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Additional Information

Influence of controlled deficit irrigation on tomato functional value

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Abstract

The effect of controlled deficit irrigation (CDI) on the accumulation of carotenoids, polyphenols and L-ascorbic acid was studied in conventional and high lycopene tomato cultivars. Plants were initially irrigated to cover 100%ET_c and after the fruit set phase, the dose was reduced to 75% or 50% of ET_c. CDI had no significant effect on the accumulation of carotenoids, while it increased the levels of the hydroxycinnamic acids chlorogenic and ferulic acids, the flavonoid rutin and L-ascorbic acid. Nevertheless, there were important interactions and this effect was highly dependent on the year and site of cultivation. Certain growing areas would be more favorable to supply high quality markets, and, fortunately, CDI would maximize polyphenol (100-75%ET_c) and L-ascorbic acid (100-50% ET_c) in these areas. A combination of the best genotype and growing area with CDI would offer high quality products, preserving a scarce resource: water.

Keywords: carotenoid, polyphenol, ascorbic acid, high-lycopene cultivar, *Solanum lycopersicum*

Chemical compounds studied in this article; Caffeic acid (PubChem CID: 689043); *p*- coumaric acid (PubChem CID: 637542); *trans*-ferulic acid (PubChem CID: 445858); Chlorogenic acid (PubChem CID: 1794427); Kaempferol (PubChem CID: 5280863); Quercetin (PubChem CID: 5280343); Myricetin (PubChem CID: 5281672); Naringenin (PubChem CID: 932); Rutin (PubChem CID: 5280805); β-carotene (PubChem CID: 5280489); Lycopene (PubChem CID: 446925); L-Ascorbic acid (PubChem CID: 54670067)

1. Introduction

The functional quality of food is determined by its ability to accumulate bioactive health-promoting compounds. In the case of tomato, it depends mainly on the content of carotenoids, vitamin C, and polyphenols. Tomato is not especially rich in these compounds, but it is an important source of these compounds in the diet due to the high levels of consumption. In fact, tomato has been reported to be the 1st source of lycopene (fresh tomato and tomato sauce represent 95% of lycopene dietary intake), the 2nd source of β-carotene (following carrots), the 2nd source of vitamin C (after oranges) and the 6th source of polyphenols, following oranges, apples, potatoes, bananas and grapefruit (Chun et al., 2005; Garcia-Closas et al., 2004).

The role of these compounds in the prevention of diseases has been reported in several epidemiological studies. Carotenoids have been linked with a lower risk of suffering some types of cancer mainly due to their antioxidant properties against Reactive Oxygen Species (ROS) and their ability to modulate several cell cycles involved in cancer progression (Martí, Roselló, & Cebolla-Cornejo, 2016). Carotenoids have been also related to a reduction of risk of suffering cardiovascular diseases by inhibiting cholesterol synthesis and increasing LDL degradation (Kris-Etherton et al., 2002). Polyphenols interfere with the initiation, promotion, and progression of cancer through different mechanisms including their ROS quenching properties, the modulation of the activity of several detoxifying enzymes, affecting enzymes involved in pro-carcinogenic metabolism and modulating NF-κB molecular pathway (Martí et al., 2016). It has also been reported that they reduce cardiovascular disease risk by inhibiting platelet aggregation and reducing prothrombotic and proinflammatory mediators (Kris-Etherton et al., 2002). Apart from its typical nutrient activity, vitamin C has been linked to a decrease in mortality due to cancer and cardiovascular disease (Carr & Frei, 1999).

These beneficial effects have spurred the interest in increasing the contents of these chemoprotective compounds either via plant breeding or agronomic management. In the case

of carotenoids, breeding programs have been focused on the use of mutants affecting single steps of the biosynthetic pathway, such as *old gold crimson, B^{ogc},* or those affecting the regulation of the pathway and chloroplast biogenesis. This last strategy has been the most successful, as the use of high pigment genes such as *hp-1* and *hp-2*, not only increases global carotenoid content but also polyphenols and vitamin C (Bino et al., 2005).

