



# Interactive Effect of Elevated Carbon Dioxide, Temperature and Nitrogen on the Growth Parameters of Rice at Active Tillering Stage

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Ever since industrialization began, carbon dioxide [CO<sub>2</sub>] concentration in the atmosphere got increased and it consecutively raised the temperature. This change in climate would affect the agricultural production and crop growth. A pot culture experiment was conducted during the Kuruvai

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season (June - September) of the year 2023 at Anbil Dharmalingam Agricultural College & Research Institute, Tiruchirappalli to assess the rice growth up to active tillering stage under elevated [CO<sub>2</sub>] and temperature conditions at different doses of nitrogen (N) fertilization. Rice seedlings were grown inside the four Open Top Chambers (OTC) at ambient (422 ppm) and elevated CO<sub>2</sub> (550 ppm) conditions as well as chamber controlled and elevated temperature (+2°C) with four different doses of N (control, 100% recommended dose of N (RDN), 150% RDN and 200% RDN). Plant growth parameters like plant height, number of tillers, leaf area, root length and root volume, root and shoot dry matter were increased significantly under elevated [CO<sub>2</sub>] (eCO<sub>2</sub>) conditions. Whereas, all the growth parameters were severely influenced by increased temperature. Increasing N concentration increased the plant growth irrespective of the treatments and the effect was more pronounced in eCO<sub>2</sub> treatment due to the fertilization effect of [CO<sub>2</sub>] as it directly necessitates photosynthesis which improves overall growth of rice plants. Under the future changing climatic conditions suitable N doses should be optimized for the better crop production.

*Keywords: Elevated carbon dioxide; temperature; rice; sodic soil; nitrogen; plant growth.*

## 1. INTRODUCTION

Carbon dioxide [CO<sub>2</sub>] is a naturally occurring chemical compound that plays an indispensable role in the Earth's ecosystem. It is often referred to greenhouse gas as it allows the sunlight to enter but restricts it to leave, thus heating the lower atmosphere. The [CO<sub>2</sub>] concentration in the atmosphere continues to rise and it increased from 270 ppm (pre industrial revolution) to 384 ppm (2009) and to 394 ppm [1,2]. Currently the average concentration of [CO<sub>2</sub>] in the atmosphere is 422 ppm [3]. Long and Feng et al. [4,5] predicted through models that the [CO<sub>2</sub>] concentration would rise to 550 ppm by 2050 and ascend up to 800 ppm by the end of the century.

The increment in anthropogenic greenhouse gas emission, especially [CO<sub>2</sub>] emission contributes to global warming and the earth's surface temperature has increased by 0.76°C since pre industrial times and if it continues to increase at the same rate, it is predicted to increase by 1.5°C between 2030 and 2052 [6]. It is projected to increase by 1.8 - 4.0°C in the next century [7]. Undoubtedly, the relationship between increasing CO<sub>2</sub> and temperature will have significant impacts on global climate change and agricultural productivity [8].

The increase in carbon dioxide levels in the atmosphere do not stress the plants, in spite, it enhances crop growth through enhanced photosynthesis [9,10]. Whereas, increase in atmospheric temperature affect the crop growth by inducing changes in water relations, accumulation of compatible osmolytes, decrease in photosynthesis, hormonal changes and cell membrane thermo stability [11,12].

Rice is the most important cereal crop and about 11% of the cultivable land is under rice cultivation [13]. Global rice production has increased from 115 M ha in 1961 to 167 M ha in 2018. In India, rice is cultivated in an area of 42.5 M ha with a production of 152.6 MT [14]. Being a predominant dietary energy source for three-fourth of India's population, it plays a key role in country's food security [15]. The net demand for rice will increase to 525 million tonnes by 2050 due to population growth in some Asian countries [16].

Climate change is expected to be a major factor in meeting this demand and ensuring food security in future. Consequently, it is essential to evaluate the sensitivity of rice to increased atmospheric [CO<sub>2</sub>] levels and temperature. Moreover, impact of climate change on rice production is location-specific, due to the varying temperature sensitivity of the crop in different agro climatic regions [17]. It has been observed by many researchers that when CO<sub>2</sub> or temperatures rises, the development stages of crops get affected [18,19]. In rice, at tillering stage, a variety of morphological signs are observed, including withering of the leaves, curling and yellowing of the leaves, decrease in the number of tillers, and a decrease in biomass [20].

