.94 cm), which was 20% higher than control plants (T4) (70.19 cm) under ambient CO_2 level.

The results summarized in (Figure 1b) showed that a similar pattern to plant height was seen for the number of branches as well. The plants grown under elevated CO_2 for one month (T3), two months (T2), and four months (T1) produced a significantly higher number of branches compared with non-CO_2-treated plants. However, in the first month after transplanting, no significant differences were seen among all the CO_2-treated plants. At 3rd month of growth, the maximum number of branches per plant was recorded for plants treated with elevated CO_2 for four months (T1) (23 branches plant $^{-1}$), 21.7% higher than T2 and T3 and 34.5% higher than control. No significant difference was seen among (T2) and (T3) plants.

Following the same pattern to plant height and number of branches, significant differences in number of leaves per branch were seen among stevia plants grown under elevated CO_2 concentration by comparing the results to those plants which were grown under ambient CO_2 (Figure 1c). At 3rd month of planting, the leaves number per plants for T1 plants was recorded 16.9% higher than control plants (T4). No difference was seen in T2 plants, while T3 plants showed only a 1.2% enhancement in leaves number compared to control. The highest leaves number per branch was recorded for CO_2-treated plants at the early growth stage, while in 4th month of planting, the plants treated with elevated CO_2 for two months (T2) and the plants grown under ambient CO_2 (T4) showed a decline in number of leaves.

Physiological parameters of leaves

All physiological parameters, including photosynthesis, stomatal conductance, transpiration rate, and internal CO_2 were positively influenced by CO_2 elevation compared to control plants grown as a control under ambient CO_2 levels. Net photosynthesis rates were significantly higher in plants grown under elevated CO2. The significant difference in photosynthesis rates between treatment and control for short- and long-term elevated CO2 has been seen. In 3rd month (Table 1), the maximum average photosynthetic rate was recorded for (T1) plants, which was 46% higher than control, followed by (T2), with 45% and T3 treated plant showed a 29% increment while comparing with control plants (T4). At

4th month of transplanting, similar patterns were seen for CO_2-treated and non-treated plants. Similar to month three, the maximum average photosynthesis rate was shown by (T1) plants with a 48% increment to control. Plants treated by T2 showed a 29.4% increment, while T3-treated plants showed 17.4% enhancement compared with control (Table 2). Overall, the trend was the same in the 3rd and 4th months of growth. However, the net photosynthesis rate was lower in the 4th month for T2, T3, and even control (T4) plants compared with the 3rd month. T1 was seen with a higher photosynthesis rate at month four as well.

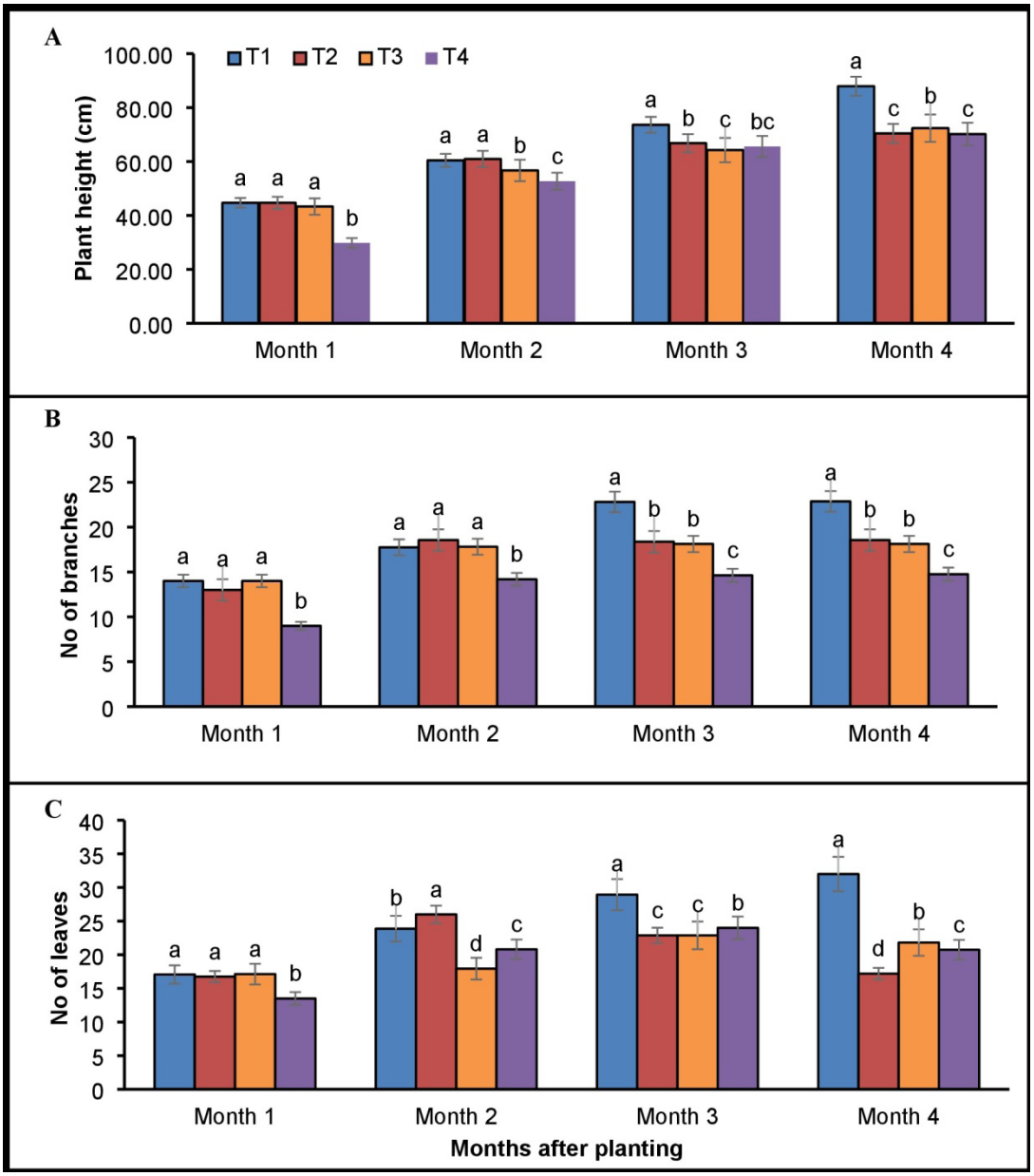

Fig. 1. Plant height (a), Number of branches per plant (b) Number of leaves per branch (c) of stevia at different months as influenced by elevated and ambient CO_2 levels.

