THALINE MARTINS PIMENTA

FRUIT SIZE, YIELD AND NUTRITIONAL QUALITY IN TOMATO: IMPLICATIONS OF ELEVATED CO_2 AND TEMPERATURE

Thesis presented to the Universidade Federal de Viçosa as part of the requirement of the Graduate Program in Plant Physiology for the obtention of the degree of *Doctor Scientiae*.

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ABSTRACT

PIMENTA, Thaline Martins, D.Sc., Universidade Federal de Viçosa, October, 2021. **Fruit size, yield and nutritional quality in tomato: implications of elevated CO_2 and temperature** Adviser: Dimas Mendes Ribeiro. Co-advisers: Agustin Zsögön and Genaina Aparecida de Souza.

The atmospheric concentration of carbon dioxide (CO_2) has increased and it could reach 1000 ppm by the end of the century. Associated with these changes, global warming occurs progressively. These factors, elevated CO_2 concentration and high temperature, are expected significantly to affect the growth, yield and quality of crops consumed worldwide. Thus, we investigated the effect of elevated CO_2 in determining fruit size, yield and nutritional quality in natural warming scenarios (Chapter 1). In addition, we investigated how high CO_2 acts in modulating the size and ripening of tomato fruits (Chapter 2). Our results show that the effects of high CO_2 on fruit yield and composition depend on plant growth temperature. In general, these factors increased the fruit fresh weight, diameter and fruit yield, however the nutritional quality was impaired. Fruits under elevated CO_2 concentration at higher temperature showed reduction of lycopene, β-carotene, ascorbic acid, Zn and Mg greater than fruits under ambient CO_2. Furthermore, heating reduced the brix, soluble sugars, total phenols and fruit dry mass, regardless of the CO_2 condition. The increase in fruit size under elevated CO_2 was related to greater availability of carbon, which provided higher growth rate. Fruit growth was modulated by greater expansion of pericarp cells, increased expression of expansin genes and nutrient concentration in fruits under high CO_2. The increase in fruit size resulted in hasty ripening. This result was associated with an increase in the concentration of carotenoids, greater ethylene production and expression of ethylene biosynthesis and signaling genes. This study brings new perspectives regarding the action of elevated CO_2 in combination with warming air temperature in determining fruit yield and nutritional quality, as well as how elevated CO_2 can act to modulate fruit size and ripening under future environmental conditions.

Keywords: CO_2 enrichment. Fruit mineral composition. Warming. Fruit development. Carotenoids. Cell expansion.

RESUMO

PIMENTA, Thaline Martins, D.Sc., Universidade Federal de Viçosa, outubro de 2021. **Tamanho do fruto, rendimento e qualidade nutricional em tomate: implicações do elevado CO_2 e temperatura.** Orientador: Dimas Mendes Ribeiro. Coorientadores: Agustin Zsögön e Genaina Aparecida de Souza.

A concentração atmosférica de CO_2 tem aumentado e poderá atingir os 1000 ppm até o fim do século. Associado a essas mudanças, o aquecimento global ocorre de forma progressiva. Esses fatores, elevado CO_2 e alta temperatura, são esperados afetar de forma significativa o crescimento, rendimento e qualidade das culturas consumidas mundialmente. Dessa forma, buscamos compreender o efeito do elevado CO_2 na determinação do rendimento dos frutos e qualidade nutricional em cenários naturais de aquecimento (Capítulo 1). Além disso, investigamos como o elevado CO_2 atua na modulação do tamanho e amadurecimento de frutos de tomate (Capítulo 2). Nossos resultados mostram que os efeitos do elevado CO_2 sobre o rendimento e composição dos frutos dependem da temperatura durante o crescimento das plantas. De forma geral, esses fatores aumentaram o peso fresco, diâmetro e rendimento dos frutos, no entanto, a qualidade nutricional foi prejudicada. Frutos sob elevado CO_2 na maior temperatura apresentaram maior redução de licopeno, β-caroteno, ácido ascórbico, Zn e Mg do que sob ambiente CO_2. Além disso, o aquecimento reduziu o brix, açúcares solúveis, fenóis totais e massa seca dos frutos, independente da condição de CO_2. O aumento no tamanho dos frutos sob elevado CO_2 está relacionado a maior disponibilidade de carbono, o que proporcionou maior taxa de crescimento. O crescimento dos frutos foi modulado pela maior expansão das células do pericarpo, aumento na expressão de genes de expansinas e concentração de nutrientes em frutos sob elevado CO_2. O aumento no tamanho dos frutos culminou em amadurecimento mais rápido. Esse resultado foi associado ao aumento na concentração de carotenoides, maior produção de etileno e expressão de genes da biossíntese e sinalização de etileno. Esse estudo traz novas perspectivas em relação a ação do elevado CO_2 em combinação com aquecimento da temperatura do ar em determinar o rendimento de frutos e qualidade nutricional, além de como o alto CO_2 pode atuar modulando o tamanho e amadurecimento dos frutos em condições ambientais futuras.

Palavras-chave: Enriquecimento de CO_2. Composição mineral de frutos. Aquecimento. Desenvolvimento do fruto. Carotenoides. Expansão celular.

SUMMARY

GENERAL INTRODUCTION

Plant growth and development is influenced by a series of environmental factors such as light, water, temperature, nutrients and atmospheric gases. Among these factors, temperature and concentration of carbon dioxide (CO_2) in the atmosphere stand out as important for crop yield since CO_2 is the substrate for photosynthesis and temperature directly affects the reactions catalyzed by Rubisco (Cen and Sage, 2005). Atmospheric CO_2 concentration has been increasing from 278 ppm in the pre-industrial period for reached 416 ppm currently (Dong et al., 2018; NOAA 2021). According to projections, the atmospheric CO_2 concentration will reach 550 ppm by the middle of the century (Smith and Myers, 2018). Greenhouse gases such as CO_2 are capable of trapping heat on the Earth's surface, keeping the temperature at levels suitable for life, called the natural greenhouse effect. However, due to the increase in CO_2 and other greenhouse gases in the atmosphere, the global surface temperature is expected to increase 1.4 - 3.1 °C by the end of the 21st century compared with the period from 1880 to 1900 (IPCC, 2014). Studies have shown that the increase in atmospheric CO_2 concentration and higher temperature in the future can alter growth and developments of tomato plants, fruit yield, nutrients concentration and important compounds to human health (Khan et al., 2013; Jagadish et al., 2016; Rangaswamy et al., 2021). In this sense, the effects of elevated CO_2 and temperature on vegetative and reproductive growth focusing on fruit size, yield and nutritional quality in tomato are discussed here.

The elevated CO_2 concentration is beneficial for plants in terms productivity gain and stress mitigation (Wei et al., 2018; Brito et al., 2020). The increase in the atmospheric CO_2 concentration positively affect photosynthetic rate and reduce the stomatal conductance (Ainsworth and Rogers, 2007). In general, the higher concentration of this gas results in an increase in carbohydrate production, making available more substrates to favor growth and crop yield (Thompson et al., 2017). Accordingly, researches has shown greater fruit fresh weight and number of fruit per plant on tomato plants grown under high CO_2 (Khan et al., 2013; Yang et al., 2020). Fruit growth is regulated by the rates of cell division and expansion that determine both cell number and size, which is driven by cellular turgor pressure formed by the accumulation of osmotically active compounds (Shameer et al., 2020; Mauxion et al., 2021) During this process occurs a remodeling of the cell wall and changes in metabolism and gene expression. Elevated CO_2 is able to influence cell division and expansion (Masle, 2000). However, the effects of these processes during fruit growth under elevated CO_2 are poorly understood. Expansins are proteins involved in cell wall loosening during growth and are distributed throughout the plant. In tomato fruits, some expansins are highly expressed during growth (*SlEXPA3*, *SlEXPA5*, and *SlEXPA6*), ripening (*SlEXPA1*) or both stages (*SlEXPA12*, *SlEXPA24*) (Lu et al., 2016).

In contrast to the beneficial effects caused by high CO_2 in crop yield, several studies have shown concern about the impact of increasing CO_2 on the nutritional quality of food crops, such as the content of essential proteins and minerals for humans (Chaturvedi et al., 2017; Smith et al., 2017; Smith and Myers, 2018; Ebi and Loladze, 2019). Researches show a 4-10% reduction in iron (Fe) and zinc (Zn) in crops consumed worldwide, such as wheat, rice, barley and maize when grown in high CO_2 concentration (Chaturvedi et al., 2017; Smith et al., 2017; Smith and Myers, 2018). Reductions in Fe and Zn levels were also observed in soybean (Köhler et al., 2019). In general, these experiments have focused on grain and cereal crops, but little is known about the changes in nutrient content in tomato fruits under elevated CO_2. The reasons for the reduction in nutrient content are still unclear and vary greatly depending on the nutrient and species analyzed (Soares et al., 2019). Some hypotheses to explain this effect are dilution by extra carbohydrates, reduced mass flow caused by less transpiration (thus less nutrient absorption), and changes in biochemical processes that alter the requirement for some nutrients (Mcgrath and Lobell, 2013; Houshmandfar et al., 2018). Conversely, higher temperatures can increase mass flow due to greater leaf transpiration and alter nutrient absorption (Sadok et al., 2020). During cultivation under ambient conditions, it is common variations in temperature to occur during plant growth, which can influence physiological responses and nutrient absorption. As demonstrated by Kohler et al. (2019), an increase in temperature can offset the negative effect in the concentration of some nutrients in soybean. Carbon dioxide absorbs light's energy and re-emits the infrared energy, about half of it returns to Earth as heat. Thus, the increase in the atmosphere CO_2 concentration contributes to the warming of the air temperature. Therefore, it is necessary to study the effects of elevated CO_2 and temperature together.

Temperature is a crucial factor that determines plant growth. Crops have temperatures boundaries to growth, represented by minimum, maximum and optimum temperatures (Hatfield and Prueger, 2015). The optimum temperatures for growing tomatoes are between 25 °C and 30 °C during the day, 20 °C at night (Camejo et al., 2005), with 35 °C defined as the maximum (Zhang et al., 2005). The reproductive stage is determinant for crops yield and the most sensitive to high temperatures due to this condition affecting pollen viability, fertilization, pollen-pistil interaction, female gametogenesis and grain or fruit formation (Ainsworth and Ort, 2010; Hatfield and Prueger, 2015). In tomatoes, higher temperature can reduce flower production and fruit set, resulting in lower fruit yield (Adams et al., 2001). On the other hand, elevated CO_2 increase fruit yield due to the higher number of fruits per plant and higher production of larger fruits (Yang et al., 2020).

Tomato (*Solanum lycopersicum* L.) is one of the most economically important crops worldwide and the second most consumed vegetable, right after potato (FAO, http://www.fao.org/land-water/databases-and-software/crop-information/tomato/en/). The fresh or processed tomato fruit is distinguished by its high nutritional value, being an important source of nutraceuticals such as minerals, phenolic compounds (phenolic acids and flavonoids), vitamins (mainly ascorbic acid) and carotenoids (lycopene, β-carotene) (Dorais et al., 2008; Salehi et al., 2019). The concentration of total carotenoids and lycopene in tomato fruits produced under high CO_2 can be increased (Zhang et al., 2014; Yang et al., 2020) or reduced (Mamatha et al., 2014), and this variation may be due to the temperature sensitivity of carotenoids synthesis (Gautier et al., 2008). Furthermore, the effects of warming on the carotenoids contents in tomatoes vary according to the stage of fruit development in which the temperature is increased (Hernández et al., 2015). Brix is an important characteristic of fruit quality that represents the proportion of all solids dissolved in water (measured as soluble solids content - SSC), including sugars, acids, phenols, amino acids, soluble pectins and minerals (Balibrea et al., 2006; Hou et al., 2020). Overall, elevated CO_2 has a positive effect on tomato fruit Brix (Zhang et al., 2014; Yang et al., 2020; Rangaswamy et al., 2021), due to increased production of carbohydrates in leaves and the transport of solutes to fruits via phloem. On the other hand, a 2°C increase during tomato growth significantly reduces Brix (Rangaswamy et al., 2021).

Ethylene is a phytohormone required during the ripening of climacteric fruits such as tomatoes, being responsible for the cascade of changes in texture, flavor, aroma and color. The early ripening process is marked by an increase in respiration of the fruit followed by the burst of ethylene production (Giovannoni et al., 2017). During this phase, there is an increased production of enzymes for the biosynthesis of colored pigments, such as carotenoids, and alterations in the structure and composition of cell wall components (Giovannoni, 2004; Tucker et al., 2017). Ethylene biosynthesis is regulated by two enzymes, 1-aminocyclopropane-1-carboxylate synthase (ACS) and ACC oxidase (ACO) (Giovannoni, 2004). Ethylene is perceived by ethylene receptors (ETR), which are negative regulators in ethylene signaling

(Kevany et al., 2007). Elevated CO_2 may influence ethylene biosynthesis by regulating the expression of *ACS* and *ACO* and the ethylene signaling pathway in a still unknown way (Guo et al., 2014; Brito et al., 2020). In tomato fruits, it was observed that elevated CO_2 may increase the production of ethylene and the concentration of carotenoids (Zhang et al., 2014). However, the responses in the ripening of fruits from plants grown in elevated CO_2 are yet unknown.

Considering the importance of tomato as a source of essential compounds for human health and recent research on possible harmful effects of climate change on food security, we investigate the interactive effects of elevated atmospheric CO_2 concentration and warm temperature on fruit size, yield and nutritional quality in tomato (Fig. 1). The thesis has been divided into two chapters, the first addresses the interactive effects of high CO_2 and temperature on the tomato plant growth, yield and fruit composition. The second chapter analyzed responses in the development of tomato fruits in elevated CO_2, addressing changes in growth and fruit ripening.

