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N Vinothini

Department of Seed Science and
Technology, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu, India

V Manonmani

Department of Seed Science and
Technology, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu, India

S Sundareswaran

Department of Seed Science and
Technology, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu, India

S Karthikeyan

Department of Bioenergy, Tamil
Nadu Agricultural University,
Coimbatore, Tamil Nadu, India

N Maragatham

Department of Farm
Management, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu, India

S Srinivasan

Department of Crop Physiology,
Tamil Nadu Agricultural
University, Coimbatore, Tamil
Nadu, India

Correspondence**N Vinothini**

Department of Seed Science and
Technology, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu, India

Biochemical changes associated with elevated carbon dioxide in rice (*Oryza sativa* L.)

N Vinothini, V Manonmani, S Sundareswaran, S Karthikeyan, N Maragatham and S Srinivasan

Abstract

The environmental change has tremendous effect in crop production in the form of increased stress all through the plant growth and development. Consequently the aim of the investigation was to assess the effect of elevated carbon dioxide under artificially induced carbon dioxide condition utilizing an open top chamber with rice genotype CO 51. Rice could provide less protein, nutrients and minerals fundamental for humans be in response to rising carbon dioxide concentrations that are involved in global warming. Increased concentrations of atmospheric carbon dioxide are anticipated to decrease the content of fundamental component, for example, protein in rice, threatening the nutrition of billions of people in the middle of the century. However, changes in plant-based nutrient substance in response to carbon dioxide have not been elucidated. The results from the present investigation revealed that the increased carbon dioxide have negative effects on soluble protein content (mg g^{-1}) and nitrate reductase activity ($\mu\text{mol NO}_2 \text{g}^{-1} \text{hr}^{-1}$) and positive effects on sucrose phosphate synthase ($\mu \text{mol mg}^{-1} \text{protein min}^{-1}$) and starch content (mg g^{-1}) in different crop developing stages.

Keywords: Rice, elevated carbon dioxide, nutrient quality, enzymes

1. Introduction

The environment is continuously changing as a result of man's activities the present investigation is to anticipate, how agriculture will react to the changing environment condition of tomorrow. One of the real main thrusts for worldwide environmental change is the rapid rise in the greenhouse gas concentration in the environment. The human activities have prompted the change of greenhouse gases, for example, methane, carbon dioxide, water vapor, nitrous oxide and chlorofluorocarbons (Gitz *et al.*, 2016; Kizildeniz *et al.*, 2018; Vinothini *et al.*, 2019) [7, 11, 20]. The sensational raise in human population since the pre-industrial period, raised concentration of these gases because of extensive scale utilization of fossil fuels, deforestation, industrialization and urbanization. The carbon dioxide content of atmosphere had been incredibly steady for a few centuries, striking at a normal concentration of around 280 ppm. With the beginning of industrial revolution 1874 the equilibrium was annoyed and the carbon dioxide content of the air started to rise in this manner to remarkable financial development (IPCC, 2014) [10].

The global climate change due to the increase in greenhouse gases, especially CO_2 , is having a direct or indirect effect on sustainable agriculture and food production. For the past 800,000 years, the CO_2 level was maintained up to a level between 180–200 and 250–280 ppm before the beginning of the industrial revolution. Once humans started to burn fossil fuels in the industrial era, things changed rapidly. In 2010, the industrialization changed the condition by rising global annual mean of CO_2 to reach the current level of 400 ppm. The intergovernmental panel on climate change (IPCC) suggests that there will be a rise of atmospheric CO_2 to a concentration of 550 ppm by 2050 due to the anthropogenic emission of greenhouse gases (IPCC, 2014) [10].

