RESEARCH ARTICLE

Carbon dioxide enrichment: a technique to mitigate the negative effects of salinity on the productivity of high value tomatoes

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Abstract

The present study was conducted to determine the mitigating influence of greenhouse CO_2 enrichment on the negative effects of salinity in Mediterranean conditions. Hybrid Raf (cv. Delizia) tomato plants were exposed to two salinity levels of the nutrient solution (5 and 7 dS/m) obtained by adding NaCl, and two CO_2 concentrations (350 and 800 μmol/mol) in which CO_2 enrichment was applied during the daytime according to a strategy linked to ventilation. Increasing water salinity negatively affected the leaf area index (LAI), the specific leaf area (SLA), the water use efficiency (WUE), the radiation use efficiency (RUE) and dry weight (DW) accumulation resulting in lower marketable yield. The high salinity treatment (7 dS/m) increased fruit firmness (N), total soluble solids content (SSC) and titratable acidity (TA), whereas pH was reduced in the three ripening stages: mature green/breaker (G), turning (T), and pink/light red (P). Also, the increase in electrical conductivity of the nutrient solution led to a general change in intensity of the sensory characteristics of tomato fruits. On the other hand, CO_2 enrichment did not affect LAI although SLA was reduced. RUE and DW accumulation were increased resulting in higher marketable yield, through positive effects on fruit number and their average weight. WUE was enhanced by CO_2 supply mainly through increased growth and yield. Physical-chemical quality parameters such as fruit firmness, TA and pH were not affected by CO_2 enrichment whereas SSC was enhanced. Greenhouse CO_2 enrichment did mitigate the negative effect of saline conditions on productivity without compromising organoleptic and sensory fruit quality.

Additional key words: *Solanum lycopersicum*; CO_2; electrical conductivity; yield; growth; radiation use efficiency.

Abbreviations used: BER (blossom end rot); dat (days after transplanting); EC (electrical conductivity); LAI (leaf area index); PAR (photosynthetically active radiation); R_g (global radiation); RUE (radiation use efficiency); SLA (specific leaf area); SSC (soluble solids content); TA (titratable acidity); VPD (vapour pressure deficit); W_U (crop water uptake); WUE (water use efficiency); WUE_S (supply WUE); WUE_U (uptake WUE).

Introduction

Air CO_2 concentration is a relevant climate variable to be controlled in greenhouses as it has a marked effect on plant CO_2 assimilation. Even at the relatively low radiation levels prevailing during winter in Mediterranean regions, CO_2 enrichment could lead to significant increases of crop photosynthesis (Nilsen *et al.*, 1983; Nederhoff, 1994; Sánchez-Guerrero *et al.*, 2005). The atmospheric CO_2 level limits the potential photosynthesis of most vegetable species and their productivity (Bowes, 1993). Although ventilation allows the renovation of greenhouse air, it is not often sufficient to replace the CO_2 consumed by crop photosynthesis. In the greenhouses of South-Eastern Spain internal CO_2 concentrations 20% lower than the external CO_2 concentration were recorded, even when the greenhouse vents were

opened (Lorenzo *et al.*, 1990) showing relatively high CO_2 depletion. The lack of climate control in many greenhouses in Mediterranean countries results in an inadequate microclimate that negatively affects yield components and input-use efficiency. Thus, better control of the greenhouse aerial environment can improve marketable yield and quality and extend the growing season (Baille, 1999).

The South-Eastern region of Spain is an important area for both production and export of high quality tomatoes for fresh consumption. The combination of unique conditions such as mild climate and saline waters or soil types, are the main causes of the exceptional fruit quality of some varieties cultivated in this region such as the hybrids of the Raf tomato, also called "flavour varieties". Despite the high value of Raf tomatoes in the Spanish national market, their productivity is relatively low and the consumer does not always get an acceptable quality because the fruit growth conditions are not adequate. Salinity could be considered as a strategic tool to improve the quality of fruits such as tomato (Dorais *et al.*, 2001). However, high salinity also lowers water potential in the plant which in turn reduces the water flow into the fruit and therefore the rate of fruit expansion (Johnson *et al.*, 1992) and also reduces the number of fruits per plant (Cuartero & Fernández, 1999). Reducing the negative effect of salinity on the tomato crop has been addressed in studies using cultural techniques or genetic engineering (Cuartero *et al.*, 2006). The increase of salinity tolerance by CO_2 enrichment has been evaluated, mainly in glass greenhouses (Li *et al.*, 1999). However, few studies have been conducted on how the saline stress can be attenuated at high CO_2 concentration in Mediterranean conditions, even though CO_2 enrichment in this area has shown yield improvements. Yield increases of the order of 12-25% for different crops (cucumber, sweet pepper, green bean and tomato) have been reported by Sánchez-Guerrero *et al.* (2005), Alonso *et al.* (2012) or Lorenzo *et al.* (2013). A remarkable improvement in water use efficiency (WUE) was reported by Sánchez-Guerrero *et al.* (2010). Since the response of plants to CO_2 enrichment and other environmental factors such as salinity, tends to be species-specific (Rozema, 1993), it would be appropriate to extend the understanding of both parameters under the specific conditions of the Mediterranean greenhouse. For this reason, the aims of this study were to investigate (i) how two environmental factors, salinity and greenhouse air CO_2 enrichment affect the growth, yield and fruit quality in three different ripening stages in a high commercial value variety of tomato and (ii) the mitigating influence of greenhouse air CO_2 enrichment on the negative effects of salinity.

