



# Combinatorial impacts of elevated CO<sub>2</sub> and temperature affect growth, development, and fruit yield in *Capsicum chinense* Jacq

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**Abstract** Hot chilli ('Bhut Jolokia') (*Capsicum chinense* Jacq.) is the hottest chilli widely grown in the North-Eastern region of India for its high pungency. However, little information is available on its physiology, growth and developmental parameters including yield. Therefore, the present research was undertaken to study the physiological responses of Bhut Jolokia under elevated CO<sub>2</sub> (eCO<sub>2</sub>) and temperature. Two germplasms from two different agro-climatic zones (Assam and Manipur) within the North-East region of India were collected based on the pungency. The present study explored the interactive effect of eCO<sub>2</sub> [at 380, 550, 750 ppm (parts per million)] and temperature (at ambient, > 2 °C above ambient, and > 4 °C above ambient) on various physiological processes, and expression of some photosynthesis and capsaicin related genes in both the germplasms. Results revealed an increase (> 1–2 fold) in the net photosynthetic rate (P<sub>n</sub>), carbohydrate content, and C: N ratio in 'Bhut Jolokia' under eCO<sub>2</sub> and elevated temperature

regimes compared to ambient conditions within the germplasms. Gene expression studies revealed an up-regulation of photosynthesis-related genes such as *CsRuBPC2* (*Ribulose biphosphate carboxylase 2*) and *CsSPS* (*Sucrose phosphate synthase*) which, explained the higher P<sub>n</sub> under eCO<sub>2</sub> and temperature conditions. Both the germplasm showed better performance under CTGT-II (Carbon dioxide Temperature Gradient Tunnel having 550 ppm CO<sub>2</sub> and temperature of 2 °C above ambient) in terms of various physiological parameters and up-regulation of key photosynthesis-related genes. An up-regulation of the *Cs capsaicin synthase gene* was also evident in the study, which could be due to the metabolite readjustment in 'Bhut Jolokia'. In addition, the cultivar from Manipur (cv. 1) had less fruit drop compared to the cultivar from Assam (cv. 2) in CTGT II. The data indicated that 550 ppm of eCO<sub>2</sub> and temperature elevation of > 2 °C above the ambient with CTGT-II favored the growth and development of 'Bhut Jolokia'. Thus, results suggest that Bhut Jolokia grown under the elevation of CO<sub>2</sub> up to 550 ppm and temperature above 2 °C than ambient may support the growth, development, and yield.

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## Introduction

Plant growth and development rely on carbon dioxide (CO<sub>2</sub>), the primary raw material for photosynthesis. The global atmospheric CO<sub>2</sub> concentrations have risen from 280 ppm (μmol mol<sup>-1</sup>) to 418 ppm in 2021 (<https://www.co2.earth/>) due to the industrial revolution and are expected to rise by another 40% by 2050 (about 550 ppm) accompanied by a 2 °C rise in the global temperature (IPCC 2013). The

increase in the global temperature is due to higher CO<sub>2</sub> and increasing concentrations of other greenhouse gases (Hanse et al. 2010). It has been reported by some researchers that due to the emission of greenhouse gases, the ecosystems and agricultural activities have been adversely affecting biodiversity, the productivity of agriculture and food security have been adversely affected as a result of global climate change (Sloat et al. 2020). Due to the sessile lifestyle, plants must endure unfavorable weather events such as high temperature, cold, drought, flood, and salinity (Ohama et al. 2017). The key determinant of plant growth is CO<sub>2</sub> (Craufurd et al. 2009), while the temperature is a key factor for the transition of various developmental phases, including flowering and yield (Zinta et al. 2018). Unfavorable weather events such as high temperature, cold, drought, flood, salinity, and an excess or deficit CO<sub>2</sub> concentration in the atmosphere affect plant growth and productivity due to an array of adjustments that are needed in the physiological processes to adapt (Sarker and Oba, 2018a, b). Elevated CO<sub>2</sub> (eCO<sub>2</sub>) influences photosynthesis, which is the entry point of carbon for plant metabolism. The expected rate of increase in photosynthesis is unclear because photosynthesis by CO<sub>2</sub> is also dependent on leaf temperature and other plant growth conditions such as water and nutrients (Zhu et al. 2017). Also, high temperatures will have a profound impact on species distribution across the globe and may affect many biological and physiological processes of temperature-sensitive plants. Therefore, plant responses would likely be prominent under eCO<sub>2</sub> and temperature due to their impacts on growth and flowering and it would be useful to study those responses in agriculturally important crops such as chilli. The impact of climate change and crop responses due to eCO<sub>2</sub> have been documented in wheat (Dais de Oliveira et al. 2013), however, the mechanisms of plant responses to combined eCO<sub>2</sub> and temperature are unclear (Zinta et al. 2018). Various workers have expressed their views regarding crop responses to climate change (Rojas-Downing et al. 2017).

Photosynthesis is a vital process necessary for crop growth, reproduction, productivity, and changes in CO<sub>2</sub> concentration or eCO<sub>2</sub> may have direct consequences which could be either positive or negative (Van der Kooi et al. 2016). Elevated CO<sub>2</sub> may cause an increase in the rate of photosynthesis (P<sub>n</sub>), which subsequently improves vegetative growth, crop biomass, and yield (Makino and Mae, 1999). This increased photosynthesis under eCO<sub>2</sub> occurs mainly due to an increase in the activity of its key enzymes, ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco). This bi-functional enzyme Rubisco catalyzes the carboxylation of RuBP and utilizes O<sub>2</sub> as a substrate to oxygenate RuBP during photorespiration (Pan et al. 2018). Under eCO<sub>2</sub> conditions, the rate of carboxylation will increase because, there will be a shift in the ratio of CO<sub>2</sub>:O<sub>2</sub> around the enzyme, whereas the rate of oxygenation

will decrease (Pan et al. 2018). In the growth and development of basil, a C3 plants, temperature and elevated CO<sub>2</sub> are considered important factors (Yuan et al. 2017). The negative consequences of eCO<sub>2</sub> would be related to an increase in temperature due to high levels of CO<sub>2</sub>. Photosynthesis is sensitive to heat stress and many crops adjust this process according to growth temperatures (Wen et al. 2005). The common stress-sensitive sites in the photosynthetic machinery are photosystem II (PSII) and its electron donor (OEC) and acceptor (QA, PQ) (Mittler et al. 2012). The plant can minimize the damage of heat stress by maintaining cellular homeostasis which enables them to function properly (Sakamoto and Murrata, 2002). The high temperature and eCO<sub>2</sub> are known to have significant effects (both positive and negative) on the growth and development of peppers or chilli (Kumari et al. 2019). The bell peppers grown in the high altitudes of Northern India showed vigorous growth and yield under 550 ppm of CO<sub>2</sub>, however, a temperature of 1 °C above ambient negated the positive effects of eCO<sub>2</sub>. A few reports also suggested vegetative and reproductive organ abscission in chilli and other crops, which seems to be regulated by growth hormones such as ethylene and auxin (Huberman et al. 1997).