Nitrogen (N) is a macronutrient that is necessary for all living things. The synthesis of many important biomolecules like amino acid, protein, nucleic acid, chlorophyll and some plant hormones depend on the availability of N. As a result, the use of N fertilizers has become a major determinant of crop yield [21]. The increase in crop growth under high [CO<sub>2</sub>] conditions will necessitate a higher rate of

nutrient uptake and assimilation. Many researchers from different part of the world have indicated that the increased yield of crops in elevated  $[CO_2]$  ( $eCO_2$ ) condition reduces the N content of the soil and demands the application of higher doses of N [22]. [23] revealed that increase in N application rate consequently increased the root and shoot biomass production of rice under  $eCO_2$ .

It is estimated that approximately 6.74 million hectares of land in India are affected by salt. Sodic soils occupy 47% of land in 11 states and salt affected soils account for approximately 5% of net cultivated area [24]. To feed the increasing population production of food crops in problem soils are also to be increased. In spite of the need for the research, only a few studies are available on the growth of rice under  $eCO_2$  and temperature conditions in sodic soil. Hence, it is important to analyze the effect of  $eCO_2$  and elevated temperature (eT) on the growth of rice at different levels of N in a sodic soil.

## 2. MATERIALS AND METHODS

### 2.1 Site

The experiment was conducted during the Kuruvai season (June - September, 2023) in the Open Top Chamber (OTC) facilities available at Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli, Tamil Nadu, India. The study site is located at  $10^\circ 45' N$  and  $78^\circ 36' E$  and 85 m above mean sea level. The climate of Tiruchirappalli is tropical and comes under a warm-humid climatic zone with the summers being rainier than the winters. The annual mean rainfall of Tiruchirappalli is 895 mm. The mean maximum temperature is  $35^\circ C$  and minimum temperature is  $24^\circ C$ .

### 2.2 Experimental Details

Open Top Chambers (OTC) (Dimension:  $3 \times 3 \times 4$  m, M/s. Genesis Technologies, Maharashtra, India) were used to study rice growth under sodic soils as induced by  $eCO_2$  concentration and or temperature at varying levels of N fertilization. In the OTC,  $[CO_2]$  gas is supplied through the gas cylinders (30 kg capacity) filled with 100% commercial grade  $[CO_2]$  and maintained at set levels using manifold gas regulators, pressure pipelines, solenoid valves, sampler, pump,  $[CO_2]$  analyzer, PC linked supervisory control and data acquisition

(SCADA). The required concentration of  $[CO_2]$ , was supplied to each chamber through the PVC pipes connected and delivered through the small pores present on the circular tubes (6 mm polyurethane tube) which were fitted inside the chamber at crop canopy level. The top of the chamber is left open in order to facilitate the circulation of air and to maintain a consistent temperature and humidity. Multilayered clear polycarbamate sheets with 85-90% light transmission were used to avoid the light hindrance for photosynthesis. The elevated temperature was maintained by infrared lamps (IR) inside the OTC and were controlled by the automatically calibrated temperature sensors. Totally four OTC were used in the experiment which facilitated the  $[CO_2]$  and temperature combinations as follows in Table 1.

**Table 1. Details of the experimental chambers**

<b>OTC 1</b>	Ambient $[CO_2]$ (422 ppm) and chamber-controlled temperature
<b>OTC 2</b>	Elevated $[CO_2]$ ( $550 \pm 25$ ppm) and chamber-controlled temperature
<b>OTC 3</b>	Ambient $[CO_2]$ (422 ppm) and elevated temperature ( $+2^\circ C$ )
<b>OTC 4</b>	Elevated $[CO_2]$ ( $550 \pm 25$ ppm) and elevated temperature ( $+2^\circ C$ )

The experiment was laid out in factorial completely randomized design (FCRD) with OTC treatments as one factor and N levels as another factor. Totally there were 16 treatments and the experiment was replicated thrice. The OTC treatments were as detailed in Table 1. The four N levels imposed were: control ( $N_0$ ), 100 % recommended dose of nitrogen (RDN) based on STCR ( $N_{100}$ ), 150 % RDN ( $N_{150}$ ) and 200 % RDN ( $N_{200}$ ).

