

Effect of elevated CO₂ and temperature on chlorophyll content and growth attributes of rice-wheat cropping system in central India

Abstract

Rice-wheat is a major cropping system in India and it is predicted that the productivity of both crops will decline due to climate change factors including elevated CO₂ and temperature. To define the mechanisms, a field experiment was carried out to evaluate the effect of elevated CO₂ and temperature on growth attributes of rice and wheat crops using a Free Air CO₂ Enrichment (FACE) system. The treatments were ambient CO₂+ambient temperature, elevated CO₂ of 600ppm+ambient temperature, ambient CO₂ + elevated temperature (+2°C), ambient CO₂+elevated temperature (+3°C), elevated CO₂600ppm +elevated temperature (+2°C) and elevated CO₂600ppm +elevated temperature (+3°C). Elevated CO₂ and elevated temperature (+2°C or +3°C) strongly affected the crop growth. Elevated CO₂ stimulated leaf chlorophyll content, root-shoot length and biomass yield. However, elevated temperature inhibited chlorophyll content in both the crops. Elevated CO₂ enhanced chlorophyll content by 12.9–19% in rice and 8.8–16.5% in wheat. Elevated temperature reduced chlorophyll content by a range of 20.5-27.3% in rice and 6.3-11.5% in wheat. Combined effect of elevated CO₂ and elevated temperature decreased the leaf total chlorophyll and plant biomass in both crops. Study highlights that elevated CO₂ concentration and rising temperature may affect the photosynthesis and productivity of rice and wheat crop in central India.

Key words: Chlorophyll, Elevated CO₂, rice-wheat, temperature, vertisol

Introduction

“Wheat (*Triticum aestivum*) and rice (*Oryza sativa*) are the two major food crops, which provides food security for more than 90% of the world’s population”(Albahri et al. 2023). “The production of food needs to increase by 70% by 2050 to meet the demands of a growing population”(Ghosh et al. 2024). “Crop production is highly affected by change in the global climate, mostly changes in CO₂ gas concentration in atmosphere. The concentration of atmospheric CO₂ is increasing at 3% per year. Models predict that even with stringent strategies to minimize greenhouse gas (GHG) emission, the concentration of CO₂ will rise to 550–700 ppm by 2050 and 650–1200 ppm by 2100”(Ishtiaque et al. 2022). “The rise in atmospheric greenhouse gasses (GHGs) will increase the mean global temperature up to 2.5 °C by 2050 and of up to 6.4 °C by the end of this century”(Yin 2013; Stocker 2014).

“Extreme climatic events (ECEs), such as warming and droughts, acts as major climate factors affecting plant growth and pose a growing threat to agriculture in coming years”(Change 2007). “To assess food security for future generation, it is necessary to find out the responses of wheat and rice to elevated CO₂ and temperature. Number of experiments have been conducted to find out the responses of wheat and rice to elevated CO₂ and increased temperature in experiments in the field and in controlled-environment chambers”(Tian et al. 2014; Wang et al. 2018, 2019; Broberg et al. 2019; Ben Mariem et al. 2021; Marcos-Barbero et al. 2021; Abdelhakim et al. 2022).

“Evaluation of climate factors on crops are best evaluated using Free Air CO₂ Enrichment (FACE) system, as crop responses to elevated CO₂ and temperature are close to natural environment under FACE than open top chamber (OTC) or greenhouse conditions”(Allen et al. 2020). “An increase in CO₂ concentration of the ambient air can enhance leaf photosynthesis of

C₃ species, including wheat, rice, and many other cereal crops, leading to enhanced growth and increased yields”(Vijayalakshmi et al. 2024). However, yield enhancement by elevated CO₂ alone should be evaluated carefully, due to two reasons. First, elevated CO₂ is associated with rise in atmospheric temperature. In general, rising temperature enhances phenological indicator of crops. Previous studies showed that warming shortens growth period of rice by 2.7 - 4.8 days/ K rise in temperature (Zhang et al. 2023). “It is unclear, whether the increase in CO₂ can compensate the negative impact of temperature on yield of wheat and rice. Second, in FACE experiments, it has been observed that crop yields are stimulated to a less extent as expected under elevated CO₂” (Yang et al. 2024). “This could be due to photosynthetic acclimation to elevated CO₂, where the initial enhancement of photosynthesis by elevated CO₂ cannot be sustained over a long period of crop cycle” (Yang et al. 2023). “Low stimulating effect of elevated CO₂ under FACE experiments could be due to photosynthetic acclimation, not only at leaf level, but also at crop canopy level. Elevated CO₂ hardly changes the quantitative relationships for plant or canopy growth in case such relationships are measured as a function of plant nitrogen status” (Gao et al. 2024). Effect of increased temperature or the combination of increased temperature and elevated CO₂ on these functional relationships need further evaluation. Elevated CO₂ may be more effective to influence growth of wheat than rice (Sloan et al. 2023), as the optimal temperatures for photosynthesis and plant growth remains at a lower level in wheat than in rice (Nagai and Makino 2009). “This response of crops to climate factors provides a difference in response to climate change. The responses of yield parameters of wheat to elevated CO₂ and temperature may be more complicated than rice. Few studies have demonstrated the differential impact of CO₂ and temperature on wheat and rice” (Wang et al. 2018, 2019). Result showed that future warming will reduce yield and this will not be mitigated by rise in atmospheric CO₂ unless

