RESEARCH ARTICLE

ABA and BAP improve the accumulation of carbohydrates and alter carbon allocation in potato plants at elevated CO_2

Mohammad Javad Ahmadi-Lahijani[1] · Mohammad Kafi[1] · Ahmad Nezami[1] ·
Jafar Nabati[2] · John E. Erwin[3]

Abstract Elevated CO_2 interactions with other factors affects the plant performance. Regarding the differences between cultivars in response to CO_2 concentrations, identifying the cultivars that better respond to such conditions would maximize their potential benefits. Increasing the ability of plants to benefit more from elevated CO_2 levels alleviates the adverse effects of photoassimilate accumulation on photosynthesis and increases the productivity of plants. Despite its agronomic importance, there is no information about the interactive effects of elevated CO_2 concentration and plant growth regulators (PGRs) on potato (*Solanum tuberosum* L.) plants. Hence, the physiological response and source-sink relationship of potato plants (*cvs.* Agria and Fontane) to combined application of CO_2 levels (400 vs. 800 µmol mol^{-1}) and plant growth regulators (PGR) [6-benzylaminopurine (BAP) + Abscisic acid (ABA)] were evaluated under a controlled environment. The results revealed a variation between the potato cultivars in response to a combination of PGRs and CO_2 levels. Cultivars were different in leaf chlorophyll content; Agria had higher chlorophyll a, b, and total chlorophyll content by 23, 43, and 23%, respectively, compared with Fontane. The net photosynthetic rate was doubled at the elevated compared with the ambient CO_2. In Agria, the ratio of leaf intercellular to ambient air CO_2 concentrations [C_i:C_a] was declined in elevated-CO_2-grown plants, which indicated the stomata would become more conservative at higher CO_2 levels. On the other hand, the increased C_i:C_a in Fontane showed a stomatal acclimation to higher CO_2 concentration. The higher leaf dark respiration of the elevated CO_2-grown and BAP + ABA-treated plants was associated with a higher leaf soluble carbohydrates and starch content. Elevated CO_2 and BAP + ABA shifted the dry matter partitioning to the belowground more than the above-media organs. The lower leaf soluble carbohydrate content and greater tuber yield in Fontane might indicate a more efficient photoassimilate translocation than Agria. The results highlighted positive synergic effects of the combined BAP + ABA and elevated CO_2 on tuber yield and productivity of the potato plants.

Keywords Dark respiration · Hydroponics · Photosynthetic rate · Photosynthetic pigments · Soluble carbohydrates · Starch

Abbreviations
CO_2	Carbon dioxide
PGR	Plant growth regulator
BAP	6-Benzylaminopurine
ABA	Abscisic acid
C_i/C_a	The ratio of leaf intercellular to ambient air CO_2 concentrations
SC	Soluble carbohydrate content
ST	Starch content
CK	Cytokinins
GA	Gibberellic acid
Chla	Chlorophyll a
Chlb	Chlorophyll b

✉ Mohammad Javad Ahmadi-Lahijani
mjahmadi@um.ac.ir

1 Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91779-48974, Mashhad, Iran

2 Research Centre of Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

3 Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA

Chl a + b	Total chlorophyll
Chl a:B	Chlorophyll a/b ratio
Car	Carotenoids
Np	Net photosynthetic rate
R_d	Dark respiration
g_s	Stomatal conductance
T_r	Transpiration rate
C_i	Intercellular CO_2
Φ	Quantum yield of photosystem II
LDM	Leaf dry matter
SDW	Stem dry matter
RDM	Root dry matter
TDM	Tuber dry matter
Y	Tuber yield
MTW	Mean tuber weight
TN	Tuber number

Introduction

Elevated CO_2 concentration increases the photosynthetic efficiency of C_3 plants through the inhibition of photorespiration (Drake et al. 1997). The growth and productivity of C_3 plants are stimulated through a higher photosynthetic rate and water use efficiency at elevated CO_2 concentrations (Reddy et al. 2010). The photosynthetic rate of potato plants have grown at an enriched CO_2 open-top chamber increased by 40% compared with the control (Katny et al. 2005). Besides, higher sucrose and starch synthesis were observed in the plants grown at elevated CO_2. The study of the source-sink relationship is important to evaluate the effect of elevated CO_2 on plants. Physiological sinks are considered as a place to store photoassimilates. At elevated CO_2, sink limitation suppresses photosynthesis due to negative feedback of starch accumulation, which can be improved through a balanced source-sink relationship (Long et al. 2004; Reddy et al. 2010).

Leaf dark respiration is widely considered as a determining factor in plant growth (Li et al. 2013). Studies revealed the important role of mitochondrial respiration in maintaining an optimal photosynthetic rate (Fuentes et al. 2011). Therefore, higher photosynthesis at elevated CO_2 can be associated with higher dark respiration. The leaf dark respiration was differently affected by the elevated CO_2 (Davey et al. 2004; Gifford 2004; Gomez-Casanovas et al. 2007). Due to the determining role of the respiratory pathway in the global carbon cycle in the cell, whole plant, and ecosystem levels (Wang et al. 2004), and its capability to return 40–50 percent of the fixed carbon into the atmosphere, a better understanding of dark respiration response to elevated CO_2 might be of great importance.

Phytohormones regulate a wide range of processes in plants. Plant growth and metabolism regulation are intricate processes and controlled by the interactions of phytohormones (LeNoble et al. 2004). Depending on their content or the ratio, phytohormones regulate the photoassimilate distribution and productivity in plants (Aksenova et al. 2012). Remarkable effects of phytohormones on potato tuberization (the process of tuber formation) have previously been reported (Ewing 1995; Rodríguez-Falcón et al. 2006; Aksenova et al. 2012). Cytokinin (CK) and abscisic acid (ABA) are phytohormones that regulate the source-sink relationships and the senescence process in plants (Pospisilova et al. 2005). Cytokinins play essential roles in regulating plant growth and the demand for carbohydrates (Ramawat and Merillon 2013). Cytokinins affect potato tuberization (Palmer and Smith 1970; Aksenova et al. 2012). Tuberization is related to cell division; CK provokes cell division in plants, which is a vital component of the tuberization process (Romanov 2009). Starch biosynthesis is also enhanced by CK, which increases the sink capacity of developing tubers (Ramawat and Merillon 2013). The application of BAP in vitro increased plant dry matter and tuber yield of potato (*Solanum tuberosum* L.) plants (Roosta et al. 2015). The tuber number was also increased by the exogenous application of CK in potato (*Solanum tuberosum* L.) plants (Ewing and Struik 2010; Ramawat and Merillon 2013). Ewing and Struik (2010) observed that the plant ABA content was increased under short days; as an essential condition for the tuber initiation. Abscisic acid has an opposite effect to gibberellic acid (GA) activity (Wang et al. 2015). Ramawat and Merillon (2013) showed that ABA inhibited GA activity in the tuberization process.

Viral diseases are known as the main limiting factor of the productivity of potato plants. Producing virus-free tubers from micro-propagated plantlets helps reduce the risk of crop yield loss. Minitubers are virus-free tubers producing on micro-propagated potato plantlets in vitro (Struik and Wiersema 2012). Potato minitubers have the least pathogens with a faster proliferation rate for a greater yield (Sharma and Pandey 2013). An increase in the tuber number and mean tuber weight is of great desire in the minituber production industry. An increase in potato tuber yield was related to either the greater tuber number or mean tuber weight at elevated CO_2 levels (Finnan et al. 2002, 2005; Sharma and Pandey 2013; Aien et al. 2014). Any possible strategy is required to enhance the quantity and quality of plant products to meet food security in the future. One of those strategies is the study of higher CO_2 levels interaction with environmental factors, resulting in various responses in different plant species in the future climate change. Although various studies have evaluated the interactive effects of enriched CO_2 and some

environmental factors on plants, e.g., temperature (Thinh et al. 2017), drought (Yuhui et al. 2017), nitrogen (Yong et al. 2000), and salinity (Piñero et al. 2014), to our knowledge, no studies evaluated the interactive effects of simultaneous application of BAP and ABA, and elevated CO_2 on potato plants. Hence, we hypothesized that the combined application of BAP and ABA under the elevated CO_2 conditions improve the physiological and biochemical traits, minituber production, and the source-sink relationship in potato plants.

