Effect of elevated CO_2 and temperature on chlorophyll content and growth attributes of rice-wheat cropping system in vertisol of 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 CO_2 and temperature. To define the mechanisms, a field experiment was carried out to evaluate the effect of elevated CO_2 and temperature on growth attributes of rice and wheat crops using a Free Air CO_2 Enrichment (FACE) system. The treatments were ambientCO_2+ambient temperature, elevated$CO_2$of 600ppm+ambienttemperature, ambient CO_2 + elevated temperature (+2°C), ambient CO_2+elevated temperature (+3°C), elevated $CO_2$600ppm +elevated temperature (+2°C) and elevated $CO_2$600ppm +elevated temperature (+3°C). Elevated CO_2 and elevated temperature (+2°C or +3°C) strongly affectedthe crop growth.ElevatedCO_2stimulatedleaf chlorophyll content, root-shoot length and biomass yield. However, elevated temperature inhibitedchlorophyll content in both the crops.Elevated CO_2enhanced chlorophyll contentby 12.9–19% in rice and 8.8–16.5%in wheat.Elevated temperaturereduced chlorophyll content by a range of 20.5-27.3% in rice and 6.3-11.5% in wheat. Combined effect of elevated $CO_2$and elevated temperature decreased the leaf total chlorophyll and plant biomass in both crops. Study highlights that elevated CO_2 concentration and rising temperature may affect the photosynthesis and productivity of rice and wheat crop in central India.

Key words: Elevated CO_2, temperature, climate change, rice-wheat, chlorophyll,biomass,Vertisol.

Introduction

Wheat (*Triticum aestivum*) and rice (*Oryza sativa*) are the two major food crops, producing food for more than 90%of the world's population(Nagai and Makino 2009). The production of food needs to increase by 70% by 2050 to meet the demands of a growingpopulation (Bruinsma 2009)Crop production ishighly affected by changein the global climate, mostly changes in CO_2gas concentration in atmosphere.The atmospheric CO_2 concentration is increasing alarmingly at 3% per year and it is predicted that even with stringent policies to curb GHG emission, the concentration of CO_2 will reach550–700 ppm by 2050 and 650–1200 ppm by 2100 (Ishtiaque et al. 2022). The rise inatmospheric greenhouse gasses (GHGs) will increase the mean global temperature up to 2.5 °C by 2050 and of up to6.4 °C by the end of this century(Change 2007; Yin 2013; Stocker 2014). Extremeclimatic events (ECEs), such as heat waves and droughts, which often affect plant growthand pose a growing threat to natural and agricultural ecosystems, are predicted to increasein frequency and severity in many cropping areas(Change 2007). To assess food security for future generation, it is necessary to find out the responses of wheat and rice to elevated $CO_2$and temperature. Number of experiments have been conducted to find out the responses of wheat and rice to elevated CO_2 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 FACE (free-air CO_2enrichment) system, as crop responses to elevated CO_2 and temperature are more realistic under such experimental conditions than under open top chamber or greenhouse conditions(Kimball 2013). An increase in CO_2 concentration of the ambient air can enhance leaf photosynthesis of C_3species, including wheat, rice, and many other cereal crops, leading to enhanced growth and increased yields(KIM et al. 2003; Sakai et al. 2006; van der Kooi et al. 2016). However, yield enhancement by elevated CO_2alone should be interpreted with caution, at least following two reasons. First, elevated CO_2under future climate change is associated with an increase in air temperature. In general, increasing temperature accelerates crop phenological development. Previous studiesshowedthat climate warming has shortened growth duration by 2.7 d/K for early rice and 4.8 d/K for late rice (Wheeler et al. 2000; Jagadish et al. 2007; Dong et al. 2011; Hatfield et al. 2011). Whether the increase in CO_2 in the field can compensate for the negative impact of the increase in temperature on yield of wheat and rice still needs to be tested. Second, in FACE experiments, crop yields are stimulated less than expected under elevated CO_2(Long et al. 2006). This might be attributed to photosynthetic acclimation to elevated CO_2 – initial enhancement of leaf photosynthesis by elevated CO_2concentration cannot be sustained over a long period during the crop cycle(Vandermeiren et al. 2002; Sakai et al. 2006). Based on an analysis using a mechanistic crop model, it was concluded that the low stimulation of elevated CO_2 concentration measured in FACE experiments was at least partly due to photosynthetic acclimation, not only at leaf level, but also at canopy level related to canopy size and senescence. Elevated CO_2hardly changes the quantitative relationships for plant or canopy growth if these relationships are expressed as a function of plant nitrogen status(Yin 2013). Whether increased temperature or the combination of increased temperature and elevated CO_2will change these functional relationships still needs to be tested. Elevated CO_2 may be more effective for wheat than for rice(Makino 2011), while the optimal temperatures for photosynthesis and plant growth are lower in wheat than in rice (Nagai and Makino 2009). This willprovidea difference in response to climate change between wheat and rice. The responses of yield components to elevated CO_2 and temperature may be more complicated in wheat than in rice. However, few studies have been conducted to explore the differences between wheat and rice in response to elevated $CO_2$and temperature(Nagai and Makino 2009; Wang et al. 2018, 2019). Result showed that future warming will reduce yield and this will not be mitigated by increased atmospheric CO_2 unless appropriate adaptation traits can be introduced into future cultivars. We hypothesize that the general positive response to elevated CO_2 in the C_3 crops wheat and rice can, to some extent, compensate for the negative impact of increased temperature. We also hypothesize that the impact of increased temperature will differ between wheat and rice, both in extent and nature, as wheat is usually grown in a cooler season than rice. Our primary objective of this study was to experimentally examine, using the FACE systems, whether the elevation in CO_2 was able to compensate for 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 CO_2 and temperature. The results for any differences between wheat and rice in response to elevated CO_2 and temperature will 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 was 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 textural composition of soil was: 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 CO_2 **Enrichment (FACE)system**

