***Pak. J. Bot.*, 56(3): 905-910, 2024.** **DOI: http://dx.doi.org/10.30848/PJB2024-3(5)**

EFFECTS OF ELEVATED CO_2 ON RICE SEEDLING ESTABLISHMENT OF MR219 AND SRI MALAYSIA 1 VARIETIES

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Abstract

Rice (*Oryza sativa* L.) is one of the most important members of the *Poaceae* family as this crop has been the staple food for people in various nations, especially in Asian countries. Current climate changes and increasing carbon dioxide (CO_2) concentration in the atmosphere have varying global impacts on crop performance. As CO_2 is one of the limiting factors in photosynthesis, adding this gas can increase carboxylation activity, hence increasing productivity and yield. Thus, this research was conducted to study the effects of elevated CO_2 (eCO_2) on rice seedlings' growth and establishment for MR219 and Seri Malaysia1 varieties. The study used a novel approach where the rice plants were treated with high CO_2 only during their early vegetative stage before being transplanted into the field. The source of CO_2 for eCO_2 condition was obtained from baker's yeast fermentation which was 600 to 800 µmol mol^{-1}. For the ambient CO_2 (aCO_2), it was 410 µmol mol^{-1} to 415 µmol mol^{-1} and control at field condition. Rice seedlings were grown in a nested design with 15 replications for four weeks in a growth chamber under Light-emitting diode (LED) lights (white, red, and blue). The seedlings in the control treatment were grown in the field. The results demonstrated that the leaf properties of rice seedlings, for instance, leaf length, leaf number per plant, and leaf area, were increased by 9.20%, 10.28%, and 25.67%, respectively, in eCO_2 compared to control. Similarly, the general growth properties such as seedling length and seedling dry weight were increased by 18.25 and 34.21% respectively, under eCO_2 compared to control.

Key words: Atmospheric CO_2, CO_2 enrichment, Elevated CO_2, *Oryza sativa* L., Rice seedling growth.

Introduction

Rice is a semi-aquatic annual grass plant with 22 species belonging to the *Oryza* genus (Khush, 1997). The two most common rice species for human consumption are *Oryza sativa* L. and *Oryza glaberrima* L. (Khush, 1997). According to Muthayya *et al*., (2014), *Oryza sativa* is the most common rice type that has become the staple food for nearly 3.5 billion people worldwide. It is one of the world's most important crops and the primary source of nutrition for a large number of populations in Asian countries (Wang *et al*., 2011). Furthermore, rice is consumed by more than half of the world's population, and 90% of rice is produced and consumed in Asian countries (Jing *et al*., 2016), where more than 60% of the world's population lives (Khush, 2005).

The population of rice-producing and consuming nations has lately increased significantly; thus, there is an urgent need to increase food production to fulfil half of the world's food demand. There is a need to increase rice production by 40% by 2030 due to the increasing population in several nations and the decrease in the supply of staple food, leading people in the developing world to suffer from malnutrition (Khush, 2005). Moreover, it was projected that the world population will reach 8.5 billion by 2030 and 9.7 billion by 2050 (Anon., 2019). Hence, the demand for rice grain will continue to rise in the coming years due to the increase in the population growth and reduction in cropland (Wang *et al*., 2011). Currently, the worldwide production of milled rice is about 495.8 million tonnes (Shahbandeh, 2021). It is estimated that, the net demand for rice will increase to 525 million tons by 2050 due to population growth in some Asian countries (Abdullah & Adhana 2006).

Global warming is a controversial modern climatic phenomenon that has been caused by the significant increase of CO_2 levels in the atmosphere (Wang *et al*., 2011). Various environmental factors affect crop production, especially rice production, such as air temperature, atmospheric CO_2, light, water, and soil nutrients (Patendol *et al*., 2015). Among the factors mentioned above, the most critical factors affecting rice production worldwide are the increase in atmospheric CO_2 concentration and temperature. CO_2 levels in the atmosphere are higher now than at any time in the past (Long *et al*., 2004), which is about 416 µmol mol^{-1} (Tans & Keeling, 2021). Crop physiological and yield performance have changed and positively impacted by eCO_2 concentration. For example, CO_2 has increased photosynthesis and crop water use efficiency in rice (Hasegawa *et al*., 2013). The plants' most important response to high CO_2 levels in the atmosphere is to increase growth and yield (Wohlfahrt *et al*., 2018).