'Bhut Jolokia' (*Capsicum chinense* Jacq.) is an orphan Solanaceous crop of semi-perennial nature grown in the North-Eastern (NE) region of India. The crop is the hottest chilli in this region with a Scoville heat unit of 1,001,304 (Bosland and Baral, 2007). This is an important spice crop of NE India and fruits have been consumed by people for a long period of time and possibly originated through the natural hybridization of *C. chinense* and *C. frutescens* (Bosland and Baral, 2007). Generally, the crop is planted in the backyard for household consumption, The crop received global attention once it was listed in the Guinness Book of World Records in (2006) as the hottest chilli in the world and since then 'Bhut Jolokia' gained commercial importance as a high valued export crop in entire Northeast India. Moreover, this triggered collection of various germplasm from NE India to study the variability and characterize the levels of capsaicin content within the available germplasm. Recently, two quantitative trait loci (QTLs) were identified in chromosomes 3 and 6 of 'Bhut Jolokia' (Lee et al. 2016). Heat stress-affected pepper transcripts, proteomes and metabolomes reported capsaicin biosynthesis genes which were positively correlated to capsaicin content (Liu et al. 2022). A study on the effects of different LED lightings on the post-harvest firmness and nutritional quality of chili peppers found that red and blue light increases the capsaicinoids content, while that of white and red light increases the amino acid content in pepper (Liu et al. 2022).

In the present study, the interactive effects of eCO<sub>2</sub> and temperature on various plant physiological processes of 'Bhut Jolokia' were studied. The two germplasms with high

pungency levels were collected from two different NE states of India, i.e., Manipur and Assam, and were further grown under varying  $e\text{CO}_2$  (at 380, 550, 750 ppm) and temperature regimes (at ambient,  $> 2^\circ\text{C}$  from ambient, and  $> 4^\circ\text{C}$  from ambient) to understand the impacts of various treatments on photosynthesis, carbohydrate content, and other physiological parameters.

## Material and methods

### Plant material and growing conditions

This study was conducted in the Department of Crop Physiology, Assam Agricultural University, Jorhat, India. The experimental site was situated at  $26^\circ 47'$  N latitude,  $94^\circ 12'$  E longitude, and an elevation of 86.6 msl. The experimental site experiences a subtropical climate with hot humid summer and a relatively dry and cold winter.

Two germplasms of 'Bhut Jolokia' were collected from Manipur (cv Manipur/ cv.1) and Assam (cv. Assam / cv. 2). The plants were exposed to four different combinations of  $\text{CO}_2$  and temperature, including natural ambient conditions. The experiment included four treatments viz. T1 = Field (Ambient  $\text{CO}_2$  condition and temperature conditions), T2 = CTGT I (380 ppm  $\text{CO}_2$  + ambient temperature), T3 = CTGT II (550 ppm  $\text{CO}_2$  +  $2^\circ\text{C}$  higher than ambient from flower bud initiation till maturity) and T4 = CTGT III (750 ppm  $\text{CO}_2$  +  $4^\circ\text{C}$  higher than ambient from flower bud initiation till maturity). Plants were raised in various Carbon dioxide temperature Gradient Tunnels (CTGTs; marketed by Genesis Technologies, Maharashtra, India). In the field conditions, plants were grown in pots kept in the open field (ambient  $\text{CO}_2$  and temperature condition). In the CTGTs, the  $\text{CO}_2$  was maintained throughout the entire crop growth period from 9 A.M. to 2 P.M. regularly. The elevation of temperature was maintained through Infra Red Heater regulated by SCADA software.

Topsoil and organic compost were collected, drenched with 0.1% Captan (WP), and covered with a plastic sheet. Later, Furadon 3G ( $3\text{ g/m}^2$ ) was mixed and covered for two days. Seeds of 'Bhut Jolokia' were also treated with Captan (@  $2.5\text{ g/kg}$  seed) fungicide agitating them for 5 min. in a container. Small earthen pots (28 cm in height and 30 cm in diameter) were filled with a mixture of soil and organic matter in an equal (50:50) ratio and the seeds were sown in those pots. Once the seedlings reached to 3–4 leaf stage, they were transplanted into big earthen pots (40 cm in height and diameter) filled with topsoil, sand, and cow dung in equal (1:1:1) parts. The pots were fertilized at the rate of N, P, and K in a ratio of 120: 60: 60 kg/ha. 60 kg N along with P and K as basal dose were applied and the remaining N fertilizer was applied as a top dressing in two split doses at 30 and

60 days after transplanting. A total of 5 pots per variety were kept in each treatment in a completely randomized manner. The data were collected from physiologically active leaves during the flowering stage and statistical analyses were performed from data obtained in the year I and II.

The structure of CTGTs were rectangular (10 m length, 2.5 m breadth, 2 m height) block, fabricated by metallic pipe and covered with polycarbonate sheet (100-micron gauge) for 85% transmission of light. Each tunnel was divided into four compartments of 2.5 m in length and within each chamber, 4 RTD temperature sensors were fitted. Humidity transmitters were placed in each chamber to get data in the control room through four core-shielded cables. Infrared heaters were mounted inside the CTGT. For measuring the ambient data, temperature sensors and humidity transmitters were placed outside the chamber. The humidity sensors were capable to measure humidity in the range of 0–100% relative humidity and  $\text{CO}_2$  gas cylinders were used for the supply of  $\text{CO}_2$  gas. An Air compressor was used to maintain the uniformity of  $\text{CO}_2$  gas.

### $\text{CO}_2$ and temperature control and monitoring

The system for monitoring and controlling the  $\text{CO}_2$  in CTGTs was fully automatic, and the desired level of  $\text{CO}_2$  was maintained in each tunnel. Data logger and the SCADA software were used to monitor and control appropriate  $\text{CO}_2$  levels in each chamber. The SCADA software facilitated the set of different concentrations of  $\text{CO}_2$  in the different CTGT chambers. Signals obtained from portable  $\text{CO}_2$  monitors were compared with an actual set value of  $\text{CO}_2$  level in ppm of each chamber. Most control actions were performed automatically by Remote Terminal Units or Data loggers. The  $\text{CO}_2$  in the chambers was controlled by a 12-channel measurement and control system using an ADC WA 526 IRGA (Infra-Red Gas Analyser). The system delivered (pure)  $\text{CO}_2$  to each chamber via 12 solenoid valves, according to a calculation based on the difference between the reading and the set point. The outlet of the  $\text{CO}_2$  regulator was connected to a manifold in which  $\text{CO}_2$  passes through distributing the system to respective CTGT. The manifold was used to distribute  $\text{CO}_2$  into hexagonal rings, and the solenoid valve is connected to each outlet of the manifold.

### Measurement of various physiological and biochemical parameters

The rate of photosynthesis ( $P_n$ ) of fully expanded 'Bhut Jolokia' leaves was measured using a portable Infrared Gas Analyzer [IRGA (Infrared Gas Analyzer, LI-6400, Lincoln, Nebraska, USA)] between 10.00 am to 12.00 noon. For leaf carbohydrate analyses, leaf samples were taken, and total

soluble sugar (TSS) and total non-structural carbohydrate (TNSC) was measured based on the methods described in McCready et al. 1950.

#### *Estimation of Carbon: Nitrogen ratio*

The C: N ratio was calculated based on the per cent carbon (C) and nitrogen (N) content of the leaves. Carbon content was estimated following the Walkley–Black's method (Walkley and Black, 1934). The dried plant samples were oxidized with a mixture of potassium dichromate and concentrated sulphuric acid using the heat of dilution of the acid. The unused potassium dichromate was estimated by back titration with ferrous ammonium sulphate.

