Journal of Tropical Plant Physiology 16(1) (2024):26-39
https://doi.org/10.56999/jtpp.2024.16.1.35

An Exploration into "Do-It-Yourself" (DIY) CO_2 Enrichment Chambers: Utilising Grey Oyster Mushroom (*Pleurotus pulmonarius* (Fr.) Quél) Respiration to Improve Okra (*Abelmoschus esculentus* L.) Seedlings Growth

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Received: 22 February 2024; Revised: 6 August 2024; Accepted: 23 February 2024; Published: 24 February 2024

ABSTRACT

The endeavour to employ CO_2 from natural sources to improve plant growth is often challenging, especially in tropical climates. While mycelium bags from the mushroom industry can generate CO_2, they need indoor conditions with controlled temperature, high humidity, and low light. These conditions are not present in open fields, leading to low CO_2 production. Inspired by the ancient clay evaporative cooling device called the zeer pot, a new solution was developed to address this problem. The eventual design was a confinement chamber that housed the mycelium bags and the okra seedlings. The chamber conditions were sufficiently conducive for the mycelium bags to produce an ambient CO_2 level of around 800 ppm during the day. The resulting okra seedlings under this high CO_2 concentration of natural origin had a notable increase in overall biomass accumulation (+ 277%), carbon assimilation rate (+103%) as well as improved water use efficiency (+95%) compared to seedlings grown under ambient CO_2 level. These promising findings not only proved the capability of mycelium as a natural CO_2 generator but also as an additional economic potential for the farmers to gain income from the CO_2-enriched crops as well as the harvested mushroom bodies at the time of mycelium bags maturity. Furthermore, this simple innovation opens the avenue for further interdisciplinary research in other agricultural efforts to improve crop yield and quality.

Keywords: CO_2 enrichment; DIY CO_2 chamber, mycelium bags; okra growth.

INTRODUCTION

Carbon dioxide (CO_2) enrichment refers to a technique of increasing the concentration of CO_2 in the air surrounding plants to enhance their growth and development (Mortensen, 1987; Mcgrath and Lobell, 2013; Reich et al., 2018). This method has emerged as a crucial tool in crop research and enhancement, aiding in the investigation of crop responses to elevated CO_2 concentrations, ultimately leading to adjustments that improve yields, stress tolerance, and nutrient content. Numerous studies have demonstrated that biomass increases by approximately 50% in C3 plants, 35% in CAM plants, and 12% in C4 plants under CO_2 enrichment conditions (Drennan and Nobel, 2000; Poorter and Navas, 2003; Reich et al., 2018).

In parallel, CO_2 enrichment technique is also widely used in commercial agricultural and horticultural applications. The technique's utility extends to greenhouse horticulture, offering enhanced productivity, higher yields per square meter, and superior product quality. However, conventional CO_2 enrichment methods relying on compressed tanks (Wang et al., 2022), fuel-burning systems (Vermeulen, 2014), or chemical reactions (Syed and Hachem, 2019) pose environmental and economic sustainability challenges. In addition to improving crop yield and quality, agricultural production systems are also under immense pressure to reduce carbon footprint to mitigate climate change. Therefore, a more sustainable, natural, and cost-effective CO_2 source is needed to mitigate the inadequacy of global food production and simultaneously meet the challenges of climate change. Thus, one promising avenue lies in harnessing CO_2 produced through fungal respiration, utilising fungal species that live on lignocellulosic waste, thereby repurposing agricultural by-products and reducing environmental harm. Embracing CO_2 from fungal respiration offers a sustainable approach to agriculture, addressing the dual imperatives of enhancing food production while mitigating climate change impacts.

Lignocellulose found in agricultural by-products poses a challenge due to its recalcitrant nature, making it resistant to degradation by chemical means or microbial enzymes (Zoghlami & Paës, 2019; Wu et al., 2022) However, certain mushroom species possess enzymes capable of breaking down lignocellulosic waste (Rajaratnam et al., 2009), such as sawdust, a common substrate in tropical regions like Malaysia (Ahmad et al., 2012; Zakil et al., 2021). Grey oyster mushrooms, for instance, can thrive on sawdust without fermentation, utilising hydrolysing and oxidising enzymes to degrade the substrate and generate CO_2 through respiration (Rajaratnam et al., 2009). While CO_2 from mycelial respiration offers a sustainable source, its concentration must be controlled to avoid inhibiting mycelial growth (Martin et al., 2017; Lin et al., 2022). Integrating plants into mushroom cultivation allows for the utilisation of CO_2 in photosynthesis, reducing its concentration while promoting sustainable crop production (Padmanabha and Streif, 2019; Jung and Son, 2021). Several studies have explored the use of CO_2 from fungal respiration to enhance plant growth. Research in Japan demonstrated increased dry weight in mint and potato plantlets grown in CO_2-enriched environments from Shiitake mushroom mycelial respiration (Jung and Son, 2021). Additionally, mixed cultivation of lettuce with king oyster mushroom led to significant increases in lettuce shoot dry weight compared to monoculture, while reducing CO_2 emissions by 80.6% (Jung and Son, 2021). This underscores the potential of harnessing CO_2 from fungal respiration to not only promote plant growth but also mitigate environmental impacts, contributing to sustainable agriculture practices.