Regarding agronomic management, deficit irrigation has been proposed as an efficient strategy to reduce the use of this scarce resource and to improve organoleptic and functional quality. In their review, Dumas, Dadomo, Di Lucca, & Grolier (2003) reported contradictory results regarding the effect of water availability on carotenoid content, though later studies tended to recognize the effect of strong water deficit with an increase in bioactive compounds. For example, Pék, Szuvandzsiev, Daood, Neményi, & Helyes (2014) comparing irrigated and rain-fed tomato production concluded that irrigation decreased total carotenoid and polyphenol concentration. Something similar concluded Pernice et al. (2010) comparing non-irrigated and irrigated samples, though these differences were limited when comparing irrigation and deficit irrigation. In the same line, our group studying continuous water deficit irrigation found no differences in lycopene content between the control (100%ET_c) and deficit irrigation (75%ET_c), but over-irrigation (125%ET_c) had a dilution effect on the levels of carotenoids (Lahoz et al., 2016a). Favati et al. (2009) found that longer irrigation interval and a reduced irrigation regime contributed significantly to increase the lycopene and β-carotene, especially those most restrictive.

Few of these studies include different genotypes and even fewer include high-lycopene cultivars (cvs.). In a previous study, we found these materials represented the main factor determining carotenoid content while continuous water deficit irrigation resulted in an excessive reduction of yield (Lahoz et al., 2016a). It seems that the timing of water deficit irrigation may be important, and Wang, Kang, Du, Li, & Qiu (2011) found that a severe reduction of irrigation dose at the flowering and fruit development stages significantly reduced crop water consumption and

enhanced fruit accumulation of lycopene and vitamin C. Consequently, continuing previous works, the objective of this study was to apply deficit irrigation only after the fruit set phase (controlled deficit irrigation) as a strategy to reduce water use and to increase the functional quality of tomato, including the effects on carotenoid, polyphenol, and L-ascorbic acid contents.

2. Material and methods

2.1. Plant material

Four processing tomato cvs. were evaluated. 'Heinz(H)-9661', 'H-9036', 'H-9997', (Heinz Seed) and 'ISI-24424' (Diamond seeds S.L.; Isi Sementi S.P.A.). The first two were selected as standard cvs. considering their good agronomical performance and their wide use by local producers in the growing areas assayed. The last two were selected as high lycopene cvs. In previous works 'ISI-24424' showed high lycopene accumulations, 'H-9997' presented intermediate accumulations while 'H-9661', 'H-9036' showed low lycopene accumulation (I. Lahoz et al., 2016b).

2.2. Growing conditions and experimental design

The selected cvs. were grown in the two main growing areas of processing tomato in Spain: Extremadura (located in the SouthWest), and Navarra (located in NorthEast) during two years: 2012 and 2013. Integrated pest management (IPM) were performed in both cultivation sites applying the fertilization doses and phytosanitary treatments typically employed in each site. In Extremadura, the plantation was carried out in the fields of the research center Finca 'La Orden-Valdesquera' (Badajoz, Extremadura) (lat. 38º 53' 26'' N, long. 6º 40' 00'' W) on April 24th in 2012 and May 2nd in 2013. In Navarra, the plantation was performed in fields of INTIA in Cadreita (lat. 42º 12' 34'' N, long. 1º 43' 1'' W) on May 10th in 2012 and May 23rd in 2013.

Initially, plants were irrigated satisfying 100% of crop evapotranspiration (ET_c). Once the first fruits were set and the fruit growth stage started, two controlled deficit irrigation doses were

applied covering 50% and 75% of ET_c. A control covering 100%ET_c was also included in the study. ET_c was calculated using the Penman-Monteith method (Allen, Pereira, Raes, & Smith, 1998).

Three replicates of 25 plants of each genotype were distributed for each irrigation dose and environment following a split plot experimental design. A spacing of 1.50 m x 0.2 m (3.33 plants m-2) was used in Extremadura and a spacing of 1.60 m x 0.35 m (3.57 plants m-2) under a 15 µm polyethylene plastic in Navarra.

Environmental conditions were recorded for further interpretation of the results. Maximum temperature and relative humidity were registered using an HMP45C probe (Vaisala, Helsinki, Finland) in both cultivation areas. Solar irradiance was recorded in Extremadura using CMP3 pyranometer (Kipp&Zonen, Delft, the Netherlands) and a 110/S pyranometer (Skye, Powys, United Kingdom) in Navarra.