Bars represent means and error bars show standard errors. This means that the same letters within a month are not significantly different at $p \leq 0.05$ according to the LSD test.

Notes: T1: Plants exposed to elevated CO_2 for one month. T2: Plants exposed to elevated CO_2 for two months. T3: Plants exposed to elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control.

Stevia plants grown under elevated CO_2 show a decrease in stomatal conductance compared to plants grown under ambient CO_2 concentration. We found from our current research that the stomatal conductance was decreased by 68% in (T1) plants, 40% in (T2) plants while the stomatal conductance was only reduced by 12% in (T3) treated by comparing with control (T4) at 3rd month after planting (Table 1). At fourth month of planting the stomatal conductance were lower down by 79% for T1 treated

plants, 47% for T2 treated plants and 40% for T3 treated plants by comparing with control plants (T4) under ambient CO_2 (Table 2).

Following a similar pattern to stomatal conductance, the transpiration rate was also found to be low for CO_2-treated plants compared with plants grown under ambient CO_2. Our results for the current study showed that the transpiration rate was decreased by 51% in (T1) plants followed by (T2) plants by 39%, and 17% decreases in transpiration were recorded for T3 treated plants by comparing with control at 3rd month (Table 1). At 4th of transplanting 30% decrease were recorded for T1, 19% for T2 and 12% for T3 (Table 2) treated plants than control plants (T4). Similarly, the level of internal CO_2 was found to be high for CO_2-treated plants. The plants grown under 100% CO_2 (T1) had 15% higher internal CO_2 than control plants. Internal CO_2 for (T2) plants was observed with 12% increment than control, while no difference was seen between (T3) plants and control at 3rd month of growth (Table 1). At month four, (T1) plants showed 15% increment in internal CO_2 followed by (T2) 08% while T3 treated plants did not show any significant to control plants (Table 2).

Table 1. Stevia plant physiological parameters at 3rd month as influenced by elevated and ambient CO_2 level

Treatments	Physiological data in 3rd month			
	PR (μmoL CO_2 m^{-2} s^{-1})	SC (μmoL CO_2 m^{-2} s^{-1})	TR (μmoL H_2O m^{-2} s^{-1})	Ci (μmoL CO_2 m^{-2} s^{-1})
T1	9.7 a	0.265 d	2.23 c	279.34 a
T2	9.6 a	0.502 c	2.79 c	270.39 b
T3	7.4 b	0.739 b	3.79 b	246.36 c
T4	5.2 c	0.843 a	4.62 a	237.26 d

Means with the same letter in a column are not significantly different According to protected Least Significance Difference (LSD) mean separation at a 5% probability level.

Footnotes: T1: Plants grown under elevated CO_2 for one month. T2: Plants grown under elevated CO_2 for two months. T3: Plants grown under elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control.

Table 2. Stevia plant physiological parameters at 4thmonth as influenced by elevated and ambient CO_2 level

Treatments	Physiological data at 4th month			
	PR (μmoL CO_2 m^{-2} s^{-1})	SC (μmoL CO_2 m^{-2} s^{-1})	TR (μmoL H_2O m^{-2} s^{-1})	Ci (μmoL CO_2 m^{-2} s^{-1})
T1	9.9 a	0.116 d	1.33 d	276.33 a
T2	7.2 b	0.303 c	1.55 c	258.66 b
T3	6.2 c	0.342 b	1.68 b	240.33 c
T4	5.1 d	0.573 a	1.92 a	233.66 d

Means with the same letter in a column are not significantly different According to protected Least Significance Difference (LSD) mean separation at a 5% probability level.

Footnotes: T1: Plants grown under elevated CO_2 for one month. T2: Plants grown under elevated CO_2 for two months. T3: Plants grown under elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control.

Biomass analysis

The elevated CO_2 concentration positively influenced the plant's performance in terms of biomass. Table 3 shows that fresh shoot weight obtained from (T1) plants was 8.16 ton/ha, which was 33% higher than control, followed by (T3) plants with a 12% increment to control. T2-treated plants did not show any increment by comparing with control on 3rd month of growth. At month four, the maximum shoot fresh weight was obtained from (T1) plants (10.16 ton/ha), which was 37.7% higher than the control, followed by (T3) plants with a 15% increment to control, while no enhancement was seen for (T2) plants to control. The shoot dry weight was found to have a similar trend. The (T1) plants showed 47% higher performance, followed by (T3) plants 35% higher than the control in 3rd month (Table 3). However, the performance for (T2) plants was recorded at 16% lower than control at month 3rd. In 4th month of growth, the total dry weight obtained from (T1) was 3.3 tons/ha, significantly higher than control. The minimum weight for root dry weight was recorded for (T2) plants which were significantly lower than control while (T3) plants were found with a 14% increment than control in terms of root dry weight at the 4th month of transplanting (Table 4).

The results, summarised in Tables 3 and 4, illustrate that plants under elevated CO_2 had higher values in root fresh and dry weight than those grown under ambient levels of CO_2 as a control. After three months of transplanting, the maximum value for root fresh weight was recorded for (T1) plants, which was 43% higher than the control. (T2) and (T3) plants show 30% increment to control. No significant difference was seen among (T2) and (T3) plants in terms of root fresh weight at 3rd month of their growth (Table 3). In 4th month of planting, (T1) plants were found with an 18.5% increment, while no significant difference was seen for (T2) and (T3) plants when the results were compared with control

(T4) (Table 4). Root dry weight followed a similar pattern to root fresh weight. The highest value of root dry weight was recorded for (T1) treated plants, which was recorded 65% higher than control, followed by T3 (56%), and 53% highest value was found for (T2) treated plants by comparing with control at 3rd month of growth. At month four, the maximum average value for root dry weight was recorded for (T1) plants, which was 14% higher than control, followed by no significant difference among (T2), (T3), and control (T4).