CO_2 enrichment is known to significantly enhance crop growth and yield (Ikawa et al., 2019; Huang et al., 2021). However, artificially increasing CO_2 levels can be costly, especially for large-scale operations (Frantz, 2011). The need for infrastructure and equipment to regulate and monitor CO_2 levels further complicates this issue (Pepin and Körner, 2002), making it challenging to conduct research in high-CO_2 environments for plants. Although CO_2 can be obtained from mycelium bags used in the mushroom industry, these mycelia require indoor conditions with room temperature, high humidity, and low light to thrive. In open fields, where these conditions are absent, respiration decreases and CO_2 production is low.

Fortunately, an ancient evaporative cooling refrigeration device made from clay, namely the zeer pot, was taken on as an inspiration. Therefore, this study was designed to address the need for sustainable and economically viable methods of CO_2 enrichment in agricultural practices, particularly in ambient tropical conditions where traditional approaches may prove challenging. This study also aimed to provide a practical and affordable solution to conduct CO_2 enrichment research, particularly targeting developing and least developed countries where access to sophisticated agricultural technologies may be limited. This approach not only addresses the economic constraints often faced by researchers or farmers in these regions but also empowers them to conduct meaningful research on improving crop yields and quality.

Okra is cultivated globally in tropical and warm temperate regions for its nutritious, fibrous fruit pods (Anwar et al., 2020). Okra's fast-growing nature and short cultivation cycle make it an attractive model crop for scientific investigations, facilitating efficient experimentation and rapid data collection. This study opens doors for broader participation in CO_2 enhancement endeavors, thereby fostering innovation and sustainable agricultural practices worldwide.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at Field 15, Faculty of Agriculture, Universiti Putra Malaysia in Serdang, Selangor. The site was located at the latitude of 3.0077 °N and longitude 101.7026 °E. The range of daily temperature inside the growing chamber used for CO_2 treatment was 29 to 40 °C with relative humidity range of 60 to 65% were recorded by a CO_2 data logger (HT-2000, Hti, Dongguan Xintai Instrument Co. Ltd., China).

Experimental Design and Growing Methods

Nested design was used to conduct the experiment where the equipment to treat the seedlings with elevated CO_2 and ambient CO_2 conditions were not replicated due to limitation in resources. The treatments were two different CO_2 concentrations, which were elevated CO_2 concentration at about 600 to 800 ppm (eCO_2) and ambient CO_2 concentration at about 350 to 400 ppm (aCO_2). The range of daily temperature inside the growing chamber used for CO_2 treatment was 29 to 40 °C with a relative humidity range of 60 to 65%. Sixteen replications of seedlings were used for each chamber used in the experiment.

The okra (*Abelmoschus esculentus* cv. MKBe 1) was planted on the 1st October 2019. MKBe 1 okra variety was released by the Malaysian Agricultural Research and Development Institute (MARDI) located in Serdang, Selangor, Malaysia. The seeds were sown in polybags (10 x 11 x 14 cm) filled with organic soil mixture by a volume of 1.4 liters per polybag. The soil was a proprietary blend of soil mixture from Kean Beng Lee Industries (M) Sdn Bhd, which consisted of coco peat, burnt soil, river sand, burnt husk, rich humus, and charcoal powder. Before sowing, the okra seeds were soaked in water for 24 h to enhance germination. Three seeds were directly sown at the depth of 4 to 5 cm in the soil at 28 °C and 70% of relative humidity. The seedlings were thinned to 1 plant per polybag at the third leaf stage.

The seedlings were watered twice a day in the morning and evening, daily until the third week. The plant spacing between the polybags was 3 cm in width and 5 cm in length. Fertiliser NPK (15:15:15) at a rate of 100kg/ha was added to each polybag of MKBe 1 seedling at 12 days after sowing (DAS).

CO_2 Chamber Construction

The DIY in-house CO_2 enrichment structure (ICES) was constructed from a clay pot and a transparent plastic sheet and a BRC welded wire mesh. The internal volume of the CO_2 chamber was approximately 0.14 m^3 and the height was 1 m. The chamber was designed with three levels where the first level was lined with one-week-old mycelium bags (mushroom bag) while the second layer was polybags containing the seedlings and the third level was the confined region with CO_2 molecules (Figure 1). The okra seeds were germinated in the polybags on the top of the mycelium bags lined with a damp cloth in the CO_2 chamber. Four mycelium bags, which acted as CO_2 supplier were placed in the clay pot where the temperature was much cooler compared to the outside environment. The whole chamber was covered with transparent plastic to contain the CO_2 inside the chamber. For the ambient CO_2 (aCO_2) condition, no mycelium bag was used in the chamber. A CO_2 logger (HT-2000, Hti, Dongguan Xintai Instrument Co. Ltd., China) was placed at the second level inside the chamber to monitor the CO_2 concentration, relative humidity, and temperature.

