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RESEARCH ARTICLE



## Individual and interactive effects of temperature, carbon dioxide and abscisic acid on mung bean (*Vigna radiata*) plants

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### ABSTRACT

We studied the effects of temperature, carbon dioxide and abscisic acid on mung bean (*Vigna radiata*). Plants were grown under 26/22°C or 32/28°C (16 h light/8 h dark) at 400 or 700  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  and received ABA application of 0 or 100  $\mu\text{l}$  (10  $\mu\text{g}$ ) every other day for three weeks, after eight days of initial growth, in growth chambers. We measured 24 parameters. As individual factors, in 16 cases temperature; in 8 cases  $\text{CO}_2$ ; in 9 cases ABA; and as interactive factors, in 4 cases, each of temperature  $\times$   $\text{CO}_2$ , and  $\text{CO}_2 \times$  ABA; and in 2 cases, temperature  $\times$  ABA were significant. Higher temperatures increased growth, aboveground biomass, growth indices, photochemical quenching (qP) and nitrogen balance index (NBI). Elevated  $\text{CO}_2$  increased growth and aboveground biomass. ABA decreased growth, belowground biomass, qP and flavonoids; increased shoot/root mass ratio, chlorophyll and NBI; and had little role in regulating temperature- $\text{CO}_2$  effects.

**Abbreviations:**  $A_N$ : net  $\text{CO}_2$  assimilation;  $E$ : transpiration;  $F_v/F_m$ : maximum quantum yield of PSII;  $g_s$ : stomatal conductance; LAR: leaf area ratio; LMA: leaf mass per area; LMR: leaf mass ratio;  $\phi\text{PSII}$ : effective quantum yield of PSII; qNP: non-photochemical quenching; qP: photochemical quenching; SRMR: shoot to root mass ratio; WUE: water use efficiency

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### KEYWORDS

Abscisic acid; carbon dioxide; mung bean; pulse crop; temperature; *Vigna radiata*

### Introduction

It is well known that greenhouse gases, such as carbon dioxide ( $\text{CO}_2$ ), methane and nitrous oxide, trap solar radiation in the atmosphere to naturally heat the Earth (Houghton 2015). However, anthropogenic activities have contributed to increased greenhouse gas emissions since the industrial revolution, and these activities are enhancing the greenhouse effect and leading to global warming. According to the report by the Intergovernmental Panel on Climate Change (IPCC), the concentration of global atmospheric  $\text{CO}_2$  has increased from 278  $\mu\text{mol mol}^{-1}$  in 1750 (Stocker et al. 2013) to 404.21  $\mu\text{mol mol}^{-1}$  in 2016 (Tans 2017), and may surpass 700  $\mu\text{mol mol}^{-1}$  by 2100 (Stocker et al. 2013). Also, the global average surface temperature has increased 0.85°C between 1880 and 2012, and may increase by up to 6.4°C by the end of this century (Stocker et al. 2013). Nine of the last 15 years have ranked among the warmest on record.  $\text{CO}_2$  concentration and temperature are progressing at alarming rates. The relationship between photosynthesis, crop growth and yield and the interactions between plant growth and abiotic factors, are posing incredible challenges for scientists around the world (Ainsworth et al. 2008).

Abiotic factors, such as temperature and  $\text{CO}_2$ , have individualistic and interactive effects on the growth and development of plants, and influence yield quantity and quality (Mooney et al. 1991; Bitá and Gerats 2013). The susceptibility of plants to higher temperatures can vary with developmental stage. Heat stress can affect plants during vegetative stages, causing inhibition of shoot and root growth, and during reproductive stage, causing pollen infertility and flower abscission

(Wahid et al. 2007; Bitá and Gerats 2013). The response of crops to increased temperature is dependent on the species-specific optimal temperatures. Physiological and biochemical responses of crops to elevated  $\text{CO}_2$  vary with their photosynthetic pathway (Qaderi and Reid 2009). Crop species with  $\text{C}_3$  pathway, such as mung bean (*Vigna radiata*), would benefit from elevated  $\text{CO}_2$ , as the carboxylation rate of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) increases, whereas its oxygenation rate decreases (Drake et al. 1997).

Previous studies have shown that increased temperatures decrease stem height, stem diameter, leaf area and overall biomass (Qaderi et al. 2006, 2010). In canola (*Brassica napus*), a temperature regime of 6°C above normal for this species decreased the rate of  $\text{CO}_2$  assimilation and water use efficiency (WUE) and increased transpiration, diminishing resource accumulation (Qaderi et al. 2010). Under higher temperatures, plants reallocate resources from shoots to roots, producing smaller leaves and a more extensive root system to offset increased water loss (Repková et al. 2009; Qaderi et al. 2010; Gliessman 2015), consequently reducing yield quantity and quality (Wahid et al. 2007). Irreversible damage to plant function or development can occur with heat stress, specifically in regard to gas exchange (Wahid et al. 2007). Heat stress-induced effects on photosynthesis have been extensively studied. It has been well documented that high temperatures can cause direct damages to the photosynthetic apparatus (especially on PSII) and multiple sites for heat-induced impairment within the chloroplast membranes have been identified (see Berry and Björkman 1980; Bukhov and Mohanty 1999; Sharkey and Schrader 2006; Allakhverdiev

et al. 2008; Mohanty et al. 2012). Heat stress induces the production of phenolic compounds, such as flavonoids, to trigger acclimation to heat stress, which can be seen in watermelons (Wahid et al. 2007). In pea (*Pisum sativum*), higher temperature has been shown to negatively affect nitrogen balance index (NBI) (Martel and Qaderi 2016).

Plants grown at elevated CO<sub>2</sub> had reduced stomatal conductance and transpiration, but increased WUE, photosynthesis and aboveground biomass (Ainsworth and Long 2005). Increased CO<sub>2</sub> concentration may offset biomass loss caused by increased temperature due to the complex relationship between photosynthesis and crop growth (Ainsworth et al. 2008).

As a plant stress hormone, abscisic acid (ABA) functions in response to several environmental stress factors, such as heat and drought. ABA rapidly accumulates in response to these abiotic stresses (Zeevaart 1999). Under stress conditions, ABA is synthesized at a higher rate than it is being degraded, and reduced growth of plants has been correlated with it (Marion-Poll and Leung 2006). Reduced growth may be a consequence of induced stomatal closure in times of water stress. Nitsch et al. (2012) discovered that tomato ABA-deficient mutants, in the absence of stress, had reduced growth, leaf surface area and fruit size, and drought-induced wilting. ABA can be regarded as a promoter or inhibitor of growth (Marion-Poll and Leung 2006). Ivanov et al. (1992) reported that exogenous ABA improved thermostability of the photosynthetic apparatus of the ABA-treated seedlings of barley (*Hordeum vulgare*) by decreasing heat damage of the chloroplast ultrastructure in this species. However, our previous study with canola revealed that higher temperatures reduced the increasing effects of water stress on ABA levels in plants (Qaderi et al. 2006).