proper adaptation strategies introduced into future cultivars. We hypothesize that the general positive response to elevated CO₂ in the C₃ crops like wheat and rice can, to some extent, compensate for the negative impact of rising or increased temperature. We also hypothesize that the impact of increased temperature will differ between wheat and rice as wheat is usually grown in a cooler season than rice. Based on the above information, experiments were conducted using a FACE system, to undermine whether the elevation in CO₂ can compensate the negative impact of the increase in temperature on leaf chlorophyll content and root-shoot length and biomass of wheat and rice under future global change. For this purpose, growth duration, leaf chlorophyll content and root-shoot length and biomass of wheat and rice over four growing seasons were assessed in response to elevated CO₂ and temperature. The results for any differences between wheat and rice in response to elevated CO₂ and temperature can be explored in the context for better designing different strategies to mitigate the negative effects of global change on wheat and rice production.

Materials and methods

Experiment site and weather conditions

The experimental site is located at ICAR-Indian Institute of Soil Science, Bhopal district, Madhya Pradesh, India. It lies in between the latitude of 21°6'N - 26°30'N. Lying in the centre of the country, the area has a semi-dry tropical monsoon climate with a mean annual temperature of 28 °C and precipitation of 1000–1200 mm during 2018–2023. The soil is black (Vertisol) and cultivated under continuous rice–wheat rotation.

Soil physico-chemical properties

The soil is a heavy clayey vertisol and the experimental site were characterized by 5.7 g kg⁻¹ organic carbon, 225 mg kg⁻¹ available N, 12.75 mg kg⁻¹ available P, and 230 mg kg⁻¹ available K. The soil had sand 15.2%, silt 30.3%, clay 54.5%. The electrical conductivity (EC) was 0.43 dS m⁻¹, and the pH was 7.5.

Free Air CO₂ Enrichment (FACE) system

The FACE experimental field had eight plots maintained under similar agronomic practices. Plots had octagonal rings for release of CO₂ to field. The CO₂ exposure system was designed and assembled with latest techniques. The CO₂ gas cylinders with two stage regulators were connected to gas mixing compressor which was directly connected to CO₂ exposure system of FACE system's rings in experimental field. It had 8-meter diameter octagonal rings and covers 50 m² per plot. CO₂ sensors (E sense) with measuring capacity of 10000 ppm (Li-COR Inc., Lincoln, NE, USA) were installed above the canopy in each plot. Evenly distributed two concentric circles established to automatically control the CO₂ pumping. The consistency of the CO₂ concentration within the ring was controlled by automatic adjustment to wind velocity. The infrared heating facilities were designed based on the principles described elsewhere (Kimball et al. 2008). Each field had 08 infrared heaters (1000 W, 240 V, 45 cm long 10 cm wide (Elestein-FSR/1, Kalglo Electronics Co, Inc., Bethlehem, PA, USA). Detailed information on the infrared heater is mentioned elsewhere (Kimball 2005). Heaters do not emit short wavelengths those might be photosynthetically or morpho-genetically active. The heaters were adjusted weekly so as to maintain their height being 1.2 m above the top of the canopy during the growth cycle. The shading of eight infrared heaters from nadir view over the open circle was about 5.5%. According to the manufacturer, the infrared thermometers were sensitive to radiation only in the range of

8.0–14 μ m, thereby minimizing interference from atmospheric absorption/emission bands below 8 μ m and above 14 μ m. However, we did not correct for radiation emitted by the heaters and reflected by the vegetation in this wave band, so our observations of canopy temperature in the heated plots are relatively high. In our experiments, CO₂ concentration above the canopy and canopy temperature Sensor (Model SI-121, Apogee instruments Inc, Logan, USA) at the top of the canopy was monitored automatically every minute and was recorded using a datalogger (TC-800, Ambetronics Scientific Inc, India).

