



Research article

Variations in assimilation rate, photoassimilate translocation, and cellular fine structure of potato cultivars (*Solanum Tuberosum* L.) exposed to elevated CO₂

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ARTICLE INFO

Keywords:

Chloroplast number
Dark respiration
Mitochondrion number
Net photosynthesis rate
Potato minituber

ABSTRACT

Rising atmospheric CO₂ concentrations are expected to impact the productivity of plants. Cultivars demonstrate different responses to CO₂ levels, hence, screening and recognizing the cultivars with a higher capacity for translocation of photoassimilates would certainly be beneficiary. To investigate the interactive impact of enhancing CO₂ on physiology, cellular fine structure and photoassimilate translocation of micro-propagated potato plantlets, plantlets (cvs. Agria and Fontane) were grown under ambient (400 ppm) or elevated (800 ppm) CO₂ concentrations in controlled environments. These high-yielding cultivars are widely cultivated in Iran and have a wide range of consumption as fresh marketing, French fries, and chips industry. Transmission electron micrographs showed an increase in the length, width, and area of chloroplasts. The number of chloroplasts per cell area was significantly increased in Agria at elevated CO₂. Also, there was an increase in mitochondria number in Agria and Fontane. Chloroplast number and Np were increased by a similar magnitude at doubled CO₂, while, mitochondria number was increased greater than the leaf Rd enhancement at elevated CO₂. Elevated CO₂ increased net photosynthesis, dark respiration (Rd), and starch concentration in leaves. However, there was no dramatic change in the leaf soluble carbohydrate content in the plants grown at elevated CO₂, apart from at 75 days after transplant (DAT) in Agria. Net photosynthesis remained relatively unchanged for each cultivar throughout the growing season at elevated CO₂, which demonstrated more efficient CO₂ assimilation to ambient CO₂. The greatest starch content was measured at 55 DAT that was accompanied by lower Np and higher Rd. The diminished starch content of leaves was contributed to a lower leaf dry matter as well as a greater tuber dry matter in Fontane. Our results highlighted a variation in photoassimilate translocation between these cultivars, in which Fontane demonstrated a more efficient photoassimilate translocation system at the elevated CO₂.

1. Introduction

Several studies have revealed that photosynthesis and sink utilization of carbohydrates are highly coordinated (Ainsworth and Bush, 2011; Kaschuk et al., 2010; Paul and Foyer, 2001). It has been reported that 50–80% of the photoassimilates are transported from a mature leaf to fulfill demands of the non-photosynthetic organs of plants (Kalt-Torres et al., 1987). Elevated CO₂ effectively increases carbohydrate accumulation in leaves and influences biomass partitioning between the source and sink organs depending on plant species (Makino and Mae,

1999).

Elevated CO₂ has been proven that directly enhances photosynthesis of C₃ plants, which leads to increase carbohydrate production and respiratory pathway, plant growth, biomass, and yield (Ainsworth and Long, 2005; Hao et al., 2013; Reddy et al., 2010). The magnitude of stimulation of photosynthesis by CO₂ enrichment depends on environmental, experimental, and genetic factors, and vary with crops, cultivars and their developmental stages (Leakey et al., 2009). The yield of tuberous and root crops are amongst the most responsive species to elevated CO₂ (Kimball et al., 2002; Miglietta et al., 2000). Such

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<https://doi.org/10.1016/j.plaphy.2018.07.019>

Received 16 March 2018; Received in revised form 24 June 2018; Accepted 16 July 2018

Available online 19 July 2018

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indeterminate crops as potato (*Solanum tuberosum* L.) are expected to respond more strongly to higher levels of CO₂ than determinate crops such as cereals (Lawlor and Mitchell, 1991). Potato and other crops with large below ground sinks for carbon and apoplastic mechanisms of phloem loading are suggested to respond best to elevated CO₂ (Komor et al., 1996). Apoplastic loaders had more flexibility. Given that carbohydrate export was being managed with adjusting by membrane-bound certain transfer proteins (Amiard et al., 2005). Acclimation and up-regulation of photosynthetic capacity under a changing environment were confirmed in pea (*Pisum sativum*), spinach (*Spinacia oleracea*), and *Arabidopsis*, species with apoplastic loading (Adams et al., 2007).

A rise in the atmospheric CO₂ concentration stimulates carbohydrate production, phloem transport, as well as the growth of C₃ plants due to the limitation of the photosynthetic rate under the current level of atmospheric CO₂ (Lemoine et al., 2013). Although elevated CO₂ stimulates photoassimilation rate for a short period of time, long-term exposure to higher CO₂ levels has been reported to suppress photosynthesis by a greater accumulation of photoassimilates (Cheng et al., 1998). Plant grown at elevated CO₂ initially shows stimulated photosynthesis, but this enhancement often does not maintain due to photosynthesis acclimations to the new environment. In spite of the greater buildup of photoassimilates in leaves, e.g. soluble carbohydrates and starch, studies have also suggested that the translocation and utilization of photoassimilates could be promoted at elevated CO₂ (Grimmer and Komor, 1999; Teng et al., 2006). Chloroplasts temporarily accumulate excess photoassimilates as starch grains at elevated CO₂ (Wolfe et al., 1998), therefore, a balanced synthesis and translocation of photoassimilates is important if plant growth and yield is to be enhanced by higher CO₂ concentrations.

Elevated CO₂ can alter the structure of chloroplast and mitochondria (Griffin et al., 2001; Sharma et al., 2014; Sun et al., 2011; Wang et al., 2004). In general, a greater number and size of chloroplast, as the primary site for conversion of CO₂ into photoassimilates, have been reported in different plant species as a result of exposure to higher atmospheric CO₂ concentrations (Sinha et al., 2009; Wang et al., 2004). Wang et al. (2004) found that chloroplast number and photosynthetic rate were increased by a similar magnitude under elevated CO₂ in *Nicotiana sylvestris*, but, by doubling mitochondria number under such conditions, dark respiration increased only by 48%. Less increase in dark respiration compared with mitochondrion numbers have been also reported in earlier studies (Griffin et al., 2001; Wang and Curtis, 2002).

Besides an increase in the number of cellular fine structures, the size of these organelles has also been reported to be affected by elevated CO₂ concentrations (Teng et al., 2006). Xu et al. (2012) concluded that an increase in chloroplast size had been mostly attributed to an increase in the chloroplast width than the length. This increment has been reported to be as a result of larger starch grains accumulated in chloroplast at elevated CO₂ (Madsen, 1971; Teng et al., 2006). However, an increase in both length and width of chloroplast in *Brassica juncea* and *Solanum tuberosum* grown at elevated CO₂ have also reported (Sun et al., 2011; Upreti et al., 2001). An increase in the number and size of starch grains in chloroplast of cucumber (Wei et al., 2002), *Arabidopsis thaliana* (Duan et al., 2014; Teng et al., 2009), *Nicotiana sylvestris* (Wang et al., 2004), and other plants grown at elevated CO₂ have also reported (Hao et al., 2013; Sinha et al., 2009).

