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Research Article

A study on elevated carbon dioxide (eCO_2), elevated temperature (eT) and their interactive effect on chickpea (*Cicer arietinum* L.) yield and seed mineral nutrients

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Abstract
Continuously increasing carbon dioxide concentration is predicted to elevate the earth's temperature. Elevated temperature is a severe problem for the cultivation of chickpea (*Cicer arietinum* L). Therefore, the present study aimed to assess the effect of elevated carbon dioxide (eCO_2), elevated temperature (eT) and their interactive effect on yield and seed mineral nutrients of two genotypes, i.e., ICC 4958 (*desi*) and Flip 90-166 (*kabuli*) of chickpea (*C. arietinum* L). The pot experiments were conducted in Open Top Chamber (OTC) for two consecutive years (2019-20 and 2020-21), along with the control placed in ambient natural conditions. The eCO_2 (650±50 µl/l) and eT (~4°C) were given individually and in combination. The gaseous exchange was measured at the flowering stage. After harvesting, yield and its parameters, seed protein and mineral nutrients were determined using standard methods. Under eCO_2, the photosynthesis of both genotypes was positively affected, ultimately converting to yield (8.8-17.5% increase). However, the effect was more prominent in ICC 4958 than Flip 90-166. Higher temperature only positively affected dry biomass, but that effect was not converted to yield; instead, a reduction occurred in yield (- 12.0 to -26.9%). In combination with eCO_2 and eT, the negative effect of high temperature was ameliorated by eCO_2 on yield, augmenting the effects on seed nutrient reduction. Among the seed mineral nutrients, Na, K, Fe and Zn were most reduced (-20.3 to -30.0%) under interactive effect. The findings will help to enhance seed yield with improved mineral nutrient content of chickpea.

Keywords: Chickpea, Climate change, Elevated carbon dioxide (eCO_2), Seed yield, Elevated temperature (eT)

INTRODUCTION

Carbon dioxide concentration (CO_2), increasing continuously in the atmosphere since the beginning of industrialization, has been well documented (IPCC 2007) and is predicted to increase up to 550ppm by 2050 (Smith and Mayers *et al.,* 2018). Such an increase in CO_2 and other greenhouse gases (CH_4, NO, CFC, etc.) may increase the global air temperature, which has already increased by about 0.74°C (IPCC 2007) during the last 100 years and is projected to rise by 0.3 to 4.8°C by the end of the century (IPCC fifth assessment report, 2014). This predicted temperature increase may decrease agricultural crop yield, particularly rabi/winter crops (Ortiz-Bobea *et al.*, 2021).

The focus on pulses was more because of two points: firstly, to fight against malnutrition, as pulses contain double the amount of protein compared to cereals, and secondly, to increase soil nutrition. Global pulse production declined before 2001, but between 2001 and 2014, pulse production increased (Rawal and Navarro, 2019). Among pulses, the chickpea is an important dry land legume of the tropics and 3rd largest pulse crop (16.9% of total world pulse production) after the common bean (*Phaseolus vulgaris L.*) and field pea (*Pisum sativum* L.) in the world. India is leading in chickpea production, contributing 75% of world production [Crop-wise pulses global scenario: 2022, (2024)]. Productivity of chickpeas is controlled by abiotic stresses (Jha *et al.,* 2014). The annual loss of chickpea by abiotic stress is 6.4million tones (Rawal and Navarro, 2019).

Several researchers have reported that elevated CO_2 in the atmosphere can enhance the photosynthetic carboxylation efficiency, particularly in C3 plants (Lamichaney *et al*., 2021). This enhanced efficiency can increase the number of branches and the number of seeds, ultimately increasing plant growth and productivity (Pal *et al.*, 2008; Bhatia *et al.*,2021); however, their nutrient quality may change (Saha *et al.*, 2015a; Bhatia *et al.*, 2021; Lamichaney *et al.*, 2021). Although chickpea is *rabi* season crop but, its productivity is affected by elevated temperature. Increasing temperature from 25^oC to $>32^oC$ reduces seed yield production in chickpea (Devasirvatham *et al.*, 2015; Awasthi *et al.,* 2017). Limited studies are available on the seed quality of chickpeas under elevated temperatures. It has been found that eCO_2 reverts the negative effects of elevated-temperature stress on biomass and yield in legumes (Abdelgawad *et al.,* 2015; Palacios *et al.*,2019). The individual effect of elevated CO_2 and high temperature is well studied in many crops, including chickpea (Vineeth *et al.,* 2015; Rai *et al.,* 2016; Lamichaney *et al*., 2021; Devi *et al.,* 2023), however, the data about their interactive effect is meagre (Liu *et al.,* 2017; Thomey *et al.,*2019; Wang *et al.,* 2020; Yuan *et al.,* 2021) and there appears to be no data available for chickpea. Therefore, it is of utmost importance to study these two factors individually and in combination as they occur in nature simultaneously and will challenge crop production. Thus, the present study aimed to assess the effect of elevated carbon dioxide (eCO_2) and elevated temperature (eT) individually and in combination on chickpea (*Cicer arietinum* L.) yield and mineral nutrient composition.