Carbon content in plant parts was estimated using ground plant material. Fifty milligrams of plant sample was placed in a dry 500 mL conical flask. Ten milliliters of  $K_2Cr_2O_7$  solution were added to it. Flask was gently swirled, and 20 mL of concentrated sulphuric acid was rapidly added to it followed by immediate swirling so that the sample and the reagent were mixed. The flask was swirled several times and allowed to stand for 30 min. This was followed by the addition of 200 mL of distilled water and 10 mL of orthophosphoric acid. After adding 1 mL of diphenylamine indicator solution, the content was titrated with ferrous ammonium sulphate solution till the colour flashes from bluish-violet to green. A blank (without sample) titration was also carried out. The carbon content was calculated using the following formula and expressed in percentage.

$$C\% = \frac{X - Y \times (\text{Normality of ferrous ammonium sulphate} \times 0.003)}{A}$$

where, A = Weight of the sample X = Volume (mL) of ferrous ammonium sulphate solution required for blank titration, Y = Volume (mL) of ferrous ammonium sulphate needed for the sample. The carbon percentage was obtained by multiplying this C per cent with a constant, 1.3, as only 77 per cent recovery was presumed from oxidation of the sample in this procedure.

Nitrogen content in plant parts was measured by Kjeltac Auto Analyzer. The dried plant material was digested with concentrated  $H_2SO_4$  in the presence of a catalyst to convert the nitrogen to ammonium sulphate. Ammonia was liberated and collected in boric acid solution as ammonium borate by steam distillation of the salt in the presence of a strong alkali which is estimated against a standard acid titration.

Free proline content in the leaves was determined following the method of Bates et al. (1973).

Leaf sample (0.5 g) was homogenized in 5 mL of sulphosalicylic acid (3%) using a mortar and pestle. Two ml of extract was taken in a test tube, and 2 mL of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in the water bath at 100° C for

30 min, and a brick-red colour was developed. After cooling the reaction mixtures, 6 mL of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorbance read at 520 nm in a spectrophotometer against a toluene blank. The concentration of the sample was estimated by referring to a standard curve and the result was expressed in  $mg\ g^{-1}\ FW$ .

#### **Chlorophyll content**

Leaf chlorophyll was estimated by a Dimethyl Sulphoxide (DMSO) based non-maceration method Hiscox and Israelstam, (1979). Fresh leaves (0.5 g) were placed in a test tube containing 5 mL of DMSO and kept in an oven at 65 °C for 4 h followed by the addition of 10 mL of DMSO. The optimal density (OD) of the extract was measured in a spectrophotometer at 663 nm and 645 nm. The chlorophyll content was determined by the formula suggested by Sarkar and Oba, 2018a, b). The amount of chlorophyll content was calculated using absorption coefficients and accordingly, the ratio of chlorophyll a/b was calculated.

#### **Estimation of fruit drop**

The fruit drop per cent was calculated by recording the number of fruit dropping at weekly intervals and the total was calculated cumulatively on per pot. The total number of fruit harvested was utilized for calculating the fruit drop per cent by the following formula.

$$\% \text{Fruit drop/plant} = \frac{\text{Total no. of fruit drop per plant}}{\text{Total no. of fruit (harvested + fruit drop)}} \times 100$$

The data obtained were transformed by the arcsine transformation, and using those values statistical analysis was performed. Meteorological data during the period of experimentation have been included (Supplementary Fig. 1 and Supplementary Fig. 2).

#### *Statistical analysis*

The experiment was laid out in a Completely Randomized Design (CRD) having 2 factors and was replicated thrice. Statistical analysis of all the parameters was done following the method of analysis of variance (ANOVA) given by Panse and Sukhatme, (1967). The critical difference (CD) values were calculated at 5 per cent probability level.

#### **Semi quantitative reverse transcriptase-PCR for gene expression**

RNA was isolated using the RNeasy Plus Minikit (Qiagen, USA) with a genomic DNA removal column. Gene-specific

primers were designed using sequences from the NCBI databases. The primer design software used was Perfect Primer Design with Invitrogen's Oligo Perfect TM Designer ([www.invitrogen.org](http://www.invitrogen.org)). Primers for the actin gene were used as an endogenous control. RNA extracted from plants grown in CTGT I, II, and III were subjected to semi-quantitative RT-PCR using gene-specific primers following the protocol outlined in the one-step RT-PCR kit (Qiagen, USA). The PCR plate was subjected to 30 cycles of the following conditions: Cycle 1: PCR activation at 50 °C for 30 min, Cycle 2: 94 °C for 90 s for denaturation Cycle 3: 94 °C for 30 s for denaturation Cycle 4: 55 °C for 30 s Cycle 5: 72 °C for 3 min for elongation Cycle 6: 72 °C for 5 min for final extension Cycle 7: 4 °C for storage. Cycle 3- Cycle 5 was repeated for 30 cycles. All the samples were run in triplicates on the RT-PCR system. PCR amplified product was mixed with gel loading buffer (1X 2 µl) and loaded into the wells of the submerged gel. The gel was run at 80 V for 1 ½ hours until the tracking dye reaches two-third of the bottom edge of the gel. The gel was visualized under UV and photographs were taken. The details of selected primers for the construction of cDNA is provided in Supplementary Table 1.

## Results

### Photosynthesis in 'Bhut Jolokia' plants under elevated CO<sub>2</sub> and temperature

To understand the photosynthetic response in 'Bhut Jolokia' grown in *e*CO<sub>2</sub> and temperature, the net photosynthetic rate ( $P_n$ ) of leaves was measured using IRGA. The  $P_n$  for various treatments is summarized in Table 1. Photosynthesis was highest in both germplasms under CTGT II conditions.  $P_n$  was significantly higher in the cv. Manipur in CTGT II compared to the field conditions, CTGT I and CTGT III. The *e*CO<sub>2</sub> of 550 ppm exhibited a stimulatory effect on the  $P_n$  in both the 'Bhut Jolokia' germplasms. On the contrary, 750 ppm of CO<sub>2</sub> and 4 °C above ambient temperature (CTGT III) compromised  $P_n$  in plants of both germplasms when compared to those grown in the CTGT II. No significant differences were found in the  $P_n$  of the plants grown in the field and CTGT I.

### Accumulation of carbohydrate under elevated CO<sub>2</sub> and temperature in 'Bhut Jolokia'

The total soluble sugar (TSS) which indicates the active assimilation of photosynthates was measured using standard protocols. The TSS in the leaves of both the germplasms was significantly higher under *e*CO<sub>2</sub> and the temperature of CTGT II. However, results also exhibited a significant decline in TSS with the increasing CO<sub>2</sub> and

**Table 1** Interactive effect of elevated carbon dioxide (*e*CO<sub>2</sub>) and high temperature on the rate of photosynthesis and total nonstructural carbohydrate content in Bhut Jolokia' germplasm

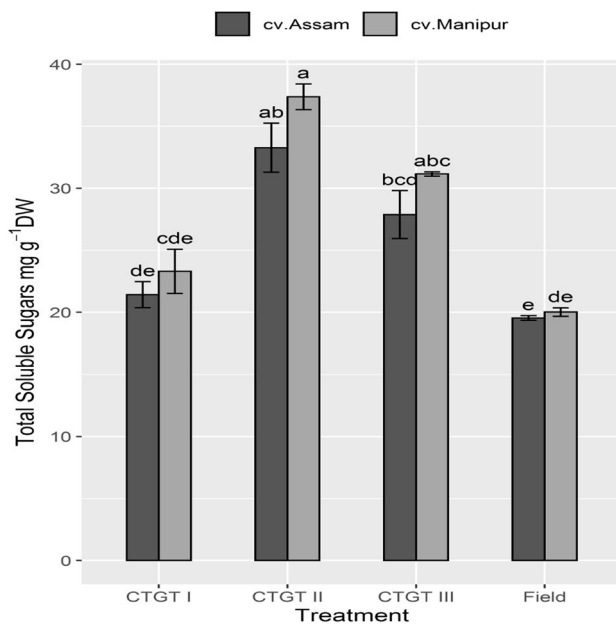
	Photosynthesis (µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )		Total Non structural Carbohydrate (mg g <sup>-1</sup> DW)	
<i>Treatments</i>				
Field	17.17		67.60	
CTGT-I (Amb)	16.45		68.86	
CTGT-II	20.87		91.61	
CTGT-III	17.93		85.16	
SEd	1.004		2.49	
CD (0.05%)	2.128		5.28	
<i>Cultivar</i>				
cv. 1 (Manipur)	18.88		81.28	
cv. 2 (Assam)	17.33		75.33	
SEd	0.71		1.76	
CD (0.05%)	1.505		NS	
<i>Treatment x Cultivar</i>				
Field	cv. 1	cv. 2	cv. 1	cv. 2
	18.37	15.97	68.00	67.20
CTGT-I (Amb)	17.80	15.10	68.57	69.16
	20.83	20.90	97.18	86.04
CTGT-II	18.50	17.37	91.37	78.94
	1.42		0.557	
SEd	1.42		0.557	
CD (0.05%)	NS		NS	