Given that applying higher levels of CO₂ to increase yield and quality of agricultural crops has received much attention amongst commercial growers (Li et al., 2013), identifying the sensitive physiological and biochemical processes of plants to elevated atmospheric CO₂ is critical. Agria and Fontane have been widely consumed and are among the most grown cultivars in Iran. A great tuber dry matter of these high-yielding cultivars makes them valuable for industrial consumption, such as French fries and chips production. Understanding how elevated CO₂ affects minituber production would generate valuable information for commercial growers to choose cultivars better responses to such conditions.

There may be some experimental evidence to assess the genetic variation available for improving responsiveness to elevated CO₂ (Ainsworth et al., 2008). Plant species and genotypes significantly vary in the response to environmental conditions, external treatments, and elevated CO₂ (Ahmadi Lahijani et al., 2018; Tang et al., 2013). Enriched CO₂ tends to enhance plant growth and reproduction, though, recognizing intraspecific variation in response to elevated CO₂ can lead to identify the cultivars better respond to such conditions (Lindroth et al., 2001). Manipulation of the existing species, e.g. overexpression of *Suc* transporters in sink cell, would enhance sink demand and photoassimilates export (Ainsworth and Bush, 2011). However, screening and recognizing the cultivars with a higher capacity for translocation of photoassimilates would certainly be simpler and cost-effective.

Given that earlier research have discovered a dramatic rise of mitochondrion numbers in an extensive variety of species at elevated CO₂ (Griffin et al., 2001; Robertson and Leech, 1995), we hypothesized that elevated CO₂ could further rise mitochondrion number in Agria and Fontane leaves. Dark respiration is an important component of the carbon cycle, hence, it is significant to a better understanding of respiratory response to elevated CO₂ and how this is related to cellular fine structural changes at elevated CO₂ (Schimel, 1995). Yet, there is no research concerning the effect of elevated CO₂ on the leaf photosynthetic physiology, biochemistry and ultra-structure, and their relationship with growth and dry matter partitioning in micro-propagated potato plantlets. This study evaluated differences between photosynthetic efficiency and the amount of starch accumulated in chloroplasts of potato cultivars, Agria and Fontane, at elevated CO₂. We also examine the simultaneous trend in chloroplast numbers and photosynthetic rates in addition to mitochondrion numbers and leaf dark respiration rates in response to increased CO₂. In addition, whether there are differences between the cultivars in term of translocation of photoassimilates out of leaves were also evaluated.

2. Materials and methods

2.1. Plant materials and growth conditions

Virus-free potato plantlets (*Solanum tuberosum* L. cvs. Agria and Fontane) were transplanted in a sterile medium containing a mixture of perlite-coco peat (1:1, v:v) in plastic pots (diameter, 15 cm; depth, 30 cm; one per pot). The plantlets were derived from micro-propagated shoot tips grown in agar culture plates containing the Murashige and Skoog medium supplement with 3% sucrose (Yekta Seed Technology Company). The environmental conditions inside the chambers (Convion, Winnipeg, Canada) were as follow: 400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ photosynthetic photon flux density (PPFD) on the leaf surface supplied by fluorescent/incandescent lamps, 12 h photoperiod, 24/16 °C day/night air temperature, and 50 \pm 5% relative humidity. Nine uniform single plantlets of each cultivar (five cm in shoot length) from the first day of transplanting were subjected to 400 \pm 10 $\mu\text{mol mol}^{-1}$ or 800 \pm 10 $\mu\text{mol mol}^{-1}$ as ambient and elevated CO₂, respectively. The CO₂-enriched air (a mixture of ambient air with commercial CO₂) from a compressed gas cylinder was injected into the chamber at a flow rate of 1 l min⁻¹, which was continuously monitored by a calibrated infrared gas analyzer (High-performance CO₂ meter, 77535, China). All plants were watered daily to prevent water stress and fertilized with the standard Hoagland's solution in five-day intervals (Hoagland and Arnon, 1950). The experiment was carried out as a completely randomized design in a factorial (two cultivars and two CO₂ concentrations) scheme with three replications.

2.2. Gas exchange measurements

Net photosynthetic rate (Np) and leaf dark respiration (Rd) were measured using a portable photosynthesis system (HCM-1000, Waltz, Germany) on the youngest mature leaves of each plant at 35, 55, and 75

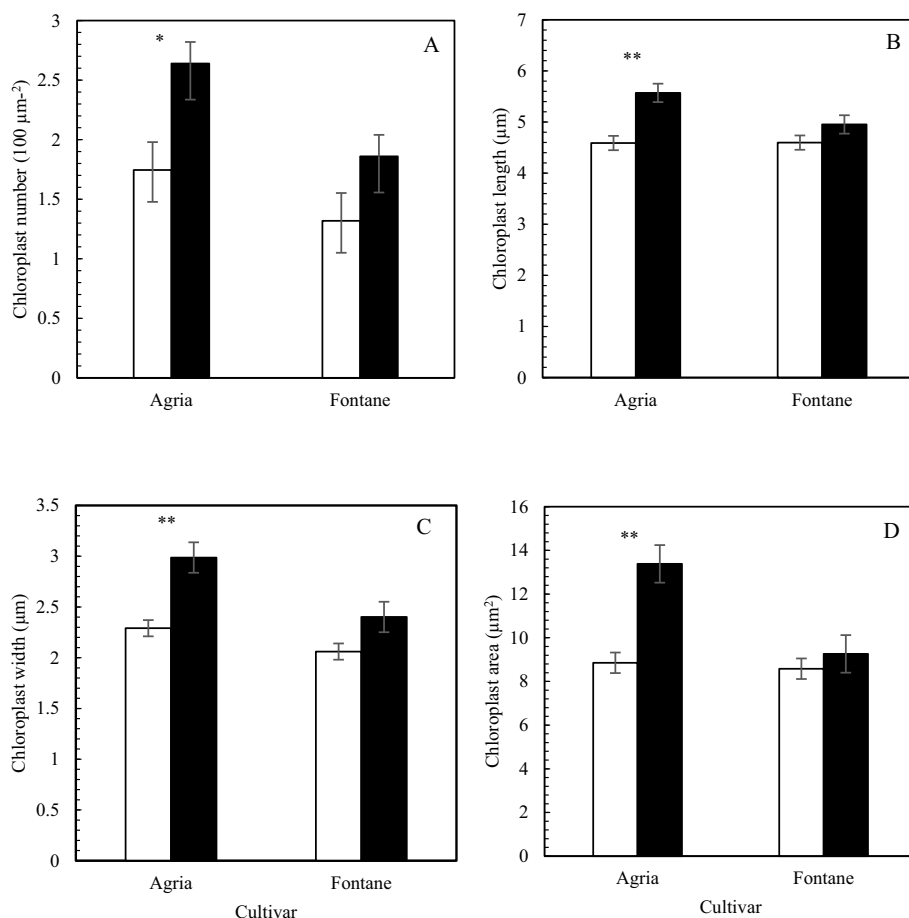


Fig. 1. Chloroplast number (A), Chloroplast length (B), Chloroplast width (C), and Chloroplast area (D) of potato cultivars grown at ambient (open bars) or elevated (closed bars) CO₂. The vertical bars represent the parameter's mean ± S.E. $n = 30$ for each CO₂ concentration. Asterisks indicate significant differences between CO₂ treatments. * and ** indicating $P < 0.05$, and $P < 0.01$, respectively.