### 2.3 Crop Management

The sodicity tolerant rice variety TRY 5 was used for the study. It is a short duration (105-110 days) variety suitable to grow under both Navarai (December - February) and Kuruvai (June - September) seasons of Tamil Nadu. Nursery sowing was taken up during June, 2023 and twenty-one days old seedlings were transplanted to the pots filled with nine kg of soil. The soil was sandy clay loam in texture with pH of 9.17 and exchangeable sodium percentage of 22.08. The soil was low in available nitrogen ( $221 \text{ kg ha}^{-1}$ ), medium in available phosphorus ( $19.3 \text{ kg ha}^{-1}$ ) and high in available potassium ( $294 \text{ kg ha}^{-1}$ ). Two seedlings were maintained for each pot. Soil

Test Crop Response - Integrated Plant Nutrition System (STCR-IPNS) equations developed for sodic soil was used for calculating the fertilizer dose and was 165:55:32 kg N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O ha<sup>-1</sup>. Nitrogen as urea, P as superphosphate and K as muriate of potash was applied to each pot on weight basis. As per the Crop Production Guide, 2020, N and K were applied in four splits (basal, active tillering, flower initiation, and heading stages) and entire quantity of P was applied as basal. All the pots received uniform application of P and K and N was applied as per the treatments. Gypsum and zinc were applied as per the recommendation. Standing water level of five cm was maintained throughout the study.

## 2.4 Plant Sampling and Measurement

The effect of the OTC treatments at varied levels of N was studied up to the tillering stage. The plants were uprooted carefully from each treatment with the help of khurpi and the adhering soil particles were washed with running water to get rid of it. The growth parameters like plant height, number of leaves, number of tillers, length of leaves, width of leaves was measured. The plants were separated into root and shoot and dried in an oven for 72 hours at 65°C till to obtain constant weight. Oven dry weight of shoot and root was recorded.

The leaf area was worked out as outlined by Palaniswamy et al. [25] using 0.75 (K) as the factor by multiplying the leaf length (L) and leaf width (W) with number of leaves hill<sup>-1</sup>(N). Partitioning coefficient of the shoot and root was calculated by dividing the oven dry shoot and root dry matter to the total dry matter.

## 2.5 Statistical Analysis

Statistical analysis of the data was done using ANOVA (analysis of variance) technique recommended [26] for the design to test whether the difference between means were statistically significant or not.

## 3. RESULTS AND DISCUSSION

### 3.1 Plant Height and Number of Tillers of Rice at Active Tillering Stage

Plant height and number of tillers showed significant variation with OTC treatments (T) and nitrogen (N) independently and also with the interaction effect (TxN) (Fig. 1). Plant height is an

important morphological trait which is associated with grain yield [27]. The number of tillers produced by the plant will result in maximum number of potential inflorescence and panicle leading to maximum yield. In the present study, the maximum plant height and number of tillers at active tillering stage was recorded under eCO<sub>2</sub> at all levels of N application. When compared to ambient, eCO<sub>2</sub> recorded 12.59 % increase in plant height and 31.16 % increase in the number of tillers. eCO<sub>2</sub> concentration might have resulted in increased photosynthesis, limited leaf respiration (carbon dioxide fertilization effect) favoring the plant growth and tiller formation.

[28] claimed that plants grown under eCO<sub>2</sub> produced more ethylene than plants grown under ambient [CO<sub>2</sub>]. Increase in ethylene production is the key feature that accelerated growth and development in rice under eCO<sub>2</sub> that improved the plant height and number of tillers. Lee et al. [21] also observed significant increase in plant height and number of tillers per plant under eCO<sub>2</sub>, when compared to that of plant grown under ambient conditions. In contrast, elevated temperature (eT) showed a negative impact and decrease in plant height and number of tillers to the tune of 7.7 % and 22.37 % was observed when compared to ambient. Number of tillers in eCO<sub>2</sub> + eT was on par with the ambient treatment. The negative effects of elevated temperature were compensated by the eCO<sub>2</sub> concentration [29]. Nitrogen fertilization showed a positive response and maximum plant height was observed in N<sub>200</sub>. Availability of more nitrogen could have resulted in more translocation of nutrients to the above ground part resulting in higher plant height and a greater number of tillers.