Materials and methods

Plant growth conditions

Micro-propagated potato plantlets (*cvs.* Agria and Fontane) were obtained from shoot cuttings grown in agar culture plates containing the Murashige and Skoog medium and supplemented with 3% sucrose, provided by the Yekta Seed Technology Company, Mashhad, Iran, in 2018. The uniform plantlets (5 cm length) were grown in plastic pots (15 and 30 cm, diameter and depth, respectively, one per pot) containing a sterile medium (perlite: coco peat, 1:1, *v:v*) in the controlled chambers (Conviron, Winnipeg, Canada). The environmental conditions inside the chambers were as follows: 400 μmol photons m^{-2} s^{-1} PPFD on the leaf surface supplied by fluorescent/incandescent lamps, 24/16 °C day/night air temperature, photoperiod 12/12 h (day/night), and 50/60 ± 5% (day/night) relative humidity. The plantlets were watered daily and fertilized by the standard Hoagland solution every 5 days (Hoagland and Arnon 1950).

CO_2 treatments

Plantlets were divided into two groups of nine, and each group was subjected to 800 ± 10 μmol mol^{-1} (elevated) or 400 ± 10 μmol mol^{-1} (ambient) CO_2 concentrations from the first day of the experiment. The enriched CO_2 air was injected into the chambers at a flow rate of 1 l min^{-1}, from a compressed gas cylinder (a mixture of ambient air with commercial CO_2). The CO_2 concentrations were continuously monitored using a calibrated infrared gas analyzer (High-performance CO_2 meter, 77,535, China).

Plant growth regulator treatments (PGR)

At the time of tuber initiation (55 days after transplant; DAT), the plantlets were foliar sprayed by 50 μM BAP + ABA (6-Benzylaminopurine and ± -Abscisic Acid, Sigma Aldrich®) (Ahmadi-Lahijani et al. 2018). The control plants were sprayed with distilled water. 10 mL of solution was applied to each plant using a handheld sprinkler. PGRs were applied at the end of the daytime, avoiding photo-degradation or -oxidation.

Collection of experimental samples

Gas exchange variables were recorded on the youngest fully expanded leaves one week after PGR treatment (62 DAT). Then, the same leaves were collected, frozen immediately in liquid nitrogen, and stored at − 80 °C for biochemical analysis (Cao et al. 1994).

Gas exchange measurements

Net photosynthetic (Np) and dark respiration (R_d) rates, stomatal conductance (g_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of leaves were measured using a photosynthesis system (HCM-1000, Waltz, Germany) at the respective CO_2 level at which the plants were grown. C_i:C_a ratio was calculated by dividing the intercellular to ambient CO_2 concentration. Nine leaflets per cultivar were analyzed (three per replication; n = 9). Leaves photosynthetic parameters were allowed to stabilize for five min to a constant rate before each measurement. The leaf temperature, relative humidity, and air-to-leaf vapor pressure deficit (ALVPD) inside the cuvette were set to 25 °C, 50%, and 50 Pa/kPa, respectively. The leaf R_d was obtained by averaging three CO_2 efflux rates at zero PPFD for each plant at the end of the daily dark period from 05:00 to 07:00 h (Li et al. 2013). The adaxial attached leaf surface was used to measure the chlorophyll fluorescence. Measurements were taken after a 15 min dark period using a handheld PEA Chlorophyll Fluorimeter (Hansatech, UK) from the same leaves. The actual quantum yield of photosystem II (Φ) was calculated according to Genty et al. (1989).

Leaf pigments and biochemical analysis

The method of Knudson et al. (1977) was used to assay the leaf pigments. 100 mg leaf fresh weight was homogenized in 98% ethanol using a mortar and pestle. The data were recorded at 665 and 649 nm using a spectrophotometer (Unico 2100, USA). The leaf soluble carbohydrate content (SC) was measured by the method of Dubois et al. (1956). Leaf fresh weight (100 mg) was homogenized in 70% ethanol using a mortar and pestle. A glucose standard curve was plotted to quantify the SC content. The residuals that remained from the SC measurement were used to assay the leaf starch content (ST); the residuals were rinsed three times by perchloric acid (Schlegel 1956). Absorbance was recorded at 485 nm spectrophotometrically. A glucose

standard curve was plotted to determine the starch content of the leaves.

Plant harvest, dry matter, and tuber yield

The plants were harvested 90 DAT at the physiological maturity and then, divided into above-media (leaf and stem) and belowground parts (root and tuber). Tuber number (TN), mean tuber weight (MTW), and tuber yield per plant were determined. The tubers were categorized based on their diameters into small (< 15 mm), medium (15–25 mm), and large (> 25 mm). Tubers and roots were rinsed, and all tissues were oven-dried at 75 °C until constant weight. Dry matter (DM) allocation to different parts of the plants was calculated as a percentage of DM dedicated to the leaves, stems, roots, and tubers concerning the whole plant DM.

Statistical analysis

The experiment was carried out as a factorial arrangement (two CO_2 concentrations, two hormonal treatments, and two cultivars) based on the completely randomized design with three replications. A two-way analysis of variance was used to specify the effects of the CO_2 levels, PGRs, cultivars, and their interactions. Tukey's Studentized Range ($_{HSD}$) test was performed to determine the mean difference at $p \leq 0.05$. Data were presented as the mean ± SE. Statistical analysis was performed with SAS (*v.* 9.4) and MINITAB (*v.* 17) statistical software.

Results

Gas exchange variables

CO_2 levels and cultivar interacted to affect Np (Table 1). The highest Np was recorded in BAP + ABA-treated Agria plants at elevated CO_2 (Fig. 1). Leaf dark respiration (R_d) was significantly affected by foliar application of BAP + ABA and CO_2 levels (Table 1). Elevated CO_2 increased leaf R_d by 22 and 40% in Agria and Fontane, respectively, compared with the ambient. The highest leaf R_d was observed when BAP + ABA was applied at the elevated CO_2. In Agria, application of BAP + ABA at elevated CO_2 increased leaf R_d 46% compared with the untreated plants (Figs. 1 and 2). Leaf transpiration rate was significantly influenced by the interaction of CO_2 levels and PGR (Table 1). Elevated CO_2 significantly decreased T_r compared with the ambient CO_2. BAP + ABA application decreased T_r at the ambient CO_2; however, leaf T_r increased when BAP + ABA applied at the elevated CO_2 (Fig. 1). CO_2 levels and the interaction of CO_2-$\times$ BAP + ABA affected g_s (Table 1). The highest g_s was recorded in untreated-Agria plants at the ambient CO_2. Foliar application BAP + ABA diminished g_s by 28 and 22% at the ambient and elevated CO_2-grown Agria, respectively. While in Fontane, BAP + ABA increased g_s by 48% at elevated CO_2 compared with the untreated plants.