days after transplant (DAT). Three leaflets per replication were analyzed ($n = 9$). Net photosynthesis was estimated at a PPFD of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Wang et al., 2004). The leaves were allowed to equilibrate for 5 min at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD before each measurement. Leaf temperature and relative humidity were set to 25°C and $50 \pm 5\%$ inside the cuvette during the measurements, respectively. Leaf dark respiration was obtained by averaging three CO₂ efflux rates at zero PPFD for each plant at the end of the daily dark period from 05:00 to 07:00 h. Leaf temperature was 22°C during the measurement of nighttime Rd. Both photosynthesis and dark respiration were measured at the respective CO₂ the plants were grown to minimize the CO₂ gradient between the cuvette and the outside air. At the same time, chlorophyll *a* fluorescence of the adaxial surface of attached leaves was measured after a 15min-dark period using a handheld PEA Chlorophyll Fluorimeter (Hansatech, UK) at room temperature and ambient CO₂. The actual quantum yield of photosystem II photochemistry, Φ , was calculated as $(F_m' - F_s)/F_m'$, where F_m' and F_s were defined as maximum fluorescence elicited by a saturating light pulse and steady-state chlorophyll fluorescence, respectively (Genty et al., 1989).

2.3. Electron microscopy

Samples from 35-day-old plantlets of the fourth full-expanded leaf from the top were collected, just after the first gas exchange measurement was made. Samples were cut into small slices and immediately fixed in 3% glutaraldehyde (w/v) in 0.05 M potassium phosphate buffer (pH 7.2). The samples were post-fixed in phosphate-buffered 2% osmium tetroxide (w/v) in the same buffer, then, they were dehydrated in a graded acetone series. Afterwards, the samples were set in catalyzed epon (TAAB resin, Energy Beam Sciences, USA). Ultrathin sections were sectioned with a Porter-Blum MT-2 ultramicrotome using a diamond

knife, following that, placed on Formvar-coated copper grids stained with lead citrate and uranyl acetate. The segments of leaf tissue derived from different plants were examined to determine the size, number, and area of chloroplasts and the number of mitochondria per cell. The size, number, and area of starch grains per chloroplast ($n = 35$) were also measured. Observations and photographs were taken using a Leo 912 AB (Zeiss, Germany) transmission electron microscope (TEM) operating at 80 kV. Images were magnified to a final enlargement of $20,000 \times$. The anatomic measurements were assessed by JMicroVision software program. Quantitative data were based on 30 measurements per sample.

2.4. Leaf soluble carbohydrates and starch content

Just after the measurements of the gas exchanges were taken, the same leaves were collected for biochemical analysis. Evaluation of the total soluble carbohydrates in leaves (SC) was assayed by the method of (DuBois et al., 1956). 100 mg leaf fresh weight was homogenized in 70% methanol using a mortar and pestle. Total soluble carbohydrates content of leaflets were measured using a glucose standard curve. To assay the starch content of leaves (ST), the method of (Sheligi, 1986) was used. The residuals of the soluble carbohydrate experiment were washed three times using Perchloric acid. Absorbance was measured by spectrophotometer at 485 nm. The starch content of leaves was determined through a glucose standard curve.

2.5. Growth parameters

The plants were harvested, rinsed and separated into stems, leaves, and tubers ($n = 9$) at 90 DAT. The above and below-ground parts of the plants were weighted and then, dried out at 75°C until constant mass

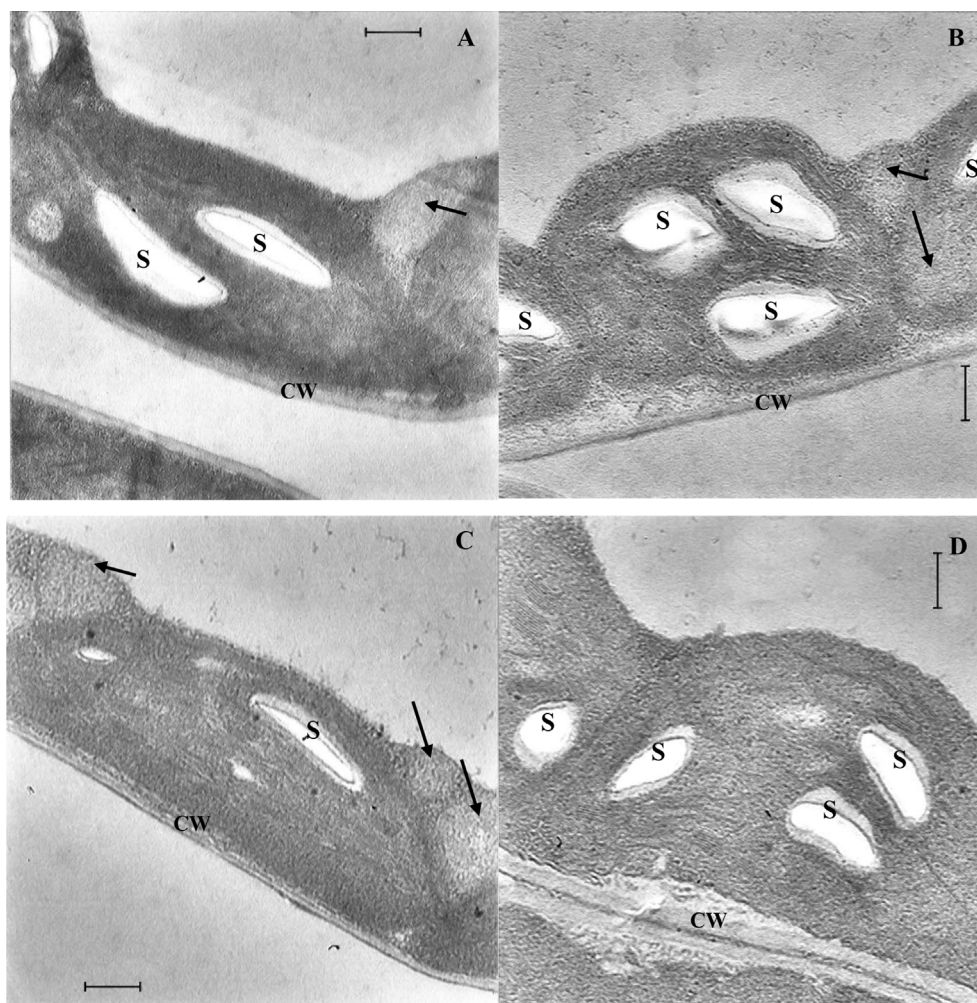


Fig. 2. Chloroplasts and mitochondria in leaf mesophyll cells of *Solanum tuberosum* L. grown at ambient (400 ppm) or elevated (800 ppm) CO₂. (A) Agria Ambient, (B) Agria Elevated, (C) Fontane Ambient, (D) Fontane Elevated. Note the different starch grains in leaf cells of two cultivars. The starch grain area in Agria was significantly greater than Fontane at both CO₂ concentrations. S: starch grains, CW: cell wall. The arrows indicate mitochondria. 1 bar = 640 nm.

and weighed. Dry matter (DM) partitioning among the plant parts was calculated as a percentage of DM accumulated in the leaves, stems, and tubers in relation to the whole plant DM. Specific leaf weight (SLW) was calculated as follows:

$$SLW = LDW/LA \quad (1)$$

Where LDW and LA are leaf dry matter and leaf area, respectively.