MATERIALS AND METHODS

Plant material

Two chickpea (*C. arietinum* L.) contrasting genotypes, i.e. ICC 4958 and Flip 90-166, with differences in phenotypes, ICC 4958: *desi* types and Flip 90-166 *Kabuli* type, were taken to study the effect of eCO_2 and eT. ICC 4958 is a short-duration crop with high yield and low productivity. It is a heat stress-tolerant genotype (Krishnamurthy *et al.*, 2011) and medium-maturity cultivar, whereas Flip 90-166 is a large seeded germplasm line from International Center for Agricultural Research in the Dry Areas *(ICARDA), Aleppo, Syria*. Seeds of both genotypes were obtained from the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi. Both types (*desi and kabuli*) of chickpea were selected for the study as their response against different climatic variables vary (Purushothaman *et al.,* 2014) and the same seeds were used in both the years of study period.

Experimental setup

The pot experiments were conducted in Open top chamber (OTC) facility at Indian Agricultural Research Institute (IARI), New Delhi (28°35′N latitude,77°12′E longitude) during *rabi* (November- March) season of two successive years, i.e. 2019-2020 (year 1) and 2020-21 (year 2) with two chickpea (*Cicer arietinum* L.) genotypes (ICC 4958 and Flip 90-166).

Six OTCs were used to perform the said experiments. Out of six OTCs, two OTCs were used for elevated CO_2 exposure (eCO_2), another two OTCs for elevated temperature (eT), and the remaining two were used for imposing combined eCO_2eT and control (C) pot were kept outside OTC in natural ambient conditions. The OTC facility was used for treatments described earlier (Chaturvedi *et al.,* 2017).

Seeds of both genotypes were sown simultaneously in plastic pots of white color (14 cm diameter and 12" cm height). In year 1 (2019-20), the effect of eCO_2 and eT stress individually and their combined impact was performed on yield parameters. Later in year 2 (2020-21), the same experiment was performed and other parameters viz., photosynthesis; and seed protein and mineral nutrients (Na , K ,Ca, Fe, P and Zn) were analyzed together with yield parameters, i.e. total biomass, pod number, seed weight, number of branches, pod fertility and 100 seed weight per plant.

In both years, each pot was filled with clay loamy soil (18 kg) supplemented with farmyard manure (in 3:1). The N: P: K was applied in the form of urea (20Kg/ha), single superphosphate (40 Kg/ha) and muriate of potash (60Kg/ha), respectively at the time of soil preparation (before sowing). The contrasting chickpea seeds were surface sterilized by washing with $HgCl_2$ (0.1%) for 2 min. After surface sterilization, seeds were rinsed with distilled water and soaked in distilled water for 6 hours. After that, seeds were dipped in 10% sugar solution and coated with *Rhizobium* culture in the shade. In each pot, six seeds were sown manually at 10cm depth. Both the varieties were sown simultaneously in different pots (10 pots of each genotype and each treatment). Six seeds of each genotype per pot were sown and 20 days after germination, thinning was performed in each pot and only three plants were kept in each pot. Weeding was performed manually throughout the growth period. The experimental design consisted of 3 treatments (eCO_2, eT, eCO_2eT) with control (C) to study the individual effects of elevated CO_2 (eCO_2) and elevated temperature (eT) together with their combined effect on chickpea genotypes. Germination date, flowering (in days) and pod emergence (in days) were recorded manually.

Elevated carbon dioxide (eCO_2) and elevated-temperature (eT) exposure in OTCs

Elevated carbon dioxide treatment was given after 20 days of seed sowing and eT treatment was started at the flower initiation stage and continued till maturity (65 days). The target for elevated eCO_2 level (~ 250ppm above ambient) in OTC and eT (4-5^oC above the ambient temperature) was achieved in the same way as described earlier (Chaturvedi *et al.,* 2017). The diameter of each OTC was 3.0m and the height was 2.5m, with the open upper end having a frustum of 0.5m. The uniform temperature and humidity were maintained by the upper end. The OTC was prepared by polyvinyl chloride (PVC) sheets with thickness of 125µm and >85% of transmittance. Elevated level of CO_2 was supplied by commercial grade CO_2 cylinders with solenoid valves (DURA, ESAB, India). The eCO_2 level was controlled by a regulator and the CO_2 was supplied during day time between 08:00-18:00. CO_2 (99.7%, v/v CO_2 and less than 10 µmol/mol CO) procured from M/S Gas Associates, New Delhi. Throughout the entire experimental period, temperature (^oC) and relative humidity (%) in all the OTCs were recorded every 30 min with the help of temperature and humidity sensors (MINCER, NIAES, Tsukuba, Japan). The eCO_2 combined with eT (eCO_2eT) treatment was imposed using the individual treatment methodology of both treatments simultaneously in the OTC.