2.3. Sampling

A single harvest for each cv. was performed when the 85% of tomato fruits were in the commercial-red stage considering common commercial practices. Two representative fruits were collected from each of the 25 plants of the replicate. Fruits were pooled and homogenized, obtaining a sample representing a biological mean of the replicate. Resulting samples were frozen at -80^{o}C until analysis.

2.4. Chemicals and reagents

Caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, chlorogenic acid, kaempferol, quercetin, myricetin, naringenin, rutin, lycopene, β-carotene, L-ascorbic acid, metaphosphoric acid, hexadimethrine bromide (HDM), butylated hydroxytoluene (BHT), formic acid, ethanol, methanol (MeOH), acetonitrile (ACN), hexane and ethyl acetate (AcEt) were purchased from Sigma-Aldrich (Syeinheim, Germany). All solvent were HPLC-grade purity. Boric acid and sodium hydroxide (NaOH) were purchased from from Panreac (Castellar del Vallés, Spain). Ultrapure water was obtained using a Milli-Q water system (Millipore, Molsheim, France).

2.5. Extraction and quantification of phenolic compounds

Polyphenols were extracted following the procedure described by Martí, Valcárcel, Herrero-Martínez, Cebolla-Cornejo, & Roselló (2015). Approximately 1 g of homogenized sample was mixed with 5 mL of MeOH/water (48:52 v/v) solution containing 1g kg^{-1} BHT. Samples were immersed in an ultrasonic bath Elmasonic S30H (Elma Electronics AG, Wetzikon, Switzerland) at 60 Hz for 177 minutes in absence of light to avoid the oxidation of target compounds. Extracts were centrifuged at 4000 rpm (2361*g*) for 5 min at 4^{o}C and the resulting supernatants were filtered through a 0.2 µm pore size PTFE filter prior their injection in the HPLC.

Phenolic compounds were quantified using a 1200 Series HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a degasser, a quaternary pump, an auto-sampler, a thermostated column, and a diode array detector (DAD). The analytical column used was a fused-core Kinetex-XB C18 (150 mm x 4.6 mm id; particle size, 2.6 µm) from Phenomenex (Torrance, CA, USA) equipped with a 4.6 mm id guard column. The chromatographic conditions employed were those described by Martí et al. (2015) with minor modifications. In brief, analytical column and guard column were thermostatically controlled at 35^{o}C. The flow rate was set at 0.8 mL min^{-1} and the sample injection volume was 10 µL. The mobile phases used consisted of water, ACN and MeOH, acidified with 1 mL L^{-1} formic acid. Separation gradient started with a 30% MeOH and 0% ACN and their concentrations were varied to 24% and 18% respectively until minute 12; then MeOH concentration was raised to 30% at minute 13 keeping constant ACN content; finally, MeOH concentration was lowered to 20% while ACN concentration was raised to 30% until minute 20; the last step recovered the conditions for the next sample injection. Rutin was quantified at 255 nm, naringenin at 290 nm, caffeic, *p*-coumaric, ferulic and chlorogenic acids at 320 nm, and kaempferol, quercetin and myricetin at 365 nm. Samples were analyzed twice.

2.6. Extraction and quantification of L-ascorbic acid

The procedure described by Galiana-Balaguer, Roselló, Herrero-Martínez, Maquieira, & Nuez (2001) with some modifications was used to analyze L-ascorbic acid. For extraction, the homogenized samples were centrifuged at 12000 rpm (10483g) for 5 min at 4^{o}C. Then, supernatants were diluted to 1/10 with a 20 g L^{-1} metaphosphoric acid solution and, finally filtered through a 0.2 µm pore size cellulose acetate (CA) filter prior injection in the CE system.

L-ascorbic acid was quantified using a 7100 capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany), equipped with a diode array detector and thermostated sample compartment. Prior its first utilization, uncoated fused silica capillaries (32cm total length, 24cm effective length, 375 µm od, 50 µm id) from Polymicro Technologies (Phoenix, AZ, USA) were rinsed with NaOH 1M at 50^{o}C for 5 min, followed by 5 min NaOH 0.1M and 10 min of water. Prior each working session, the capillaries were flushed with running buffer for 30 min at 25^{o}C and, between runs, during 3 min at 25^{o}C. Running buffer was made up of a 400mM boric acid solution containing 1 g L^{-1} HDM adjusted to pH 8. All buffers were filtered through a 0.2 µm pore size CA filter. Hydrodynamic injection at 3400Pa for 5s and a voltage of -15kV were applied at 25^{o}C. Electropherograms at 254 nm were recorded for detection and quantification. Samples were analyzed twice.

