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Effects of CO_2 elevation and irrigation regimes on leaf gas exchange, plant water relations, and water use efficiency of two tomato cultivars

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ABSTRACT

We investigated the effects of elevated CO_2 concentration ([CO_2]), different irrigation regimes, and their interactions on leaf gas exchange, water relations, biomass production, and water use efficiency in tomato plants. In spring 2014, two tomato cultivars (CV1, which is potentially drought tolerant, and CV2 which is potentially heat tolerant) were grown in two separate greenhouse cells at [CO_2] of 380 and 590 μmol L^{-1} (ppm) located at the experimental farm, Taastrup, Denmark. Plants were either irrigated to 18% of volumetric soil water content (FI, full irrigation), or irrigated with 70% water of the fully-irrigated control, delivered to either the whole pot (DI, deficit irrigation) or alternately to only half of the pot (PRD, partial root-zone drying). The experiment was a completed factorial design with four replications per treatment. The two cultivars showed a similar response to soil water deficits, but their water consumption responded differently to high [CO_2]. Intrinsic water use efficiency (WUE_i, photosynthetic rate/stomatal conductance) and plant water use efficiency (WUE_p, aboveground biomass/plant water use) were both significantly increased by reduced irrigation treatments and elevated [CO_2], although no significant reduction of stomatal conductance was detected under high [CO_2]. There was a positive interaction between CO_2 enrichment and water deficits on plant water use efficiency. Root water potential was negetatively affected by reduced irrigation but positively influenced by elevated [CO_2], while leaf water potential was significantly decreased only by reduced irrigation. CO_2 enrichment increased flower number without affecting fruit number, thereby reducing fruit set. Reduced irrigation in combination with elevated [CO_2] caused a significant improvement in plant water use efficiency in both tomato cultivars.

1. Introduction

The patterns of changes in climate and the mechanisms driving plant responses to such changes are important for the development of agricultural practices and crops that are better adapted to future growing conditions (Ainsworth et al., 2008). It is predicted that atmospheric CO_2 concentration ([CO_2]) will rise globally to 550 ppm in the middle of the present century (Kirtman et al., 2013). On the other hand, the short- and long-term projected changes in temperature and precipitation patterns show a great regional – and sometimes seasonal – variability (Kirtman et al., 2013). Mid-latitude regions, including large parts of Europe, are likely to experience reductions in summer precipitation and increases in temperature, which will result in higher frequency of seasonal drought (Christensen et al., 2007). Therefore, an understanding of plant responses to rising [CO_2] and limited water availability is necessary for maximizing crop yield and quality under future climate scenarios.

Declining freshwater resources have stimulated research into developing novel irrigation strategies to use the water more efficiently. Alternate partial root-zone drying irrigation (PRD) and deficit irrigation (DI) are water-saving irrigation techniques being intensively studied in many regions on different crop species, including tomatoes (Sun et al., 2013). Deficit irrigation consists of delivering plants a reduced amount of water (typically 30–50% less) relative to full irrigation (FI) that usually compensates 100% plant evapotranspiration (Sezen et al., 2008). Although photosynthetic rates are often lowered in deficit-irrigated plants (Liu et al., 2006), the reduction of irrigation water generally improves water use efficiency (WUE), either at the stomatal level (photosynthetic

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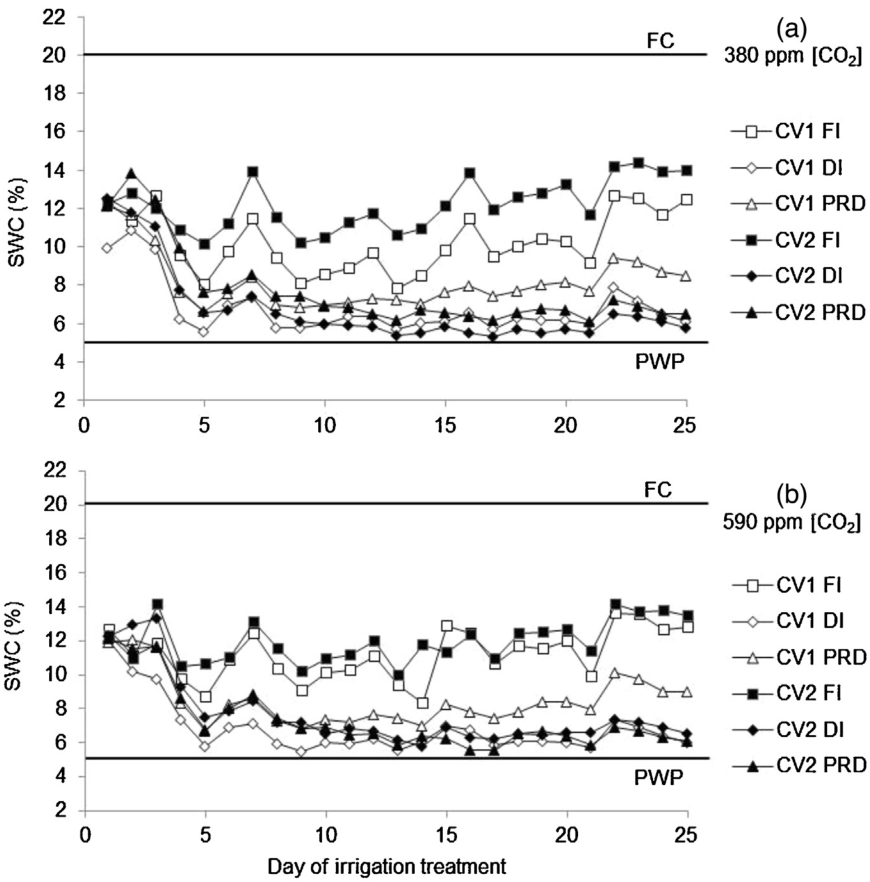