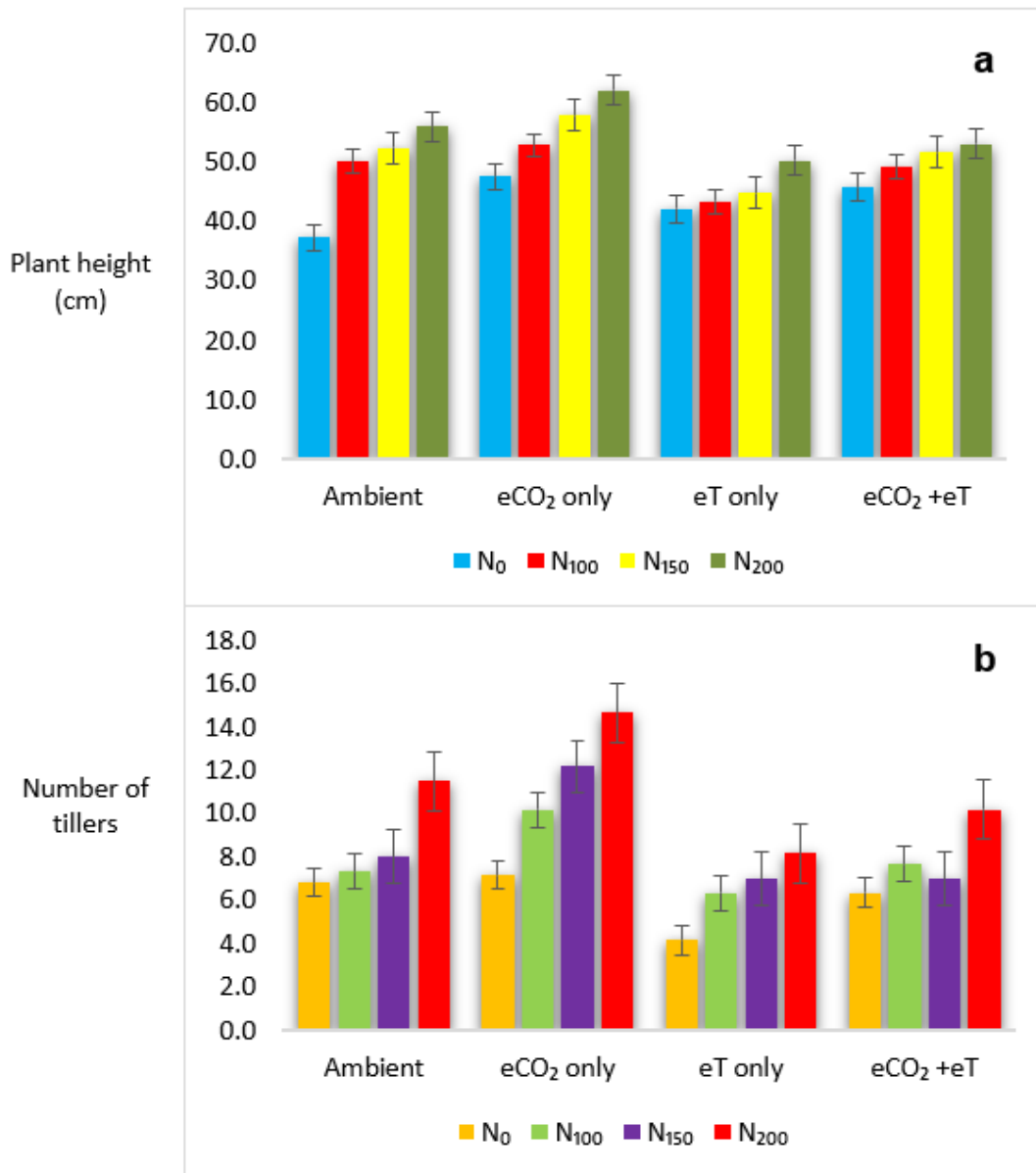
### 3.2 Leaf and Root Parameters of Rice at Active Tillering Stage

Leaf length and leaf width of the rice plants increased significantly under eCO<sub>2</sub> concentration and decreased under eT condition. Leaf area is a fundamental measure of the conversion of light energy to chemical energy in plant leaves. Many researchers from different part of the world [30-32] found that increase in [CO<sub>2</sub>] concentration increased the growth parameters due to [CO<sub>2</sub>] fertilization effect.