Leaf intercellular CO_2 concentration was affected by the interaction of CO_2 levels with cultivar and BAP + ABA (Table 1). Hormonal treatment increased C_i by 46% at the ambient CO_2 in Fontane. Elevated CO_2 increased C_i by 10 and 28% in BAP + ABA-treated and untreated Agria plants, respectively, compared with the ambient CO_2 (Fig. 1). At the ambient CO_2, C_i was 28% higher in Agria than Fontane, while Fontane C_i was 34% higher compared

Table 1 ANOVA results of growth regulators (PGR) and CO_2 levels on leaf gas exchange variables of potato cultivars

Variables	S.O.V[‡]							C.V.[§]
	PGR (H)[†]	Cultivar (C)	CO_2 concentration (G)	H × C	H × G	G × C	H × G × C	
N_p	NS	**	**	NS	NS	**	NS	19.9
R_d	*	NS	**	NS	NS	NS	NS	17.9
T_r	**	NS	**	NS	**	NS	NS	24.5
g_s	NS	NS	*	*	NS	NS	NS	25.1
C_i	NS	NS	**	NS	*	**	NS	11.7
$C_i:C_a$	*	NS	NS	NS	NS	**	NS	13.8
Φ	NS	NS	NS	NS	NS	NS	NS	0.43

[*]Significant at $P < 0.05$; [**]Significant at $P < 0.01$, *ns* Not significant at $P > 0.05$. [‡]*S.O.V* source of variation, [§]*CV* coefficient of variation, [†] *H* PGR, *C* Cultivar, *G* CO_2 concentration, N_p net photosynthetic rate, R_d dark respiration, g_s stomatal conductance, T_r transpiration rate, C_i intercellular CO_2, $C_i:C_a$ intercellular to ambient partial pressure of CO_2, Φ quantum yield of photosystem II
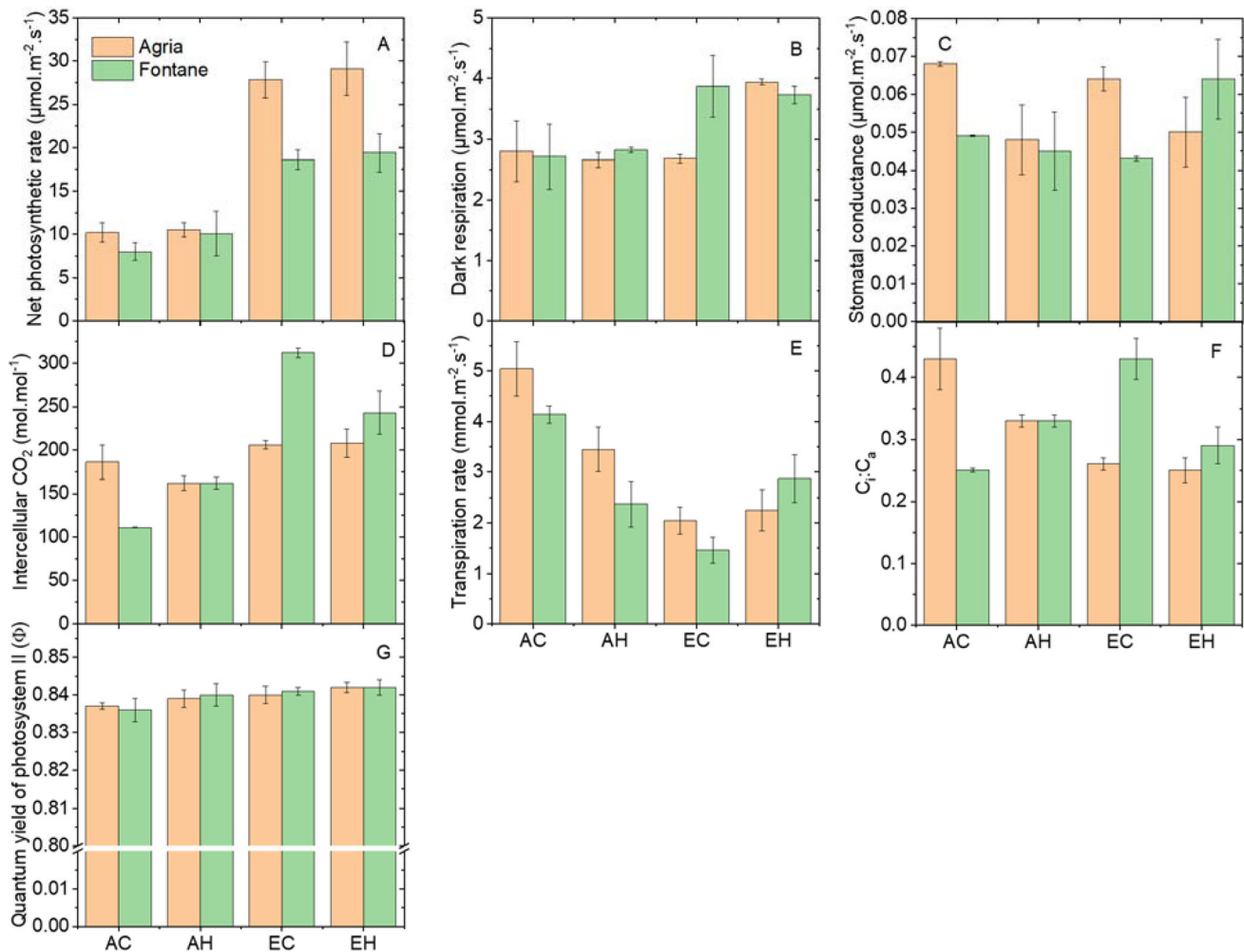
Fig. 1 Effects of growth regulators (PGR) and CO_2 levels on gas exchange variables of potato cultivars. A: net photosynthetic rate, B: dark respiration, C: stomatal conductance, D: intercellular CO_2, E: $C_i:C_a$; intercellular to the ambient partial pressure of CO_2, F: transpiration rate, G: quantum yield of photosystem II. AC; ambient CO_2-no PGR, AH; ambient CO_2-BAP + ABA (50 μM, tuberization), EC; elevated CO_2-no PGR, EH; elevated CO_2- BAP + ABA (50 μM, tuberization). PGR; plant growth regulator (55 days after transplant). Columns are means of three replicates (n = 9). Vertical bars are ± SE

with Agria at the elevated CO_2. BAP + ABA and CO_2-× cultivar interacted to affect $C_i:C_a$. Elevated CO_2 decreased $C_i:C_a$ in BAP + ABA-treated and untreated plants by 40 and 24%, respectively, compared with the ambient (Fig. 2). In Fontane, $C_i:C_a$ increased by 31% when BAP + ABA applied at the ambient CO_2, while $C_i:C_a$ decreased by 32% in BAP + ABA-treated plants at the elevated CO_2. Quantum yield was not affected by any of the treatments. However, BAP + ABA-treated and elevated CO_2-grown plants slightly stimulated Φ compared with the untreated and ambient-grown plants, respectively (Figs. 1 and 2).

Biochemical analysis

Cultivars were different in leaf chlorophyll a (Chla), b (Chlb), and a + b (Chla + b) (Table 2). Generally, Agria had higher Chla, Chlb, and Chla + b by 23, 43, and 23%, respectively, compared with Fontane (Fig. 3). On the other hand, leaf carotenoid content was affected by the CO_2 levels, cultivars, and BAP + ABA. In Agria, leaf carotenoid content decreased in BAP + ABA-treated plants by 21 and 18%, respectively, at the ambient and elevated CO_2 compared with the untreated plants. Generally, Agria leaf carotenoid content was 20% greater than Fontane; however, in Fontane, elevated CO_2 increased leaf carotenoid content by 30% compared with the ambient.