Table 3. Effect of elevated CO_2 on biomass (fresh and dry weight) on stevia at 3rd month of planting

Treatments	Biomass data at 3rd month			
	SFW (ton/ha)	SDW (ton/ha)	RFW (ton/ha)	RDW (ton/ha)
T1	8.16 a	2.66 a	1.06 a	0.63 a
T2	5.33 c	1.25 c	0.86 b	0.48 b
T3	6.16 b	2.31 b	0.87 b	0.51 b
T4	5.41 c	1.49 c	0.60 c	0.22 c

Means with the same letter are not significantly different According to protected Least Significance Difference (LSD) mean separation is at a 5% probability level.

Footnotes: T1: Plants grown under elevated CO_2 for one month. T2: Plants grown under elevated CO_2 for two months. T3: Plants grown under elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control

Table 4. Effect of elevated CO_2 on biomass (fresh and dry weight) on stevia at 3rd month of planting

Treatments	Biomass data at 3rd month			
	SFW (ton/ha)	SDW (ton/ha)	RFW (ton/ha)	RDW (ton/ha)
T1	10.16 a	3.33 a	1.24 a	0.77 a
T2	5.83 c	1.73 c	1.01 b	0.66 b
T3	6.83 b	2.73 b	1.00 b	0.65 b
T4	6.33 bc	2.35 c	1.01 b	0.66

Means with the same letter are not significantly different According to protected Least Significance Difference (LSD) mean separation is at a 5% probability level.

Footnotes: T1: Plants grown under elevated CO_2 for one month. T2: Plants grown under elevated CO_2 for two months. T3: Plants grown under elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control

The subsequent effect of elevated CO_2 on glycosides in stevia

The results from the current showed that the elevated CO_2 has positively influenced the glycoside content in stevia plants. Glycosides (steviosides & rebaudiosides a) were found in greater amounts in CO_2-treated plants than in plants grown under ambient CO_2 as a control. The highest amount of stevioside was found in (T1) plants, which was 33% greater than control, followed by (T2) plants (28.83%). In comparison, T3-treated plants showed 11% higher values than control (Figure 2). A similar pattern was followed by rebaudiosides A, where the maximum average values were recorded for (T1) plants 32.75% higher than control, followed by (T2) plants with 25% increment. In comparison, T3-treated plants were found with 15% higher values than the control (Figure 3).

DISCUSSION

Stevia plants follow C_3 photosynthetic pathway and could be influenced by atmospheric CO_2 levels. The growth and development of plants are directly and indirectly influenced by CO_2 through altering a variety of physiological processes. As we know CO_2 has a direct effect on photosynthesis and stomatal conductance which is why plant growth altered with CO_2 elevation in the atmosphere. (Seneweera & Conroy, 2005; Ainsworth *et al*., 2008; Singh & Reddy, 2016). However, besides the higher carbon supply to the growth of roots and shoots under elevated CO_2, the overall growth of plants at elevated CO_2 also depends on the post-photosynthetic process, which may alter nitrogen and carbon metabolism, cell cycle characteristics, and hormone metabolism. In our current research, we found that elevated CO_2 has significantly enhanced the overall growth pattern of our stevia plants by comparing them with plants grown under ambient CO_2 levels. The result from our current study shows similarity with Ainsworth and Long (2005), where they mention 17% growth enhancement for C_3 crops like rice, wheat, and soybean from their meta-analytic review of elevated CO_2 (475-600 ppm with ambient temperature). Being C_3 crop stevia followed a similar pattern of growth enhancement under elevated CO_2. Our current results are also similar to Degraaff *et al*. (2006) in terms of plant height, where they reported C_3 plant height enhancement by 10-12% under 700 ppm of elevated CO_2. Gene expression related to cell growth, division, and cell wall characteristics is influenced by elevated CO_2. The set of processes that occurs inside a cell before the division of the cell is known as the cell cycle. Various environmental factors,

including CO_2, influence the cell cycle (Kinsman *et al*., 1997; Dong *et al*., 2018). It has been proposed that if there is be high carbon supply under higher CO_2 concentrations in the environment it may speed up the division of the cell and meristematic tissue expansion which will promote the growth and development of the plants at early stages. (Thilakarathne *et al*., 2015). Plants have been seen to be more productive under elevated CO_2 concentration because photosynthesis depends on using sunlight's energy to synthesize sugar from CO_2 and water. In general, the overall growth performance of stevia was positively affected by both short and long-term CO_2 by comparing with plants grown under ambient CO_2 as control. However, the plants exposed to elevated CO_2 for the entire growth period showed maximum values in the number of branches per plant, plant height, and number of leaves (Figure 1a, 1b & 1c) in the 3rd and 4th months after planting. The current results also showed that plants perform better at early growth stages than in maturity. The enhancement in photosynthesis under elevated CO_2 results in higher sugar production, together with fructose, glucose, and raffinose (Watanabe *et al*., 2013; Aranjuelo *et al*., 2015). The production of these sugars in excess amounts enables the development of new sink organs, in which leaves, stems, tiller, and seeds are included. The final growth response to CO_2 is determined by these organs' developmental plasticity. However, early vegetative stages of plants exhibit significantly stronger responses to elevated CO_2 than later stages. (Seneweera *et al*., 2002). Leaky *et al*. (2009) also suggest that elevated CO_2 causes an increase in plant photosynthesis; however, the response's strength can fluctuate depending on the stage of plant development.