Interaction between elevated CO<sub>2</sub> and higher temperatures has been explored in earlier studies, which indicated that elevated CO<sub>2</sub> can partially alleviate the detrimental effects of higher temperatures on plants (Morison and Lawlor 1999; Qaderi et al. 2006). However, the role of exogenous ABA in the interactive effects of temperature and CO<sub>2</sub> on growth, development and physiological characteristics of plants has not been studied. We therefore studied the effects of temperature and CO<sub>2</sub> on mung bean growth and development and examined the role of exogenous ABA in such interaction. Mung bean was used because of its potential to be grown in Canada in areas adapted for soybean (Olson et al. 2011; Goenther 2012) and its global market value, as it is the third most important pulse crop in the world. Mung bean is grown for human consumption (Nair et al. 2013) and crop rotation, as it improves the sustainability of cropping systems by increasing available nitrogen in the soil (De Costa et al. 1999; Anjum et al. 2015) via symbiotic fixation of atmospheric nitrogen (Gao et al. 2015).

We hypothesized that higher temperature adversely affects mung bean growth, elevated CO<sub>2</sub> mitigates this effect, and ABA reduces stress effects by mediating the interactive effects of temperature and CO<sub>2</sub>. Our objective was to investigate if exogenous ABA increases the mitigating effects of CO<sub>2</sub> on mung bean response to higher temperature stress by interacting with these two environmental factors. This study should have important implications for crop responses to the key components of global climate change.

## Materials and methods

### Plants and growth conditions

In this study, we used mung bean (*V. radiata* (L.) Wilczek; Halifax Seed, Halifax, NS). First, 50 seeds were placed in each of three 9-cm glass Petri dishes on one layer of blue filter paper (Anchor Paper Co., St Paul, MN), and moistened with 10 ml of distilled water. The seeds were germinated under 22/18°C (16 h light/8 h dark) in a growth chamber (model ATC26, Conviron, Controlled Environments, Winnipeg, MB). A mix of incandescent lamps (Litemor, Boston, MA) and cool white fluorescent tubes (Master TL-D-58W/840, Philips, Amsterdam) was used as light source. Light intensity (photosynthetic photon flux density) was measured with a quantum LI-250A radiometer/photometer (LI-COR Biosciences, Lincoln, NE) at the shoot apex, and it was 300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . A thermohygrometer (WD-35612-00, Oakton Instruments, Vernon Hills, IL) was used to measure relative humidity of the chamber. After five days of germination, the seedlings were transplanted into 1.32 liter pots (containing mixture of peat moss, Perlite and Vermiculite; 2:1:1 ratio by volume). Each pot was supplied with about 30 pellets of Nutricote® slow release fertilizer (NPK, 14-14-14, Chisso-Asahi Fertilizer Co., Tokyo). Plants were kept in this chamber for another three days, and then randomly assigned to eight treatments (eight plants per treatment) with the following combinations: two temperature regimes (lower, 26/22°C, 16 h light/8 h dark; higher, 32/28°C, 16 h light/8 h dark); two CO<sub>2</sub> concentrations (ambient, 400  $\mu\text{mol mol}^{-1}$ ; elevated, 700  $\mu\text{mol mol}^{-1}$ ); and two ABA applications (not applied, 0  $\mu\text{l}$  of ABA; applied, 100  $\mu\text{l}$  of ABA solution containing 10  $\mu\text{g}$  ABA in 90 ml of distilled water and 10 ml of 95% ethanol every other day). Higher temperatures and elevated CO<sub>2</sub> were selected to follow IPCC predictions (Stocker et al. 2013), and ABA application to evaluate its role in plant stress response (Sah et al. 2016) to combination of temperature and CO<sub>2</sub>. Experiments were conducted in 2 Conviron growth chambers, with either lower or higher temperature regime for 21 days. Each chamber contained two equal-sized (50 cm height  $\times$  65 cm width  $\times$  60 cm depth) Plexiglas cabinets (GE Polymershapes, Dartmouth, NS). In each chamber, one cabinet was supplied with ambient CO<sub>2</sub> and the other with elevated CO<sub>2</sub>, which were kept constantly circulated inside the cabinets by electrical fans, and regularly monitored, using a pSense portable CO<sub>2</sub> meter (CO<sub>2</sub> Meter, Inc., Ormond Beach, FL). A pressure gauge, a solenoid valve and a flow meter were used to regulate CO<sub>2</sub> flow. In the cabinets, growth conditions (photoperiod, light intensity, relative humidity) were fairly similar to that of the initial conditions. Experiments had three trials, which were conducted under different combinations of chamber and cabinet to reduce effects of experimental enclosures.

### Measurement of plant growth

Growth rate was calculated from the plant height data obtained on four 7-day intervals. After 21 days, from each treatment, 3 plants with average height were harvested to measure stem height, diameter and mass; leaf area and mass; and root mass per plant. Before harvest, stem diameter was measured with a Digimatic caliper (Mitutoyo Corp, Kanagawa). Then, each plant sample was cut and properly

placed on one sheet of paper within a folder and dried in a forced-air Fisher Isotemp Premium oven (model 750F, Fisher, Nepean, ON) at 60°C for 72 h. Mass of each plant part was measured with an analytical balance (model ED224s, Sartorius, Goettingen). Leaf area was measured with an area meter (Delta-T Devices, Cambridge). Plant dry mass and leaf area were used to calculate growth indices: leaf mass per area (LMA ( $\text{g m}^{-2}$ ) = leaf dry mass(DM)/leaf area), leaf mass ratio (LMR = leaf DM/plant DM), leaf area ratio (LAR ( $\text{cm}^2 \text{g}^{-1}$ ) = leaf area/plant DM) and shoot/root mass ratio (SRMR = shoot DM/root DM; Slauenwhite and Qaderi 2013).

### Measurement of gas exchange

From each treatment, three fully expanded leaves were used to measure gas exchange parameters ( $A_N$ , net  $\text{CO}_2$  assimilation;  $E$ , transpiration; and  $g_s$ , stomatal conductance), using a LI-6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE). Before taking measurements, the photosynthesis system was calibrated with a known  $\text{CO}_2$  concentration of either 400 or 700  $\mu\text{mol mol}^{-1}$ . By dividing  $A_N$  by  $E$ , WUE was calculated. All parameters were measured under relevant temperature and light conditions, and reported as  $A_N$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ;  $E$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ;  $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ; and WUE,  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$  (Lambers et al. 2008).

### Measurement of chlorophyll fluorescence

A Fluorpen FP 100 portable fluorometer (Photon Systems Instruments, Drásov) was used to measure leaf chlorophyll fluorescence on 21-day-old plants grown under experimental conditions. Measurements were taken inside the growth chamber on three fully grown leaves from each treatment, using respective temperature and light intensity. First, the  $\phi\text{PSII}$  ( $F_q'/F_m'$ ) was determined for light-adapted leaves, which were then dark-adapted for 30 min, using the fluorometer clamp. Measurement of dark-adapted leaves included maximum quantum yield of PSII ( $F_v/F_m$ ), non-photochemical quenching (qNP;  $(F_m - F_m') - 1$ ) and photochemical quenching (qP;  $F_q'/F_v'$ ; Baker 2008). The saturating light pulse was delivered at 2100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for 1 s.