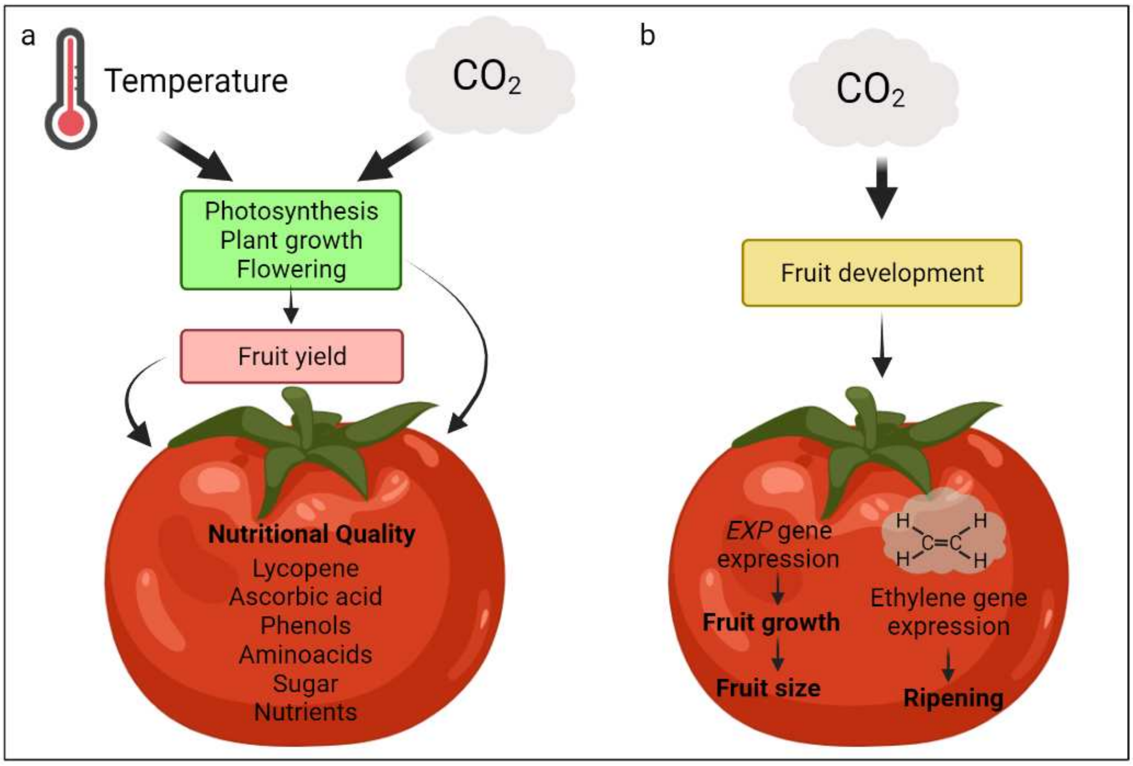

Fig. 1 Simplified overview showing the effects of the interaction between temperature and elevated CO_2 on plants and the responses in fruit development under elevated CO_2. Here we study the combined effect of high CO_2 and temperature in determining responses in photosynthesis, growth and flowering of tomato plants, inducing changes in fruit yield. Finally, we investigated whether elevated CO_2 and high temperature could modify the nutritional quality of tomato fruit (a). As second hypothesis, we investigated whether the increase in fruit size under elevated CO_2 could be regulated by the expression of the *EXPANSINS* genes. Furthermore, we investigated whether elevated CO_2 may affect fruit ripening through changes in gene expression of ethylene biosynthesis and signaling (b). Figure was created using the software Biorender (app.biorender.com).

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CHAPTER 1

The combination of elevated CO_2 concentration and high temperature impacts fruit size, yield and nutritional quality in tomato

Abstract

Rising atmospheric CO_2 concentration and temperature have the potential to modify fruit size and nutritional composition in horticultural crops. However, how elevated CO_2 concentration (eCO_2) regulates fruit size and quality in response to fluctuating temperature during the growing season is less well understood. Here, we show that under eCO_2, elevation of air temperature increased size but decreased nutritional content in tomato fruits. In general, fruit concentrations of the antioxidants lycopene, β-carotene and ascorbic acid, and the minerals Zn and Mg were influenced by warm temperature, with a more pronounced decrease under eCO_2 than under ambient CO_2 concentration (aCO_2). Moreover, there was an increase in concentrations of B, Mn and Ca in fruit of tomato plants at high relative to low temperature regimes, but this effect was less pronounced under eCO_2. Overall, our results revealed that many of the effects of eCO_2 in tomato fruit size and composition are due to a shift in the temperature regime during the growing season. These findings have implications concerning the action of rising CO_2 and warming air temperature in determining yield and tomato fruit quality in the future environmental conditions.

Keywords: Carotenoids. CO_2 enrichment. Fruit mineral composition. Warming

1. Introduction

The increase of atmospheric CO_2 has the potential to increase tomato fruit size, measured as either weight or diameter, via a positive effect on the whole-plant photosynthesis, which allows better carbohydrate availability for fruit (Jiao et al., 2019). In addition to elevated CO_2 concentration (eCO_2), high temperatures may reduce the number of flower and fruits in tomato plants, decreasing plant productivity (Harel et al., 2014). For instance, the rates of flower opening and fruit set were lower in tomato plants grown at 26 °C compared to those grown at 22 °C (Adams et al., 2001). It has recently been shown that the inhibitory effect of higher temperatures on number of flower and fruit set was overcome when tomato plants were grown under eCO_2, thereby increasing fruit yields (Rangaswamy et al., 2021). These results suggest a certain developmental flexibility of tomato plants under eCO_2, which improves their yield potential even at high temperatures. However, responses underlying the interaction between CO_2 and temperature are still not fully understood. Since plants growing in natural conditions are exposed to frequent temperatures fluctuations, it is important to test whether the growth flexibility of tomato plants under eCO_2 behaves in different temperature regimes.

The combination of high CO_2 and elevated temperature not only influences productivity, but also affects nutritional composition of crops (Beach et al., 2019; Dusenge et al., 2019). In tomato fruits, total soluble solids (Brix), sugars, ascorbic acid and lycopene increase under eCO_2 and decrease under elevated temperatures (Yang et al 2020; Rangaswamy et al., 2021). Thus, physiological studies have shown that high CO_2 and elevated air temperature may alter the composition of tomato fruit. In many cases, however, the combined effect of high CO_2 and elevated temperature on tomato fruit composition has been investigated in moderately limiting temperature. As example, high CO_2 (700 µmol mol^{-1} air) in combination with an elevation of 2 ºC above that of the canopy temperature reduced Brix and concentrations of reducing sugars, ascorbic acid and lycopene in tomato fruits (Rangaswamy et al., 2021).

Brix is a particularly important factor for tomato fruit quality used in the processing industry as it is positively correlated with the amount of product that can be extracted from a fixed quantity of freshly harvested fruits (Liabeuf and Francis, 2017). Most tomato cultivars used by the processing industry have determinate growth habit to allow mechanical harvesting (Robbins et al., 2011), and their fruits ripen simultaneously, but tend to have a low Brix (Rosseaux et al., 2005). As eCO_2 may affect tomato fruit composition in an opposite manner to elevated temperature, it is possible that the ability of eCO_2 to regulate fruit quality in tomato plants with a determinate growth habit depends on the temperature regime during the growing season. However, it remains unclear how eCO_2 and climate differences between different growing seasons are involved in the regulation of the fruit quality of tomato.

Elevated CO_2 and elevated temperature affect mineral composition in edible part of the plant, with most of the information obtained from several seed crops (Loladze et al., 2014; Myers et al., 2014; Köhler et al., 2019). In this context, eCO_2 resulted in a lower concentration of minerals in soybean seeds, which were restored by elevated temperature (Köhler et al., 2019). The tomato fruit is a source of macro- and micronutrients important for human health (Guil-Guerrero et al., 2009). In tomato plants, eCO_2 reduced the concentrations of Mg, N, Zn and Mn in the fruit, but increased the concentrations of Ca, Fe and Cu at 35/14 ºC maximum/minimum temperature conditions (Khan et al., 2013). In this temperature range, there were no differences in concentrations of K in tomato fruit under eCO_2 (Khan et al., 2013). On the other hand, K, Ca and Mg showed higher accumulation in the fruit of tomato plants grown under ambient CO_2 concentration (aCO_2) at 25/15 ºC day/night temperature conditions (Inthichack et al., 2013). These results show that, when assessing fruit mineral composition of tomato plants, it is necessary to take the combined CO_2 conditions and temperature into account. However, information on the interactive effect of eCO_2 and growing-season air temperature on mineral composition of tomato fruits is limited. In this study, we test the hypothesis that effects of eCO_2 conditions in tomato fruit size and composition are evident when there is also an increase in temperature during the growing season.

2. Materials and methods

2.1 Plant material and experimental setup

Seeds of tomato plants (*Solanum lycopersicum* L. cv Teteia) with determinate growth habit were sown into trays containing commercial substrate (Tropstrato HT®, Mogi Mirim, Brazil) and germinated in a greenhouse at the Universidade Federal de Viçosa (20º 45'S, 42º 15'W, 650 m altitude), Minas Gerais State, Brazil. When the first true leaf appeared, seedlings were pricked out in 3.5 L pots containing commercial substrate supplemented with 1 g L^{-1} 10:10:10 NPK and 4 g L^{-1} dolomite limestone. After five days, plants grown singly in pots were selected for uniformity and then moved to open-top chambers (1.2 m diameter and 1.4 m high; 8 plants per chamber) with either ambient CO_2 [aCO_2] (410 ± 20 µmol mol^{-1} air) or elevated CO_2 [eCO_2] (650 ± 50 µmol mol^{-1} air) as described by Brito et al. (2020). Routine practices for tomato cultivation were used, including 2 g N (as urea), 1.5 g P (as single super phosphate) and 5 g K (as KCl) per plant applied with irrigation water weekly starting to 15 days after transplanting and subdivided according to the phenological stage. Experiments were carried out with tomato plants over two years (2019 and 2020) in open-top chambers inside the greenhouse under natural photoperiod to investigate the combined effect of rising CO_2 and warming growing-season temperature on fruit size and composition of tomato. The daily light integrals, vapor pressure deficit and air temperature inside the chambers during the two growing seasons are shown in Table S1. The mean day/night air temperature over second year growing season was 4 °C higher than the first year (Table S1).

2.2 Phenotypic measurements

Individual flowers were tagged on the day of anthesis, and fruit set percentage of tomato plants was calculated as the ratio of number of fruits to total number of flowers. The diameter of fruits at red ripe stage was measured using a Vernier Caliper and fresh weight of tomato fruits was determined directly after harvest. For determination of dry weight, slices of fruits pericarp were oven-dried at 70 °C until constant weight. Total soluble solids content was measured with a digital refractometer (model RTD 45, Instrutherm®, São Paulo State, Brazil).

2.3 Gas exchange measurements

Gas exchange was measured on the third fully expanded leaf using an open-flow gas exchange system infrared gas analyzer (LI-6400XT, LICOR, Lincoln, NE, USA). The analyses were performed under artificial PAR of 1000 μmol photons m^{-2} s^{-1} and the reference CO_2 concentration was maintained at 410 μmol mol^{-1} air (for plants under aCO_2) and 650 μmol mol^{-1} air (for plants under eCO_2) using a CO_2 injector and compressed CO_2 cartridge. Measurements were made between 09:00 and 11:30 h under relative humidity 50-60% and temperature 27-32 °C corresponding to the top of the chambers.

2.4 Biochemical analysis

Six fruits from each sample were harvested at red ripe stage (56 days after anthesis) and then fruit pericarp samples were ground to a fine powder in liquid nitrogen and stored at –80 °C until analysis. The extraction of sucrose, glucose, fructose and total amino acids was performed according to Cross et al. (2006), with 100 mg frozen fruit material. Ascorbic acid content was determined as described by Stevens et al. (2008) and total phenolics compounds as described by Fu et al. (2011). For carotenoids measurements, frozen fruit material was extracted and analyzed for concentration of lycopene, β-carotene and lutein using high performance liquid chromatography (HPLC, Agilent 1200, NY, USA, equipped with an Eclipse XDB-C_{18} column) as described by Zhang et al. (2014).

2.5 Mineral analysis

Six fruits from each sample were harvested at red ripe stage and fruit pericarp samples were dried at 65 °C until a constant weight, ground to a fine powder using a pestle and mortar and then digested in concentrated nitric acid. Concentrations of P, K, Ca, Mg, S, Cu, B, Fe, Mn, Zn and Mo were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer, Shelton, CT, USA) as described by Wheal et al. (2011). Concentrations of N in fruit pericarp samples were determined by the Dumas combustion method (Jung et al., 2003).

2.6 Statistical analysis

The experiments were designed in a completely randomized distribution, in a factorial scheme 2 x 2 (CO_2 concentration x temperature). Two-way analysis of variance (ANOVA, $P \leq 0.05$) was applied to compare the means of the measured parameters with the factors temperature and CO_2 concentration. The *F*-test was used to assess the differences between CO_2 concentrations within each temperature condition and *vice versa*. All statistical analyses were performed using the R program version 4.0.2 (R Core Team 2020).

3 Results

3.1 Changes in the productive traits of tomato plants in response to CO_2 and temperature

To characterize the responses of vegetative and reproductive development in tomato plants to changes in CO_2 concentration and temperature, tomato plants were grown in a 2 × 2 factorial design of aCO_2 (410 µmol mol^{-1} air) and eCO_2 (650 µmol mol^{-1} air) at both 23/18 °C and 27/22 °C day/night temperature regimes (Fig. 1a, b). No differences were found in biomass accumulation and allocation of tomato plants between treatments (Fig. 1c, d). After 45 days, there were significant differences in the gas exchange parameters determined in fully expanded leaves of plants (Fig. 1e-h). The net CO_2 assimilation rate (A) increased by 20% at 23/18 °C and 18% at 27/22 °C in plants grown under eCO_2 compared with aCO_2, a result that was associated with increased internal CO_2 concentration (C_i) (Fig. 1e, f). The results also showed that stomatal conductance (g_s) and transpiration rate (E) increased at 27/22 °C compared with plants at 23/18 °C under eCO_2 but not under aCO_2, leading to a temperature x CO_2 interaction (Fig. 1g, h). It is interesting to note that no differences were found on the time taken for the first flower appears between temperature and CO2 treatments, as indicated by arrows (Fig. 1a, b). We next examined the effect of CO_2 and temperature during on agronomic traits reproductive development. The number of flowers per plant was higher at elevated temperature regime than at 23/18 °C regardless of the CO_2 conditions (Fig. 2a). The plants grown at 27/22 °C displayed decreased fruit set percentage under both CO_2 conditions but no difference from the plants at 23/18 °C in number of fruits per plant (Fig. 2b, c).

Overall, eCO_2 significantly increased the fruit fresh weight, fruit yield and fruit diameter across temperature treatments (Fig. 2d-f). Compared with 23/18 °C, fruit fresh weight increased by 25% at aCO_2 and 18% at eCO_2 in the high temperature regime (Fig. 2d). The tomato plants grown at 27/22 °C displayed higher fruit yield (24% at aCO_2; 22% at eCO_2) when compared with plants at 23/18 °C (Fig. 2e). Moreover, fruit fresh weight and fruit yield increased by approximately 15% and 13%, respectively, in plants grown under eCO_2 compared with aCO_2, when averaged across temperature regimes (Fig. 2d, e). The fruit diameter was higher at 27/22 °C compared with 23/18 °C across CO_2 conditions (Fig. 2 f). The fruit dry weight content did not differ between CO_2 conditions at both temperature regimes (Fig. 2g). However, fruit dry weight content decreased by 27% in plants grown at 27/22 °C compared with 23/18 °C, when averaged across CO_2 conditions. Additionally, Brix in tomato fruits was lower at 27/22 °C that at 23/18 °C regardless of CO_2 conditions (Fig. 2h). Comparing the temperature regime, Brix yield decreased by 29% under aCO_2 and 25% under eCO_2 at 27/22 °C compared with 23/18 °C (Fig. 2i). Moreover, Brix yield was higher at eCO_2 compared with aCO_2 at both temperature regimes.