Treatment conditions

Under ambient conditions, where control pots were kept, the average maximum (max) day (0700 to1900 hrs) temperature was 21.4 ±5.0 ^oC in year 1, while it was 24.5 ±5.9 ^oC in year 2 (Fig 1A &B). Similarly, the average minimum (min) night temperature (1900 to 0700 hrs) was 8.9 ±4.1 ^oC and 9.0±4.8 ^oC in years 1 and 2, respectively (Fig. 1 C&D). In contrast to this, under eT treatment, the average maximum temperature was 24.0±4.2 ^oC in year 1 and 27.3±7.4^oC in year 2, Under this treatment condition, the average minimum temperature was 10.1±4.7 ^oC and 10.6±5.5 ^oC in year 1 and year 2, respectively. The level of eCO_2 in two consecutive experimental years were 688.9 ±16.1 µl/l (in year 1; ambient was 392.6±10.7 µl/l) and 662.7±21.8 µl/l) (in year 2; ambient was 388.1±10.0 µl/l). Meteorological observations of both years during the treatments and control are presented in Fig.1.

Determination of yield and yield attributes

On maturity after 128 days, the plants were randomly selected in five replicates for yield components analysis. Their seed yield, pod fertility, pod weight, total biomass, number of branches, and 100 seed weight were recorded as described by Saha *et al.* (2015b).

Measurement of gaseous exchange

In year 2, net photosynthesis (µmol CO_2m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}) and transpiration rate (mmol m^{-2} s^{-1}) were measured after 7 days of flowering in randomly selected fully expanded third leaf using LI-6400XT (Li-Cor, Inc., Lincoln, NE, USA) in between 10:00-12:00 hrs. The CO_2 concentration was kept at 400 µmol CO_2 mol^{-1} with a constant flow rate of 500µmol s^{-1} and photosynthesis photon flux density (PPFA) of 1000 µmol $m^{-2}s^{-1}$ (by a red-blue LED light source) (Vineeth *et al.,*2015).

Determination of protein and seed mineral nutrients under different treatments

Protein (%)

Total protein content in chickpea seeds was determined indirectly by estimating nitrogen content following Semi-micro-Kjeldahl method, and Kjeldahl conversion coefficient of 6.25 was used for the analysis (Juliano, 1993).

Mineral nutrients

Both macro (Na, K, P, Mg and Ca) and micronutrients (Zn and Fe) were estimated according to Bhargava and Raghupathi (1993). Seeds from different treatments were ground homogenized into flours and dried at 80°C for 24 hrs. Finely ground dried seed samples (0.5g) were digested in 10 ml digestion mixture (HNO_3 and $HClO_4$, 9:4). The digested solution was cooled and washed in a 50 ml volumetric flask. The solution was again filtered and analyzed for all the macro and micronutrients using Atomic absorption spectrophotometer (AAS 4141, Electronics Corporation of India Limited, Hyderabad).

Statistical analysis

Data obtained in the experiments were analyzed using SPSS v.16 for Windows (SPSS Inc., Chicago, USA). The data was statistically verified with analysis of variation (ANOVA) and the significance of difference using Tukey and the significance of difference was measured using Tukey's post-hoc test at 5%.

RESULTS AND DISCUSSION

Pulses are important crops, firstly to fight against malnutrition and secondly to increase soil fertility. Chickpea is the most important pulse crop in India. However, its productivity and quality are altered by present climatic changes. Although the yield of chickpea is enhanced by eCO_2 . The research on eCO_2 and eT individually reveals the quality deterioration affecting human nutrition (Lamichaney *et al.,* 2021; Devi *et al.,* 2023). The study on combined effect of eCO_2 eT in