Fig 1. Daily volumetic soil water content (SWC, %) before irrigation in the pots of the two tomato cultivars exposed to three irrigation regimes (FI, DI, and PRD) and two atmospheric [CO_2] (380 and 590 ppm) treatments. FC indicates field capacity and PWP denotes permanent wilting point.

rate/stomatal conductance, WUE_i) or the plant level (aboveground biomass/plant water use, WUE_p). Indeed, stomatal conductance (g_s) is more sensitive than photosynthetic rate (A_n) to decreases in water availability, leading to higher WUE_i under moderate soil water deficits (Liu et al., 2005). Similarly, under mild and moderate droughts, the proportional reduction in biomass production is often consistently lower than that in delivered water, and WUE_p is therefore increased (Liu et al., 2006).

PRD is an irrigation strategy based on the delivery of water (usually 50–70% of the full irrigation requirements) to only one half of the root system, while the other is left to dry. The irrigated side is generally alternated during the treatment in order to avoid death of the roots, and to maintain root-to-shoot signaling (Liu et al., 2008). The intensity of drought-signaling was reported to be larger in PRD than in DI for various species, and many studies describe benefits of PRD relative to DI in increasing WUE (Wang et al., 2010). The alternation of drying and re-wetting cycles in the soil under PRD leads to increased root production (Liu et al., 2006; Mingo et al., 2004) and to an increased release of inorganic N into the soil solution (Birch effect—Birch 1958) resulting in beneficial effects on plant N nutrition (Wang et al., 2010; Wang et al., 2013).

A number of experiments have been conducted in this sense during the last two decades, involving different CO_2 concentrations and ranges of irrigation reduction (*e.g.* Fleisher et al., 2013; Nackley et al., 2014; Xu et al., 2013). The increase in internal CO_2 concentration (C_i) caused by a rise in ambient [CO_2] leads to a reduction in stomatal aperture (Ainsworth and Rogers, 2007), and a positive interaction between elevated [CO_2] and drought in decreasing g_s is generally reported (Kang et al., 2002). In C_3 plants, an increase in atmospheric [CO_2] increases the CO_2:O_2 ratio at the chloroplast, improving the efficiency of net carbon gain through acceleration of the carboxylation reaction and inhibition of the oxygenation reaction (Ogren, 2003). Significant enhancement of photosynthesis and growth by elevated [CO_2] occurs in C_4 plants only under water stress conditions (Conley et al., 2001; Leakey et al., 2006; Morgan et al., 2011), and in C_3 plants the relative stimulation under drought is generally larger than in well-watered conditions (*e.g.* Poorter and Pérez-Soba 2001). Indeed, CO_2 enrichment may extend assimilation periods during moderate (Nackley et al., 2014) and temporary (Vu and Allen 2009) drought. Therefore, elevated [CO_2] may further increase WUE of plants under reduced irrigation (Kumar et al., 2014).

The objective of this study was to investigate the independent and combined effects of CO_2 enrichment and reduced irrigation on two tomato cultivars with potentially different responses to drought and heat stress. Three different irrigation regimes (FI, DI, and PRD) in combination with two CO_2 concentrations (380 and 590 ppm) were investigated for the two tomato cultivars. We hypothesized that both reduced irrigation and elevated [CO_2] will increase WUE, and that the combination of the two factors would further enhance WUE of tomato plants.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted from March to June 2014 at the experimental farm of University of Copenhagen located in Taastrup, Denmark (55N40′6.61″; 12E18′25.62″). Tomato plants were grown in two cells (50 m^2 each) of a recently built greenhouse, designed to allow control of temperature, humidity, and CO_2 concentration, and the use of supplementary lights. The CO_2 concentration in the greenhouse cells was monitored every six seconds by a CO_2 Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland). The climatic conditions in the greenhouse cells were detailed by Zhu et al. (2016). Briefly, the two glasshouse cells set at $25/16 \pm 2\,^\circ C$ day/night air temperature, 60% relative humidity, 16 h photoperiod, and >500 $\mu mol\,m^{-2}\,s^{-1}$ photosynthetic active radiation (PAR) supplied by sunlight plus meta-halide lamps.

Two tomato (*Solanum lycopersicum* L.) cultivars were studies: one potentially drought tolerant (ST 22, CV1 hereafter), and one thought to be heat tolerant (ST 52, CV2). The seeds were provided by SEAN Seed Service Centre Ltd., Kathmandu, Nepal. On the 7th March, 300 seeds were sown in 100 pots (0.5 L). 50% germination was reached in 7 days by CV1 and in 8 days by CV2. As seedlings emerged, only the most robust one was left to grow in each pot. On the 7th April, the 24 healthiest plants for each cultivar (total 48 plants) were transplanted into pots with 10 L capacity (diameter 17 cm; depth 50 cm), filled with approximately 14.5 kg of air-dried soil. The soil used is classified as sandy loam, with a pH of 6.7, total C 10.3 $g\,kg^{-1}$, total N 1.0 $g\,kg^{-1}$, $NH4^+$ 0.1 $mg\,kg^{-1}$, $NO3^-$ 5.3 $mg\,kg^{-1}$; soil water content at full pot water-holding capacity and permanent wilting point of 20.0% and 5.0% (vol.), respectively. Fertilizers were added to each pot in the form of NH_4NO_3 (5.71 g), KPO_4 (3.80 g) and K_2SO_4 (1.23 g) to give 2.00 g N, 0.87 g P, and 1.66 g K per pot, respectively. The plants were prevented from bending by being tied to bamboo sticks placed into the pots. Side shoots were removed, and all plants were trimmed at the third internode above the 4th inflorescence in order to increase uniformity. Towards the end of May, many plants started developing symptoms of chloroses and leaf desiccation, regardless of irrigation or CO_2 treatment (but with a clear tendency of CV1 plants to appear healthier than those of CV2), which could be caused by either nutrients (e.g. Iron) deficiency or diease infection. Such condition led the decision to harvest the plants on the 7th June, although fruit maturation was not yet complete.