3.2 Effects of CO_2 and temperature on tomato fruit nutritional composition

Concentrations of total phenols, glucose, fructose and sucrose were affected only by temperature regimes (Fig. 3a-d). In this context, concentrations of total phenols, glucose, fructose and sucrose decreased in fruits of plants grown at 27/22 °C compared with 23/18 °C regardless of the CO_2 (Fig. 3a-d). On the other hand, concentration of lutein in fruit were significantly affected by either temperature or CO_2 treatments (Fig. 3e). Compared with 23/18 °C, concentration of lutein in fruit decreased by 35% under ambient CO_2 and 41% under elevated CO_2 at 27/22 °C, respectively (Fig. 3e). There was a significant temperature x CO_2

interaction for concentrations of lycopene, β-carotene, total amino acids and ascorbic acid (Fig. 3f-i). The fruit lycopene concentration was increased by eCO_2 under 23/18 °C but not under 27/22 °C temperature regime (Fig. 3f). Concentrations of β-carotene and total amino acids in fruits were lower at 27/22 °C compared with 23/18 °C across the CO_2 treatments (Fig. 3g, h). The results also showed a decrease in lycopene and ascorbic acid concentration of 34% under elevated eCO_2 in fruits of plants grown at 27/22 °C compared with 23/18 °C (Fig. 3f, i).

3.3 Changes in the fruit mineral accumulation in response to CO_2 and temperature

Irrespective of the CO_2 conditions, high temperature regime resulted in a positive effect on concentration of P in the tomato fruits but a negative effect on the concentration of N and K (Fig. 4a-c). The fruit Ca concentration was increased by eCO_2 under the 23/18 °C temperature regime (Fig. 4d). On the other hand, elevated temperature increased fruit Ca concentration irrespective of CO_2 conditions. Elevated CO_2 decreased concentrations of Mg in fruits of plants grown at 27/22 °C compared with 23/18 °C (Fig. 4e). The ability of the tomato fruit to accumulate S and B depended both on the temperature and CO_2, but the effects of these environmental factors were independent, *i.e.*, eCO_2 increased S and B at both temperature regimes (Fig. 4f, g). There was a significant temperature x CO_2 interaction for concentrations of Zn and Mn in tomato fruits (Fig. 4h, i). Under eCO_2, fruit Zn concentration decreased by 27% at 27/22 °C compared with 23/18 °C (Fig. 4h). Under aCO_2, fruit Mn concentration increased by 37% at 27/22 °C compared with 23/18 °C (Fig. 4i). On the other hand, elevated temperature regime decreased fruit Mn concentration by 20% under eCO_2 compared with aCO_2. Elevated CO_2 itself significantly increased the concentration of Fe in fruits, when compared to fruits that developed under aCO_2 in both growing seasons (Fig. 4j). The main effect of the treatments (temperature and CO_2) was significant for fruit Cu concentration, showing a decreased at 27/22 °C compared with 23/18 °C but an increase under eCO_2 compared with aCO_2 (Fig. 4k). There were no differences in fruit Mo concentration across treatments (Fig. 4l).

4 Discussion

The increases of atmospheric CO_2 concentration and temperature are expected to have strong effects on agronomic parameters of crops (Dusenge et al., 2019; Moore et al., 2021). However, little is known about the consequences of their combined effects. Here, we investigated the effect of eCO_2 on the vegetative growth and reproductive development of tomato plants at natural warming scenarios. The results showed that many alterations in fruit yield and nutritional quality are produced by a combination of eCO_2 and increased average growing-season air temperature (Fig. 5). This has implications for horticultural production in the face of rising CO_2 and global warming on yield and nutritional quality of tomato fruit.

Temperature and CO_2 are important environmental factors influencing the time intervals between vegetative and reproductive events in tomato plants (Raza et al., 2019). Our results revealed that the high temperature regime combined with eCO_2 did not alter the time of developmental process in tomato plants, as evidenced by the unchanged number of days needed for tomato plants to reach the flowering stage (Fig. 1). Interestingly, the number of flowers and the rate of fruit set in the tomato plants were not affected by CO_2 conditions (Fig. 2), whereas there was an optimum temperature for early reproductive development. This resulted in a positive effect on number of flowers but a negative effect on the rate of fruit set in the high temperature regime (Fig. 2), suggesting that temperature is the primary signal that offer the necessary flexibility for control fruit set within a given atmospheric CO_2 concentration.

Floral development and fruit set are known to be at least partly under the control of nutrient availability of source leaves (Quinet et al., 2019). In our experimental setup, the increase in number of flowers in plants grown at high temperature regime was balance by a smaller proportion of fruit set, regardless of CO_2 conditions (Fig. 2). However, photosynthetic capacity of plants under eCO_2 was higher than in plants under aCO_2 (Fig. 1), suggesting that the elevated temperature modulates early stage of fruit development, not through an effect on source capacity.

Previous work has shown that B concentration in source leaves play an important role in the flower process, whereas K concentration is important to support fruit set (Sainju et al., 2003). We observed that elevated temperature had a positive effect on B concentration and a negative effect on K concentration in tomato leaves (Fig. S1), which may explain why the high temperature regime increased the number of flowers, but reduced the rate of fruit set (Fig. 2). In this context, the early fruit development in tomato plants of determinate growth habit was not associated with a greater photosynthetic capacity (i.e. greater source capacity) of the plants grown under eCO_2 (Figs 1 and 2). These responses may be related to the reported reduction in leaf photosynthesis at mild heat stress that could not limit the development of tomato fruits at early stages (Sato et al., 2000).

The later stages of tomato fruit development rely on a continuous supply of carbohydrates from source leaves (Ho et al., 2019). Our study revealed that the increase in fruit size, measured as either weight or diameter, depended on both temperature regime and CO_2 conditions, but the effects of these factors were independent (Fig. 2). The observation that the increase in fruit fresh weight as a result of eCO_2 was larger in 27/22 ^oC than in 23/18 ^oC suggest the increased in yield is not a response in the photosynthetic capacity of tomato plants, nor is it an effect of fruit number per plant (Figs 1 and 2). The increase in yield response to high temperature regime may be due to increased water content of the fruits, as evidenced by the decrease in dry weight content of the fruit irrespective of the CO_2 conditions (Fig, 2). This conclusion is further supported by the lower concentration of solid soluble content in fruits of plants at elevated temperature regime grown under both CO_2 conditions (Fig. 2). Reduced irrigation regime combined with high CO_2 cause an increase in soluble solid content in tomato fruit (Yang et al., 2020). Therefore, interactions of elevated temperature regime with the soil water content in the tomato fruit quality must be considered in the context of climate change. This is indeed realistic when considering the elevated temperature regime coupled to the significant drop in the product of Brix x ripe yield (BRY), an important agronomic parameter for tomato plants of determinate growth, under eCO_2 (Fig. 2).

Tomato fruits are important source of minerals elements and functional metabolites that are important for human nutrition (Guil-Guerrero et al., 2009). In this context, antioxidants in tomato fruits such as lycopene, β-carotene, ascorbic acid and phenolic compounds play a significant role in their nutritional quality for humans (Ali et al., 2021). In our experiments, elevated temperature regime (27/22 ^oC) counteracted the increase in concentrations of lycopene, β-carotene, lutein, and ascorbic acid in fruits driven by the combination of eCO_2 with 23/18 ^oC temperature regime (Figs 3 and 5). The elevated temperature regime was also shown to have a negative effect on the concentration of total phenols (Fig. 3). These results imply that high temperature regime may reduce the nutritional quality of tomato fruit under future eCO_2 by decreasing the concentrations of antioxidant compounds. Dilution by enhanced fruit size can explain the decrease in concentrations of antioxidant compounds in fruit of plants treated with eCO_2 grown at 27/22 ^oC temperature regime compared with 23/18 ^oC, since fruit water content increased with increasing temperature regime. Another noteworthy point is that Mg, Zn and Cu are important for the synthesis of antioxidant enzymes (Sainju et al., 2003). Our results showed that concentration of Mg and Zn in fruit (based on dry weight) decreased with eCO_2 under high temperature regime but not at 23/18 ^oC air temperature, leading to an interaction between temperature regime and CO_2 (Figs 4 and 5). Moreover, plants grown at 27/22 °C had lower Cu concentration in fruit dry weight under both CO_2 conditions (Fig. 4). These results suggest that growth of tomato plants under high CO_2 and elevated temperature is not an effective means of obtained higher antioxidant capacity of fruits. Therefore, there seems to be expected that the cultivation of tomato plants under future climate conditions will be positively effect in fruit size, but with a negative effect in terms of food safety via decrease in antioxidant capacity (Fig. 5).

Temperature and CO_2 may affect total soluble solids content and mineral accumulation in fruits of some plant species (Moretti et al., 2010). The product of Brix x yield (BRY) is particularly important in the tomato processing industry (Nemeskéri et al., 2019). Our results show that high temperature regime reduced BRY in fruits of tomato plants with determinate growth habit under both CO_2 conditions (Fig. 2). Since most industrial cultivars are determinate, the decreased in BRY under elevated temperature could lead to potential consequences for the processing industry. In addition, concentrations of Mg and Zn in dry fruit weight was significantly lower under eCO_2 compared to aCO_2 in the high temperature regime (Figs 4 and 5). The amounts of minerals in tomato fruits are the result of the balance between uptake by roots, distribution and partition to the fruits (Barickman et al., 2019). Elevated CO_2 combined with high temperature regime did not cause Mg and Zn deficiency in leaves of tomato plants (Fig. S1). Therefore, we think it is reasonable to assume that the effect eCO_2 and high temperature on concentrations of Mg and Zn in dry fruit weight are associated with the reduction in import from the phloem. Variations in concentrations of B, Mn and Ca were also noted, *e.g*. a smaller increases in concentrations of these minerals in fruits of tomato plants under eCO_2 at 27/22 °C relative to 23/18 °C (Fig. 5). This observation is somewhat at odds with a previous study showing that elevated temperature counteracted the reductions of minerals in soybean seeds (Köhler et al., 2019). Several factors could explain this contrasting trend, including the fact that we are studying different species subject to different nutrition and temperature regimes. Irrespective of the reason underlying the different conclusion of this study, our work indicates that eCO_2 combined with high temperature regime could have a negative effect on tomato fruit quality as a result of reduced BRY and minerals concentrations. The exception to this generalization was fruit Fe concentration, which was higher in fruits of tomato plants grown under eCO_2 at 27/22 °C relative to 23/18 °C (Fig. 5).

5 Conclusions

Given that the factors high temperature and elevated CO_2 will condition agricultural production in the coming decades, it is important to understand their interaction. Our work sheds light on the ability of tomato plants to maintain a higher photosynthetic rate under eCO_2, while allowing flexibility in the early development of fruit under elevated temperature regime may play an important role in optimizing fruit size of tomato plants of determinate growth habit. However, the increase in fruit size and yield caused by combining elevated CO_2 with high temperature was accompanied with marked decreases in the fruit nutritional quality of tomato. Concentrations of the main tomato antioxidant compounds (lycopene, β-carotene, ascorbic acid) and essential minerals (Zn and Mg) in fruits were influenced by the higher temperature, with a more pronounced decrease under eCO_2 than under aCO_2 (Fig. 5). In addition, eCO_2 resulted in lower accumulation of Ca, B and Mn in fruits of tomato plants at 27/22 °C than 23/18 °C (Fig. 5). The eCO_2 treatment only partially compensated the negative effect of temperature stress on concentrations of total amino acids (Fig. 5). Thus, these findings could raise a concern about future warming, since tomato plants grown at elevated temperature conditions may not safeguard the fruit nutritional composition under eCO_2, which raises urgent questions about food security under future climate conditions.

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Figures

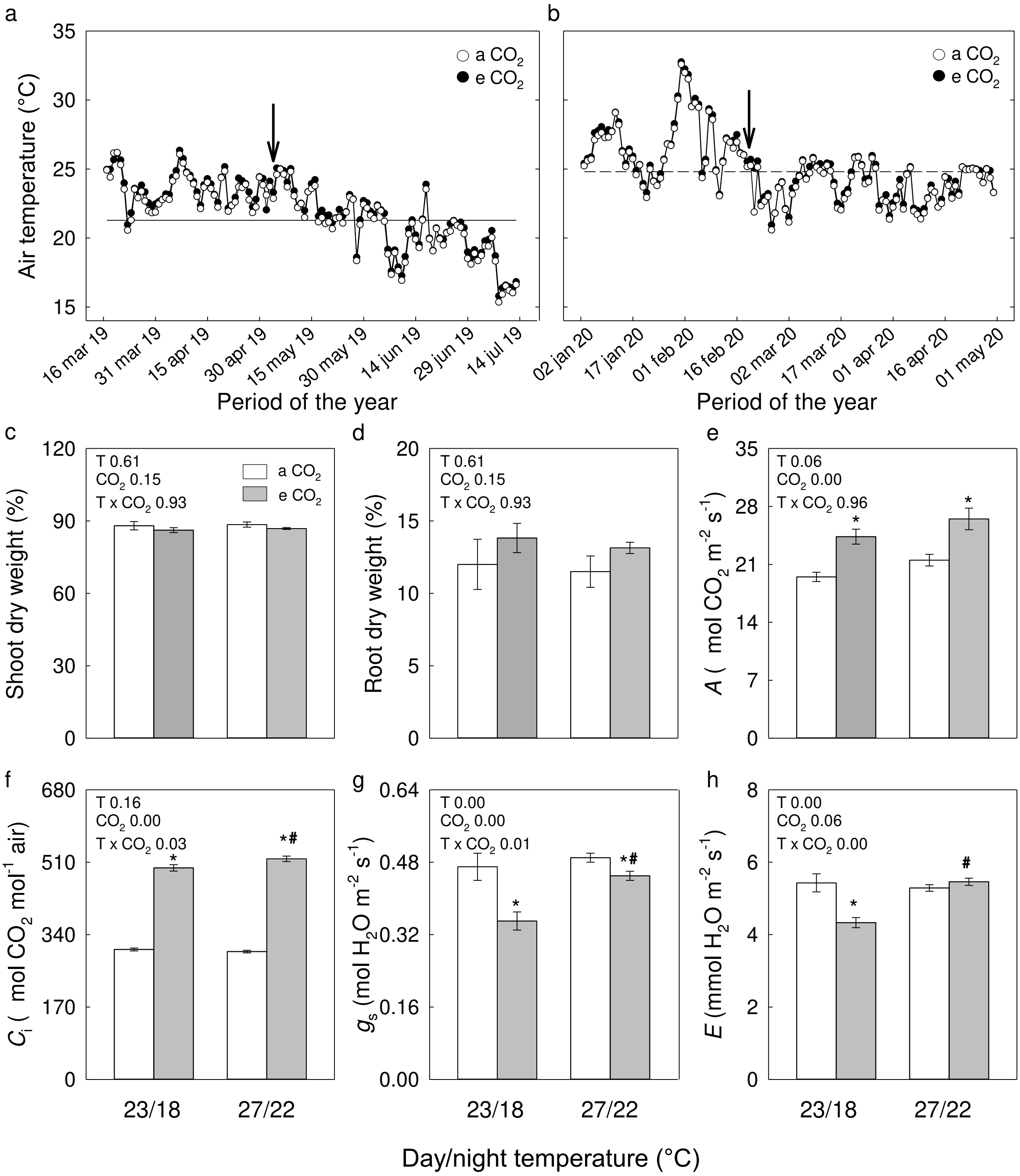

Fig. 1 Effects of CO_2 conditions and temperature during development of tomato plants. **a, b** Fluctuation of daily air temperature inside the open-top chambers supplemented with ambient (open circle) or elevated (filled circle) CO_2 during the course of experiments. Solid and dashed lines represent data at 23/18 °C and 27/22 °C day/night temperature conditions throughout March to July 2019 and January to May 2020, respectively. Arrows indicate days after planting that the first flower appears. **c** Biomass accumulation. **d** Net CO_2 assimilation rate. **e** Internal CO_2 concentration. **f** Stomatal conductance. **g** Transpiration rate. Asterisks indicate statistically different means between plants grown under ambient and elevated CO_2 within the same temperature regime ($P \leq 0.05$). Hashtags indicate statistically different means between plants grown under 23/18 °C and 27/22 °C temperature regime within the same CO_2 ($P \leq 0.05$). Values are means ± SEM ($n = 10$).

