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The Impact of Selected Ecological Factors on the Growth and Biochemical Responses of Giza Faba Bean (*Vicia faba* L.) Seedlings

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Abstract

The climate is changing, warming and drying, and how plants will fare under such deteriorating climate is presently a prime question in plant ecology research. Giza faba bean (*Viacia faba* L.) is one essential crop of the world, and reductions in its biomass and yield are expected in response to global climate change, particularly in stressful habitats. A greenhouse study was conducted from March to April 2022 to examine the impacts of the main components of climate change. temperature, elevated CO_2, and water stress on the growth and biochemical responses of Giza faba bean seedlings. Seedlings were grown under two temperature regimes (22/18°C and 28/24°C), 12/12h light/dark photoperiod, two CO_2 concentrations (400 and 800 μmol mol^{-1}), and two watering regimes (well-watered and water stressed). Twelve-day-old seedlings were assigned in random to experimental conditions where they were grown for 14 days. Upon harvest, growth and biochemical parameters were measured. Overall, higher temperatures and water stress, individually, decreased growth parameters, leaf moisture content, dry and fresh matter of all plant parts, leaf mass area, nitrogen balance index and anthocyanin, but increased electrolyte leakage, chlorophyll measured by Dualex, flavonoid content, and Chl *a*, carotenoids, total chlorophyll, and Chl*a/b* ratio. Moreover, higher temperatures increased proline content and water stress increased malondialdehyde (MDA) content. Elevated CO_2 increased growth parameters, leaf moisture content, fresh matter of stems and leaves and dry matter of all plant parts, leaf area ratio, flavonoid content, but decreased MDA and electrolyte leakage. Interactions among the three components of climate change primarily affected leaf number, moisture content, root dry mass, MDA and flavonoid contents. Elevated CO_2 alleviated the negative impacts of temperature and water stress on Giza faba bean seedlings through decreasing oxidative stress and increasing plant water status. Our study showed that Giza faba bean has the potential to decrease negative impacts of the main components of climate change in the future.

Keywords: Abiotic stress, Climate change, Elevated CO_2, Giza faba bean (*Vicia faba* L.), Plant growth, Temperature, Water stress.

1. Introduction

For more than a decade, climate change has been considered as a chief issue of international concern because of increased emissions of greenhouse gases (primarily CO_2) and warming of the Earth (Triacca *et al.,* 2014; Kesaulya and Vega, 2019). Human modifications of the global environment, through burning of fossil fuels and destruction of plants natural habitats, are key causes of climate deterioration and altered behavior and distribution of organisms at the individual, population and community levels of ecological organization (Kesaulya and Vega, 2019). For this reason, climate change ecology has become a core research topic in different laboratories of life sciences around the world (Timpane-Padgham *et al.,* 2017).

Since plant growth and food production, as measured by plant biomass, are generally enhanced by atmospheric CO_2 enrichment, a central question in plant ecology today is, "how will plants fare under increases in atmospheric CO_2 in the future?" Attentions have thus been turned toward investigating how unavoidable increases in atmospheric CO_2 affects plants performance and structure of the Earth's vegetation, particularly in stressful environments (Marcilly *et al.,* 2021). The main components of climate change (temperature, drought) associated with elevated atmospheric CO_2 influence plants growth and productivity on a global scale and so have received special research attention by plant ecologists (IPCC, 2013). Global warming and a drier climate result from increased emissions and accumulation of greenhouse gases in the atmosphere (Triacca *et al.,* 2014). With such drastic climatic changes, plants have to develop the ability to adapt to external and frequently harmful environmental stresses of increased temperatures and decreased soil water availability associated with increased levels of greenhouse gases in the atmosphere (Moretti *et al.,* 2010; Lacis *et al.,* 2013).

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Temperature stress can cause plants to go through morphological and biochemical changes in order to adapt to adverse conditions (Moretti *et al.,* 2010; Qaderi *et al.,* 2012; Xu *et al.,* 2012; Prasch and Sonnewald, 2015; Martel and Qaderi, 2016; Abo Gamar *et al.*, 2019, 2021). DaMatta *et al.* (2010) stated that, in regions of low latitude, a modest increase of 1-2^{o}C would have negative impacts on the yield of crops. Rising atmospheric temperature is continuous due to the amount of carbon dioxide present in the atmosphere and could bring a temperature increase in earth's temperature from 1-6^{o}C, depending on the climate model (DaMatta *et al.,* 2010; IPCC, 2013).

Carbon dioxide concentrations rise by an average of 0.005 ppm per year; in our current climate they rise by an average of 2 ppm per year (Sukumaran *et al.*, 2018). Anthropogenic activities have quickly increased the concentration of CO_2 in the atmosphere, and the present concentration of CO_2 is 400 ppm is likely to reach to 700 ppm by 2100 (IPCC, 2013). However, on the basis of the current anthropogenic emission rates of CO_2 to the atmosphere, other studies suggested that it may exceed 1000 µmol mol^{-1} by 2100 (Solomon, 2007; Diatta *et al.,* 2020). This places stress on existing ecosystems, and allows forest lines to retreat while other species move into their former territory (Jiang *et al.*, 2013). This elevation in the concentration of CO_2 in the atmosphere could positively and negatively affects plants (Qaderi *et al.*, 2006).

Water stress is a major abiotic stress inhibiting plant growth and development and causing enormous losses in many crops (Mishra and Singh, 2010). Global warming has been shown to increase its incidence in many parts of the world, particularly in the warm regions of the globe (Qaderi *et al.,* 2012; Okorie *et al.*, 2019; Abo Gamar *et al.,* 2021). This poses a problem for areas that are already dry, and may lead to a need for frequent irrigation in agricultural areas (Liu *et al.,* 2013). Previous studies have shown that plant growth and biomass are considerably retarded during periods of water stress (Sangtarash *et al.,* 2009; Qaderi and Reid, 2009; Qaderi *et al.,* 2012; Prasch and Sonnewald, 2015; Abo Gamar *et al.,* 2021). Water stress enhances plants to make adjustment for their physiological processes to help them make an adaption to moderate levels of stress factors (Sangtarash *et al.,* 2009; Kaur *et al.,* 2015).

Earlier studies have looked at temperature (Cline, 2007; Kaur *et al.,* 2015; Mekonnen *et al.,* 2016) or carbon dioxide (Taub, 2010; Munir *et al.*, 2014; Váry *et al.*, 2015) or watering regime (Hu *et al.,* 2006; Ihuoma and Madramootoo, 2019); some have examined a combination of two of them (Farrar and Gunn, 2017; Ulfat *et al.*, 2021); and only few studies examined the interactive effects of the main components of climate change on plant (Qaderi *et al.,* 2011; Padhy *et al.,* 2018; Abo Gamar *et al.,* 2019, 2021; Singh *et al.,* 2021; Fallah *et al.,* 2022).

Faba bean (*Vicia faba* L.) belongs to the family Fabaceae or Leguminosae, and it is among the oldest crops grown worldwide (Osman *et al.,* 2010). Faba bean (*Vicia faba* L.) is also known as broad bean, horse bean and field bean and it is the fourth most main crop in the world (Tiwari and Singh, 2019). The seeds of faba bean contain high protein content of 24-33% (Tiwari and Singh, 2019). In Jordan, the crop is cultivated either under irrigation or rainfed for the purposes of fresh pod utilization and dry seed production (Thalji, 2015).

We hypothesized that growth of Giza faba bean seedlings would be negatively influenced by high temperature and water stresses, but positively enhanced by elevated CO_2, and that the elevated CO_2 could alleviate the detrimental impacts of high temperature and water stresses on Giza faba bean seedlings. The objectives of this study were to: (1) examine how Giza faba bean plants respond to the single and interactive effects of temperature, carbon dioxide and water stress and (2) find if stress responses in Giza faba bean plants are alleviated by elevated CO_2.