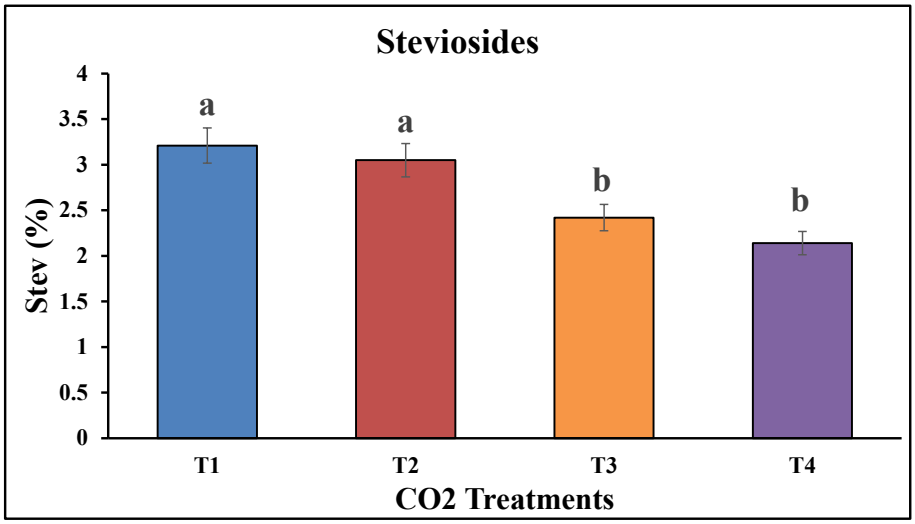

Fig. 2. Effect of elevated CO_2 treatments on stevioside content in stevia leaves

Bars represent means and error bars show standard errors. This means that the same letters within a month are not significantly different at $p \leq 0.05$ according to the LSD test.

Footnotes: T1: Plants exposed to elevated CO_2 for one month. T2: Plants exposed to elevated CO_2 for two months. T3: Plants exposed to elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control.

Carbon dioxide is playing important role in photosynthesis. Higher levels of CO_2 positively enhance photosynthetic rates, and the primary factor contributing to this increment in photosynthesis is an enhancement in carboxylation efficiency, which is comparatively low at the current level of CO_2 in the atmosphere. Therefore, if the concentration of CO_2 rises in the atmosphere, it will increase the CO_2/O_2 ratio; as a result, the rate of photorespiration will be slowed down and will promote Rubisco's carboxylation efficiency (Bowes, 1991; Chang *et al*., 2016). In our current study, we found that the plants grown under elevated CO_2 showed the highest photosynthesis rate for the entire growth cycle (T4) followed by plants exposed to elevated CO_2 for two months (T2) and one month (T1). The photosynthetic rate is positively associated with the efficiency of photosystems I and II (PSI & PSII), which also respond positively to elevated CO_2. Higher CO_2 concentration also increases the production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), which are the two essential substances needed to activate photosynthetic enzymes (Vanheerden *et al*., 2007; Zhang *et al*., 2008). The photosynthetic rate at 4th month of transplanting was significantly higher for all CO_2-treated plants compared with control plants under ambient CO_2. However, it was noticed that at month four, the photosynthesis rate was not as high as month 3rd and it was possibly due to the maturity stage of plants, as at 4th month the stevia plants were fully matured and were ready to harvest. Our

findings agree with Ainsworth and Rogers (2007), who reported a 40% enhancement in photosynthesis for their C_3 crops under elevated CO_2 of 700 ppm. Our results also show similarity to Vanderkooi *et al*. (2016) and Engineer *et al*. (2016), who reported a 40-50% enhancement in photosynthesis for C_3 crops like rice, wheat, and soybean under elevated CO_2.

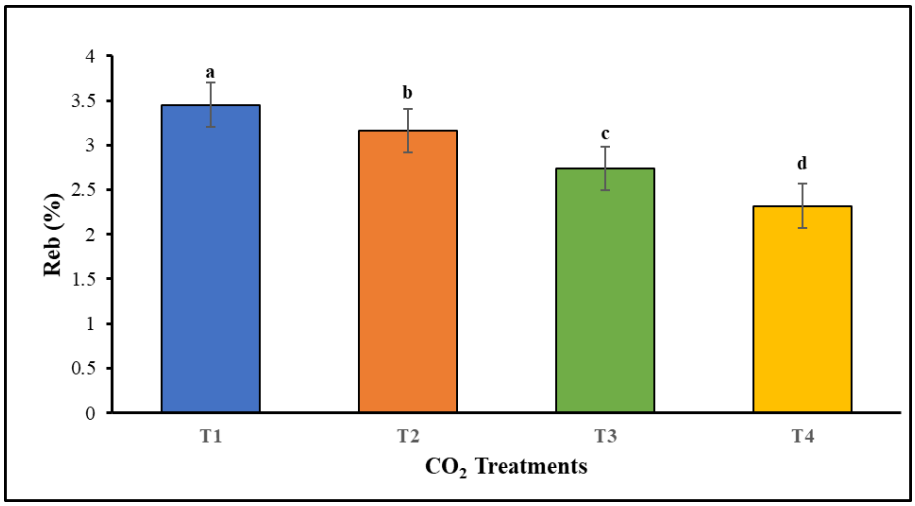

Fig. 3. Effect of elevated CO_2 treatments on rebaudioside content in stevia leaves

Bars represent means and error bars show standard errors. This means that the same letters within a month are not significantly different at $p \leq 0.05$ according to the LSD test.

Footnotes: T1: Plants exposed to elevated CO_2 for one month. T2: Plants exposed to elevated CO_2 for two months. T3: Plants exposed to elevated CO_2 for four months. T4: Plants grown under ambient CO_2 as a control.