temperature in the CTGT III compared to CTGT II. In comparison to cv. 2, the plants of cv. 1 grown under CTGT II accumulated more TSS (Fig. 1). In addition, the total non-structural carbohydrate content (TNSC) of plants grown in various *e*CO<sub>2</sub> and temperature regimes was also measured. Results suggested that plants of both germplasms grown in CTGT II and III accumulated the highest TNSC compared to the plants grown in the field and ambient CO<sub>2</sub> conditions (Table 1). However, cv. Manipur exhibited higher TNSC accumulation in the CTGT II and III compared to cv. 2 (Table 1).

### Carbon: nitrogen ratio in leaves of 'Bhut Jolokia'

The C: N ratio in the leaves of two different germplasms of 'Bhut Jolokia' varied significantly under *e*CO<sub>2</sub> and temperature. The leaf C: N ratio of both the germplasms was the highest in CTGT II and III compared to the plants grown in the field and ambient conditions. A significantly higher C: N ratio ranging from 6.92 to 5.91 was recorded in CTGT II and CTGT III, respectively (Fig. 2). A significant drop in the C: N values were recorded in Field and



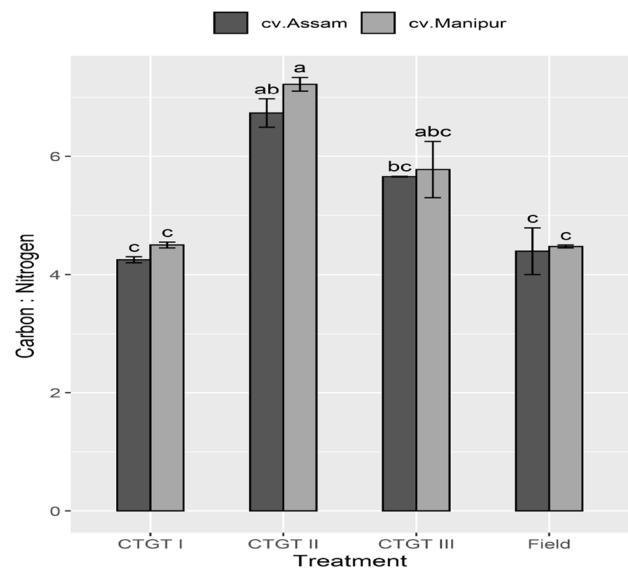


**Fig. 1** Interactive effect of elevated CO<sub>2</sub> and temperature on total soluble sugar. Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on the endogenous content of total soluble sugars (mg. g<sup>-1</sup> dry weight) on two germplasm of Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub>+ambient temperature), CTGT II (550 ppm CO<sub>2</sub>+2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub>+4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other ( $n=6$ )

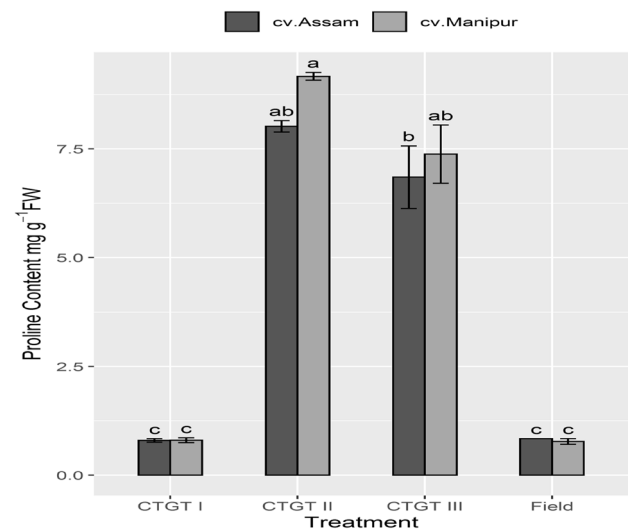
CTGT I compared to CTGT II and CTGT III. The  $e$ CO<sub>2</sub> and temperature favored vigorous vegetative growth of ‘Bhut Jolokia’ that is reflected in the C: N values.

### Leaf proline content in ‘Bhut Jolokia’

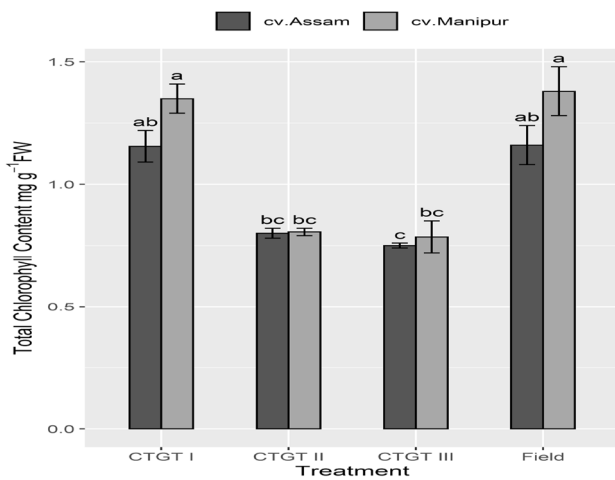
It is well known that the amino acid proline accumulates to protect plants from various stresses. Therefore, the proline levels in the plants grown in various CO<sub>2</sub> and temperature regimes were measured. As expected, results showed higher levels of proline accumulation in the plants grown under  $e$ CO<sub>2</sub> and temperature (CTGT II and III). The increment in the proline content of both the germplasm at CTGT II and CTGT III was significantly higher than that of CTGT I and Field. CTGT II and CTGT III recorded variations in proline accumulation within the germplasm. Interestingly, the proline content of leaves of ‘Bhut Jolokia’ cv. 1 was more compared to the cv. 2 under CTGT II and III (Fig. 3).