2.2. Treatments

The experiment was conducted in two cells with different CO_2 concentrations: the control cell with ambient $[CO_2]$ and a cell with elevated $[CO_2]$. It should be noted that the set point of the $[CO_2]$ was 700 ppm in the CO_2 elevated cell, which was maintained only during the first three weeks of the experimental period, and fluctuated between 500 and 650 ppm thereafter. Thus, the overall average of $[CO_2]$ during the experimental period was 590 ppm in the high-$[CO_2]$ cell. The CO_2 enrichment was achieved by emission of pure CO_2 from a bottled tank, released in one point and distributed in the cells through internal ventilation.

The pots used for the experiment enabled us to separate plant root-zone into two equal parts. A plastic sheet was used to divide the pots into two equal sides (S1 and S2 hereafter), allowing no water exchange between them, thus creating two separated irrigation compartments. Before the beginning of irrigation treatment, plants were irrigated every 3–5 days, depending on weather conditions. The amount of water delivered before the beginning of irrigation treatment was the same for each plant (8.5 L), and distributed equally between the two sides of the pot. On the 13th May ("Day 0", 60 days after 50% emergence) all pots were irrigated to 18% of volumetric soil water content, and the following day the irrigation treatment started and continued until plant harvest on the 7th June. During the treatment, pots were irrigated daily (between 15:00–16:00 h) according to three different watering regimes, each assigned to 8 plants per cell (4 plants per cultivar): (a) full irrigation (FI), representing the control for irrigation treatments, where pots were irrigated to 18% of volumetric soil water content and water was delivered in equal amounts to S1 and S2; (b) deficit irrigation (DI), where pots were irrigated with 70% of the water used for FI of the corresponding cultivar, and water was delivered in equal amounts to S1 and S2; (c) partial root-zone drying (PRD), where pots were irrigated with the same water as DI, but water was delivered all to one side while the other was left to dry until reaching a soil water content of approximately 6–7%. At this point the irrigated side was shifted. During the 24 days of irrigation treatment, a total of 4 shifts of side took place.

2.3. Measurements

Volumetric soil water content (SWC, %) was measured daily (between 13:00–15:00) with a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment Corp., CA, USA). Two probes (35 cm in length) were installed in each soil compartment and allowed separate evaluation of S1 and S2. Data obtained from FI pots was used for calculating their water requirements, which served in turn as the reference for determining irrigation volume of DI and PRD plants. The change of daily volumetric soil water content (SWC, %) before irrigation in the pots exposed to different treatments is shown in Fig. 1.

Leaf gas exchange rates including stomatal conductance (g_s) and photosynthetic rate (A_n) were measured twice during the irrigation treatment, on days 15 and 21 after treatment starts. Measurements were conducted at 11:00–12:00 am on one fully expanded upper canopy leaf of each plant (four plants per treatment) with CIRAS-2 Portable Photosynthesis System (PP Systems Inc., Amesbury, MA, USA) at a light intensity (PAR) of 500 $\mu mol\,m^{-2}\,s^{-1}$ provided by the LED light source of the leaf vuvette (PP Systems, Amesbury, MA, USA). The temperature in the vuvette was set to 25 °C and the vapour pressure deficit (VPD) was kept steady at ca. 1.2 kPa. Plants of each cell were measured at the respective CO_2 concentration initially set for each cell (*i.e.* plants in control cell were measured at 400 ppm while plants in high-$[CO_2]$ cell at 700 ppm). Data obtained from gas exchange measurements were used for the calculation of intrinsic water use efficiency (WUE_i) as A_n/g_s.

Leaf water potential (LWP) was measured using a Scholander-type pressure chamber (Model 3000F01H12G2P40, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Three measurements were conducted during the irrigation treatment (at 10, 17, 24 days after treatment start), between 09.00–11.00 h on healthy fully expanded upper leaflets with four replicates per treatment. Root water potential (RWP) was measured on the day of harvest (24 days after treatment start with four replicates per treatment), with a Scholander-type pressure chamber (Liu et al., 2005). The whole pots were inserted into the pressure chamber, which was then sealed leaving out only the above-soil part of the plants. Stem was then cut with a scalpel at about 10 cm height from soil surface, and the edge of the cut was carefully dried with blotting paper. Pressure was increasingly applied into the chamber until xylem sap started to appear from the cut. At this point, the corresponding pressure level was recorded as the equivalent of RWP.

At harvest, stem, leaves and fruits were collected, and subsequently dried in an oven at 70 °C for 48 h (leaves and stem) or 72 h (fruits) before weighing. Plant water use efficiency (WUE_p) was calculated as: aboveground biomass/plant water use during the experimental period.