2. Materials and methods

2.1. Plant materials and growth conditions

A greenhouse study was conducted from March to April 2022 at Yarmouk University to investigate the effects of the main climate change components; temperature, CO_2, and watering regimes and their interactions on growth and biochemical responses of Giza faba bean (large seed) seedlings. In this study, Giza faba bean seeds (obtained from the National Agricultural Research Center [NARC] in Amman, Jordan) were sawn in 200 cm^3 pots containing a mixture of vermiculite, perlite and peat moss (1:1:1, by volume). One faba bean seed was sown in each pot and left for germination in a naturally illuminated greenhouse under the conditions of 12/12h light/dark photoperiod, photon flux density (PFD) of 600 photons m^{-2} s^{-1}, relative humidity (RH) 45.9% ± 6.5%, and 22/18^{o}C day/night thermoperiod. Each pot was given 60 mL of tap water to begin germination; afterwards, seedlings were watered using a fine-spray watering, which was gradually increased in spray amount as plants grow larger and hardier. The germination process, in total, was lasted roughly 7 days. After germination, seedlings were left to grow under the previous mentioned greenhouse conditions for a period of 5 days until established and produced their first foliage leaves. Seedlings were fertilized with NPK fertilizer (18-18-18, Type 100, Chissor-Asahi® Fertiliser Co. Ltd., Tokyo, Japan). After that, seedlings were randomly placed in four equal size Plexiglas cabinets of 60 cm depth, 65 cm width, and 50 cm height built up in the greenhouse. Same previous conditions were established inside cabinets in terms of light density, photoperiod, thermoperiod and relative humidity. However, two cabinets were selected to represent the normal temperature treatment (22/18^{o}C day/night; the greenhouse normal temperature) and the other two to represent the high temperature treatment (28$^{/}$24^{o}C day/night; the high temperature was established and maintained by lamps covered with aluminum foil connected to heat sensors). Temperatures were chosen to represent the current average temperature values (22/18°C) in Jordan from March to April, whereas the higher temperatures represent the expected average temperature by 2100 if temperatures increase by the highest expected value of 6.4°C (28/24°C, IPCC, 2013). Under each temperature treatment, one cabinet was supplied with ambient CO_2 (400 ppm) and the other one with elevated CO_2 (800 ppm), and half of the seedlings were watered to field capacity (well-watered) and the other half was water-stressed. A split-split-plot design with three factors (temperature, CO_2, and watering regime) was applied to get eight experimental conditions as shown in table 1. An electrical fan was used to keep CO_2 circulation constant in each cabinet and the relative humidity was kept at 45.9% ± 6.5% based on the greenhouse relative humidity. The flow of gas from the CO_2 cylinder to the Plexiglas cabinet was regulated by pressure gauge, solenoid valve and flow meter, and regularly monitored by a pSense portable CO_2 meter (CO_2 Meter, Inc., Ormond Beach, FL, USA).

Table 1. Experimental conditions under which Giza faba bean seedlings were grown.

Normal temperatures regime (22°C/18°C)				High temperatures regime (28°C/24°C)			
Ambient CO_2 (400 μmol mol^{-1})		Elevated CO_2 (800 μmol mol^{-1})		Ambient CO_2 (400μmol mol^{-1})		Elevated CO_2 (800 μmol mol^{-1})	
Well-watered	Water-stressed	Well-watered	Water-stressed	Well-watered	Water-stressed	Well-watered	Water-stressed

Seedlings were grown under their respective treatment for 14 days in cabinets. Pots were rotated within the cabinets every second day to minimize the variation in growth due to placement within the cabinets and shading effects of the greenhouse. The experiment was replicated three times in order to show reproducibility.

2.2. Determination of growth parameters

After 14 days of growth, the following growth parameters for all experimental conditions were measured: stem length and diameter, dry and fresh mass of leaves, stems and roots, and leaf area and moisture content. Measurement of stem length was performed from the surface of soil to apical meristem of each plant using a ruler, whereas stem diameter was measured using a Digimatic caliper (Mitutoyo Corp. Kanagawa, Japan) placed at the midway point between soil and the apical meristem. Three plants, per condition, were harvested to determine leaf, stem, and root fresh masses using a digital electronic balance (Model GD603, Sartorius, Gottingen, Germany). Then, same plants were dried at 60 °C for 72 h in a forced air oven in order to determine leaf, stem, and root dry masses using the same balance. To assess leaf moisture content, an average-sized leaf from each plant was removed and weighed before and after drying using the previous mentioned balance. Leaf area of each dried plant was determined using an image J software (http://rsb.info.nih.gov/ij/).

2.3. Stress indicators

2.3.1. Measurement of proline content

Proline content of three different samples per treatment for each experimental condition were estimated according to Bates *et al.* (1973). Proline concentrations were measured using a standard curve of proline on a fresh mass basis (μmol g^{-1} FM).

2.3.2. Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) using 2-thiobarbituric acid assay procedure following Abo Gamar *et al.* (2019). Three fresh leaf samples (50 mg) from three separate plants were collected from each treatment, frozen in liquid nitrogen and homogenized using a mortar and a pestle in a solution composed of 1.5 ml 0.5% 2-thiobarbituric acid and 1.5 ml 0.1% trichloroacetic acid. Following centrifugation at 4000g for 15 min at 4°C, the supernatant was boiled for 10 min and cooled on ice. Afterwards, the absorbance at 532 nm and 600 nm using A UV/visible spectrophotometer (Olympus, Tokyo, Japan). The 0.5% 2-thiobarbituric acid and 0.1% trichloroacetic acid were used as a blank. The concentration of MDA (nmol g^{-1} FM) was calculated by using the following formula: $[((A_{532} - A_{600}) \times v) \times 1000]/(\varepsilon \times M)$. 'ε' represents the specific extinction coefficient (= 155 mM^{-1} cm^{-1}), 'v' is the volume of extraction medium, 'M' is the leaf fresh mass, and 'A_{600}' and 'A_{532}' are absorbance at 600 and 532 nm, respectively.

2.3.3. Measurement of membrane permeability

Membrane permeability was estimated by measuring the electrical conductivity of three samples per treatment according to Abo Gamar *et al.* (2019). Three leaf samples (100 mg) from three different plants per treatment were washed thoroughly and soaked in 15 ml distilled water at room temperature for 24 h. The initial conductivity (C1) of the fresh tissue was measured using an HI 98311 DiST® 5 EC/TDS/Temperature Tester. Samples were then boiled for 1 hour at 100°C to release the electrolytes. The maximum conductivity of the dead tissue (C2) was measured, and the electrolyte leakage was calculated as a ratio of C1 and C2 expressed in percentage.

2.4. Measurement of nitrogen balanced index (NBI), chlorophyll, flavonoids and anthocyanins by dualex machine

Three leaves from separate plants per treatment were used for determination of nitrogen balance index (NBI), chlorophyll, flavonoids, and anthocyanins contents using Dualex Scientific® (Dualex Scientific, Force-A, Orsay Cedex, France).

2.5. Analysis of photosynthetic pigments

The concentrations of chlorophyll (Chl) *a*, Chl *b*, and carotenoids, as well as total Chl and Chl *a*:*b* ratio were measured according to Hiscox and Israelstam (1979). For each treatment, three leaf samples (~ 50 mg) were harvested from three different plants and were incubated in 5 ml of dimethylsulfoxide at room temperature for 24 h in the dark until the pigments were completely bleached. The absorbance of Chl *a, b,* and carotenoids was then measured at 664 nm, 648 nm, 470 nm using a UV-visible spectrophotometer (model V5800, Shanghai Metash Instruments Co. Ltd (China), respectively. The concentrations of Chl *a*, Chl *b*, carotenoids, as well as total Chl, and Chl *a/b* ratio were calculated per gram of fresh weight according to Chappelle et al. (1992).

2.6. Data analysis

The impacts of temperature, CO_2, and watering regime on growth and biochemical features of Giza faba bean were analyzed, using ANOVA for split-split-plot design (SAS Institute, 2011). For the split–split–plot analysis, temperature, CO_2, watering regime and cabinets were considered, respectively, as the main plot, subplot, split-subplot, and replications. A one-way ANOVA was used to find variations among single factors, using Schefé's test at the 5% probability level (SAS Institute 2011). For all parameters, three trials were used.

3. Results

3.1. Plant growth

Elevated CO_2 significantly increased stem length, leaf area and number, leaf moisture content and root length of Giza faba bean seedlings, while water and high temperature stresses significantly decreased them, in addition to the stem diameter (Table 2; Figure 1A-F).