Experimental set up

The experiment was carried out with treatments representing the future climate (2040-50). It is based on the IPCC(2007) projection for CO₂ of 550-650 ppm and global mean surface air temperature rise of 2-3 °C under the A2 emission scenario. Treatments details of the experiment are mentioned in Table 1. The treatments were as follow: T1- Ambient CO₂ & ambient Temperature, T2 - Elevated CO₂ & ambient Temperature, T3 - Ambient CO₂ & elevated Temperature, T4- Ambient CO₂ & elevated Temperature, T5- Elevated CO₂ & elevated Temperature, T6 - Elevated CO₂ & elevated Temperature. “In first two years (2019-20 and 2020-21) each treatment (T1, T2, T3, and T5) was replicated into two rings with the same infrastructure, based on block split-plot design. After two years, (2021-22 and 2022-23) experiments were carried out in six treatments (T1-T6) with single replication. CO₂ was enriched at daytime and canopy temperature was maintained both at day and night. To avoid artifacts caused by any heterogeneity present among crop seedlings in the winter phase of wheat and any effect of transplanting shock in rice, we started the CO₂ and temperature treatments in the FACE system

only after crops were well established and became homogeneous within a plot. This was achieved at the early tillering stage” (Ruiz- Vera et al. 2015).

Crop cultivation and agronomic practices

In each experimental season, winter wheat, HI-1605 (Pusa Ujala) and rice, PB-1 (Pusa Basmati-1), both local cultivars, were grown. Standard cultivation practices, performed in the area, were followed in all experimental plots. For wheat, seeds were sown in rows 10-12 cm apart at a rate of 120 kg seed per ha resulting in a plant density of approximately 250-300 plants m⁻². For rice, DRS method was used. Spacing of hills was maintained at 12 x 22.4 cm (equivalent to 37 hills m⁻²).

Sampling and observations

Leaf chlorophyll content measurements

In order to extract chlorophyll, 0.3 g of fresh third leaves from top were stored in 15 ml of 80% (v/v) acetone for more than 2 days in the dark at 4°C. The absorbance of the solution was measured using a multi-wavelength spectrophotometer (Spectronic-20) at 645 and 663 nm. The total chlorophyll content was calculated using the absorbance values (Arnon 1949) as below.

$$\text{Chlorophyll 't'} = ((20.29 \times A_{645}) + (8.05 \times A_{663})) \times V/1000 \times 1/W$$

Where,

A₆₄₅, A₆₆₃ are absorbance at 645 nm and 663 nm, respectively

V = volume of solvent used (ml)

W = Weight of sample used (g)

Root-shoot length and Biomass

Plant samples were harvested at full vegetative stage from all treatments and fresh weight and root-shoot length were observed immediately. Plant samples were kept in hot air oven, after drying the plant sample were further measured for dry weight.

Results

Chlorophyll content

Compared to the control, elevated CO_2 ($e[\text{CO}_2]$) resulted in significant increases in leaf total chlorophyll [T. chl] in rice (Fig 1) and wheat (Fig 2), ranging from 12.9-19% and 8.8-16.5%, respectively. Compared to the control, [T. chl] significantly decreased under elevated temperature ($e(\text{Temp.})$) by 20.5-27.3% and 6.3-11.5% in both rice and wheat, respectively.

Compared to the control, combined effect of $e(\text{CO}_2)$ plus $e(\text{Temp.})$ had decreased the [T. chl] from 6.5-14.4% and 5.9-9.6% in rice and wheat crop, respectively.

Plant root-shoot length

Growth of rice and wheat crop under elevated CO_2 ($e[\text{CO}_2]$), in terms of root and shoot length was increased by 0.0-12.9% and 2.1-9.9%, and 7.2-11.9% and 1.7-19.3% in rice (Fig 3) and wheat (Fig 4), respectively. Plant root length decreased from 6-14.8% over control in rice due to climate factors. In wheat, root length declined from 5.5-10.3% over control due to climate factors. Shoot length also decreased by 0.4-4.1% and 0.7-5.5% in rice and wheat, respectively. In case of combined effect of $e[\text{CO}_2]$ plus $e[\text{Temp.}]$ positive effect observed in rice root length by 0.5-17% and negative effect on wheat root length by 0-5.9%. There was no negative effect on plant height in both crop under co-elevation of CO_2 and temperature.