2.4. Statistical analysis

Three-way ANOVA was performed for the independent variables cultivar (CV), CO_2 concentration ($[CO_2]$), and irrigation regime; as well as for their interactions. Data were analyzed with SPSS version 22.0 (IBM Electronics).

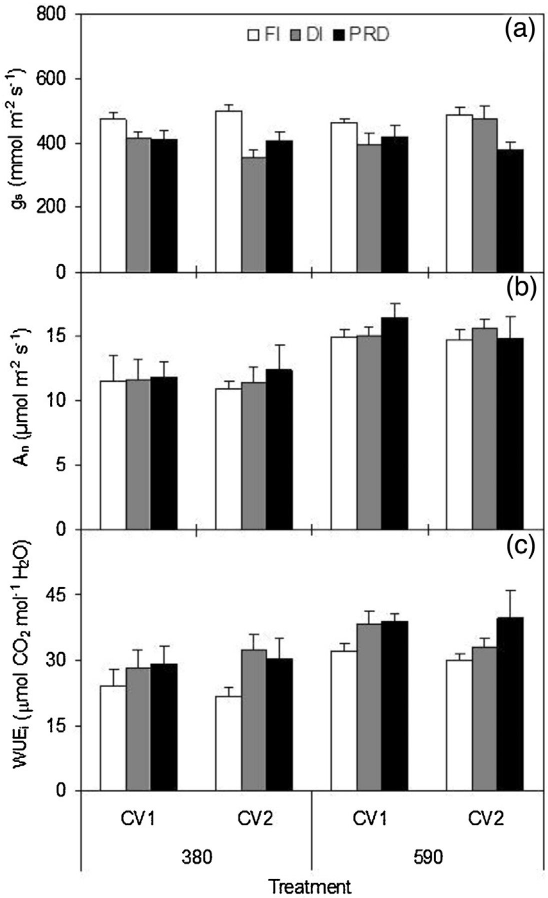

Fig. 2. Stomatal conductance (g_s), photosynthetic rate (A_n), and intrinsic water use efficiency (WUE_i) of tomato plants as affected by the CO_2 concentration (380 ppm, 590 ppm), cultivar (CV1, CV2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; partial rootzone drying, PRD). Error bars indicate standard error of the mean (n = 4). Statistical comparisons among the treatments are presented in Table 1.

3. Results

3.1. Leaf gas exchange and intrinsic water use efficiency

Stomatal conductance (g_s) was only significantly affected by irrigation ($P < 0.001$; Fig. 2a; Table 1), being 15% and 16% lower in DI and PRD, respectively, than that of the FI plants. While photosynthetic rate (A_n) was only affected by [CO_2] ($P < 0.001$; Table 1) being 30% higher in plants grown at 590 ppm compared to those grown at 380 ppm (Fig. 2b).

Intrinsic water use efficiency (WUE_i) calculated as A_n/g_s was influenced by both [CO_2] and irrigation (Fig. 2c; Table 1). Plants under DI and PRD showed an average increase in WUE_i of 14% and 22%, respectively, compared to FI plants. WUE_i was significantly higher in plants grown at 590 ppm compared to those grown at 380 ppm.

3.2. Leaf and root water potential

Leaf water potential (LWP) was significantly affected by irrigation ($P < 0.01$; Table 1), with the fully irrigated plants showing higher LWP than plants under reduced irrigation (Fig. 3a). Root water potential (RWP) was also significantly affected by irrigation ($P < 0.001$; Fig. 3b; Table 1) being significantly higher in FI than in DI and PRD plants. [CO_2] affected RWP significantly ($P < 0.05$; Table 1), with plants grown at 590 ppm showing higher values than those grown at 380 ppm.

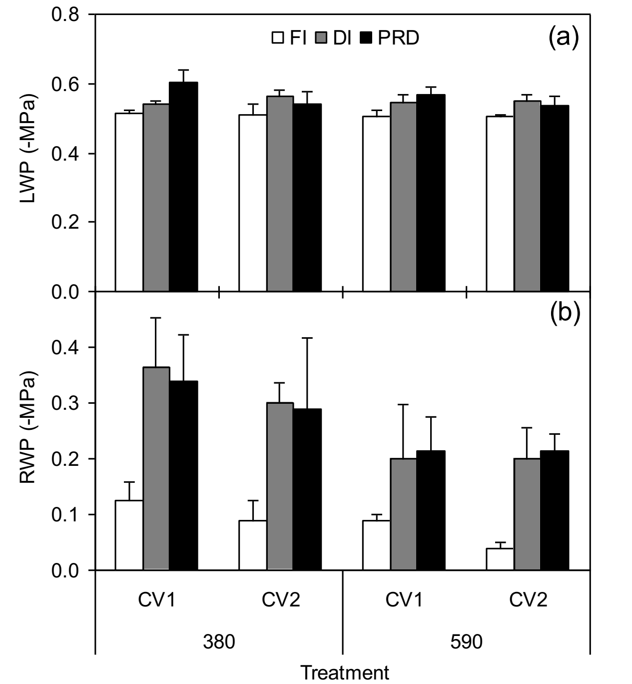

Fig. 3. Leaf water potential (LWP) and root water potential (RWP) of tomato plants as affected by the CO_2 concentration (380 ppm, 590 ppm), cultivar (CV1, CV2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; partial rootzone drying, PRD) measured on 24 days after treatment. Error bars indicate SE (n = 4). Statistical comparisons among the treatments are presented in Table 1.