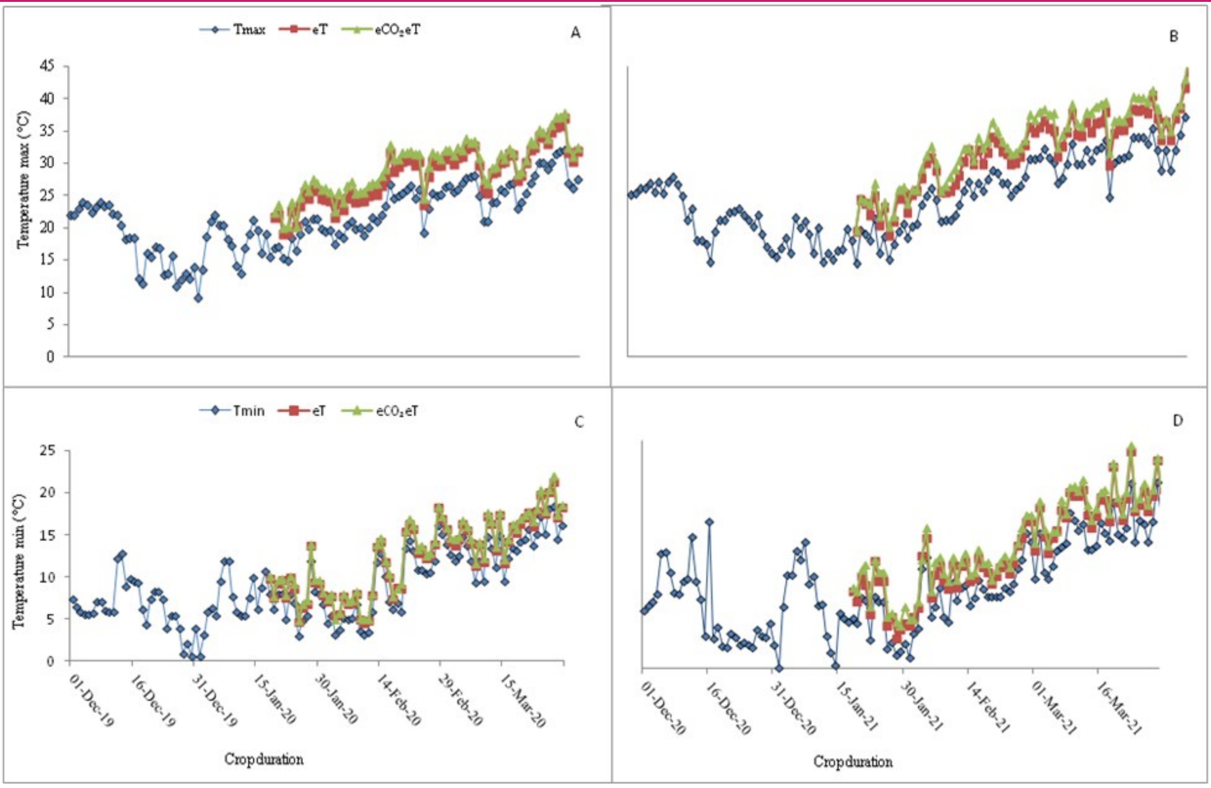

Fig. 1. *Daily air temperature for control [Tmax (A&B) and Tmin (C&D)], elevated temperature (eT), and their interaction (eCO_2eT) recorded inside Open top chamber in 2019-20 and 2020-21*

crops is limited (Thomey *et al*., 2019;Wang *et al*.,2020; Yuan *et al*., 2021), and there appears to be no data on Chickpea.

Yield of chickpea

In the present study, eCO_2 alone increased total dry biomass, number of branches/ plant, number of seeds/ plant and seed weight in both genotypes (Table 1). The number of branches increased significantly (P<0.001) from 4.6-4.4 to 6.2-5.8 in ICC 4958 and from 3.4-3.8 to 4.2-4.8 in Flip 90-166. The number of seeds/ plant enhancement was 37.4-35.7% in ICC 4958 and 29.1-28.8% in Flip 90-166. Seed weight /plant under eCO_2 was significantly higher (P<0.001) in both genotypes, ICC4958 and Flip 90-166 (15.3-17.5% and 8.8-11.1%, respectively, in both years, i.e., 2019-20 and 2020-21) as compared to seed yield in ambient condition (control). The present results are concurrence with earlier findings made in chickpeas under eCO_2 (Singh *et al*., 2013; Saha *et al.,* 2015b; Rai *et al.,* 2016; Lamichaney *et al*., 2021; Singh *et al*., 2021). The seed size was also increased in the present study, similar to Lamichaney *et al.,* (2021), but on the contrary, Saha *et al.* (2015b) reported no effect on seed size. It is reported that the enhanced yield of crops under eCO_2 indicates that more biomass accumulation under eCO_2 favouring plants that become taller and fruit more. Biomass accumulation also helps to store more carbon in seed and enhance seed number and size (Lamichaney *et al.,* 2021; Singh *et al.,* 2021).

Under eT, all yield parameters (total dry biomass, number of branches/ plant, number of seeds/ plant, pod fertility and seed weight) decreased significantly (P<0.001) except dry biomass. The number of branches was reduced from 4.6-4.4 branches/plant to 4.0-3.8 branches/ plant in ICC 4958 and from 3.8-3.4 to 2.6-2.4 branches/ plant in Flip 90-166 in 2019-20 and 2020-21 respectively. Reduction in the number of branches resulted in reduced pod number (P<0.001) and seed yield. The highest decrease in seed yield was observed in Flip 90-166 (22.0-26.9%) compared to ICC4958 (~12.0 %) in 2019-20 and 2020-21 respectively. An increase in dry biomass has been reported in chickpea due to increased photosynthesis rate and enhancement in rubisco activity under a mild increase in temperature (Devi *et al.,* 2023). However, as the temperature increased above 32^{o}C at the flowering stage, it reduced seed yield in chickpea (Gaur *et al.,* 2019). Decrease in dry matter allocation towards the seed can also be another reason for the decrease in yield (Rai *et al*., 2016). Elevated temperatures badly affect pollen germination, pollen tube growth, and stigma receptivity, resulting in a low yield of chickpeas (Devasirvatham, 2012). Reduction in pollen viability and pollen number is a key factor for legume yield under eT (Prasad *et al.,* 2002). The seed size was also reduced in the present study, evident by a 6.3-11.1% decrease in 100 seed weight. It is reported that elevated temperature subsequently reduces the activities of several proteins/enzymes related to the conversion of sugar to starch (Wardlaw and Mon-

Table 1. Effect of treatments on yield attributes of chickpea crop raised inside OTC (Open Top Chamber) along with control during 2019–2020 and 2020–2021 (Mean values ± SE of 5 replicates)