BAP + ABA, cultivar, and CO_2 × cultivar affected the leaf soluble carbohydrate content (SC) (Table 2). Agria leaf SC was 17% greater than Fontane. In Agria, foliar application of BAP + ABA increased leaf SC by 20% compared with the untreated plants (Fig. 3). Elevated CO_2 increased Agria leaf SC by 20% compared with the ambient; however, in Fontane, leaf SC remained
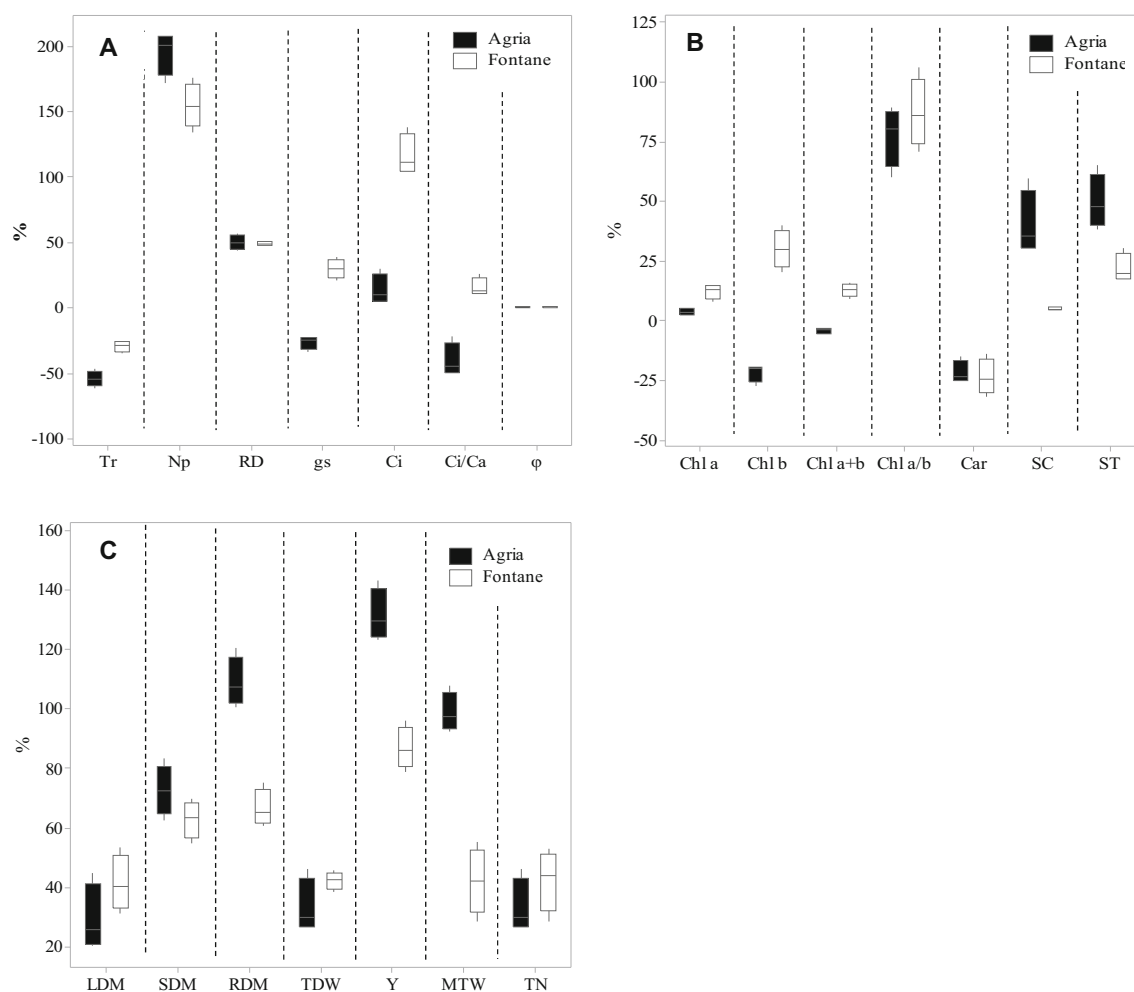
Fig. 2 Percent changes of gas exchange variables (**a**), biochemical traits (**b**), and growth and tuber yield parameters (**c**) of potato cultivars affected by a combination of elevated CO_2 and BAP + ABA application compared with the control

Table 2 ANOVA results of growth regulators (PGR) and CO_2 levels on leaf pigments and biochemical parameters of potato cultivars

Variables	S.O.V[‡]							C.V.[§]
	PGR (H)[†]	Cultivar (C)	CO_2 concentration (G)	H × C	H × G	G × C	H × G × C	
Chla	NS	**	NS	NS	NS	NS	NS	7.8
Chlb	NS	*	NS	NS	NS	NS	NS	18.8
Chla + b	NS	**	NS	NS	NS	NS	NS	10.8
Chla:b	NS	NS	NS	NS	NS	NS	NS	25.3
Car	*	**	**	NS	NS	NS	NS	10.9
SC	*	**	NS	NS	NS	*	NS	10.9
ST	**	NS	NS	NS	NS	NS	NS	19.2

[*]Significant at $P < 0.05$; [**]Significant at $P < 0.01$, *ns* Not significant at $P > 0.05$. [‡]*S.O.V* source of variation, [§]*CV* coefficient of variation, [†] *H* PGR, *C* Cultivar, *G* CO_2 concentration, *Chla* chlorophyll a, *Chlb* chlorophyll b, *Chla + b* total chlorophyll, *Chla:b* chlorophyll a/b ratio, *Car* carotenoids, *SC* soluble carbohydrates, *ST* starch

unchanged at the elevated CO_2. Leaf Starch content (ST) was only affected by BAP + ABA (Table 2). Foliar application of BAP + ABA increased leaf ST by 26 and 36% in Agria and by 15 and 10% in Fontane, respectively, at the ambient and elevated CO_2 compared with the untreated plants (Fig. 3).
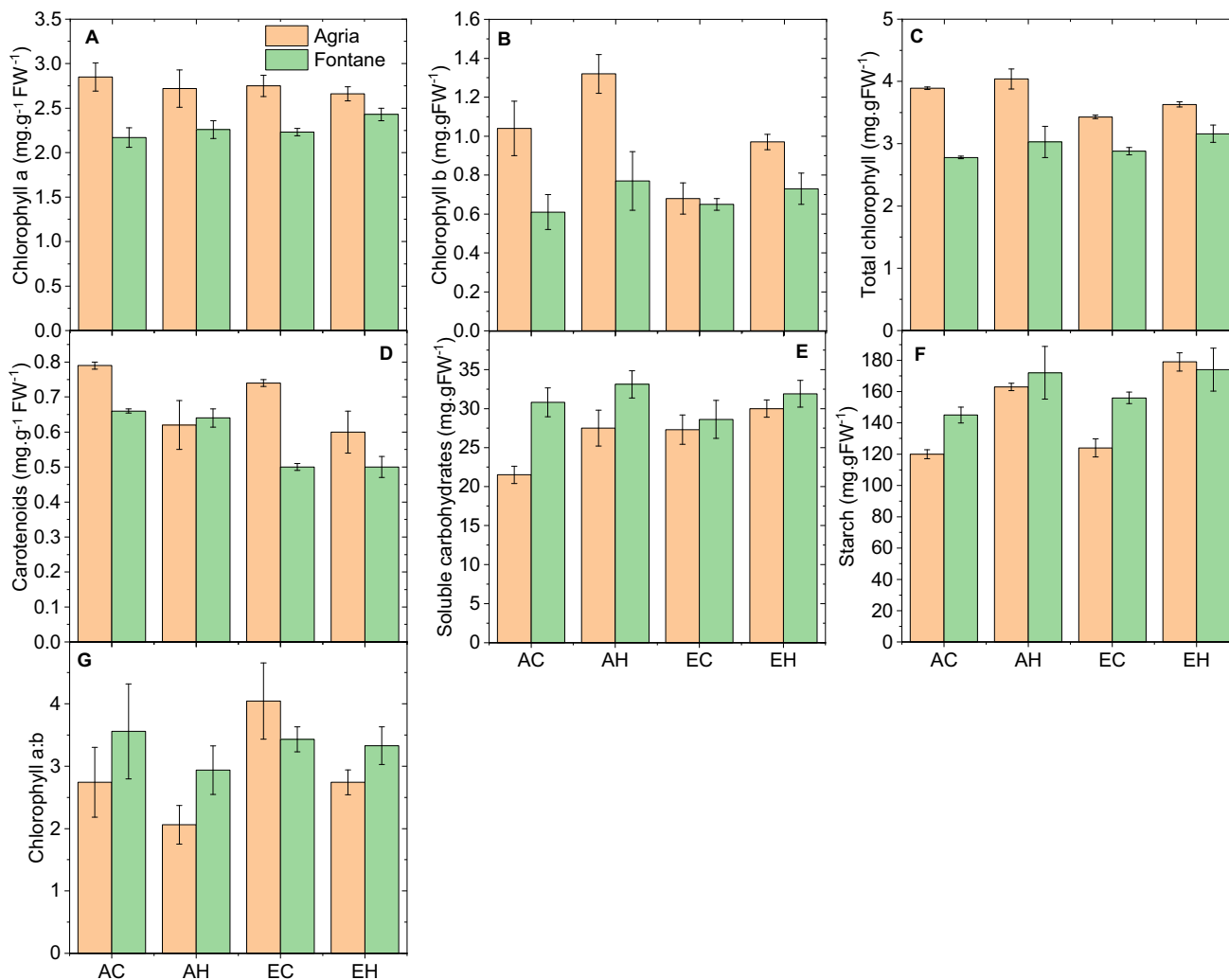
Fig. 3 Effects of growth regulators (PGR) and CO_2 levels on the leaf biochemistry of potato cultivars. A: chlorophyll a, B: chlorophyll b, C: total chlorophyll, D, carotenoids, E: soluble carbohydrates, F: starch G: chlorophyll a:b ratio. AC; ambient CO_2-no PGR, AH; ambient CO_2-BAP + ABA (50 μM, tuberization), EC; elevated CO_2-no PGR, EH; elevated CO_2- BAP + ABA (50 μM, tuberization). PGR; plant growth regulator (55 days after transplant). Columns are means of three replicates (n = 9). Vertical bars are ± SE