The FACE experimental field had eight plots maintained under similar agronomic practices. Plots had octagonal rings for release of CO_2 to field. The CO_2 exposure systemwas designed and assembled with latest techniques.CO_2 gas cylinders with two stage regulatorswere connected to gas mixing compressor which was directly connected to CO_2 exposure system of FACE system's rings in experimental field. It had 8-meterdiameter octagonal rings and covers50 m^2 per plot. CO_2sensors(E sense) with measuring capacity of 10000ppm (Li-COR Inc., Lincoln,NE, USA) were installed above the canopy in each plot. Evenly distributed two concentric circles established to automaticallycontrol the CO_2 pumping. The consistency of the CO_2 concentrationwithin the ring was controlled by automatic adjustmentto wind velocity. The infrared heating facilities were designed based on the principles described elsewhere (Kimball et al. 2008).Ecah field had 08 infrared heaters(1000 W, 240 V, 45cm long 10cm wide (Elestein-FSR/1,Kalglo Electronics Co, Inc., Bethlehem, PA, USA). Detailed information on the infrared heater is mentioned elesewhere(Kimball 2005). Heatersdo not emit short wavelengths those might be photosynthetically or morphogeneticallyactive. The heaters were adjusted weekly so as to maintaintheir height being 1.2 m above the top of the canopy during the growth circle. The shading of eight infrared heaters from nadir view over the open circle was about 5.5%.According to the manufacturer, the infrared thermometers weresensitive to radiation only in the range of 8.0–14μm, therebyminimizing interference from atmospheric absorption/emission bands below 8 μm and above 14μm.However, we did notcorrect for radiation emitted by the heaters and reflected bythe vegetation in this wave band, so our observations of canopy temperature in the heated plots are relatively high. Inour experiments, CO_2 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 ScientificInc, India).

Experimental set up

The experiment was carried out with treatmentsrepresenting the future climate (2040-50). It is based on the IPCC(2007) projection for $CO_2$of 550-650 ppm and global mean surface air temperature rise of 2-3 °C underthe A2 emission scenario.Treatments details of the experiment are mentioned in Table 1. The treatments were as follows : T1- AmbientCO_2 &ambient Temperature, T2 - Elevated CO_2 & ambient Temperature, T3 - Ambient CO_2& elevated Temperature, T4- Ambient CO_2& elevated Temperature, T5- Elevated CO_2& elevated Temperature, T6 - Elevated CO_2& elevated Temperature. In first two years(2019-20 and 2020-21) each treatment (T1, T2, T3, and T5) was replicated intwo 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. $CO_2$was enriched at day time and canopy temperature was maintained both at day and night. To avoid artifacts caused by any heterogeneity present amongcrop seedlings in the winter phase of wheat and any effect oftransplanting shock in rice, we started the CO_2 and temperaturetreatments in the FACE system only after crops were wellestablished and became homogeneous within a plot. This wasachieved 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 thearea, 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 haresulting in aplant 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 topwere 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 chlorophyllcontent was calculated using the absorbance values(Arnon 1949) as below.