Material and methods

Greenhouse conditions and climate control

The experiment was conducted in two adjacent, identical multispan greenhouses of 720 m^2 clad with polyethylene plastic film, located at the IFAPA Research Center (La Mojonera, Almería, Spain, latitude 36º30'N, longitude 2º18'W). The greenhouses were equipped with a pipe water heating system, roof and sidewall vents, external mobile shading screen and a system for the injection of pure CO_2. All vents were permanently covered with insect-proof nets (38% porosity). The heating, ventilation and shading set-points were the same for the two greenhouses. The heating systems were activated when the greenhouse air temperature fell below 10 ºC. The set-points for the activation of ventilation were: temperature > 25 ºC and/ or relative humidity > 70/80% (day/night). Temperature and relative humidity were measured with ventilated platinum resistance and capacitive sensors (HMP45C; Vaisala, Helsinki, Finland) placed in a central position inside the greenhouses 2 m above the ground. The global radiation (R_g) incident on the crop in each greenhouse was measured by SP1110 pyranometers (Sky Instruments, Richmond, Canada). The external mobile shading screen OLS 35 ABRI (Ludving Svensson, Kinna, Sweden) was operated to close, whenever the solar $R_g > 650$ W/m^2 at the beginning of the growth cycle (September-October), and when solar $R_g > 500$ W/m^2 from February until the end of the growth cycle. Overnight, the external shading screen was used as a thermal screen during the winter months. After transplanting, pure CO_2 was supplied in one of the greenhouses (C), distributed through a pipe network with one outlet below each plant. CO_2 enrichment was applied during the daytime according to a strategy linked to ventilation (800 µmol/mol when the roof and side vents were closed and 350 µmol/mol when vent opening was > 20%). The period of CO_2 supply during the daytime began 15 min before sunrise and ended 75 min before sunset. CO_2 concentration was continuously monitored through an infrared gas analyser IRGA (GMD20; Vaisala, Helsinki, Finland). The second greenhouse was not CO_2 enriched and served as the reference (R) in this experiment. The climatic variables (temperature, humidity and CO_2 concentration) were controlled by means of a commercial climate-control system (CDC; INTA, Águilas, Spain) connected to a PC and data were recorded at 1 min intervals.

Crop and cultural techniques

Hybrid Raf tomato plants (*Solanum lycopersicum* L. cv. Delizia), grafted on tomato plants (*Solanum lycoper-*

sicum L. cv. Rambo) were transplanted in 27 L pots containing perlite substrate on 20 September 2012. The pots were placed in north-south rows and there were two stems on each plant giving a stem density of 2.5 stems/m^2. Pests and diseases were controlled using an integrated control strategy thought the use of beneficial insects and low impact chemicals. In both greenhouses, the nutrient solution was adjusted to two salinity levels, 5 dS/m (EC5) and 7 dS/m (EC7), by adding 25 mM and 45 mM of NaCl respectively to the irrigation water (Table 1). The nutrient solution was provided by an automated fertigation system (CDN; INTA, Águilas, Spain). The EC of the nutrient and drainage solutions were measured manually three times per week, early in the morning, using a portable EC-meter (CM35 81839, Crison Instruments, Barcelona, Spain). The mean EC values of the drainage solutions were ~ 7.5 and ~ 7.2 dS/m for nutrient solution EC5 and 12.2 and 12.2 dS/m for nutrient solution EC7 in the reference and enriched crops, respectively. The crop ended 203 days after transplanting (dat).

Crop growth and yield

Shoot biomass was sampled 6 times through the growing period to determinate fresh and dry weight, on 4 October (14 dat), 18 October (28 dat), 20 November (61 dat), 9 January (111 dat), 26 February (159 dat) and 11 April 2013 (203 dat). A two-factor experimental design was used, with samples consisting of six plants randomly selected from homogeneous zones in each greenhouse. Each plant was separated into three fractions: leaf, stem and fruit to measure their fresh and dry weights, drying at 80 ºC in a forced ventilation oven until constant weight. All pruning (axillary stems and old leaves) and harvested fruits, removed before the sampling date, were included in the corresponding fraction (total fresh and dry weight).

The leaf area of each plant was measured with a leaf area meter (LI-3100; LI-COR Biosciences, Lincoln, NE, USA). The values were multiplied by the crop density to determine the leaf area index (LAI, m^2/m^2). The specific leaf area (SLA, leaf area per unit leaf dry weight, m^2/g) was also calculated.

Fruit yield (fresh weight) of 8 plants (6 repetitions of each treatment) was routinely measured. Ripe fruits in each plant were harvested during the experimental pe-

riod, twice a week, from 21 November 2012 to 11 April 2013. The harvested fruits were counted, weighed, and scored for marketable or non-marketable yield. Non-marketable fruits were those with evidence of blossom end rot (BER) or with visible external defects. Marketable fruits were classified by size grades (82-102 mm, 67-82 mm, 57-67 mm, 47-57 mm) according to the Council Regulations (EC) Nº 1221/2008 (OJ, 2008) about marketing standards of fruits and vegetables.

PAR interception by the canopy and radiation use efficiency

To measure the photosynthetically active radiation (PAR) interceptance of the canopy (i), nine measurements were made during the growing season, on 2 October, 23 October, 23 November, 11 December, 27 December 2012, 23 January, 6 February, 8 March and 11 April 2013. On each occasion, 6 repetitions of each factor and level were routinely recorded. Measurements were made near solar noon on clear days, using a linear quantum sensor (LI-191; LI-COR Biosciences) connected to a portable microprocessor (LI-1000; LI-COR Biosciences). A sampling protocol was established in order to get representative values of the PAR intercepted by the crop: the linear sensor was positioned completely horizontal above the plant and afterwards below the plant, representing an area half in the path and half between the rows of plants, perpendicularly to the crop row (Nederhoff, 1984; Giménez *et al*., 1995). Two measurements were made below the plant, at the base and between adjacent plants, to calculate the average.

The intercepted PAR was calculated as 47% of the incident daily global radiation (MJ/m^2 · d) (Challa *et al*., 1995), multiplied by the PAR interceptance. The radiation use efficiency (RUE, g/MJ) was defined as the ratio of accumulated aerial dry biomass and accumulated intercepted PAR (Monteith, 1977).

Crop water supply, uptake and water use efficiency

Crop water uptake was calculated as the difference between the amounts of water supplied and the leached

Table 1. Electrical conductivity (EC; dS/m) and nutrient/ion concentration (mmol/L) of irrigation water and nutrient solution used to adjust the two salinity treatments to 5 dS/m (EC5) and 7 dS/m (EC7)

	EC	pH	NO$_3^-$	H$_2$PO$_4^-$	SO$_4^-$	Cl$^-$	Na$^+$	K$^+$	Ca^{2+}	Mg^{2+}
Irrigation water	1.20	7.72	0.02	0.00	1.00	7.00	4.50	0.10	1.80	2.10
Nutrient solution (EC5)	5.25	6.63	8.16	1.39	4.15	32.57	29.57	6.89	4.87	2.30
Nutrient solution (EC7)	7.55	6.58	9.61	1.82	4.86	51.77	47.02	9.13	5.70	2.38

nutrient solution. The leached solution was monitored using a collecting tray containing three pots with three plants (2 stems per plant) and collected in a 20 L drainage tank. There were four drainage tanks per treatment and they were measured three times per week, early in the morning.