Growth and Physiological Measurements

Plant height (cm) was measured using a measuring tape while leaf thickness of okra leaves was measured using a thickness gauge micrometer (QST, China). The total chlorophyll content was measured by using the SPAD 502 chlorophyll meter (Minolta, Japan) on the third fully expanded leaf of the sample. For each plant, leaf thickness and SPAD index readings were taken around the midpoints of the leaf 3 times and the average values were recorded. The measurements were taken at the third week of the experimental period, which was at the end of elevated CO_2 treatment. The total leaf area was also determined at the third week by using the Li-31000 leaf area meter (LI-COR Inc., Lincoln, NE, USA). Specific leaf area (SLA) is calculated by using the formula below: Where LA is the area of leaves, and DL is the dry mass of the leaves.

$$\text{SLA (cm}^2\text{g}^{-1}) = \frac{\text{LA (cm}^2)}{\text{DL(g)}} \quad [1]$$

Fresh and dry matter of shoot and root of seedlings were determined on the third week of the seedling growth. Shoot and root fresh weights were measured separately using an electronic scale (Sartorious A and D FX200Iwp, Germany). The shoots were then cut into small pieces and placed in paper bags and dried for 48 hours at 60 °C in an oven. The dried seedlings were weighed using the electronic scale. Root to shoot ratio (R:S) was calculated on the dry weight basis using the following formula (Reynolds and Thornley, 1982):

$$\text{R:S} = \frac{\text{Root dry weight (g)}}{\text{Shoot dry weight (g)}} \quad [2]$$

Leaf gas exchange including photosynthesis rate (µmol $m^{-2}s^{-1}$) and stomatal conductance (mol m^{-2} s^{-1}) were measured using the LI-6800 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). Measurements were taken on leaf no. 5 from 8.00 a.m. to 12.00 p.m. at 21 days after sowing (DAS). The entire leaf attached to the plant was inserted into the leaf chamber. The LI-6800 portable photosynthesis system provided irradiance by using the LED RGB (Red, Green, Blue) light and the irradiance levels can be changed as needed. The irradiance was set at 10% blue light to promote stomata opening and 90% red light. The temperature inside the measuring chamber was maintained at 30 °C with 70% relative humidity while the flow setpoint was set at 400 µmol s^{-1}. The CO_2 cartridge delivered CO_2 and the CO_2 injection system delivered pure CO_2 at 400 µmol CO_2 $m^{-2}s^{-1}$ into the leaf chamber. Intrinsic water use efficiency (iWUE) was calculated as the ratio of assimilation rate to stomatal conductance and calculated using the following formula:

$$\text{iWUE} = \frac{\text{Assimilation rate } (A_{400})}{\text{Stomatal conductance } (g_{sw})} \qquad [3]$$

Statistical Analysis

All data were analysed in GraphPad Prism version 9.2.0 for Windows (GraphPad Software, San Diego, California USA). The data were also subjected to normality and equality of variance testing. Student’s two sample t-test at P = 0.05 were performed for normally distributed and had equal variance data while Mann-Whitney non-parametric test was used to analyse the data that were non-normal and had unequal variance. Analysis of non-parametric correlation between growth and physiology parameters was also performed for each CO_2 treatment at P = 0.05 to determine the strength and direction of correlation. Spearman’s rank correlation was used due to non-normality in most of the data.

RESULTS AND DISCUSSION

CO_2 Chamber Environment

The DIY in-house CO_2 enrichment structure (ICES) was designed with three levels, where the first level was lined with one-week-old mycelium bags (grey oyster mushroom bag), served as the source of CO_2 while the second layer was polybags containing the seedlings and the third level was the confined region with CO_2 molecules (Figure 1). Mushrooms offer a cost-effective and environmentally friendly alternative for CO_2 enrichment due to their ubiquitous presence across continents and continuous production (FAOSTAT, 2023; Singh et al., 2021), thus it is possible for farmers to adopt mushrooms as a cost-effective CO_2 source for enriching the growing environment in both agricultural and research settings. However, integrating mushrooms and plants within the same system presents challenges, given their divergent growth requirements. While mushrooms thrive in dark and cool environments conducive to respiration and CO_2 production, plants necessitate light for photosynthesis and warmth from sunlight.

Hence, an invention that combines the zeer pot concept with a clear plastic cover on top to capture the CO_2 from mushrooms can be used as a tool to treat plants in a high CO_2 condition. The mushroom was kept in the earthen pot, which was darker, and cooler compared to the top part creating a favourable environment for mushroom to respire, and plants placed on the top part of the pot can get enough light for photosynthesis. The CO_2 from mushroom was trapped inside the system covered with a clear plastic creating an elevated CO_2 condition for the plants inside the structure. A small opening at the top part was required to regulate the heat and gasses inside the structure.