	Genotype	Treatment	Total Biomass (g/plant)	Pod Fertility (%)	Total Pod Number/plant (No.)	Pod Wt (g/plant)	Seed wt (g/plant)	Test Wt (g/100seeds)	Number of Branches per plant (No.)	Grain number/plant (No.)
Year 2019-20	ICC 4956	C	12.36±0.57a	77.40±1.83d	29.60±2.38bc	4.63±0.28ab	3.85±0.30ab	28.24±0.45b	4.60±0.24bc	23.00±2.21b
		eCO_2	15.60±0.65ab	89.49±0.93e	34.80±2.67c	5.63±0.40bc	4.44±0.35abc	31.13±0.27c	6.20±0.37c	31.20±2.60d
		eT	14.20±0.72a	70.94±1.92d	26.60±1.81ab	4.12±0.30a	3.39±0.22a	26.44±0.44a	4.00±0.32ab	18.80±1.11ab
		eCO_2eT	15.94±0.58ab	74.92±1.93d	30.20±1.77ab	4.17±0.24a	3.74±0.31ab	27.81±0.22ab	4.20±0.37ab	22.60±1.40b
	Flip 90-166	C	19.77±0.63bc	62.25±2.15bc	27.60±2.25ab	6.04±0.16cd	5.24±0.20cd	32.39±0.32c	3.80±0.37ab	17.20±1.50ab
		eCO_2	22.88±1.75cd	70.31±1.48cd	31.60±0.81ab	7.24±0.17d	5.82±0.0.31d	34.27±0.40d	4.80±0.58bc	22.20±0.58b
		eT	21.80±0.90cd	52.83±2.82a	22.00±2.07ab	4.37±0.22a	4.09±0.10abc	28.81±0.20b	2.60±0.24a	11.60±1.21a
		eCO_2eT	24.04±1.00d	55.24±1.25ab	26.40±2.38a	5.19±0.27abc	4.67±0.22bcd	30.87±0.48c	3.20±0.37ab	14.60±1.36a
		LSD (TRT)	1.89***	3.81***	4.60***	0.54***	0.53***	0.74***	0.76***	3.28***
Year 2020-21	ICC 4956	C	13.10±1.30a	72.98±2.82cd	35.60±2.25bc	6.15±0.19ab	4.56±0.24ab	27.58±0.53b	4.40±0.24c	26.20±2.62bc
		eCO_2	17.17±0.42ab	88.32±1.34e	44.20±2.67c	7.41±0.42bc	5.35±0.26bc	30.16±0.31c	5.80±0.37d	36.00±1.58d
		eT	14.62±0.64ab	68.21±1.72bc	34.00±2.07a	5.44±0.28a	3.98±0.10a	25.82±0.28a	3.80±0.37bc	23.20±1.59abc
		eCO_2eT	18.10±0.56cd	70.36±2.45bc	38.20±2.06ab	5.65±0.35a	4.16±0.13a	26.79±0.15ab	4.00±0.32bc	26.80±1.32bc
	Flip 90-166	C	21.40±0.97cd	71.21±1.75bcd	32.80±4.22abc	7.46±0.25bc	5.76±0.19cd	33.39±0.28d	3.40±0.24abc	23.60±3.54abc
		eCO_2	23.70±1.33d	80.13±2.09de	38.00±2.41abc	8.61±0.45c	6.27±0.16d	34.74±0.30d	4.20±0.20bc	30.40±1.86cd
		eT	22.80±0.62d	57.30±0.32a	27.60±1.17ab	5.79±0.38a	4.21±0.27a	30.15±0.33c	2.40±0.24a	15.80±0.58a
		eCO_2eT	24.62±1.05d	62.73±2.64ab	31.20±1.46ab	6.20±0.32ab	4.72±0.15ab	30.24±0.28c	3.00±0.32ab	19.60±1.36ab
		LSD (TRT)	1.87***	4.14***	5.01***	0.69***	0.40***	0.66***	0.60***	4.23***

[C=control, eCO_2=Elevated CO_2, eT=Elevated temperature, eCO_2eT= Elevated CO_2 and Elevated temperature] (Comparison of means was obtained from Fisher's least significant difference (LSD) test) cur, 1995; Dong and Beckles, 2019). Further reduction in seed size under eT might be due to reduced crop duration that affects the seed size in pulses (Chakrabarti *et al.,* 2013 in chickpea; Jumrani and Bhatia, 2014 in chickpea; Baidya *et al.,* 2021 in lentil).

Under combined conditions (eCO_2eT treatment), the present study showed that crop yield reduction was improved in both genotypes, Flip 90-166 and IC4958, compared to eT. The amelioration was more in ICC 4958 compared to Flip 90-166. However, total biomass did not show a similar trend to seed yield and was higher in both genotypes than in the control and eCO_2 (15.1 -21.6% in Flip 90-166 and 26.3-38.2% in ICC 4958) (Table 1). Similar to yield other yield parameters were also improved. The present results of eCO_2eT conform to Palacios et al. (2019) and Thomey (2019), who worked for soybeans. However, Prasad *et al.* (2002) in kidney beans and Delahunty *et al.* (2018) in lentil did not observe any interaction between eCO_2 eT on seed yield and its components. It may be that eT might be a problem in reducing the yield of chickpea in both genoyptyes, which may be due to pollen germination and pod abortion as reported by Baidya et al. (2021) in the case of lentil.