**Fig. 2** Interactive effect of elevated CO<sub>2</sub> and temperature on C: N ratio. Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on carbon, C: nitrogen, N (C:N) ratio of the two germplasm of Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub>+ambient temperature), CTGT II (550 ppm CO<sub>2</sub>+2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub>+4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other ( $n=6$ )



**Fig. 3** Elevated CO<sub>2</sub> and temperature altered proline content (mg g<sup>-1</sup> fresh weight). Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on proline content (mg. g<sup>-1</sup> fresh weight) of the two germplasm of Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub>+ambient temperature), CTGT II (550 ppm CO<sub>2</sub>+2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub>+4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other ( $n=6$ )



**Fig. 4** Total chlorophyll content changes under elevated CO<sub>2</sub>. Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on total chlorophyll content (mg. g<sup>-1</sup> fresh weight) of the two germplasm of Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub>+ambient temperature), CTGT II (550 ppm CO<sub>2</sub>+2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub>+4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other (n=6)

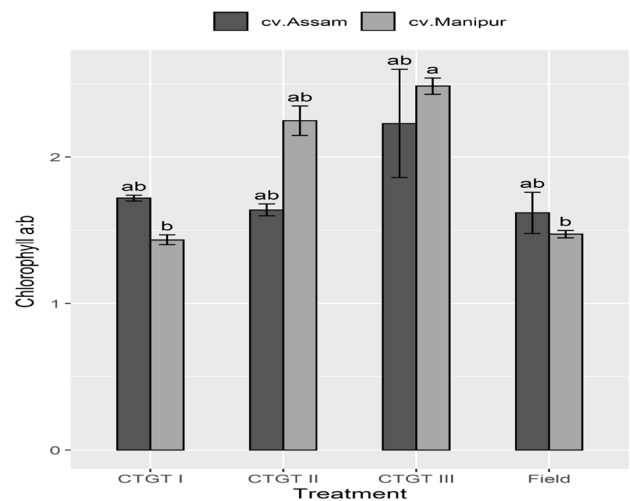
**Chlorophyll content and chlorophyll a: b ratio of ‘Bhut Jolokia**

The leaf chlorophyll content in the ‘Bhut Jolokia’ plant of both the germplasms under treatment was lower than those grown in the field and ambient conditions (Fig. 4). A significant decline in chlorophyll content under eCO<sub>2</sub> and temperature was recorded. When compared with cv. 1, chlorophyll content in leaves of cv. 2 recorded a decrease of 8%. Stress-induced reduction in chlorophyll content ranged from 42% (CTGT II) to 47% (CTGT III) compared to ambient conditions. The interaction effect of treatments and germplasm also recorded a significant difference.

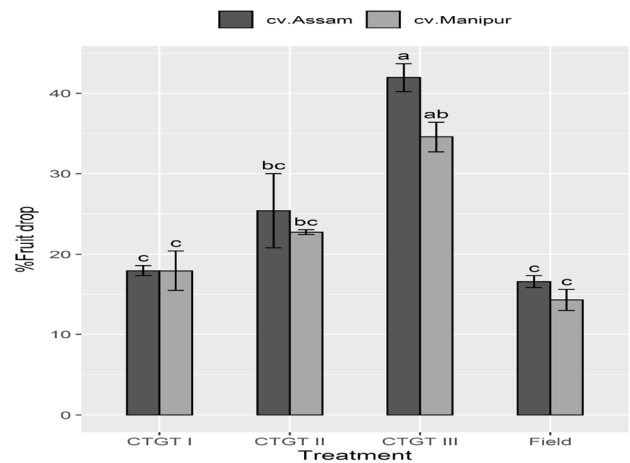
In addition, eCO<sub>2</sub> and temperature brought about a significant difference in chlorophyll a/b ratio in cv. 1 (Fig. 5). Cv. 1 recorded a significant variation in chlorophyll a/b ratio in CTGT III with that of CTGT I and the field. A non-significant variation within treatments was recorded in cv. 2.

**Fruit drop of ‘Bhut Jolokia’ under eCO<sub>2</sub> and temperature**

The elevated CO<sub>2</sub> and temperature could also influence the yield. One of the major problems of the local ‘Bhut Jolokia’ germplasms is fruit drop; therefore, in the current study, the intensity of fruit drop was calculated due



**Fig. 5** Interactive effect of elevated CO<sub>2</sub> and temperature on chlorophyll a:b ratio. Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on Chl a:b ratio of the two germplasm of Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub>+ambient temperature), CTGT II (550 ppm CO<sub>2</sub>+2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub>+4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other (n=6)



**Fig. 6** Interactive effect of elevated CO<sub>2</sub> and temperature on percent fruit drop. Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on %fruit drop of the two germplasm of Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub>+ambient temperature), CTGT II (550 ppm CO<sub>2</sub>+2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub>+4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other (n=6)

to various treatments. Results showed that the intensity of fruit drop was higher under elevated CO<sub>2</sub> and temperature (CTGT III) compared to CTGT I and field. The percent fruit drop was 40% in cv. Assam and 35% in cv. Manipur grown under CTGT III (Fig. 6). Data revealed that 550 ppm of CO<sub>2</sub> and 2°C above ambient temperature had a marginal effect on per cent fruit drop of cv. Manipur.

### Expression of photosynthesis-related genes in ‘Bhut Jolokia’

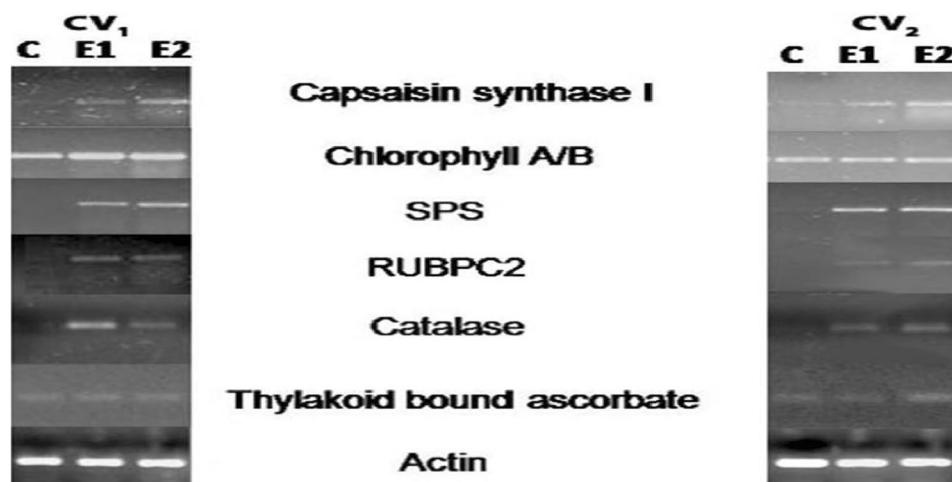
The expression of four photosynthesis-related genes such as *chlorophyll A/B*, *sucrose phosphate synthase (SPS)*, *RuBPC2*, *thylakoid bound ascorbate peroxidase*, and two other genes, *catalase* for defence-related and *capsaicin synthase I* for capsaicin were studied. Expression of the gene in plants grown CTGT II (E1) and CTGT III (E2) with CTGT I (control; C) was compared. Samples from plants grown in CTGT I was used as a control instead of plants grown in the field to avoid the additive effects of various environmental conditions, including soil on gene expression. The transcript abundance of the photosynthesis-related genes viz. *chlorophyll A/B*, *SPS*, *RuBPC2*, *thylakoid bound ascorbate peroxidase* revealed differences between the two germplasm. The expression of *RuBPC2* was higher at *eCO*<sub>2</sub> and temperatures in both germplasms. Under the elevated CO<sub>2</sub>, and temperature, *RuBPC2* and *SPS* transcript was up regulated in comparison to the ambient. The gene expression of *Chlorophyll A/B* and *thylakoid bound ascorbate peroxidase* was not affected by the elevated climatic conditions in comparison

to ambient. In both germplasms, the expression of *SPS* was highly up regulated under elevated conditions. The expression of *catalase*, an enzyme that expresses during stress was also studied. Up-regulation of *catalase* in both the germplasm was recorded (Fig. 7). The influence of *eCO*<sub>2</sub> and temperature on the expression of *capsaicin synthase I* in ‘Bhut Jolokia’ showed up-regulation under CTGT II and III conditions compared to CTGT I.