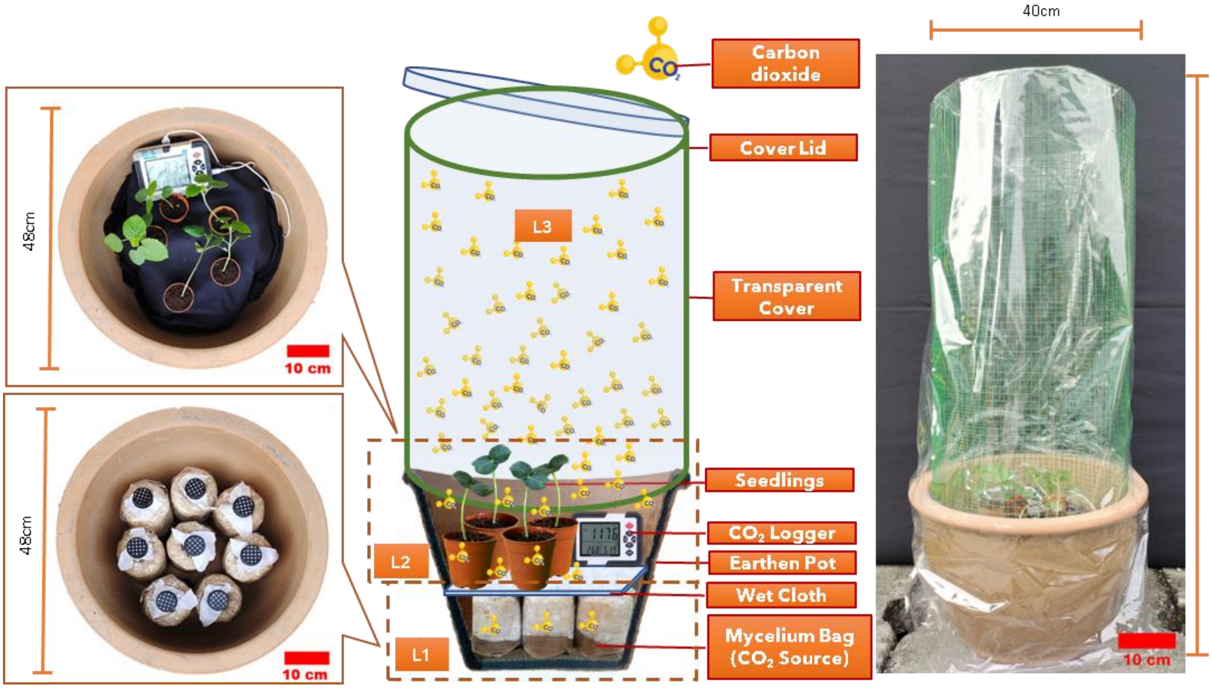


Figure 1. DIY in-house CO_2 enrichment structure (ICES) assembly and set up. CO_2-producing mycelium bags reside in the bottom layer (L1) of the earthen pot while the okra seedlings located immediate above it in layer 2 (L2). A cylindrical and transparent tunnel sits on the top layer (L3) whose role is to trap CO_2 while maintaining a conducive growing condition for the okra seedlings in it.

CO_2, Temperature, and Relative Humidity Profiles

This system (ICES) was invented so that it could be easily reproducible by anyone, anywhere in the world. It is also reliable in producing a large amount of CO_2 with acceptable temperature rise as shown in Figure 2. The average CO_2 reading for the eCO_2 treatment was recorded at 750 ppm with a maximum reading of 882 ppm and a minimum of 641 ppm (Figure 2A). On the other, the average CO_2 reading for the aCO_2 treatment was recorded at 444 ppm with a maximum reading of 487 ppm and a minimum of 414 ppm (Figure 2A). The average temperature for the eCO_2 and aCO_2 treatments were similar with readings of 30.7 °C and 31.5 °C, respectively (Figure 2B). The maximum temperature for the eCO_2 and aCO_2 treatments was 40.3 °C and 35.3 °C, respectively while the minimum readings were recorded at 26.1 °C and 27.4 °C, respectively. The average relative humidity (RH) for both eCO_2 and aCO_2 treatments was recorded at 84 % and 75 %, respectively (Figure 2B). The maximum RH for the eCO_2 and aCO_2 treatments was 92 % and 83 %, respectively while the minimum readings were recorded at 69 % and 66 %, respectively.