As a result of increase in plant growth parameters leaf area of rice increased significantly under eCO<sub>2</sub> conditions (Table 2). An

increase in leaf area (45%) was observed under eCO<sub>2</sub> conditions. [33] also observed increase in leaf area at early stage of the crop in eCO<sub>2</sub> treatment. Contrary, a decrease of 40% in leaf area was observed under eT showing the inhibitory effect of eT on leaf length, leaf width and leaf area in rice at active tillering stage. This might be due to the reason that temperature inside the chamber increased more than the

optimum temperature for normal growth and development of rice. This increase in temperature could interrupted the normal physiological and biochemical function of rice resulting in decreased leaf area [8]. Positive interaction of OTC treatments and N was observed. Higher N availability facilitated the growth and tiller production resulting in increased leaf area.



**Fig. 1. Effect of open top chamber treatments and nitrogen levels on plant height (a) and number of tillers (b) of rice at active tillering**

Ambient: Ambient [CO<sub>2</sub>] + chamber-controlled temperature, eCO<sub>2</sub>= elevated [CO<sub>2</sub>], eT = elevated temperature. N<sub>0</sub> = no N fertilizer, N<sub>100</sub> = 100% of the RDN, N<sub>150</sub> = 150% of RDN, N<sub>200</sub> = 200% of RDN.

**Table 2. Effect of OTC treatments and varied levels of nitrogen on leaf and root parameters of rice at active tillering stage**

<b>Leaf and Root parameters</b>	<b>N levels</b>	<b>Ambient</b>	<b>eCO<sub>2</sub></b>	<b>eT</b>	<b>eCO<sub>2</sub> + eT</b>
<b>Leaf length (cm)</b>	N <sub>0</sub>	27.6	33.8	29.9	31.8
	N <sub>100</sub>	35.1	34.7	30.8	33.4
	N <sub>150</sub>	36.6	36.6	33.5	35.2
	N <sub>200</sub>	37.4	38.8	34.1	36.5
	<b>Source</b>	<b>T</b>	<b>N</b>	<b>T x N</b>	
	<b>SEd</b>	<b>0.55</b>	<b>0.55</b>	<b>1.10</b>	
	<b>CD (p=0.05)</b>	<b>1.10</b>	<b>1.10</b>	<b>2.20</b>	
<b>Leaf Width (cm)</b>	N <sub>0</sub>	0.66	0.76	0.70	0.76
	N <sub>100</sub>	0.73	0.90	0.76	0.81
	N <sub>150</sub>	0.75	0.95	0.85	0.86
	N <sub>200</sub>	1.00	1.05	0.86	0.98
	<b>Source</b>	<b>T</b>	<b>N</b>	<b>T x N</b>	
	<b>SEd</b>	<b>0.02</b>	<b>0.02</b>	<b>0.05</b>	
	<b>CD (p=0.05)</b>	<b>0.05</b>	<b>0.05</b>	<b>NS</b>	
<b>Leaf Area (cm<sup>2</sup>)</b>	N <sub>0</sub>	381.8	553.4	272.0	531.2
	N <sub>100</sub>	565.9	973.5	475.3	863.9
	N <sub>150</sub>	653.3	1293.9	603.9	1153.8
	N <sub>200</sub>	1234.1	1794.5	733.8	1561.6
	<b>Source</b>	<b>T</b>	<b>N</b>	<b>T x N</b>	
	<b>SEd</b>	<b>38.80</b>	<b>38.80</b>	<b>77.61</b>	
	<b>CD (p=0.05)</b>	<b>77.23</b>	<b>77.23</b>	<b>154.47</b>	
<b>Root length (cm)</b>	N <sub>0</sub>	13.0	15.3	12.9	16.4
	N <sub>100</sub>	14.5	17.5	15.9	18.8
	N <sub>150</sub>	18.7	20.8	18.5	19.3
	N <sub>200</sub>	21.9	23.0	20.6	22.8
	<b>Source</b>	<b>T</b>	<b>N</b>	<b>T x N</b>	
	<b>SEd</b>	<b>0.47</b>	<b>0.47</b>	<b>0.95</b>	
	<b>CD (p=0.05)</b>	<b>0.95</b>	<b>0.95</b>	<b>NS</b>	
<b>Root volume (g/cc)</b>	N <sub>0</sub>	1.3	2.1	1.6	1.8
	N <sub>100</sub>	2.3	2.6	2.3	2.5
	N <sub>150</sub>	2.8	3.1	2.6	2.8
	N <sub>200</sub>	3.0	3.6	2.8	3.1
	<b>Source</b>	<b>T</b>	<b>N</b>	<b>T x N</b>	
	<b>SEd</b>	<b>0.46</b>	<b>0.46</b>	<b>0.94</b>	
	<b>CD (p=0.05)</b>	<b>0.94</b>	<b>0.94</b>	<b>NS</b>	