### Measurement of NBI, chlorophyll and flavonoids

From each treatment, at least three fully expanded leaves were used to determine NBI, chlorophyll content and flavonoids with the Dualex Scientific® (Dualex Scientific, Force-A,

Orsay Cedex, France). NBI was measured as the ratio of chlorophyll and flavonoid (Cerovic et al. 2012; Martel and Qaderi 2016).

### Data analysis

First, data were analyzed by means of a three-way analysis of variance (ANOVA) to determine the effects of main factors and their interactions on growth and physiological parameters of mung bean plants. Then, data were subjected to a one-way ANOVA to show differences between treatments, using Scheffé's multiple-comparison procedure at the 5% confidence level (SAS Institute 2011).

## Results

### Plant growth

Temperature,  $\text{CO}_2$ , ABA, and the two-way interaction of  $C$  ( $\text{CO}_2$ )  $\times$   $A$  (ABA) affected stem height (Table 1), which was significantly increased by higher temperatures, elevated  $\text{CO}_2$  or the absence of exogenous ABA (Table 4). Under higher temperatures, plants grown at elevated  $\text{CO}_2$  were taller than those grown at ambient  $\text{CO}_2$ . Within  $\text{CO}_2$  concentrations, plants grown under higher temperatures were taller than those grown under lower temperatures (Figure 1(a)). On the basis of  $C \times A$  interaction, the tallest plants were those grown at elevated  $\text{CO}_2$  and received no ABA treatment, whereas the shortest plants were those grown at ambient  $\text{CO}_2$  and received ABA treatment.

Similarly, temperature,  $\text{CO}_2$ , ABA, and the two-way interaction of  $C \times A$  affected growth rate (Table 1), which was significantly increased by higher temperatures, elevated  $\text{CO}_2$  or absence of exogenous ABA (Table 4). Under both temperature regimes, plants grown at elevated  $\text{CO}_2$  had faster growth than those grown at ambient  $\text{CO}_2$ . Within  $\text{CO}_2$  concentrations, plants grown under higher temperatures had faster growth than those grown under lower temperatures (Figure 1(b)). The  $C \times A$  interaction revealed that the non-ABA-treated plants grown at elevated  $\text{CO}_2$  had faster growth, whereas the ABA-treated plants grown at ambient  $\text{CO}_2$  had slowest growth.

$\text{CO}_2$  affected stem diameter (Table 1). Elevated  $\text{CO}_2$  significantly increased stem diameter (Table 4), which was not different among treatments (Figure 1(c)).

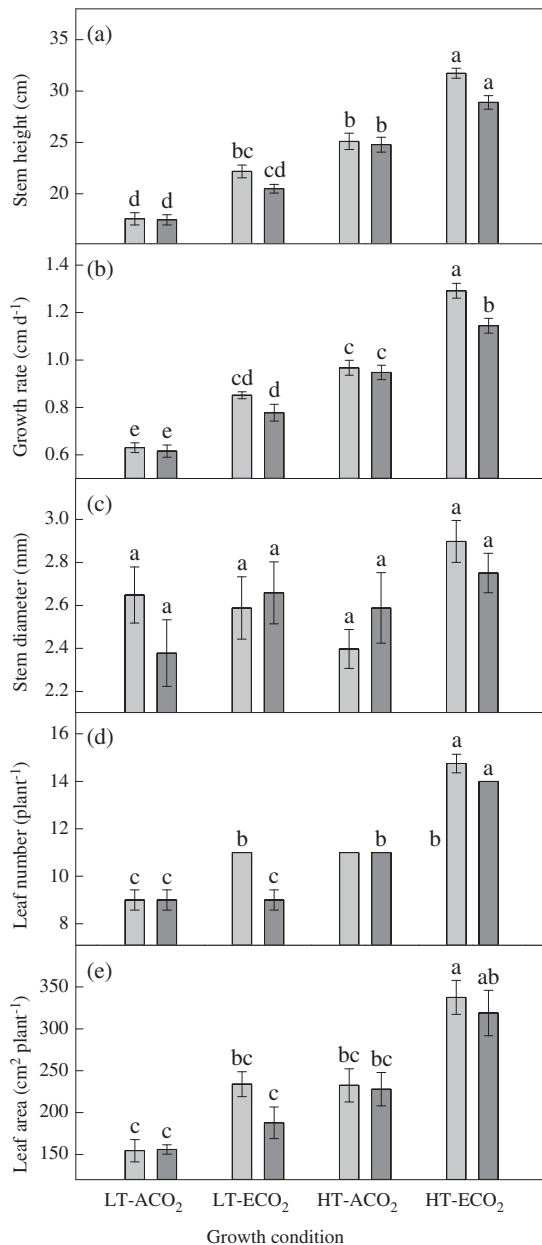
Temperature,  $\text{CO}_2$ , ABA, and the two-way interactions of  $T \times C$  and  $C \times A$  affected leaf number (Table 1), which was significantly increased by higher temperatures, elevated  $\text{CO}_2$  or absence of exogenous ABA (Table 4). Under higher temperatures, plants grown at elevated  $\text{CO}_2$  had more leaves

**Table 1.** ANOVA ( $F$  value) for effects of temperature,  $\text{CO}_2$ , ABA and their interactions on plant growth and dry mass of one-month-old mung bean (*V. radiata*) plants.

Treatment	Plant growth							Dry mass				
	d.f.	Stem height	Growth rate	d.f.	Stem diameter	Leaf number	Leaf area	d.f.	Leaf	Stem	Root	Total
Temperature ( $T$ )	1	353.8****	387.4****	1	1.0	232.6****	54.5****	1	34.1****	74.3****	10.3**	6.1*
$\text{CO}_2$ ( $C$ )	1	111.1****	145.2****	1	5.7*	109.6****	34.7****	1	46.8****	22.1****	2.2	13.0****
ABA ( $A$ )	1	8.1**	11.6***	1	0.2	10.8**	1.7	1	1.0	0.1	8.2**	2.9
$T \times C$	1	3.2	3.4	1	1.4	32.3****	2.6	1	7.5**	21.2****	0.6	0.6
$T \times A$	1	0.6	1.1	1	0.4	2.2	0.2	1	0.7	2.9	6.9*	3.0
$C \times A$	1	5.6*	6.3*	1	0.0	10.8**	1.4	1	0.1	4.4*	0.6	2.9
$T \times C \times A$	1	0.3	0.8	1	3.4	2.2	0.4	1	0.1	0.3	0.0	0.2
Error	312	–	–	88	–	–	–	88	–	–	–	–

Notes: Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient (400  $\mu\text{mol mol}^{-1}$ ) or elevated (700  $\mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  concentration and treated with 0 or 100  $\mu\text{l}$  (10  $\mu\text{g}$ ) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient  $\text{CO}_2$ . Significant values: \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ ; \*\*\*\* $P < .0001$ .

than those grown at ambient CO<sub>2</sub>, whereas under lower temperatures, it was true only for the non-ABA-treated plants. Within each CO<sub>2</sub> concentration, plants grown under higher temperatures produced more leaves than those grown under lower temperatures (Figure 1(d)). On the basis of  $T \times C$  interaction, plants grown under higher temperatures at elevated CO<sub>2</sub> had most leaves, whereas plants grown under lower temperatures at ambient CO<sub>2</sub> had fewest leaves. The  $C \times A$  interaction showed that the non-ABA-treated plants grown at elevated CO<sub>2</sub> had most leaves, whereas the ABA-treated plants at ambient CO<sub>2</sub> had fewest leaves.