The uptake water use efficiency (WUE_U) was defined as the ratio of aerial biomass to crop water uptake, and the supply water use efficiency (WUE_S) as the ratio of aerial biomass to water supply. Also, WUE was calculated in terms of marketable fruit yield.

Fruit selection

To measure the quality parameters, four sampling dates were established through the growing season: 18 December, 24 January, 4 March and 11 April. The common stage for commercialization of this tomato type is the green/breaker stage (mature, full size, firm and green), but they should be consumed at the turning or even at the red mature stages (Sánchez Pérez *et al.*, 2011). To determine the standard quality parameters, tomatoes were hand-picked and classified into the three ripening stages according to the OECD-standardized ripeness classes (OECD, 2002): mature green/breaker, G (not more than 10% of the surface showing red color, color index 2-3), turning, T (more than 10% but less than 30% of the surface showing red color, color index 4-5) and pink/light red, P (red color on almost all the surface but firm, color index 7-8). One hundred and eighty tomatoes without defects (three replicates per treatment, each of five tomatoes) showing uniformity in external appearance, size and shape, were selected and harvested. The selection of fruits was based on colour at harvest using a Minolta spectrophotometer (Konica Minolta CM-2600d/2500d Ramsey, NJ, USA) to measure the Hue angle (hº) at three different points located in the equatorial area. In this study only uniformly coloured fruits were selected with hº values higher than 100 for stage G, between 90-100 for stage T and between 70-80 for stage P, enabling comparison between treatments according to preliminary studies (Sánchez-González *et al.*, 2014).

Physical-chemical quality parameters

Tomato fruit firmness was determined on each fruit at two orthogonal spots on the equatorial plane, using a Texture analyzer (TA-XT-Plus, Stable Micro System, Surrey, UK) equipped with two flat plates. The maximum force (N) required to produce a deformation of 5 mm at a speed of 30 mm/min was recorded (Artés *et al.*, 1999).

Then, the three replicates of tomatoes were liquefied in a commercial Turmix blender (Moulinex, Barcelona, Spain) and the juice was filtered. The pH, titratable acidity (TA) and soluble solids content (SSC) were measured. The titratable acidity was expressed as (% citric acid) after a titration with 0.1 mol/L NaOH (AOAC, 1984). The SSC was measured with an Abbe digital refractometer (WYA-S, China) and expressed as ºBrix.

Sensory evaluation

One hundred and three untrained panellists of different age, sex and profession took part to assess the fruits obtained from the crop subject to two levels of salinity: 5 dS/m (EC5) and 7 dS/m (EC7) and two inside greenhouse conditions: CO_2 enriched (C) and without CO_2 enrichment (R). All samples were selected without defects showing uniformity in ripening stage (external colour). Sánchez Pérez *et al*. (2011) found that hybrids of the Raf tomato as 'Delizia', seem to achieve the best organoleptic quality at the turning or even at the red mature stages. For this reason, to carry out the sensory evaluation we selected the pink/light red stage.

Individual panellists were presented with four white plates (one representing each treatment) containing whole tomato fruits, halved-fruits and sliced-fruits for the sensory analysis. All tests were conducted under the same environmental conditions and without time limit. Panel tests were conducted on isolated tables in the same room to prevent interchange between panel members. Three number codes were used to name samples in order to avoid the identification of the applied treatments. Following Artés *et al*. (1999), panellists were asked to assess visual quality (size, shape, internal and external appearance, aroma and preference) and organoleptic quality (firmness by mouth, juiciness, crunchiness, sweetness, acidity and flavour), using scales (values of acceptance) with 5 points (5= excellent, 4= good, 3= acceptable-limit of marketability, 2= poor and 1= inedible).

Experimental design and statistical analysis

To analyze the crop growth, yield, WUE and RUE, we established a two-factor statistical design: (CO_2 concentration: C, enriched greenhouse; R, reference greenhouse) and EC of the nutrient solution (EC5, 5 dS/m; EC 7, 7 dS/m), each with two levels. To study the quality parameters a third factor (stage of maturity) was incorporated into the design. The data compiled were subject of an analysis of variance (ANOVA) and the differences between means were compared by least significant difference (LSD) at

Results

Climatic conditions

Climatic parameters such as radiation, temperature and vapour pressure deficit (VPD) were similar in the R and C greenhouses. The average values of incident PAR over the crop canopy ranged from 4 to 3 MJ/m^2·d in the period from September to December and from 4 to 6 MJ/m^2·d from January to April (Fig. 1). At the end of the cycle, the total incident PAR in the R greenhouse was 781 MJ/m^2 and in the C greenhouse was 756 MJ/m^2. The averages over the growth cycle of maximum, mean and minimum temperatures and VPD in the R and C greenhouses, respectively, were: 28.3/28.0 °C, 2.3/2.1 kPa; 17.7/17.8 °C, 0.7/0.6 kPa and 12.3/12.5 °C, 0.2/0.2 kPa. In the C greenhouse, the CO$_2$ supply enabled a concentration close to 800 μmol/mol to be maintained when the vents were closed and near the outside level during ventilation periods (380 μmol/mol). In the R greenhouse the minimum average values were around 300 μmol/mol. The average values of daytime CO$_2$ concentration (Fig. 1) remained around 550 μmol/mol throughout the crop cycle in the C greenhouse and around 350 μmol/mol in the R greenhouse. The total amount of CO$_2$ injected over the whole cycle was 4.6 kg CO$_2$/m^2.