3.3. Biomass production

Stem dry weight (DW) was affected by all factors (Fig. 4a; Table 1) and a [CO_2] × CV interaction ($P < 0.05$). Stem DW of CV1 plants was on average 83% higher than that of CV2 plants. The two cultivars responded differently to CO_2 elevation, with stem DW of CV2 increasing by 48% and that of CV1 only by 1%. DI and PRD resulted in decreased stem DW by 17% and 19%, respectively, compared to FI. Leaf DW was affected by [CO_2] ($P < 0.001$) and cultivar ($P < 0.001$; Fig. 4b; Table 1). CV2 showed lower leaf DW compared to CV1 (30% less at 380 ppm and 22% less at 590 ppm). Compared to plants grown under ambient [CO_2], elevated [CO_2] increased leaf DW in both cultivars (24% in CV2 and 11% in CV1). Fruit DW of CV2 was significantly greater than that of CV1 (37% in average, $P < 0.001$; Fig. 4c; Table 1). A marginally significant [CO_2] × CV × irrigation interaction was observed on fruit DW. At 380 ppm, all plants under reduced irrigation treatments showed lower fruit DW than the FI plants. Elevated [CO_2] exacerbated such difference in CV2, while in CV1 fruit DW was higher in plants under reduced irrigation than in FI plants. Total above ground dry weight was significantly affected by [CO_2], CV, and irrigation (Fig. 5a; Table 1). CV1 showed 21% higher total DW than CV2. Both cultivars produced greater biomass at 590 ppm than at 380 ppm. Irrigation treatment had a smaller, but significant effect on above ground biomass and being lower in in the DI and PRD as compared to the the FI plants.

3.4. Plant water use and water use efficiency

Across the irrigation treatment, PWU was significantly affected by CV ($P < 0.001$): CV1 used 23% more water than CV2. Elevated [CO_2] affected the PWU of the two cultivars in opposite directions (*i.e.* decreasing PWU of CV1 while slightly increasing that of CV2), and resulted in a significant [CO_2] × CV interaction on PWU ($P < 0.01$; Table 1). Plant water use efficiency (WUE_p) was significantly affected by [CO_2] and irrigation (Fig. 5c; Table 1). Plants

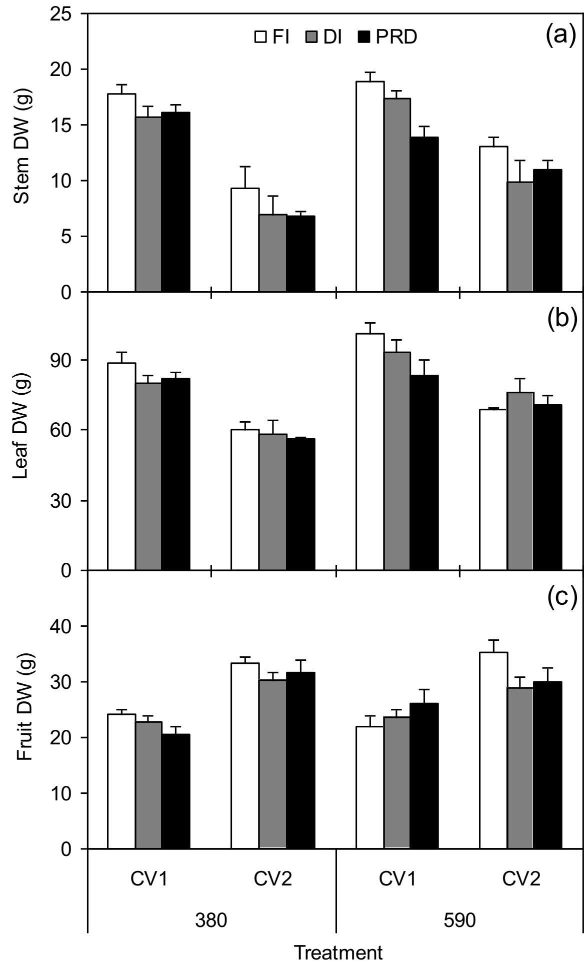

Fig. 4. Dry weight (DW) of stem, leaf, and fruit of tomato plants as affected by the CO_2 concentration (380 ppm, 590 ppm), cultivar (CV1, CV2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; partial rootzone drying, PRD). Error bars indicate SE ($n = 4$). Statistical comparisons among the treatments are presented in Table 1.