High CO_2 and high temperature have impacted rice growth stages, especially tillering and grain filling, which is considered vulnerable concerning other growth stages at higher CO_2 and high temperatures (Liu *et al*., 2017). Carbon dioxide enrichment for 33 days earlier to flowering enhanced yield components, including the number of grains and grain weight by 30%, but CO_2 enrichment for one month after flowering enhanced yield components including grain weight and filled grain percentage by 10% (Yoshida, 1973). Based on the above obtained results from the previous reports it is hypothesized that eCO_2 will affect positively rice seed establishment and will increase rice seedling growth. Previous studies on eCO_2 on rice seedling establishment have also been limited; however, the current study was designed to assess the impact of eCO_2 on rice seedling growth and performance in the first month of seedling establishment. CO_2 is among the constraints of photosynthetic activity, so increasing the amount could speed up the carboxylation process. The present work proposes a new approach in which eCO_2 only enriches rice seedlings during their early vegetative phase before being transplanted into a specific cultivation area, rather than regularly applying CO_2 during the growing period. The objective of the present study was to evaluate how eCO_2 influenced rice seedling establishment before they could be transplanted into the field for MR219 and Seri Malaysia1 rice varieties.

Materials and methods

Experimental location, design, and treatments: The experiment was performed in the Physiology Laboratory, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (latitude 2° 59′ 22.812″ N and longitude 101° 43′ 33.2256″ E). A nested design was used to conduct the study with varieties nested within the CO_2 treatment. Three levels of CO_2 treatment were evaluated, which were (i) eCO_2 (600 - 800 µmol mol^{-1}), (ii) aCO_2 (410 - 415 µmol mol^{-1}), and (iii) control (410 - 415 µmol mol^{-1} CO_2). Both ambient and control treatments had the same CO_2 level (410-415 µmol mol^{-1}) except that aCO_2 used LED as the source of light, and the growing phase was carried out in the laboratory. The control had grown in a rain shelter in Field 15, Faculty of Agriculture, UPM. There were 15 replications of the treatment combinations of varieties and CO_2 treatments. The rice plants were grown in plastic containers for four weeks.

Chamber design and construction: The seedlings were grown in eCO_2 and aCO_2 conditions inside two large plastic containers (70 cm height, 50 cm length, and 40 cm depth). The boxes were covered with plastic film and placed on the top section of the growth chamber. To circulate air and prevent the formation of a gas potential gradient during the experiments, a small portable ventilator operated using 12V DC was installed in the plastic container used for ambient treatments. The CO_2 source was located on the top of the chamber in a 2.5-liter plastic tank, and a pipe was connected from the CO_2 source tank to the eCO_2 box to supply the CO_2 for seedlings grown in the container.

Seedlings establishment: Every plastic cup (12 cm width and 6 cm diameter) was filled with 240 g paddy field soil from Tanjung Karang, Selangor, on March 8, 2019. The seeds were washed with water to clean the sources and remove the dead and empty seeds. Then, at a depth of 2-2.5 cm in the soil, five seeds were directly sown in each cup. During the third leaf stage, the plants were thinned to three plants per cup.

Prior to the sowing of the seeds, 15 replications of each rice variety were randomly placed in the plastic container in both aCO_2 and eCO_2 treatments. To prevent CO_2 leakage, the top part of the box was protected with plastic film, particularly the eCO_2 box. A 10 cm gap was cut on the outer side of the eCO_2 container for gas exchange. The ambient box, on the other hand, was covered entirely as this box had a small electronic ventilator fan to circulate air in the container.

The seedlings were illuminated using ten cool white light-emitting diodes (LED) tube lamps (PHILIPS, 1600 lumens, 16-watt,9290011846c, China). The distance between seedlings and light was set at 30 to 50 cm for uniform light interception by the plants. Additional seven rows of blue and red LED strip lights (Get home Da7339, China) were installed between the white tube lights to provide adequate photosynthetic active radiation (PAR) for plant growth. The plants were exposed to the light for 12 hours. This duration is equivalent to a day from 7 a.m. to 7 p.m., and an electronic timer was used to switch on and off the light at the precise time during the experiment.