Gas exchange

Net photosynthesis (p_n) was high under eCO_2, while stomatal conductance (*gS*), and transpiration (*E*) were low (Figure 2). eCO_2 conditions increased ($P<0.001$) the rate of photosynthesis by 36.4% in ICC4958 and 21.7% in Flip 90-166 in 2020-21 compared to ambient control conditions. The rate of photosynthesis was increased due to the enhancement effect on ribulose bisphosphate carboxylase-oxygenase (Rubisco) activity. The present concentration of CO_2 may not be sufficient to completely saturate the Rubisco enzyme as reported in C3 crop (Ainsworth and Rogers 2007). So, when the condition of eCO_2 was given, p_n was enhanced. The enhanced photosynthesis results in more biomass accumulation in plants. The present study also exhibited an increase in biomass accumulation. The results of biomass accumulation were consistent with the study of Mishra and Agrawal (2014) in mungbean, Jumrani *et al.,* (2017) in soybean and Soba *et al.* (2020) in soybean. In the present study, the reduction in stomatal conductance was 13.7% in ICC4958 and 17.8% in Flip 90-166 and the transpiration rate decreased ($P<0.001$) by 21.5% in ICC4958 and 28.1% in Flip 90-166 as compared to control. The concentration of CO_2 was higher inside the stomata, which kept p_n at the higher side to maintain the constant ratio of internal CO_2 to external CO_2 level, reducing stomatal conductance. Soba *et al.* (2020) also reported a reduction in stomatal conductance in soybeans and Mishra and Agrawal (2014) in mungbean under eCO_2 conditions. Reduction in stomatal conductance ultimately reduces transpiration and conserve water. Water conservation aids plants in growing in more favourable conditions (Mishra and Agrawal, 2014).

Like eCO_2, eT also increased photosynthesis ($P<0.001$). The maximum induction was observed in ICC 4958 (9.5%) compared to Flip90-166 (8.4%). Increase in P_N was due to Rubisco enhancement under a mild increase in temperature. Similar to eCO_2, stomatal conductance was also decreased under eT. The maximum reduction in stomatal conductance observed was Flip 90-166 (30.4%) compared to ICC4958 (27.7%). Under eT, for cooling the plants, the transpiration rate was not affected in ICC 4958; however, 17.3% reduction was observed in Flip90-166.The present results were similar to those of Jumrani and Bhatia (2014) and Kumar *et al.* (2020) in study on chickpea and Suarez *et al.* (2021) in common beans. The result showed that ICC 4958 had more effective machinery to maintain the rate of photosynthesis under eT compared to Flip90-166. It is reported that elevation in temperature significantly increases photosynthesis and reduces transpiration; this effect, in turn, increases biomass accumulation in rice (Wang *et al.*, 2020).

Under the combination of eCO_2eT, photosynthesis was not affected significantly ($P<0.05$); however, stomatal conductance and transpiration reduction occurred (Fig. 2 B&C). eCO_2eT caused the maximum reduction in transpiration (19.4% in ICC 4958 and 23.7% in Flip 90-166) in both genotypes. The reduction in transpiration was less in ICC 4958, showing that this genotype has effective cooling machinery compared to Flip 90-166. The plants close their stomata to reduce transpiration and ultimately to save water. However, the eCO_2eT condition fixed carbon at a rate similar to the control but lower than the eCO_2 condition. In combined condition eCO_2eT reduced transpiration and maintained photosynthesis at the normal rate as reported by Yuan *et al.* (2021) in rice and Vanaja *et al.* (2024) in maize. The interactive effect of eCO_2eT is beneficial for photosynthesis, but an increase in photosynthesis is not the result in terms of yield in rice, as reported by Wang *et al.* (2020).

Seed protein and mineral nutrients

Protein and nutrient contents (Na, K, Ca, Fe, P and Zn) of seed were decreased significantly ($P<0.01$) under all three treatments. Reduction in seed protein was recorded to be 5.3-11.0% under eCO_2 compared to control (Table 2). The reduction in protein was also observed by Mishra and Agrawal (2014) in mungbean (9.3-15.5%), Saha *et al.* (2015a) in chickpea (9-10%) and by Li *et al.,* (2018) in soybean (2.4-6.1%).Under eCO_2, the decrease in net protein content might be due to an increase in the carbon concentration that causes protein dilution. Inhibition in nitrate assimilation could be another reason for the reduction in seed protein content in leguminous crops, as reported earlier (Hao *et al.*,2014; Myers *et al.*, 2014; Saha *et al.*, 2015a; Jin *et al.*, 2019; Soba *et al.*, 2020). All analyzed macro and micronutrients were decreased under eCO_2, except phosphorus, but the reduction was more noticeable in *kabuli* type genotype Flip 90-166 as compared to ICC4958. Overall, the reduction in macronutrients across the genotypes was 6.0-25.4%, whereas in the case of micronutrients (Fe and Zn), the reduction ranged from 6.0-12.9% compared to ambient conditions. Like protein dilution, other nutrients were diluted by more carbon fixation in grains. However, the mineral dilution was not in the same ratio in both genotypes. The P was not affected by eCO_2, as Saha et al. (2015a) reported in chickpea whereas Na, K and Ca were the worst affected minerals in both genotypes. An increase in photosynthesis under eCO_2 is linked to Rubisco enzyme concentration. This will require more P for synthesis in enzyme; thus, the P assimilation rate will increase and will not affect the P content in seeds (Jin *et al.*, 2015; Zhu *et al.*, 2019).