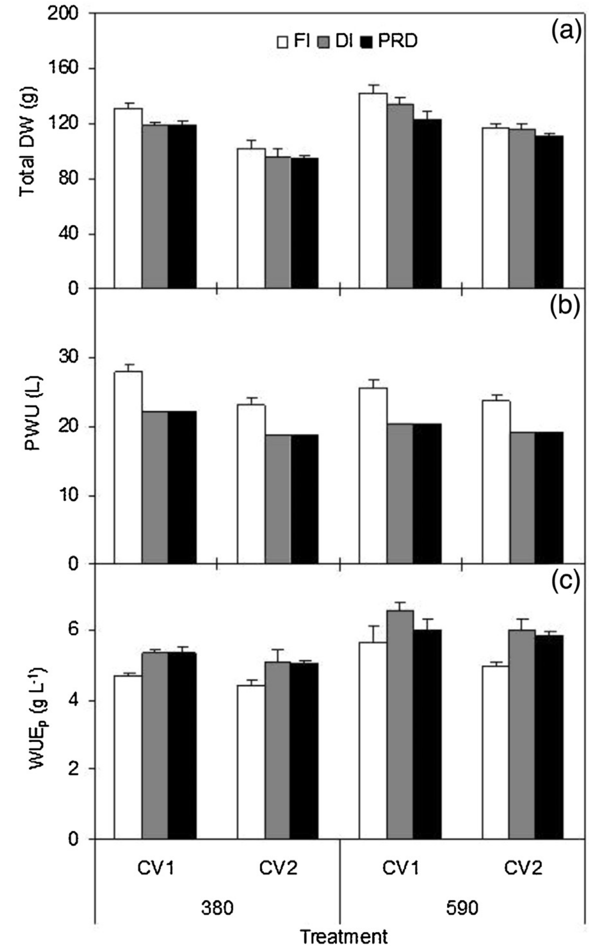

Fig. 5. Total aboveground dry weight (TDW), plant water use (PWU), and plant water use efficiency (WUE_p) of tomato plants as affected by the CO_2 concentration (380 ppm, 590 ppm), cultivar (CV1, CV2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; partial rootzone drying, PRD). Error bars indicate SE ($n = 4$). Statistical comparisons among the treatments are presented in Table 1.

grown under reduced irrigation had higher WUE_p than those under full irrigation. In relation to plants grown at ambient $[CO_2]$, plants grown at elevated $[CO_2]$ showed an increase in WUE_p by 25% in CV1 and by 13% in CV2.

When pooling all the data of the twelve treatments, there was a significant positive linear relationship between WUE_p and WUE_i (Fig. 6).

3.5. Flower number, fruit number and fruit set

Flower number was affected significantly by $[CO_2]$ and slightly by the interaction of $[CO_2] \times$ irrigation (Fig. 7a; Table 1). At 590 ppm, plants of both cultivars had more flowers than grown at 380 ppm, mainly as a result of significant increases of flower number in the 4th and 2nd inflorescences (data not shown). Fruit number was significantly affected only by a cultivar × irrigation interaction (Fig. 7b; Table 1). Reduced irrigation treatments decreased fruit number of CV2 at both $[CO_2]$ concentrations, while not affecting (at 380 ppm)

Table 1
Out put of three-way analysis of variance (ANOVA).

Factors	g_s	A_n	WUE_i	LWP	RWP	SDW	LDW	FDW	TDW	PWU	WUE_p	FLN	FRN	FRset
$[CO_2]$	ns	***	***	ns	*	**	***	ns	***	ns	***	*	ns	**
CV	ns	ns	ns	ns	ns	***	***	***	***	***	*	ns	ns	ns
IRRI	***	ns	*	**	***	**	ns	ns	**	***	***	ns	ns	ns
$[CO_2] \times$ CV	ns	ns	ns	ns	ns	*	ns	ns	ns	**	ns	ns	ns	ns
$[CO_2] \times$ IRRI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV × IRRI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
$[CO_2] \times$ CV × IRRI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

The table reports the significance results of the three-way ANOVA on stomatal conductance (g_s), photosynthetic rate (A_n), intrinsic water use efficiency (WUE_i), leaf water potential (LWP), root water potential (RWP), stem dry weight (SDW), leaf dry weight (LDW), fruit dry weight (FDW), total dry weight (TDW), plant water use (PWU), plant water use efficiency (WUE_p), flower number (FLN), fruit number (FRN), and fruit set (FRset) of tomato plants as affected by the CO_2 concentration ($[CO_2]$), cultivar (CV) and irrigation regimes (IRRI) and their interactions (data in Figs. 2–5 and 7).
*, **, *** Indicate significance levels at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; ns denotes no significance.

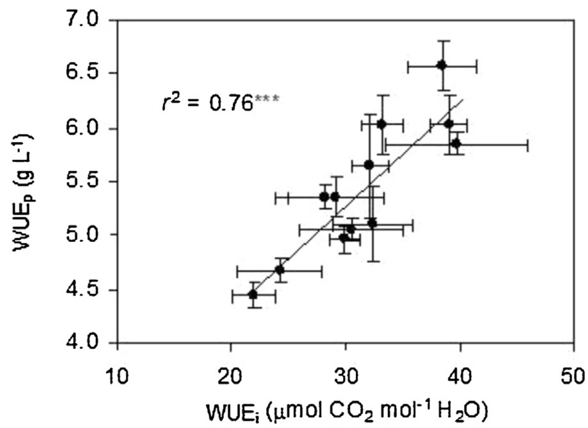

Fig. 6. Relationship between intrinsic water use efficiency (WUE_i) and plant water use efficiency (WUE_p) of two tomato cultivars grown under different irrigation regimes and atmospheric CO_2 concentrations. Error bars indicate SE. ***Indicates the significance of the regression line ($P < 0.001$).