Biomass production and yield

Carbon dioxide enrichment significantly increased (+15%) the aerial plant dry biomass at the end of the crop cycle (203 dat). All plant fractions were significantly increased by CO$_2$ enrichment from February 26 (159 dat) onwards. At the end of the crop (203 dat) this increase was +21%, +19% and +16% for leaf, stem and fruit, respectively. The generative part represented 73% on the total dry weight of the plant (Table 2). Conversely, the high salinity treatment (7 dS/m) had significantly reduced dry matter production. The vegetative fraction was reduced from 61 dat and the generative fraction from 159 dat onwards (Table 2). At the end of the crop cycle, there were a reduction in the aerial plant dry biomass of -15%, with reductions of -16%, -25% and -15% in the leaf, stem and fruit fractions, respectively. In this study, the CO$_2$ supply did not affect the values of LAI although at 28 dat it was higher in the greenhouse R than in the greenhouse C (Table 3). However, salinity reduced significantly (p<0.001) the values of LAI from 61 dat. At the end of
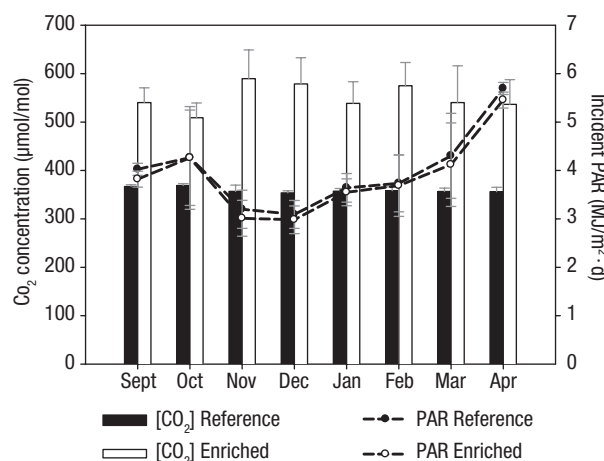
Figure 1. Average values of incident photosynthetically active radiation (PAR) and mean daytime CO$_2$ concentration inside the CO$_2$ enriched and reference greenhouses during the crop growing cycle of cv. Delizia (hybrid Raf).

the crop cycle (203 dat), LAI was reduced (-32%) by high salinity (7 dS/m). SLA was significantly lower in plants grown under elevated CO$_2$ concentration (-20%) than in plants grown under low CO$_2$ concentration (Table 3). High salinity also reduced SLA significantly at the end of the crop cycle (-16%).

Marketable yield was significantly higher (+13%) for the enriched crop than for the reference one (Table 4). At the end of the cycle, salinity decreased significantly the marketable yield (-31%). CO$_2$ enrichment increased significantly fruit number and average weight of marketable fruits and salinity decreased these parameters (Table 4). A significant interaction was observed between the effects of salinity and CO$_2$ concentration on these yield parameters. CO$_2$ enrichment increased the number of fruits only in the high salinity treatment while it only increased the average weight of marketable fruits in the lower salinity treatment. In relation to the distribution of marketable fruits in the size grades (82-102 mm, 67-87 mm, 57-67 mm, 47-57 mm), salinity reduced significantly the yields in the 82-102 mm grade (-56%) and the 67-87 mm grade (-16%) in favour of the 57-67 mm and 47-57 mm grades (Table 4). In return, CO$_2$ enrichment gave significant increases in the 82-102 mm grade (+23%) and 67-87 mm grade (+38%) in the high salinity treatment. There was a significant interaction between salinity and CO$_2$ concentration in the 57-67 mm grade. Also, the 47-57 mm grade was increased by CO$_2$ enrichment only in the high saline conditions (+56%) resulting in a significant interaction. On the other hand, salinity increased significantly the incidence of BER, though this cultivar was not affected at 5 dS/m nutrient solution. CO$_2$ enrichment did not affect the incidence of this symptom (Table 4).

Table 2. Analysis of variance of the effect of salinity (EC: 5 and 7 dS/m) and carbon dioxide (CO_2) on dry matter accumulation in leaf, stem, fruit and total, of cv. Delizia (hybrid Raf) for the two greenhouses (Reference: R, CO_2 enriched: C)

Treatment		Days after transplanting					
		14	28	61	111	159	203
Leaf (g/m^2)	EC5-R	3.1	22.0	58.0	61.5	53.4	78.4
	EC5-C	3.4	19.5	58.4	60.3	71.2	93.2
	EC7-R	3.1	22.2	46.0	47.0	42.9	65.0
	EC7-C	4.0	19.3	52.3	57.8	57.3	80.0
	ANOVA						
	EC	ns	ns	*	*	*	**
	CO_2	ns	ns	ns	ns	**	**
	EC × CO_2	ns	ns	ns	ns	ns	ns
Stem (g/m^2)	EC5-R	2.8	22.5	60.7	70.4	82.0	126.5
	EC5-C	3.1	20.7	56.3	63.6	101.3	144.7
	EC7-R	3.1	21.0	45.7	53.0	65.9	89.4
	EC7-C	3.4	19.1	44.9	58.8	72.0	113.1
	ANOVA						
	EC	ns	ns	**	**	**	***
	CO_2	ns	ns	ns	ns	*	**
	EC × CO_2	ns	ns	ns	ns	ns	ns
Fruit (g/m^2)	EC5-R		2.3	163.2	218.4	755.8	944.9
	EC5-C		2.6	179.7	215.6	880.8	1098.2
	EC7-R		1.4	146.8	218.9	686.3	799.1
	EC7-C		2.9	163.8	245.8	757.9	931.8
	ANOVA						
	EC		ns	ns	ns	***	***
	CO_2		ns	ns	ns	***	***
	EC × CO_2		ns	ns	ns	ns	ns
Total (g/m^2)	EC5-R	5.7	47.3	283.3	403.6	997.9	1309.0
	EC5-C	6.8	43.5	295.7	387.6	1146.1	1488.9
	EC7-R	6.0	44.6	239.4	368.1	893.8	1101.0
	EC7-C	7.4	41.6	262.0	430.1	988.8	1278.5
	ANOVA						
	EC	ns	ns	*	ns	***	***
	CO_2	ns	ns	ns	ns	***	***
	EC × CO_2	ns	ns	ns	ns	ns	ns

ns, not significant. * Significant at $p \leq 0.05$. ** Significant at $p \leq 0.01$. *** Significant at $p \leq 0.001$

Intercepted radiation and radiation use efficiency

The intercepted PAR by the crop was highest under the lower salinity condition, with values of 358 MJ/m^2 with CO_2 enrichment (EC5-C) and slightly higher 380 MJ/m^2 in the reference crop (EC5-R). Under higher salinity (7 dS/m), the intercepted PAR by the enriched (EC7-C) and reference (EC7-R) crops were similar, 333 and 332 MJ/m^2, respectively.