Temperature, CO_2, watering regime and the two–way interactions between T × W significantly affected stem height (Table 3). Temperature, watering regime and the two–way interactions between T × W were significantly affected stem diameter (Table 3). The T × W interaction revealed that seedlings grown under normal temperatures and well–watered had the highest and thickest stem, while those grown under high temperatures and water–stressed had the shortest and thinnest stem (Figure 1A,B). Temperature, carbon dioxide, watering regime, and the two–way interactions between T × CO_2 and T × W significantly affected leaf area (Table 3). For the T × CO_2 interaction, seedlings grown under low temperatures and elevated CO_2 had significantly the highest leaf area, whereas seedlings grown under high temperatures and ambient CO_2 had significantly the lowest leaf area (Figure 1C). The T × W interaction revealed that seedlings grown under normal temperatures and well–watered had significantly the highest leaf area, while those grown under high temperatures and water-stressed had significantly the lowest leaf area (Figure 1C). Leaf number was significantly affected by the main factors and their interactions with exception to the two–way interaction between T × CO_2 (Table 3). The T × W interaction revealed that seedlings grown under normal temperatures and well–watered had the highest leaf number, while those grown under high temperatures and water-stressed had the lowest leaf number (Figure 1D). The CO_2 × W interaction showed that leaf number was significantly the highest for well-watered seedlings at elevated CO_2, but significantly the lowest for water-stressed seedlings at ambient CO_2 (Figure 1D). Based on the T × CO_2 × W interaction, leaf number was significantly highest for seedlings grown under the combination of normal temperature, elevated CO_2 and well-watered, but significantly lowest for seedlings grown under the combination of high temperatures, ambient CO_2 and water-stressed (Figure 1D). Leaf moisture content was significantly affected by the main factors and their interactions except for the two–way interactions between T × CO_2 and CO_2 × W (Table 3). The T × W interaction indicated that seedlings grown under normal temperatures and well-watered had significantly the highest leaf moisture content, whereas seedlings grown under high temperatures and water-stressed had significantly the lowest leaf moisture content (Figure 1E). On the basis of the T × CO_2 × W interaction, leaf moisture content was significantly highest for seedlings grown under normal temperatures, elevated CO_2 and well-watered, but significantly lowest for seedlings grown under high temperatures, ambient CO_2 and water-stressed (Figure 1E). Root length was significantly affected by the carbon dioxide and the two–way interaction between T × W (Table 3). The T × W interaction showed that seedlings grown under high temperatures and water-stressed had significantly the tallest root, while those grown under normal temperatures and water–stressed had significantly the shortest root (Figure 1F).

Table 2. Individual impacts of temperature, CO_2 and watering regime on growth and biochemical parameters of Giza faba beans (*Vicia faba* L.) seedlings.

	Temperature		Carbon dioxide		Watering regime	
Parameter	Normal	High	Ambient	Elevated	Well-watered	Water-stressed
Stem length (cm)	37.3 ± 3.6A	26.1 ± 1.4B	29.9 ± 3.1B	33.4 ± 3.3A	39.7 ± 2.9A	23.7 ± 0.8B
Stem diameter (mm)	0.47 ± 0.04A	0.27 ± .02B	0.38 ± 0.03A	0.36 ± 0.04A	0.46 ± 0.04A	0.29 ± 0.02B
Leaf area (cm^2 $plant^{-1}$)	107.7 ± 12A	73.9 ± 8.6B	74.7 ± 9.3B	107.1 ± 11.6A	119.9 ± 9.3A	61.8 ± 5.5B
Leaf number ($plant^{-1}$)	11.7 ± 1.3A	6.9 ± 0.4B	8.04 ± 0.8B	10.5 ± 1.4A	11.6 ± 1.3A	6.9 ± 0.4B
Leaf moisture (%)	81.4 ± 2.8A	70.9 ± 4.8B	72.9 ± 4.5B	79.5 ± 3.7A	88.1 ± 1.3A	64.3 ± 2.9B
Root length (cm)	25.5 ± 1.48A	27.4 ± 1.5A	24.2 ± 1.2B	28.7± 1.5A	26.5± 1.04A	26.4± 1.9A
Stem fresh mass (g)	3.9 ± 0.4A	2.5 ± 0.2B	2.8 ± 0.3B	3.7 ± 0.5A	4.2 ± 0.4A	2.3 ± 0.1B
Leaf fresh mass (g)	3.9 ± 0.5A	2.5 ± 0.4B	2.8 ± 0.4B	3.6 ± 0.6A	4.6 ± 0.4A	1.9 ± 0.2B
Root fresh mass (g)	4.34 ± 0.3A	4.11 ± 0.1A	4.1 ± 0.2A	4.38 ± 0.3A	4.8 ± 0.2A	3.7 ± 0.1B
Total fresh biomass (g)	12.2 ± 1.3A	9.2 ± 0.6B	9.7 ± 0.8B	11.7 ± 1.3A	13.6 ± 0.9A	7.8 ± 0.2B
Stem dry mass (g)	2.3 ± 0.2A	1.2 ± 0.1B	1.67 ± 0.2B	1.98 ± 0.2A	2.2 ± 0.3A	1.4 ± 0.1B
Leaf dry mass (g)	1.6 ± 0.24A	0.89 ± 0.16B	1.1 ± 0.2B	1.4 ± 0.2A	1.9 ± 0.2A	0.6 ± 0.1B
Root dry mass (g)	1.3 ± 0.1A	0.85 ± 0.1B	0.98 ± 0.1B	1.2 ± 0.1A	1.4 ± 0.08A	0.78 ± 0.09B
Total dry biomass (g)	5.3 ± 0.5A	2.9 ± 0.3B	3.7 ± 0.5B	4.5 ± 0.6A	5.4 ± 0.5A	2.8 ± 0.3B
Proline (µmole g^{-1} FM)	34.9 ± 4.6B	55.6 ± 2.2A	45.7 ± 5.1A	44.8 ± 4.3A	40.9 ± 4.4A	49.5 ± 4.7A
MDA (µmole g^{-1} FM)	0.23 ± 0.03A	0.3 ± 0.04A	0.3 ± 0.1A	0.18 ± 0.01B	0.16 ± 0.01B	0.32 ± 0.04A
Electrical conductivity (%)	16.9 ± 1.4B	31.4 ± 1.8A	27.5 ± 2.6A	20.9 ± 2.4B	20.8 ± 2.2B	27.7 ± 2.8A
Nitrogen balance index	80.8 ± 8.1A	69.1 ± 1.9B	77.7 ± 7.3A	72.3 ± 4.6A	85.9 ± 6.7A	64.1 ± 3.03B
Chlorophyll (µg cm^{-2} FM)	25.7 ± 1.08B	29.5 ± 1.05A	26.7 ± 0.8A	28.5 ± 1.4A	24.9 ± 0.86B	30.2 ± 0.96A
Flavonoids (µg cm^{-2} FM)	0.37 ± 0.04B	0.54 ± 0.01A	0.4 ± 0.03B	0.52 ± 0.01A	0.32 ± 0.02B	0.59 ± 0.04A
Anthocyanin (nmol/cm^2)	0.07 ± 0.005A	0.05 ± 0.004B	0.06 ± 0.004A	0.05 ± 0.006A	0.07 ± 0.005A	0.04 ± 0.003B
Chl *a* (µg mg^{-1} FM)	1.9 ± 0.2B	2.6 ± 0.1A	2.28 ± 0.2A	2.29 ± 0.1A	2.5 ± 0.17A	2.1 ± 0.2A
Chl *b* (µg mg^{-1})	1.3 ± 0.1A	1.8 ± 0.2A	1.8 ± 0.2A	1.3 ± 0.1A	1.7 ± 0.21A	1.4 ± 0.17A
Carotenoids (µg mg^{-1} FM)	0.42 ± 0.06B	0.65 ± 0.07A	0.48 ± 0.07A	0.6 ± 0.08A	0.54 ± 0.07A	0.54 ± 0.08A
Total Chl (µg mg^{-1} FM)	3.2 ± 0.4B	4.4 ± 0.3A	4.1 ± 0.4A	3.6 ± 0.3A	4.2 ± 0.35A	3.5 ± 0.4A
Chl *a/b*	1.5 ± 0.1B	2.1 ± 0.2 A	1.7 ±0.17A	1.9 ± 0.15A	1.77± 0.15A	1.8 ± 0.17A

Giza faba bean seedlings were grown under normal (22/18°C) or high (28/24°C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions. Data are means ± SE of at least 9 samples from three different experiments. Means (± SE) followed by different upper-case letters within rows and factors are significantly different ($P < 0.05$) according to Scheffé's test.