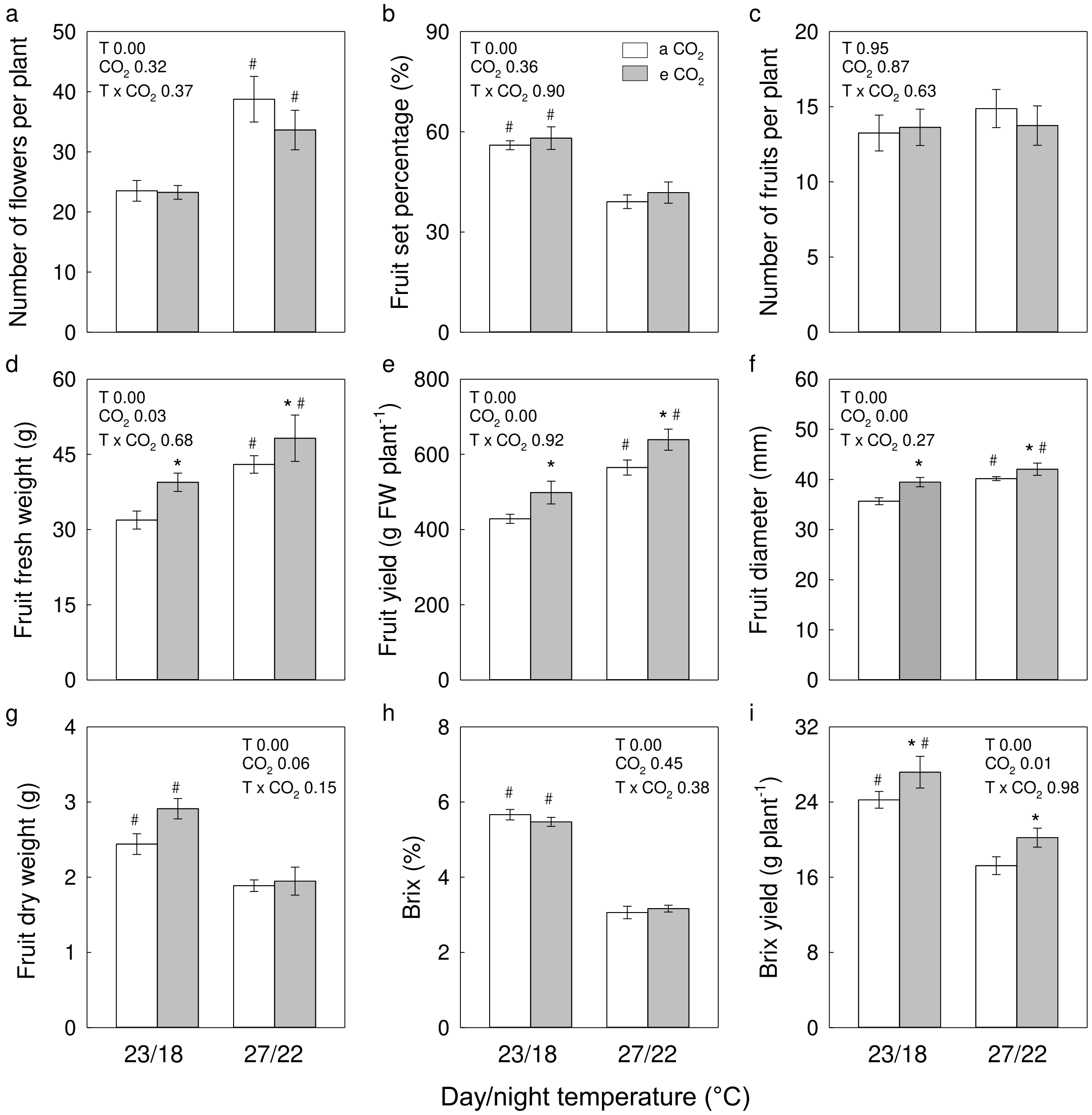

Fig. 2 Changes in physiological parameters observed in tomato plants in response to CO_2 and temperature treatments. **a** Number of flowers per plant. **b** Fruit set percentage. **c** Number of fruits per plant. **d** Fruit fresh weight. **e** Fruit yield. **f** Fruit diameter. **g** Fruit dry weight. **h** Brix. **i** Brix yield. Asterisks indicate statistically different means between plants grown under ambient and elevated CO_2 within the same temperature regime ($P \leq 0.05$). Hashtags indicate statistically different means between plants grown under 23/18 ºC and 27/22 ºC temperature regime within the same CO_2 ($P \leq 0.05$). Values are means ± SEM ($n = 10$).

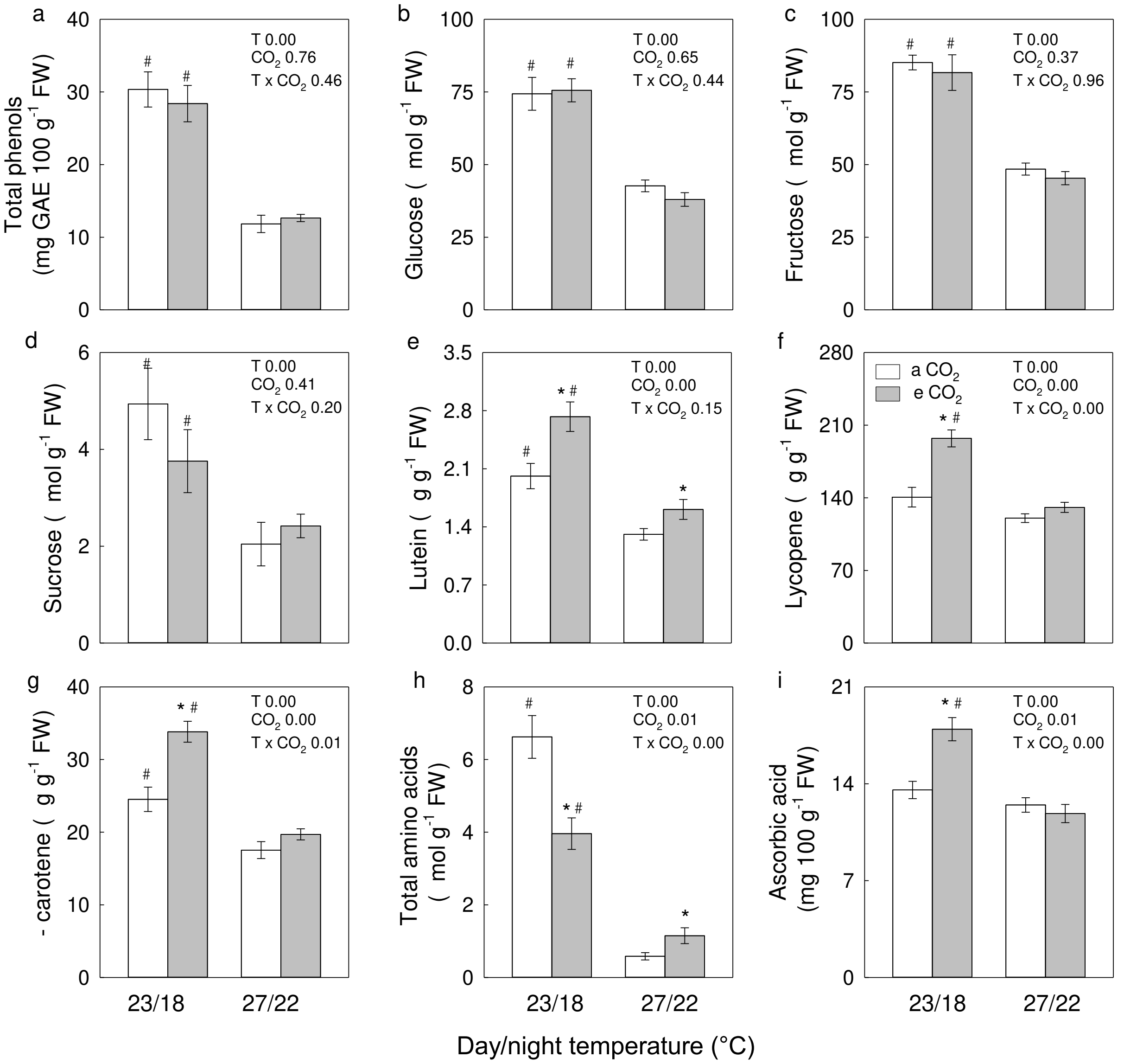

Fig. 3 Changes in tomato fruit composition in response to CO_2 and temperature treatments. **a** Total phenols. **b** Glucose. **c** Fructose. **d** Sucrose. **e** Lutein. **f** Lycopene. **g** β-Carotene. **h** Total amino acids. **i** Ascorbic acid. Asterisks indicate statistically different means between plants grown under ambient and elevated CO_2 within the same temperature regime ($P \leq 0.05$). Hashtags indicate statistically different means between plants grown under 23/18 °C and 27/22 °C temperature regime within the same CO_2 ($P \leq 0.05$). Values are means ± SEM (*n* = 6). GAE, Galic acid equivalents.

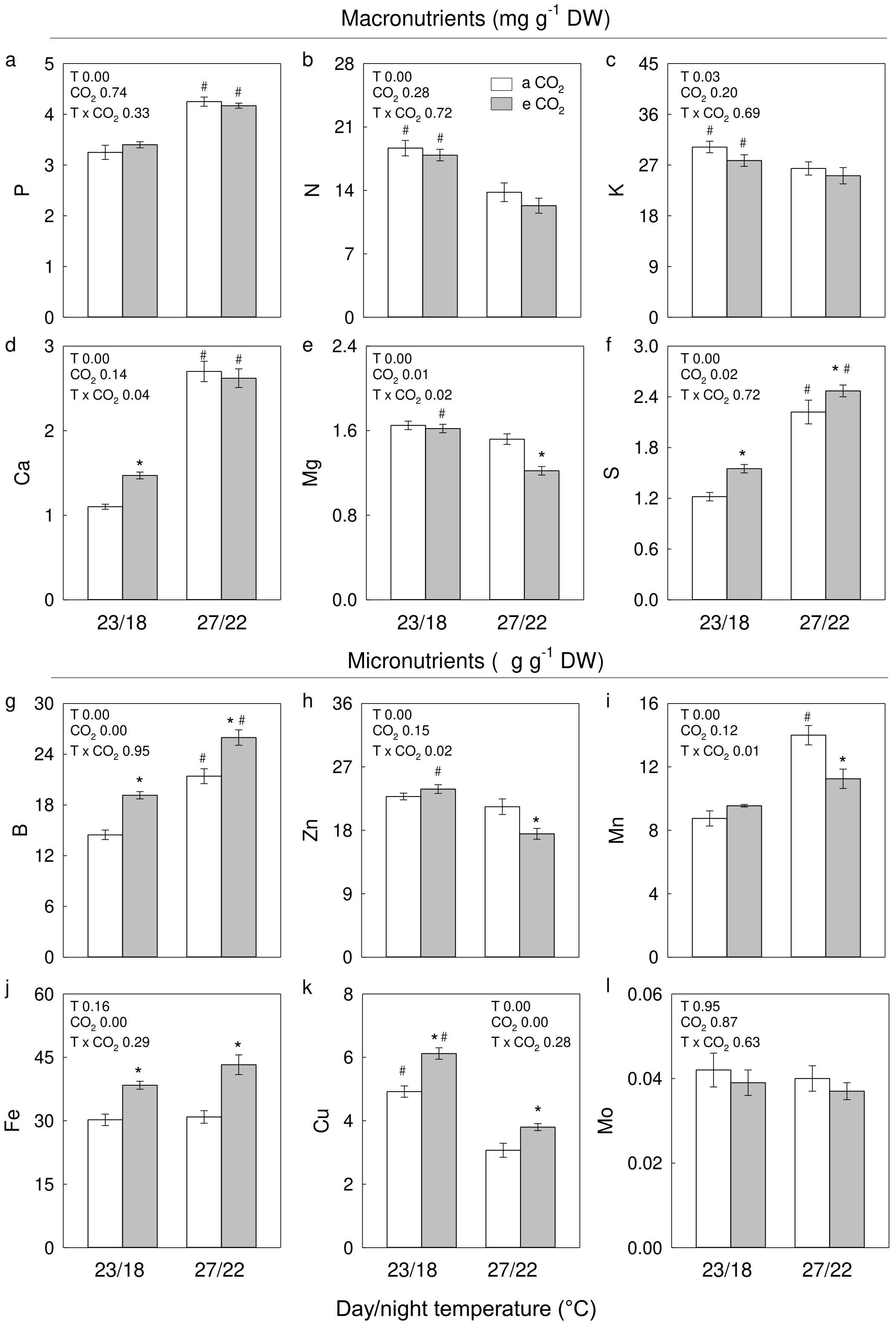

Fig. 4 Macro and microelement concentrations in fruits of tomato plants grown under aCO_2 and eCO_2 at both 23/18 ºC and 27/22 ºC temperature regimes. **a** Phosphorus. **b** Total nitrogen. **c** Potassium. **d** Calcium. **e** Magnesium. **f** Sulphur. **g** Boron. **h** Zinc. **i** Manganese. **j** Iron. **k** Copper. **l** Molybdenum. Asterisks indicate statistically different means between plants grown under ambient and elevated CO_2 within the same temperature regime ($P \leq 0.05$). Hashtags indicate statistically different means between plants grown under 23/18 ºC and 27/22 ºC temperature regime within the same CO_2 ($P \leq 0.05$). Values are means ± SEM ($n = 4$).