From our current finding, we found that stevia plants grown under elevated CO_2 were seen with low stomatal conductance, transpiration rate, and intercellular CO_2 when the results were compared with those plants that were grown under elevated CO_2 concentration. It has been reported by Engineer *et al*. (2016) that higher than ambient CO_2 concentrations mediate a closer of stomatal pores in plants while low CO_2 concentrations trigger the opening of stomatal pores. Turgor pressure of the guard cell also determined the size of the stomatal aperture, which is mediated through ion concentration (Fernie *et al*., 2011). According to reports, increased CO_2 causes stomatal defects because it upsurges the activity of apparent repairing K^+ channels comparatively to inward rectifying K^+ channels. (Brearley & Blatt, 1997). Moreover, increased CO_2 causes guard cells to release Cl^- and concentrate more Ca_2+ inside of them. By depolarizing the membrane potential of guard cells, these modifications aid in the closing of stomata which is commonly happening under elevated CO_2 (Hanstein & Felle, 2002). Our current results follow a similar pattern to that of Ainsworth and Rogers (2007), where stomatal conductance was reduced by an average of 22% with CO_2 elevation in C_3 crops (rice, wheat, soybean). By diffusing down a concentration gradient from the bulk air outside to the intercellular spaces inside, carbon dioxide enters the leaves. It then diffuses to the fixation sites in the stomas' chloroplast after dissolving in the liquid on mesophyll surfaces. Since this path's center is the intercellular CO_2 concentration (Ci), it plays a central role in the availability of CO_2 for photosynthesis (Boyer, 2015). McDonald *et al*. (2002) reported decreased transpiration rate and stomatal conductance while increased internal CO_2 for C_3 crops under eCO2 which are similar to our results. Our current research finding also follows a similar trend to Vandarkoi *et al*. (2016) and Engineer *et al*. (2016), where they found increased Ci by treating plants with elevated CO_2 concentration.

It has been well explained in the earlier section that elevated CO_2 always increases the photosynthetic rate and lowers transpiration by decreasing stomatal conductance. Elevated CO_2 causes a wide range of secondary effects on plant physiology during the growth period because photosynthesis and stomatal behavior are essential to the metabolism of plant carbon and water. The accessibility of additional photosynthate enables most plants to grow faster under elevated CO_2 and enhances plant growth parameters like plant height, number of branches, and leaf area. This increase in plant morphological parameters by altering the plant physiology ultimately increased the plant biomass. As we know Plant biomass (W) is the total weight of below and above-ground plant material at the unit of ground surface area at a given point in time. Our results, summarised in Tables 3 and 4, show the increment in biomass from stevia plants with CO_2 elevation. According to several studies, plants grown under elevated CO_2 showed an average increment in dry matter production of 17% for the aboveground

portion of the plants and more than 30% for the belowground portion (Ainsworth & Long 2005; Degraaff *et al*., 2006). This growth enhancement is also redirected in the yield of other harvestable crops, with wheat, rice, and soybean all showing increases in yield of 12–14% under elevated CO_2 (Long *et al*., 2006; Ainsworth, 2008). Lamichaney *et al*. (2021) reported that under eCO2 conditions the total dry weight of chickpea (C3 crop) was 28-29% higher which is in support of our results from the current experiment. Canada thistle (*Cirsium arvense*) showed a 180% increase in biomass under elevated CO_2 which is widely considered one of the most invasive species in the continental United States, (Ziska, 2003). Ainsworth and Long (2005) and Degraaff *et al*. (2006) reported a 30% increment in biomass in C_3 crops like rice, wheat, and soybean under elevated CO_2 of 750 ppm, which supports the results from the current study.

The results from the current research showed that elevated CO_2 has positively influenced the glycoside content in stevia plants. Glycosides (steviosides & rebaudiosides) were found in greater amounts in CO_2-treated plants than in plants grown under ambient CO_2 as a control. This is because higher levels of CO_2 in the atmosphere promote soluble sugar accumulation in the edible parts of C_3 vegetables. Triose phosphate, which can be further converted into other carbohydrates, is synthesized in leaves more readily due to the enhanced CO_2 fixation under elevated CO_2 levels (Long *et al*., 2004). The meta-analysis from Dong *et al*. (2018) showed that elevated CO_2 increased glucose concentrations by 13.2%, fructose by 14.2%, sucrose by 3.7%, and total soluble sugar by 17.5% in terms of all selected vegetables for their study. The results are summarised in Figures 2 and 3, which show similarities to Dong *et al*. (2018) regarding sugar increment. Mostly the chemical structures of all soluble sugars are similar. Jin *et al*. (2009) also concluded from their study that the increment in total soluble sugar in leaves under elevated CO_2 was the greatest (36.2%) among all the classes of vegetables. This enrichment in soluble sugar can reach from 38 to 188% in Chinese cabbage leaves and 16–53% in oily sow thistle leaves. The total soluble sugar in strawberry fruits was seen to be enhanced by 20% under elevated CO_2 of 950 ppm comparatively to ambient CO_2 level (Wang & Bunce, 2004). Similarly, under 1,000 ppm of CO_2 as opposed to 400 ppm as a control, the total soluble sugar increment was 13% in radish and 20% in turnip (Azam *et al*., 2013). The results from our current study show similarities to all the above findings in terms of soluble sugar. All the above findings support our results from the current study as stevia is a C_3 crop and most of the above studies have been carried out on C_3 species.

CONCLUSION

The current results show that elevated CO_2 generally positively enhances growth and biomass by enhancing the physiological parameters, especially photosynthesis, and reducing the stomatal conductance and transpiration rate. However, it is also clear from the above findings that exposing the stevia plants to long-term elevated mean until the final harvest produces more biomass than short-term exposure to CO_2.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abzar, A., Ahmad, W.J.W., Said, M.N.M., Doni, F., Zaidan, M.W.A.M., Fathurahman, F. & Zain, C.R.C.M. 2018. Enhanced growth, yield and physiological characteristics of rice under elevated carbon dioxide. In AIP Conference Proceedings, 1940: 020064. https://doi.org/10.1063/1.5027979

Abzar, A., Said, M.N.M., Ahmad, W.J.W., Mohtar, W.Y.W., Doni, F., Fathurahman, F. & Zain, C.R.C.M. 2017. Elevated CO_2 concentration enhances germination, seedling growth and vigor of rice. Ecology, Environment and Conservation, 23(3): 41-45.