Possible upcoming climatic change coming about because of expanded atmospheric carbon dioxide concentration and other greenhouse gases, particularly the increase in temperature, hampers our capacity to anticipate worldwide rice production and raises serious inquiries concerning food security for the near future (Myers *et al.*, 2017) [16]. Higher concentrations of carbon dioxide are related with decreases in protein and different key nutrients in rice. Rice is the essential source of nourishment for in excess of 2 billion individuals. Diminishes in the nourishing substance of rice could disproportionate affect wellbeing results in the poorest

rice-dependent countries (Ebi and Zizka, 2018) [3]. This investigation gives important insights into how higher CO₂ concentrations could influence the protein content of rice. Along these lines, this experiment was to study about impact of elevated CO₂ on biochemical changes in rice.

2. Materials and Methods

The field experiments were carried out in open top Chamber (OTC) with elevated CO₂ and ambient condition, established at Wetland Farm, Department of Farm Management, Tamil Nadu Agricultural University, Coimbatore. The air enriched CO₂ is distributed through a circular tube and the uniform distribution of the carbon dioxide within the chamber was ensured by air blowers. The CO₂ analyzer established in the OTC measures the concentration of carbon dioxide within and the computer regulated inlet valves will control the flow of the gas (Vinothini *et al.*, 2019) [20].

To study the effect of elevated CO₂ on biochemical parameters, the seeds of rice variety CO 51 were sown in a nursery bed and after 20 days, the seedlings were transplanted in the open top chamber with elevated CO₂ and in ambient condition. The field trial was laid out in Randomized Block Design with eight replications. The following observations were recorded at three stages of crop growth and development *viz.*, i) Active tillering stage (24 to 42 DAT), ii) Heading stage (55 to 60 DAT) and iii) Grain filling stage (80 to 85 DAT) in the plants raised in the open top chamber chamber as well as under ambient conditions. The details of the treatments are as follows.

2.1 Treatment details

Environmental condition		Level of CO ₂ (ppm)
E ₁	Ambient condition	400±9
E ₂	Open Top Chamber + Ambient CO ₂	400±9
E ₃	Open Top Chamber + Elevated CO ₂	550

The following biochemical parameters were recorded.

2.2 Starch content

Starch content was determined by the Anthrone method (Mcready *et al.*, 1950) [15].

2.3 Soluble protein content (mg g⁻¹)

Leaf soluble protein was estimated by the method suggested by Lowry *et al.* (1951) [13].

2.4 Sucrose phosphate synthase (μ mol mg⁻¹ protein min⁻¹)

Sucrose phosphate synthase activity was measured by the method of Huber (1992) [9].

2.5 Nitrate reductase activity (μmol NO₂ g⁻¹ hr⁻¹)

Nitrate reductase activity in the leaves at different stages was estimated by the method suggested by Evans and Nason (1953) [4].

2.6 Statistical analysis

The analysis of variance was carried out and comparison was done by Duncan's multiple range test (DMRT). The mean difference is significant at the P-values < 0.05. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., Chicago, USA).

3. Result and Discussion

3.1 Soluble protein content (mg g⁻¹)

Soluble protein content was showed a significant difference due to a different environmental condition. The increase in soluble protein content was observed from tillering stage to the heading stage and a gradual decrease towards the grain filling stage. The highest soluble protein content (5.07, 8.03 and 7.11) registered in ambient condition at 30, 60 and 85 DAT followed by OTC + ambient CO₂ (4.95, 7.29 and 6.74) and the lowest was registered in OTC + elevated CO₂ (4.52, 6.98 and 6.24) (Table 1). Longer exposure to raised CO₂ results in acclimation of photosynthesis with down-guideline of the degree of Rubisco protein. This coarse control of the degree of Rubisco protein serves to improve CO₂ acquiring with use of the fixed carbon. Notwithstanding coarse control of Rubisco protein, there are fine controls, which react all the more quickly to changes in environmental conditions. In such manner, two important components are known to manage Rubisco action. One includes the reversible carboxylation of lysine buildup in the dynamic site to actuate the enzyme, while the different operates by the reversible binding of 2-carboxyarabinitol-1 phosphate, the Rubisco dark inhibitor, to the carboxylates site (Zhu *et al.*, 2018) [21]. Nitrogen deficiency brought about the diminishing in the content of protein, including Rubisco, and chlorophyll, and decrease in photosynthetic capacity and carboxylation effectiveness. Under this hypothesis, diminishes observed in Rubisco may reflect a general decrease of leaf protein, because of reallocation of nitrogen inside the plant (Makino, 2003; Taub, 2008) [14, 19].