The RUE, expressed as g dry weight per MJ intercepted PAR, was positively affected by CO_2 supply. Under the lower salinity conditions, the RUE was 4.2 and 3.5 (g/MJ) and under the high saline conditions, the RUE was 3.8 and 3.3 (g/MJ) for the enriched and reference crops, respectively. Increased salinity reduced the RUE of this cultivar (-6%).

Crop water supply, uptake and water use efficiency

The total water supply to the crop was significantly reduced by CO_2 supply (-8%) and salinity (-25%) (Table 5). CO_2 enrichment did not significantly affect plant water uptake. However, salinity significantly reduced the total crop water uptake (-17%). CO_2 enrichment enhanced significantly WUE$_S$ (+26% and +23% referred to the aerial dry biomass and marketable fruit yield, respectively) (Table 5). Also, the WUE$_U$ was increased significantly by CO_2 enrichment, with gains of (+24% and +20% for aerial dry biomass and marketable fruit yield, respectively). On the other hand, salinity increased significantly the WUE$_S$ (+12%) for aerial dry biomass, however the WUE$_S$ for marketable fruit yield was reduced (-9%). Also, WUE$_U$ was reduced signifi-

Table 3. Analysis of variance of the effect of salinity (EC: 5 and 7 dS/m) and carbon dioxide (CO$_2$) on leaf area index (LAI) and specific leaf area (SLA) of cv. Delizia (hybrid Raf) for the two greenhouses (Reference: R, CO$_2$ enriched: C)

Treatment		Days after transplanting					
		14	28	61	111	159	203
LAI (m^2/m^2)	EC5-R	0.10	0.60	1.88	1.76	1.66	1.75
	EC5-C	0.13	0.48	1.75	1.78	1.65	1.76
	EC7-R	0.11	0.55	1.46	1.22	1.10	1.21
	EC7-C	0.12	0.49	1.41	1.39	1.17	1.19
	ANOVA						
	EC	ns	ns	***	***	***	***
	CO$_2$	ns	**	ns	ns	ns	ns
	EC × CO$_2$	ns	ns	ns	ns	ns	ns
SLA (m^2/g)	EC5-R	0.032	0.027	0.032	0.029	0.031	0.022
	EC5-C	0.038	0.025	0.030	0.030	0.023	0.019
	EC7-R	0.035	0.025	0.032	0.026	0.026	0.019
	EC7-C	0.030	0.025	0.027	0.024	0.020	0.015
	ANOVA						
	EC	ns	ns	ns	ns	**	**
	CO$_2$	ns	ns	**	ns	***	***
	EC × CO$_2$	ns	ns	ns	ns	ns	ns

ns, not significant. ** Significant at $p \leq 0.01$. *** Significant at $p \leq 0.001$.

Table 4. Effect of salinity (EC: 5 and 7 dS/m) and carbon dioxide (CO$_2$) on marketable accumulated yield, distribution of marketable yield fruits per size grade, fruit affected by blossom end rot (BER), marketable fruits number and average weight of marketable fruits of cv. Delizia (hybrid Raf) for the two greenhouses (Reference: R, CO$_2$ enriched: C) measured at 203 dat

Treatments	Distribution of marketable fruits yield (kg/m^2) per size grade				Marketable yield (kg/m^2)	Marketable fruits number (nº/m^2)	Average weight of marketable fruits (g)	BER (kg/m^2)
	82-102 (mm)	67-87 (mm)	57-67 (mm)	47-57 (mm)				
EC5-R	6.86	5.02	1.08 c	0.17 ab	13.1 ± 0.3	76.4 ± 1.7 a	172.1 ± 4.3 b	0
EC5-C	8.53	5.16	0.92 d	0.09 c	14.7 ± 0.8	77.0 ± 3.6 a	190.8 ± 5.3 a	0
EC7-R	3.09	4.26	1.46 b	0.16 bc	8.9 ± 0.5	60.9 ± 2.8 c	144.1 ± 5.5 c	0.2 ± 0.1
EC7-C	3.72	4.25	2.01 a	0.25 a	10.2 ± 0.3	70.3 ± 2.9 b	145.4 ± 2.7 c	0.4 ± 0.2
ANOVA								
EC	***	**	***	**	***	***	***	***
CO$_2$	**	ns	*	ns	***	***	***	ns
EC × CO$_2$	ns	ns	***	**	ns	**	**	ns

ns, not significant. * Significant at $p \leq 0.05$. ** Significant at $p \leq 0.01$. *** Significant at $p \leq 0.001$

Table 5. Water supply (W$_S$), water uptake (W$_U$) and water use efficiency (WUE), referred to aerial dry biomass or marketable fruit yield, per unit of water supply (WUE$_S$) and water uptake (WUE$_U$) of cv. Delizia (hybrid Raf), for the two greenhouses (Reference: R, CO$_2$ enriched: C) and two salinity treatments (EC: 5 and 7 dS/m)

Treatment	W$_S$ (L/m^2)	WUE$_S$ (g/L)		W$_U$ (L/m^2)	WUE$_U$ (g/L)	
		Aerial dry biomass	Marketable fruit yield		Aerial dry biomass	Marketable fruit yield
EC5-R	457	2.9	28.9	234	5.7	57.0
EC5-C	420	3.6	35.2	216	7.0	69.2
EC7-R	342	3.2	26.2	194	5.8	49,4
EC7-C	320	4.1	32.5	179	7.2	57.9
ANOVA						
EC	***	***	***	**	ns	***
CO$_2$	***	***	***	ns	***	**
EC × CO$_2$	ns	ns	ns	ns	ns	ns

ns, not significant. * Significant at $p \leq 0.05$. ** Significant at $p \leq 0.01$. *** Significant at $p \leq 0.001$

cantly by salinity (-15%) for marketable fruit yield, but it had no effect on that referred to aerial dry biomass.