### 3.3 Root Length and Root Volume at Active Tillering Stage

Root length and root volume in eCO<sub>2</sub> and eCO<sub>2</sub> + eT increased significantly with the control. Increase in nitrogen doses further increased the root growth and volume. It has been well shown by various workers that CO<sub>2</sub> enrichment showed a positive effect on root growth, adventitious root number, root hair development and root elongation [34,35].

### 3.4 Shoot and Root Dry Matter Production at Active Tillering Stage

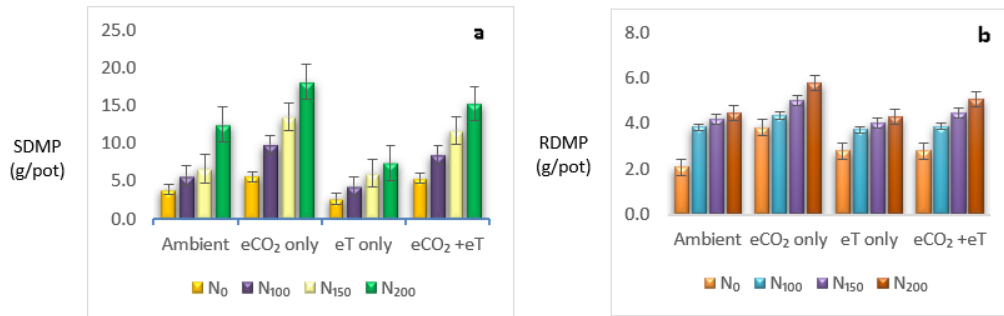
At active tillering stage, both shoot dry matter production (SDMP) and root dry matter production (RDMP) was influenced by OTC

treatments, N levels and their interactions. The SDMP recorded was in the order of eCO<sub>2</sub> (11.76 g /pot) > eCO<sub>2</sub>+ eT (10.17 g /pot) > ambient CO<sub>2</sub>+ chamber-controlled temperature (7.16 g/pot) > eT (5.11 g /pot). The primary mechanism favoring enhanced crop growth under eCO<sub>2</sub> followed by eCO<sub>2</sub>+eT is increased photosynthesis rate due to the [CO<sub>2</sub>] fertilization effect. The stimulation of above ground biomass appears to be proportional to the N supply in all the OTC treatments and RDMP also showed similar increase [3] observed that the above ground biomass of rice at 62 days after transplanting (DAT) increased significantly in eCO<sub>2</sub> treatment at different levels of N supplementation. Similar results were obtained by [36].