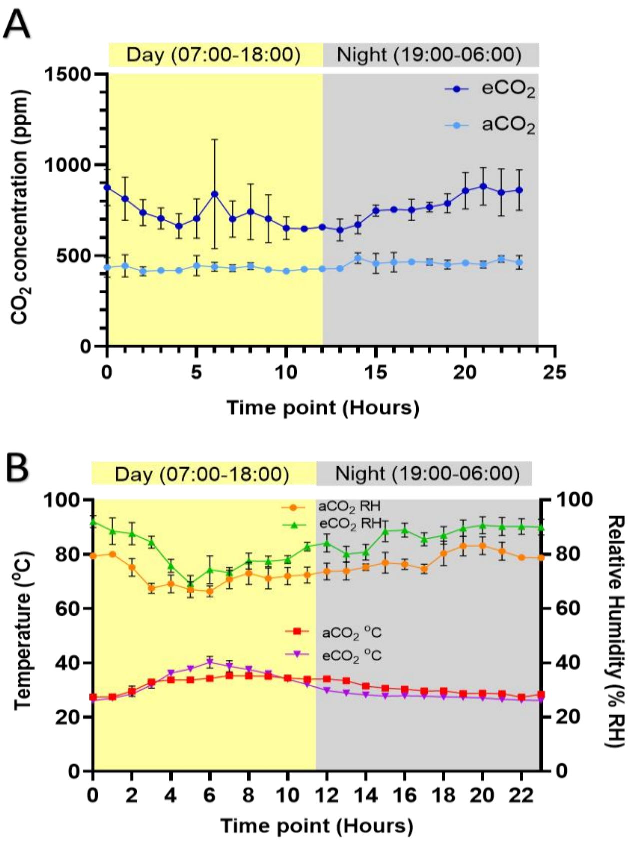


Figure 2. Comparison of 24-hour profiles for environmental conditions in the ICES (elevated CO_2 condition) (eCO_2) with a chamber under ambient CO_2 condition lacking mycelium bags (aCO_2). Each figure covers the hourly reading of CO_2 (A) as well as temperature and relative humidity (B), starting with daytime at 7am to 6pm then nighttime at 7pm to 6am. Error bars indicate standard error of means where n = 3.

Growth and Appearance

The plant height of the okra seedlings (Figure 3A) with eCO_2 treatment (29.02 cm) compared to aCO_2 treatment (16.45 cm) (Figure 3B) was significantly higher by 77% (Figure 4). The increased plant height of okra under eCO_2 treatment was potentially due to enhanced primary shoot growth, which resulted from increased cell division and a shorter cell cycle in the shoot tips (Kinsman et al., 1997). This finding aligned with recent eCO_2 experiments in rice, where plants exposed to eCO_2 were found to be 42% taller than those grown under aCO_2 conditions (Sloan et al., 2023). In terms of biomass accumulation capacity, the okra seedlings with eCO_2 treatment resulted in a remarkably higher total dry weight by 227% (Figure 4) when compared to aCO_2 treatment (Figure 3F). The significant increase in biomass under eCO_2 treatment is closely related to the CO_2 concentration, which directly increases the carbon assimilation rate and resulting in higher biomass production (Thompson et al., 2019). In addition, eCO_2 treatment resulted in a significantly higher root-to-shoot ratio (Figure 3G) with an increment of 59% (Figure 4). Notably, the data points in the plant height, total dry weight, and root-to-shoot ratio variables were clustered closely together, indicating a high level of confidence in these measured variables that eCO_2 seedlings resulted in higher values than aCO_2 seedlings. A similar pattern was observed in the leaf area variable, with okra seedlings that received eCO_2 treatment exhibiting a larger leaf area (44.97 cm^2) compared to a leaf area of aCO_2 (38.28 cm^2) (Figure 3C). The eCO_2 treatment has increased the leaf area of okra seedlings by 17% (Figure 4).

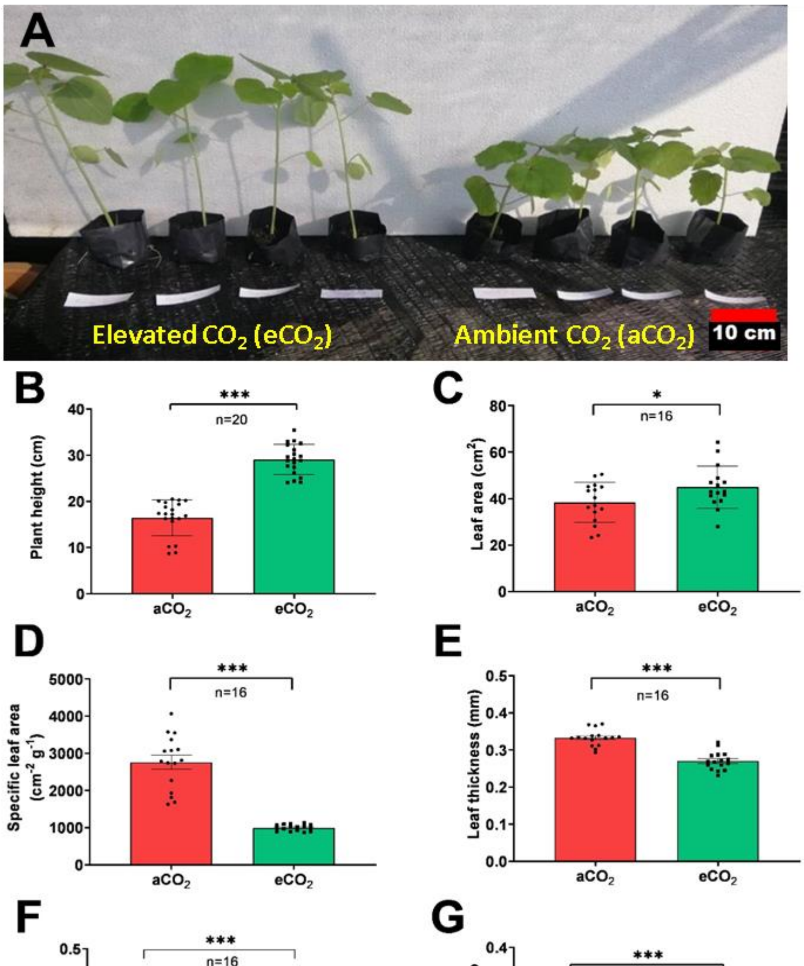
In contrast, the eCO_2 treatment resulted in a notably lower specific leaf area (SLA) value (652.6 cm^2g^{-1}) by 76% lower (Figure 4) when compared to aCO_2 (2762 cm^2g^{-1}) (Figure 3D). SLA was calculated as the ratio of leaf area to leaf weight. Leaves with a lower SLA value are thicker, while leaves with a higher SLA are thinner or less dense. Thus, in relation to that, the leaf thickness was significantly lower by 19% (Figure 4) in eCO_2 treatment when compared to aCO_2 treatment (Figure 3E).