Physical quality parameters: firmness

Firmness was significantly affected by salinity (Table 6), where fruits grown with high EC (EC7) were firmer than fruits grown with low EC (EC5). The stage of maturity significantly affected fruit firmness, so that this quality parameter was highest in green-breaker fruit (G stage). A significant interaction ($p<0.01$) was observed between EC and stage of maturity when the influence of salinity on fruit firmness decreases during ripening. The fruits grown with high EC (7 dS/m) and at G stage showed the highest values of firmness, 37 N on average for both greenhouses, then decreased during ripening, and was lowest (24 N) in pink-light red fruit (P stage). On the other hand, CO_2 enrichment did not affect fruit firmness (Table 6).

Chemical quality parameters: SSC, TA and pH

The SSC, TA and pH values were significantly affected by the salinity but only the first showed a sig-

nificant response to CO_2 (Table 6). Fruits grown with high EC (EC7) had higher SSC and TA than fruits grown with low EC (EC5). The opposite effect of EC was found for pH. The stage of maturity significantly affected SSC, TA and pH. The highest values of acidity (0.53% as averaged from both greenhouses) were found in fruits grown with high EC (7 dS/m) at stage G. The ripening process slightly reduced TA, especially under conditions of high salinity. On the other hand, the pH of tomato fruit juice followed a mirror-like curve with respect to TA, with a minimum value of 4.3 (averaged from both greenhouses) at the green-breaker stage (G) under high saline conditions (7 dS/m). The SSC was slightly increased with CO_2 supply (+4%). However, the SSC was largely improved by salinity, especially in the stages of maturity T (+26%) and P (+27%).

Sensory evaluation

Sensory evaluation revealed substantial differences between treatments (Figs. 2A and 2B). Most of the panellist (96%) had previously consumed Raf tomato and 41% had consumed it frequently (Table 7). The panellists found no differences between treat-

Table 6. Analysis of variance of the effect of salinity (EC: 5 and 7 dS/m), carbon dioxide (CO_2) and the stage of maturity (G: green mature/breaker; T: turning; P: light red) on firmness, soluble solids content (SSC), titratable acidity (TA) and pH of cv. Delizia (hybrid Raf) at harvest for the two greenhouses (Reference: R, CO_2 enriched: C)

Greenhouse	EC	Stage of maturity	Firmness (N)	SSC (ºBrix)	TA (% Citric acid)	pH
C	EC5	G	32.6 ± 7.0	4.3 ± 0.1	0.41 ± 0.01	4.44 ± 0.03
		T	27.2 ± 4.7	4.5 ± 0.1	0.42 ± 0.01	4.42 ± 0.02
		P	22.6 ± 4.1	4.6 ± 0.2	0.39 ± 0.01	4.43 ± 0.02
	EC7	G	38.2 ± 9.2	5.3 ± 0.2	0.53 ± 0.02	4.33 ± 0.02
		T	31.1 ± 5.7	5.7 ± 0.2	0.49 ± 0.02	4.34 ± 0.03
		P	24.1 ± 4.4	5.8 ± 0.2	0.47 ± 0.02	4.38 ± 0.03
R	EC5	G	32.9 ± 6.6	4.2 ± 0.1	0.42 ± 0.02	4.43 ± 0.03
		T	28.3 ± 5.2	4.4 ± 0.1	0.42 ± 0.03	4.40 ± 0.02
		P	21.8 ± 4.4	4.3 ± 0.3	0.39 ± 0.02	4.44 ± 0.02
	EC7	G	36.4 ± 7.9	5.1 ± 0.3	0.52 ± 0.03	4.30 ± 0.03
		T	29.9 ± 7.1	5.5 ± 0.2	0.51 ± 0.01	4.35 ± 0.02
		P	23.8 ± 4.7	5.5 ± 0.3	0.49 ± 0.03	4.38 ± 0.02
ANOVA						
EC			***	***	***	***
CO_2			ns	***	ns	ns
Stage of maturity			***	***	***	***
EC × CO_2			ns	ns	ns	ns
EC × Stage of maturity			**	*	*	***
CO_2 × Stage of maturity			ns	ns	ns	ns
EC × CO_2 × Stage of maturity			ns	ns	ns	ns

Values are the mean ± standard deviation of three replicates of five fruits. Firmness was analyzed in each sample presented in the replicate. ns, not significant. * Significant at $p \leq 0.05$. ** Significant at $p \leq 0.01$. *** Significant at $p \leq 0.001$.
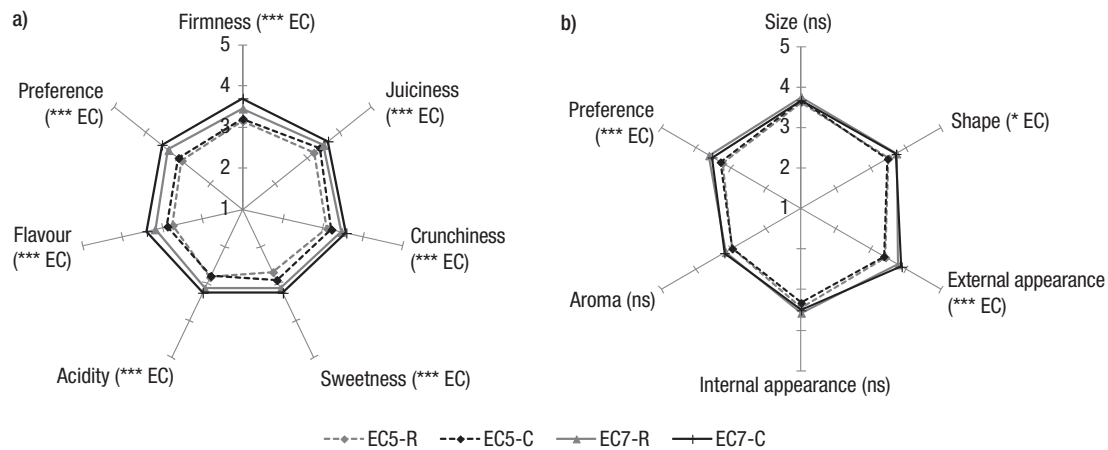
Figure 2. Quantitative analysis of the effect of salinity (EC: 5 and 7 dS/m) and carbon dioxide levels (Reference: R, CO$_2$ enriched: C) on (A) organoleptic quality parameters and (B) visual quality parameters of cv. Delizia (hybrid Raf). Values represent mean of assessments made by 103 panellists per treatment. Only significant effects of analysis of variance are shown. ns, not significant, *Significant at $p \leq 0.05$, ***Significant at $p \leq 0.001$.