Under eT, the concentration of all analyzed seed nutrients decreased. Elevated temperature exposure also reduced 6.7-9.3% seed protein in both genotypes. The results are in agreement with Mourtzinis *et al.* (2017); Nakagawa *et al.* (2020) in soybean; a 4.5-6.7% decrease in protein content was reported by Sehgal *et al.* (2019) in lentil and 17-57 % Devi *et al.* (2023) in chickpea. The reduction in protein content was due to interruptions in biosynthesis and the import of precursors in leaves (Devi *et al.,* 2023) or nitrogen assimilation in roots (Nakagawa *et al.,* 2020). Among analysed nutrients, Na and P in ICC 4958 and Na and Zn were the most affected in Flip 90-166 under eT. The reduction in seed nutrient concentration may be due to reduction in seed filling duration, which is the most critical stage for grain nutrient concentration and it is related to various factors such as disruption in the mobilization of photosynthates, resulting in small and wrinkled seeds (Egli *et al.*,2005; Farooq *et al.*, 2017; Devi *et al.,* 2023). A higher transpiration rate might be another reason for the reduction, required to cool down the leaf temperature and convert most of the sugar into energy to accelerate the process of transpiration (Lin *et al.,* 2017).

Under the interactive effect of eCO_2eT in chickpea, seed mineral nutrients were more deteriorated in both genotypes except P than in their individual effect, i.e., eCO_2 and eT. The P content was not affected under the eCO_2 eT in both genotypes. However, an increase in P uptake was observed by Guo *et al*. (2022) in soybean and rice. Similar results of reduction in protein are also observed by Chaturvedi *et al.* (2017) (4-6 %) and Liu *et al.* (2017) in rice (~7%). In the present study, the maximum reduction in macronutrients *viz.* K, Na, Ca and Mg were recorded in Flip 90-166. In the case of micronutrients *viz.* Fe and Zn (20.3% and 18.9%, re-

Table 2. Effect of treatments on Micro and macronutrient of chickpea crop raised inside OTC (Open Top Chamber) with control during 2020–2021(Mean values ± SE of 5 replicates)

Genotype	Treatment	Macronutrient (mg/g)					Micronutrient (µg/g)		Protein (%)
		Mg	Ca	Na	K	P	Fe	Zn	
ICC 4958	C	1.60±0.01c	1.42±0.01e	1.22±0.01bc	7.52±0.08e	1.70±0.07a	69.77±0.67b	33.23±0.43abc	23.74±0.63c
	eCO_2	1.50±0.01b	1.29±0.02cd	0.99±0.01ab	6.29±0.02cd	1.86±0.07a	63.54±4.01ab	31.23±0.81ab	22.48±0.63abc
	eT	1.55±0.03bc	1.30±0.03de	1.03±0.02ab	6.85±0.05d	1.54±0.03a	61.43±2.19ab	30.54±1.07a	22.13±0.60abc
	eCO_2eT	1.49±0.02b	1.23±0.11c	0.96±0.11a	6.03±0.04bc	1.73±0.05a	58.87±2.33a	29.18±0.83a	21.26±0.58abc
Flip 90-166	C	1.49±0.02b	0.92±0.01b	1.28±0.02c	6.31±0.05cd	3.34±0.06bc	113.20±1.15e	41.97±0.27d	22.95±0.38bc
	eCO_2	1.33±0.01a	0.78±0.03a	0.95±0.02a	4.83±0.28a	3.59±0.14c	99.87±2.40d	36.53±0.35c	20.42±0.63ab
	eT	1.37±0.03a	0.80±0.02ab	1.03±0.05ab	5.46±0.12b	2.92±0.24b	95.34±1.85cd	35.13±0.57bc	20.82±0.59ab
	eCO_2eT	1.28±0.01a	0.74±0.07a	0.93±0.04a	4.41±0.08a	3.20±0.02bc	90.23±2.28c	34.03±1.64abc	20.19±0.25a
	LSD (TRT)	**0.04*****	**0.06*****	**0.10*****	**0.25*****	**0.23****	**4.66*****	**2.18*****	**1.17****

[C=control, eCO_2=Elevated CO_2, eT=Elevated temperature, eCO_2eT= Elevated CO_2 and Elevated temperature] (Comparison of means was obtained from Fisher's least significant difference (LSD) test)