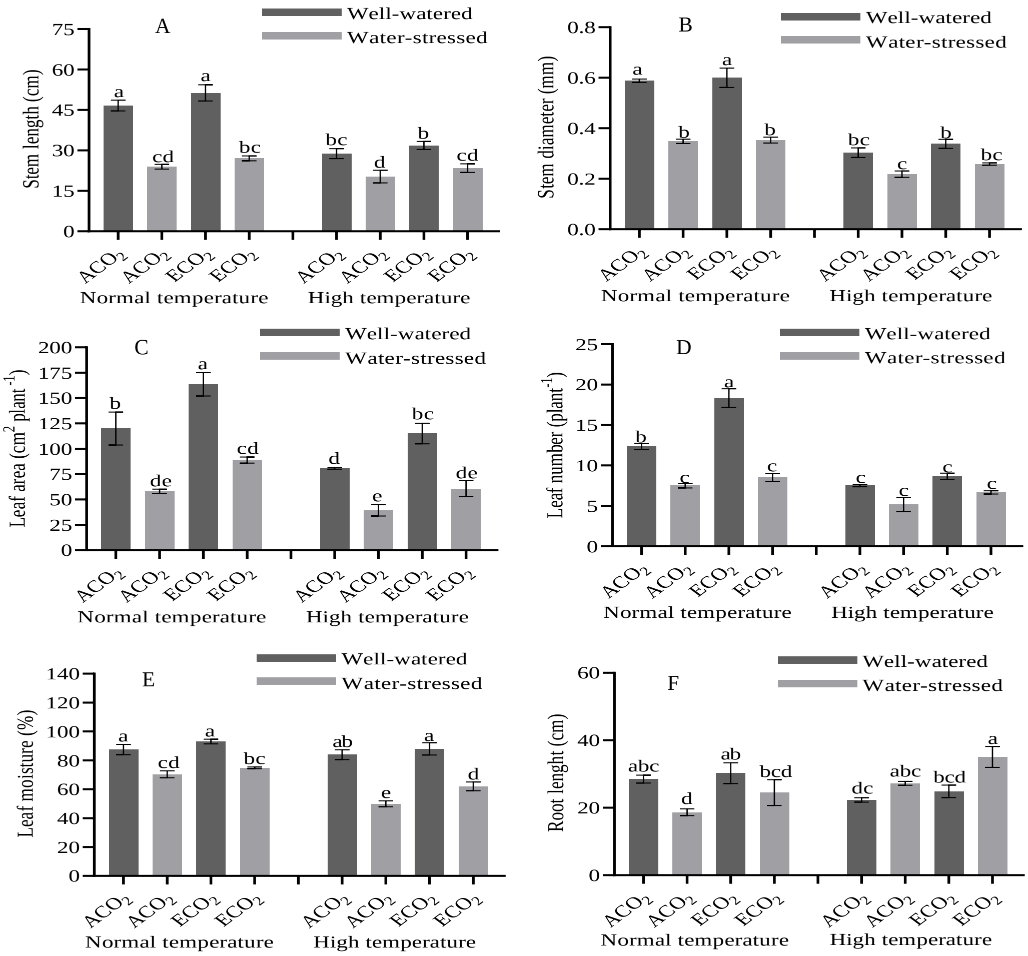

Figure 1. Stem height (A), stem diameter (B), leaf area (C), leaf number (D), moisture content (E) and root length (F) for 26-day-old Giza faba bean (*Vicia faba* L.) seedlings grown in Plexiglas cabinets for 14 days under eight experimental conditions after 12 days of initial germination and growth under greenhouse conditions. Error bars denote standard error (n = 9). Means followed by different letters are significantly different at P < 0.05 according to Scheffé's test. *ACO₂* ambient CO_2, *ECO₂* elevated CO_2.

Table 3. Analysis of variance for the individual impacts of temperature, CO_2 and watering regime and their interactions on stem height, stem diameter, leaf area, leaf number, moisture content and root length of Giza faba bean (*Vicia faba* L.) seedlings.

		Stem height (cm)	Stem diameter (mm)	Leaf area (cm^2 $plant^{-1}$)	Leaf number ($plant^{-1}$)	Moisture content (%)	Root length (cm)
Source	df						
Temperature (T)	1	2240.22***	590.55**	43.04*	609.19**	31.43*	3.44
Main plot error	2	-	-	-	-	-	-
Carbon dioxide (CO_2)	1	12.49*	3.56	765.26****	31.76**	20.86*	68.89**
T x CO_2	1	0.19	1.44	15.87*	5.08	1.11	1.66
Subplot error	4	-	-	-	-	-	-
Watering regime (W)	1	376.60****	169.55****	250.63****	96.49****	725.49****	0.01
T x W	1	83.28****	41.31***	7.58*	31.51***	48.66***	52.40****
CO_2 x W	1	0.20	0.00	3.11	6.92*	3.86	4.89
T x CO_2 x W	1	0.25	0.05	0.00	6.92*	6.49*	0.08
Split-subplot error	8	-	-	-	-	-	-

Note: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Giza faba bean seedlings were grown under normal (22/18°C) or high (28/24°C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions.

3.2. Biomass accumulation

3.2.1. Fresh mass accumulation

High temperatures and water stress reduced stem (Table 2; Figure 2A), leaf (Table 2; Figure 2B), and total (Table 2; Figure 2D) fresh biomass. Water stress also decreased root fresh mass (Table 2; Figure 2C). On the other hand, elevated CO_2 increased stem (Table 2; Figure 2A), leaf (Table 2; Figure 2B) and total (Table 2; Figure 2D) fresh biomass.

All measured fresh biomass parameters were significantly affected by the three main factors (Table 4). Stem and total fresh biomass were significantly affected the two–way interactions between T × W and CO_2 × W (Table 4). The T × W interaction revealed that seedlings grown under normal temperatures and well–watered had significantly the highest stem and total fresh biomass, whereas seedlings grown under high temperatures and water–stressed had significantly the lowest stem and total fresh biomass (Figure 2A,D). The CO_2 × W interaction showed that well-watered seedlings at elevated CO_2 had the highest stem and total fresh biomass, whereas water-stressed seedlings at ambient CO_2 had the lowest stem and total fresh biomass (Figure 2A,D). Root fresh mass was significantly affected the two–way interactions between T × CO_2 and T × W (Table 4). The T × CO_2 interaction showed that elevated CO_2 significantly caused the highest root fresh mass for seedlings grown under normal temperatures, but ambient CO_2 significantly caused the lowest root fresh mass for seedlings grown under normal temperatures (Figure 2C). For the T × W interaction, well-watered seedlings grown under normal temperatures had significantly the highest root fresh mass, whereas water-stressed seedlings grown under normal temperatures had significantly the lowest root fresh mass (Figure 2C).