Ahmad, J., Khan, I., Blundell, R., Azzopardi, J. & Mahomoodally, M.F. 2020. *Stevia rebaudiana* Bertoni.: an updated review of its health benefits, industrial applications and safety. Trends in Food Science

& Technology, 100: 177-189. https://doi.org/10.1016/j.tifs.2020.04.030

Ainsworth, E.A. & Rogers, A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant, Cell & Environment, 30(3): 258-270. https://doi.org/10.1111/j.1365-3040.2007.01641

Ainsworth, E.A. 2008. Rice production in a changing climate: A meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biology, 14(7): 1642-1650. https://doi.org/10.1111/j.1365-2486.2008.01594

Ainsworth, E.A. & Long, S.P. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist, 165(2): 351-372. https://doi.org/10.1111 /j.1469-8137.2004.01224

Aranjuelo, I., Erice, G., Sanz-Sáez, A., Abadie, C., Gilard, F., Gil-Quintana, E., Avice, J.C., Staudinger, C., Wienkoop, S., Araus, J.L., Bourguignon, J., Irigoyen, J.J. & Tcherkez, G. 2015. Differential CO_2 effect on primary carbon metabolism of flag leaves in durum wheat (*Triticum durum* Desf.). Plant, Cell & Environment, 38(12): 2780-2794. https://doi.org/10.1111/pce.12587

Azam, A., Khan, I., Mahmood, A. & Hameed, A. 2013. Yield, chemical composition and nutritional quality responses of carrot, radish and turnip to elevated atmospheric carbon dioxide. Journal of the Science of Food and Agriculture, 93(13): 3237-3244. https://doi.org/10.1002/jsfa.6165

Bowes, G. 1991. Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. Plant, Cell & Environment, 14(8): 795-806. https://doi.org/10.1111/j.1365-3040.1991.tb01443

Boyer, J. S. 2015. Turgor and the transport of CO_2 and water across the cuticle (epidermis) of leaves. Journal of Experimental Botany, 66(9): 2625-2633. https://doi.org/10.1093/jxb/erv065

Brearley, J., Venis, M. A. & Blatt, M. R. 1997. The effect of elevated CO_2 concentrations on K^+ and anion channels of *Vicia faba* L. guard cells. Planta, 203: 145-154.

Chang, S., Chang, T., Song, Q., Zhu, X.G. & Deng, Q. 2016. Photosynthetic and agronomic traits of an elite hybrid rice Y-Liang-You 900 with a record-high yield. Field Crops Research, 187: 49-57.

Cruz, M.M. 2015. Bibliographic review. *Stevia rebaudiana* (Bert.) Bertoni. A Revision. Cultivos Tropicales, 36(5): 5-15.

Degraaff, M.A., Vangroenigen, K.J., Six, J., Hungate, B. & van Kessel, C. 2006. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Global Change Biology, 12(11): 2077-2091. https://doi.org/10.1111/j.1365-2486.2006.01240

Dong, J., Gruda, N., Lam, S. K., Li, X. & Duan, Z. 2018. Effects of elevated CO2 on nutritional quality of vegetables: A review. Frontiers in Plant Science, 9: 924.

Doni, F., Zain, C.R.C.M., Isahak, A., Fathurrahman, F., Anhar, A., Mohamad, W.N.A.W. & Uphoff, N. 2018. A simple, efficient, and farmer-friendly *Trichoderma*-based biofertilizer evaluated with the SRI Rice Management System. Organic Agriculture, 8: 207-223.

Dusenge, M.E., Duarte, A.G. & Way, D.A. 2019. Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. New Phytologist, 221(1): 32-49. https://doi.org/10.1111/nph.15283

Engineer, C.B., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordstrom, M., Azoulay-Shemer, T., Rappel, W.J., Iba, K. & Schroeder, J.I. 2016. CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions. Trends in Plant Science, 21(1): 16-30. https://doi. org/10.1016/j.tplants.2015.08.014

Fernie, A. R., Aharoni, A., Willmitzer, L., Stitt, M., Tohge, T., Kopka, J. & DeLuca, V. 2011. Recommendations for reporting metabolite data. The Plant Cell, 23(7): 2477-2482.

Foyer, C.H. & Shigeoka, S. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiology, 155(1): 93-100. https://doi.org/10.1104/pp.110.166181

Garcia-Plazaola, J.I., Hernandez, A. & Becerril, J.M. 2003. Antioxidant and pigment composition during autumnal leaf senescence in woody deciduous species differing in their ecological traits. Plant Biology, 5(05): 557-566. https://doi.org/10.1055/s-2003-44791

Gill, S.S. & Tuteja, N. 2010. Polyamines and abiotic stress tolerance in plants. Plant Signaling & Behavior, 5(1): 26-33. https://doi.org/10.4161/psb.5.1.10291

Hanstein, S.M. & Felle, H.H. 2002. CO_2-triggered chloride release from guard cells in intact fava bean leaves. Kinetics of the onset of stomatal closure. Plant Physiology, 130(2): 940-950. https://doi. org/10.1104/pp.004283

Ibrahim, M.H. & Jaafar, H.Z. 2017. Impact of carbon dioxide and increasing nitrogen levels on growth, photosynthetic capacity, carbohydrates and secondary metabolites in kacip fatimah (*Labisia pumila*). Annual Research & Review in Biology, 19(6): 1-16. https://doi.org/10.9734/