Plant DM and DM partitioning

Leaf dry matter (LDM) was affected by the CO_2 levels and cultivar (Table 3). Generally, Fontane LDM was 53% greater compared with Agria (Fig. 4). At the elevated CO_2, Agria and Fontane LDM were 32 and 21% greater, respectively, compared to those at the ambient CO_2. Cultivar × BAP + ABA and cultivar × CO_2 interacted to affect stem dry matter (SDW). In Agria, foliar application of BAP + ABA increased SDW by 32% at both CO_2 levels compared with the untreated plants. Under the ambient CO_2, Agria had a greater SDW than Fontane by 12%; however, elevated CO_2 increased Fontane SDW by 20% higher compared to Agria (Fig. 4). Root dry matter (RDM) was affected by the interactions of elevated CO_2 and cultivar (Table 3). Fontane RDM was higher at both

CO_2 levels compared with Agria. Elevated CO_2 increased RDM by 52 and 67% in Agria and Fontane, respectively, compared with the ambient CO_2 (Fig. 4). CO_2 levels and cultivar interacted to affect the tuber dry matter (TDM). Fontane TDM was greater by 64% compared to Agria; however, elevated CO_2 increased Agria TDM by 90% compared with the ambient CO_2 (Fig. 4).

Elevated CO_2 affected the DM partitioning pattern of the potato plants (Fig. 5). Elevated CO_2 increased the above-media and belowground DM of the plants; generally, the roots and tubers gained more DM compared with the above-media parts of the plants. While above-media DM increased by 32 and 38%, belowground DM increased by 71 and 40% in Agria and Fontane, respectively, at the elevated CO_2. Foliar application of BAP + ABA also increased DM partitioning toward stems, roots, and tubers.

Table 3 ANOVA results of growth regulators (PGR) and CO_2 levels on plant dry matter, tuber yield, and yield component of potato cultivars

Variables	S.O.V[‡]							C.V.[§]
	PGR (H)[†]	Cultivar (C)	CO_2 concentration (G)	H × C	H × G	G × C	H × G × C	
LDM	NS	**	**	NS	NS	NS	NS	15.3
SDM	**	NS	**	*	NS	*	NS	11.3
RDM	NS	**	**	NS	NS	*	NS	18.6
TDM	NS	**	**	NS	NS	**	NS	18.3
TY	*	**	**	NS	NS	NS	NS	16.9
TN	NS	NS	NS	NS	NS	NS	NS	20.6
MTW	NS	**	**	NS	NS	NS	NS	17.3
ST (< 15 mm)	NS	*	*	NS	NS	NS	NS	23
MT (15-25 mm)	*	*	NS	NS	NS	NS	NS	19.9
LT (> 25 mm)	NS	NS	**	NS	NS	NS	NS	20.6

[*]Significant at $P < 0.05$; ** Significant at $P < 0.01$, *ns* Not significant at $P > 0.05$. [‡]*S.O.V* source of variation, [§]*CV* coefficient of variation, [†] *H* PGR, *C* Cultivar, *G* CO_2 concentration, *LDM* leaf dry matter, *SDM* stem dry matter, *RDM* root dry matter, *TDM* tuber dry matter, *TY* tuber yield, *MTW* mean tuber weight, *TN* tuber number, *ST* small-size tuber, *MT* medium-size tuber, *LT* large-size tubers

For instance, BAP + ABA significantly decreased DM accumulation in Agria leaves compared with the untreated plants at the ambient CO_2. The greatest tuber and root DM were observed in BAP + ABA-treated and untreated Fontane plants, respectively, at the elevated CO_2, while the lowest TDM was observed in untreated Agria plants at the ambient CO_2.

The tuber yield per plant (TY) was affected by the CO_2 levels, cultivar, and BAP + ABA (Table 3). Fontane TY was 22% greater than Agria. Foliar application of BAP + ABA increased Agria and Fontane TY by 48 and 14%, respectively, compared with the untreated plants (Fig. 4). Tuber yield was also increased by 70 and 61%, respectively, in Agria and Fontane at the elevated compared with the ambient CO_2. Tuber yield per plant was more affected by the foliar application of BAP + ABA at the ambient compared with the elevated CO_2. Mean tuber weight (MTW) was affected by CO_2 levels and cultivar. Fontane MTW was greater by 40% compared with that of Agria. Mean tuber weight enhanced by 48 and 36% in Agria and Fontane, respectively, at elevated compared with the ambient CO_2. Percent distribution of tubers was also affected by experimental factors (Table 3). Generally, hormonal treatment and elevated CO_2 increased the medium- and large-size tubers. Elevated CO_2 increased the percent distribution of large-size tubers by 54 and 59% in Agria and Fontane, respectively, compared with the ambient CO_2. BAP + ABA also enhanced the medium-size tubers by 32 and 18% in Agria and Fontane, respectively, compared with the untreated plants (Fig. 6).

Discussion

The photosynthesis of C_3 plants is limited under the present atmospheric CO_2 concentration. Carbon dioxide enrichment enhances the photosynthetic rate and stimulates crop growth and productivity (Reddy et al. 2010; Fleisher et al. 2014). Plant growth regulators are widely used to improve the physiological processes in plants (Aksenova et al. 2012). It has been reported that the application of BAP and ABA improved photosynthesis and increased leaf chlorophyll content of potato (*Solanum tuberosum* L.) and soybean (*Glycine max*) plants (Caldiz et al. 1998; Reinoso et al. 2011). While BAP + ABA-treated plants showed a lower T_r at the ambient CO_2, the foliar application of BAP + ABA stimulated T_r at the elevated CO_2. A similar behavior was also observed in the leaf g_s. It seems T_r was not decreased by the hormonal treatment under elevated CO_2; however, BAP + ABA tended to diminish the subtractive effects of the higher CO_2 concentration on g_s and T_r. Pospíšilová (2003) observed that stomatal conductance could be inhibited by higher concentrations of BAP, although the effects depend on the CK type, concentration, and method of application. Furthermore, the inhibitory effect of ABA on stomatal aperture has already been reported (Davies et al. 2005). Reinoso et al. (2011) observed that ABA application decreased g_s in soybean (*Glycine max*) plants.