2.6. Statistical analysis

The experiment was carried out as a completely randomized design in a factorial (two cultivars and two CO₂ concentrations) scheme with three replications (n = 9). All experimental data were analyzed by analysis of variance at 0.05 probability level using SAS System 9.1. Comparisons of means for CO₂ levels and cultivars were made by Tukey's Studentized Range (HSD) Test at 0.05 probability level.

3. Results

3.1. Cellular fine structure

Elevated CO₂ significantly increased the chloroplast length and width in Agria by 17 and 23%, respectively. However, the differences in Fontane were not significant (Fig. 1). The number of chloroplast per 100 μm² cell area was significantly increased in Agria at elevated CO₂,

nevertheless, the number of mitochondria per 100 μm² cell area was increased in both cultivars (Fig. 3). Also, the ratio of chloroplast to mitochondria was decreased at the elevated CO₂ in both cultivars (Fig. 3). There were 1.14 and 1.23 chloroplasts for each mitochondrion in Agria and Fontane at the ambient CO₂, respectively; nonetheless, the numbers were decreased to one for both cultivars at the elevated CO₂.

While the number of starch grains per chloroplast were significantly increased in the leaf cells of Fontane, it was unaffected in Agria by the elevated CO₂ (Figs. 2 and 4). The area of starch grains was significantly increased by 42 and 35% in Agria and Fontane at the elevated CO₂, respectively (Fig. 4), nevertheless, the ratio of starch to chloroplast area was enhanced by 33 and 66% in Agria and Fontane at the doubled CO₂, respectively (Fig. 3). The chloroplasts in the elevated CO₂ plants were filled with starch grains by 17 and 13%, but, it was 11 and 4% at the ambient CO₂ in Agria and Fontane, respectively (Fig. 2). The most striking impact of higher CO₂ concentration was the significantly greater starch width in the elevated CO₂ plants, at which significantly widened the chloroplasts in Agria, but not in Fontane. The elevated CO₂ significantly increased the starch width by 45 and 41% in Agria and Fontane, respectively, however, starch length remained unaffected by higher CO₂ concentration (Figs. 2 and 4).

3.2. Gas exchange

The results of analysis of variance showed that gas exchange

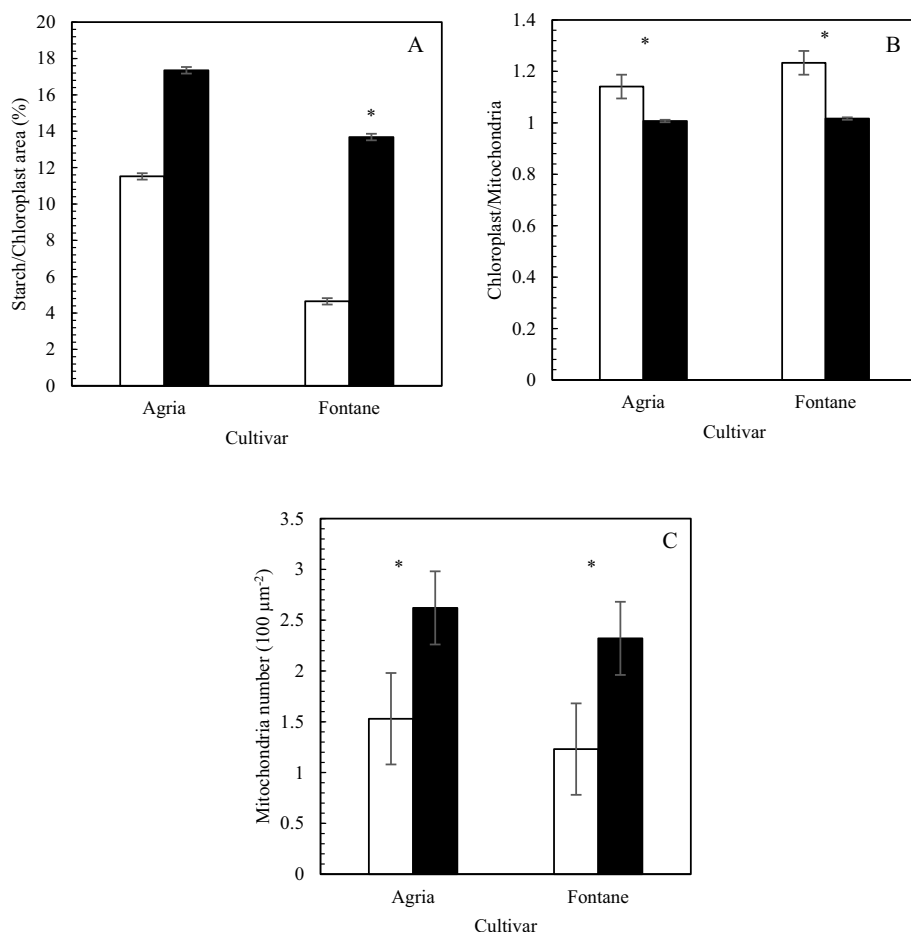


Fig. 3. Starch to chloroplast area ratio (A), chloroplast to mitochondria ratio (B), and mitochondria number (C) of potato cultivars grown at ambient (open bars) or elevated (closed bars) CO₂. The vertical bars represent the parameter's mean \pm S.E. $n = 30$ for each CO₂ concentration. Asterisks indicate significant differences between CO₂ treatments. * and ** indicating $P < 0.05$, and $P < 0.01$, respectively.

variables were influenced by cultivar, CO₂ concentration, and their interactions at different sampling dates (Table 1). The elevated CO₂ significantly increased Np during the growth season (Fig. 5). The highest Np was recorded during the first measurement date (35 DAT) by 36 and 30% higher than the control in Agria and Fontane at the elevated CO₂, respectively, just before the samples were taken for electron microscopy. The results showed that Np of the ambient plants was decreased over the growth season, in which the lowest Np was observed at 75 DAT, but, the elevated CO₂ enhanced Np compared to the ambient at 75 DAT.

Leaf dark respiration (Rd) was significantly increased by 31 and 27% at 35 DAT in Agria and Fontane at the elevated CO₂, respectively (Fig. 5). The lowest leaf Rd was recorded on the first day of measurement, but it was dramatically increased afterwards. Even though the greatest Rd was observed at 55 DAT in both cultivars, there was no difference between two CO₂ treatments. The ratio of Np to Rd was changed throughout the growth season (Fig. 5). The relationship of Np and leaf Rd showed little difference in the first measurement date for both cultivars, though, it was increased over time in Agria (Fig. 5). The ratio of Np to Rd was increased by 43 and 64% at 55 and 75 DAT in Agria at the elevated CO₂, respectively, but it was increased by 38% only at 75 DAT for Fontane under such conditions. Growth at the ambient or elevated CO₂ had no influences on the quantum yield (Φ), therefore, no photosynthetic acclimation was observed in the elevated CO₂ plants.