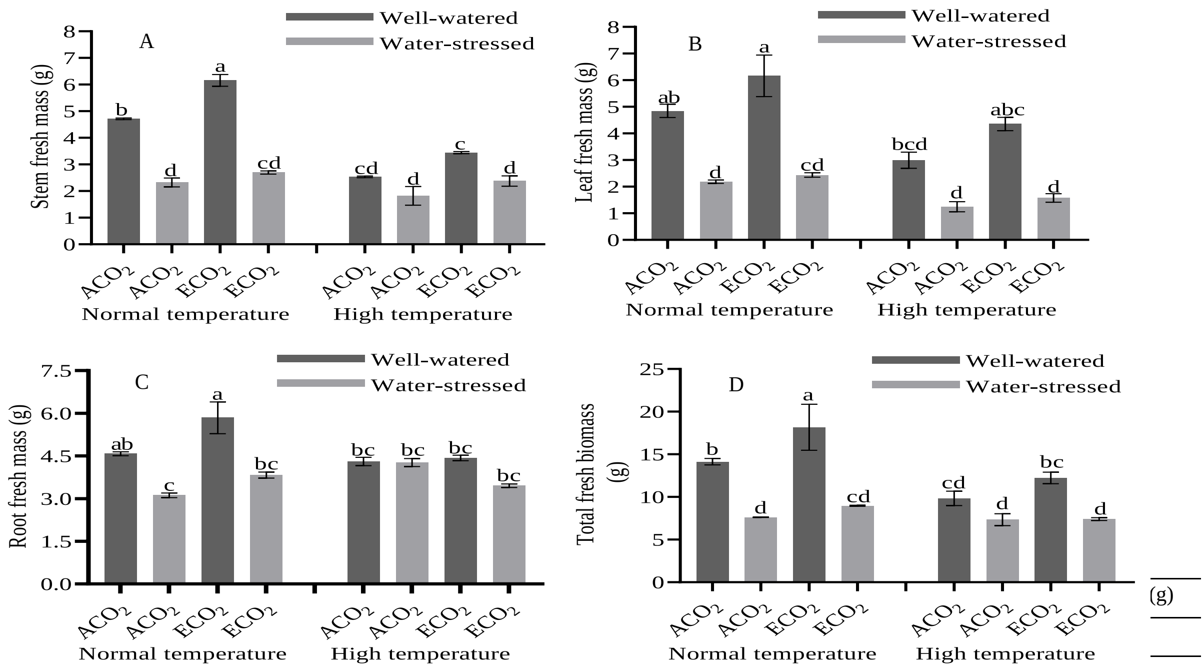

Figure 2. Stem fresh mass (A), leaf fresh mass (B), root fresh mass (C) and total fresh mass (D) for 26-day-old Giza faba bean (*Vicia faba* L.) seedlings grown in Plexiglas cabinets for 14 days under eight experimental conditions after 12 days of initial germination and growth under greenhouse conditions. Error bars denote standard error (n = 9). Means followed by different letters are significantly different at P < 0.05 according to Scheffé's test. *ACO₂* ambient CO_2, *ECO₂* elevated CO_2.

Table 4. Analysis of variance for the individual impacts of temperature, CO_2 and watering regime and their interactions on stem fresh mass, leaf fresh mass, root fresh mass and total fresh biomass of Giza faba bean (*Vicia faba* L.) seedlings.

Main plot error	2	-	-	-	-
Carbon dioxide (CO_2)	1	22.99**	34.85**	7.82*	28.87**
T x CO_2	1	0.26	0.04	33.48**	3.95
Subplot error	4	-	-	-	-
Watering regime (W)	1	254.98****	126.95****	43.71***	164.28****
T x W	1	72.37****	3.70	13.13**	21.80**
CO_2 x W	1	8.87*	4.68	4.80	7.76*
T x CO_2 x W	1	2.25	0.00	0.33	0.04
Split-subplot error	8	-	-	-	-

Note: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Giza faba bean seedlings were grown under normal (22/18°C) or high (28/24°C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions.

3.2.2. Dry mass accumulation

High temperature and water stresses decreased stem, leaf, root and total dry, while elevated CO_2 increased them (Table 2; Figure 3A,B,C,D). All measured dry biomass parameters were significantly affected by the three main factors (Table 4). Moreover, stem dry mass was significantly affected by the two–way interactions between T × W (Table 5). The T × W interaction showed that seedlings grown under high temperatures and water–stressed had significantly the highest stem dry mass, whereas those grown under normal temperatures and well-watered had significantly the lowest stem dry mass (Figure 3A). Leaf dry mass and total dry biomass were significantly affected by the two-way interactions between T × CO_2 and T × W (Table 5). The T × CO_2 interaction revealed that seedlings grown under normal temperatures at elevated CO_2 resulted in significantly the highest leaf dry mass and total dry biomass, but high temperatures at ambient CO_2 resulted in significantly the lowest leaf dry mass and total dry biomass in seedlings (Figure 3B,D). The T × W interaction indicated that seedlings grown under normal temperatures and well–watered had significantly the highest leaf dry mass and total dry biomass, whereas seedlings grown under high temperatures and water–stressed had significantly the lowest leaf dry mass and total dry biomass (Figure 4B,D). Root dry mass was significantly affected by the three–way interaction among T × CO_2 × W (Table 5). On the basis of the three-way interaction among the three main factors, seedlings grown under normal temperatures, elevated CO_2, and well-watered conditions had significantly the highest root dry mass, whereas those grown under high temperatures, ambient CO_2 and water-stressed conditions had significantly the lowest root dry mass (Figure 3C).

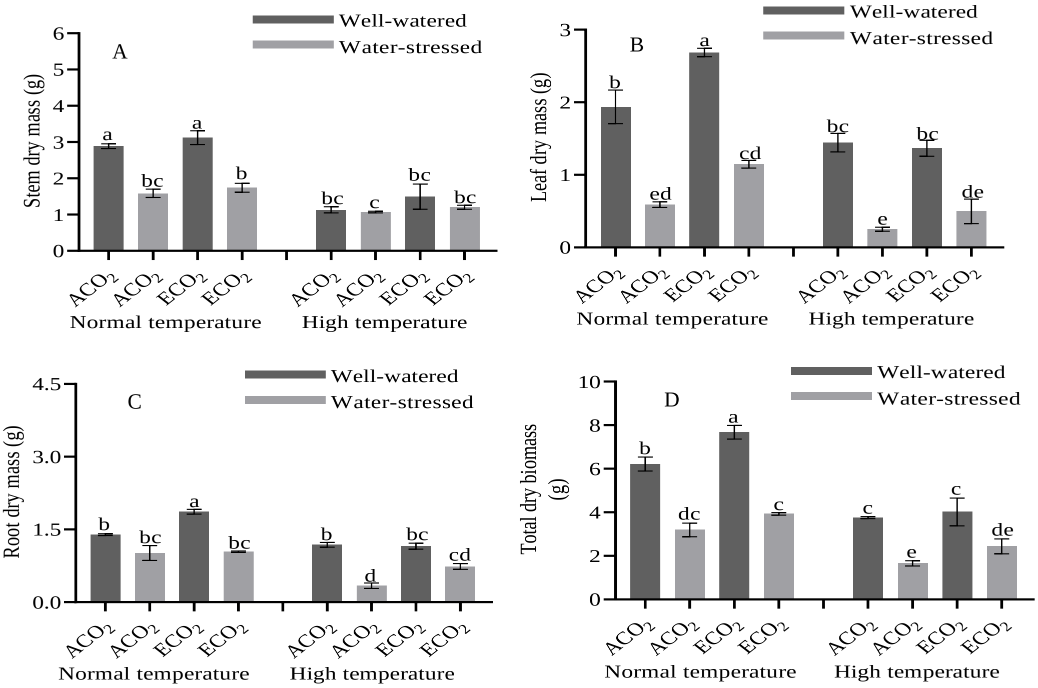

Figure 3. Stem dry mass (A), leaf dry mass (B), root dry mass (C) and total dry mass (D) for 26-day-old Giza faba bean (*Vicia faba* L.) seedlings grown in Plexiglas cabinets for 14 days under eight experimental conditions after 12 days of initial germination and growth under greenhouse conditions. Error bars denote standard error (n = 9). Means followed by different letters are significantly different at P < 0.05 according to Scheffé's test. *ACO₂* ambient CO_2, *ECO₂* elevated CO_2.

Table 5. Analysis of variance for the individual impacts of temperature, CO_2 and watering regime and their interactions on stem dry mass, leaf dry mass, root dry mass and total dry biomass of Giza faba bean (*Vicia faba* L.) seedlings.

		Stem dry mass (g)	Leaf dry mass (g)	Root dry mass (g)	Total dry biomass (g)
Source	Df				
Temperature (T)	1	2005.35***	286.21**	88.72*	4539.80***
Main plot error	2	-	-	-	-
Carbon dioxide (CO_2)	1	17.28*	62.71**	147.55***	85.81***
T x CO_2	1	0.26	37.15**	3.29	11.05*
Subplot error	4	-	-	-	-
Watering regime (W)	1	213.27****	196.97****	107.84****	229.58****
T x W	1	125.24****	5.50*	0.06	20.19**
CO_2 x W	1	2.24	0.14	0.01	0.09
T x CO_2 x W	1	0.49	2.15	13.45**	3.24
Split-subplot error	8	-	-	-	-

Note: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Giza faba bean seedlings were grown under normal (22/18^{o}C) or high (28/24^{o}C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions.

3.2.3. Proline, lipid peroxidation and electrical conductivity

Proline content was increased by high temperatures Giza faba bean seedlings (Table 2; Figure 4A). Significant effect of the two–way interaction between T × CO_2 was observed on proline content (Table 6). For the T × CO_2 interaction, seedlings grown under high temperatures at ambient CO_2 had significantly the highest proline content, while those grown under normal temperatures at ambient CO_2 had significantly the lowest proline content (Figure 6A).