ARRB/2017/36673
Jin, X., Zhang, X., Wu, Z., Teng, D., Zhang, X., Wang, Y., Wang, Z. & Li, C. 2009. Amphiphilic random glycopolymer based on phenylboronic acid: synthesis, characterisation, and potential as glucose-sensitive matrix. Biomacromolecules, 10(6): 1337-1345. https://doi.org/10.1021/bm8010006
Khiraoui, A., Hasib, A., Al Faiz, C., Amchra, F., Bakha, M. & Boulli, A. 2017. *Stevia rebaudiana* Bertoni (Honey Leaf): A magnificent natural bio-sweetener, biochemical composition, nutritional and therapeutic values. Journal of Natural Sciences Research, 7(14): 75-85.
Kinsman, E.A., Lewis, C., Davies, M.S., Young, J. E., Francis, D., Vilhar, B. & Ougham, H.J. 1997. Elevated CO_2 stimulates cells to divide in grass meristems: a differential effect in two natural populations of *Dactylis glomerata*. Plant, Cell & Environment, 20(10): 1309-1316.
Lamichaney, A. & Maity, A. 2021. Implications of rising atmospheric carbon dioxide concentration on seed quality. International Journal of Biometeorology, 65(6): 805-812.
Leakey, A.D., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. & Ort, D.R. 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany, 60(10): 2859-2876. https://doi.org/10.1093/jxb/erp096
Lee, T.D., Tjoelker, M.G., Ellsworth, D.S. & Reich, P.B. 2001. Leaf gas exchange responses of 13 prairie grassland species to elevated CO_2 and increased nitrogen supply. New Phytologist, 150(2): 405-418. https://doi.org/10.1046/j.1469-8137.2001.00095
Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L. & Ah-Hen, K. 2012. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chemistry, 132(3): 1121-1132. https://doi.org/10.1016/j.foodchem.2011.11.140
Long, S.P., Ainsworth, E.A., Rogers, A. & Ort, D.R. 2004. Rising atmospheric carbon dioxide: plants FACE the future. Annual Review of Plant Biology, 55: 591-628. https://doi.org/10.1146/annurev.arplant.55.031903.141610
Long, S.P., Zhu, X.G., Naidu, S.L. & Ort, D.R. 2006. Can improvement in photosynthesis increase crop yields. Plant, cell & environment, 29(3): 315-330. https://doi.org/10.1111/j.1365-3040.2005.01493
Madan, S., Ahmad, S., Singh, G.N., Kohli, K., Kumar, Y., Singh, R. & Garg, M. 2010. *Stevia rebaudiana* (Bert.) Bertoni-A review. Indian Journal of Natural Products and Resources, 1(3): 267-286.
McDonald, E.P., Erickson, J.E. & Kruger, E.L. 2002. Research note: Can decreased transpiration limit plant nitrogen acquisition in elevated CO_2. Functional Plant Biology, 29(9): 1115-1120. https://doi.org/10.1071/FP02007
Misra, H., Soni, M., Silawat, N., Mehta, D., Mehta, B.K. & Jain, D.C. 2011. Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats. Journal of Pharmacy and Bioallied Sciences, 3(2): 242. https://doi.org/10.4103/0975-7406.80779
Morelli, L. & Capurso, L. 2012. FAO/WHO guidelines on probiotics: 10 years later. Journal of Clinical Gastroenterology, 46, S1-S2.
Morgan, J.A., Milchunas, D.G., LeCain, D.R., West, M. & Mosier, A.R. 2007. Carbon dioxide enrichment alters plant community structure and accelerates shrub growth in the short grass steppe. Proceedings of the National Academy of Sciences, 104(37): 14724-14729. https://doi.org/10.1073/pnas.0703427104
Moroney, J.V., Jungnick, N., DiMario, R.J. & Longstreth, D.J. 2013. Photorespiration and carbon concentrating mechanisms: Two adaptations to high O_2, low CO_2 conditions. Photosynthesis Research, 117: 121-131. https://doi.org/10.1007/s11120-013-9865-7
Pal, P., AbuKashabeh, A., Al-Asheh, S. & Banat, F. 2015. Role of aqueous methyldiethanolamine (MDEA) as solvent in natural gas sweetening unit and process contaminants with probable reaction pathway. Journal of Natural Gas Science and Engineering, 24: 124-131. https://doi.org/10.1016/j.jngse.2015.03.007
Poorter, H. & Perez-Soba, M. 2002. Plant growth at elevated CO_2. Encyclopedia of Global Environmental Change, 2: 489-496.
Prior, S.A., Runion, G.B., Marble, S.C., Rogers, H.H., Gilliam, C.H. & Torbert, H.A. 2011. A review of elevated atmospheric CO_2 effects on plant growth and water relations: implications for horticulture. HortScience, 46(2): 158-162. https://doi.org/10.21273/HORTSCI.46.2.158
Prior, S.A., Watts, D.B., Arriaga, F.J., Runion, G.B., Torbert, H.A. & Rogers, H.H. 2010. Influence of elevated atmospheric CO_2 and tillage practice on rainfall simulation. Conservation Agriculture Impacts–Local and Global, Volume(Issue): 83-88.
Rai, A., Mohanty, B. & Bhargava, R. 2016. Supercritical extraction of sunflower oil: A central

composite design for extraction variables. Food Chemistry, 192: 647-659. https://doi.org/10.1016/j.foodchem.2015.07.070

Robredo, A., Perez-Lopez, U., Lacuesta, M., Mena-Petite, A. & Munoz-Rueda, A. 2010. Influence of water stress on photosynthetic characteristics in barley plants under ambient and elevated CO_2 concentrations. Biologia Plantarum, 54(2): 285-292. https://doi.org/10.1007/s10535-010-0050-y

Rogers, M.J., Ji, X., Russell, R.G.G., Blackburn, G.M., Williamson, M.P., Bayless, A.V., Ebetino, F.H. & Watts, D.J. 1994. Incorporation of bisphosphonates into adenine nucleotides by amoebae of the cellular slime mould *Dictyostelium discoideum*. Biochemical Journal, 303(1): 303-311. https://doi.org/10.1042/bj3030303

Salvucci, M.E. & Crafts-Brandner, S.J. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiologia plantarum, 120(2): 179-186. https://doi.org/10.1111/j.0031-9317.2004.0173

Sanoubar, R., Cellini, A., Veroni, A. M., Spinelli, F., Masia, A., Vittori Antisari, L., Orsini, F. & Gianquinto, G. 2016. Salinity thresholds and genotypic variability of cabbage (*Brassica oleracea* L.) grown under saline stress. Journal of the Science of Food and Agriculture, 96(1): 319-330. https://doi.org/10.1002/jsfa.7097