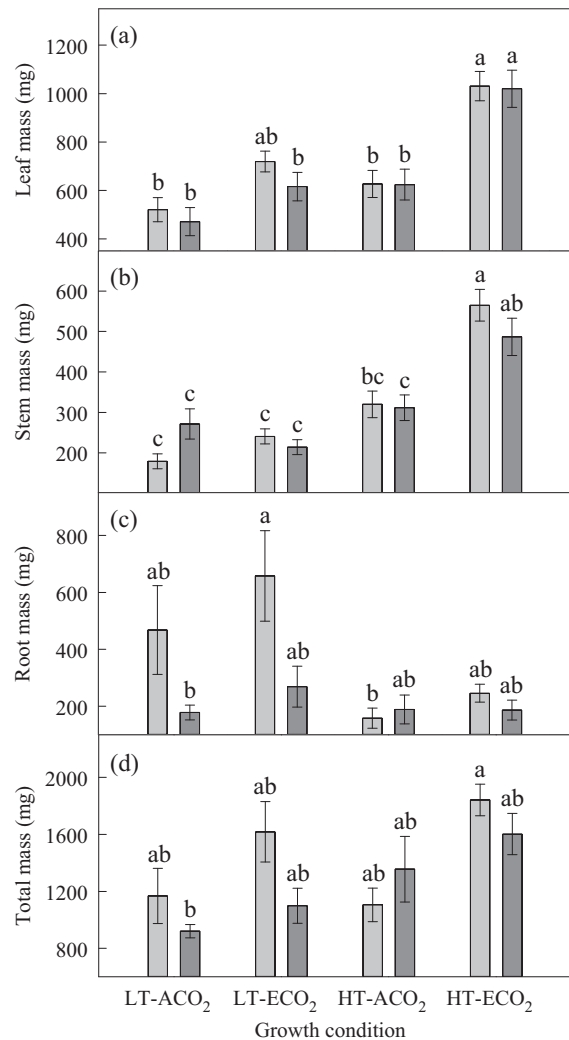


**Figure 1.** Growth of one-month-old mung bean (*V. radiata*) plants. Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient (400  $\mu\text{mol mol}^{-1}$ ) or elevated (700  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> concentration and treated with 0 or 100  $\mu\text{l}$  (10  $\mu\text{g}$ ) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient CO<sub>2</sub>. Light gray bars represent the non-ABA-treated plants and dark-gray bars, the ABA-treated plants. (a) stem height; (b) growth rate; (c) stem diameter; (d) leaf number and (e) leaf area. Data are means  $\pm$  SE of 30 (for stem height and growth rate) and 12 (for stem diameter, leaf number and leaf area) samples from 3 trials. Bars surmounted by different letters within each panel are significantly different ( $P < .05$ ) according to Scheffé's multiple-comparison procedure.

Temperature and CO<sub>2</sub>, but not exogenous ABA, affected leaf area (Table 1), which was significantly increased by higher temperatures or elevated CO<sub>2</sub> (Table 4). Under higher temperature regime, the non-ABA-treated plants grown at elevated CO<sub>2</sub> had larger leaves than the non-ABA-treated plants grown at ambient CO<sub>2</sub>. Within elevated CO<sub>2</sub>, plants with similar ABA application grown under higher temperatures had larger leaves than those grown under lower temperatures (Figure 1(e)).

### Dry mass

Temperature, CO<sub>2</sub> and the two-way interaction of these factors affected leaf mass (Table 1), which was significantly increased by higher temperatures and elevated CO<sub>2</sub> (Table 4). Regardless of ABA treatment, under higher temperatures, plants grown at elevated CO<sub>2</sub> had greater leaf mass than those grown at ambient CO<sub>2</sub> (Figure 2(a)). The  $T \times C$  interaction revealed that plants grown under higher temperatures at



**Figure 2.** Dry mass of one-month-old mung bean (*V. radiata*) plants. Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient (400  $\mu\text{mol mol}^{-1}$ ) or elevated (700  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> concentration and treated with 0 or 100  $\mu\text{l}$  (10  $\mu\text{g}$ ) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient CO<sub>2</sub>. Light gray bars represent the non-ABA-treated plants and dark-gray bars, the ABA-treated plants. (a) leaf mass; (b) stem mass; (c) root mass and (d) total mass. Data are means  $\pm$  SE of 12 samples from 3 trials. Bars surmounted by different letters within each panel are significantly different ( $P < .05$ ) according to Scheffé's multiple-comparison procedure.

elevated CO<sub>2</sub> had greatest leaf mass, whereas plants grown under lower temperatures at ambient CO<sub>2</sub> had least leaf mass.

Temperature, CO<sub>2</sub> and the two-way interactions of  $T \times C$  and  $C \times A$  affected stem mass (Table 1), which was significantly increased by higher temperatures and elevated CO<sub>2</sub> (Table 4). Within ABA application, under higher temperatures, plants grown at elevated CO<sub>2</sub> had higher stem mass than those grown at ambient CO<sub>2</sub> (Figure 2(b)). On the basis of  $T \times C$  interaction, plants grown under higher temperatures at elevated CO<sub>2</sub> had greatest stem mass, whereas plants grown under lower temperatures at ambient CO<sub>2</sub> had least stem mass. The  $C \times A$  interaction revealed that the non-ABA-treated plants grown at elevated CO<sub>2</sub> had highest stem mass, whereas the non-ABA-treated plants grown at ambient CO<sub>2</sub> had lowest stem mass.

Temperature, ABA and the two-way interaction of these factors affected root mass (Table 1), which was significantly reduced by higher temperatures or ABA application (Table 4; Figure 2(c)). The  $T \times A$  interaction showed that the non-ABA-treated plants grown under lower temperatures had greatest root mass, whereas the ABA-treated plants grown under higher temperatures had the least root mass.