Leaf Physiology

Photosynthesis rate (A_{400}), stomatal conductance (g_{sw}) and intrinsic water use efficiency (iWUE) measurements were used to assess the performance of leaf no. 5 at 21 DAS. The chlorophyll content was significantly reduced by 10% (Figure 4) with eCO_2 treatment (22.4 a.u.) compared to aCO_2 treatment (24.8 a.u.) (Figure 3H). This is potentially attributed to a larger leaf area under eCO_2 treatment, leading to a dilution effect. When leaves expand faster while nitrogen supply remains constant, the concentration of chlorophyll per unit area may decrease, even if the total amount of chlorophyll in the leaf remains the same or increases. Consequently, SPAD meter readings, which measure the relative concentration of chlorophyll in a specific leaf area, may show lower values.

Interestingly, the photosynthesis (assimilation rate) in eCO_2 seedlings was 103% higher (Figure 4) compared to those grown under aCO_2 conditions (Figure 3I). This increase in photosynthesis under eCO2 is primarily due to the enhanced carboxylation activity of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) (Thompson et al., 2017). The increased atmospheric CO_2 levels increase the CO_2 surrounding Rubisco, shifting the ratio of CO_2:O_2 and thereby increasing the rate of carboxylation. A higher trend g_{sw} values was observed in eCO_2 treatments (0.65 mol H_2O m^{-2} s^{-1}) compared to the aCO_2 treatments (0.58 mol H_2O m^{-2} s^{-1}) (Figure 3J). Despite the non-significant difference between both treatments, eCO_2 treatment has a great tendency to increase the g_{sw} value by 12% (Figure 4). When both A_{400} and g_{sw} were combined as a ratio to assess intrinsic water use efficiency (iWUE), interestingly, the iWUE value was significantly higher in eCO_2 treatment (31 µmol CO_2 mol H_2O^{-1}) compared to aCO_2 treatment (16 µmol CO_2 mol H_2O^{-1}). The eCO_2 treatment significantly increased the iWUE by 95% (Figure 4). A high-water use efficiency value in eCO_2 treatment indicated that these plants can tolerate drought conditions well (Toh et al., 2024). It is worth noting that there was a highly significant level observed in the total chlorophyll content, photosynthesis, and iWUE respectively, resulting in high statistical confidence and low standard mean of error in these measured variables. A graph with high statistical confidence provides a strong basis for making inferences and decisions based on the data.

Figure 3. Okra seedlings image and bar graphs of t-test. *, **, *** significantly different at P < 0.05, 0.01 and 0.001, respectively using Student's t-test (E, H, I, J) and Mann Whitney test (B, C, D, F, K), ns not significantly different at P > 0.05 using Student's t-test.



Impact of Elevated CO_2 Condition on Okra Seedlings: A Summary of Percentage Changes in Growth and Physiological Parameters

Under CO_2 enrichment conditions, various variables including plant height, leaf area, total dry weight, root-to-shoot ratio, photosynthesis, stomatal conductance, and intrinsic water use efficiency increased remarkably compared to ambient CO_2 conditions. Conversely, specific leaf area and total chlorophyll content decreased under CO_2 enrichment conditions. These findings provide strong evidence of the efficacy of the current CO_2 enrichment system, indicating its ability to elicit positive responses in plants exposed to elevated CO_2 levels.

While CO_2 enrichment technique has been widely used in both research and commercial crop production, with major crops such as rice, wheat, and sorghum benefiting from this technique (Ainsworth and Long, 2020). However, there are still many underexplored crops that could benefit from CO_2 enrichment (Ebi et al., 2021). Despite its advantages, challenges such as the high cost of implementing and maintaining CO_2 enrichment systems persist, particularly in developing or underdeveloped countries (Chakrabarti et al., 2012). Therefore, there is a pressing need for simpler CO_2 enrichment systems that can benefit indigenous species and improve crop yields and food security in these regions.

The versatility of ICES can potentially extend its application to various regions and crops worldwide. In this study, okra, a tropical plant species was shown to be improved in various variables related to growth and physiology (Figures 3 and 4) under elevated CO_2 conditions. Therefore, this study shows interdisciplinary aspects of knowledge from different fields into a working and functional high CO_2 system for both mycelium development and plant growth. The present study opens up an avenue for future investigations on the impact of CO_2 enrichment on plants, presenting a promising avenue for further research in this field. In other words, the ICES system presents a simple, sustainable, practical, reproducible, versatile, and reliable solution for studying the effects of elevated CO_2 on plant physiology and growth.