Seneweera, S.P. & Conroy, J.P. 2005. Enhanced leaf elongation rates of wheat at elevated CO_2: is it related to carbon and nitrogen dynamics within the growing leaf blade?. Environmental and Experimental Botany, 54(2): 174-181. https://doi.org/10.1016/j.envexpbot.2004.07.002

Seneweera, S.P., Ghannoum, O., Conroy, J.P., Ishimaru, K., Okada, M., Lieffering, M., Kim, H.Y. & Kobayashi, K. 2002. Changes in source–sink relations during development influence photosynthetic acclimation of rice to free air CO_2 enrichment (FACE). Functional Plant Biology, 29(8): 947-955. https://doi.org/10.1071/PP01250

Shivanna, N., Naika, M., Khanum, F. & Kaul, V.K. 2013. Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. Journal of Diabetes and its Complications, 27(2): 103-113. https://doi.org/10.1016/j.jdiacomp.2012.10.001

Singh, S.K. & Reddy, V.R. 2016. Methods of mesophyll conductance estimation: its impact on key biochemical parameters and photosynthetic limitations in phosphorus-stressed soybean across CO_2. Physiologia Plantarum, 157(2): 234-254. https://doi.org/10.1111/ppl.12415

Sivaramanan, S. 2015. Global Warming and climate change, causes, impacts and mitigation. Central Environmental Authority, 2(4): 1-26. https://doi.org/10.13140/RG.2.1.4889.7128

Thilakarathna, S.M.C.R., Kirthisinghe, J.P., Gunathilaka, B.L. & Dissanayaka, D.M.P.V. 2015. Influence of gypsum application on yield and visual quality of groundnut (*Arachis hypogaea* L.) grown in Maspotha in Kurunegala district of Sri Lanka. Tropical Agricultural Research, 25(3): 432-436.

vanderKooi, C.J., Reich, M., Low, M., De Kok, L.J. & Tausz, M. 2016. Growth and yield stimulation under elevated CO_2 and drought: A meta-analysis on crops. Environmental and Experimental Botany, 122: 150-157. https://doi.org/10.1016/j.envexpbot.2015.10.004

Vanheerden, P.D.R., Swanepoel, J.W. & Kruger, G.H.J. 2007. Modulation of photosynthesis by drought in two desert scrub species exhibiting C_3-mode CO_2 assimilation. Environmental and Experimental Botany, 61(2): 124-136. https://doi.org/10.1016/j.envexpbot.2007.05.005

Vazquez-Baxcajay, L., Robledo-Paz, A., Muratalla-Lua, A. & Conde-Martínez, V. 2014. Micropropagation of *Stevia rebaudiana* Bertoni, and steviosides detection. Bioagro, 26(1): 49-56.

Walker, A.P., De Kauwe, M.G., Bastos, A., Belmecheri, S., Georgiou, K., Keeling, R.F. *et al*. 2021. Integrating the evidence for a terrestrial carbon sink caused by increasing atmospheric CO_2. New Phytologist, 229(5): 2413-2445. https://doi.org/10.1111/nph.16866

Wand, S.J., Midgley, G.F., Jones, M.H. & Curtis, P.S. 1999. Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biology, 5(6): 723-741. https://doi.org/10.1046/j.1365-2486.1999.00265

Wang, S.Y. & Bunce, J.A. 2004. Elevated carbon dioxide affects fruit flavour in field-grown strawberries (*Fragaria ananassa* Duch). Journal of the Science of Food and Agriculture, 84(12): 1464-1468. https://doi.org/10.1002/jsfa.1824

Watanabe, H., Arai, F. & Okazaki, K. 2013. Role of CO_2 in the CH_4 oxidation and H_2 formation during fuel-rich combustion in O_2/CO_2 environments. Combustion and Flame, 160(11): 2375-2385. https://doi.org/10.1016/j.combustflame.2013.05.022

Wood, A.J. & Roper, J. 2000. A simple & non-destructive technique for measuring plant growth & development. The American Biology Teacher, 62(3): 215-217. https://doi.org/10.1662/0002-7685(2000)062[0215:ASNTFM]2.0.CO;2

Yu, J., Chen, L., Xu, M. & Huang, B. 2012. Effects of elevated CO_2 on physiological responses of tall

fescue to elevated temperature, drought stress, and the combined stresses. Crop Science, 52(4): 1848-1858. https://doi.org/10.2135/cropsci2012.01.0030

Zhang, L., Wu, D., Shi, H., Zhang, C., Zhan, X. & Zhou, S. 2011. Effects of elevated CO_2 and N addition on growth and N_2 fixation of a legume subshrub (Caragana microphylla Lam.) in temperate grassland in China. PLoS ONE, 6(10): 26842. https://doi.org/10.1371/journal.pone.0026842

Zhang, X., Wollenweber, B., Jiang, D., Liu, F. & Zhao, J. 2008. Water deficits and heat shock effects on photosynthesis of a transgenic Arabidopsis thaliana constitutively expressing ABP9, a bZIP transcription factor. Journal of Experimental Botany, 59(4): 839-848.

Zheng, Y., Li, F., Hao, L., Yu, J., Guo, L., Zhou, H., Ma, C. & Xu, M. 2019. Elevated CO_2 concentration induces photosynthetic down-regulation with changes in leaf structure, non-structural carbohydrates and nitrogen content of soybean. BMC Plant Biology, 19: 255. https://doi.org/10.1186/s12870-019-1788-9

Zinta, G., AbdElgawad, H., Domagalska, M. A., Vergauwen, L., Knapen, D., Nijs, I., janssens, I. A., Beemster, G.T.S & Asard, H. 2014. Physiological, biochemical, and genome-wide transcriptional analysis reveals that elevated CO_2 mitigates the impact of combined heat wave and drought stress in Arabidopsis thaliana at multiple organisational levels. Global Change Biology, 20(12): 3670-3685. https://doi.org/10.1111/gcb.12626

Ziska, L.H. 2003. Evaluation of yield loss in field sorghum from a C_3 and C_4 weed with increasing CO_2. Weed Science, 51(6): 914-918. https://doi.org/10.1614/WS-03-002