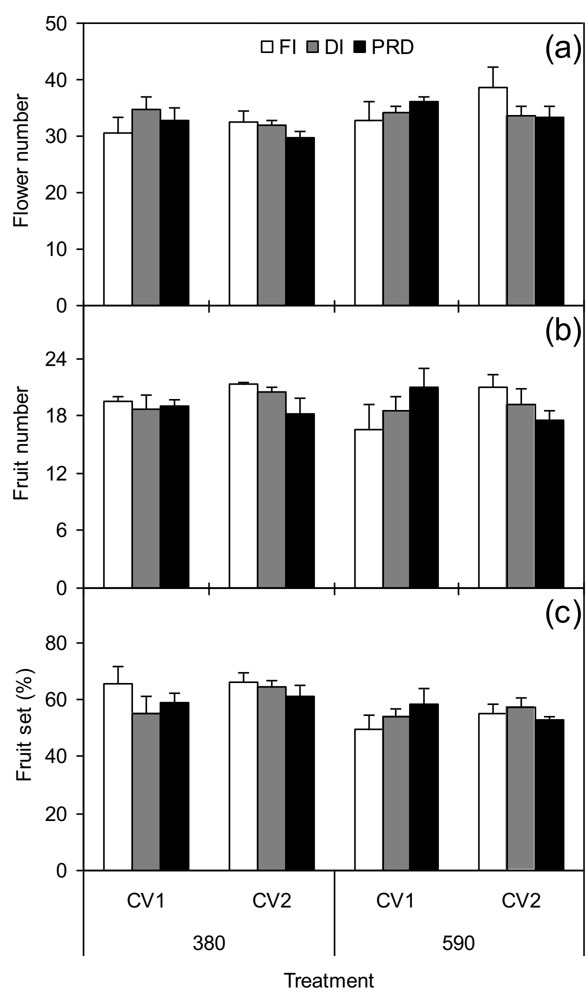

Fig. 7. Flower number, fruit number, and fruit set of tomato plants as affected by the CO_2 concentration (380 ppm, 590 ppm), cultivar (CV1, CV2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; partial rootzone drying, PRD). Error bars indicate SE ($n = 4$). Statistical comparisons among the treatments are presented in Table 1.

or even stimulating it (at 590 ppm) in CV1. There was a significant effect of [CO_2] on fruit set (Fig. 7c; Table 1). Plants grown at 590 ppm showed lower fruit set than grown at 380 ppm, across all CVs and irrigation treatments. The overall reduction was 12%, being higher in fully irrigated plants (20%) than in plants under reduced irrigation (7%).

4. Discussion

4.1. Leaf gas exchange

Decrease in g_s is one of the most evident and ubiquitous responses to water stress (Liu et al., 2005; Liu et al., 2008; Wang et al., 2012), and has been reported for deficit-irrigated tomato in a number of studies (Sun et al., 2013; Wang et al., 2012). Accordingly, in the present experiment, g_s of plants grown under reduced irrigation was consistently lower than that of the fully irrigated plants, especially at ambient [CO_2]. The reduction in g_s was less significant at 590 ppm, mostly as a consequence of high g_s of DI plants of CV2 (Fig. 2a; Table 1). Most previous studies report significant decreases in g_s in response to elevated [CO_2] (Long et al., 2004 and literature therein). A 57% reduction in g_s was reported in tomatoes grown at 900 ppm in relation to that grown under ambient [CO_2] (Yelle and Beeson 1990). By contrast, g_s was hardly affected by [CO_2] in the current experiment (Fig. 2a). However, a decrease in g_s must have occurred in plants growing at 590 ppm, at least in CV1, to explain the observed reductions in plant water use (PWU) coupled with greater photosynthetic rate and biomass production. Photosynthetic rate (A_n) of plants grown at high [CO_2] was on average 31% greater than for those grown at ambient [CO_2] (Fig. 2b). Previous studies on tomato (Yelle and Beeson 1990) also reported short-term increases in A_n by 29.6% and 36% in plants grown at CO_2 concentration of 900 ppm as compared to plants grown at ambient [CO_2]. A_n is generally decreased in reduced irrigation regimes (Topcu et al., 2007), and this decrease is usually slightly larger under DI than under PRD (Topcu et al., 2007). In the present experiment, however, no significant differences in A_n were observed between FI, DI, and PRD plants (Fig. 2b; Table 1).

Increased intrinsic water use efficiency (WUE_i) under reduced irrigation has been reported in previous experiments on tomato (Wang et al., 2012) and other C_3 species (Liu et al., 2005), as a result of significant reductions in g_s coupled with smaller decreases in A_n. Consistent with this, in the present experiment, plants grown under reduced irrigation showed a significantly increased WUE_i, as an effect of decreases in g_s which were not coupled with a reduction in A_n (Fig. 2c). Plants grown under DI and PRD showed similar WUE_i, but a slight tendency towards higher WUE_i in PRD compared to DI plants was detected, in agreement with earlier studies (Wang et al., 2010). Growth at elevated [CO_2] was expected to increase WUE_i as noticed in previous experiments on tomato and other species (*e.g.* Yelle and Beeson 1990). Accordingly, plants grown at high [CO_2] had significantly higher WUE_i than those grown at ambient [CO_2] (Fig. 2c). However, cited literature often reports increased WUE_i being the result of both an increase in A_n and a decrease in g_s, In the present study, since g_s was unaffected by CO_2 enrichment, the observed increase in WUE_i was therefore solely due to A_n enhancement. It has been hypothesized that elevated [CO_2] will increase WUE_i of plants suffering from drought more than that of well-watered plants, as a result of larger decreases in g_s and smaller reductions in A_n relative to ambient [CO_2] (Kang et al., 2002; Xu et al., 2013). The present results are not consistent with this hypothesis, due to the apparent lack of responsiveness of g_s to high [CO_2].