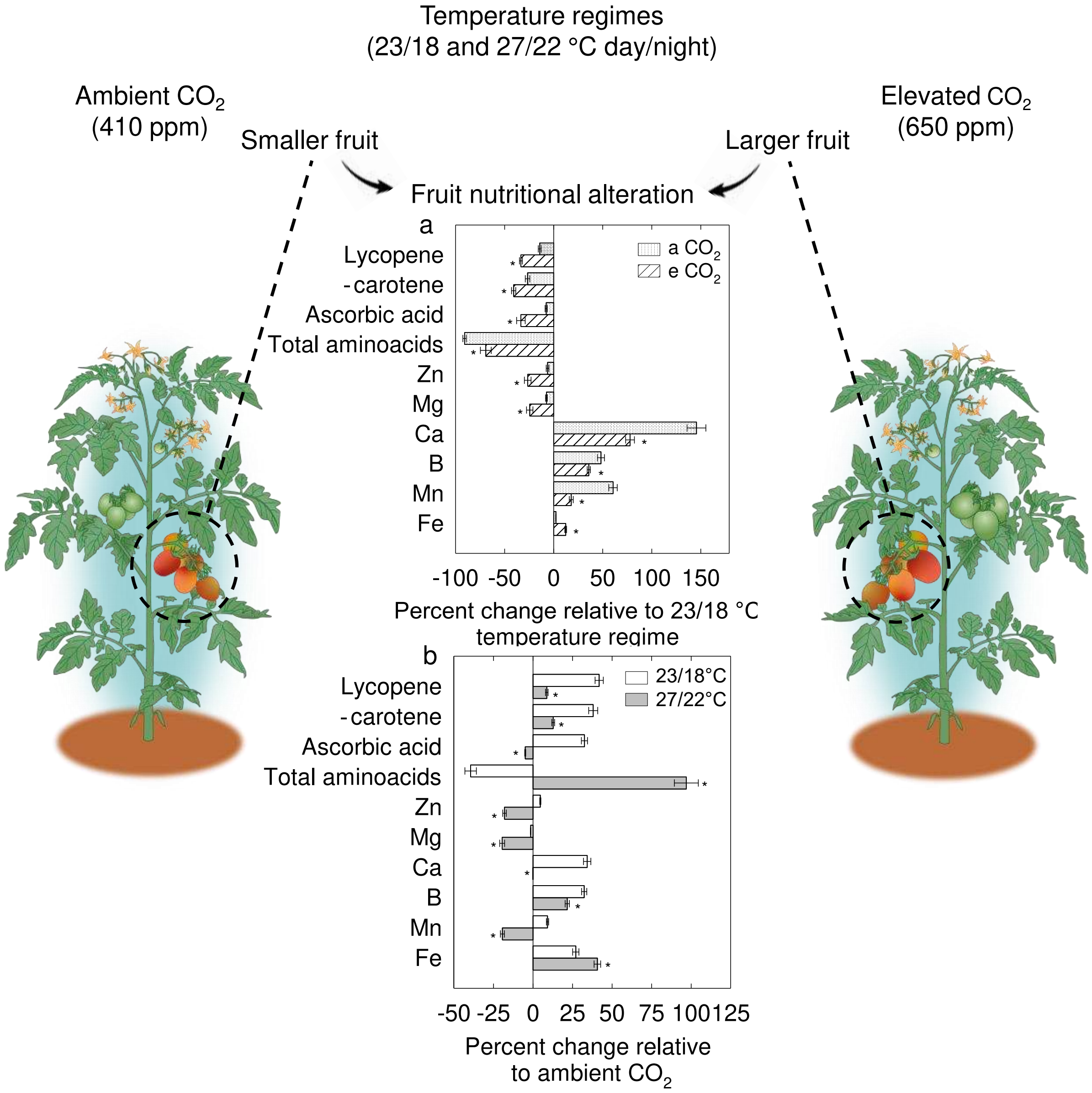

Fig. 5 Summary of CO_2 and temperature effects on fruit size and fruit nutritional quality. Overall, high temperature regime (27/22 ºC) and elevated CO_2 increased fruit size and yield of tomato plants, independently. The combined effect of eCO_2 and elevated temperature affect the fruit nutritional quality of tomato plants. a Percent change in fruit nutritional composition of tomato plants grown at 27/22 ºC relative to 23/18 ºC temperature regime. Asterisks indicate statistically different means between plants grown under aCO_2 and eCO_2 ($P \leq 0.05$). **b** Percent change in fruit nutritional composition of tomato plants grown under eCO_2 relative to aCO_2. Asterisks indicate statistically different means between plants grown at 27/22 ºC and 23/18 ºC ($P \leq 0.05$). Data are derived from figures 3 and 4. Values are means ± SEM.

Supplementary data

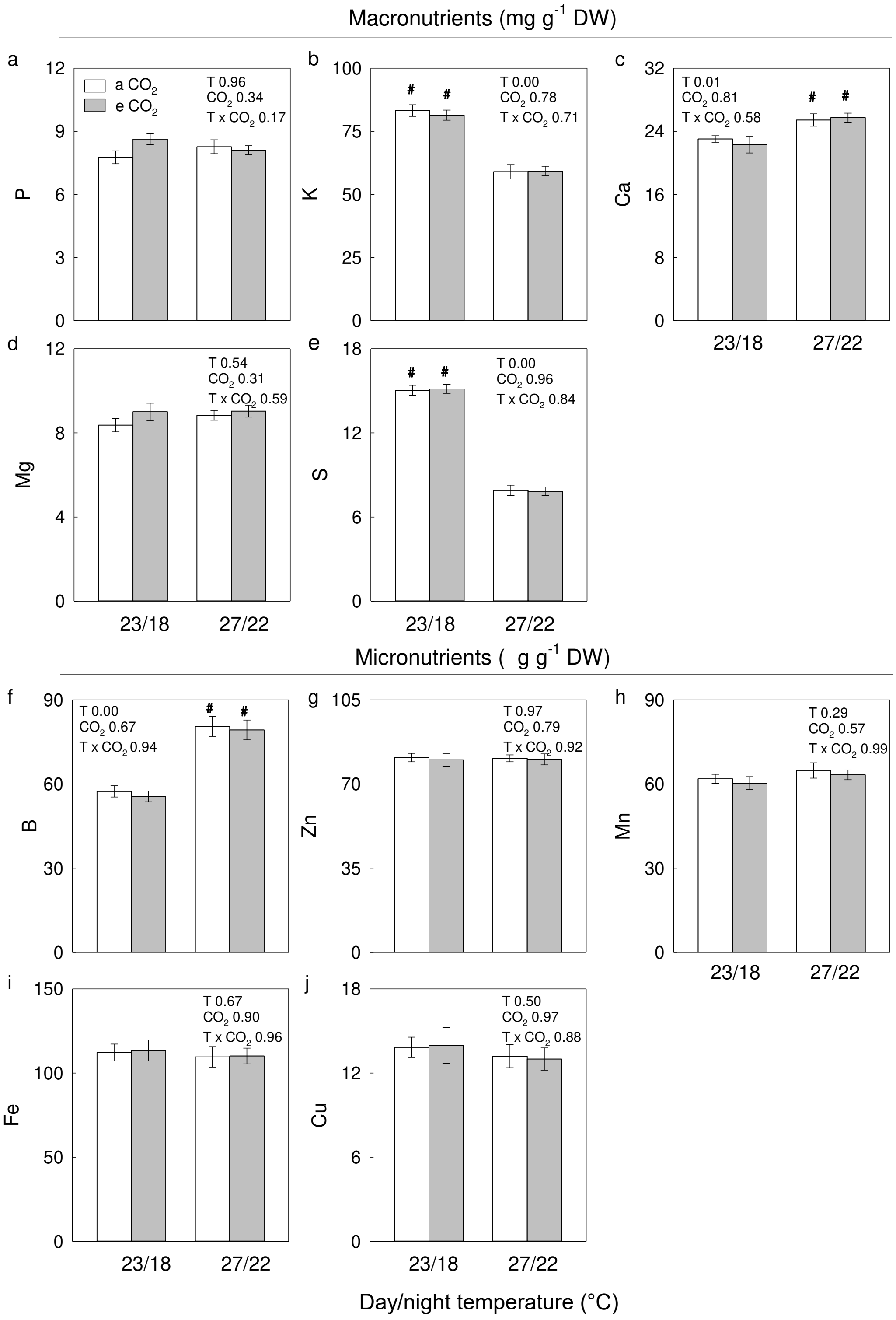

Fig. S1 Macro and microelement concentrations in leaf of tomato plants grown under aCO_2 and eCO_2 at both 23/18 ºC and 27/22 ºC temperature regimes. **a** Phosphorus. **b** Potassium. **c** Calcium. **d** Magnesium. **e** Sulphur. **f** Boron. **g** Zinc. **h** Manganese. **i** Iron. **j** Copper. Asterisks indicate statistically different means between plants grown under aCO_2 and eCO_2 within the same temperature regime ($P \leq 0.05$). Hashtags indicate statistically different means between plants grown under 23/18 ºC and 27/22 ºC temperature regime within the same CO_2 condition ($P \leq 0.05$). Values are means ± SEM ($n = 4$).

Table S1 Climatic properties inside the open-top chambers supplemented with aCO_2 or eCO_2 during the two growing seasons.

	Year	
	2019	2020
PAR (µmol m² dia^{-1})	5.19	7.18
VPD (kPa)	0.64	0.91
Temperature Day (°C)	23.6	27.4
Temperature Night (°C)	18.5	22.1

CHAPTER 2

Elevated CO_2 accelerates ripening and stimulates the production of larger fruits in tomato

Abstract

Elevated atmospheric CO_2 concentration is able to stimulate the tomato fruit yield by increasing of the size and fruit fresh weight. However, the mechanisms involved in the fruit development under elevated CO_2 are still poorly understood. Here, we investigate how elevated CO_2 stimulates tomato fruit growth and whether changes associated with elevated CO_2 can alter fruit ripening. Therefore, we cultivated tomato plants in open-top chambers under ambient and elevated CO_2 conditions and analyzed the fruit growth rate, gene expression, anatomy, carotenoids content and ethylene production in the fruits. Fruits under elevated CO_2 had a larger diameter as a result of increased fruit expansion rate, that was coupled with increase in photosynthetic rate. The increased in carbon availability led to a greater pericarp cells area, it was consistent with increase in *EXP* genes expression in fruits. Moreover, larger fruit size under high CO_2 was associated with a general increase in nutrient concentration and reduction in fruit dry matter. The increase in fruit size under high CO_2 was accompanied by accelerated fruit ripening, which was the result of increased ethylene production and increased expression of biosynthesis and signaling genes. Thus, our results bring new insights into the potential of elevated CO_2 to increase the size and to modify the ripening pattern of tomato fruits. Our findings are relevant to understanding the development of fruit in the context of climate change.

Keywords: Expansins. Fruit development. Cell expansion. Anatomy. Carotenoids

1 Introduction

The current atmospheric CO_2 concentration is around 416 ppm and continues to increase significantly, it may achieve 1000 ppm by the end of the century (Smith and Myers, 2018; NOAA, 2021). Responses to elevated CO_2 commonly observed in plants include increased photosynthetic rates, growth, yield, water use efficiency and improved tolerance to environmental stresses (Zhang et al., 2019; Brito et al., 2020; Yang et al., 2021). Tomato plants grown in elevated CO_2 have a greater carboxylation efficiency, which increases the photosynthetic capacity, providing more carbon for fruit growth (Pan et al., 2020). Plants are able to utilize the greater carbon availability while the sink capacity remains high, otherwise photosynthesis inhibition may occur (Thompson et al., 2017). In tomato, the fruit size is determinant for yield (Renau-Morata et al., 2020). Thus, studies have shown an increase in fruit yield per plant due to the higher fruit fresh weight in plants grown under high CO_2 (Yang et al., 2020; Rangaswamy et al., 2021). However, the mechanisms involved in the development tomato fruits under elevated CO_2 that lead to increased growth are still poorly understood.

The fruit development is regulated by the rates of cell division and cell expansion (Quinet et al., 2019). Tomato fruit consists of the pericarp, septum, placenta and the locule tissue surrounding the seeds (Renaudim et al., 2017). At the anatomical level, most of the pericarp is formed by the mesocarp, composed of large thin wall cells (Rančić et al., 2010). Elevated CO_2 may stimulate leaf growth by increased of cell expansion and cell production (Ferris et al., 2001). These changes are associated with increased enzyme activity and gene expression associated with cell wall properties (Ferris et al., 2001; Gamage et al., 2018). Cell expansion plays an essential role in determining the final fruit size (Rančić et al., 2010). As demonstrated by Renaudim et al. (2017), mesocarp cells undergo a dramatic expansion, about 1550-fold from fruit set up to 20 days post anthesis. In order to support this large growth, it is necessary to remodel the cell wall by changes in the expression *EXPANSIN* (*EXP*) in the pericarp of immature tomato fruits (Lu et al., 2016).

The potential to improve production under high CO_2 depends on the greater allocation of carbon to the fruits, with the sink strength being important to avoid photosynthetic acclimation (Ruiz-Vera et al., 2020). In tomatoes, the sink strength in developing fruits is a function of the activity and size of the sink (Quinet et al., 2019). In addition to the greater allocation of carbon, the growth of tomato fruits requires adequate amounts of macro and micronutrients. For example, potassium (K) concentration has a high positive correlation with fruit size and soluble solids content (Fontes et al., 2000; Luo et al., 2020), while calcium (Ca) is important in maintaining the structural strength of the cell wall in fruits (Gao et al., 2019). Elevated CO_2 may alter nutrient concentration in plants due to effects on transpiration and changes in nutrient requirement (McGrath and Lobell, 2013). Khan et al. (2013) reported increase in Ca, Cooper (Cu) and Iron (Fe) levels in tomato fruits under high CO_2, however, there was reduction in Magnesium (Mg).

Ripening is the last phase of fruit development and starts when the final fruit size is reached (Azzi et al., 2015). Ethylene is essential for the ripening of climacteric fruits such as tomato, used as a model plant for studying the role of ethylene in this process (Iqbal et al., 2017). The beginning of ripening is marked by a burst of ethylene production, triggering changes in color and firmness through the chlorophyll reduction, carotenoid accumulation (lycopene and β-carotene) and cell wall softening enzymes activity (Giovannoni et al., 2017). Thus, the ethylene action is a target point for the regulation of fruit ripening, being usual the utilization of inhibitors of ethylene biosynthesis and signaling (Schaller and Binder, 2017). Tomato fruits can increase ethylene production under elevated CO_2 during ripening (Zhang et al., 2014). Investigating factors that may affect the ethylene production in fruits are relevant to deal with possible changes in postharvest losses.

Here we tested the hypothesis that elevated CO_2 alters tomato fruit size by driving expression of expansin genes during fruit development. We also examined whether the elevated CO_2-associated process affect tomato fruit ripening.

2 Material and Methods

2.1 Plant material and growth conditions

The experiments were conducted in open top chambers (OTCs) installed in a greenhouse at the Universidade Federal de Viçosa (20º 45’S, 42º 15’W, 650 m altitude), Minas Gerais State, Brazil. Tomato seeds (*Solanum lycopersicum* L. cv Teteia CNPH1143) were sown on polyethylene trays with commercial substrate Tropstrato HT®, for the production of seedlings. After the appearance of the first pair of true leaves, approximately two weeks after sowing, the seedlings were transferred to 3.5 L polyethylene pots containing the commercial substrate Tropstrato HT® plus 5 g of NPK 4-14-8 and 5 g dolomite limestone per pot. Three days after transplanting, plants grown singly in pots were transferred to two identical open top chambers (1.2 m diameter and 1.4 m high; 8 plants per chamber) at ambient CO_2 concentration (410 ± 20 μmol mol^{-1} air) or elevated CO_2 (650 ± 50 μmol mol^{-1} air) as described by Brito et al. (2020). Topdressing fertilization of the plants consisted of 2 g N (as urea), 1.5 g P (as single super phosphate) and 5 g K (as KCl) per plant applied via irrigation water weekly at a volume of 250 mL per pot, starting up to 15 days after transplanting and subdivided according to the phenological stage. Air temperature, vapor pressure deficit (VPD) and photosynthetically active radiation inside the chambers were recorded at 30 min intervals by means of sensors connected to a data acquisition system (Li-1400, LI-COR, Lincoln, NE, USA). The CO_2 concentration in the chamber was monitored once a week at plant height using an infrared gas analyzer (CO277, Akso, Brazil).