Even though, the number of chloroplasts and Np were increased with the same magnitude (Figs. 1 and 5), the number of mitochondria and Rd increased variously (Figs. 3 and 5). Chloroplast number and Np were increased by 33 and 36% in Agria, and 35 and 31% in Fontane at the doubled CO₂, respectively. On the other hand, mitochondria

number increased by 41 and 46% in Agria and Fontane, respectively, but the leaf Rd was slightly increased by 31 and 27% in Agria and Fontane, respectively, at the doubling CO₂, on average 51% lower than the mitochondria number enhancement.

3.3. Leaf soluble carbohydrates and starch

Cultivar, CO₂ concentration, and their interaction affected leaf soluble carbohydrates and starch content at different sampling dates (Table 1). Leaf soluble carbohydrates content (SC) showed a similar seasonal pattern to Rd, even though, SC was not affected by elevated CO₂ except from the last measurement date (Fig. 6). At 75 DAT, however, SC of the elevated CO₂ of Agria was increased by 30% compared to the control. A sharp increase in SC occurred at 55 DAT at both CO₂ concentrations compared to the first measurement date, but it reversed and started to decrease afterwards (Fig. 6). Biochemical analysis revealed that the CO₂ elevation significantly increased ST during the experiment (Fig. 6). The same pattern of SC was also observed for leaf starch content (ST). Greater ST was observed in Agria to Fontane over the growth season, but, there was no significant difference between the cultivars except from the second measurement date at the ambient CO₂. Leaf starch content also showed similar changes throughout the growth season to Rd, so that the greatest ST was recorded at 55 DAT.

3.4. Dry matter partitioning

The results of analysis of variance of the growth parameters is shown in Table 2. The elevated CO₂ significantly increased dry matter (DM) allocation to different parts of the plants (Fig. 7). Tuber dry matter was increased in Agria (by 62%) more than Fontane at elevated

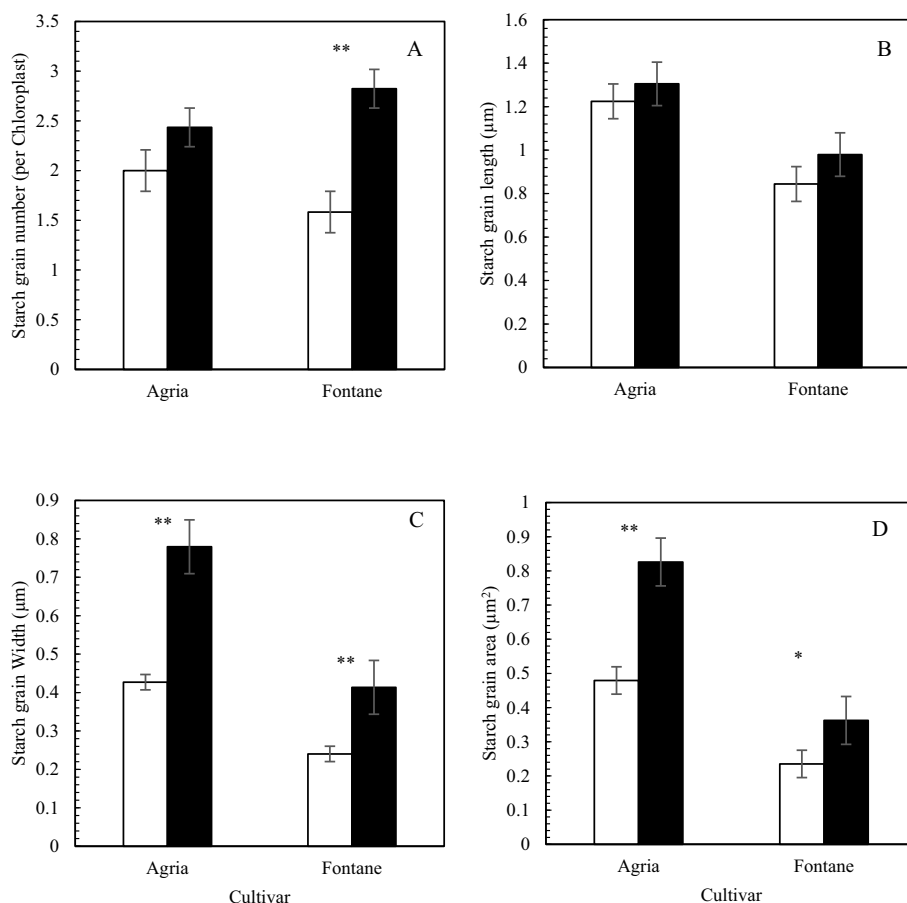


Fig. 4. Starch number (A), Starch length (B), Starch width (C), and Starch area (D) of potato cultivars grown at ambient (open bars) or elevated (closed bars) CO₂. The vertical bars represent the parameter's mean ± S.E. $n = 30$ for each CO₂ concentration. Asterisks indicate significant differences between CO₂ treatments. * and ** indicating $P < 0.05$, and $P < 0.01$, respectively.

Table 1

ANOVA results of gas exchange and biochemical parameters at 35, 55, and 75 days after transplanting.

Variables	Cultivar (V)	CO ₂ concentration (C)	V × C	C.V.
35 DAT				
Leaf soluble sugars (SC)	NS	NS	NS	8.1
Leaf starch content (ST)	NS	*	NS	13.2
Net photosynthesis rate (Np)	**	**	**	11.2
Dark respiration (R _D)	NS	**	NS	16.4
Quantum yield (Q)	NS	**	NS	1.1
55 DAT				
Leaf soluble sugars (SC)	NS	NS	NS	10.6
Leaf starch content (ST)	**	**	*	7.7
Net photosynthesis rate (Np)	**	**	NS	9.8
Dark respiration (R _D)	NS	**	NS	16.4
Quantum yield (Q)	NS	NS	NS	2.3
75 DAT				
Leaf soluble sugars (SC)	NS	*	*	10.3
Leaf starch content (ST)	NS	**	NS	6.6
Net photosynthesis rate (Np)	**	**	*	13.6
Dark respiration (R _D)	NS	NS	NS	16.7
Quantum yield (Q)	NS	NS	NS	0.4

*Significant at $p < 0.05$, **Significant at $p < 0.01$, NS, not significant. DAT, day after transplanting, 35 DAT (stolonization), 55 DAT (tuberization), and 75 DAT (tuber bulking).

CO₂, but, Fontane slightly allocated more DM to the tubers in both CO₂ treatments. Dry matter of stem was significantly increased by 36% in Fontane at doubled CO₂ concentration. However, there were no differences between two cultivars. The elevated CO₂ increased leaf dry

matter of cultivars compared to the control. Nevertheless, SLW was not affected by the elevated CO₂, Agria showed slightly greater SLW than Fontane at both CO₂ levels (Fig. 7).