4.2. Plant water relations

Consistent with previous literature (*e.g.* Vivin et al., 1996), leaf water potential and particularly root water potential of plants grown under reduced irrigation were significantly lower (*i.e.* more negative) than those of fully irrigated plants (Fig. 3). In the present study, DI and PRD plants had a similar LWP (ca. −0.50 MPa), which disagrees with earlier findings on tomato by Tahi et al. (2007) who reported higher values for PRD (ca. −0.30 MPa) and lower values for DI (up to −0.92 MPa) on tomato plants. In the above-mentioned studies, however, the degree of irrigation reduction was 50% rather than 30% here. Based on the majority of previous experiments (Wullschleger et al., 2002), we hypothesized that CO_2 elevation would improve plant–water relations, leading to higher RWP and LWP values. In good agreement with this, elevated [CO_2] consistently increased RWP across all cultivars and irrigation treatments. On the other hand, no significant differences in LWP were observed between the two CO_2 treatments, more similarly to a recent study on potato (Fleisher et al., 2013) that also reported non-univocal effects of [CO_2] on LWP, with CO_2-enriched plants showing either higher, lower, or unchanged midday LWP.

4.3. Plant water use and dry weight

Plant water use (PWU) was higher for CV1 than for CV2 at both CO_2 concentrations (Fig. 5b). However, CO_2 enrichment influenced the two CVs differently, increasing PWU of CV2 while decreasing that of CV1. Lowered PWU at elevated [CO_2] is attributed to decreases in stomatal conductance (Leakey et al., 2009; Long et al., 2004). Nevertheless, plants grown at high [CO_2] usually have larger leaf area, which increases transpiration and consistently diminishes – and in some cases totally offsets – the effects of the lowered g_s per unit area (Fleisher et al., 2008). Although leaf area was not measured in the present experiment, the larger increase in leaf dry weight in plants grown at 590 ppm in CV2 relative to CV1 likely reflects a larger increase in leaf area, and may partly explain the observed differences among cultivars in their water use response to CO_2 enrichment.

The two cultivars behaved very differently in their biomass production and allocation. CV1 plants showed greater stem and leaf DW than CV2 plants (83% and 36% greater, respectively). On the other hand, fruit DW was 37% larger in CV2 than in CV1. Despite the 30% water reduction relative to the fully irrigated plants, total DW was decreased only by 7% in average in the reduced irrigation treatments (slightly more in PRD than in DI), which coincides with the small decrease in A_n observed under reduced irrigation. Total plant DW at 590 ppm was increased by an average of 13.5% relative to plants grown at ambient [CO_2], with enhancement twice as great for CV2 (+18%) than CV1 (+9%). Increases of up to 30% in total DW of tomatoes grown at high [CO_2] have been reported in literature (Hartz et al., 1991), in good agreement with the results of this experiment (considering the much larger differences between control and high [CO_2] in the cited studies). The additional biomass produced at 590 ppm was primarily partitioned to leaves (in both cultivars) and stem (only in CV2). In contrast with other studies (Hartz et al., 1991), fruit DW was not significantly affected by CO_2 enrichment, but the premature harvest might have influenced such result since high [CO_2] could also have affected plant developmental stage.

Elevated [CO_2] significantly increased WUE_p across all cultivars and irrigation treatments (Fig. 5c). Although total DW at 590 ppm increased more in CV2 than in CV1, the different water use response resulted in a greater increase in WUE_p in CV1 (+19%) than CV2 (+15%). Literature on tomato and other species has reported that reductions in irrigation volume ranging from 30% to 50% increased WUE_p consistently relative to full irrigation treatment (*e.g.* Wang et al., 2010). Accordingly, in the present experiment WUE_p of plants under reduced irrigation was in average 15% higher than that of fully irrigated plants. In some studies, slight but significant differences are reported between DI and PRD plants, with the latter showing higher WUE_p (Wang et al., 2010). Here, similar values of WUE_p were observed at 380 ppm, while at 590 ppm plants under DI had a slightly higher WUE_p than those under PRD. Our results suggest that the amount of irrigation water, rather than the irrigation method, determines WUE_p. Moreover, the significant positive linear relationship between WUE_i and WUE_p (Fig. 6) indicates that the improvement of WUE at the whole plant level was closely associated with that of the stomatal.

4.4. Reproductive development

The two cultivars showed a similar number of flowers (Fig. 7a). Although flower number was in large part already determined by flower bud formation when the irrigation treatment was initiated, large differences emerged in the upper (more recent) inflorescence. Indeed, flower number of the 4^{th} inflorescence of plants under reduced irrigation decreased by 22% in CV2, whilst it was increased by 10% in CV1, possibly indicating a better overall condition of the latter under water deficit (presumably due to its potential drought tolerance). CV1 also had a positive response to reduced irrigation in terms of fruit number, especially at 590 ppm (Fig. 7b). On the other hand, in CV2 fruit number was decreased by reduced irrigation in all circumstances (more in PRD than in DI), for an average reduction of 9% under ambient [CO_2], and 13% under elevated [CO_2], in relation to their fully irrigated counterparts. Reduced irrigation treatments decreased fruit set only at 380 ppm (Fig. 7c). Whereas, fruit set was significantly decreased by elevated [CO_2] across all cultivars and irrigation treatments (−12% in average), with bigger reductions in fully irrigated plants than in those under reduced irrigation (Fig. 7c).

5. Conclusion

Our results show that plant water status was negatively affected by reduced irrigation regimes but positively influenced by elevated [CO_2]. Water use efficiency, at both stomatal and whole plant levels, was enhanced individually by the reduced irrigation regimes and high [CO_2]. Moreover, there was a synergistic effect of the two factors on WUE_p, but not on WUE_i. Elevated [CO_2] increased flower number without affecting fruit number, and therefore reduced fruit set. Despite large differences between the cultivars, both of them showed significant improvements in plant water use efficiency under both reduced irrigation and CO_2 enrichment, as well as under the combination of the two treatments.

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