2.2 Photosynthetic parameters and vegetative growth assessment

The parameters of gas exchange were evaluated in fully expanded leaves during the vegetative stage of tomato plants grown under ambient and elevated CO_2 concentration at 40 days after germination (DAG). Gas exchange parameters were determinate using an open-flow gas exchange system infrared gas analyzer (LI-6400-40, LI-COR, Lincoln, NE, USA). The leaf chamber conditions consisted of a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1}, with 10% blue light provided by a blue/red LED light source. Measurements were made under relative humidity 50-60% and temperature 27-32 °C corresponding to the top of the chambers. The net rate of carbon assimilation (A), internal CO_2 concentration (C_i), transpiration rates (E) and stomatal conductance (g_s) were measured between 9:30 and 11:30 h. The reference CO_2 concentration was 410 μmol CO_2 mol^{-1} air for the plants in ambient CO_2 and 650 μmol CO_2 mol^{-1} air for the plants under elevated CO_2 concentration using a gas injector coupled to a compressed CO_2 cylinder.

Plant growth was evaluated in the final of vegetative stage at 40 DAG. Leaves of each plant were separated and the total leaf area was determined by using a Li-Cor 3100 area meter (LI-COR, Lincoln, NE, USA). Afterwards, the total biomass (roots, stems and leaves) of each plant were were oven-dried at 70 ^{o}C until constant weight. Subsequently the dry weight (DW) of plants grown under ambient and elevated CO_2 was determined.

2.3 Fruit growth and yield parameters

Diameter growth was measured on tomato fruits from five until forty-five days after anthesis (DAA). Measurements were made every three days on the second proximal fruit of the first truss chosen as the average position. Changes in fruit diameter were measured using a Vernier Caliper. The fruit expansion rate and duration of fruit expansion were obtained from the data of the growth curves as described by Ribeiro et al. (2012). Productive parameters of plants were assessed at 100 DAG. Fruits were harvested and the number of fruits per plant, fruit fresh weight and fruit yield per plant were determined. Subsequently, diameter of the fruits was determined and classified into the following diameter classes: fruits with a diameter less than 20 mm, fruits with a diameter of 20 to 30 mm, 30 to 40 mm, 40 to 50 mm and fruits with a diameter greater than 50 mm. From this we established the frequency (% of total fruits per plant) of fruits by diameter class.

2.4 Fruit anatomy

Pericarp sections (0.5 cm²) of the fruit equatorial region were collected at 56 DAA and immediately fixed in FAA50 (formaldehyde, acetic acid, 50% ethanol - 1: 1: 18 - volume: volume) for 48 h under vacuum and subsequently stored in 70% ethanol (Johansen, 1940). The sample processing and image capture were performed as described by De Souza et al. (2018). Subsequently, mesocarp cell area and cell number per area were determined using Image-Pro® Plus software (version 4.1, Media Cybernetics, Inc., Silver Spring, MD, USA).

2.5 Ethylene quantification

Fruits were collected at 56 DAA, corresponding to the ripe red stage, and placed in 0.5 L capacity glass flasks for 12 hours in a growth chamber (Forma Scientific Inc, OH, USA) at a temperature of 25 °C. After a gas sample 1 mL was taken from the headspace of the flask with a tight syringe and ethylene concentration was quantified by gas chromatography with a flame ionization detector, as described by Ribeiro et al. (2010).

2.6 Carotenoids and nutrients quantification

The nutrients concentration was determined in fruits pericarp at the ripe red stage. Samples of four fruits per plant were oven-dried at 65 °C until a constant weight, ground to a fine powder using a pestle and mortar. For quantification of nutrients (P, K, Ca, Mg, S, Fe, Cu, Zn and B) samples were subjected to digestion in concentrated nitric acid, followed by analysis in inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer, Shelton, CT, USA) as described by Wheal et al. (2011). For carotenoids measurements, frozen fruit material was extracted and analyzed for concentration of lycopene, β-carotene and lutein using high performance liquid chromatography (HPLC, Agilent 1200, NY, USA, equipped with an Eclipse XDB-C_{18} column), as described by Zhang et al. (2014).

2.7 Gene expression quantification

Total RNA was extracted from fruits pericarp at 35 DAA and 56 DAA using a RNeasy Plant Mini Kit (Qiagen) and cDNA was synthesized from 2 µg of total RNAs using SuperscriptTM III reverse transcriptase (Invitrogen), according to the manufacturer's instructions. Quantitative real-time reactions were performed as described by Zanor et al. (2009). Relative transcript abundance was calculated by the comparative cycle threshold (CT) method (Livak and Schmittgen, 2001). Each sample was normalized using *ACTIN* as an internal control. We selected candidate genes from the literature that have been shown to regulate biosynthesis and receptors of ethylene and cell expansion in tomato fruit (Lu et al. 2016; Mata et al., 2018). The gene-specific primers used for qRT-PCR are shown in Table S1.

2.8 Statistical analyses

The experiments were designed in a completely randomized distribution. The *t*-test was applied to evaluate difference among CO_2 concentration treatment. Statistical analyses were performed using the R program version 4.0.2 (R Core Team 2020).

3 Results

3.1 Elevated CO_2 improves photosynthetic rate and fruit yield

In order to investigate the growth and development of fruits under elevated atmospheric CO_2 concentration, we cultivated plants from seedlings until fruit harvest under ambient CO_2 conditions (410 µmol mol^{-1} air) and elevated CO_2 (650 µmol mol^{-1} air). Overall, elevated CO_2 significantly increased fruit size and yield but not plants growth (Fig. 1, 2). We did not observe difference in the total biomass accumulation and total leaf area under elevated CO_2 at the vegetative stage (Fig. 1a, b). However, analyzing gas exchanges we observed that elevated CO_2 increased A by 20% compared with plants under ambient CO_2 conditions, which was accompanied by 38% increase in C_i (Fig. 1c, d). On the other hand, g_s and E reduced by approximately 25% and 20%, respectively, in plants grown under elevated CO_2 compared with ambient CO_2 (Fig. 1e, f). In relation to productive parameters, we observed that tomato plants grown at elevated CO_2 showed higher fruit fresh weight (20%) and fruit yield (25%) compared to plants grown under ambient CO_2 condition (Fig. 2b, c). However, we did not observe changes in the number of fruits per plant under elevated CO_2 (Fig. 2d). Elevated CO_2 increased the production of larger diameter fruits, with 52% of the fruits belonging to the 40 - 50 mm class compared to 37% under ambient CO_2 (Fig. 2e). In addition, there was a lower frequency of fruits in the class of smaller diameter (20 – 30 mm) in elevated CO_2 compared to fruits from plants grown in ambient CO_2 (Fig. 2e). Moreover, elevated CO_2 reduced fruit dry matter by 16% compared to fruits under ambient CO_2 (Fig. 2f).

3.2 Elevated CO_2 increases the fruit expansion rate and positively regulates the expression EXPs genes in fruits

In order to characterize differences in development of tomato fruits under elevated CO_2, the diameters of fruits were recorded until reaching the stabilization of the growth curve. Elevated CO_2 increased fruit growth compared to fruits from plants grown in ambient CO_2, observed by the larger diameter from 11DAA (Fig. 3a). The expression of expansins genes *SlEXPA3, SlEXPA5, SlEXPA6, SlEXPA8, SlEXPA12* and *SlEXPA24* was upregulated in immature tomato fruits under elevated CO_2 (Fig. 3b). In order to understand the changes in cell expansion caused by elevated CO_2 we analyzed the anatomy of the fruit pericarp at maturity. Elevated CO_2 increased by 22% the area of mesocarp cells while reduced by 21% the number of cells per area (Fig. 4a-c). The final fruit diameter was increased by approximately 15% in plants grown under elevated CO_2 compared with CO_2 ambient (Fig. 4d). Moreover, we observed that the fruit expansion rate was higher under elevated CO_2 but without changes in the fruit expansion duration (Fig. 4e, f).

3.3 Elevated CO_2 increases nutrients concentration in fruits

In general, elevated CO_2 significantly increased the concentration of nutrients in tomato fruits (Fig. 5). Among the macronutrients, we observed increase in P (14%), Ca (29%), Mg (20%) and S (27%) in fruits of plants grown under elevated CO_2 compared with ambient CO_2 (Fig. 5a-d). However, we did not observe differences in K levels in fruits under elevated and ambient CO_2 (Fig. 5e). Elevated CO_2 increased micronutrient levels B (17%), Fe (21%) and Cu (17%) in tomato fruits (Fig. 5f-h). We did not observe effect of the elevated CO_2 on the Zn concentration in the fruits (Fig. 5i).

3.4 Elevated CO_2 accelerates fruit ripening by modifying ethylene biosynthesis and signaling

Our results showed that elevated CO_2 caused the fruits ripening hasty in relation to the ambient CO_2 (Fig. 6a). Fruits under elevated CO_2 showed changes in color from 49 DAA onwards (*breaker* stage) while fruits of plants grown under ambient CO_2 still maintained their green color. At 50 DAA fruits in elevated CO_2 showed orange color and at 54 DAA they reached the light red stage. Meanwhile, fruits under ambient CO_2 started to ripen and showed pink color at 50 DAA and 54DAA respectively. However, the fruits of the elevated CO_2 and ambient CO_2 reached the ripe red stage at 56 DAA (Fig. 6a). Elevated CO_2 increased ethylene production (~28%) in fruits at 56 DAA (Fig. 6b). The expression ethylene biosynthesis genes *SlACS2*, *SlACS4, SlACO1, SlACO3* and ethylene signaling genes *SlETR1, SlETR2, SlETR4* was upregulated in ripe fruits under elevated CO_2. Moreover, the expression of *SlEXPA1, SlEXPA12* and *SlEXPA24* genes was increased in fruit of tomato plants grown under elevated CO_2 compared with ambient CO_2 (Fig. 6c). Compared with ambient CO_2, high CO_2 condition increased lycopene concentration (35%), β-carotene (26%) and lutein (25%) in ripe fruits (Fig. 6d-f).

4 Discussion

Elevated atmospheric CO_2 concentration can lead to an increase in tomato fruit size (Rangaswamy et al., 2021). However, the mechanisms that increase fruit growth rate in response to high CO_2 condition remain hitherto unclear. The results of the present study showed that the elevated CO_2 increased tomato fruit size in a manner that was coupled to increased expression of expansin genes. Moreover, elevated CO_2 that had a positive effect on ethylene biosynthesis in tomato fruit resulted in hastening maturity.

Tomato fruit development depends on an adequate import of carbohydrates from source organs (Osorio et al., 2014). Kinematic analysis of fruit growth revealed that the first significant difference in fruit diameter under elevated CO_2 occurs 11 days after anthesis (Fig. 3), coinciding with the initial cell expansion phase of tomato fruit development (Bertin, 2005; Quinet et al., 2019). Interestingly, the duration of fruit expansion and the total fruit number were not affected by CO_2 conditions (Figs 2 and 4). However, the fruit fresh weight was increased in plants under elevated CO_2 as a result of increased fruit expansion rate (Figs 2 and 4). These results imply that elevated CO_2 results in increased levels of carbohydrate added to the fruit per day, which triggers tomato fruit growth through stimulation of cell expansion. Consistent with this view, elevated CO_2 stimulated A by increasing C_i, a result that was associated with increase in pericarp cell area (Figs 1 and 4). However, it is important to note that the increase in pericarp cell size under elevated CO_2 was associated with a reduction in the number of pericarp cells. In tomato fruit, a large number of pericarp cells lead to a greater competition for carbohydrates (Bertin, 2005). In this context, treatment that increase carbohydrate supply, as does elevated CO_2, is expected to reduce competition between cells on pericarp. In fact, elevated CO_2 condition resulted in fewer pericarp cells but of a large size, which in turn increased fruit size (Figs 3 and 4). The increase in cell size in the pericarp of fruits under elevated CO_2 was consistent with an increase in the expression of *EXP* genes during fruit expansion period (Fig. 3). Thus, changes in the expression of genes involved in cell-wall remodeling could alter the competition for carbohydrates among tomato pericarp cells. The accumulation of fresh matter in fruits is also linked to increases in water flux driven to growing cells (Liu et al., 2007). The water content in fruits was higher under elevated CO_2, which is evidenced by a reduction in dry matter of the fruit (Fig. 2). These change in fruit water status under elevated CO_2 is likely to be related to reduction in g_s and E in leaves (Fig. 1). Collectively, these findings indicate that modulations in mechanical properties of growing cell walls associated with increasing water flux to growing cells are part of the control mechanism regulating tomato fruit size under elevated CO_2 condition.

Tomato fruit development is also dependent on a continuous supply of minerals (Kim et al., 2020). Given that elevated CO_2 did not affect the total leaf area (Fig. 1), the reduction in g_s and E by high CO_2 condition could lead to a decrease of the uptake of minerals that are acquired through mass flow (McGrath and Lobell, 2013). In our experiments, elevated CO_2 increased concentrations of minerals that move by mass flow (e.g., Ca, Mg and S) in tomato fruits as well as nutrients translocated by diffusion (e.g., Fe, Cu, P and B) (Fig. 5). The absence of alteration in concentrations of minerals translocated by mass flow in tomato fruits makes it tempting to speculate that mineral partitioning and fruit size are tightly controlled under elevated CO_2 in a manner that allows the tomato plants to prioritize reproductive organs. This observation is consistent with previous reports that elevated CO_2 increases size and Ca concentration in tomato fruit (Khan et al., 2013).

The increase in fruit size under elevated CO_2 culminated in a generally accelerated fruit ripening (Fig. 6). Tomato fruit ripening is controlled by the action of hormones, among which ethylene has been shown to be an important role in regulating carotenoid accumulation during ripening (Kou et al., 2021). Our analyses revealed that ethylene production increasing in fruits developed under elevated CO_2 (Fig. 6). It seems possible, therefore, that elevated CO_2 accelerates tomato fruit ripening in a manner that was coupled to increased levels of ethylene. Consistent with this proposition, the expression of genes associated with the biosynthesis and signaling of ethylene increased in tomato fruits of plants grown under elevated CO_2 (Fig. 6). This observation was also supported by the demonstration that the concentrations of carotenoid increased in fruits under elevated CO_2 compared with ambient CO_2 (Fig. 6). It has been pointed out that EXPs may contribute to the fruit softening during ripening process (Minoia et al., 2016). In this context, EXPA1 is a key element linking the ethylene to the tomato ripening process (Lu et al., 2016). Moreover, it was demonstrated that EXPA12 and EXPA24 transcript abundance increased in the tomato fruit at the red stage (Lu et al., 2016). The fact that the expression of *EXPA1*, *EXPA12* and *EXPA24* increased in tomato fruit under elevated CO_2 strengthens the idea that rising atmospheric CO_2 concentration tunes the relationship between ethylene and tomato fruit ripening.

5 Conclusions

In summary, elevated CO_2 increases tomato fruit size through carbon-dependent mechanisms, which in turn positively regulates the expression *EXPs* genes in fruits (Fig. 7). Consistent with a positive regulation of cell-wall related genes, high CO_2 results in increased cell size and larger fruits, while the number of cells is reduced. This alters the competition for carbohydrates among tomato pericarp cells, establishing the means to control fruit growth in tomato plants under elevated CO_2 conditions. In addition, the effect of elevated CO_2 on the carbohydrate availability accelerates fruit ripening in a manner consistent with the increased ethylene biosynthesis and signaling pathway components. Thus, our study highlights the potential role of elevated CO_2 in determining the tomato fruit size by controlling cell expansion in pericarp, leading to new pattern of fruit ripening.