2.7. Extraction and quantification of carotenoids

In the case of carotenoids, 200 mg of homogenized sample were extracted with a mixture made of 8 mL of ethanol and 6 mL of hexane with 0.1% BHT (w/v) during 24 h in a horizontal shaker (STR6, Bibby Sterlin LTD, Staffordshire, UK) at 4^{o}C. All extraction procedure was performed in absence of light to avoid the degradation of target carotenoids. The upper hexane layer was separated by adding 1 mL water, and after concentrated to dryness using a SpeedVac SPD-121 P and refrigerated vapor trap RVT-4104 (Thermo Scientific, Waltham, MA, USA). The concentrate was re-suspended in 0.5 mL of hexane containing 0.1% BHT (w/v).

188 The procedure described by Garcia-Plazaola & Becerril (1999) with some modifications was
189 followed to determine lycopene and β-carotene contents using a 1200 Series HPLC system
190 (Agilent Technologies, Waldbronn, Germany), equipped with a degasser, quaternary pump, an
191 auto-sampler, a thermostated column, and a diode array detector (DAD). A reversed-phase
192 column Zorbax ODS (250 mm x 4.6 mm id; particle size, 5 µm) from Agilent (Agilent Technologies,
193 Waldbronn, Germany) equipped with a 4.6 mm id guard column was used at 20^{o}C. The flow rate
194 used was 1.2 mL min^{-1} and the sample injection volume was 20 µL. Samples were previously
195 filtered through a 0.2 µm pore size PTFE filter. The mobile phases consisted of a mixture of 84:9:7
196 ACN/MeOH/water (v/v/v) (solvent A), and a mixture of 68:32 MeOH/AcEt (v/v) (solvent B).
197 Linear separation gradient was performed varying from 100% A to 100% B until minute 12. Then
198 concentrations were kept isocratic until minute 19. Finally, a linear decrease of B from 100% to
199 0% was performed to prepare the column for next sample injection. The DAD detector with
200 spectra recoding was used for detection and quantification of lycopene and β-carotene at 470
201 nm and 445 nm, respectively. Samples were analyzed twice.

202 ***2.8. Statistical analysis***

203 MANOVA test was performed using the SPSS 22.0 software (NYSE: IBM, Armonk, NY, USA) to
204 evaluate the effects of the site of cultivation, genotype, irrigation dose, and their interactions
205 on polyphenol profile. *P*-value was calculated using the Pillai trace test. The effect of cv. and
206 irrigation doses on phenolic profile for both years and sites of cultivation was further studied
207 with a graphical MANOVA Biplot representation (freeware licensed software by Vicente-
208 Villardon, 2015). Bonferroni circles were plotted to represent the confidence intervals ($\alpha = 0.05$),
209 using their projection on each variable for the identification of statistically significant differences
210 between groups. In the MANOVA Biplot, dashed lines were used to indicate non-significant
211 effects. To complete the analysis, individual ANOVAs and Tukey B multiple range test were
212 performed. These tests were also used for the rest of compounds.

3. RESULTS

Environmental conditions were recorded as they exert a clear influence in the accumulation of bioactive compounds. Global solar irradiance was higher in 2012 than in 2013 especially in Navarra (Fig.1). In 2012, the irradiance in Extremadura was higher than in Navarra, and notably during the last part of the growing cycle. The conditions of 2013 were not ideal for tomato cultivation, with a bad weather that conditioned a late transplant in Navarra. Although during the growing cycle the differences in irradiance between both sites were lower, during the weeks prior to harvest irradiance was much lower in Navarra. The differences in maximum temperature between Extremadura and Navarra were limited in 2012, but in 2013, higher maximum temperatures were recorded in Extremadura.

3.1. Polyphenol profile

All studied factors (year, site of cultivation, irrigation dose and genotype) showed a significant effect on the polyphenol profile (MANOVA Pillai trace $p < 0.01$). In order to identify specific effects, separate ANOVA tests were run for each compound. The effect of year of cultivation, representing climatic differences significantly affected the accumulation of caffeic acid, rutin, and naringenin (Table 1). Higher accumulations of caffeic acid were found in 2013, while for rutin and naringenin the conditions of 2012 were more favorable. On the other hand, the site of cultivation, representing differences in climatic conditions and agricultural management, was significant for the accumulation of caffeic, *p*-coumaric and ferulic acids, with higher concentrations obtained in Navarra.