Chlorophyll ‘t’ = ((20.29 x A645) + (8.05 x A663)) x V/1000 x 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 i.e $_E[CO_2]$ resulted in significant increases in leaf total chlorophyll [T. chl] in rice (Fig 1) and wheat (Fig 2), ranging from12.9-19% and8.8-16.5%, respectively. Compared to the control, [T. chl] significantly decreased under elevated temperature$_E$(Temp.) by 20.5-27.3% and 6.3-11.5% in both rice and wheat, respectively. Compared to the control, combined effect of$_E$(CO_2)plus$_E$(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 cropunder elevated CO_2e[CO_2], in terms of root and shoot length wasincreased 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[CO_2] plus e[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[CO_2] 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

High-temperature stress causes photosynthesis reduction through disruptions in the structure and function of chloroplasts and reductions in chlorophyll content in wheat leaves(Brestic et al. 2016).In the C3 crop, eCO_2 induced photosynthesis to increase, resulting in Rubisco's two-properties transition. First, the CO_2 enzyme's Km (Michaelis constant), which measures the kinetics of the Rubisco, is the relative presence of CO_2 concentration; thus, eCO_2 improves carboxylation level. Second, CO_2 prevented the oxygenation reaction, glycolate production, and CO_2 released in the photo respiratory phase(Long and Ort 2010).

Elevated CO_2 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 CO_2. Impact of elevated temperature was more pronounced in case of wheat than rice. Elevated temperature inhibited the both root and shot length of wheat plant by a factor of 11% grown in 2021 and 16% during 2022 and 2023 (Fig).

Temperatures above the optimum and increased atmospheric CO_2 concentration will have opposite effects on net photosynthesis and will interact strongly(Sage and Sharkey 1987; Long 1991). Elevated temperature often reduces rice dry matter accumulation due to enhanced plant respiration, shortens growth duration and increases floral sterility(Ziska et al. 1996; Dong et al. 2011; Cai et al. 2016). Additionally, there is substantial evidence that e[CO_2] positively impacts rice dry matter accumulation which was consistent with the present study(Sakai et al. 2001; Roy et al. 2012; Cai et al. 2018; Wang et al. 2020).In wheat crop, total dry matter increased by 15% when the CO_2 concentration of the atmosphere was increased from 350 to 700 umol mol^{-1}(Delgado et al. 1994), For example, the rice yield increased by 15% under e[CO_2] (550 ppm) in a free-air [CO_2] enrichment study (Wang et al. 2018). The results showed that while warming, slightly reduced rice yield, the increase in yield under e[CO_2] plus warming was less than that under e[CO_2]. Nevertheless, considering the possible increase in rice biomass caused by the co-elevation of e[CO_2] and temperature in this region, the varieties adapting to e[CO_2] 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 CO_2 treatment (Fig 5). But increase in CO_2 concentration significantly increased straw weight of the crops. Elevated CO_2 level along with high temperature was able (some extend) to compensate the loss of temperature rise due to the CO_2 fertilization effect. Elevated CO_2 plus elevated temperature treatment straw weight in rice and wheat from 7.1-14.45g and 5.58-8.12 g $hill^{-1}$(Singh et al. 2013) also indicated that elevated CO_2 could alleviate the negative impact of high temperature but the effect is crop and region specific.

Root weight of rice increased in elevated CO_2 treatment while high temperature causes 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 (Fig 5). Earlier studies also showed that increased root growth contributes to higher root biomass and root dry weight under elevated CO_2condition. Elevated temperature often reduces rice dry matter accumulation due to enhanced plant respiration, shortens growth duration(Ziska et al. 1996; Dong et al. 2011; Cai et al. 2016).