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Figures

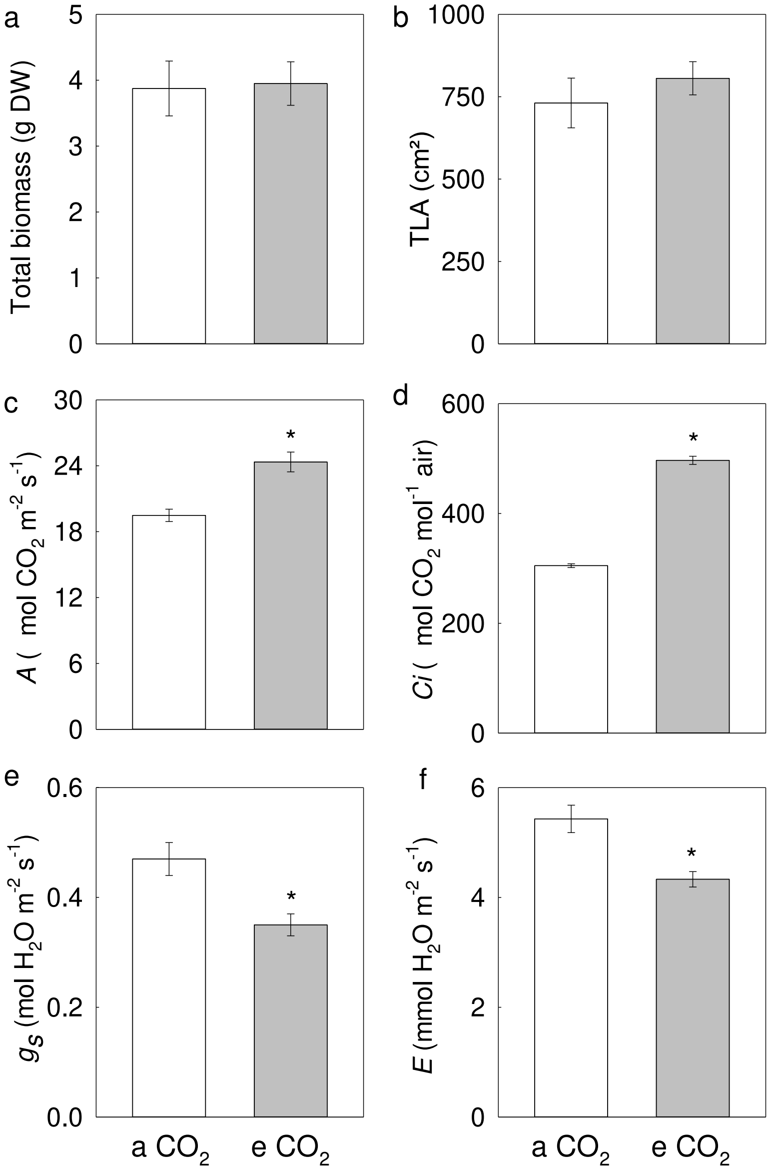

Fig. 1 Effects of elevated CO_2 on growth and gas exchange of plants at 40 days after sowing. **a** Total biomass. **b** Total leaf area. **c** Net CO_2 assimilation rate. **d** Internal CO_2 concentration. **e** Stomatal conductance. **f** Transpiration rate. Asterisks indicate statistically different means by Student's t-test ($P \leq 0.05$). Values are means ± SEM ($n = 8$).

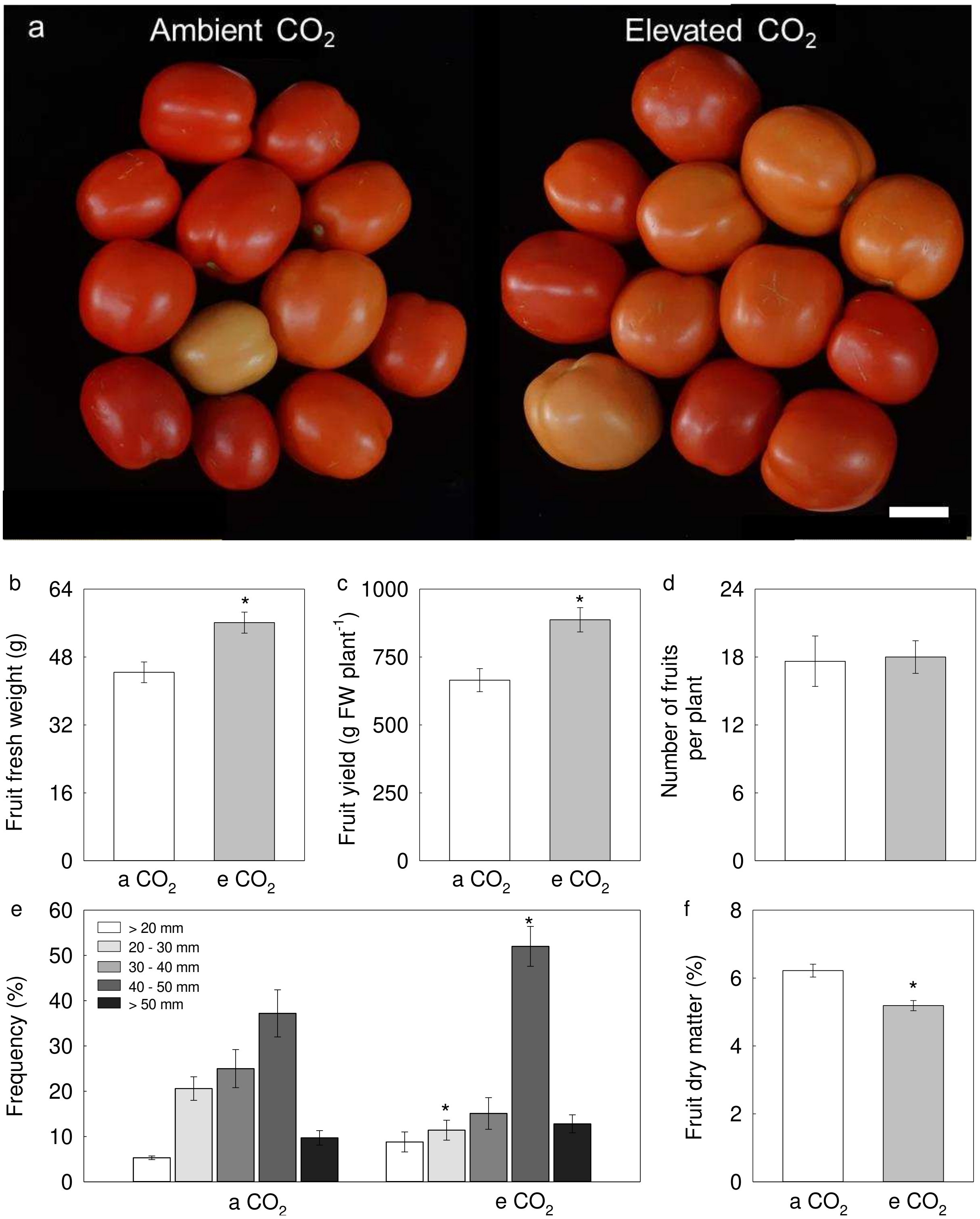

Fig. 2 Effects of elevated CO_2 on productive parameters of tomato fruits. **a** Phenotype of fruits produced under ambient CO_2 and elevated CO_2. Representative photo of the fruits at 56 DAA. The scale bar represents 3 cm. **b** Fruit fresh weight. **c** Fruit yield. **d** Number of fruits per plant. **e** Frequency of fruits by diameter class. **f** Fruit dry matter. Asterisks indicate statistically different means by Student's t-test ($P \leq 0.05$). Values are means ± SEM ($n = 8$).

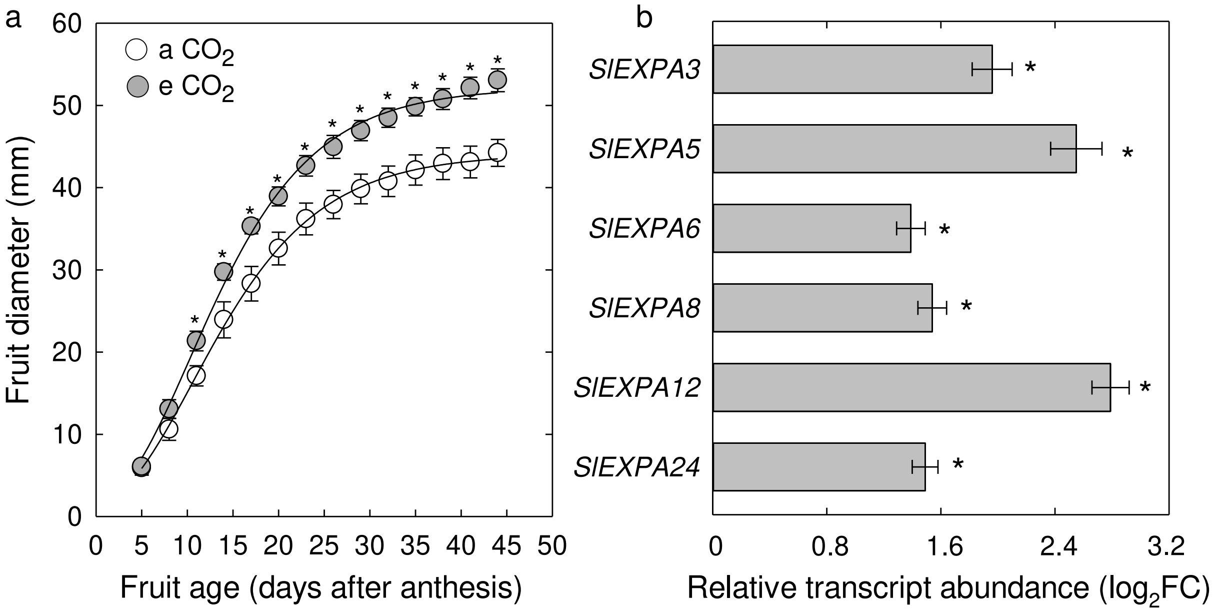

Fig. 3 Effects of elevated CO$_2$ on tomato fruit expansion and change on the expression of *EXPs* genes in fruit of plants grown under ambient CO$_2$ and elevated CO$_2$. **a** Time course of fruit growth of plants under ambient CO$_2$ and elevated CO$_2$. Fruit growth was fitted to three-parameter Gompertz function. **b** Gene expression in fruits at 35 days after anthesis under elevated CO$_2$. Data were normalized in relation to the mean response obtained in the ambient CO$_2$. Asterisks indicate statistically different means by Student's t-test ($P \leq 0.05$). Values are means ± SEM, with eight replicates for growth curve and three replicates for gene expression.

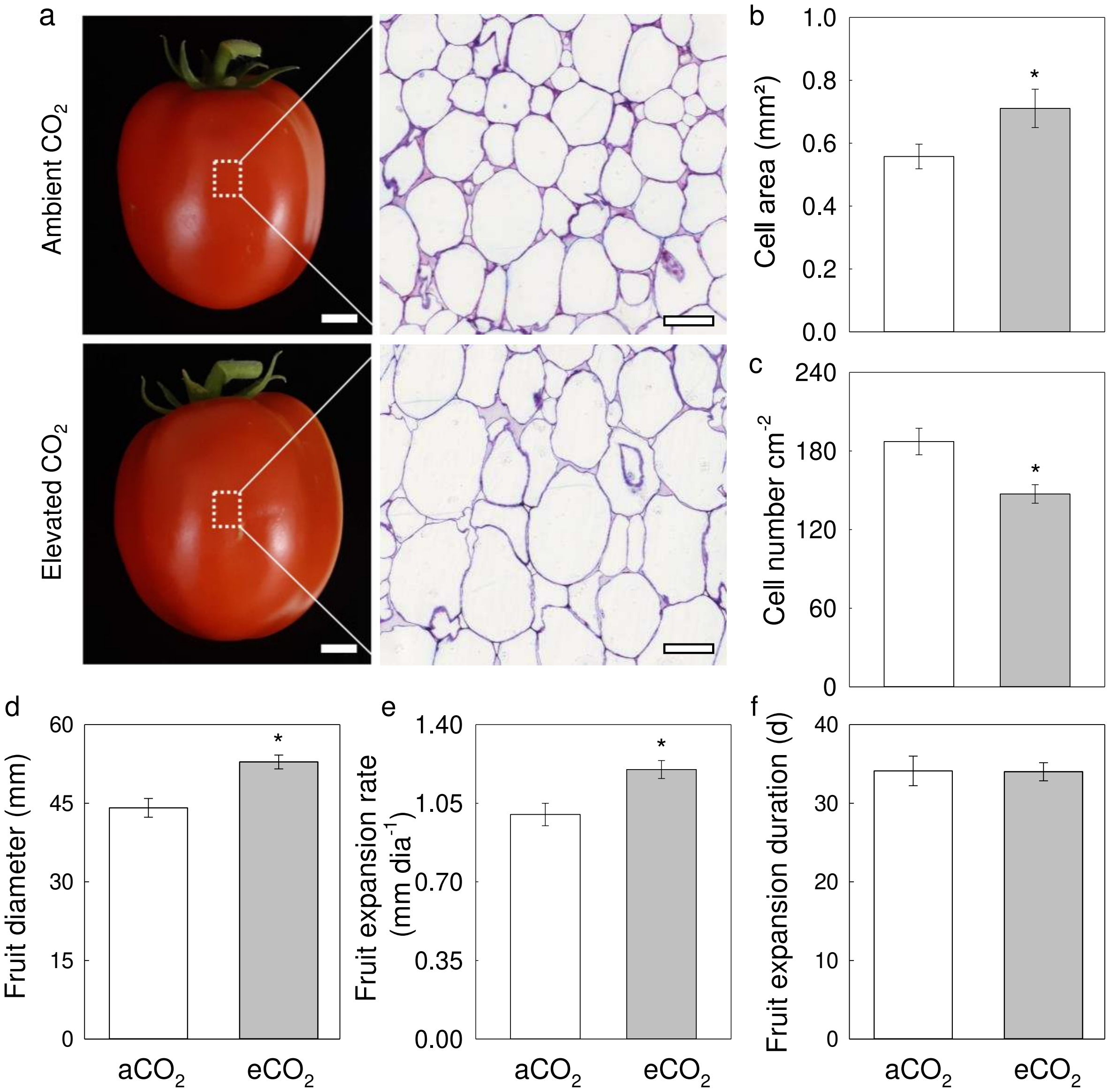

Fig. 4 Mesocarp structure and components of fruit growth dynamics of plants grown under ambient CO$_2$ and elevated CO$_2$. **a** Cross section of tomato mesocarp from ripe fruits grown in ambient and elevated CO$_2$. The scales bar represents 1 cm in tomato fruit and 250 µm in mesocarp micrographs. Dotted frame represents the portion of the fruits sampled for anatomy. **b** Cell area. **c** Cell number. **d** Fruit diameter. **e** Fruit expansion rate. **f** Fruit expansion duration. Asterisks indicate statistically different means by Student's t-test ($P \leq 0.05$). Values are means ± SEM, with five replicates for mesocarp anatomy and eight replicates for growth parameters.