Malondialdehyde (MDA) generation increased under water-stressed, but decreased by elevated CO_2 in Giza faba bean seedlings (Table 2; Figure 4B). MDA generation was significantly affected by carbon dioxide, watering regime, the two–way interactions between T × CO_2, CO_2 × W and the three-way interactions among T × CO_2 × W (Table 6). The T × CO_2 interaction revealed that seedlings grown under high temperatures at ambient CO_2 resulted in significantly the highest MDA content, but high temperatures at ambient CO_2 resulted in significantly the least MDA content in Giza faba bean seedlings (Figure 4B). The CO_2 × W interaction showed that seedlings grown at ambient CO_2 and water-stressed had significantly the highest MDA content, whereas seedlings grown at ambient CO_2 and well-watered had significantly the lowest MDA content (Figure 4B). On the basis of the T × CO_2 × W interactions, seedlings grown under high temperatures, ambient CO_2, and water–stressed conditions had significantly the highest MDA content, whereas those grown under high temperatures, ambient CO_2 and well-watered conditions had significantly the lowest MDA content (Figure 4B).

Electrical conductivity was increased by high temperatures and water stresses, but decreased by elevated CO_2 in Giza faba bean seedlings (Table 2; Figure 4C). Effects of temperature, CO_2, watering regime and the two–way interactions between CO_2 × W were significant on the electrical conductivity (Table 6). The CO_2 × W interaction showed that seedlings grown at ambient CO_2 and water–stressed conditions had significantly the highest electrical conductivity, whereas those grown at ambient CO_2 and well-watered conditions had significantly the lowest electrical conductivity (Figure 4C).

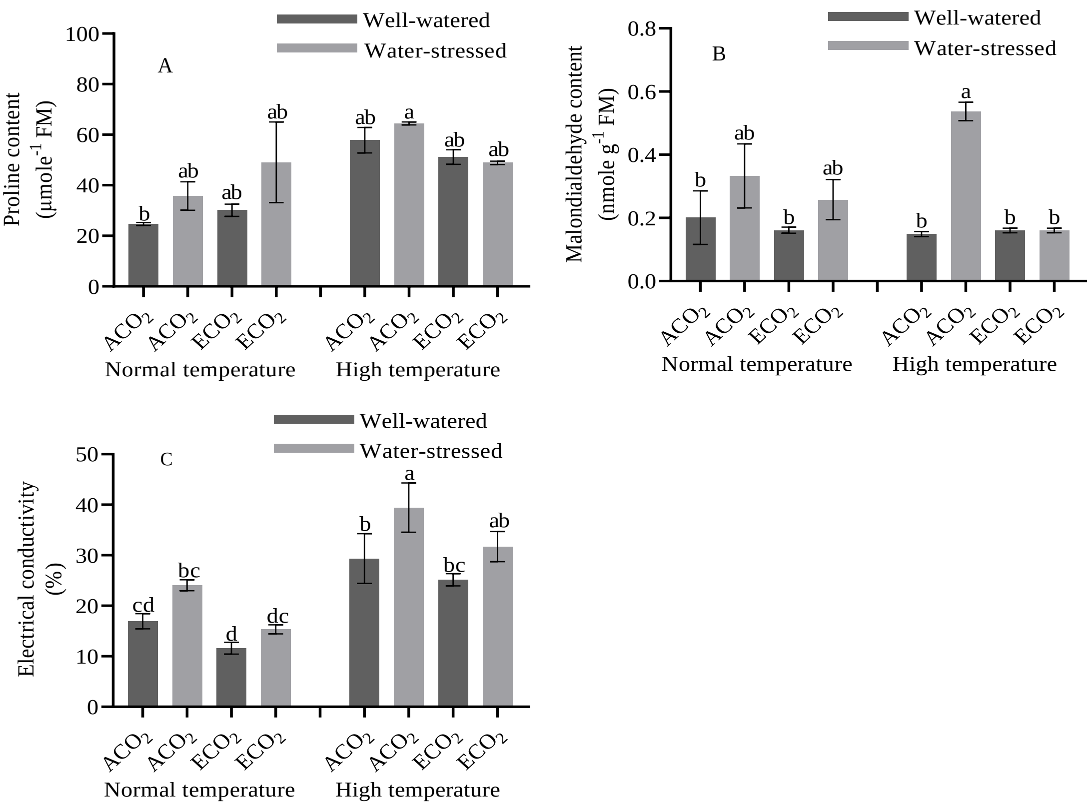

Figure 4. Proline content (A), Malondialdehyde content (B) and electrical conductivity (C) for 26-day-old Giza faba bean (*Vicia faba* L.) seedlings grown in Plexiglas cabinets for 14 days under eight experimental conditions after 12 days of initial germination and growth under greenhouse conditions. Error bars denote standard error (n = 9). Means followed by different letters are significantly different at $P < 0.05$ according to Scheffé's test. *ACO_2* ambient CO_2, *ECO_2* elevated CO_2.

Table 6. Analysis of variance for the individual impacts of temperature, CO_2 and watering regime and their interactions on proline content, MDA content and electrical conductivity of Giza faba bean (*Vicia faba* L.) seedlings.

		Proline content (µmole g $^{-1}$ FM)	MDA content (µmole g $^{-1}$ FM)	Electrical conductivity (%)
Source	Df			
Temperature (T)	1	11.27	0.10	99.86**
Main plot error	2	-	-	-
Carbon dioxide (CO_2)	1	0.08	46.14**	15.43*
T x CO_2	1	11.98*	12.52*	0.10
Subplot error	4	-	-	-
Watering regime (W)	1	4.69	23.89**	108.16****
T x W	1	2.64	1.61	4.74
CO_2 x W	1	0.00	11.27*	6.85*
T x CO_2 x W	1	1.13	7.83*	0.00
Split-subplot error	8	-	-	-

Note: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. Giza faba bean seedlings were grown under normal (22/18°C) or high (28/24°C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions.

3.2.4. Nitrogen balance index (NBI), chlorophyll, flavonoids and anthocyanin

NBI decreased under high temperatures and water-stressed condition in seedlings (Table 2; Figure 7A). NBI was significantly affected by watering regime and the two–way interactions between T × CO_2 and T × W (Table 7). The T × CO_2 interaction revealed that seedlings grown under normal temperatures at ambient CO_2 resulted in significantly the highest NBI, but normal temperatures at elevated CO_2 resulted in significantly the lowest NBI in Giza faba bean seedlings (Figure 7A). The T × W interaction indicated that seedlings grown under normal temperatures and well–watered had significantly the highest NBI, whereas seedlings grown under normal temperatures and water–stressed had significantly the lowest NBI (Figure 7A).

Chlorophyll content was significantly increased by high temperatures and water stresses (Table 2; Figure 7B). Chlorophyll content was only significantly affected by temperature and watering regime (Table 7).

Flavonoid content was significantly increased by the three main factors (Table 2; Figure 7C). Flavonoid content was significantly affected by the three main factors, the two–way interaction between CO_2 × W and the three–way interaction among T × CO_2 ×W (Table 7). The CO_2 × W interaction showed that flavonoid content was significantly highest for water-stressed seedlings at elevated CO_2, but significantly lowest for well-watered seedlings at ambient CO_2 (Figure 7C). On the basis of the T × CO_2 × W interaction, flavonoid content was significantly highest for seedlings grown under high temperatures, elevated CO_2 and water-stressed, but significantly lowest for seedlings grown under normal temperatures, ambient CO_2 and well-watered (Figure 7C).

Anthocyanin was significantly decreased by high temperatures and water stresses (Table 2; Figure 7D). Anthocyanin was significantly affected by watering regime and the two–way interaction between T × CO_2 (Table 7). The T × CO_2 interaction revealed that seedlings grown under normal temperatures at elevated CO_2 resulted in significantly the highest anthocyanin, but high temperatures at elevated CO_2 resulted in significantly the lowest anthocyanin in Giza faba bean seedlings (Figure 7A).