Plant root-shoot biomass

Compared to the control, the plant root and shoot biomass significantly increased under e[CO₂] condition, Plant root and shoot biomass increased from 0.5-30.4% and 15.3-41.5% respectively in rice (Fig 5). Plant root and shoot biomass increased from 14.9-26.3% and 12.1-28.3% respectively in wheat (Fig 6). Elevation of e[Temp.] had significantly negative effect on plant root and shoot biomass from 1.2-12.4% and 11.2-15.1% and 1.7-15.7% and 4.1-16.6% in both rice and wheat, respectively.

Discussion

Hightemperature stress inhibits photosynthesis through disrupting the structure and function of chloroplasts and reductions in chlorophyll content in leaf tissues(Brestic et al. 2016).In the C3 crop, eCO₂stimulates photosynthesis, resulting in transition of Rubisco's two-properties. First, the Michaelis constantof Rubisco, where elevated CO₂ (eCO₂) improves carboxylation level. Second, CO₂ prevents oxygenation reaction, glycolate production, and CO₂ release through photo respiration (Bauwe 2023).

Elevated CO₂ increased root and shoot length of both rice and wheat crop. Elevated temperature inhibited the growth parameters. The trend was similar over the years (2019 to 2022). There was a positive response in case of rice plant height (+7 %) Similarly there was a factor of at least 10% increase in shoot height under elevated CO₂. Impact of elevated temperature was more pronounced in case of wheat than rice. Elevated temperature inhibited the both root and shoot length of wheat plant by a factor of 11% grown in 2021 and 16% during 2022 and 2023.

Elevated atmospheric CO₂ (eCO₂) concentration can increase root exudation into soils. In a study on the impacts of elevated CO₂ on plant resistance to nutrient deficiency and toxic ions via root exudates, it was observed that elevated CO₂ increased production of sugars, carboxylic

acids, and citric acids by 47%, 111%, and 16%, respectively. The increased carbon exudation, increased plant requirements of nutrients, and plant growth (Sardans et al. 2023).

Temperature above the ambient and increased atmospheric CO₂ concentration have opposite effects on net photosynthesis and interact strongly (Sage and Sharkey 1987; Long 1991).

Elevated temperature often inhibits dry biomass of rice due to enhanced plant respiration, shortens growth period and increases floral sterility (Cai et al. 2016; Ren et al. 2023).

Additionally, there are reports which suggest that, e[CO₂] positively impacts rice dry matter accumulation which was consistent with the present study (Roy et al. 2012; Cai et al. 2018; Wang et al. 2020). In wheat crop, total dry matter increased by 15% when the CO₂ concentration of the atmosphere was increased from 350 to 700 $\mu\text{mol mol}^{-1}$ (Delgado et al. 1994). For example, the rice yield increased by 15% under e[CO₂] (550 ppm) in a free-air [CO₂] enrichment study (Wang et al. 2018). The results showed that while warming, slightly reduced rice yield, the increase in yield under e[CO₂] plus warming was less than that under e[CO₂]. Nevertheless, considering the possible increase in rice biomass caused by the co-elevation of e[CO₂] and temperature in this region (central India), the varieties adapting to e[CO₂] plus warming should receive more attention to improve rice and wheat production in the future.

Rise in temperature led to reduced growth of the crop. Straw weight over control of rice and wheat reduced from 11.2-24.9 % and 4.1-18.2 % respectively, in high temperature and ambient CO₂ treatment. But increase in CO₂ concentration significantly increased straw weight of the crops. Elevated CO₂ level along with high temperature was able to compensate the loss of temperature rise due to the CO₂ fertilization effect. Elevated CO₂ plus elevated temperature treatment, straw weight in rice and wheat varied from 7.1-14.45g and 5.58-8.12 g hill⁻¹ (Singh et

al. 2013), indicated that elevated CO₂ could alleviate the negative impact of high temperature but the effect is crop and region specific.

Root weight of rice increased under elevated CO₂ while high temperature caused reduced root weight of the crops. Root weight reduced from 1.2-25.9 and 1.7-20.7 % in both (rice and wheat) crops respectively, in high temperature treatment. Earlier studies also showed that increased root growth contributes to higher root biomass and root dry weight under elevated CO₂ condition.

Elevated temperature often reduces rice dry matter accumulation due to enhanced plant respiration, shortens growth duration (Cai et al. 2016; Oh et al. 2023).

Conclusion

Results of the current experiment showed that growth of rice and wheat crop was influenced under the influence of climate factors (elevated CO₂ and temperature). In general, elevated CO₂ stimulated growth parameters. However, the growth of crops was reduced under elevated temperature. The growth parameters were chlorophyll content, plant length and biomass yield. Experiment was conducted for four years to define the trend. Data indicated that the effect was similar over the years. Study highlighted that increased CO₂ concentration can compensate the crop growth due to elevated temperature to some extent. Further research is essential to understand the nutrient cycling process in rhizosphere of both crops to better understand the negative effect of climate factors on rice wheat cropping system.