Production and source of CO_2: The carbon dioxide source in this experiment was obtained from the fermentation of baker's yeast (Saccharomyces cerevisiae) in a sugar solution. A 500 g of sugar and 10 g of baker's yeast were mixed in 2 L of tap water. This solution produced about 600-800 µmol mol^{-1} of CO_2 within a period of one week to treat the seedlings. Throughout the experiment, a CO_2 meter (OEM, TEMP/RH, data logger, China) was used to monitor and record the level of carbon dioxide, temperature, and humidity inside the boxes.

Plant sampling, measurements, and data collection

Leaf morphology: The fully expanded leaf 5th was used to conduct the leaf morphology. A ruler was used to measure the leaf length (cm) from the tip to the basal end of the leaf that reached the sheath. A ruler was also used to measure the leaf width (mm) in the middle of the leaf. In comparison, the leaf thickness was determined with a thickness gauge (Aluminum Alloy, DTNR-0055, China) from around the middle parts of the left and right sides of the leaf, and the average thickness from both sides was calculated. Finally, for all procedures, the total number of leaves on the seedling was manually counted. Then, the same leaf was cut near the sheath crown for image capture. The Image J software (Version 1.52 v) was used to determine the leaf area (cm^2) (Schneider *et al.*, 2012)

General growth properties measurements: The seedling length (cm) was measured with a ruler from the soil surface to the tip of the fully expanded leaf 5. The plants were removed from the soil, and the roots were washed to remove the soil. After drying for 24 hours at 50ºC in an oven, the roots and shoots were weighed separately using an electronic weighing scale (Kern & Sohn, PCE-ABT, China). Then, the root to shoot dry weight ratio was calculated.

Physiological attributes

Relative chlorophyll content (SPAD value), chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* ratio, and total chlorophyll measurements: The chlorophyll content of the fully expanded leaf 5 was measured three times using a chlorophyll meter in the canter of the leaf (SPAD-502, Minolta, Japan) and the average relative chlorophyll content (SPAD values) were calculated. Chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* ratio, and total chlorophyll contents were determined using leaf 5 samples using the method described in detail by Coombs *et al*., (1986). For each treatment, three-one-cm^2 samples were prepared. The samples were placed in bottles containing 20 ml of acetone (80%) and stored in the dark for one week. The chlorophyll was then measured using a spectrophotometer (Model Shimazu, Japan) at 647 nm and 664 nm with 3.5 ml of the solution (Coombs *et al*., 1986). Total chlorophyll content on the same samples was calculated by adding the chlorophyll *a* and *b* calculated previously.

Data analysis

All data were subjected to analysis of variance (ANOVA) for nested design in SAS (Statistical Analysis Software, Version 9.4) (SAS Institute Inc, NC, USA). The ANOVA assumptions (normality and constant variance) were tested to ensure that the data were appropriate for ANOVA. The ANOVA for nested design tested the effects of factor A (CO_2 treatment) and factor B, the nested factor (Var (CO_2)). Due to the nested structure, where each level of one factor is only present with one level of the other factor, thus the interaction effects between the factors could not be estimated. Finally, post-hoc LSD (least significant difference) testing was conducted where the means were significantly different at $p<0.05$.

Results

The experiment used a nested design, with the variety nested in the CO_2, and was denoted Var (CO_2). Due to the nested structure, the interaction effects between the factors could not be estimated. Instead, the effects of each CO_2 treatment and variety nested within each CO_2 treatment were assessed. Leaf properties, general growth properties, and physiological properties were all measured in this experiment. Leaf length, width, thickness, area, and number are all parameters for leaf properties. Leaf width and leaf thickness, for both varieties and treatments were not statistically significant ($p>0.05$). Physiological properties include relative chlorophyll content, chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* ratio, and total chlorophyll of leaf 5. No significant differences ($p>0.05$) in physiological properties parameters were observed.

