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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.

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

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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, and 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, and 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., 2016). 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., 2016). It seems that the timing of water deficit irrigation may be important, and Wang, Kang, Du, Li, and 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 (Lahoz et al., 2016).

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. 38° 53′ 26″ N, long. 6° 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. 42° 12′ 34″ N, long. 1° 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 × 0.2 m (3.33 plants m-2) was used in Extremadura and a spacing of 1.60 m × 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 °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 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, and Roselló (2015). Approximately 1 g of homogenized sample was mixed with 5 mL of MeOH/water (48:52 v/v) solution containing 1 g kg^{-1} BHT. Samples were immersed in an ultrasonic bath Elmasonic S30H (Elma Electronics AG, Wetzikon, Switzerland) at 60 Hz for 177 min in absence of light to avoid the oxidation of target compounds. Extracts were centrifuged at 4000 rpm (2361*g*) for 5 min at 4 °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 × 4.6 mm id; particle size, 2.6 μm)