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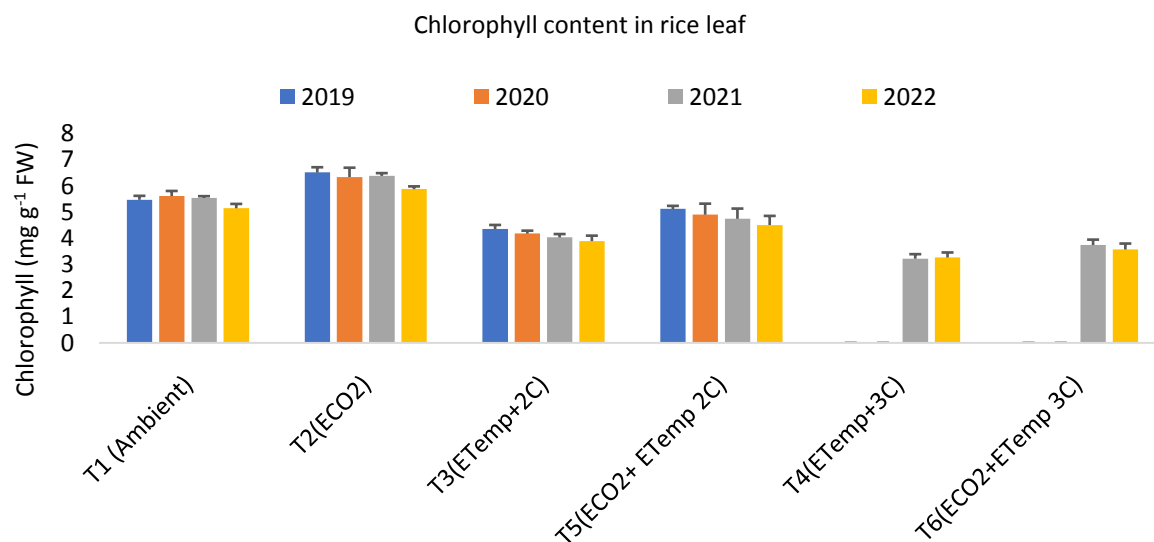


Figure 1. Effect of climate factors on chlorophyll content in rice leaf. The climate factors were elevated CO₂ (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO₂ + ambient temperature), T2 - elevated CO₂ (ECO₂), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO₂ (600 ppm) + Elevated temperature (ambient temperature +2C), T6 – Elevated CO₂ (600 ppm) + Elevated temperature (+3C). Rice and wheat cropping system was maintained following standard agronomic practices. Chlorophyll content in leaf biomass was estimated (mg g⁻¹ fresh weight or FW) during vegetative stage. X axis represents different treatments and Y axis represents chlorophyll content. Each data point represents arithmetic mean with standard deviation as error bar of three replicates.