### Discussion

#### Photosynthesis under elevated CO<sub>2</sub> and temperature

An *eCO*<sub>2</sub> can have both positive and negative effects on the productivity of major crops. One of the reasons for a positive impact is the fact that the carboxylation of RuBP is not saturated in the current CO<sub>2</sub> levels in the atmosphere. The sensitivity of the rate of carboxylation of RUBP to temperatures > 35 °C has been reported (Perdomo et al. 2017). Therefore, an increase in the rate of photosynthesis can be expected with *eCO*<sub>2</sub> combined with a moderately higher temperature regime. Data from our present study also indicate that *eCO*<sub>2</sub> levels up to 550 ppm with a rise in temperature by 2 °C (CTGT-II) above ambient temperature favored photosynthetic rate (Pn) in hot chilli (‘Bhut Jolokia’). The Bhut jolokia, being a C<sub>3</sub> plant species undergoes photorespiration; however, with *eCO*<sub>2</sub> by way of suppression of photorespiration rate and Pn might have increased as noted in the current study. Elevated CO<sub>2</sub> decreases the allocation



**Fig. 7** Semi-quantitative gene expression analysis of some photosynthesis and capsaicin-related genes under elevated CO<sub>2</sub> and temperature. Effects of different levels of carbon dioxide (CO<sub>2</sub>) and temperatures (°C) on semiquantitative RT-PCR expression of key genes of photosynthesis *Capsicum chinense chlorophyll A/B*, *Ccapsaisin synthase I*, *CcSPS*, *CcRUBP*, *Cccatalase* of the two germplasm of

Bhut Jolokia subjected to different treatments. Different treatments given were: CTGT I (380 ppm CO<sub>2</sub> + ambient temperature), CTGT II (550 ppm CO<sub>2</sub> + 2 °C higher than ambient from flower bud initiation till maturity) and CTGT III (750 ppm CO<sub>2</sub> + 4 °C higher than ambient from flower bud initiation till maturity), normal field conditions. Bars with different letters are statistically different from each other (*n* = 6)



of electron transport to photorespiration and increases the electron flow to RuBisCO carboxylation

(Pan et al. 2018). Such mechanisms potentially functioned in the current study, which may result in the promotion of photosynthesis. Ahammed et al. (2022), reported the effect of elevated atmospheric  $\text{CO}_2$  on physiological processes viz. photosynthesis, stomatal movement, and ethylene production as a result of response under abiotic stress. They have reported the role of elevated  $\text{CO}_2$  in delaying senescence. In current study, the CTGT III, the temperature-induced reduction in photosynthesis was observed with a regime of 750 ppm of  $\text{CO}_2$  and a temperature 4 °C above the ambient (CTGT-III). The reduced fixation rate of  $\text{CO}_2$  at high temperature could be due to a reduction in the stomatal aperture in ‘Bhut Jolokia’ as observed in the present investigation. Qaderi et al. (2006) opined that stomatal closure reduces  $\text{CO}_2$  diffusion to the leaf mesophyll which is the prime  $\text{CO}_2$  fixation site as observed in *Brassica*. Although a positive effect of  $e\text{CO}_2$  in ‘Bhut Jolokia’ was found in our study, adverse effects of  $e\text{CO}_2$  on  $P_n$  were reported when plants were exposed to  $e\text{CO}_2$  for a longer period in growth chambers (Warren et al. 2014). A study on clover plants exposed to 600 ppm of  $\text{CO}_2$  for 8 years showed a 37% increase in photosynthesis after acclimation (Ainsworth et al. 2003). This lower rate of  $P_n$  at CTGT III could also be due to the accompanying rise in temperature. Moore et al. (2021) reported that the inhibition of photosynthetic rate in plants grown under heat stress may be due to a reduction in RuBP regeneration rate and inactivation of oxygen-evolving enzymes. In both  $C_3$  and  $C_4$  plants, inhibition of net photosynthesis which is related to a decrease in active Rubisco accounts for the temperature response of net photosynthesis (Salvucci and Brandner, 2003). Increase in photosynthesis under  $e\text{CO}_2$  (500 ppm and 2 °C) elevation of temperature from ambient might also be linked to the proper maintenance of some physiological processes like stomatal conductance (Supplementary Table 2) with a reduced level of transpiration rate (Supplementary Table 3). Ahammed et al. (2022), reported the effect of elevated atmospheric  $\text{CO}_2$  on photosynthesis, stomatal movement, ethylene production. They have reported the role of elevated  $\text{CO}_2$  in delaying senescence. Habermann et al. (2019) also reported the association of stomatal properties with the positive combined effect of  $e\text{CO}_2$  (600  $\mu\text{mol mol}^{-1}$ ) and elevated temperature (+2 °C) in a  $C_3$  plant *Stylosanthes capitata*. The  $e\text{CO}_2$  treatment up to 550 ppm could reduce the stomatal density, stomatal index, and stomatal conductance ( $g_s$ ), resulting in reduced transpiration rate, thus maintaining the favorable plant water status during the growing period. Elevated  $\text{CO}_2$  could stimulate photosynthesis, starch content, water use efficiency, and PSII photochemistry in the leaves (Habermann et al. 2019). According to them, both  $e\text{CO}_2$  and elevated temperature caused an increase in leaf temperature by negatively

affecting transpiration, however, the stomatal functioning was independent of the rise in temperature. Das et al. (2016) reported higher carotenoid content in cv Manipur as compared to cv Assam under both the elevated  $\text{CO}_2$  and temperature regimes. Therefore cv Manipur showed better heat dissipation, thereby minimizing the impact of increased leaf temperature. Elevated  $\text{CO}_2$  and high temperature induce the expression of respiratory burst oxidase homologs (RBOHs) and reactive oxygen species (ROS) which plays a critical role as a signaling molecule in stomatal movement, for optimizing gas exchange and water use efficiency (Ahammed et al. 2021) The results of the present study are consistent with the findings of Habermann et al. (2019) in Tropical Forage Legume. Similarly, Zhang et al. (2019) in tomato and Chavan et al. 2019 in wheat, suggested that the combined been reported due to the influence effects of  $e\text{CO}_2$  and temperature not only control the stomatal opening but also improve functioning. Driesen et al. (2020) also reported that the varied stomatal response could be observed under changing environments, which could affect the stomatal conductance and photosynthetic rate. Stomatal development and its opening are significantly influenced by environmental factors both in the short and long term. A reduction in stomatal conductance might decrease the transpiration rate under  $e\text{CO}_2$ , thereby conserving soil water (Purcell et al. 2018).

### Carbohydrates accumulation under elevated $\text{CO}_2$ and temperature

In the present study, an increase in total non-structural carbohydrate (TNSC) content has been reported due to the influence of  $e\text{CO}_2$  and temperature. Such an increase in TNSC might have increased the osmotic potential as a means to combat the high temperature stress by improving the osmoregulation of cells. This high sugar concentration could also change the temperature response of respiration by eliminating the increased respiration rate. Thus enhanced leaf sugar concentrations or enhanced osmotic potential may protect leaf cells from heat stress, i.e. higher sugar concentrations significantly modified the temperature-response curve of respiration by eliminating the increased respiration rate. Moreover, the increase in osmotic potential under the influence of sugars might protect the respiratory membranes and reduce starch degradation and consumption in respiratory processes (Huve et al. 2012). At CTGT III, the elevated temperature might have caused an impact on water stress due to higher temperature, which might be causally related to lower photosynthetic capacity in plants grown under CTGT III compared to that of CTGT II. Accordingly (Pan et al. 2018) high temperature might have a negative impact on, carboxylation rate, RuBP regeneration rate and maximal photochemical efficiency of PSII. Das et al. (2020b) recorded a significant increase in plant growth parameters

viz. root: shoot ratio, leaf area index (LAI), leaf area duration (LAD), specific leaf weight (SLW) and a reduction in specific leaf area (SLA) under elevated CO<sub>2</sub> and temperature in hot chilli due to elevated CO<sub>2</sub>. They explained that the negative effect of high temperature was counteracted by a higher level of CO<sub>2</sub> when plants were grown under ambient conditions. Similar effect was also observed by Das (2020a) in *Brassica species* when grown under elevated CO<sub>2</sub> under moisture stress conditions in free air CO<sub>2</sub>.