Table 1: Changes in soluble protein content (mg g⁻¹) of rice under ambient and elevated CO₂ condition

Treatments	Soluble protein content (mg g ⁻¹)		
	Tillering stage	Heading stage	Grain filling stage
E ₁ - Ambient condition	5.07±0.02 ^a	8.03±0.05 ^a	7.11±0.05 ^a
E ₂ - OTC + Ambient CO ₂	4.95±0.01 ^b	7.29±0.08 ^b	6.74±0.06 ^b
E ₃ - OTC + Elevated CO ₂	4.52±0.04 ^c	6.98±0.02 ^c	6.24±0.03 ^c

Data presented are means from eight replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at *P*<0.05

3.2 Starch content (mg g⁻¹)

The environmental condition had a significant influence on starch content at various growth stages of the plant. Among the environmental condition, the highest starch content was recorded in OTC + elevated CO₂ (52.6, 69.6 and 54.6) followed by OTC + ambient CO₂ (42.4, 54.6 and 46.3), while

the lowest was in ambient condition (32.8, 49.5 and 38.7) at 30, 60 and 85 DAT (Table 2). Under elevated CO₂ condition, a major storage carbohydrate starch is also increased in plants (Aranjuelo *et al.*, 2008) [2]. This increase in starch likely contributes to the high levels of sucrose observed with elevated CO₂, due to the conversion of starch to sucrose overnight. For normal plant growth conversion of starch to sucrose is essential and increased in starch likely contributes to the high level of sucrose (Zhu *et al.*, 2016) [22]. However, under elevated CO₂ it may contribute to the accumulation of sucrose. In plants grown under ambient CO₂ the starch

content builds up during the day and disappears overnight. The increased production of starch under elevated CO₂, however, means that not all of the plant's starch reserves are

depleted during the night, leading to a gradual accumulation in leaves over time (Aoki *et al.*, 2003) [11].

Table 2: Changes in starch content (mg g⁻¹) of rice under ambient and elevated CO₂ condition

Treatments	Starch content (mg g ⁻¹)		
	Tillering stage	Heading stage	Grain filling stage
E ₁ - Ambient condition	32.8±0.09 ^c	49.5±0.7 ^c	38.7±0.5 ^c
E ₂ - OTC + Ambient CO ₂	42.4±0.09 ^b	54.6±0.4 ^b	46.3±0.6 ^b
E ₃ - OTC + Elevated CO ₂	52.6±0.04 ^a	69.6±0.1 ^a	54.6±0.7 ^a

Data presented are means from eight replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$

3.3 Sucrose phosphate synthase ($\mu\text{mol mg}^{-1}\text{ protein min}^{-1}$)

Sucrose phosphate synthase was significantly influenced by environmental condition. The increase in soluble protein content was observed from tillering stage to the heading stage and a gradual decrease towards the grain filling stage. Highest sucrose phosphate synthase of (23.1, 25.3 and 23.9) in OTC + elevated CO₂ was followed by OTC + ambient CO₂ (20.8, 23.1 and 21.0) and the lowest in ambient condition (20.1, 22.4 and 20.2) at 30, 60 and 85 DAT (Table 3). The raised CO₂ up controlled activities of the sugar metabolizing enzymes, sucrose phosphate synthase (SPS) and adenosine-5'-diphosphoglucose pyrophosphorylase (AGP), bringing about more prominent accumulation and export of