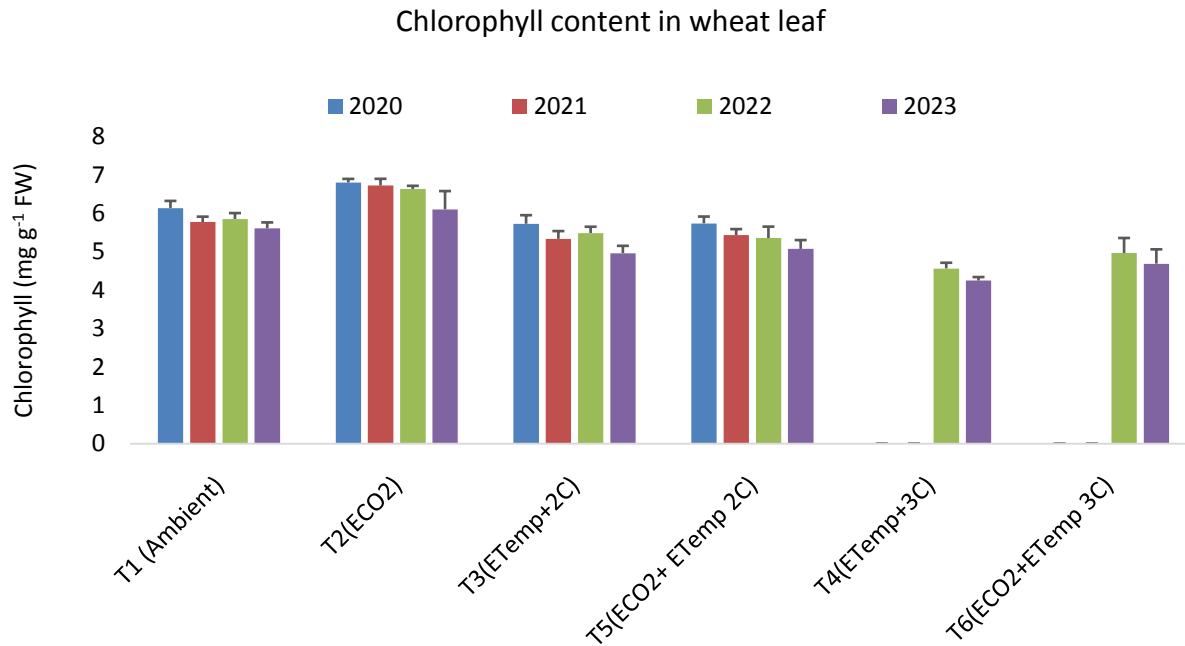


Figure 2. Effect of climate factors on chlorophyll content in wheat leaf. The climate factors were elevated CO₂ (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO₂ + ambient temperature), T2 - elevated CO₂ (ECO₂), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO₂ (600 ppm) + Elevated temperature (ambient temperature +2C), T6 – Elevated CO₂ (600 ppm) + Elevated temperature (+3C). Rice and wheat cropping system was maintained following standard agronomic practices. Chlorophyll content in leaf biomass was estimated (mg g⁻¹ fresh weight or FW) during vegetative stage. X axis represents different treatments and Y axis represents chlorophyll content. Each data point represents arithmetic mean with standard deviation as error bar of three replicates.

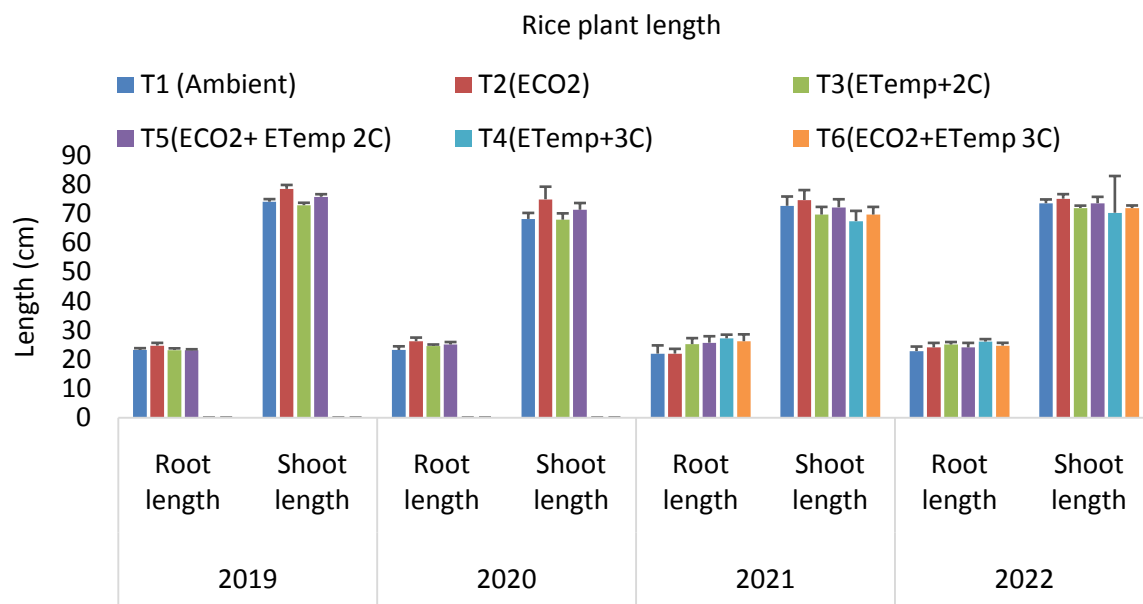


Figure 3. Effect of climate factors on growth of rice plant (Root length and Shoot length). The climate factors were elevated CO₂ (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO₂ + ambient temperature), T2 - elevated CO₂ (ECO₂), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO₂ (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO₂ (600 ppm) + Elevated temperature (+3C). Rice and wheat cropping system was maintained following standard agronomic practices. X axis represents different treatments and Y axis represents length of root or shoot. Each data point represents arithmetic mean with standard deviation as error bar of three replicates.