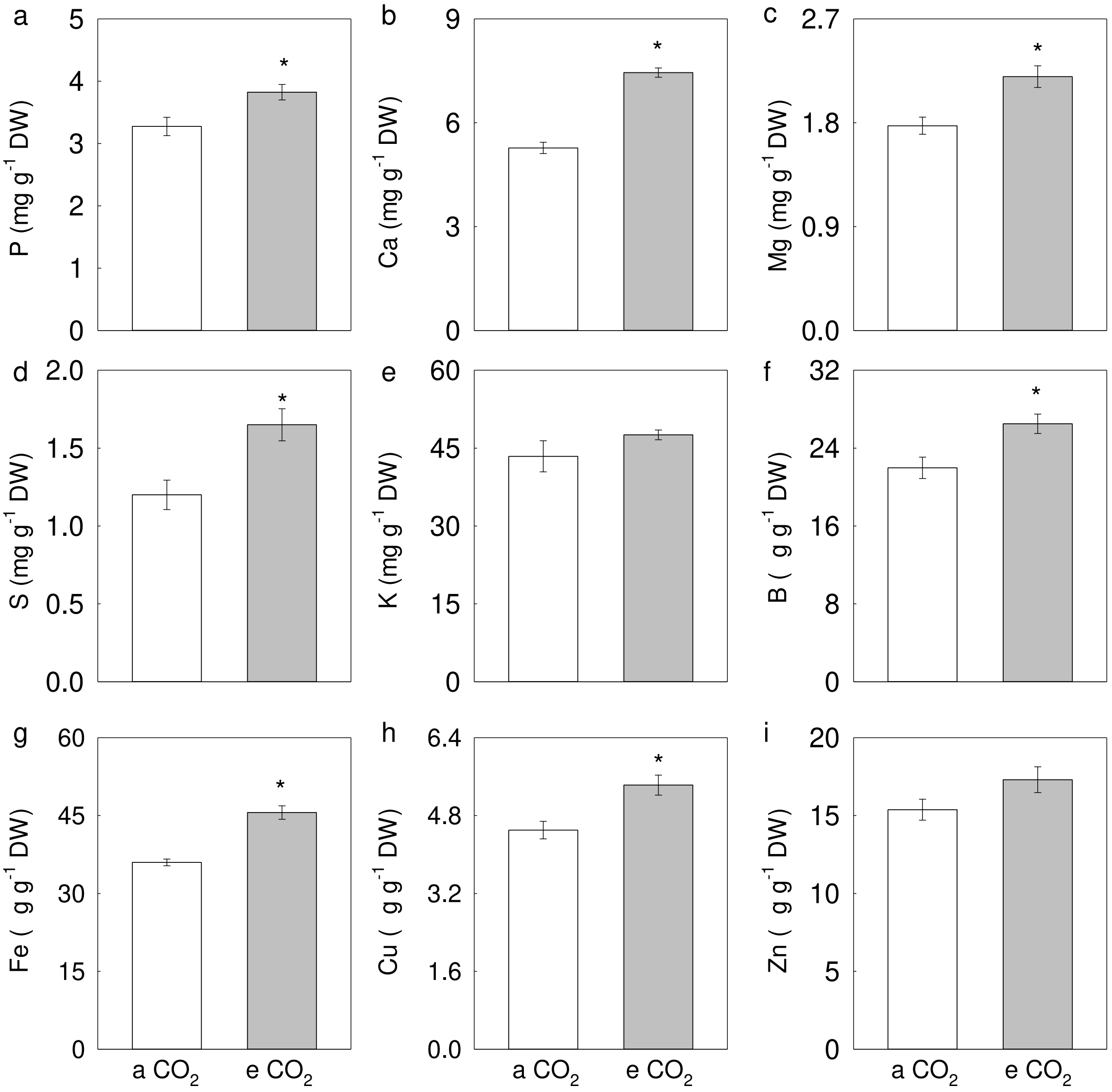

Fig. 5 Effect of elevated CO_2 on fruit mineral composition. **a** Phosphorus. **b** Calcium. **c** Magnesium. **d** Sulphur. **e** Potassium. **f** Boron. **g** Iron. **h** Copper. **i** Zinc. Asterisks indicate statistically different means by Student's t-test ($P \leq 0.05$). Values are means ± SEM ($n = 4$).

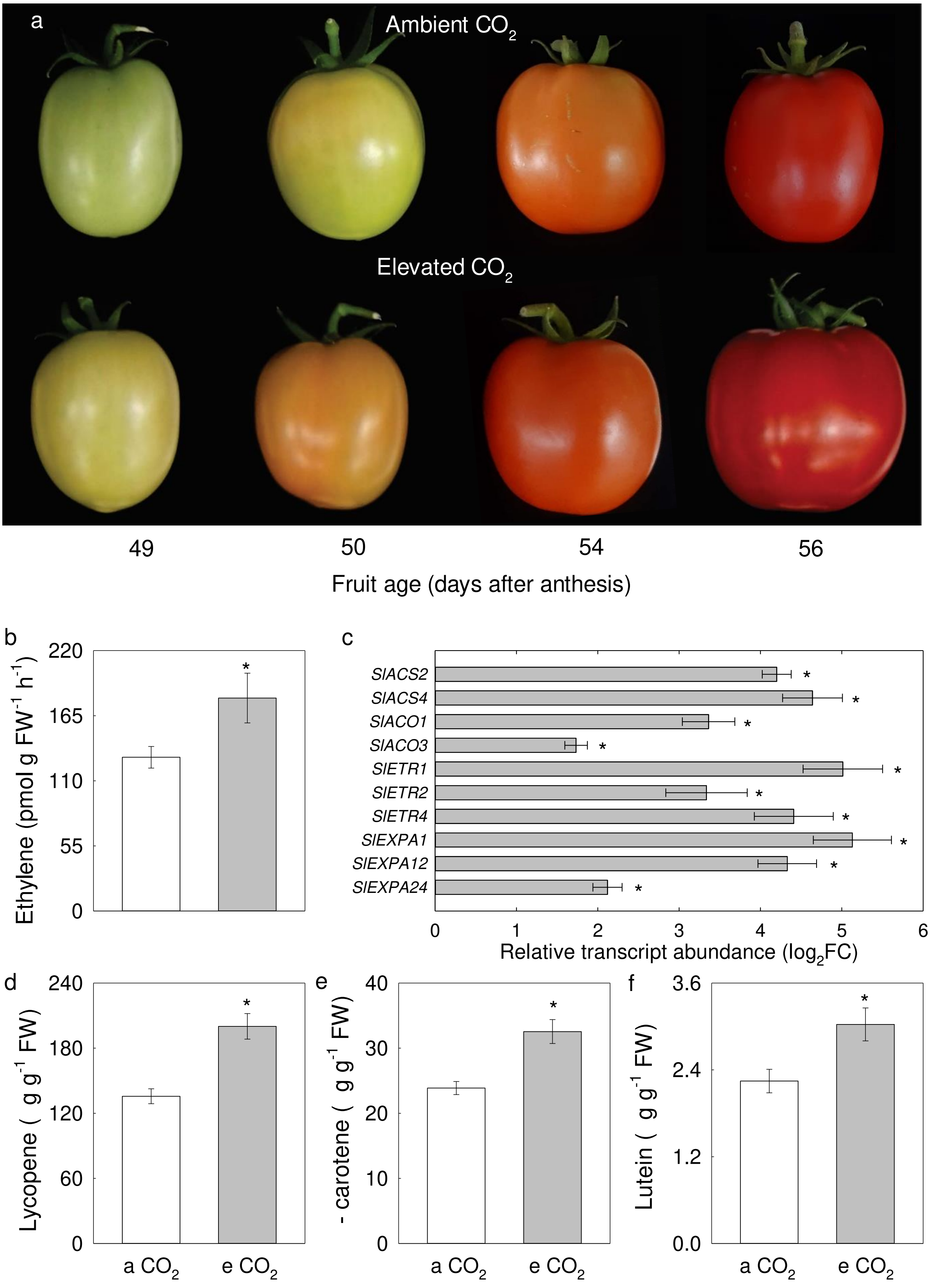

Fig. 6 Effects of elevated CO_2 on tomato fruit ripening. **a** Ripening fruit phenotype of tomato plants grown under ambient CO_2 and elevated CO_2 at 40, 50, 54 and 56 DAA. **b** Ethylene production in fruits at 56DAA. **c** Gene expression in fruits at 56 DAA under elevated CO_2. Data

were normalized in relation to the mean response obtained in the ambient CO_2. **d** Lycopene. **e** β-carotene. **f** Lutein. Asterisks indicate statistically different means by Student's t-test ($P \leq 0.05$). Values are means ± SEM, with six replicates for ethylene and carotenoids and three replicates for genes expression.

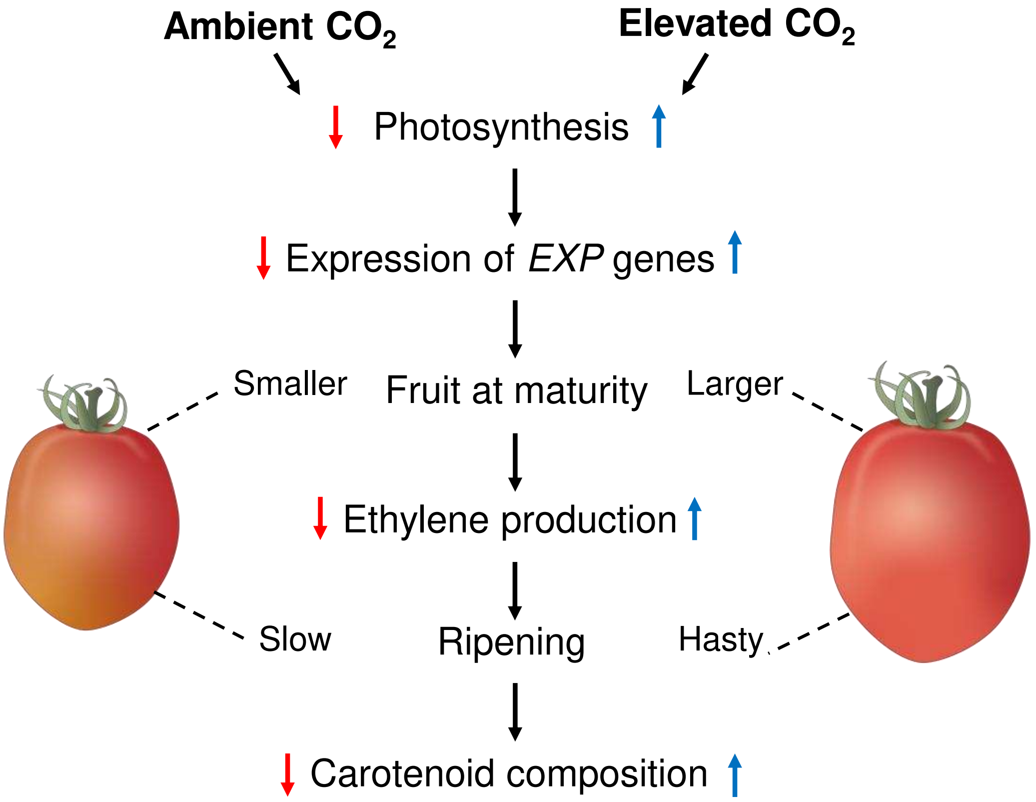

Fig. 7 Scheme summarizing the effect of elevated CO_2 on size and ripening of tomato fruit. Elevated CO_2 increases the photosynthetic rate and expression of expansin genes, stimulating the production of larger fruits at maturity compared to fruits under ambient CO_2. High CO_2 increases ethylene production in tomato fruits promoting hasty ripening and higher concentration of carotenoids in fruits compared to ambient CO_2.

Supplementary data

Table S1. Primers sequences used for RT- qPCR analysis

Gene	Primer Sequences	
	Forward (5'-3')	Reverse (5'-3')
ACS2	TGGAGAAAACAAGAGGAGGAAGA	GGCACCACCAGCCATAACA
ACS4	CCATCTTGTTTGCGACGAAATA	CGATGCTAACGAATTTTGGAGAA
ACO1	AAATCATGAAGGAGTTTGCTGATAAA	TTTTCACACAGCAAATCCAACAG
ACO3	ACGGGAAGTACAAGAGCGTGAT	CTAGTGACATCCGAGTCCCATCT
ETR1	GGCATTCCTGGACGTGCAAATG	TTTGGTTACCATCCTGCTAACACC
ETR2	TGGCATTCCTGGTCGCTTAAATG	CTTCGTTACCATCCTGCTAAACCC
ETR4	TGTGCAGAAAGCTGGTTCAGTTG	AGTTGAAGCCCAAGAACGACAGC
EXPA1	CCAAATGACAATGGTGGCTGGTGT	TCTGATTCCTCCTTGCTTTCGGCA
EXPA3	GTTCAAGAACAGGATGGCTTCCCA	AGAACAGCCTACGATTCGAACTGC
EXPA5	GGCAGAATAACGCTTACCTTAACGGC	GACCAAGAACTAGGAGCTGCATTG
EXPA6	GGTGGGCAGAAATTGGTTCCTGTT	ATGGGAATCTGTACAGCCAAGGGT
EXPA8	GGTACCTTGCAAGAAACATGGTGG	GCATTTGATTGCCAACTTGCTCCC
EXPA12	AGAAGTGGAAAGCAGTGGTGGACT	CAAAGTCCATCTTCAGCTACAGGC
EXPA24	CTCTGCGATTGTGCGGTATGTTGT	TGCACGTGGCAAGTTTCTTCTCTG

GENERAL CONCLUSIONS

This study shows how temperature and atmospheric CO_2 concentration, the main factors associated with global climate change, can affect production and nutritional quality of tomato fruits. In general, the warming air temperature and elevated CO_2 improve the productive aspects of tomato plants as yield, weight and fruit diameter compared to control condition. On the other hand, the nutritional quality of the fruits is impaired, with reduction in the levels of antioxidants important to human health (lycopene, β-carotene, ascorbic acid) and nutrients Zn and Mg. The effects of elevated CO_2 on fruit yield are associated with an increase in photosynthetic rates, while high temperature reduces fruit dry weight and Brix. Thus, these results may raise concerns in tomato growing about future environmental conditions, since tomato plants grown under warming temperatures and elevated CO_2 are not able to maintain the nutritional quality of fruits. In addition to the effects on fruit yield and quality, the results sheds light on the possible mechanisms regulating the fruit size under elevated CO_2. The larger diameter of fruits under elevated CO_2 is associated with upregulation of expression *EXP* genes in fruits. The greater photosynthetic rate of plants under elevated CO_2 results in higher carbon availability, that combined with smaller number of cells per area in fruits reduces competition between cells. These factors allow the greater expansion of pericarp cells and results in grater fruit growth rate. Changes in fruit growth culminate in accelerated ripening, with greater expression of ethylene biosynthesis and signaling genes in ripe fruit under elevated CO_2 compared to the ambient CO_2. These results show how tomato plants growing under elevated CO_2 adjust fruit growth according to carbon availability.