Conclusion

Results of the current experiment showed that growth of rice and wheat crop was influenced under the influence of climate factors (elevated CO_2 and temperature). In general, elevated CO_2stimulated 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 CO_2 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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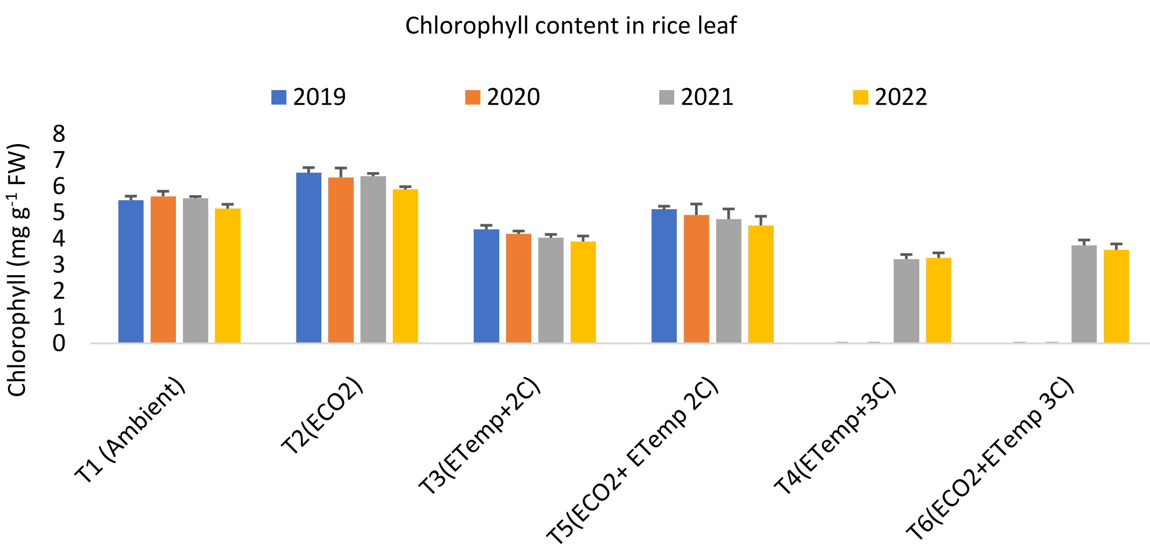

Fig 1. Effect of climate factors on chlorophyll content in rice leaf. The climate factors were elevated CO_2 (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO_2 + ambient temperature), T2 - elevated CO_2 (ECO_2), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO_2 (600 ppm) + Elevated temperature (ambient temperature +2C), T6 – Elevated CO_2 (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) 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.