Leaf properties: Regardless of rice varieties, there were significant differences in rice seedling leaf length between CO_2 treatments at p<0.05. In contrast to the control, eCO_2 resulted in a 9.20% increase in leaf length. When compared to the control (26.93 cm) and ambient (25 cm) treatments, the eCO_2 treatment had a longer leaf length (29.41 cm). There were significant differences p<0.05 between the eCO_2 and ambient treatments, but no differences between the eCO_2 and control with ambient treatments (Table 1).

There was no significant difference ($p>0.05$) in leaf width between CO_2 treatments at. However, the leaf widths for the ambient, control, and eCO_2 treatments were ranged from 0.46 cm to 0.48 cm, respectively (Table 1). There were no significant differences ($p>0.05$) in leaf thickness between CO_2 treatments. For all treatments, leaf thickness ranged from 0.045 mm to 0.057 mm.

There were significant differences between CO_2 treatment on leaf area at $p<0.05$. In comparison to the control, leaf area increased by 25.67%. As shown in Table 1, the eCO_2 treatment had the highest mean value leaf area (7.49 cm^2), while the ambient (6.39 cm^2) and control (5.96 cm^2) treatments had no significant differences.

There were also significant differences ($p<0.05$) in the number of leaves per seedling between CO_2 treatments. In the eCO_2 condition, the leaf number increased by 10.28% compared to the control. The eCO_2 treatment had the highest leaf number per seedling (5.79 leaves), followed by ambient (5.46 leaves) and control (5.25 leaves). At the same time, no significant differences ($p>0.05$) were found between eCO_2 and aCO_2 treatments, as well as control and aCO_2 treatments (Table 1).

General growth properties: Significant differences between the eCO_2 treatments were found for seedling length at p<0.05. In comparison with the control treatment, seedling length increased by 9.20% under the eCO_2 treatment. The eCO_2 treatment had the highest mean value for the seedling length of 48.72 cm, followed by the control treatment with 41.20 cm, and ambient 38.75 cm. However, there was no statistically significant difference (p>0.05) between ambient and eCO_2 treatments (Table 2). The difference in seedling dry weight between the eCO_2 treatments was also significant at p<0.05. In the eCO_2 condition, seedling dry weight increased by 34.21% compared to the control. The eCO_2 treatment had the highest mean value for the seedling dry weight (0.51 g), followed by ambient (0.40 g) and control (0.38 g), with no significant difference between ambient and control treatments. The seedling root to shoot dry weight ratio did not differ significantly between treatments p>0.05. However, for all treatments, the root-to-shoot dry weight ratio ranged from 0.69 to 0.80 g. (Table 2).

Physiological properties

Relative chlorophyll content (SPAD value), chlorophyll *a*, *b*, *ab* ratio, and total chlorophyll: For all treatments, there were no significant differences ($p>0.05$) in the relative chlorophyll content (SPAD value). However, the relative chlorophyll content ranged from 27.01 to 28.23 SPAD values (Table 3). There were no significant differences ($p>0.05$) in chlorophyll *a* level between CO_2 treatments in both varieties. Chlorophyll *a* value ranged from 2.59 to 2.95 µmol (Table 3). Furthermore, no significant differences ($p>0.05$) in chlorophyll *b* levels were found between CO_2 treatments. However, there were significant differences ($p<0.05$) for Var (CO_2), and there were significant differences ($p<0.05$) between treatments for the MR219 variety. At the same time, there were no significant differences ($p>0.05$) between control and eCO_2 treatments, and similar results were observed for Var (CO_2) and Sri Malaysia 1 variety as presented in (Table 3). Furthermore, the chlorophyll *ab* ratio did not vary significantly ($p>0.05$) between CO_2 treatments at $p<0.05$. Nonetheless, there was a trend for eCO_2 treatment to increase chlorophyll *ab* ratio, which was 1.08 µmol compared to 1.07 µmol for control and 0.93 µmol for ambient (Table 3). Finally, no significant differences ($p>0.05$) in total chlorophyll were found between treatments. On the other hand, total chlorophyll ranged from 6.32 to 6.67 ml/cm^2 (Table 3).

Table 1. The effects of elevated CO_2 on leaf properties measured on leaf 5 of rice seedlings grown in the Physiology Laboratory, Faculty of Agriculture, UPM, Serdang, Selangor in 2019.