The effect of irrigation dose was significant for the accumulation of chlorogenic acid, ferulic acid, and rutin. The highest concentrations of polyphenols were obtained with a controlled deficitary irrigation regime of 100-75%ET_c (Table 1).

The genotype had a significant effect on the concentration of all phenolic compounds with the exception of *p*-coumaric and ferulic acids (Table 1). The cultivar 'H-9661' outstood for high

238 contents of rutin and naringenin, especially the first one. However, the concentrations of
239 hydroxycinnamic acids (chlorogenic and caffeic acids) were lower compared to other cultivars.
240 Cultivar ‘H-9997’ outstood for high contents of chlorogenic and caffeic acids and intermediate
241 contents of rutin and naringenin. Cultivar 'H-9036' showed the highest content of chlorogenic
242 acid, the lowest contents of rutin and naringenin and the lowest accumulations of polyphenols.
243 Finally, cultivar ISI-24424 showed an intermediate accumulation of polyphenols, with only high
244 contents of caffeic acid.

245 Strong double interactions were detected between the considered factors. Especially, in the
246 case of year and site of cultivation, as this interaction was significant for all the compounds.
247 Consequently, it was considered necessary to evaluate the effect of irrigation dose separately
248 for each environment. MANOVA biplots were used for this purpose (Fig. 2). In Extremadura
249 controlled deficitary irrigation did not show an increase in the accumulation of polyphenols in
250 neither year. In fact, the projections of the Bonferroni confidence circles overlapped on the
251 variable vector. A certain year x genotype interaction could be observed while analyzing the
252 whole MANOVA Biplot model (Supp. Fig. 1) as in 2012 a clear increase in the accumulation of
253 rutin was obtained with a controlled deficit irrigation of 100-75%ET_c in ‘H-9661’. But this effect
254 was not observed for this cultivar in 2013, and rutin contents with this regime increased only in
255 the case of ‘H-9997’.

256 On the contrary, a dramatic effect was observed in Navarra. In 2012 controlled deficitary
257 irrigation resulted in higher polyphenol content, especially of hydroxycinnamic acids (Fig. 2 and
258 Supp. Table 1). Between 100-75% and 100-50%ET_c the differences were limited. In 2013, the the
259 polyphenol profile of the 100-75%ET_c dose overlapped with the control 100-100%ET_c, and only
260 the most restrictive irrigation dose increased polyphenol content (Fig. 2). A strong cultivar x
261 irrigation dose appeared in this environment and the global model (Supp. Fig. 1) made clear that
262 the distinctive performance in 2013 of the 100-50%ET_c dose was evident for ‘ISI-24424’ and ‘H-
263 9997’ but not for the rest of cultivars.

The effect of cultivar was more pronounced compared to irrigation strategy. The general performance detected with the global ANOVAs could be confirmed in the MANOVA biplots of each environment, where 'H-9661' outstood for rutin accumulation, while 'H-9036' tended to show lower contents of rutin and naringenin. On the other hand, 'H-9997' and 'ISI-24424' showed intermediate profiles that varied among environments, confirming a certain cultivar x environment interaction.

3.2. Carotenoid profile

The effect of year had a significant influence on the concentration of both carotenoids, with higher accumulations of β-carotene in 2013 and lycopene in 2012 (Table 2). On the other hand, the effect of the site of cultivation only was significant for lycopene content, being more favorable for its accumulation the conditions of Navarra. Controlled deficit irrigation did not affect the content of these carotenoids. Again, the genotype had a major and significant effect, 'ISI-24424' outstanding for the highest levels of both carotenoids. 'H-9997' also showed high lycopene content, but the accumulation of β-carotene was remarkably lower. 'H-9661' and 'H-9036' showed low lycopene accumulation, but differed in the accumulation of β-carotene, being lower in 'H-9036'. The only significant interaction included year and site of cultivation. An independent analysis for each year and site of cultivation (Fig. 3), also showed that the differences were mainly defined by genotype independently of the year, site or irrigation dose used.