Temperature and CO<sub>2</sub> affected total mass (Table 1), which was significantly increased by higher temperatures and elevated CO<sub>2</sub> (Table 4; Figure 2(d)).

### Growth index

Temperature affected LMA and LMR (Table 2). Higher temperatures significantly decreased LMA, but increased LMR (Table 4). Both LMA and LMR did not show significant differences among treatments (Figure 3(a,b)).

Temperature and the  $T \times A$  interaction affected LAR (Table 2), which was significantly increased by higher temperatures (Table 4). At ambient CO<sub>2</sub>, within the non-ABA treatment, plants grown under higher temperatures had higher LAR than those grown under lower temperatures (Figure 3(c)). The  $T \times A$  interaction showed that LAR was highest for the non-ABA-treated plants grown under higher temperatures, whereas it was lowest for the non-ABA-treated plants grown under lower temperatures.

Temperature and ABA application affected SRMR (Table 2), which was significantly increased by higher temperatures and exogenous ABA (Table 4). At elevated CO<sub>2</sub>, within non-ABA treatment, plants grown under higher temperatures had higher SRMR than those grown under lower temperatures (Figure 3(d)).

### Gas exchange

Except  $E$ , other photosynthetic parameters were not significantly affected by the main factors or their interactions (Tables 2 and 4). Transpiration was significantly affected by the interaction of  $T \times C$  (Table 2). This interaction revealed that plants grown under higher temperatures at elevated CO<sub>2</sub> had highest  $E$ , whereas plants grown under higher temperatures at ambient CO<sub>2</sub> had lowest  $E$ . None of the gas exchange parameters revealed significant differences among treatments (data not shown).

### Chlorophyll fluorescence

None of the factors or their interactions affected  $\phi$ PSII and maximum quantum yield of PSII ( $F_v/F_m$ ; Tables 2 and 4).

Temperature affected qNP of dark-adapted leaves (Table 2), which was significantly decreased by higher temperatures (Table 4).

Temperature and ABA application affected qP of dark-adapted leaves (Table 2), which was significantly increased by higher temperatures, but decreased by exogenous ABA (Table 4).

None of the chlorophyll fluorescence parameters showed significant differences among treatments (data not shown).

### Chlorophyll content, flavonoids and NBI

Temperature and ABA application affected flavonoids and NBI, whereas only ABA influenced chlorophyll content (Table 3), which was higher in the leaves of ABA-treated plants than in the leaves of non-treated plants (Table 4; Figure 4). Higher temperature and exogenous ABA decreased flavonoids and, in turn, increased NBI (Table 4; Figure 4).

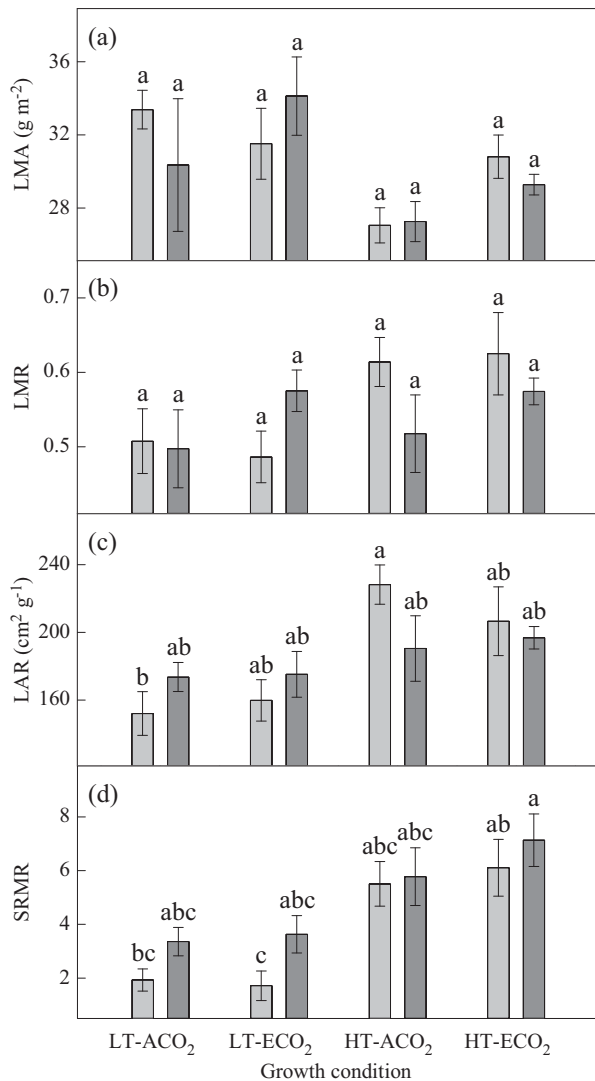
### Discussion

In this study, we examined the individual and interactive effects of temperature, CO<sub>2</sub> and exogenous ABA on the growth and physiology of mung bean (*V. radiata*) plants. In total, we determined the effects on 24 parameters (Tables 1–4). Although the combined effects of temperature and CO<sub>2</sub> on plants have been extensively studied, the role of exogenous ABA in such interaction has received little attention. In this study, temperature in 16 cases, CO<sub>2</sub> in 8 cases, ABA in 9 cases, the 2-way interactions of  $T \times C$  and  $C \times A$  each in 4 cases and of  $T \times A$  in 2 cases were significant. However, the three-way interactions were not significant for any of the measured parameters (Tables 1–3).

**Table 2.** ANOVA ( $F$  value) for effects of temperature, CO<sub>2</sub>, ABA and their interactions on growth index, gas exchange and chlorophyll fluorescence of one-month-old mung bean (*V. radiata*) plants.

Treatment	Growth index					Gas exchange				Chlorophyll fluorescence					
	d.f.	LMA	LMR	LAR	SRMR	d.f.	$A_N$	$E$	$g_s$	WUE	d.f.	$\phi$ PSII	$F_v/F_m$	qNP	qP
Temperature ( $T$ )	1	8.5**	5.1*	16.9****	37.4****	1	0.2	0.1	0.1	1.8	1	2.7	3.4	6.3*	11.0**
CO <sub>2</sub> ( $C$ )	1	2.2	1.1	0.0	0.8	1	1.7	1.3	0.2	0.2	1	2.0	0.0	0.6	0.3
ABA ( $A$ )	1	0.1	0.3	0.1	4.2*	1	1.2	0.1	0.0	0.5	1	2.7	0.6	0.1	6.0*
$T \times C$	1	0.6	0.0	0.4	0.7	1	0.0	4.4*	1.3	2.7	1	0.2	0.1	0.5	0.3
$T \times A$	1	0.0	3.7	4.6*	0.8	1	0.7	1.2	0.4	0.2	1	0.0	0.4	0.0	0.8
$C \times A$	1	0.6	1.5	0.3	0.3	1	0.1	0.0	0.1	0.6	1	1.7	0.0	0.0	0.1
$T \times C \times A$	1	2.1	0.2	0.8	0.0	1	0.4	0.0	0.1	2.3	1	0.5	0.5	1.4	0.1
Error	88	–	–	–	–	64	–	–	–	–	64	–	–	–	–

Notes: Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient (400  $\mu\text{mol mol}^{-1}$ ) or elevated (700  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> concentration and treated with 0 or 100  $\mu\text{l}$  (10  $\mu\text{g}$ ) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient CO<sub>2</sub>. Significant values: \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ ; \*\*\*\* $P < .0001$ .