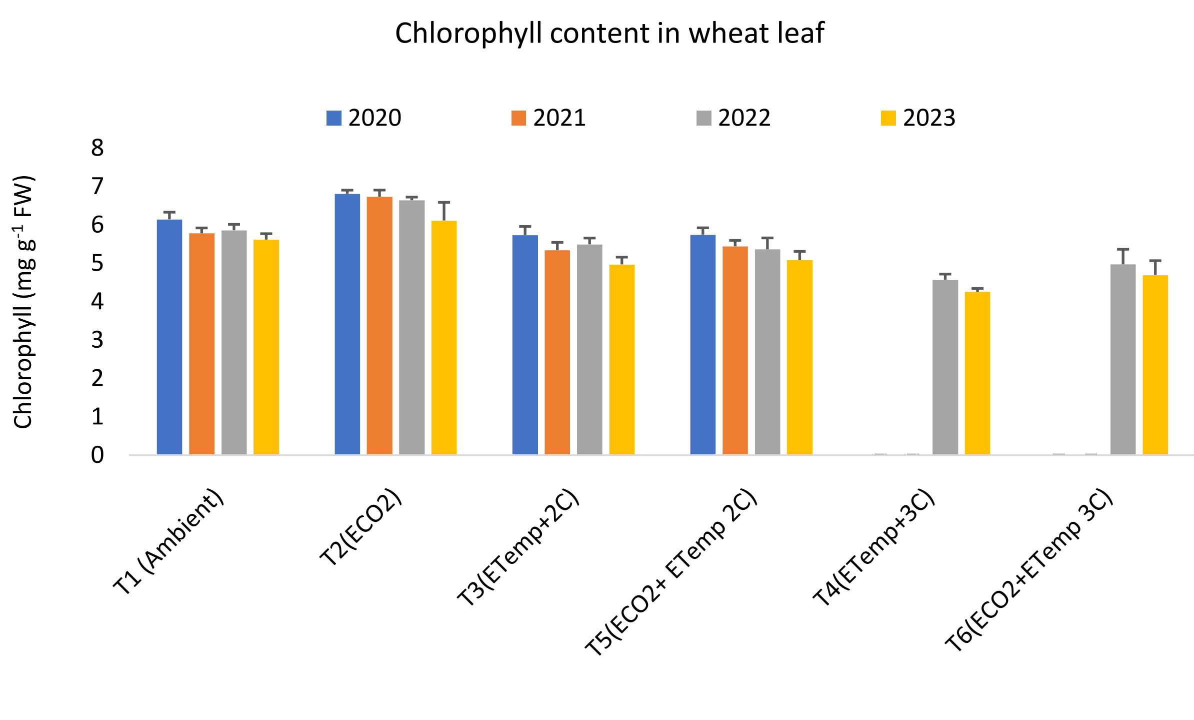

Fig 2. Effect of climate factors on chlorophyll content in wheat leaf. The climate factors were elevated CO_2 (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO_2 + ambient temperature), T2 - elevated CO_2 (ECO_2), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO_2 (600 ppm) + Elevated temperature (ambient temperature +2C), T6 – Elevated CO_2 (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) 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.

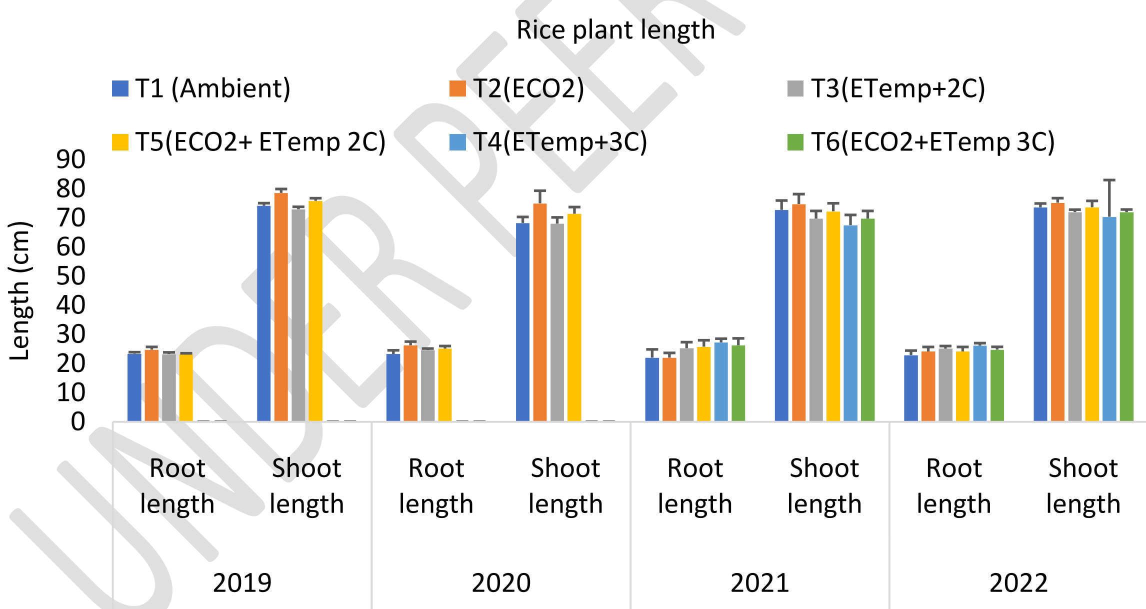

Fig 3. Effect of climate factors on growth of rice plant (Root length and Shoot length). The climate factors were elevated CO_2 (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO_2 + ambient temperature), T2 -

elevated CO_2 (ECO_2), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO_2 (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO_2 (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.