Soluble sugars act as signaling molecules and play an important role in the expression of various genes, hence controlling plant growth and metabolism (Li et al. 2018). Plants grown at elevated atmospheric CO<sub>2</sub> require coordination of photosynthate production and sink demand. Elevated CO<sub>2</sub> stimulates photosynthesis; this will lead to an increase in carbohydrate content and thereby respiratory metabolism. This leads to an increase in plant growth and biomass and economic yield (Leakey et al. 2009).

The higher accumulation of TSS and total non-structural carbohydrate in both the ‘Bhut Jolokia’ germplasm under CTGT II could have resulted from high P<sub>n</sub>. Analyses showed a highly significant positive correlation between photosynthesis and TSS ( $r=0.892$ ) as shown in Table 2. The higher accumulation of sugar in ‘Bhut Jolokia’ in our study could be due to the stimulatory effect of elevated temperature as observed by Han et al. (2013) in lettuce. They reported that the accumulation of soluble sugar was higher in heat-resistant varieties than that in non-heat-resistant varieties.

A large accumulation of non-structural carbohydrates (TNSC) in the leaf has been reported under elevated CO<sub>2</sub> (Yoshinaga et al. 2020). During plant growth and metabolism, non-structural carbohydrate (NSC) has been reported to act as a substrate for respiration (Pan et al. 2002). NSC accumulation in the stem acts as a reserve and is directly related to sink strength and seed setting in rice (Fu et al. 2011). Due to the greater accumulation of soluble sugars, and non-structural carbohydrates in the cell sap, favourable water status could be maintained through osmoregulation. In the present study also, the higher accumulation of NSC in the CO<sub>2</sub>-enriched plant might have possibly helped in the maintenance of water status by osmoregulation. Long-term exposure of the plant to eCO<sub>2</sub> could enhance growth and ameliorate the negative effect of stress (Wullschleger et al. 2002).

In the present study, higher NSC could have played a defensive role during the stress condition. Das (2020) reported in *Brassica spp.*, that the increase in NSC might be related to the higher photosynthetic rate of the treated plants. Ulfat et al.

(2021) reported a better assimilation rate of CO<sub>2</sub> in plants grown under higher CO<sub>2</sub> levels by enhancing antioxidant potential and due to a higher activities of carbohydrate metabolic enzymes. Under elevated CO<sub>2</sub>, high concentration of total NSC and N was reported in the stems when compared to ambient condition (Cao et al. 2020). From the present study, it was clear that the elevated CO<sub>2</sub> could ameliorate the deleterious effects of high temperature on plants by augmented rates of net photosynthesis. This may lead to provide more substrate for osmotic adjustment during drought and high temperature. Zhuo et al. (2000) also reported that abundant soluble carbohydrates could enhance cell division and wall expansion under a higher level of CO<sub>2</sub>. There may be an alternation of C metabolism and growth due to interactive effects of elevated CO<sub>2</sub> and high temperatures. Song et al. 2014, also reported that in C<sub>3</sub> species for example, Kentucky bluegrass (*Poa pratensis*) under elevated CO<sub>2</sub> could promote plant growth, whereas high temperature leads to an inhibitory effect when plants were exposed to a higher regime of temperatures (25 °C and above). Under such condition, a decrease in the rate of photosynthesis, shoot and root growth was evident along with an increase in leaf respiration rate leading to a negative C balance and a decline in soluble sugar content under ambient CO<sub>2</sub>. According to them, eCO<sub>2</sub> could ameliorate the adverse effects of high temperatures (30 and 35 °C) which could be associated with the maintenance of a positive C balance and the accumulation of soluble sugars and TNSC by stimulating photosynthesis and suppressing respiration.

The accumulation of soluble sugars, as well as NSC in the cell-sap, might have helped in the maintenance of osmoregulation in ‘Bhut Jolokia’ leaves and avoiding sink limitations by developing new sinks. This might have resulted in higher productivity under CTGT II conditions. Interestingly, the up-regulation of SPS transcript under eCO<sub>2</sub> and temperature (CTGT-II) in ‘Bhut Jolokia’ suggests the enhanced activity of carbohydrate metabolizing enzymes such as sucrose phosphate synthase which might have resulted in greater accumulation and export of carbohydrates to the development of new sinks. This might be the reason behind high P<sub>n</sub> in ‘Bhut Jolokia’ plants grown in CTGT II. As compared to CTGT II, plants grown at CTGT III experienced a decline in P<sub>n</sub> and reduced accumulation of TSS and TNSC. The reason for the decline in P<sub>n</sub> at 750 ppm could be related to the decreased mobilization of carbohydrates due to less number and size of the sink, which results in the inhibition of photosynthesis (feedback inhibition).

### Enhanced biomass under elevated CO<sub>2</sub> and temperature

The CO<sub>2</sub> enrichment and elevated temperature brought about a significantly higher C: N ratio in ‘Bhut Jolokia’ grown in CTGT II. This higher C:N ratio indicates that plant tissue

**Table 2** Correlation study between Total non-structural carbohydrate (TNSC), total soluble sugar (TSS) and rate of photosynthesis

	Photosynthesis	TSS
TNSC	0.892*	0.964*
TSS	0.932	

accumulated more carbohydrates compared to nitrogen, which may improve above-ground biomass, rate of decomposition and alter the balance of vegetative and reproductive growth (Fageria and Baligar, 2005). Marcos-Barbero et al. (2021) found that when  $e\text{CO}_2$  was combined with higher temperatures, it increased biomass and yield in wheat, counteracting the negative effects of temperature on grain yield. (Das 2021) reported the favourable effect of elevated  $\text{CO}_2$  on dry matter partitioning in the Brassica crops. The increment in C: N ratio in 'Bhut Jolokia' may be due to a higher accumulation of carbohydrates in the leaf tissues under  $e\text{CO}_2$  and temperature. Interestingly, the change in the C: N ratio in soybean (*Glycine max*) occurred due to increased structural and NSC and decreased protein content (Allen et al. 1988). The increase in C: N ratio could be due to a reduction in the relative concentration of N in plants compared to C when grown under  $e\text{CO}_2$ . Under elevated  $\text{CO}_2$ , inhibition in the assimilation of leaf nitrate has also been reported by Bloom et al. (2014). Enhanced  $\text{CO}_2$  raised the C:N ratio in rice and wheat crops, with the changes owing to an increase in C concentration and a decrease in N concentration, implying that the beneficial effect of elevated  $\text{CO}_2$  could offset the detrimental impact of warming on the C:N ratio Bloom et al. (2014). Due to  $e\text{CO}_2$ , a depression in the glycolate pathway is apparent and because of that, there will be less production of the amino acids (glycine and serine) leading to alteration in C:N ratio. Moreover, a decline in the photo-respiratory pathway may lead to a reduced breakdown of stored carbon.