**Figure 3.** Growth indices of one-month-old mung bean (*V. radiata*) plants. Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient ( $400 \mu\text{mol mol}^{-1}$ ) or elevated ( $700 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  concentration and treated with 0 or  $100 \mu\text{l}$  ( $10 \mu\text{g}$ ) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient  $\text{CO}_2$ . Light gray bars represent the non-ABA-treated plants and dark-gray bars, the ABA-treated plants. (a) LMA; (b) LMR; (c) LAR and (d) SRMR. Data are means  $\pm$  SE of 12 samples from 3 trials. Bars surmounted by different letters within each panel are significantly different ( $P < .05$ ) according to Scheffé's multiple-comparison procedure.

### Effects of temperature

Mung bean plants under higher temperatures grew faster and were significantly taller, had more larger leaves, and consequently greater leaf, stem and total mass, but less root mass than plants under lower temperatures. Decreased belowground and increased aboveground biomass, under higher temperature regime, increased LMR, LAR and SRMR (Table 4; Figures 1–3). This shift in resource allocation was expected, as growth temperatures usually affect SRMR (Osmond et al. 1980; Qaderi and Reid 2009). Due to decreased photosynthesis and stomatal conductance under higher temperatures, typically plants produce small leaves (Wahid et al. 2007; Repková et al. 2009). However, in the current study, plants under higher temperatures had more large leaves that utilized more  $\text{CO}_2$  and led to increased growth rate and biomass, although relatively lower light intensity, somewhat smaller pots, and limited

**Table 3.** ANOVA ( $F$  value) for effects of temperature,  $\text{CO}_2$ , ABA and their interactions on chlorophyll content, flavonoids and NBI of one-month-old mung bean (*V. radiata*) plants.

Treatment	d.f.	Chlorophyll	Flavonoids	NBI
Temperature ( $T$ )	1	2.8	19.0****	18.8****
$\text{CO}_2$ ( $C$ )	1	0.2	3.1	2.7
ABA ( $A$ )	1	4.1*	5.2*	7.4**
$T \times C$	1	0.0	0.1	0.1
$T \times A$	1	1.0	0.7	1.5
$C \times A$	1	0.7	0.7	1.0
$T \times C \times A$	1	1.4	0.0	0.4
Error	64	–	–	–

Notes: Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient ( $400 \mu\text{mol mol}^{-1}$ ) or elevated ( $700 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  concentration and treated with 0 or  $100 \mu\text{l}$  ( $10 \mu\text{g}$ ) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient  $\text{CO}_2$ . Significant values: \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ ; \*\*\*\* $P < .0001$ .

growing space within the cabinets might have influenced plant responses.

In this study, there were no significant differences in photosynthetic parameters between temperature regimes (Tables 2 and 4). Although higher temperatures generally decrease  $A_N$  and increase  $E$  and  $g_s$  (Lambers et al. 2008; Qaderi and Reid 2009), this was not the case in this study. The effects of leaf-to-air vapor pressure deficit (Lambers et al. 2008) within the cabinets cannot be ruled out here.

Effective and maximum quantum yield of PSII were not affected by temperature. However, plants under higher temperatures had higher  $q_P$  and lower  $q_{NP}$  than plants under lower temperatures (Tables 2 and 4). It has been shown that plants use  $q_{NP}$  to avoid photo-oxidation (Jahns and Holzwarth 2012; Lambrev et al. 2012). A decrease in  $q_{NP}$  increases damage to PSII of plants grown under higher temperatures (Jahns and Holzwarth 2012). However, in our study, increased  $q_P$  in plants grown under higher temperatures reflects their ability to absorb excess energy and utilize it for metabolism and growth. As shown, there is an increase in the degradation of photosynthetic apparatus under higher temperature (Nobel 2009), but in this study, it seems that mung bean, with a wide range of growth temperature (Malaviarachchi et al. 2016), could have properly coped with it.

In our study, the chlorophyll content was relatively higher, although not significant, in plants grown under higher temperatures than in plants grown under lower temperatures (Table 4). Increased plant biomass under higher temperatures might have been related to greater leaf number and size with more chlorophyll and, in turn, higher photosynthetic activities on whole-plant basis, as growth analysis is an excellent method of estimating net  $\text{CO}_2$  assimilation (Jones 2014). However, plants under higher temperatures produced significantly lower flavonoids than those under lower temperatures (Table 4; Figure 4(b)). It is possible that biosynthesis of flavonoids in plants was partially inhibited by somewhat supra-optimal temperatures, likely outside the optimal range of plants (Jaakola and Hohtola 2010). On the other hand, plants under higher temperatures had significantly higher NBI than plants under lower temperatures (Table 4; Figure 4(c)). NBI indicates changes in the allocation of carbon/nitrogen in the leaves due to N-deficiency rather than leaf N content (Cerovic et al. 2012). In this case, decreased flavonoids and slightly increased chlorophyll content resulted in increased NBI (chlorophyll/flavonoids ratio) in plants grown under higher temperatures.

**Table 4.** Effects of temperature, CO<sub>2</sub> and ABA on plant growth, dry mass, growth index, gas exchange, chlorophyll fluorescence, chlorophyll content, NBI and flavonoids of mung bean (*V. radiata*) plants.