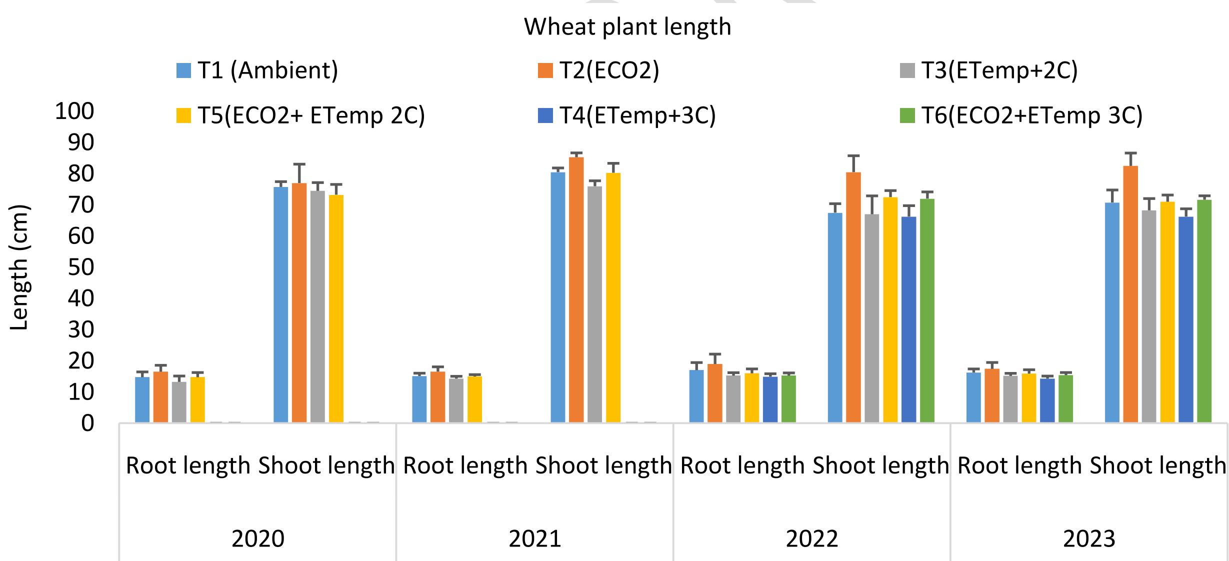

Fig 4. Effect of climate factors on growth of wheat plant (root length and shoot length). The climate factors were elevated CO_2 (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO_2 + ambient temperature), T2 - elevated CO_2 (ECO_2), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 –

Elevated CO_2 (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO_2 (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.