4. Discussion

The elevated CO₂ plants showed higher Np compared to the control. The improvement of photosynthesis at elevated CO₂ is an outcome of the carboxylation rate and inhibition of oxygenation of Rubisco, yet the effects of CO₂ may vary with plant species, CO₂ levels, developmental stage, and environmental conditions (Hao et al., 2013). Higher photosynthesis of plants at elevated CO₂ has also been reported by other researchers (Ainsworth and Long, 2005; Hao et al., 2013), which could be due to adjustment of the photosynthetic apparatus, such as chloroplast number, to such conditions. The results revealed that the higher Np was accompanied by a greater chloroplast number per unit cell area, although, there were no significant differences in Fontane. The enhancement of the chloroplast number and Np occurred with a similar magnitude. One possible reason for the greater chloroplast number of plants grown at higher CO₂ has been assumed to be stimulation of chloroplast biogenesis (Bockers et al., 1997; Wang et al., 2004).

Since the greater size of chloroplasts was as a result of greater starch grain size, it seems the number of chloroplasts per unit cell area had a greater impact on photosynthesis. A close relationship between chloroplast number and photosynthesis has been also reported by Wang et al. (2004). Our results revealed that chloroplast to mitochondrion ratio was decreased by 11 and 17% in Agria and Fontane, respectively, by doubling the CO₂ concentrations, suggesting that less chloroplast per mitochondrion was required to meet a higher Np under such conditions. Therefore, it might be concluded that the efficiency of photosynthetic apparatus increased in the elevated CO₂ grown plants.

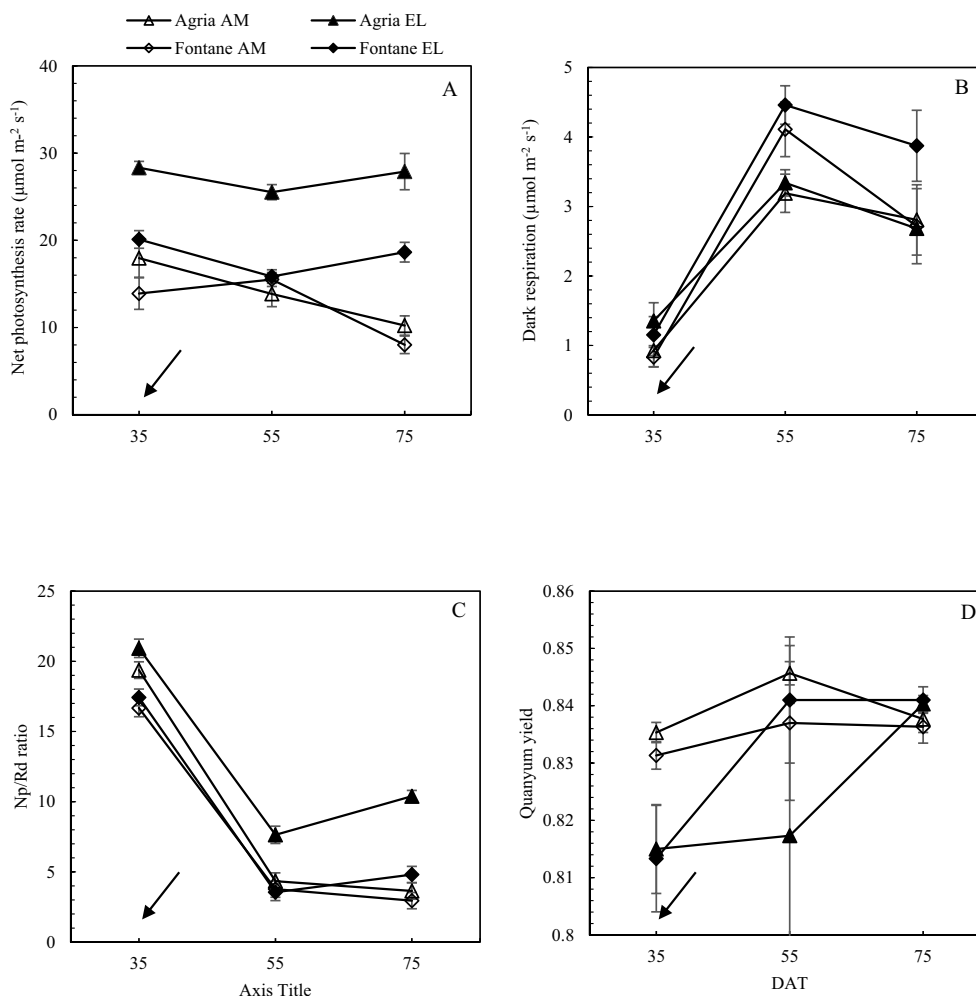


Fig. 5. Net photosynthetic rate (A), Dark respiration (B), Net photosynthesis to dark respiration ratio (C), and Quantum yield (D) of potato cultivars grown at ambient (open symbols) or elevated (closed symbols) CO₂. AM: 400 ppm CO₂, EL: 800 ppm CO₂. Arrows indicate sampling day for electron microscopy (EM). The vertical bars represent the parameter's mean \pm S.E. $n = 9$.

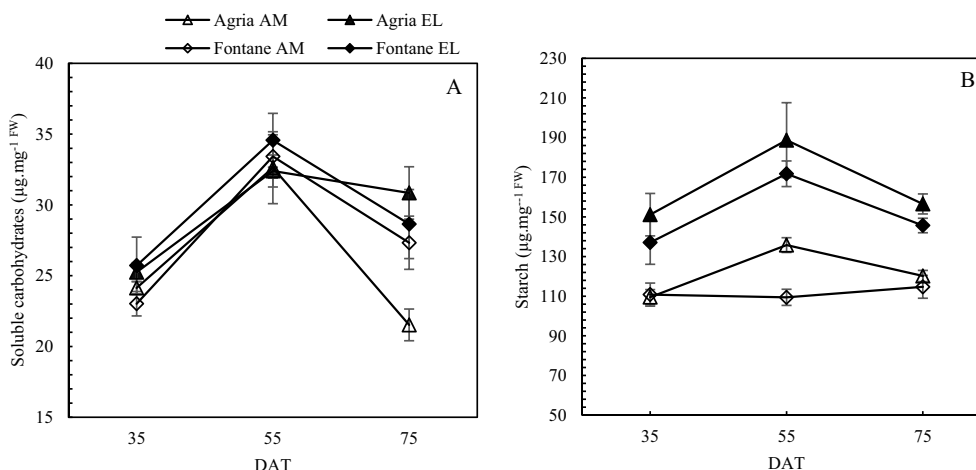


Fig. 6. Leaf soluble carbohydrate (A) and starch content (B) of potato cultivars grown at ambient (open symbols) or elevated (closed symbols) CO₂. AM: 400 ppm CO₂, EL: 800 ppm CO₂. Arrows indicate sampling day for electron microscopy (EM). The vertical bars represent the parameter's mean \pm S.E. $n = 9$.