Plant properties	Parameters	Temperature		CO <sub>2</sub>		ABA	
		Lower	Higher	Ambient	Elevated	Not applied	Applied
Growth	Stem height (cm)	19.4 ± 0.31b	27.6 ± 0.41a	21.2 ± 0.44b	25.8 ± 0.46a	24.1 ± 0.52a	22.9 ± 0.45b
	Growth rate (cm d <sup>-1</sup> )	0.72 ± 0.01b	1.09 ± 0.02a	0.79 ± 0.02b	1.02 ± 0.02a	0.94 ± 0.02a	0.87 ± 0.02b
	Stem diameter (mm)	2.6 ± 0.07a	2.7 ± 0.06a	2.5 ± 0.07b	2.7 ± 0.06a	2.6 ± 0.06a	2.6 ± 0.07a
	Leaf number (plant <sup>-1</sup> )	9.5 ± 0.22b	12.7 ± 0.27a	10.0 ± 0.20b	12.2 ± 0.36a	11.4 ± 0.33a	10.8 ± 0.33b
	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	183.1 ± 8.2b	279.3 ± 12.9a	192.8 ± 9.4b	269.6 ± 13.4a	239.7 ± 12.7a	222.7 ± 13.0a
Dry mass	Leaf (mg)	581.7 ± 29.0b	825.5 ± 42.8a	560.8 ± 29.3b	846.5 ± 39.8a	724.5 ± 37.7a	682.8 ± 43.2a
	Stem (mg)	226.4 ± 12.9b	420.8 ± 24.2a	270.5 ± 17.0b	376.7 ± 27.3a	326.3 ± 25.5a	321.0 ± 22.4a
	Root (mg)	393.2 ± 63.0a	194.8 ± 19.4b	248.3 ± 45.0a	339.8 ± 51.4a	382.5 ± 62.0a	205.5 ± 24.3b
	Total (mg)	1201.3 ± 85.1b	1476.4 ± 86.5a	1137.4 ± 82.4b	1540.3 ± 84.1a	1433.2 ± 91.6a	1244.4 ± 82.2a
	LMA (g m <sup>-2</sup> )	32.34 ± 1.17a	28.60 ± 0.51b	29.51 ± 1.04a	31.43 ± 0.81a	30.69 ± 0.72a	30.25 ± 1.12a
Growth index	LMR	0.52 ± 0.02b	0.58 ± 0.02a	0.53 ± 0.02a	0.57 ± 0.02a	0.56 ± 0.02a	0.54 ± 0.02a
	LAR (cm <sup>2</sup> g <sup>-1</sup> )	165.2 ± 6.0b	205.5 ± 7.8a	186.1 ± 7.8a	184.6 ± 7.3a	186.7 ± 8.5a	184.0 ± 6.5a
	SRMR	2.7 ± 0.30b	6.1 ± 0.49a	4.1 ± 0.43a	4.7 ± 0.51a	3.8 ± 0.47b	5.0 ± 0.47a
Gas exchange	A <sub>N</sub> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	7.9 ± 0.57a	8.4 ± 0.94a	7.5 ± 0.90a	8.9 ± 0.57a	8.8 ± 0.61a	7.6 ± 0.90a
	E (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	3.6 ± 0.41a	3.4 ± 0.46a	3.2 ± 0.46a	3.9 ± 0.40a	3.6 ± 0.35a	3.4 ± 0.50a
	g <sub>s</sub> (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.14 ± 0.02a	0.14 ± 0.03a	0.13 ± 0.03a	0.15 ± 0.02a	0.14 ± 0.02a	0.14 ± 0.02a
	WUE (μmol CO <sub>2</sub> mmol <sup>-1</sup> H <sub>2</sub> O)	2.6 ± 0.16a	2.9 ± 0.20a	2.8 ± 0.19a	2.7 ± 0.17a	2.9 ± 0.20a	2.7 ± 0.15a
	Chlorophyll fluorescence	φPSII	0.75 ± 0.00a	0.75 ± 0.00a	0.75 ± 0.00a	0.75 ± 0.00a	0.75 ± 0.00a
Photosynthetic pigment	F <sub>v</sub> /F <sub>m</sub>	0.77 ± 0.00a	0.77 ± 0.00a	0.77 ± 0.00a	0.77 ± 0.00a	0.77 ± 0.00a	0.77 ± 0.00a
	qNP	0.59 ± 0.02a	0.52 ± 0.01b	0.55 ± 0.02a	0.57 ± 0.02a	0.56 ± 0.02a	0.56 ± 0.02a
	qP	0.02 ± 0.00b	0.04 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.00b
	Chlorophyll (μg cm <sup>-2</sup> )	39.11 ± 0.89a	41.67 ± 1.25a	40.74 ± 0.97a	40.03 ± 1.22a	38.83 ± 0.88b	41.95 ± 1.24a
	Flavonoids (μg cm <sup>-2</sup> )	0.88 ± 0.03a	0.71 ± 0.03b	0.76 ± 0.03a	0.83 ± 0.03a	0.84 ± 0.03a	0.75 ± 0.03b
Nutrient ratio	NBI	46.55 ± 2.23b	62.75 ± 3.23a	57.74 ± 3.28a	51.56 ± 2.81a	49.55 ± 2.59b	59.75 ± 3.32a

Notes: Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient (400 μmol mol<sup>-1</sup>) or elevated (700 μmol mol<sup>-1</sup>) CO<sub>2</sub> concentration and treated with 0 or 100 μl (10 μg) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient CO<sub>2</sub>. Data are means ± SE (n = 9, for gas exchange, chlorophyll fluorescence, chlorophyll content, flavonoids and NBI; n = 12, for stem diameter, leaf number and area, dry mass and growth index; n = 30, for stem height and growth rate) of samples from three trials. Means followed by different letters within rows and factors are significantly different (P < .05) according to Scheffé's multiple-comparison procedure.

### Effects of CO<sub>2</sub>

CO<sub>2</sub> can play a beneficial or a detrimental role in plant development depending on species and phenological stage (Reekie and Bazzaz 1991). In our study, plants at elevated CO<sub>2</sub> grew faster, were taller, and had thicker stems, more larger leaves than plants at ambient CO<sub>2</sub> (Table 4; Figures 1 and 2) and, in turn, greater aboveground biomass. This is consistent with earlier findings in some C<sub>3</sub> plants (Kimball et al. 2002; Qaderi et al. 2006, 2013). In C<sub>3</sub> crops, photosynthesis is stimulated by elevated CO<sub>2</sub>, as Rubisco fixes CO<sub>2</sub> and photorespiration is inhibited (Ainsworth et al. 2008). Although there were no differences in photosynthesis per single leaf area, due to greater number and size of leaves at elevated CO<sub>2</sub> (see Table 4), photosynthesis could have been higher in these plants compared to plants at ambient CO<sub>2</sub>. As shown, photosynthesis is typically accompanied by an increase in WUE and a decrease in transpiration at elevated CO<sub>2</sub> (Lambers et al. 2008; Qaderi et al. 2013). In a FACE (free-air CO<sub>2</sub> enrichment) experiment with mung bean, elevated CO<sub>2</sub> (550 ± 17 μmol mol<sup>-1</sup>) increased A<sub>N</sub>, WUE and qNP of the upper most fully expanded leaves, but decreased g<sub>s</sub>, φPSII and qP, compared to the ambient CO<sub>2</sub> (400 μmol mol<sup>-1</sup>; Gao et al. 2015). Findings from these authors differ from ours, because plant responses to the environmental factors could have been affected by the experimental growth conditions (cabinet vs. field).

### Effects of ABA

In this study, the ABA-treated plants had slower growth, and were shorter with fewer leaves and smaller root mass than the non-ABA-treated plants. Smaller root mass resulted in greater SRMR in the ABA-treated plants. ABA controls aspects of growth and development, especially during abiotic

stress, slowing shoot growth and maintaining root growth (Chen et al. 2006).