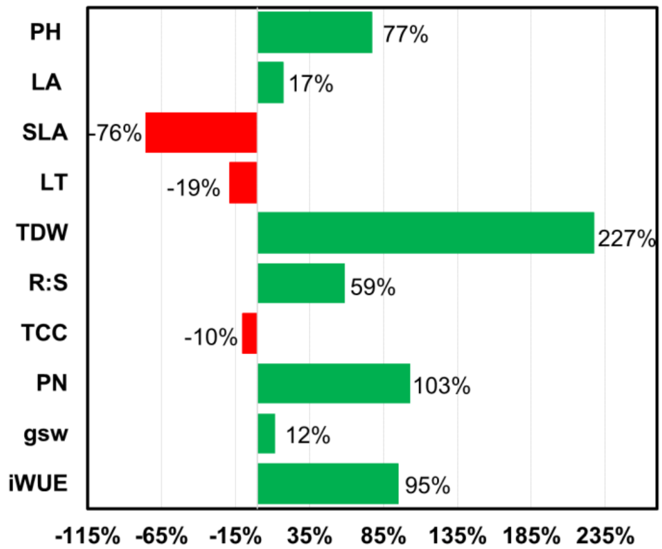


Figure 4. Summary for percentage changes in growth and physiological parameters of eCO_2 compared to aCO_2 seedlings. The response ratio was calculated as a relative variation of each parameter under eCO_2 treatment using the aCO_2 as a control. Value bars facing the left and right indicate decreases and increases in properties compared to the value of aCO_2 treatment, respectively. PH plant height, LA leaf area, SLA specific leaf area, LT leaf thickness, TDW total dry weight, R:S root to shoot ratio, TCC total chlorophyll content, PN photosynthesis, GSW stomatal conductance, iWUE intrinsic water use efficiency.

Correlation Between Plant Growth and Leaf Physiology Parameters

Spearman's rank correlation was performed to determine the relationship between all growth and physiological parameters for aCO_2 and eCO_2 treatments at P = 0.05 (Figure 5). The parameter in the correlation analysis revealed interesting relationship among the tested variables. Among the growth parameters for aCO_2, significant strong correlations were observed between 3 pairs of variables, namely between TDW and SLA, between TDW and R:S, and between R:S and SLA. Among these, negative correlations were observed between TDW and SLA, and between TDW and R:S. On the other hand, R:S shows a significant positive correlation with SLA. Only two pairs of variables were observed for eCO_2 showing significant strong correlations that existed between TDW and SLA, and between TDW and LA. Among these variables, TDW showed significant negative correlation with SLA but positive strong correlation with LA. These findings suggested that under aCO_2 conditions, plants allocate resources differently compared to eCO_2 conditions. A higher TDW might be associated with thicker leaves (lower SLA) and a greater allocation of resources to roots (lower R:S) in aCO_2. Under eCO2, increased TDW might be linked to larger leaf size (higher LA) without necessarily affecting leaf thickness. Other growth variables showed no significant correlation with moderate to weak relationships for both aCO_2 and eCO_2.

Between the physiological parameters of aCO_2 treatment, only iWUE and GSW showed a significant negative correlation. For eCO_2, iWUE and GSW showed a significant strong negative correlation, and PN and GSW were observed to show a positive strong correlation. These results indicated that under both CO_2 conditions, plants tend to improve water use efficiency by reducing stomatal conductance. In addition, enhanced stomatal conductance facilitated increased gas exchange within the leaf, thereby promoting CO_2 uptake and subsequently boosting photosynthetic rates. The results aligned with previous research demonstrating a close relationship between photosynthetic rate and stomatal conductance, with higher rates of photosynthesis typically associated with increased stomatal conductance (Yin et al., 2020).

Between growth and physiological parameters, the correlation analysis revealed four pairs of variables with significantly strong correlations from aCO_2 treatment while none of eCO_2 showed a significant strong correlation. For aCO_2, among the significant strong correlations, LA and iWUE showed negative correlations. larger leaves often exhibit higher stomatal density (Maylani et al., 2020), leading to increased stomatal conductance and, consequently, lower iWUE. On the other hand, LA and GSW showed positive correlations. The observed positive correlation provided further support for the hypothesis that larger leaves tend to have higher stomatal densities. This was consistent with previous findings by Maylani et al. (2020). Non-significant variables with moderate negative correlation were between PH and iWUE, between LA and TCC, between SLA and iWUE, between TDW and TCC, and between TDW and GSW. Positive moderate relationships were shown between SLA and PN, between SLA and GSW, between LT and TCC, and between R:S and GSW. Other variables of aCO_2 and eCO_2 were either moderate to weak, or uncorrelated.

The observed differences in correlations between growth and physiological parameters under aCO_2 and eCO_2 conditions highlight the complex responses of plants to elevated CO_2. While increased CO_2 availability can enhance photosynthesis and growth, it can also affect plant water use efficiency and resource allocation. Further research is needed to elucidate the underlying mechanisms driving these correlations and to assess the potential implications for plant productivity and water use under future climate scenarios.