Treatment	Leaf length (cm)	Leaf width (cm)	Leaf thickness (mm)	Leaf area (cm^2)	Leaf number
Ambient CO_2	25.34 ± 0.72^{b}	0.48 ± 0.02	0.045 ± 0.006	6.39 ± 0.22^{b}	5.46 ± 0.12^{ab}
Control	26.93 ± 0.49^{ab}	0.49 ± 0.03	0.048 ±0.006	5.96 ± 0.14^{b}	5.25 ± 0.12^{b}
Elevated CO_2	29.41 ± 1.08^{a}	0.46 ± 0.03	0.057 ± 0.005	7.49 ± 0.31^{a}	5.79 ± 0.17^{a}

Within each column, means with the same letter are not significantly different (p>0.05) using LSD. Values are the mean ± SE of three plants and four replications (n=12)

Table 2. The effects of elevated CO_2 on general growth properties of rice seedlings grown in the Physiology Laboratory, Faculty of Agriculture, UPM, Serdang, Selangor in 2019.

Treatment	Seedling length (cm)	Seedling dry weight (g)	Root to shoot dry weight ratio
Ambient CO_2	38.75 ± 1.01^{b}	0.40 ± 0.02^{b}	0.69 ± 0.07
Control	41.20 ± 0.72^{b}	0.38 ± 0.04^{b}	0.76 ± 0.07
Elevated CO_2	48.72 ± 0.85^{a}	0.51 ± 0.02^{a}	0.80 ± 0.06

Within each column, means with the same letter are not significantly different at p>0.05 using LSD. Values are the mean ± SE of three plants and four replications (n=12)

Table 3. The effects of elevated CO_2 on physiological properties of rice seedlings leaf 5, grown in the Physiology Laboratory, Faculty of Agriculture, UPM, Serdang Selangor in 2019.

Treatment	Relative chlorophyll content (SPAD value)	Chlorophyll *a* (µmol)	Chlorophyll *b* (µmol)^{a}		Chlorophyll *ab* ratio	Total chlorophyll (mg/cm^2)
			MR219	SRM1		
Ambient CO_2	27.01 ± 0.85	2.59 ± 0.13	2.85 ± 0.27^{b}	2.79 ± 0.03^{a}	0.93 ± 0.01	6.32 ± 0.31
Control	28.23 ± 0.71	2.95 ± 0.24	3.16 ± 0.03^{a}	2.37 ± 0.04^{b}	1.07 ± 0.02	6.67 ± 0.55
Elevated CO_2	27.36 ± 0.68	2.84 ± 0.16	3.08 ± 0.04^{a}	2.25 ± 0.05^{b}	1.08 ± 0.03	6.38 ± 0.36

Within each column, means with the same letter are not significantly different at p<0.05 using LSD. Values are the mean ± SE of three plants and four replications (n=12)

[a] for this parameter we explain the effects of variety because the Var (CO_2) is significant. SRM1 Sri Malaysia 1

Discussion

The leaf length of the MR219 and Sri Malaysia1 varieties increased by 9.20% compared to the control. The increase in leaf length could be attributed to the high cell number and an increase in cell length, as Tsutsumi *et al.*, (2014) reported similar results. Similarly, Li *et al.*, (2008) reported that a short-term eCO_2 treatment of 700 µmol mol^{-1} significantly increased the leaf 7th elongation rate.

The leaf area of rice seedlings of both varieties increased by 25.67% compared to the control in the current research. This is likely due to an increase in cell division and elongation. An increase in leaf area index by 8% was observed in eCO_2 by Wang *et al.*, (2015), who conducted a meta-analysis study on the production of rice when exposed to aCO_2 (330-420 µmol mol^{-1}), and eCO_2 (500-800 µmol mol^{-1}).

The number of leaves was 10.28% higher throughout the eCO_2 treatments than in the control. A high number of leaves is associated with a faster rate of growth, as illustrated by greater leaf length in eCO_2 compared to control. In addition, leaf width and leaf thickness were not significantly different in this research. However, some studies reported that leaf width decreased while leaf thickness mainly increased in the uppermost fully expanded leaves (leaf 8–13) when grown at various levels of N supply (very low, low, and excess N) (Tsutsumi *et al.*, 2014). Therefore, this finding may suggest that the difference in leaf width and thickness could be due to the age of the leaf and N levels.