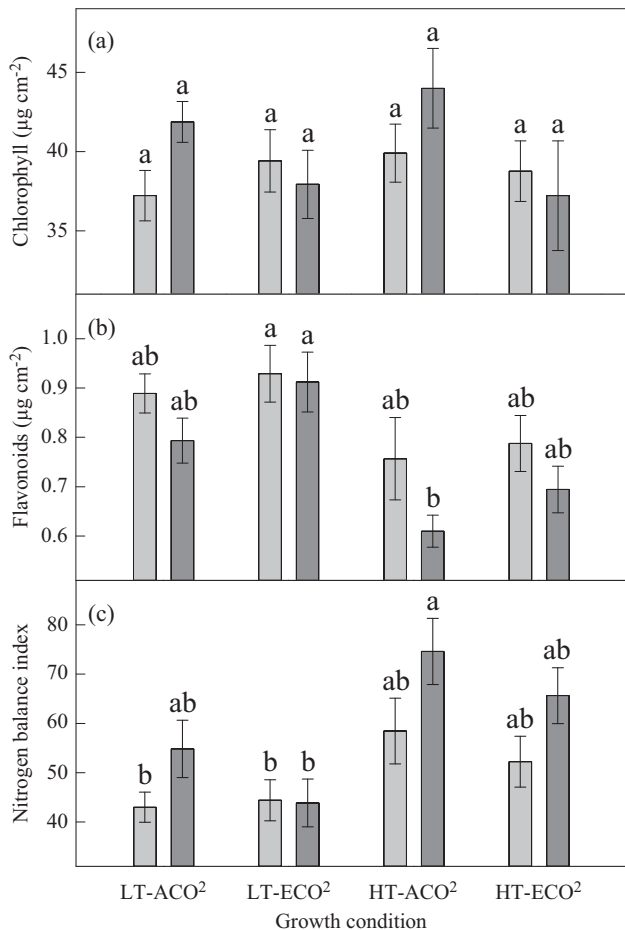
Exogenous application of ABA negatively affected qP in plants (Table 4). Reduced qP inhibits the capacity of plants to utilize excess absorbed energy for metabolism and growth and, in turn, leads to the degradation of photosynthetic apparatus (Hopkins and Hüner 2009). Decreased qP might have contributed to reduced growth of the ABA-treated plants.

The ABA-treated plants had higher chlorophyll and NBI, but lower flavonoids, than the non-ABA-treated plants (Table 4). High NBI indicates that in the ABA-treated plants resources were directed less towards the production of flavonoids, which could have still played a protective role against heat stress (Bitra and Gerats 2013).

### Interactive effects of temperature, CO<sub>2</sub> and ABA

As shown previously, elevated CO<sub>2</sub> can partially alleviate the detrimental effects of increased temperatures on plants (Qaderi et al. 2006; Wahid et al. 2007; Qaderi and Reid 2008, 2009; Song et al. 2014). Findings from this study coincide well with earlier studies in regard to the interactive effects of temperature and CO<sub>2</sub>. Plants grown under higher temperatures at elevated CO<sub>2</sub> had most leaves and greatest leaf and stem mass, whereas plants grown under lower temperatures at ambient CO<sub>2</sub> had fewest leaves and least leaf and stem mass (Figures 1 and 2). It is interesting to note that higher temperatures at elevated CO<sub>2</sub> lead to greater transpiration than higher temperatures at ambient CO<sub>2</sub>. This could have been related to both endogenous ABA level and exogenous ABA effectiveness under the former conditions than under the latter ones. Our previous study has shown that canola plants grown under higher temperatures at elevated CO<sub>2</sub> produced somewhat less endogenous ABA than plants grown under higher temperatures at ambient CO<sub>2</sub> (Qaderi et al. 2006). Also, in a study with *Arabidopsis*





**Figure 4.** Chlorophyll content, flavonoids and NBI of one-month-old mung bean (*V. radiata*) plants. Plants were grown under temperature regime of 26/22°C (16 h light/8 h dark) or 32/28°C (16 h light/8 h dark) at ambient (400 µmol mol<sup>-1</sup>) or elevated (700 µmol mol<sup>-1</sup>) CO<sub>2</sub> concentration and treated with 0 or 100 µl (10 µg) of ABA every other day in controlled-environment growth chambers for three weeks, after eight days of initial growth under 22/18°C at ambient CO<sub>2</sub>. Light gray bars represent the non-ABA-treated plants and dark gray bars, the ABA-treated plants. (a) chlorophyll content; (b) flavonoids and (c) NBI. Data are means ± SE of nine samples from three trials. Bars surmounted by different letters within each panel are significantly different ( $P < .05$ ) according to Scheffé's multiple-comparison procedure.

*thaliana*, elevated CO<sub>2</sub> significantly decreased the concentration of ABA (Teng et al. 2006). It is well known that ABA triggers stomatal closure and reduces transpiration (Lambers et al. 2008); however, elevated CO<sub>2</sub> can affect stomatal response to ABA, leading to reduced night closure and, in turn, increased transpiration (Levine et al. 2009), which might have been the case in our current study. Overall, interaction between higher temperatures and elevated CO<sub>2</sub> increased total biomass of plants (Table 4), which confirms some earlier findings (Long 1991).

ABA responds to abiotic factors, such as temperature and CO<sub>2</sub>, by inhibiting plant growth and development until conditions improve (Marion-Poll and Leung 2006; Yang et al. 2014). Root mass was lowest in the ABA-treated plants under higher temperatures, but highest in the non-ABA-treated plants under lower temperatures. This indicates that, under increased temperature, ABA negatively affects root growth, although it marginally affects other plant organs as well (Table 4). Also, greatest LAR in the non-ABA-treated plants under higher temperatures and lowest LAR in the non-ABA-treated plants under lower temperatures indicate that temperature influences hormonal regulation of plant growth (Davies 2004).

The ABA-treated plants at ambient CO<sub>2</sub> had slowest growth, were shortest with fewest leaves, whereas the non-ABA-treated plants had fastest growth and were tallest with most leaves. On the other hand, the non-ABA-treated plants had smallest stem mass at ambient CO<sub>2</sub>, but greatest stem mass at elevated CO<sub>2</sub>. These findings indicate that extra amount of ABA in plants than the normal level can enhance growth inhibition of naturally occurring hormone (Marion-Poll and Leung 2006), adversely affects plant growth through full or partial closure of stomata (Davies 2004), and leads to reduced gas exchange and, in turn, plant growth (Ainsworth et al. 2008). In this study, no significant three-way interactions were found for any measured plant parameters, indicating that ABA has little role in mediating the interactive effects of CO<sub>2</sub> and temperature on mung bean.

## Conclusions

Higher temperature increases mung bean growth and biomass and its effect is enhanced by elevated CO<sub>2</sub>. ABA has little role in regulating the interactive effects of these two environmental factors on plants during vegetative stage when they respond positively to higher growth temperatures. It is most likely that, under future climate conditions, mung bean plants benefit from the interactive effects of elevated CO<sub>2</sub> and increased temperature during vegetative stage. However, the effects of these two important components of climate change on the reproductive yield of mung bean need to be explored.

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