Scientia Horticulturae 211 (2016) 203–212

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Salinity and ripening on/off the plant effects on lycopene synthesis and chlorophyll breakdown in hybrid Raf tomato

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ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 28 August 2016
Accepted 30 August 2016
Available online 2 September 2016

Keywords:
Remittance spectroscopy
STAY-GREEN proteins
Colour physiology
Maturity

ABSTRACT

The aim of this study was to describe the physiology of fruit colour in tomato as affected by salinity and ripening on and off the plant. Chlorophyll and lycopene levels were repeatedly measured in ninety Raf tomatoes over a period of eight days using remittance spectroscopy. Fruits were subjected to three salinity levels and were measured either on or off the plant.

The physiology of tomato colour was described by a kinetic model centred on the role of STAY-GREEN proteins (SGR) that was calibrated simultaneously on chlorophyll and lycopene data with a percentage variance explained for of 91%. Lycopene precursor and transcript *SGR* levels were estimated considerably higher for on-plant than for off-plant ripened fruits which indicates ongoing expression while attached to the plant. There is less inhibition of the lycopene precursor by SGR in on plant ripened tomatoes which results in higher maximum lycopene levels and less chlorophyll breakdown causing residual chlorophyll levels. Effects of salinity treatments on chlorophyll breakdown and lycopene synthesis are small, but higher salinity levels strongly diminish fresh weight. Ripening on and off the plant strongly affects colour physiology of tomato fruit and is described well by the proposed model.

1. Introduction

Cultivation practices have mainly been used to optimise crop characteristics and yield, but little attention has been paid to effects on fruit quality. For instance, limiting water supply and increasing salinity results in stimulation of the secondary metabolism which increases compounds that improve taste and health (Ripoll et al., 2014). Deficit irrigation reduced fresh fruit yield, but led to higher sugar and acid levels. Irrigation with saline water had no effect on marketable red fruit yield × percent soluble solids (Mitchell et al., 1991). In other words, salinity can be considered a strategic tool to improve the quality of tomato fruits (Petersen et al., 1998; Lin and Block, 1999; Dorais et al., 2001; De Pascale et al., 2001). Tomato fruits grown at high salinity (0.7 S m^{-1}) showed an increase in fruit firmness, soluble solids content, titratable acidity and dry matter percentage compared to fruits grown at 0.5 S m^{-1} (Sánchez-González et al., 2015). Another cultivation tool to increase the quality of tomatoes is to allow fruits to ripen on the vine instead of harvesting in an immature state and allow ripening during storage, transport and retail life. Field-ripened fruits have a higher flavour quality than those harvested as mature-green and ripened off the plant (Bisogni et al., 1976). Tomatoes ripened on the plant were firmer and had significantly higher levels of lycopene, ß-carotene and soluble solids (Arias et al., 2000a). Higher lycopene and ß-carotene levels are not always encountered for vine ripened tomato fruits. Giovanelli et al. (1999) found that lycopene and β-carotene levels in post-harvest ripened 'Moneymaker' tomatoes were almost twice as high as in vine-ripened tomatoes having the same colour. How colour is quantitatively affected by cultivation practices is unknown. Nevertheless, colour affects consumer acceptance, taste and flavour perception (Francis, 1995; Crisosto et al., 2003).

Chlorophyll and carotenoids are responsible for the colour of tomatoes (Arias et al., 2000b). One of the intriguing properties of the chloroplast to chromoplast transition in tomato fruits is the way synchronisation between chlorophyll breakdown and

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http://dx.doi.org/10.1016/j.scienta.2016.08.030
0304-4238/

Table 1
Electrical conductivities (S m^{-1}) and nutrient concentrations (mmol L^{-1}) for the salinity treatments at 0.4 S m^{-1} (EC4), 0.8 S m^{-1} (EC8) and 1.2 S m^{-1} (EC12).