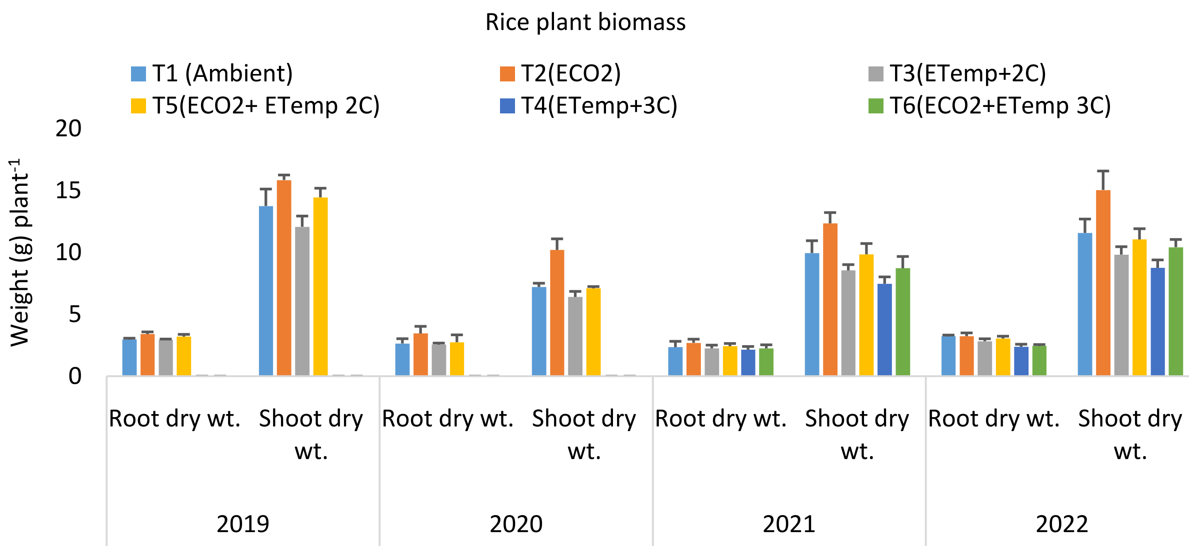

Fig 5. Effect of climate factors on biomass of rice plant (root length and shoot length). The climate factors were elevated CO_2 (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO_2 + ambient temperature), T2 - elevated CO_2 (ECO_2), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO_2 (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO_2 (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.

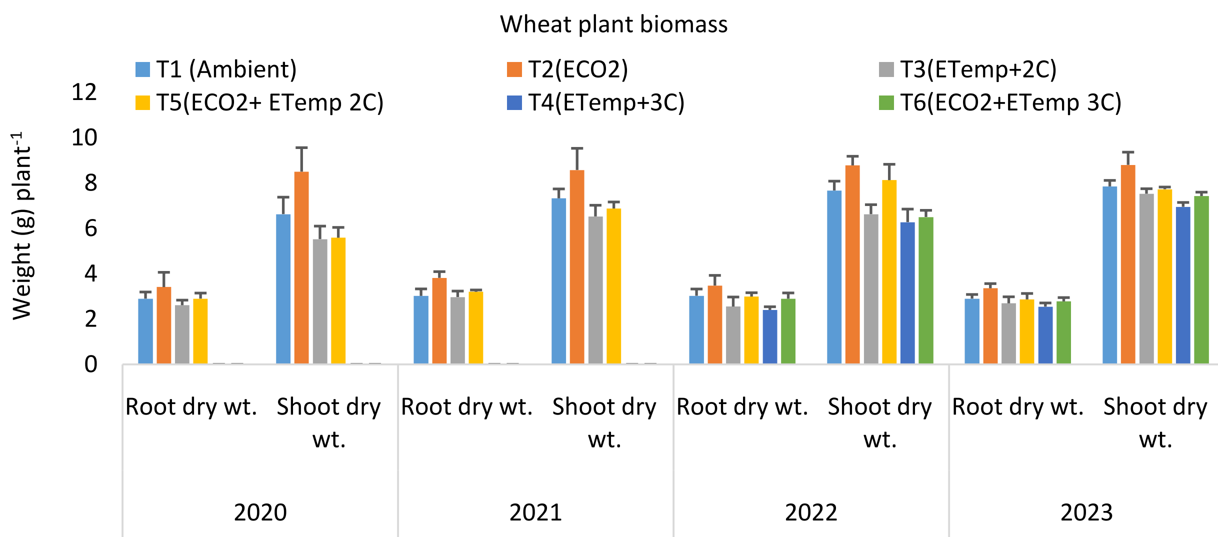

Fig 6. Effect of climate factors on biomass of wheat plant (Root length and Shoot length). The climate factors were elevated CO_2 (600 ppm), elevated temperature (either ambient +2C or ambient +3C). The treatments were T1 – ambient (ambient CO_2 + ambient temperature), T2 -

elevated CO_2 (ECO_2), T3 - Elevated temperature (+ 2C), T4 - Elevated temperature (+ 3C), T5 – Elevated CO_2 (600 ppm) + Elevated temperature (ambient temperature + 2C), T6 – Elevated CO_2 (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. 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.

Treatments	Field IDs	Climate combinations	Abbreviation
T_1	A_0B_0	Ambient CO_2 & ambient Temperature	control
T_2	A_1B_0	Elevated CO_2 & ambient Temperature	$_E[CO_2]$ 600ppm
T_3	A_0B_1	Ambient CO_2 & elevated Temperature	$_E$[Temp.]+2°C
T_4	A_0B_2	Ambient CO_2 & elevated Temperature	$_E$[Temp.]+3°C
T_5	A_1B_1	Elevated CO_2 & elevated Temperature	$_E[CO_2$&Temp.]+2°C
T_6	A_1B_2	Elevated CO_2 & elevated Temperature	$_E[CO_2$&Temp.]+3°C