Stomatal conductance has been reported to be suppressed at higher CO_2 concentrations (Ainsworth and Rogers 2007). According to the results of Vandermeiren et al. (2002), we also found that potato (*Solanum tuberosum* L.) plants grown at elevated CO_2 had lower g_s and higher C_i. Generally, the photosynthetic rate is regulated by
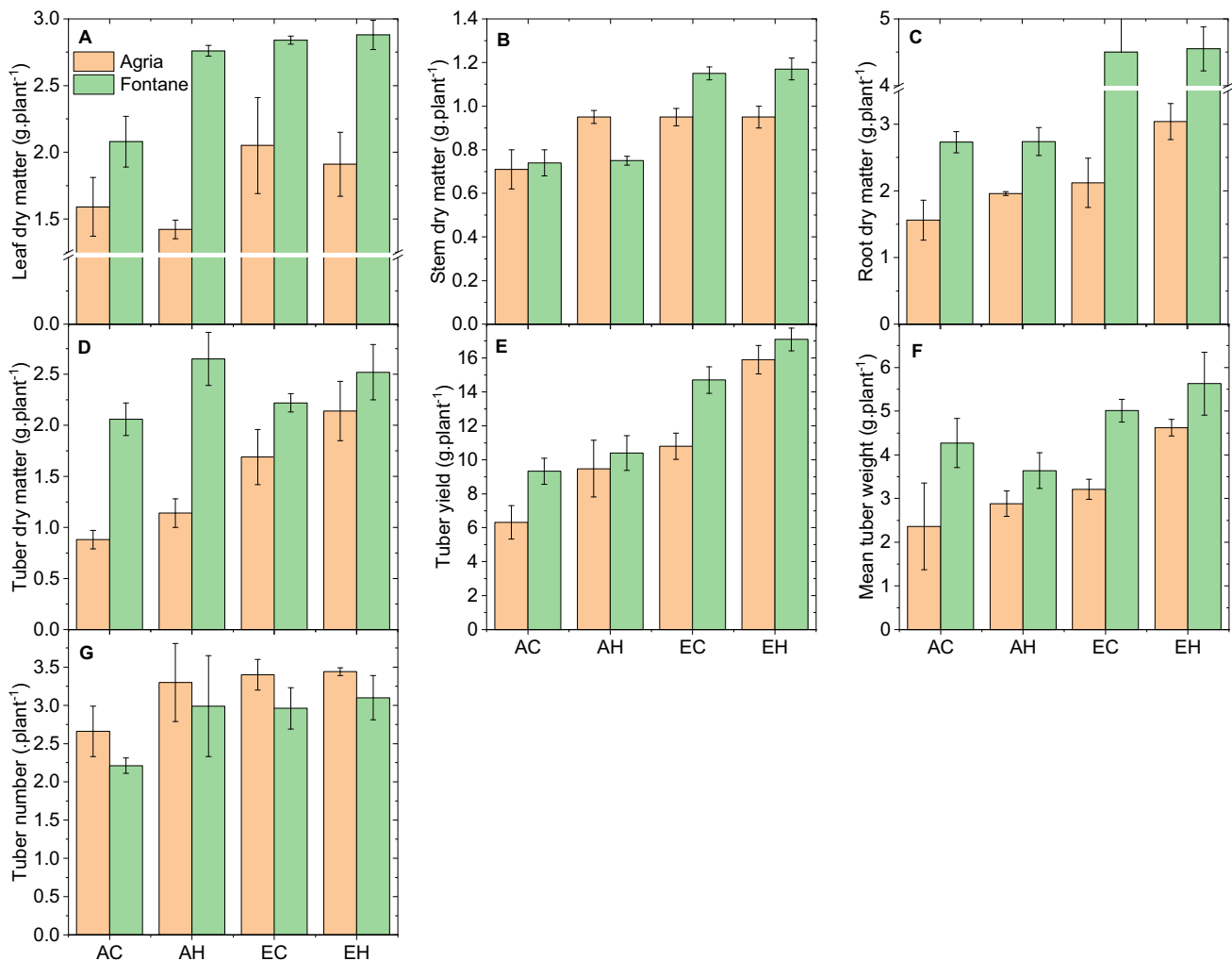
Fig. 4 Effects of growth regulators (PGR) and CO_2 levels on dry matter, tuber yield, yield components of potato cultivars. A: leaf dry matter, B: stem dry matter, C: root dry matter, D, tuber dry matter, E: tuber yield, F: mean tuber weight G: tuber number. AC; ambient CO_2- no PGR, AH; ambient CO_2-BAP + ABA (50 μM, tuberization), EC; elevated CO_2-no PGR, EH; elevated CO_2- BAP + ABA (50 μM, tuberization). PGR; plant growth regulator (55 days after transplant). Columns are means of three replicates (n = 9). Vertical bars are ± SE

both stomatal and non-stomatal factors. In this experiment, the higher Np was accompanied by a higher C_i, which indicates that the photosynthesis was mainly controlled by the stomatal factors. At the elevated CO_2, while C_i:C_a tended to decrease in Agria, it was increased in Fontane. The decreased C_i:C_a in Agria indicated the role of the stomatal factor in photosynthetic regulation. It has already been reported that elevated CO_2 decreased C_i:C_a (Long et al. 2004). However, no changes (Bunce 2001) and an increase (Kaminski et al. 2014) in C_i:C_a have also been reported in potato (*Solanum tuberosum* L.) plants at elevated CO_2. Bunce (2001) believed that a higher C_i:C_a at prolonged elevated CO_2 probably indicates the photosynthetic acclimation to such conditions.

Agria R_d was increased by the foliar application of BAP + ABA at the elevated CO_2. It has been observed that leaf R_d was stimulated at elevated CO_2, indicating a

higher demand for maintenance due to the accelerated senescence (Donnelly et al. 2001; Vandermeiren et al. 2002). A simultaneous increase in the leaf R_d and SC and ST content was observed in our experiment. An increase in the leaf SC content and R_d was also reported in tomato (*Solanum lycopersicum*), and potato (*Solanum tuberosum* L.) plants grown under elevated CO_2 (Li et al. 2013). They concluded that greater availability of carbohydrates and energy demand resulted in a higher leaf dark respiration rate at elevated CO_2. The higher accumulation of carbohydrates due to higher photosynthesis might be due to the greater availability of substrate for stimulating the respiratory pathway (Aranjuelo et al. 2011).

Elevated CO_2 accelerates the senescence process in plants, mainly due to the accumulation of starch granules in chloroplasts and lower stomatal conductance that increase the leaf temperature. The trend of leaf Chl content changes

Fig. 5 Effect of elevated CO_2 and BAP + ABA on dry matter partitioning of potato cultivars. Ambient CO_2; 400 µmol mol^{-1}, Elevated CO_2; 800 µmol mol^{-1}, Control; untreated plants, PGR; BAP + ABA-treated plants (50 µM, tuberization, 55 days after transplant). Columns with the same letters are not significantly different at $P < 0.05$, according to the Tukey test
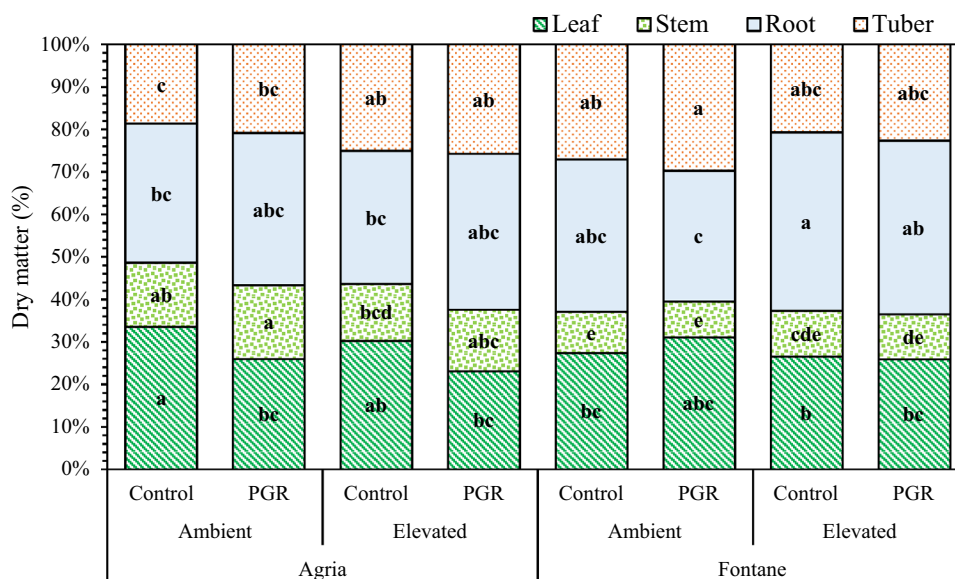
Fig. 6 Effect of elevated CO_2 and BAP + ABA on the percentage distribution of tubers produced per size category in potato cultivars. Ambient CO_2; 400 µmol mol^{-1}, Elevated CO_2; 800 µmol mol^{-1}, Control; untreated plants, PGR; BAP + ABA-treated plants (50 µM, tuberization, 55 days after transplant). Size category (mm). The vertical bar shows the parameter's mean ± SE. n = 9
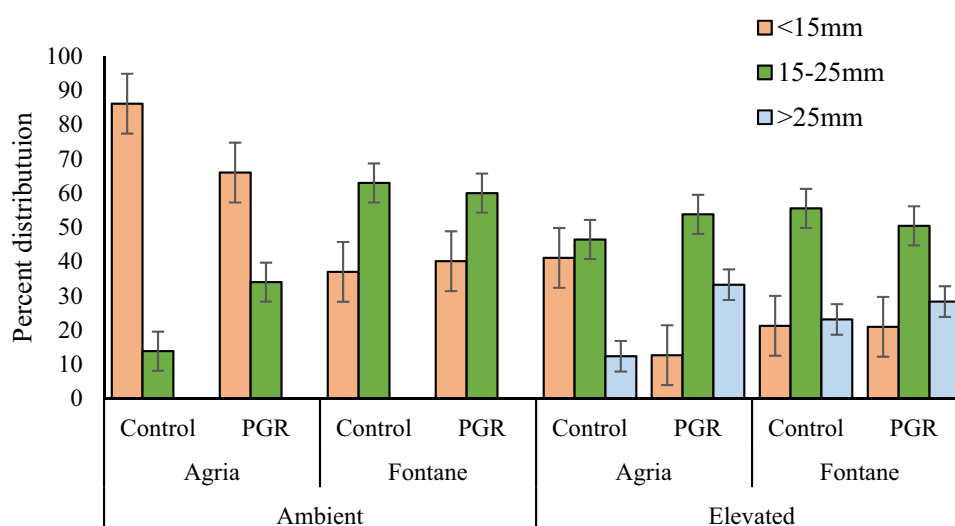
of the cultivars was different. Agria senescence started sooner than Fontane at the elevated CO_2, which could be observed through a slight decrease in the leaf Chl content of Agria exposed to the elevated CO_2. It might be due to the greater accumulation of SC and ST content in Agria leaves. Furthermore, Fontane TY was greater than Agria regardless of lower Np, which might indicate that Fontane translocated the photoassimilates more efficiently than Agria from the leaves to the tubers, which has been proved by a lower leaf SC content of Fontane. Therefore, the higher accumulated carbohydrates in Agria leaves tended to more rapid senescence onset compared with Fontane. On the other hand, it might also indicate that Fontane was more affected by ABA, as a stimulator of dry matter remobilization, compared with Agria.