The greater starch accumulation altered the shape of chloroplast within leaf mesophyll cells of potato plantlets at the elevated CO₂, nevertheless, the changes were not significant in Fontane. In Agria, the area of chloroplasts was expanded due to a greater area of the starch grains. It has been observed that a greater starch accumulation altered

cell ultrastructure, e.g. swelling of thylakoids (Utriainen et al., 2000), separation and distortion of internal membrane system in chloroplasts (Sinha et al., 2009), changes in the amount of stroma thylakoid (Griffin et al., 2001), and widened chloroplasts (Teng et al., 2006; Wang et al., 2004). However, no changes in the shape of chloroplasts within

Table 2
ANOVA results of growth parameters.

Variables	Cultivar (V)	CO ₂ concentration (C)	V × C	C.V.
Leaf dry matter (LDM)	**	**	NS	10.3
Stem dry matter (SDM)	NS	**	NS	14.3
Tuber dry matter (TDM)	NS	**	NS	25.5
Specific leaf weight (SLW)	NS	NS	NS	8.6

*Significant at $p < 0.05$, **Significant at $p < 0.01$, NS, not significant.

mesophyll cells of young wheat leaves at elevated CO₂ have also been reported (Robertson and Leech, 1995). It assumed that the different fine cell structure responses to higher CO₂ concentration may be due to the different genetic background of species, length of exposure to elevated CO₂, or the time of taking samples for electron microscopy (Wang et al., 2004).

Even though, the starch grains occupied lower than 11 and 4% of the chloroplast area in Agria and Fontane at the ambient CO₂, respectively, the elevated CO₂ enhanced the greater occupation of chloroplast by starch grains by 17 and 13% in Agria and Fontane, respectively. Earlier studies have also discovered that CO₂ enrichment triggered a greater buildup of starch in mesophyll cells (Hao et al., 2013; Oksanen et al., 2001; Utriainen et al., 2000; Wang et al., 2004). In contrast, our results demonstrated that the greater impact of elevated CO₂ on chloroplasts size caused less effect on starch:chloroplast ratio. However, Wang et al. (2004) found that starch grains were occasionally detected in ambient CO₂ *N. sylvestris*, due to the differences in plant species and the time of sampling for electron microscopy.

The average leaf Rd was increased by 29%, which was 51% less compared to the increase of the mitochondria number at the elevated CO₂. This result is consistent with Wang et al. (2004) which found more

than a doubling of the mitochondria number in *N. sylvestris* at elevated CO₂, while leaf dark respiration increased by a much smaller magnitude at elevated compared to ambient CO₂. Working on nine plant species, Griffin et al. (2001) also found that mitochondria number increased by 30–140% when CO₂ concentration elevated. Larger number of less-efficient and non-functional mitochondria (Wang et al., 2004), less CO₂ efflux as a result of more re-fixed CO₂ through enhanced PEP-carboxylase (Amthor, 1997; Griffin et al., 1999), and reduced activity of cytochrome C oxidase and succinate dehydrogenase (González-Meler et al., 1996; Palet et al., 1991; Reuveni et al., 1995) are amongst the feasible reasons for the various magnitude of enhancement between mitochondrion number and dark respiration rate at elevated CO₂. Mitochondrion is the major organelle providing required ATP for cell growth and maintenance. Given that the size of mitochondria has been widely reported to show no changes at elevated CO₂ (Griffin et al., 2001, 2004; Wang et al., 2004), it seems the number of mitochondria is the determinate factor to influence Rd. The metabolic activity of the tissue is directly pertaining to mitochondria number in a plant cell; therefore, cells with a greater demand for energy have a greater mitochondria number (Moyes and Battersby, 1998; Taiz and Zeiger, 2012; Weibel et al., 1992).

It has been reported that leaf dark respiration stimulated over the growth period at elevated CO₂, which indicates higher demand for maintenance and survival as a result of sped-up senescence (Donnelly et al., 2001; Vandermeiren et al., 2002). Energy demand and substrate supply are able of controlling dark respiration rate (González-Meler et al., 2009). One explanation for increasing Rd over the growth period might be greater carbohydrate content, which provides more substrates for dark respiration in the experiment. A common consequence of plants grown at elevated CO₂ is a higher production of photoassimilates and a higher concentration of carbohydrates in the plant tissues. Li

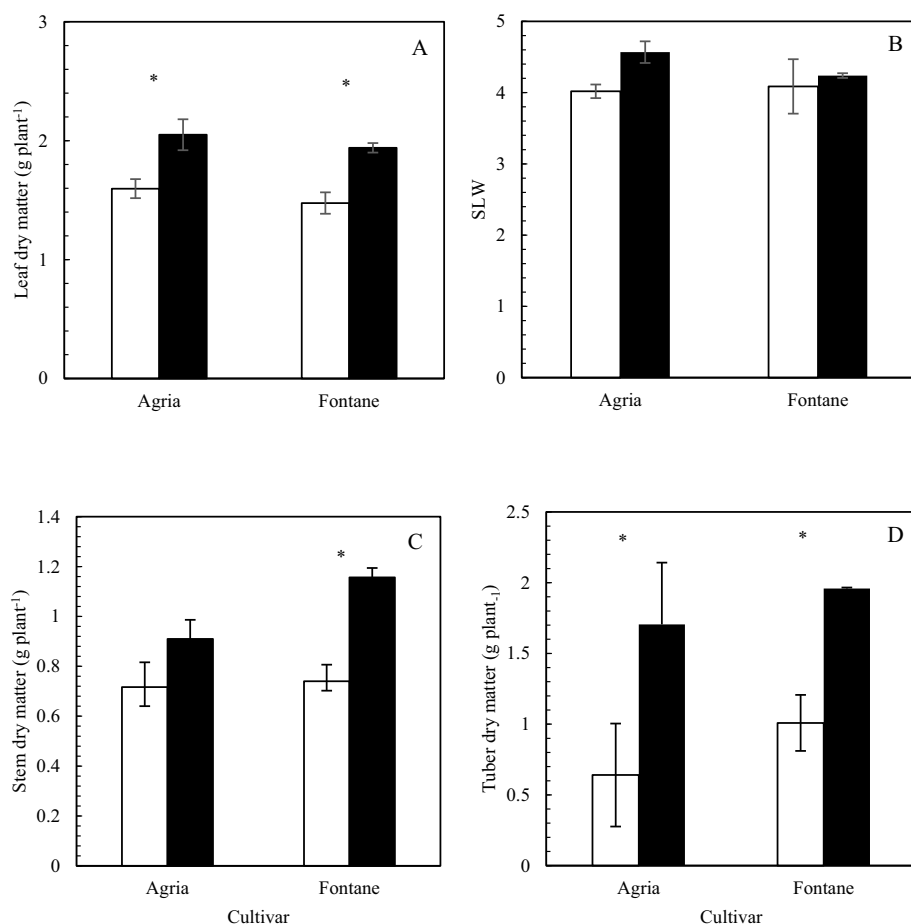


Fig. 7. Leaf dry matter (A), specific leaf weight (B), stem dry matter (C), and tuber dry matter (D) of potato cultivars grown at ambient (open bars) or elevated (closed bars) CO₂. The vertical bars represent the parameter's mean \pm S.E. $n = 9$ for each CO₂ concentration. Asterisks indicate significant differences between CO₂ treatments. * and ** indicating $P < 0.05$, and $P < 0.01$, respectively, according to Tukey's post hoc test.

et al. (2013) also reported a significant increase in the leaf carbohydrate content and dark respiration in tomato plants when exposed to elevated CO₂.