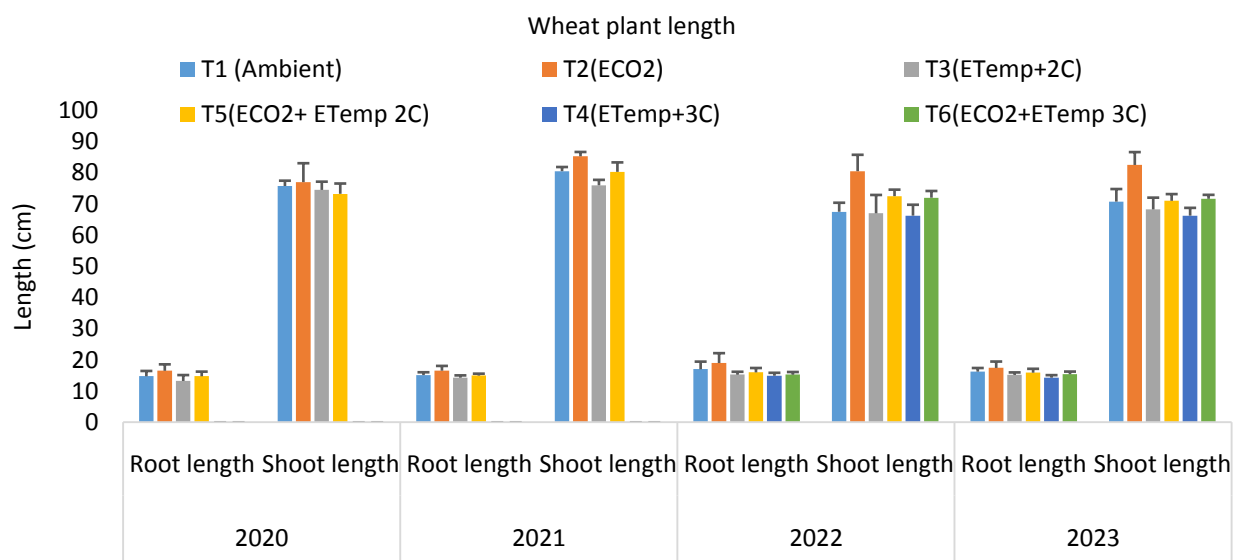


Figure 4. Effect of climate factors on growth of wheat plant (root length and shoot length). The climate factors were elevated CO₂ (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO₂ + ambient temperature), T2 - elevated CO₂ (ECO₂), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO₂ (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO₂ (600 ppm) + Elevated temperature (+3C). Rice and wheat cropping system was maintained following standard agronomic practices. X axis represents different treatments and Y axis represents length. Each data point represents arithmetic mean with standard deviation as error bar of three replicates.

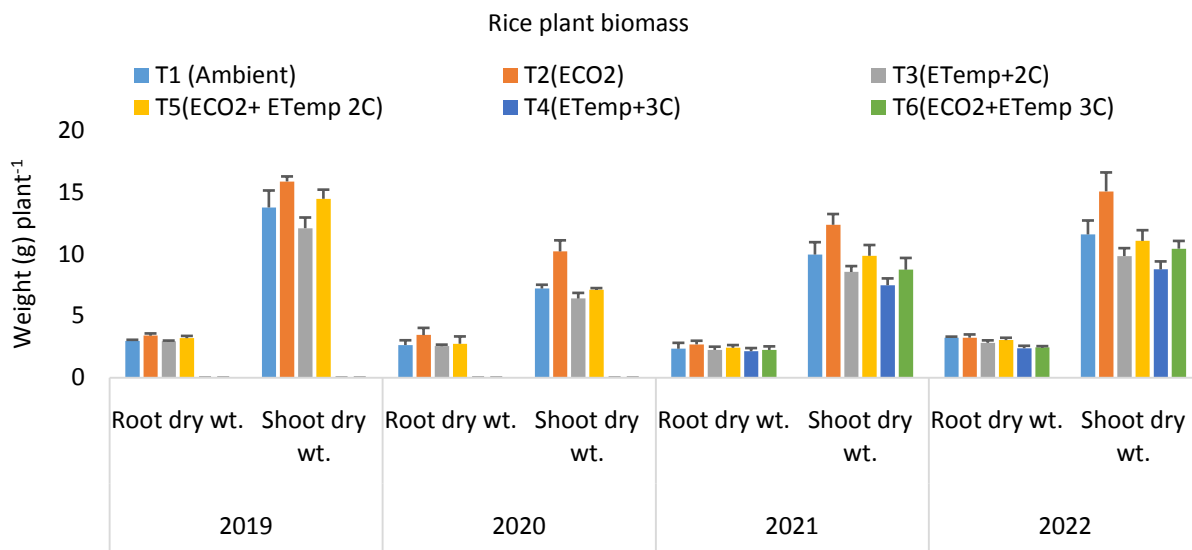


Figure 5. Effect of climate factors on biomass of rice plant (root length and shoot length). The climate factors were elevated CO₂ (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO₂ + ambient temperature), T2 - elevated CO₂ (ECO₂), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO₂ (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO₂ (600 ppm) + Elevated temperature (+3C). Rice and wheat cropping system was maintained following standard agronomic practices. X axis represents different treatments and Y axis represents length. Each data point represents arithmetic mean with standard deviation as error bar of three replicates.