3.3. L-ascorbic acid

All the environmental factors (year, site, irrigation dose) affected significantly L-ascorbic accumulation (Table 2). Higher concentrations were obtained with the conditions of 2013 and in Extremadura. Only the most restrictive controlled deficit irrigation dose (100-50%ET_c) favored higher accumulations of L-ascorbic. Regarding the genotype no significant differences were found among the rest of cultivars.

289 Considering that several interactions were significant, the accumulation of L-ascorbic acid was
290 again analyzed separately for each year and site of cultivation. In 2012, higher contents were
291 obtained in Navarra (144.0 mg kg^{-1} vs 118.4 mg kg^{-1}; $p<0.001$). In this year, irrigation dose did
292 not have, in general, any significant effect (Fig, 4). Only in 'H-9997' a higher accumulation was
293 detected in the control 100-100%ET_c dose. In 2013, higher concentrations were obtained in the
294 conditions of Extremadura (186.1 mg kg^{-1} vs 123.8 mg kg^{-1}; $p<0.001$), where the controlled deficit
295 irrigation did not affect significantly L-ascorbic acid contents. On the other hand, in Navarra
296 controlled deficit irrigation, independently of the dose, dramatically increased the accumulation
297 of L-ascorbic acid for all cultivars except for 'H-9036'.

298 **4. Discussion**

299 The growing concern of consumers on how to prevent diseases and improve their health has
300 placed the focus on the accumulation of bioactive compounds in food. Especially, since it is
301 becoming clearer that the positive effect of these molecules is higher when they are ingested in
302 their natural matrix (Burton-Freeman & Sesso, 2014). In this context, it is important to spur the
303 accumulation of these compounds, increasing the functional value of food, in order to take
304 advantage of the price premium that can offer this demand and to satisfy the consumer
305 necessities.

306 Tomato has become one of the main sources of bioactive compounds such as L-ascorbic acid,
307 carotenoids and polyphenols, considering that the food supply of tomatoes reaches up to 35.8
308 kg $capita^{-1}$ $year^{-1}$ in North America or 34.7 kg $capita^{-1}$ $year^{-1}$ in Southern Europe (FAO, 2013; last
309 data available 2013). Thus, it has become an ideal model to tackle the development of high
310 functional value produces. The increase in the levels of these compounds can be achieved
311 manipulating either the environment or the genotype. In our study, the comparison between
312 years offered information on the effect of climatic conditions, while the comparison between
313 sites was more complex to asses, as not only climatic conditions changed, but also the

314 agricultural management. It should be considered that in order to evaluate real commercial
315 situations, the specific practices followed at each site of cultivation were adopted. Thus,
316 different soils and climates entailed different ET_c, yields and consequently different irrigation
317 doses, fertilizations and even mulches or plant densities.

318 The effect of the year is not foreseeable and escapes the intervention of the grower. Therefore,
319 even knowing the most appropriate climatic conditions to maximize the accumulation of
320 bioactive compounds, the capacity to alter them is limited. This being said, it seems that certain
321 growing areas may be more appropriate for producing high functional value tomatoes. It would
322 be the case in our study in Navarra, where with optimal climate conditions higher contents of
323 carotenoids and hydroxycinnamic acids can be obtained.

324 The importance of the site of cultivation becomes clear when comparing the highest and lowest
325 levels of accumulation of the different compounds for each significant factor. A 9% higher caffeic
326 acid content was obtained in the best site and a 97% in the case of ferulic acid, 45% in the case
327 of lycopene and 14% for L-ascorbic acid. These values were similar or higher than those obtained
328 with the best year of cultivation.

329 In the case of polyphenols, previous assays in the same years and cultivation sites indicated that
330 the accumulation of rutin (major polyphenol) was maximized with intermediate conditions,
331 while high irradiance and temperature levels would limit the accumulation of this flavonoid, with
332 a lower effect on hydroxycinnamic acids (Martí, Leiva-Brondo, Lahoz, Campillo, Cebolla-Cornejo,
333 & Roselló, 2018). On the other hand, low irradiance levels would also limit the accumulation of
334 rutin. In the present study, the more favorable climatic conditions in terms of irradiance also
335 favored the accumulation of rutin, but no significant difference was observed between sites of
336 cultivation. Nevertheless, it seems clear that the lower irradiance levels of Navara 2013 had a
337 negative impact on the accumulation of this polyphenol (Supp. Table 1).