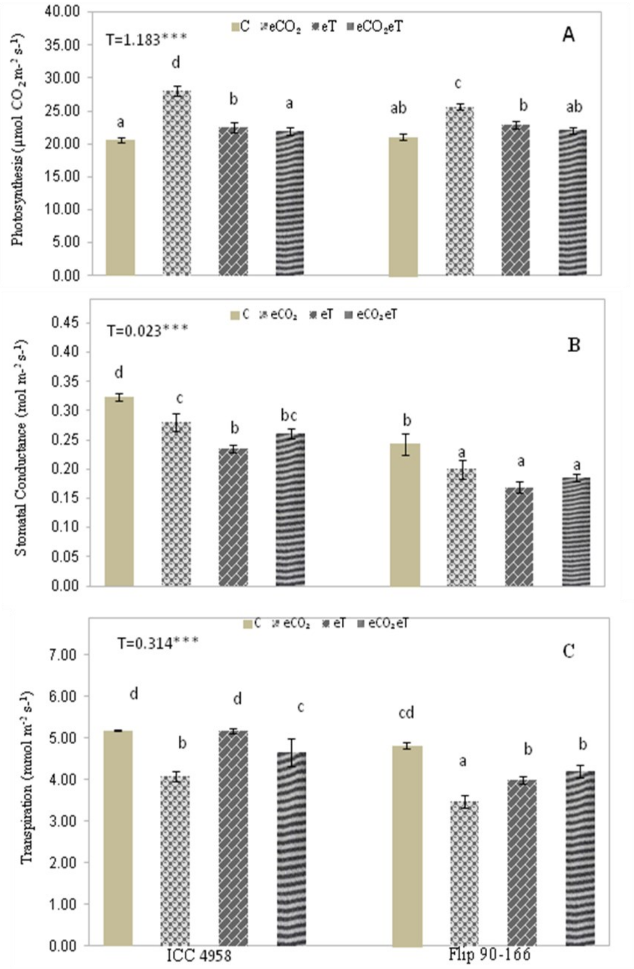

Fig. 2. *(A)Photosynthesis (B) Stomatal conductance and (C) Transpiration of two chickpea genotypes during flowering stage in year 2020-21. Each bar represents mean value with ±SE. Different Small Letters (a, b, c, d ..etc.) indicate significant differences at the 0.05 level Significant Difference at P<0.05 using Tukey's Post Hoc Test. Comparison of means was obtained from Fisher's least significant difference (LSD) test. [C=control, eCO_2=Elevated CO_2, eT=Elevated temperature, eCO_2eT= Elevated carbon dioxide and Elevated temperature]*

spectively) were highly reduced in Flip 90-166 under eCO_2eT compared to control conditions. An aggregated reduction in all nutrients was observed compared to control in both genotypes. The reduction in protein content was in the range of 10.4-16.4% in both the genotypes.

The present study indicated that in Chickpea, a C3 crop, the present concentration of CO_2 was not optimum for crop growth. An increased concentration of CO_2 favoured a higher rate of photosynthesis, resulting in higher growth, i.e. total biomass, including plant height and branches of the plant increased. Increased biomass furnished more space for flowers and pods on plants and better yields. Despite the positive effect on growth under eCO_2, seeds became more carbonaceous and mineral nutrients were diluted. Chickpea grows more favourably under ~ 28°C and at the vegetative stage in Delhi condition, the temperature was very low (2-15°C) compared to optimum grown temperature. eT showed a positive effect on growth, but when the onset of flowers came, the temperature was higher than the optimum temperature (~35°C), which affected pollen germination and pod development, resulting in yield reduction. The grain filling stage became shorter to avoid plants' high temperature and reduction in mineral adsorption duration, resulting in the seed's low protein and mineral nutrient content. Under the interactive effect eCO_2eT, the growth of chickpea was better than control; however, showing an additive effect of reduction of mineral nutrient.

Conclusion

The present study focused on the individual effect of eCO_2 and eT and their interactive effect on two chickpea genotypes, i.e., ICC 4958 and Flip 90-166. The results showed that the yield of chickpea increased in both genotypes by increasing the number of branches and seeds of the plant under eCO_2. eT (~4.0 °C) showed a positive effect on plant growth, suggesting that chickpea growth was not affected by a bit of rise in temperature in the studied climatic conditions. However, under eT, might have reduced pollen germination resulted in pod abortion and consequently, a reduction in the yield. The eCO_2 eT interaction significantly affected yield and seed quality parameters. The rate of photosynthesis showed a synergistic effect, so the plants' biomass increased but did not turn into yield. However, the yield was reduced compared to control but improved as compared to eT. The inference from this result was that eCO_2 (650±50μl/l) ameliorated the negative effect of high temperature ~4°C on yield. However, an augmenting effect was observed under the interactive effect of eCO_2eT for their protein and seed nutrient content except P. The reduction in seeds' protein and other mineral nutrients such as Ca, Fe, Zn, K and Na in both genotypes suggested that all these factors viz. eCO_2, eT and eCO_2eT were unfavourable for their nutrient levels. The uneven decrease in both genotypes showed that *desi* and *kabuli* genotypes may vary against different abiotic stress. The study provides useful information on agricultural planning and management to enhance yield because of climate challenges. A systematic investigation is suggested to study the yield and the mineral nutrients with more genotypes and different climatic conditions.

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Conflict of interest

The authors declare that they have no conflict of interest

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