#### Effect of elevated $\text{CO}_2$ and temperature on proline content

The amino acid proline accumulates in plants in almost all adverse growing conditions such as drought, high temperature, low temperature, nutrient deficiency, diseases, etc. (Sarker and Oba, 2018a, b). The accumulation of proline helps maintain the protoplasm and osmotic balance in the plant, which reduced water loss and provide an N reserve for the plant to recover from the stress (Sarker and Oba, 2018a, b). High-temperature stress also induces physiological and biochemical changes and accumulation of compatible osmolytes (Wang et al. 2008). A rapid increase in proline content with an increase in  $\text{CO}_2$  and temperature was reported in leaf lettuce (*Lactuca* sp) (Han et al. 2013). In the present study, an increase in proline and soluble sugar offered osmotic adjustment to the stressed plants. The increase in the concentration of compatible solutes facilitated the adaptability by maintaining membrane function at high temperatures. Results depicted that 'Bhut Jolokia' plants grown under  $e\text{CO}_2$  and temperature (CTGTIII) can perform with better yield over CTGTIII. Das and Uprety (2006) reported that elevated  $\text{CO}_2$  reduced the accumulation of active oxy radicals and increased the activity of antioxidant enzymes ameliorating the drought-induced

oxidative stress effects in *Brassica* species when grown under the FACE facility. Many scientists believe that the first reaction of most plants responds to drought stress by closing their stomata in a bid to conserve moisture. Stomatal closure can cause a progressive decline in PSII electron transport, triggering proline and malondialdehyde (MDA) accumulation in plants. Malondialdehyde is produced from membrane lipid oxidative damage, which causes an increase in the activity of antioxidant enzymes, namely POD and CAT Mardinata et al. (2021).

#### Effects of elevated $\text{CO}_2$ and temperature on chlorophyll content and chlorophyll a:b ratio

The total amount of leaf chlorophyll content and the ratio of Chl *a/b* directly influence the photosynthetic capacity of plants (Li et al. 2018). In the present study, a decrease in chlorophyll content was recorded in CTGT III as compared to CTGT II. At the same time, the ambient condition showed the highest content of chlorophyll in both cultivars. There was an increase in chlorophyll *a/b* under  $e\text{CO}_2$  and high-temperature stress, which might be attributed to the faster degradation of chlorophyll *b*. A link between the decrease in chlorophyll content and the C: N ratio was reported by Lee et al. (2020).

In contrast, the concentration of chlorophyll declined in the later stages. Photosynthetic pigments also declined under unfavourable environments/environmental stress like drought and salinity, corroborating the present findings (Sarker and Oba, 2018a, b). An increase in the Pn indicates that NADP is reduced to NADPH, where the electron source is from an excited chlorophyll molecule. The higher chlorophyll content observed in the Bhut Jolokia in CTGT II appears to be at the cost of decreased synthesis of chlorophyll *b*. For accepting electrons, a more core complex of photo-system might have been produced in the lipid bilayer by converting chlorophyll *b* to chlorophyll *a*. Released chlorophyll *b* from LHC *a/b*-protein photo-system II can be converted to chlorophyll *a* for forming core complexes of photo-systems (Ohtsuka et al. 1997).

#### Expression of photosynthesis-related genes

One of the important observations is the expression of *RuBPC2* under  $e\text{CO}_2$  and elevated temperature in both germplasms. A common response of  $\text{C}_3$  plants grown at elevated  $\text{CO}_2$  is photosynthetic acclimation, implemented by downregulation of the *RbcS* transcript abundance, compared with the *RbcS* transcript abundance of plants grown at ambient  $\text{CO}_2$ . However, results indicated that both germplasms showed more *RuBPC* transcript under elevated climatic conditions. Ainsworth et al. (2003) reported that elevated temperature resulted in the transcription level of these genes as in soybean. Similar results

were reported in *Arabidopsis* (Li et al. 2008). In the present study, the *SPS* transcript was up-regulated in CTGT II when compared to the ambient. A higher in vivo SPS activity and an increase in leaf blade elongation rate were observed under  $e\text{CO}_2$  where the growing elongated blades acted as strong carbohydrate sinks (Seneweera et al. 1995). The up-regulation of the *catalase* was observed, which could be due to prolonged exposure of plants to  $e\text{CO}_2$  concentrations. Some researchers conducted research on genome-wide gene expression analysis under heat stress in poplar, they observed a number of transcription factors and gene that were differentially expressed (Song et al. 2014 and Kim et al. 2021). In response to heat stress, these factors interact and regulate the expression of photosynthesis-related genes, affecting carbon fixation and stomatal conductance (Song et al. 2014). Capsaicin is the key metabolite in terms of its commercial importance of Bhut Jolokia, therefore the levels of the Capsaicin forming gene, *capsaicin synthase I* was studied and over-expression of *capsaicin synthase I* transcripts in plants grown under CTGT II and III was recorded. Elevated  $\text{CO}_2$  might have increased the capsaicin content (a secondary metabolite) thereby showing upregulation of the *capsaicin synthase* gene as a result of more incorporation of the  $\text{CO}_2$ . A similar result of an increase in tea quality has been reported by Ahammed et al. (2021).

### Fruit drop under elevated $\text{CO}_2$ and temperature

The primary factor for the decrease in fruit production has been attributed to high temperatures. The decrease in fruit production is again attributed to flower and immature fruit abortion and not because of decreased flower initiation or plant growth. Amongst the studied germplasms, cultivar Assam showed higher fruit drop as compared to cultivar Manipur under CTGT-III. High-temperature stress is known to cause a profound impact on reproduction resulting in a reduced number of pollen grains, pollen infertility, and stigma deformation (Das et al. 2021) and lower fruit setting (Das et al. 2020b) and reducing yield in hot chilli (Das et al. 2020b) and reduced the fruit quality in hot chili (Das et al. 2016). Aloni et al. (1996) suggested that an accumulation of sugars and starch in flowers during daytime is an important factor in determining their retention and fruit set. Prasad et al. (2001) in their study on the effect of high temperature in peanut (*Arachis hypogaea*) has also reported that the plant was especially sensitive to elevated temperature from a few days before pollen maturation through fertilization of the ovule.

In the present investigation, an increase in premature fruit drop under CTGT III was observed as compared to CTGT II, which might be due to the formation of the lesser amounts of photosynthates in the form of TNSC leading to unbalanced nutrition and water transport under CTGTIII causing formation in abscission layer. The high temperature at CTGT III might have caused water stress, leading to abscisic acid (ABA)

accumulation and subsequently causing senescence and abscission of immature fruits.

### Conclusion

It is concluded that  $\text{CO}_2$  increment up to 550 ppm with a rise of 2 °C temperature has a positive effect on *Capsicum chinense*. A higher photosynthetic and carbohydrate status and lower fruit drop were observed in cv. Manipur at CTGT II. Both the germplasms showed up-regulation of transcripts related to photosynthesis, carbohydrate metabolism and capsaicin production, which correlates with biochemical responses in the present study. Germplasm Manipur (cv 1) was better adapted to the  $e\text{CO}_2$  and temperature compared to cv. Assam (cv.2) indicating its potentiality to adapt to the future climate change phenomenon. The present findings will help identify cultivars, develop the models, and in modifying cultivation and nutrient application technologies for future environments of higher  $\text{CO}_2$  and temperature stress.

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**Author contribution** SD performed experiments and analyzed data with the guidance from RD. SA helped to do semi quantitative RT-PCR in her laboratory. SD and SA co-wrote the manuscript. RD obtained grants for this research work. SK, SP, PK and AJN wrote and reviewed the manuscript.

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### Declarations

**Conflict of interest** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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