The effect of environment on the accumulation of carotenoids was more dramatic. As stated in other assays, the higher values of lycopene obtained in Navarra may be related to the maximum temperatures obtained during the ripening phase (Fig. 1), as temperatures higher than 30-32°C block lycopene synthesis, while it continues being cyclisated (Brandt, Pék, Barna, Lugasi, & Helyes, 2006; Tomes, 1963).

In the case of L-ascorbic acid the results are difficult to interpret due to the presence of important interactions. In 2012 the higher values detected in Navarra compared to Extremadura maybe related to lower maximum temperatures. Although light exposure is favorable to vitamin C accumulation (Dumas et al., 2003), for similar irradiance condition, total ascorbate concentration is lower with stressing temperatures, 27°C vs. 32°C (Gautier et al., 2008). The low levels of irradiance in 2013 in Navarra would explain the dramatic lower contents obtained in this location. On the contrary, the high contents obtained in Extremadura 2013 are difficult to explain, as temperatures in Extremadura in 2012 and 2013 were similar, and the irradiance levels of 2013 were lower during the growing cycle, but similar during the last phase of the cycle.

Deficit irrigation did not offer differences in the levels of polyphenols, carotenoids or ascorbic acid contents as important as the rest of environmental conditions (year and site of cultivation). Nevertheless, it can be used as an effective strategy to further improve the functional value of tomatoes.

In the case of polyphenols, few studies are available regarding the effect of water deficit on the individual accumulation of flavonoids and hydroxycinnamic acids. Sánchez-Rodríguez, Moreno, Ferreres, Rubio-Wilhelmi, & Ruiz (2011) described that water stress resulted in a decrease of the shikimate pathway, with reduced levels of quercetin and kaempferol in sensitive cultivars but it was induced in tolerant material. That study was conducted in a growth chamber with a perlite-vermiculite mix and the results are not comparable, but they indicate the existence of an

362 important cultivar interaction. In other species such as grapes or apples, water stress seems to
363 stimulate the flavonoid biosynthesis pathway (Stefanelli, Goodwin, & Jones, 2010)

364 The comparison of irrigated vs. non irrigated field tomato showed in the case of Barbagallo, Di
365 Silvestro, & Patanè (2013) an increase of 13% in total phenolic content, though total flavonoid
366 content was not affected. On the contrary, also comparing irrigated vs. rain-fed tomatoes, Pék
367 et al. (2014) found that irrigation decreased flavonoid content, especially rutin, while the effect
368 on phenolic acids depended on the harvest date. Similarly, Pernice et al. (2010) also found that
369 with reduced irrigation or none irrigation total flavonoids increased. Nevertheless, in one of the
370 cv. analyzed, this increase was only observed with reduced irrigation, as with none-irrigation,
371 the levels decreased. This result seems to agree with our results, as deficit irrigation increased
372 the levels of chlorogenic and ferulic acids, but in the case of rutin this increase was only obtained
373 with the intermediate dose of 100-75%ET_c and a further reduction in irrigation decreased the
374 levels to contents similar to the control. Nevertheless, this effect is highly dependent on the
375 environment, with a clear effect in milder climates such as Navarra and no effect in climates
376 with higher irradiance and temperature such as Extremadura. A detailed analysis in each
377 environment also confirmed a higher effect on hydroxycinnamic acids. On the other hand, a
378 clear effect of the genotype was also observed, in agreement of the commented results of
379 Sánchez-Rodríguez et al. (2011) suggesting that the promotion of the biosynthetic pathway
380 depended on the cultivar.

381 More information is available in the case of carotenoids. In fact, in a previous study using
382 continuous deficit irrigation and similar cultivars, our group found that deficit irrigation did not
383 increase the level of lycopene, while an over-dose (125%ET_c) had a dilution effect (Lahoz et al.,
384 2016a). Accordingly, a milder treatment as the controlled deficit irrigation applied in the present
385 study had no significant effect on carotenoid content. It seems that in order to increase the
386 levels of carotenoids, a higher water stress should be applied. In this sense, the mentioned study

387 of Pernice et al. (2010) showed that reduced irrigation had no effect on total carotenoids, that
388 could be increased only with none irrigation.