Leaf carotenoid content decreased when BAP + ABA was applied, or CO_2 was enriched. Carotenoids, as the auxiliary pigments, protect the photosynthetic apparatus from the extra sunlight energy through the nonphotochemical quenching (NPQ) process (Taiz and Zeiger 2006). Foyer and Shigeoka (2011) found that a lower CO_2 concentration led to an increase in the reactive oxygen species (ROS), under which the carotenoids may protect the photosynthetic apparatus against the ROS damages. In the present study, the lower carotenoid content of leaves might probably be due to the role of elevated CO_2 in decreasing ROS production. The slightly higher leaf Chl content due to the BAP + ABA application might also decrease the demand for a greater carotenoid content.

The availability of physiological sinks is a critical factor in enhancing the photosynthesis of C_3 plants (Chen and Setter 2012). The elevated CO_2 enhanced MTW of the plants. Finnan et al. (2005) observed that the higher levels of CO_2 stimulate the tuberization of potato plants and

increase the physiological sink strength to store higher photoassimilates, leading to reduce inhibition feedback of starch accumulation on photosynthesis. Aranjuelo et al. (2011) believed that an increase in soluble sugar accumulation indicates an impairment in the sink strength. It has been reported that the allocation of photoassimilates is regulated by the plant hormones, environmental conditions, and developmental stage (Geiger and Servaites 1991). BAP and ABA, by stimulating the photosynthesis and mobilization of photoassimilates to economic organs, provoke the tuberization process (Ewing and Struik 2010; Aksenova et al. 2012). Sansberro et al. (2004) observed that the DM accumulation enhanced when ABA was foliar sprayed on *Ilex paraguariensis*. Fischer et al. (2008) believed that a higher accumulation of carbohydrates could be due to a higher photosynthetic rate of PGR-treated potato (*Solanum tuberosum* L.) plants. The enhancement of the TY in our experiment might be due to the positive effects of the elevated CO_2 and BAP + ABA on leaf Np and R_d, leading to higher carbohydrate production. Carbohydrates play vital roles in tuber growth and starch biosynthesis and are known as the tuber initiation stimulator (Ramawat and Merillon 2013). Kloosterman et al. (2005) stated that higher soluble carbohydrate activate many genes involved in starch biosynthesis, leading to an increase in the tuber DM accumulation capacity.

Various studies have shown that DM allocation was increased more to belowground organs than to shoots at elevated CO_2 levels (Fleisher et al. 2008; Högy and Fangmeier 2009). Agria LDM and TDM comprised 33 and 18%, respectively, of the total biomass of untreated plants at the ambient CO_2, while it altered to 23 and 26%, respectively, when BAP + ABA and elevated CO_2 were applied. It indicated that the elevated CO_2 and hormonal treatment altered DM allocation toward the plant's belowground organs. The tuber classification results also showed that the proportion of large- and medium-size tubers increased at elevated compared with the ambient CO_2. Högy and Fangmeier (2009) also reported that the size of tubers increased at elevated CO_2. They concluded that the stimulation of potato tuber yield was mainly due to an increase in the tuber weight compared to the tuber number.

Conclusion

Our results showed that the elevated CO_2 and BAP + ABA foliar application at the tuberization stage affected physiobiochemical traits, tuber yield, and source-sink relationship in two potato cultivars. The lower T_r and higher C_i were coupled with a moderate decrease in g_s at the elevated CO_2. Agria C_i:C_a was declined at the elevated

CO_2, indicating that the stomata may become more conservative under the higher CO_2 concentrations. On the other hand, the increase in Fontane C_i:C_a showed a stomatal acclimation to the elevated CO_2. Elevated CO_2 and BAP + ABA shifted DM partitioning to belowground organs; lower DM was allocated to the leaves compared with the roots and tubers. Manipulating the plant's ability to benefit from the higher photoassimilates, alleviates the negative effects of photosynthetic acclimation and increases the productivity of plants at elevated CO_2. Our results showed that the application of BAP + ABA with doubling the CO_2 level could positively increase MTW and TY in potato minituber production. The greater tuber yield of Fontane might indicate the role of a greater leaf number (data not shown), as the photosynthetic area, than photosynthesis per leaf area unit, and a more efficient photoassimilates translocation. The variations between the cultivars might help to identify those cultivars which are better adapted to elevated CO_2 and to improve the productivity of plants to meet the food demand and security in the future climate change.

References

Ahmadi-Lahijani MJ, Kafi M, Nezami A, Nabati J, Erwin J (2018) Effect of 6-benzylaminopurine and abscisic acid on gas exchange, biochemical traits, and minituber production of two potato cultivars (*Solanum tuberosum* L.). J Agric Sci Tech 20:129–139

Aien A, Pal M, Khetarpal S, Kumar Pandey S (2014) Impact of elevated atmospheric CO_2 concentration on the growth, and yield in two potato cultivars. J Agric Sci Technol 16:1661–1670

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Aksenova N, Konstantinova T, Golyanovskaya S, Sergeeva L, Romanov G (2012) Hormonal regulation of tuber formation in potato plants. Russ J Plant Physiol 59:451–466

Aranjuelo I et al (2011) Does ear C sink strength contribute to overcoming photosynthetic acclimation of wheat plants exposed to elevated CO_2? J Exp Bot 62:3957–3969

Bunce JA (2001) Direct and acclimatory responses of stomatal conductance to elevated carbon dioxide in four herbaceous crop species in the field. Glob Change Biol 7:323–331

Caldiz D, Clua A, Beltrano J, Tenenbaum S (1998) Ground cover, photosynthetic rate and tuber yield of potato (*Solanum tuberosum* L.) crops from seed tubers with different physiological age modified by foliar applications of plant growth regulators. Potato Res 41:175–185

Cao W, Tibbitts T, Wheeler R (1994) Carbon dioxide interactions with irradiance and temperature in potatoes. Adv Space Res 14:243–250