In terms of general growth properties, eCO_2 increased seedling length and dry weight in both varieties, resulting in higher above-ground biomass. The height of the seedlings increased by 18.25% for both varieties compared to the control. This result is similar to Abzar *et al.*, (2017), who reported that seedling length was higher in the eCO_2 (800 µmol mol^{-1}) treatments than in the ambient (400 µmol mol^{-1}) treatments. However, Lamichaney *et al.*, (2019) reported that seedling length did not significantly differ between the CO_2 levels used in the study (ambient, 510 µmol mol^{-1}, 610 µmol mol^{-1}, and 720 µmol mol^{-1}).

Elevated CO_2 increased seedling dry weight (g) by 34.21% in both varieties. The seedling root-to-shoot dry weight ratio was not affected by the eCO_2 condition. Seedling growth was enhanced by eCO_2, as predicted, by increasing seedling length and dry weight. At the same time, Vu *et al.*, (1997) found that eCO_2 increased leaf photosynthetic CO_2 assimilation in rice.

Furthermore, CO_2 had no significant effect on physiological properties such as relative chlorophyll content (SPAD value), chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* ratio, and total chlorophyll. Conversely, Zhang *et al.*, (2012) reported that chlorophyll *a*, b, and total chlorophyll were significantly reduced by 26.2%, 13.3%, and 13.6%, respectively, when *Impatiens hawkeri* was exposed to eCO_2 at 380 µmol mol^{-1} and 760 µmol mol^{-1} for ten weeks. Despite no significant changes in the overall chlorophyll contents in rice seedlings observed in this study, the rice seedlings significantly enhanced some general growth parameters with the presence of eCO_2.

It was implied that increasing the vigour of seedlings, the response, and the early development of tillers after transplanting in rice fields is the basis for coordinating the rice source-sink relationship in hybrid rice (Bai *et al*., 2016). Vigorous seedlings of hybrid rice tend to produce more tillers earlier and rapidly turn green after transplanting due to the high number of tillers and root production during the seedling stage (Bai *et al*., 2016). The photosynthates movement from source to sink (grains) also improves in that of the panicles that emerged from the early developed tillers that possess improved vascular systems. Moreover, the capacity of the source for photosynthates has improved, which resulted from increased leaf area and longer vegetative growth duration in hybrid rice. In the current study, a short duration of eCO_2 treatment exposed to rice seedlings before transplanting to the field enhanced the growth of the seedlings. It is hypothesized that these vigorous seedlings will have a better growth performance in the field and improve grain yield production.

Conclusion

It is observed that elevated CO_2 (eCO_2) treatment during the early stage of seedling growth has significant effects on many rice growth parameters for both MR219 and Sri Malaysia1 rice varieties. Compared to control, rice seedlings grown in eCO_2 have improved leaf properties, such as leaf area and leaf number. On the other hand, the CO_2 treatments have no impact on leaf width and thickness. Furthermore, eCO_2 increases general growth properties such as seedling length and dry weight compared to the control. Rice seedling's establishment of both rice varieties under the study was positively affected by eCO_2 treatment than the control. Rice seedling's eCO_2 treatment enhanced leaf length by 9.20%, leaf area by 25.67%, leaf number 10.28%, seedlings height by 18.25%, and dry weight by 34.21%. Other parameters are not significantly affected by eCO_2 treatments, including seedling root-to-shoot dry weight ratio and physio-biochemical properties, namely relative chlorophyll content (SPAD value), chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* ratio, and total chlorophyll. Based on the observation during the experiment, especially on leaf and general growth parameters, it is suggested that the eCO_2 treatment can produce vigorous rice seedlings. As a result, this will shorten the seedling establishment duration in the nursery, leading to faster field transplanting. Thus, it is also recommended that further study be carried out to determine the optimum amount of CO_2 concentration and different time duration for seedlings establishment under eCO_2 treatment.

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(Received for publication 15 January 2023)