389 Favati et al. (2009) also compared continuous or controlled deficit irrigation, but in that case,
390 they found that a limitation of the irrigation dose in whole cultivation cycle (50-50%ET_c) or in
391 the last part of the cultivation cycle (100-50%ET_c) favored the accumulation of lycopene and β-
392 carotene, especially with the most restrictive treatments. It seems, then, that the specific
393 environment would play an important role in determining the effect of deficit irrigation on
394 carotenoid content.

395 In that study, Favati et al. (2009) also found that continuous (50%ETc) or controlled (100-50%)
396 deficit irrigation increased the levels of vitamin C. In our case, we also found that the most
397 restrictive dose (100-50%) increased the levels of L-ascorbic acid, while this effect was not found
398 with the 100-75% dose. Nonetheless, this effect was dependent on the environment, as it was
399 clearly marked in Navarra and in 2013, but not in the rest of conditions.

400 Genotype exerted an even higher effect on the content of functional compounds. Accordingly,
401 the selection of the best genotype should be an essential step in order to target the production
402 to high quality markets, able to pay a price-premium for high functional value. Within the
403 materials tested, the joint selection of increased levels of the three types of compounds is
404 difficult. Nonetheless, cv. 'H-9661', would offer high rutin and L-ascorbic acids contents and 'H-
405 9997' would combine high lycopene and rutin levels.

406 'H-9997' and 'ISI-24424' have been classified previously as high lycopene cultivars and it was
407 suggested that the profile of the first one is compatible with mutation such as *Og*^{c} altering single
408 steps of the carotenoid pathway while the second was linked with the possible presence of a
409 high lycopene, *hp*, gene, which affect chloroplast biogenesis and pathway regulation (reviewed
410 by Cebolla-Cornejo, Roselló, & Nuez, 2013). This, would explain the different β-carotene to
411 lycopene ratios in each material. It has been described that materials carrying *hp-1* or *hp-2* genes

tend to show high contents of carotenoids, polyphenols and vitamin C. Although the carotenoid profile of 'ISI-24424' seemed compatible with one of these mutations (Martí et al., 2018), the levels of major polyphenols were lower than 'H-9997' or 'H-9661'. In addition, although it also presented relatively high L-ascorbic contents in certain conditions, in general, the differences with the rest of cvs. were not significant. It seems, then, that it either does not have this mutation or that the genetic background would affect this pleiotropic effect. Considering that the yield of 'H-9997' is remarkably higher than 'ISI-24424' (Lahoz et al., 2016b), its higher polyphenol profile and good lycopene accumulation levels, it would be a good candidate for high quality markets.

In conclusion, controlled deficit irrigation can have a positive impact on the functional value of tomato, especially via its effects on the polyphenol profile and L-ascorbic acid accumulation. Nevertheless, a high quality production should be obtained in a favorable growing area with the best genotypes in order to assure this effect. This strategy would not only have a synergic effect on fruit quality but would also help to preserve water, an increasingly scarce resource.

Conflict of interest

The authors declare no conflict of interest.

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Figure 1. Global solar irradiance and maximum temperature for Extremadura (Ext) and Navarra (Nav) in 2012 and 2013.

Figure 2. MANOVA Biplot for polyphenol accumulation in Extremadura and Navarra in 2012 and 2013. Effect of irrigation dose (left) and cultivar (right). Circles represent Bonferroni confidence intervals. Solid vector lines correspond to significant effect of the variable and dashed lines to non-significant effect ($p<0.05$). Significance of differences among conditions is inferred by the projection of the confidence circles on each vector.

Figure 3. Carotenoid content for each cultivar and irrigation doses in different cultivation sites and years. Irrigation dose is represented by color codes: white (100-50%ET_c), grey (100-75%ET_c), black (100-100%ET_c).

Figure 4. L-ascorbic acid contents with control conditions (100-100%ETc; black bars) and controlled deficit irrigation (100-75%, dark grey and 100-50% ETc, light grey) for Extremadura (Extr) and Navarra (Nav) in 2012 (12) and 2013 (13). Different letters for each cultivar indicate significant differences at $p<0.05$ (Tukey B test).

Supp. Figure 1. MANOVA Biplot for Extremadura and Navarra in 2012 and 2013 representing global effect on polyphenol content. Circles represent Bonferroni confidence intervals. Solid vector lines correspond to significant effect of the variable and dashed lines to non-significant effect ($p<0.05$). Significance of differences among conditions is inferred by the projection of the confidence circles on each vector.