ments for aroma, internal appearance and size. The following sensory attributes: visual quality (shape, external appearance) and organoleptic quality (firmness by mouth, juiciness, crunchiness, sweetness, acidity and flavour) showed differences between tomatoes from different EC treatments. According to the chemical quality results tomatoes produced under the highest EC (7 dS/m) had significantly more sweetness and acidity and therefore more "tomato taste", more flavour and firmness-by-mouth, more juiciness and crunchiness and better external appearance than fruit grown under low EC treatment (5 dS/m). In relation to CO$_2$ enrichment, although the panellists had higher preference for the organoleptic quality of CO$_2$

enriched and saline crop (EC7-C) fruit, the differences between EC7-C and EC7-R fruit were not significant.

Discussion

Effect of salinity and CO$_2$ enrichment on growth and yield

In the present study, the adverse effects of salinity were apparent even at an early developmental stage (61 dat). The plants responded to increasing salinity by a gradual decline of vegetative growth and leaf area (Tables 2 and 3). This is in agreement with the observations of Magán (2005) for a tomato crop in the SE region of Spain for an autumn-winter cycle, who observed a 5% decrease of aerial dry matter content for each dS/m that the EC exceeded 4. The increased EC resulted in a 32% decrease in LAI at the end of the crop cycle (Table 3). Li & Stanghellini (2001) obtained a lower decrease in LAI (about 20%) at the highest EC (9 dS/m) and Magán (2005) had a 6.5% decrease of LAI for each dS/m that EC exceeded 4. With respect to SLA, this parameter was reduced 16% by salinity indicating thicker leaves in the high saline treatment. A similar trend was observed by Magán (2005) with a 1.4% decrease of SLA for each dS/m that EC exceeded 4. The differences found in our study could be explained for a high saline concentration accumulated in the root medium (12 dS/m), in this experiment. Increasing the salinity of the nutrient solution significantly decreased the fresh marketable yield of tomatoes by about -31%, mainly reducing fruit size, number and average weight (Table 4). Saranga *et al.* (1991) found

Table 7. Composition of tasting panel based on sex, age, profession and consumption preferences of Raf tomato (n=103)

Sex (%)	Men	48
	Women	52
Age (%)	20-30	10
	30-40	24
	40-50	42
	50-60	22
	>60	2
Profession (%)	Farmer	10
	Student	1
	Consumer	18
	Researcher	30
	Agricultural technician	30
	Others	11
Do you consume Raf tomato? (%)	Yes	96
	No	4
How often do you consume Raf tomato? (%)	Sporadic	49
	Frequent	41
	Habitual	10

a threshold between 2.0 and 2.5 dS/m and a reduction in yield of 9 to 10% with an increase of 1 dS/m beyond the threshold.

In the crop grown under high saline conditions, the generative part represented 73% of the total dry weight of the plant. Such findings agree with De Koning (1993) in tomato, although other authors as Cuartero & Fernández (1999) found a normal distribution of dry matter for fruits, shoots and roots in a tomato crop of 52, 44 and 4%, respectively. In this study, the additional biomass partitioned to fruits with elevated CO_2 concentration was about 16% (Table 2). Similar results were reported by Sánchez-Guerrero *et al.* (2005) for cucumber (19%) and by Alonso *et al.* (2012) for sweet pepper (20%). These authors found no significant effect on vegetative dry weight while in this study, leaf and stem dry weights were significantly increased by +21% with CO_2 enrichment. However, the LAI was about equal in the R and C greenhouses and the SLA was reduced. A reduced SLA can be regarded as characteristic of morphological adaptation of a canopy to assimilate abundance, which can be a consequence of high CO_2. These results were observed by Nederhoff (1994) who reported that a too low sink/source ratio in tomato severely reduces SLA. Thus, a method to minimize the harmful effects of the increased source could be to increase the shoot density.

On the other hand, the increase of marketable yield by CO_2 enrichment (+13%) is within the range of values obtained by Sánchez-Guerrero *et al.* (2005) for a cucumber crop and Alonso *et al.* (2012) for a sweet pepper crop, in Mediterranean greenhouses. Özçelik & Akilli (1999) found that CO_2 enrichment increased the yield of a tomato crop (+37%), for a long growing cycle. Lorenzo *et al.* (2013) also found an increase of marketable yield (+19%) in tomato by CO_2 enrichment in high salinity conditions (7 dS/m), where increases in the number and mean weight of fruits were found. These results meant an attenuation of the adverse effect of salinity on marketable yield under high CO_2 conditions.

The incidence of BER was not very high despite a high salinity in the root medium (12 dS/m), so it seems that this is a less BER sensitive cultivar (Table 4).

Effect of salinity and CO_2 enrichment on intercepted radiation and its efficiency

In the present study, small differences in the quantity of intercepted PAR throughout the growth season were detected between the C and R greenhouses. The PAR intercepted by the reference crop was +3% higher than the enriched crop. This result could be due to

slightly differences in the incident PAR found between greenhouses. At the end of the cycle crop, large differences were found on LAI between high and low saline treatments, whose average between greenhouses was 1.20 and 1.75, respectively. As a consequence, salinity reduced the intercepted PAR by the canopy about 10%. Heuvelink (1996) validated a model of light interception for tomato, $I=1-exp^{(-0.75 \ LAI)}$, which predicts that a reduction in LAI of 3 to 2 would cause at most a decrease in light interception of about -15%. The large effect that salinity had on the LAI, the PAR intercepted by the canopy and the RUE, could explain the decrease in the vegetative and generative dry weights.