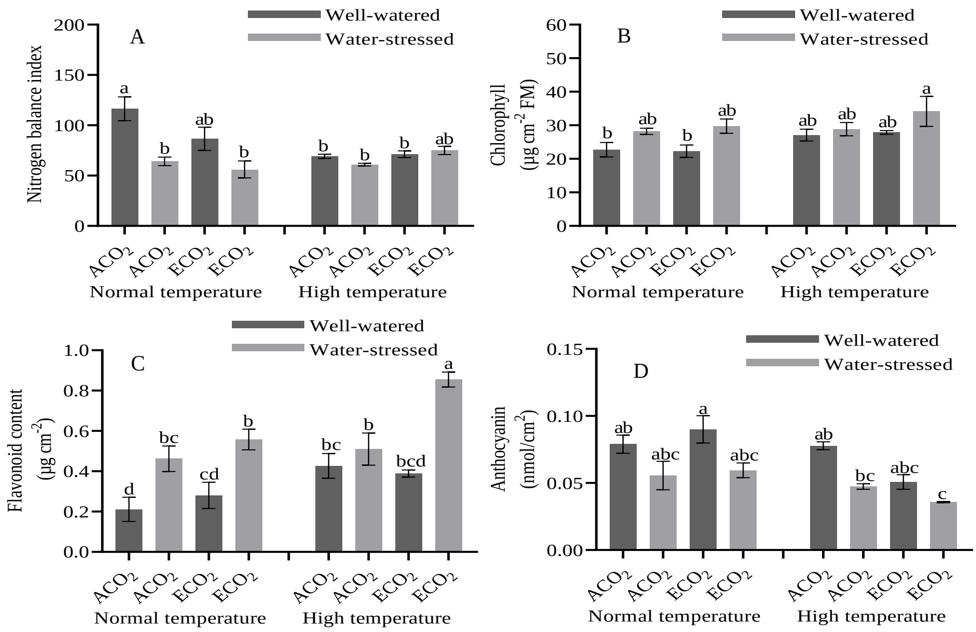

Figure 5. NBI (A), chlorophyll content (B) flavonoid content (C) and anthocyanin (D) measured by Dualex machine for 26-day-old Giza faba bean (*Vicia faba* L.) seedlings grown in Plexiglas cabinets for 14 days under eight experimental conditions after 12 days of initial germination and growth under greenhouse conditions. Error bars denote standard error (n = 9). Means followed by different letters are significantly different at P < 0.05 according to Scheffé's test. *ACO_2* ambient CO_2, *ECO_2* elevated CO_2.

Table 7. Analysis of variance for the individual impacts of temperature, CO_2 and watering regime and their interactions on NBI, chlorophyll, flavonoids and anthocyanin of Giza faba bean (*Vicia faba* L.) seedlings.

		NBI	Chlorophyll (µg cm^{-2} FM)	Flavonoids (µgcm^{-2} FM)	Anthocyanin(nmol/cm^2)
Source	df				
Temperature (T)	1	9.91	113.72**	45.07*	4.60
Main plot error	2	-	-	-	-
Carbon dioxide (CO_2)	1	1.54	3.81	24.71**	2.42
T x CO_2	1	9.64*	1.87	2.25	12.50*
Subplot error	4	-	-	-	-
Watering regime (W)	1	14.14**	43.91***	186.61****	63.33****
T x W	1	11.33**	2.40	0.07	0.47
CO_2 x W	1	2.13	4.17	26.82***	0.43
T x CO_2 x W	1	0.18	0.63	20.53**	3.29
Split-subplot error	8	-	-	-	-

Note: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. Giza faba bean seedlings were grown under normal (22/18°C) or high (28/24°C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions. NBI, chlorophyll content, flavonoids and anthocyanin were measured by Dualex machine.

3.2.5. Photosynthetic pigments

High temperatures significantly increased Chl *a*, carotenoids, total Chl and Chl *a/b* ratio (Table 2; Figure 8A,C,D,E). Chl *a* was significantly affected by watering regime, while Chl *b* and Chl *a/b* ratio were significantly affected by CO_2 (Table 8). However, none of the interactions among the three main factors had significant effect on the photosynthetic pigments (Table 8).

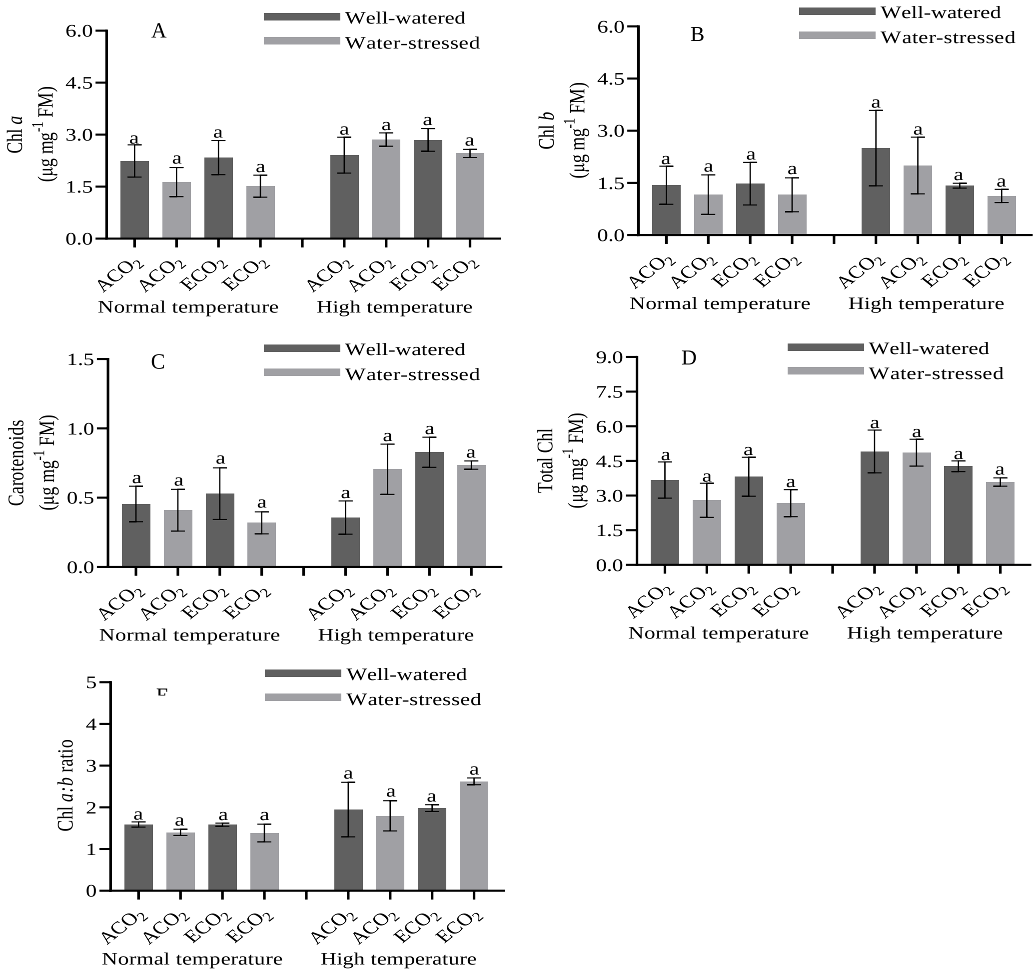

Figure 6. Chl *a* (A), Chl *b* (B), carotenoids (C), total Chl (D) and Chl *a:b* ratio (E) for 26-day-old Giza faba bean (*Vicia faba* L.) seedlings grown in Plexiglas cabinets for 14 days under eight experimental conditions after 12 days of initial germination and growth under greenhouse conditions. Error bars denote standard error (n = 9). Means followed by different letters are significantly different at P < 0.05 according to Scheffé's test. *ACO_2* ambient CO_2, *ECO_2* elevated CO_2.

Table 8. Analysis of variance for the individual impacts of temperature, CO_2 and watering regime and their interactions on Chl *a*, Chl *b*, carotenoids, total Chl and Chl *a/b* ratio of Giza faba bean (*Vicia faba* L.) seedlings.