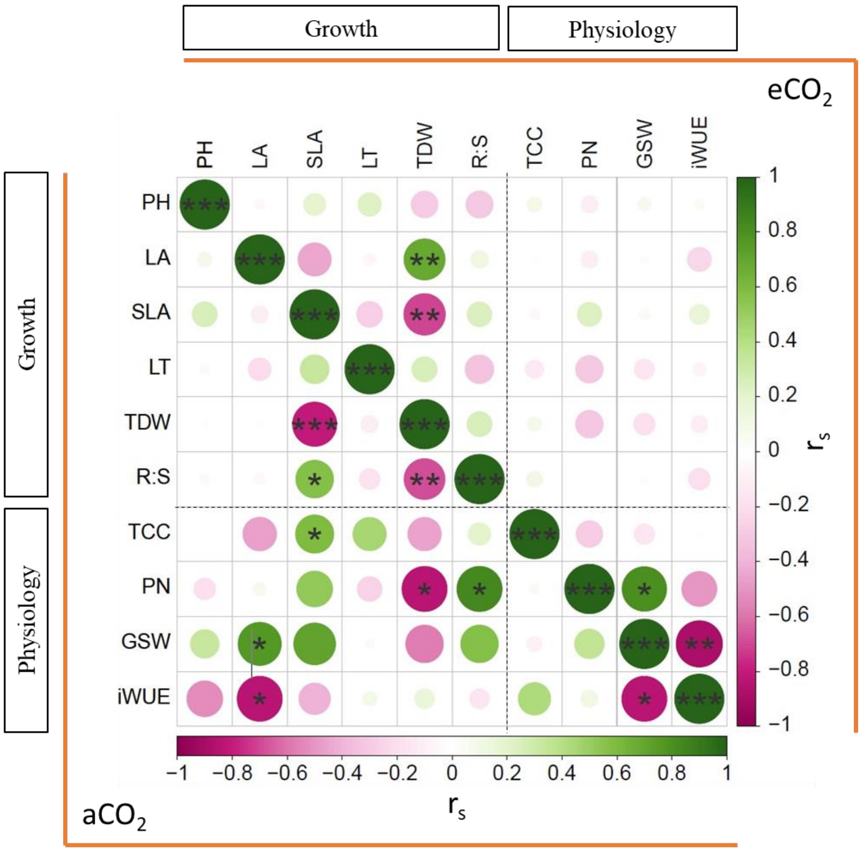


Figure 5. Spearman's rank correlation coefficients between growth and leaf physiology parameters of okra seedling (MKBe 1). *, **, *** Statistically significant at $P < 0.05$, 0.01 and 0.001, respectively. r_s Spearman's rank correlation coefficient. Non-star circle means not statistically significant at $P = 0.05$. Green and pink circles indicated positive and negative correlations, respectively. The size and colour intensity of circle indicated the relative strength of correlation between parameters. Large circles mean strong correlation and small circle means weak correlation. PH plant height, LA leaf area, SLA specific leaf area, LT leaf thickness, TDW total dry weight, R:S root to shoot ratio, TCC total chlorophyll content, PN photosynthesis, GSW stomatal conductance, iWUE intrinsic water use efficiency.

CONCLUSION

In conclusion, CO_2 enrichment techniques have long been recognised for their value in enhancing crop yield and quality, offering pivotal insights into physiological processes like photosynthesis, respiration, and transpiration. However, conventional CO_2 sources pose challenges due to costliness and environmental impact. Thus, this study introduces the potential of leveraging natural CO_2 enrichment through fungal respiration to boost okra plant growth, demonstrated through our In-House CO_2 Enrichment Structure (ICES). The resulting okra seedlings under this high CO_2 concentration of natural origin showed a notable increase in overall biomass accumulation (+277%), carbon assimilation rate (+103%), and improved water use efficiency (+95%) compared to ambient CO_2 levels setting. These promising findings not only highlight the capability of mycelium as a natural CO_2 generator but also its economic potential for farmers. The versatility and reliability of ICES make it an attractive option for enhancing crop growth across diverse settings. By providing valuable insights into innovative and sustainable CO_2 enrichment techniques, this study paves the way for further research in this field. Ultimately, our findings contribute to the exploration of cost-effective solutions aimed at enhancing agricultural productivity and sustainability on a global scale

ETHICAL APPROVAL

Not applicable.

AUTHORS CONTRIBUTION

TLS, MAK and MJ performed the analysis and wrote the paper. KSC conducted the experiment, collected the data, and performed the analysis. AAMM and KK performed the analysis and checked and approved the submission. AF contributed data or analysis tools and provided valuable insights and expertise in the interpretation of the results. NY conceived and designed the analysis, provided valuable insights and expertise in the interpretation of the results and wrote the paper.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

FUNDING

This project was supported by a Royal Society GCRF Challenge-Led grant CHL\R1\18007 "Breeding Rice Resilient to a High CO_2 Future" awarded to N.Y. and AJF.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Professor Andrew Fleming from the University of Sheffield, UK, for his insightful comments and suggestions, which greatly enriched the scope and quality of this work. We also express our gratitude to Madam Surina Saad from Nas Agro Farm for her valuable contributions to this study. Her guidance and support in providing advice and information on mushroom cultivation were indispensable in ensuring the success of our research.

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