Chen C-T, Setter TL (2012) Response of potato dry matter assimilation and partitioning to elevated CO_2 at various stages of tuber initiation and growth. Environ Exp Bot 80:27–34

Davey PA, Hunt S, Hymus GJ, DeLucia EH, Drake BG, Karnosky DF, Long SP (2004) Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO_2], but is increased with

long-term growth in the field at elevated [CO_2]. Plant Physiol 134:520–527

Davies WJ, Kudoyarova G, Hartung W (2005) Long-distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought. J Plant Growth Regul 24:285–295

Donnelly A, Craigon J, Black CR, Colls JJ, Landon G (2001) Elevated CO_2 increases biomass and tuber yield in potato even at high ozone concentrations. New Phytol 149:265–274

Drake BG, Gonzàlez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Ann Rev Plant Biol 48:609–639

Dubois M, Gilles KA, Hamilton JK, Rebers P, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Ewing EE (1995) The role of hormones in potato (*Solanum tuberosum* L.) tuberization. In: Plant Hormones. Springer, pp 698–724

Ewing E, Struik P (2010) Tuber formation in potato: induction, initiation, and growth. Hort Rev 14:197

Finnan JM, Donnelly A, Burke JI, Jones MB (2002) The effects of elevated concentrations of carbon dioxide and ozone on potato (*Solanum tuberosum* L.) yield. Agric Ecosyst Environ 88:11–22

Finnan J, Donnelly A, Jones M, Burke J (2005) The effect of elevated levels of carbon dioxide on potato crops: a review. J Crop Improv 13:91–111

Fischer L, Lipavska H, Hausman J-F, Opatrny Z (2008) Morphological and molecular characterization of a spontaneously tuberizing potato mutant: an insight into the regulatory mechanisms of tuber induction. BMC Plant Biol 8:117

Fleisher DH, Barnaby J, Sicher R, Resop JP, Timlin D, Reddy V (2014) Potato gas exchange response to drought cycles under elevated carbon dioxide. Agron J 106:2024–2034

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100. https://doi.org/10.1104/pp.110.166181

Fuentes D et al (2011) A deficiency in the flavoprotein of Arabidopsis mitochondrial complex II results in elevated photosynthesis and better growth in nitrogen-limiting conditions. Plant Physiol 157:1114–1127

Geiger DR, Servaites JC (1991) Carbon allocation and response to stress. In: Mooney H, Winner W, Pell E (eds) Response of plants to multiple stresses. Academic Publishers, San Diego, pp 103–127

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta Gen Subj 990:87–92

Gifford RM (2004) The CO_2 fertilising effect–does it occur in the real world? New Phytol 163:221–225

Gomez-Casanovas N, Blanc-Betes E, Gonzalez-Meler MA, Azcon-Bieto J (2007) Changes in respiratory mitochondrial machinery and cytochrome and alternative pathway activities in response to energy demand underlie the acclimation of respiration to elevated CO_2 in the invasive *Opuntia ficus-indica*. Plant Physiol 145:49–61

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. vol 347. Circular. California Agricultural Experiment Station, vol 2nd edit.

Högy P, Fangmeier A (2009) Atmospheric CO_2 enrichment affects potatoes: 1. Aboveground biomass production and tuber yield. Europ J Agron 30:78–84

Kaminski KP, Kørup K, Nielsen KL, Liu F, Topbjerg HB, Kirk HG, Andersen MN (2014) Gas-exchange, water use efficiency and yield responses of elite potato (*Solanum tuberosum* L.) cultivars to changes in atmospheric carbon dioxide concentration, temperature and relative humidity. Agric Forest Meteorol 187:36–45

Katny MAC, Hoffmann-Thoma G, Schrier AA, Fangmeier A, Jäger H-J, van Bel AJ (2005) Increase of photosynthesis and starch in potato under elevated CO_2 is dependent on leaf age. J Plant Physiol 162:429–438

Kloosterman B, Vorst O, Hall RD, Visser RG, Bachem CW (2005) Tuber on a chip: differential gene expression during potato tuber development. Plant Biotechnol J 3:505–519

Knudson LL, Tibbitts TW, Edwards GE (1977) Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol 60:606–608

LeNoble ME, Spollen WG, Sharp RE (2004) Maintenance of shoot growth by endogenous ABA: genetic assessment of the involvement of ethylene suppression. J Exp Bot 55:237–245

Li X et al. (2013) Stimulated leaf dark respiration in tomato in an elevated carbon dioxide atmosphere. Sci Rep 3

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants FACE the Future*. Ann Rev Plant Biol 55:591–628

Palmer C, Smith O (1970) Effect of kinetin on tuber formation on isolated stolons of *Solanum tuberosum* L. cultured *in vitro*. Plant Cell Physiol 11:303–314

Piñero MC, Houdusse F, Garcia-Mina JM, Garnica M, del Amor FM (2014) Regulation of hormonal responses of sweet pepper as affected by salinity and elevated CO_2 concentration. Physiol Plant 151:375–389

Pospíšilová J (2003) Participation of phytohormones in the stomatal regulation of gas exchange during water stress. Biol Plant 46:491–506

Pospisilova J, Vagner M, Malbeck J, Travnickova A, Batkova P (2005) Interactions between abscisic acid and cytokinins during water stress and subsequent rehydration. Biol Plant 49:533–540

Ramawat KG, Merillon J-M (2013) Bulbous plants: biotechnology. CRC Press

Reddy AR, Rasineni GK, Raghavendra AS (2010) The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. Curr Sci pp 46–57

Reinoso H, Travaglia C, Bottini R (2011) ABA increased soybean yield by enhancing production of carbohydrates and their allocation in seed. INTECH Open Access Publisher

Rodríguez-Falcón M, Bou J, Prat S (2006) Seasonal control of tuberization in potato: conserved elements with the flowering response. Ann Rev Plant Biol 57:151–180

Romanov G (2009) How do cytokinins affect the cell? Russ J Plant Physiol 56:268–290

Roosta H, Vazirinasab S, Raghami M (2015) Effect of 6-benzy-laminopurine and cycocel on minituber production in two potato cultivars *In Vitro*. Iran J Hotr Sci 46:141–156

Sansberro PA, Mroginski LA, Bottini R (2004) Foliar sprays with ABA promote growth of *Ilex paraguariensis* by alleviating diurnal water stress. Plant Growth Regul 42:105–111

Schlegel H-G (1956) Die verwertung organischer säuren durch Chlorella im licht. Planta 47:510–526

Sharma AK, Pandey K (2013) Potato mini-tuber production through direct transplanting of *in vitro* plantlets in green or screen houses—a review. Potato J 40

Struik PC, Wiersema SG (2012) Seed potato technology. Wageningen Academic Publication

Taiz L, Zeiger E (2006) Plant Physiology, 4 eds. Sinauer Associates, Inc., Sunderland, Massachusetts

Thinh NC, Shimono H, Kumagai E, Kawasaki M (2017) Effects of elevated CO_2 concentration on growth and photosynthesis of Chinese yam under different temperature regimes. Plant Prod Sci 20:227–236

Vandermeiren K, Black C, Lawson T, Casanova M, Ojanperä K (2002) Photosynthetic and stomatal responses of potatoes grown

under elevated CO_2 and/or O_3—results from the European CHIP-programme. Europ J Agron 17:337–352

Wang X, Anderson OR, Griffin KL (2004) Chloroplast numbers, mitochondrion numbers and carbon assimilation physiology of *Nicotiana sylvestris* as affected by CO_2 concentration. Environ Exp Bot 51:21–31

Wang X et al (2015) Effects of exogenous GA_3 on wheat cold tolerance. J Agric Sci Technol 17:921–934

Yong JW, Wong SC, Letham DS, Hocart CH, Farquhar GD (2000) Effects of elevated [CO_2] and nitrogen nutrition on cytokinins in the xylem sap and leaves of cotton. Plant Physiol 124:767–780

Yuhui W, Denghua Y, Junfeng W, Yi D, Xinshan S (2017) Effects of elevated CO_2 and drought on plant physiology, soil carbon and soil enzyme activities. Pedosphere 27:846–855