The RUE was about +18% higher as a result of CO_2 supply. Thus, the increased productivity with high CO_2 concentration must have been caused by an increased rate of assimilation, a result which is indicated by the elevated RUE. Such findings agree with De Koning (1993) in tomato, who observed RUE data of 3 to 5 g/MJ with intercepted PAR ranging from about 1 to 8 MJ/d at high CO_2.

Effect of salinity and CO_2 enrichment on water use and its efficiency

This study shows that WUE, calculated on the basis of crop water supply and uptake for the Raf tomato crop, was enhanced by CO_2 supply independently of saline conditions (Table 5), mainly through increased growth and yield (Tables 2 and 4). No effect of CO_2 supply was observed on crop water uptake (W_U), which was similar in both enriched and reference crops and mainly dependent on the amount of intercepted radiation (Table 5). Sánchez-Guerrero *et al.* (2009) found no effect of CO_2 supply on water uptake for a cucumber crop.

The increase in biomass resulting from CO_2 supply jointed to a decrease in irrigation requirements (W_S) compared to the reference treatment, resulted in larger WUE for the high CO_2 concentration treatment. High CO_2 may improve the WUE by increased photosynthesis and reduced transpiration (Nederhoff, 1994), which is favorable if good water is scarce. The high salinity reduced the water supply (W_S) and uptake (W_U), and mainly affected the WUE for marketable fruit yield. Maggio *et al.* (2002) observed for a tomato crop that water use did not proportionally decrease with increasing root zone salinity and that the total water use was restricted more by the effects of reduced canopy growth than by the effects of nutrient solution osmotic potential on water uptake. Summarizing, these aspects, the reduced water supply and increased productivity, indicate that when grown in high saline conditions, CO_2 supply leads to a more efficient tomato production.

Effect of salinity and CO$_2$ enrichment on physical-chemical quality and sensory

Physical-chemical composition and sensory (organoleptic and visual quality) differences were observed in the tomatoes under the different EC treatments (Table 6). The measurements relating to the physical-chemical composition revealed that salinity increased fruit firmness, SSC and TA at the different stages of maturity. Such findings are consistent with Del Amor *et al*. (2001) who observed that tomato fruit firmness increased when the EC of the irrigation water was increased; Petersen *et al*. (1998), Cuartero & Fernández (1999), Krauss *et al*. (2006) and Escobar *et al*. (2012) also showed that high EC treatments increased the concentration of sugars, organic acids and percentage of dry matter in tomato fruits. Zushi & Matsuzoe (2011) reported that SSC and TA in tomato, were closely related to the sweetness and acidity of the fruits, respectively. An increase in the concentration of both, led to better tasting tomatoes (Petersen *et al*., 1998) meaning that fruits grown under high EC were preferred by consumers (Fabre *et al*., 2011). A useful indicator of tomato taste is the SSC:TA ratio (maturity index) where minimum values of SSC and TA of 5 and 0.4%, respectively (SSC:TA of 12.5) are considered necessary to produce a good-tasting table tomato (Kader *et al*., 1978). In this study, the fruit produced in the high saline condition was characterized by and SSC of more than 5 and a TA of more than 0.4% giving a SSC:TA ratio >12, which indicates tomatoes of high quality.

On the other hand, fruits grown with high EC showed lower pH values than from the low EC treatment (EC5). This was also observed by Magán *et al*. (2008) in a tomato crop grown in Mediterranean greenhouses.

Fruits of cv. Delizia showed a progressive increase of SSC and decrease of TA from the mature-green to the red ripe stages. Sánchez Pérez *et al*. (2011) showed a similar trend for Raf tomatoes at different stages of maturity. The combination of reducing sugars and organic acids content found for Raf fruits explains the unique and exceptional taste of this variety. All fruits tested had firmness values signally higher than 8 N. Artés *et al*. (1999) considered 8-10 N as the marketability and consumption limit for tomatoes for fresh market.

With respect to the effects of CO$_2$ enrichment on the physical-chemical quality parameters, it had no effect on fruit firmness, TA or pH (Table 6). In contrast, the SSC was significantly higher in the CO$_2$ enriched greenhouse and this quality parameter increased from the mature-green to the red ripe stages. Islam *et al*.

(1996) obtained similar findings. This was possibly due to increased photosynthesis in the CO$_2$ enriched plants, resulting in a greater translocation of photosynthate into the fruit and increased sugar concentration. Thus, CO$_2$ could maintain the positive effect on SSC in the enriched greenhouse.

The increase in EC led to a general change of intensity of sensory characteristics of tomato fruits (Figs. 2A and 2B) and to an increase in sugar and acid contents (Table 7). In this study, high EC tomatoes had higher firmness (flesh firmness), juiciness, crunchiness (peel firmness), sweetness, acidity, flavour and better external appearance than the tomatoes produced with the lower EC. Such findings concur with Auerswald *et al*. (1999) who reported that mouthfeel (fruit flesh firmness, fruit flesh juicy and peel firmness) and flavour of tomatoes increased with elevated EC values. On the other hand, Petersen *et al*. (1998) observed that the greater Na$^+$ content of fruits could enhance taste and the perception of sweetness, thus improving flavour balance and overall aroma balance. Previous studies on mineral composition of this hybrid Raf cultivar, showed that fruits grown under high EC (7 dS/m) had higher Na$^+$ content values (Sánchez-González *et al*., 2014). Panel trials revealed an overwhelming preference for fruits grown under high EC during choice tests, based on both organoleptic and sensory evaluations.

In conclusion, the results show that growing hybrid Raf tomatoes under high salinity conditions (7 dS/m) has clear benefits on fruit quality, by increasing flavour and improving firmness. However, there is also a decrease in marketable yield and less vegetative growth resulting in lower radiation use and water use efficiencies. The negative result was countered by enriching the greenhouse with CO$_2$ which increased the radiation use efficiency giving a significant increase in yield and also reduced the crop water requirement and increased the water use efficiency. This finding has important implications as water is a scarce and limited resource in the Mediterranean area. Therefore, adequate climate management, through greenhouse CO$_2$ enrichment based on a strategy linked to ventilation, could have an important and positive influence in mitigating the negative effect of salinity on productivity of this high value cultivar without compromising fruit quality.

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