		Chl *a* (µg mg^{-1} FM)	Chl *b*(µg mg^{-1} FM)	Carotenoids (µg mg^{-1} FM)	Total Chl (µg mg^{-1} FM)	Chl *a/b* ratio
Source	df					
Temperature (T)	1	3.53	2.10	3.03	2.86	15.69
Main plot error	2	-	-	-	-	-
Carbon dioxide (CO_2)	1	0.00	30.09**	5.70	2.90	12.63*
T x CO_2	1	0.01	32.45	6.52	3.01	13.78
Subplot error	4	-	-	-	-	-
Watering regime (W)	1	5.95*	1.53	0.00	2.91	0.01
T x W	1	7.01	0.03	2.00	0.63	0.71
CO_2 x W	1	3.50	0.02	2.82	0.31	0.56
T x CO_2 x W	1	1.19	0.05	0.58	0.05	0.60
Split-subplot error	8	-	-	-	-	-

Note: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. Giza faba bean seedlings were grown under normal (22/18^{o}C) or high (28/24^{o}C) temperatures, ambient CO_2 (400 ppm) or elevated CO_2 (800 ppm) and well-watered or water-stressed conditions. Plants were grown under experimental conditions in Plexiglas cabinets for 14 days, following an initial germination period of 7 days and growth period of 5 days under greenhouse conditions.

4. Discussion

There is still a gap in our understanding about the adaptability or plasticity of species regarding their growth behavior in response to the short-term combination effect of main ecological components of climate change. The purpose of this study was to examine the effects of the individual and combined impacts of temperature, CO_2, and watering regime on growth and biochemical responses of Giza faba bean (*Vicia faba* L.).

4.1. Effects of temperature

Seedlings grown under high temperatures exhibited lower stem height, stem diameter, leaf area, leaf number, leaf moisture than those grown under normal temperatures (Table 2; Figure 1A-F). This coincides with the results of previous research showing declines on growth parameters and leaf moisture content under high temperatures in *Arabidopsis thaliana* (Abo Gamar *et al.,* 2019, 2021). Fresh and dry biomass accumulation except root fresh mass were negatively affected by high temperatures (Table 2; Figures 2A-D, 3A-D), which are consistent with earlier findings on different plant species (Hamidou *et al.,* 2013; Sehgal *et al.,* 2017; Qaseem *et al.,* 2019) including faba bean (Qaderi and Reid, 2009). Seedlings accumulated more proline under high temperatures (Table 2; Figure 4A). This result is consistent with previous findings related to the effects of high temperature on *Prunus persica* (Shin *et al.,* 2016). Proline has a role in protecting and stabilizing different antioxidant enzymes and plasma membranes (Abo Gamar *et al.,* 2019). High temperatures increased electrolyte leakage in seedlings (Table 2; Figure 4C). Electrolyte leakage is an indication of an enhancement in membrane permeability and reduction in cell abilities to tolerate temperature (Cottee *et al.,* 2007). Chlorophyll (measured by Dualex) (Table 2; Figure 5B) and flavonoids (Table 2; Figure 5C) were increased in seedlings grown under high temperatures, while nitrogen balance index (NBI) (Table 2; Figure 5A), which measures the ratio between chlorophyll to flavonoids, decreased. This is an indication that nitrogen nutrition is an important factor in regulating growth of plants, as nitrogen is an important constituent in the structure of chlorophyll molecule (Taiz *et al.,* 2014). Plants under stress conditions produce different antioxidant compounds, including flavonoids, to protect them against ROS (Fini *et al.,* 2011; Naheed *et al.,* 2022; Akhter *et al.,* 2022). We found that high temperature caused a reduction in anthocyanin concentration (Table 2; Figure 5D), which is consistent with previous studies on Jaguar' rose (Dela *et al.,* 2003). Chl *a*, carotenoids, total Chl and Chl *a/b* ratio were all increased by high temperatures (Table 2, Figure 6A, C, D, E). In contrast to our results, chlorophyll content has been found to decrease in mulberry plants grown under high temperature (Chaitanya *et al.,* 2001). On the other hand, other reports reported that, total chlorophyll content was increased in plants grown under stress factors (Romero-Aranda *et al.,* 2001). Synthesizing more carotenoids under high temperatures might be an indicative of the increase in the antioxidant abilities of plants (Abo Gamar *et al.,* 2019).

4.2. Effects of CO_2

In our study, elevated CO_2 increased growth parameters (Table 2; Figure 1A, B, C, D, F), leaf moisture content (Table 2; Figure 1E), and fresh and dry biomass (Table 2; Figures 2A-D, 3A-D). Elevated CO_2 has been shown to increase growth and biomass accumulation in plants by increasing leaf photosynthetic rate and the efficiency of water usage by plants (Jones, 1992). The reduction in MDA content (Table 2; Figure 4B) and electrolyte leakage (Table 2; Figure 4C) in seedlings grown under elevated CO_2 is consistent with a previous study on *Arabidopsis thaliana* (Abo Gamar *et al.*, 2019). Elevated CO_2 increased flavonoid content (Table 2; Figure 5C), which indicated that elevated CO_2 helps plants to accumulate more antioxidant materials, such as flavonoids (Abo Gamar *et al.,* 2019). Furthermore, this explained the reduction in the MDA content and electrolyte leakage in faba bean seedlings grown at elevated CO_2.

4.3. Effects of watering regime

Our results showed that water stress decreased growth parameters (Table 2; Figure 1A, B, C, D, F), leaf moisture content (Table 2; Figure 1E) and fresh and dry biomass (Table 2; Figures 2A-D, 3A-D). These significant effects on growth parameters and biomass might be due to stomatal closure, which has been shown to reduce photosynthesis in water-stressed plants, including faba bean (Qaderi and Reid, 2009; Sangtarash *et al.,* 2009; Prasch and Sonnewald, 2015). Our results showed an increase in MDA content (Table 2; Figure 4B) and electrolyte leakage (Table 2; Figure 4C) in water-stressed seedlings. This agrees with past research, which showed that water stress increased lipid peroxidation, which caused damage in the plasma membrane and, in turn, increased electrolyte leakage and MDA content (Hajihashemi and Ehsanpour, 2013). Water stress decreased NBI (Table 2; Figure 5A), which is consistent with a previous study on pea (*Pisum sativum*) seedlings (Abdulmajeed *et al.,* 2018). Anthocyanin content was reduced in water-stressed plants (Table 2; Figure 5D). During water stress, the usage of antioxidant enzymes to anthocyanin as co-substrates molecules to capture for ROS resulting in decreased anthocyanin content (Maritim *et al.*, 2021).

4.4. Effects of the combination of the climate change main components.

In this study, from 38 cases, 33 of two-way (see Tables 3, 4, 5, 6 and 7) and 5 of three-way interactions (Tables 3, 5, 6 and 7) were significant. Plant parameters were significantly affected by T × CO_2 in 12 cases (see Tables 3, 4, 5 and 6), by T × W in 14 cases (see Tables 3, 4, 5 and 7), by CO_2 × W in 7 cases (see Tables 3, 4, 6 and 7), and by T × CO_2 × W in 5 cases (Tables 3, 5, 6 and 7).

The interaction among the three main factors (T × CO_2 × W) had significant effects on leaf number (Table 3; Figure 1D), moisture content (Table 3; Figure 1E), root dry mass (Table 5; Figure 3C), MDA (Table 6; Figure 4B) and flavonoids (Table 7; Figure 5C). Water-stressed plants grown under higher temperatures at elevated CO_2 had highest leaf number, moisture content and root dry mass, whereas well-watered plants grown under lower temperatures at elevated CO_2 had lowest leaf number (Table 3; Figure 1D), moisture content (Table 3; Figure 1E) and root dry mass (Table 5; Figure 3D). Seedlings grown under higher temperature, elevated CO_2, and water stress conditions had the highest flavonoid content (Table 7; Figure 5C). Moreover, higher temperature and water stress, individually and together, increased MDA content (Table 6; Figure 4B), but elevated CO_2 decreased it under stress conditions. These results indicated that the stress-reducing effect of elevated CO_2 was clearly observed for biomass, plant water status and stress indicator parameters, as shown by increasing some growth and biomass parameters, moisture content and flavonoids and decreasing MDA. Moreover, these results are consistent with previous study on *Arabidopsis thaliana* (Abo Gamar *et al.,* 2019). Thus, it is important to study the effects of the main ecological climate change factors (temperature,

CO_2 and watering regime) associated with climate change along with their combined effects on plants.

5. Conclusions

This study showed that higher temperature and water stress had negative effects, while elevated CO_2 had positive effects, on Giza faba bean seedlings. Elevated CO_2 decreased stresses on Giza faba bean seedlings by increasing their growth, water content and antioxidant activity. The capability shown in Giza faba bean to potentially survive under climate change main components may predict that this plant will resist such conditions in the future.

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Conflicts of Interest

Authors declare no conflict of interests.

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