Np/Rd ratio tended to decline as the potato plants grew over time, that might be due to higher Rd, although elevated CO₂ enhanced the ratio specially in Agria compared to the ambient. Wang et al. (2004) found that the A/Rd ratio was slightly affected by elevated CO₂ in *N. sylvestris*. They found that while the photosynthesis remained relatively unchanged, leaf dark respiration at elevated CO₂ was greatly decreased to ambient CO₂ throughout the growing season. It is also consistent with our results that reported, although, Np did not dramatically vary for each cultivar during the growing season, there was higher dark respiration at elevated to the ambient CO₂. It might demonstrate more efficient CO₂ assimilation at the elevated compared to ambient CO₂. Dark respiration has been reported to have significant variation compared to photosynthesis for both herbaceous as well as woody species over time (Curtis et al., 2000; Poorter et al., 1988; Wang et al., 2004; Wang and Curtis, 2002). The dynamic relationship between photosynthesis and dark respiration, which lead to more efficient CO₂ assimilation, will have an crucial impact on carbon cycling in terrestrial ecosystems (Wang et al., 2004).

The results showed no significant changes in Φ as a result of photosynthetic acclimation at the elevated CO₂. Our result is in accordance with the results of Hao et al. (2013), which observed no photosynthetic acclimation in medicinal plant *I. indigotica* at elevated CO₂ due to developed new carbon sinks. A significant increase in photosynthetic rate of potato leaves was observed at elevated CO₂ (Donnelly et al., 2001), however, Lawson et al. (2001) and Katny et al. (2005) reported that a long term exposure to such conditions resulted in photosynthetic acclimation and then, decrease photosynthesis. Chen and Setter (2012) concluded that cell division in sink organs is highly responsive to higher levels of CO₂ and it triggers attraction of more photoassimilates. Nonetheless, previous studies have well-documented photosynthetic acclimation of a wide range of C₃ plant species at elevated CO₂ (Aranjuelo et al., 2011; Hao et al., 2012; Huang et al., 2003; Seneweera et al., 2011; Sicher et al., 1995), it has been stated that the different response of plants to higher levels of CO₂ could be related to plant species, cultivars, developmental stages, and environmental conditions (Ainsworth and Bush, 2011; Hao et al., 2012). One possible reason for photosynthetic acclimation is the equal day and night hours, at which the plants had enough time to re-allocate photoassimilates toward underground parts. Stutte et al. (1996) found that higher Np in potato plants was recorded under 12/12 day/night photoperiod, but by reducing the night hours to 6 h, photosynthesis rate substantially decreased. They concluded that the shorter night did not allow photoassimilates move properly out of the leaves. In addition, such crops as potato with large belowground organs to store carbon (Farrar, 1996) and apoplastic loading for photoassimilates (Komor et al., 1996) have known as the best alternatives for a wide response to elevated CO₂.

We observed that leaf starch content significantly increased at elevated CO₂. This is consistent with Katny et al. (2005) who found a dramatic increase in starch content of young leaves of potato plants at elevated CO₂. The greatest carbohydrates and starch content were recorded at 55 DAT, which led to a lower Np and higher Rd at both CO₂ conditions. At this stage, tubers have not formed properly yet and do not have enough capacity to store assimilates, therefore, lack of sufficient attraction for photoassimilates caused extra soluble carbohydrates and starch be stored in the leaves resulting in a negative feedback on the photosynthesis. Accumulation of carbohydrates in source leaves enhance the expression of genes involved in carbohydrate storage, and it suppresses photosynthetic gene expression (Stitt et al., 2010). The results of different experiments on the source-sink relationship revealed that photosynthesis and utilization of carbohydrates by sinks are tightly coordinated (Kaschuk et al., 2010; Paul and Foyer, 2001). Davey et al. (2006) found that fast-growth poplar (*Populus* spp.) trees are capable to maintain a higher photosynthesis rate at elevated CO₂ by exporting

more than 90% of photoassimilates throughout the day. Therefore, upkeep of a higher photosynthesis rate at elevated CO₂ is directly determined by the capacity of sinks to attract, utilize, or store more carbohydrates (Leakey et al., 2009). By the beginning of the tuber bulking (at 75 DAP) and increasing the sinks demand for the photoassimilates, the carbohydrates content was decreased. This indicated that the magnitude of photosynthesis under the elevated CO₂ varies with potato cultivars and developmental stages. It has been elucidated that over-expression of sucrose transporters in sink cells would enhance sink demand at elevated CO₂, and alleviate negative feedback of carbohydrates accumulation on photosynthesis (Ainsworth and Bush, 2011).

The results of the biochemical analysis showed that Fontane had lower leaf starch content compared to Agria at both CO₂ concentrations. Also, the results of electron microscopy revealed that Fontane had lower starch grain size compared to Agria. The lower starch content in Fontane was associated with a greater stem and tuber dry matter. Since the samples for biochemical analysis were taken soon after the dark period, the lower leaf starch content and greater tuber dry matter were likely indicating the better translocation of the photoassimilates from leaves toward underground parts, especially tubers in Fontane. Working on 94 Arabidopsis accessions, Sulpice et al. (2009) found that the starch content at the end of the light period was negatively correlated with biomass accumulation. This observation suggests that plants with greater growth rate have better carbon use efficiency, in contrary, plants that conserve more starch have lower growth rates (Ainsworth and Bush, 2011). Hence, it seems that Agria with higher leaf starch content and lower tuber dry matter has a more conservative starch-accumulating strategy, and slightly greater SLW also confirms that this cultivar transported fewer carbohydrates out of the leaves.

5. Conclusion

The greater Np/Rd ratio at the elevated CO₂ grown plants likely indicated a higher efficiency of photosynthesis. Although, chloroplast number and Np were increased by the same magnitude at the elevated CO₂ in both cultivars, the average number of mitochondria was increased by 51% greater than Rd. Although, the starch grain area was greater in Agria, the starch number was more increased in Fontane in comparison to Agria at elevated CO₂. The lower starch content associated with greater tuber dry matter suggested that Fontane transported more photoassimilates out of the leaves. In the future globe with higher CO₂ concentration, increasing the capacity for utilization and transportation of photoassimilates would be of a great importance to alleviate negative feedback on photosynthesis, and to stimulate biomass and yield. Considering that there were differences between the cultivars in transportation and partitioning of dry matters, it might be able to benefit from these variations to improve the productivity of crops and identifying cultivars better responded to elevated CO₂. However, Fontane demonstrated a more efficient photoassimilate translocation system at the elevated CO₂.

Acknowledgment

This work was supported by the Ferdowsi University of Mashhad financially and technologically. We thank Dr. Xianzhong Wang for technical revision of the manuscript.

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