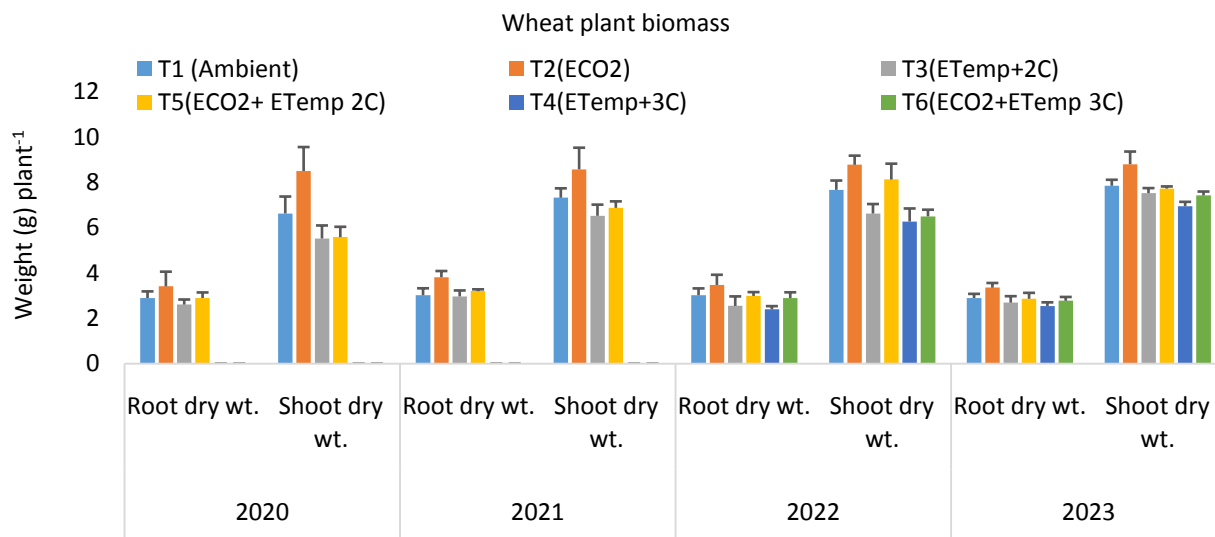


Figure 6. Effect of climate factors on biomass of wheat plant (Root length and Shoot length). The climate factors were elevated CO₂ (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO₂ + ambient temperature), T2 - elevated CO₂ (ECO₂), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO₂ (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO₂ (600 ppm) + Elevated temperature (+3C). Rice and wheat cropping system was maintained following standard agronomic practices. X axis represents different treatments and Y axis represents length. Each data point represents arithmetic mean with standard deviation as error bar of three replicates.

Table 1. Experimental parameters to study the effect of elevated CO₂ and elevated temperature on growth of rice and wheat crops.

Treatments	Field IDs	Climate combinations	Abbreviation
T ₁	A ₀ B ₀	Ambient CO ₂ & ambient Temperature	control
T ₂	A ₁ B ₀	Elevated CO ₂ & ambient Temperature	_E [CO ₂] 600ppm
T ₃	A ₀ B ₁	Ambient CO ₂ & elevated Temperature	_E [Temp.]+2°C
T ₄	A ₀ B ₂	Ambient CO ₂ & elevated Temperature	_E [Temp.]+3°C
T ₅	A ₁ B ₁	Elevated CO ₂ & elevated Temperature	_E [CO ₂ &Temp.]+2°C
T ₆	A ₁ B ₂	Elevated CO ₂ & elevated Temperature	_E [CO ₂ &Temp.]+3°C

Experiment was conducted in a Free Air CO₂ Enrichment (FACE) facility. The FACE system was equipped with octagonal rings for CO₂ release, overhead infra-red heaters to increase the temperature of crop canopy. Concentration of CO₂ and temperature was maintained using CO₂ and temperature sensors. Fields were cultivated with rice and wheat during rainy and winter season following recommended doses of fertilizer.