carbohydrates related with photosynthetic activities, in spite of decrease in rubisco activity and Rubisco protein content (Sun *et al.*, 2011). Gesch *et al.* (2002) [18, 6] revealed that raised CO₂ altogether increased the activity of sucrose phosphate synthase (a key protein in the production of sucrose) and ADP glucose phosphorylase (a key enzyme in starch synthesis in rice). Lee *et al.* (2005) [12] likewise revealed an increase in the sucrose phosphate synthase activity in rice at raised CO₂. The increased sucrose phosphate synthase activity was because of the reallocation of nitrogen far from Rubisco and into sucrose phosphate synthase, along these lines prompting a more prominent sucrose synthesis and export of photosynthates from the leaves. These investigations infer that rice responds to increasing CO₂ concentrations by moving the priority of allocation of sugars more towards export than starch storage, thus preventing the feedback inhibition.

Table 3: Changes in sucrose phosphate synthase ($\mu\text{mol mg}^{-1}\text{ protein min}^{-1}$) of rice under ambient and elevated CO₂ condition

Treatments	Sucrose phosphate synthase ($\mu\text{mol mg}^{-1}\text{ protein min}^{-1}$)		
	Tillering stage	Heading stage	Grain filling stage
E ₁ - Ambient condition	20.1 ± 0.24 ^c	22.4 ± 0.30 ^c	20.2 ± 0.23 ^b
E ₂ - OTC + Ambient CO ₂	20.8 ± 0.14 ^b	23.1 ± 0.07 ^b	21.0 ± 0.20 ^b
E ₃ - OTC + Elevated CO ₂	23.1 ± 0.20 ^a	25.3 ± 0.31 ^a	23.9 ± 0.20 ^a

Data presented are means from eight replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$.

3.4 Nitrate reductase activity ($\mu\text{mol NO}_2\text{ g}^{-1}\text{ hr}^{-1}$)

The difference in nitrate reductase activity was significant due to an environmental condition. The gradual decrease in nitrate reductase activity was observed during the grain filling stage. The highest nitrate reductase activity was observed in ambient condition (1.35, 3.42 and 2.62) at 30, 60 and 85 DAT followed by OTC + ambient CO₂ (1.02, 3.11 and 2.13) while

the lowest (0.93, 2.38 and 1.42) was registered in OTC + elevated CO₂ (Table 4). Raised CO₂ prolonged accumulation of carbon in various plant parts. In spite of the point that the nitrate reductase action increased, the nitrogen concentration and the soluble protein content in the plant parts were diminished. Raise in temperature decreased the nitrate reductase activity and the accumulation of carbon and nitrogen in various plant parts. Though, this decrease was more under raised CO₂. Raised CO₂ positively influenced the nitrate reductase activity (Fujita *et al.*, 2002; Rakshit *et al.*, 2012; Hofmann *et al.*, 2013) [5, 17, 8].

Table 4: Changes in nitrate reductase activity ($\mu\text{mol NO}_2\text{ g}^{-1}\text{ hr}^{-1}$) of rice under ambient and elevated CO₂ condition

Treatments	Nitrate reductase activity ($\mu\text{mol NO}_2\text{ g}^{-1}\text{ hr}^{-1}$)		
	Tillering Stage	Heading Stage	Grain filling Stage
E ₁ - Ambient condition	1.35 ± 0.004 ^a	3.42 ± 0.05 ^a	2.62 ± 0.04 ^a
E ₂ - OTC + Ambient CO ₂	1.02 ± 0.007 ^b	3.11 ± 0.03 ^b	2.13 ± 0.02 ^b
E ₃ - OTC + Elevated CO ₂	0.93 ± 0.001 ^c	2.38 ± 0.03 ^c	1.42 ± 0.01 ^c

Data presented are means from eight replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$.

4. Conclusion

The anthropogenic increase in the atmospheric carbon dioxide concentration will influence the crop growth both by rising

the atmospheric temperature in addition to by changing the biological processes in the plant system. Response of rice under elevated CO₂ using the open top chamber exhibited a significant increase in growth and development. The sucrose phosphate synthase along with the content of starch was increased. Elevated CO₂ positively affected the soluble protein content and nitrate reductase activity of the rice.

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