### 3.5 Shoot and Root Partitioning Coefficients at Active Tillering Stage

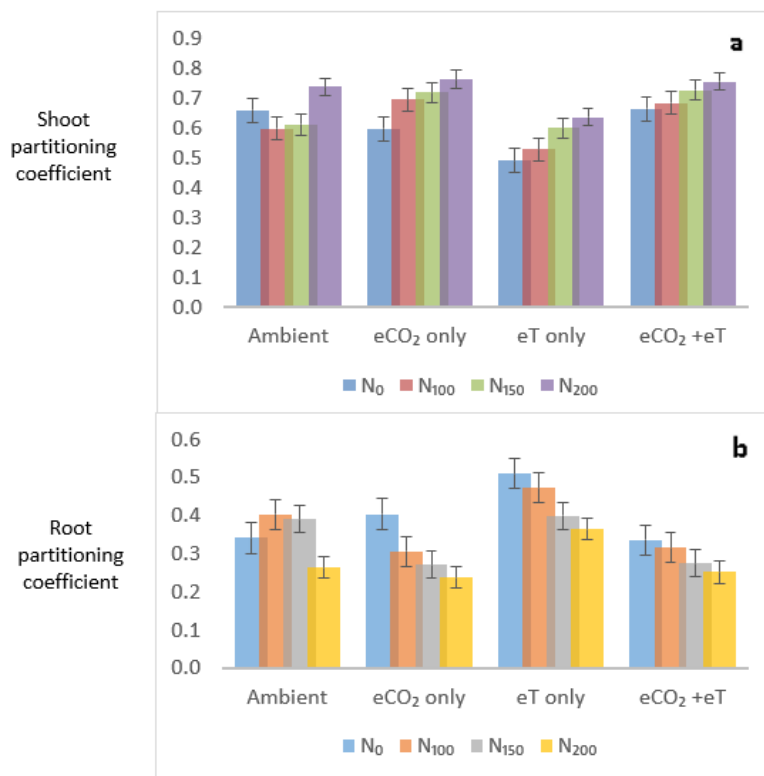
Shoot partitioning coefficient of rice increased in both eCO<sub>2</sub> and in eCO<sub>2</sub> + eT treatments. Plants grown under these conditions invested more assimilates for shoot growth than for root growth. But the root partitioning coefficient was higher

under elevated temperature conditions [37,38]. The reduced plant growth in the elevated temperature conditions resulted in the high root partitioning coefficient. Similar results were obtained from the studies conducted by [21]. Favorable effect of N fertilization on shoot partitioning coefficient was observed and the values increased from N<sub>0</sub> to N<sub>200</sub>.



**Fig. 2. Effect of open top chamber treatments and nitrogen levels on shoot dry matter (a) and root dry matter production (b) of rice at active tillering**

*Ambient: Ambient [CO<sub>2</sub>] + chamber-controlled temperature, eCO<sub>2</sub>= elevated [CO<sub>2</sub>], eT = elevated temperature. N<sub>0</sub> = no N fertilizer, N<sub>100</sub> = 100% of RDN, N<sub>150</sub> = 150% of RDN N<sub>200</sub> = 200% of RDN.*



**Fig. 3. Effect of open top chamber treatments and nitrogen levels on shoot partitioning coefficient (a) and root dry partitioning coefficient (b) of rice at active tillering**

*Ambient: Ambient [CO<sub>2</sub>] + chamber-controlled temperature, eCO<sub>2</sub>= elevated [CO<sub>2</sub>], eT= elevated temperature. N<sub>0</sub> = no N fertilizer, N<sub>100</sub> = 100% of RDN, N<sub>150</sub> = 150% of RDN N<sub>200</sub> = 200% of RDN*

#### 4. CONCLUSION

Rice at active tillering stage, under sodic soil, with the eCO<sub>2</sub> concentration resulted in increasing the growth due to carbon fertilization effect which ultimately resulted in higher dry matter production. The elevated temperature retards the growth and dry matter production in rice. Whereas, in combined effect of elevated temperature and elevated carbon dioxide, the negative effect of higher temperature was partly compensated by increased [CO<sub>2</sub>] concentration. Further, the crop growth was accelerated by the additional doses of N fertilizer especially in eCO<sub>2</sub> conditions. But the effect of increased N doses in growth parameters of eT and eCO<sub>2</sub>+eT is not prominent. Hence, here comes the significance of optimizing suitable N doses for rice under future climatic conditions.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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