	EC	pH	NO_3^-	$H_2PO_4^-$	SO_4^-	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
Irrigation water	1.20	7.72	0.02	0.00	1.00	7.0	4.5	0.1	1.80	2.10
EC4	4.28	6.63	9.20	1.39	4.15	22.3	19.4	10.1	4.87	2.30
EC8	8.12	6.58	8.77	1.82	4.86	58.4	54.8	10.0	5.70	2.38
EC12	12.3	6.58	7.78	1.75	4.52	109.9	104.5	9.3	5.20	2.38

lycopene synthesis is arranged. Recently, a lycopene synthesis and chlorophyll breakdown model was presented that linked both processes postulated to be STAY-GREEN proteins (SGR) (Schouten et al., 2014). SGR1 affects H_2O_2 production that initiates chlorophyll degradation by inhibiting the recovery of β-carotenes that protect chlorophyll (Barry et al., 2008; Hörtensteiner, 2009; Hu et al., 2011). Down-regulation of *SGR1* increases the β-carotene content in ripening tomato fruits (Luo et al., 2013). SGR proteins affect, surprisingly, also lycopene accumulation. SGR proteins interact directly with phytoene synthase (PSY1), the rate-limiting enzyme responsible for the net synthesis of carotenoids. *PSY1* antisense reduction resulted in ripe tomato fruit with only 3% of the normal carotenoid content (Ray et al., 1992). Transcript levels of *PSY1* increased drastically during fruit ripening in *SGR1*-RNAi transgenic lines similar to those of *PSY1* over-expressing tomato fruits (Luo et al., 2013). It appears therefore that both PSY1 activity and gene expression are regulated by the SGR1 protein. Synchronisation of chlorophyll breakdown and lycopene accumulation might therefore be regulated by SGR proteins: first inducing chlorophyll breakdown but at the same time blocking lycopene synthesis until SGR protein levels decrease to a level that allows synthesis of lycopene to start. Additionally, SGR proteins likely affect the final lycopene level by limiting the lycopene precursor production. When the synchronisation of chlorophyll breakdown and lycopene accumulation is absent, such as in the *green-flesh* mutant that has a point mutation in the SGR protein, brown tomatoes are observed with no chlorophyll breakdown but with lycopene synthesis (Barry et al., 2008).

The aim of this study is to investigate the effects of salinity and ripening on/off the plant on chlorophyll decay and lycopene synthesis in tomato fruit. A kinetic model is developed that describes chlorophyll breakdown and lycopene synthesis based on remittance spectroscopy measurements of chlorophyll and lycopene in Raf tomatoes. Raf tomatoes are grown commercially in high soil salinity and often harvested early, often immature green or at the latest at the turning stage. Nevertheless, these irregular shaped tomatoes are appreciated in Spain as a much-praised variety whose exquisite flavour commands high market prices. Remittance spectroscopy is a technique that allows repeated non-destructive quantitative assessments of chlorophyll and lycopene levels (Farneti et al., 2012; Schouten et al., 2014). The development and calibration of a chlorophyll and lycopene model as a function of ripeness, salinity level and ripening on/off the plant for Raf tomatoes is presented.

2. Material and methods

2.1. Cultivation and growth chamber conditions

Tomato plants of a hybrid Raf variety (*Solanum lycopersicum* L. cv. Dumas) were grafted on rootstock (*Solanum lycopersicum* L. cv. Arbiore) and transplanted on September 22, 2014 in 10 L pots containing perlite substrate. Plants were arranged in four rows with eight plants each. Spacing between plants and rows was 15 and 32 cm, respectively. The tomato plants were grown with a single stem until four trusses fully developed. Pollination was carried out manually. Plant nutrition was in accordance with commercial practices in southern Spain. Treatments were maintained up to 147 days after transplanting.

The experiment was conducted in a 12 m^2 growth chamber (3.2 × 3.7 × 1.94 m), located at the IFAPA Research Center (La Mojonera, Almería, Spain, latitude 36°30′N, longitude 2°18′W). The growth chamber was equipped with a temperature, humidity and CO_2 injection control system (FITOCLIMA 23000 EHV; Aralab, Albarraque, Portugal) and was illuminated with fluorescent lamps (L 58W/840 LUMILUX Cool White and 230V/760W HALOLUX 64472 BT; Osram, Munich, Germany; Osram, Munich, Germany). Climate management was arranged by a ClimaPlus V (Aralab, Portugal) controller connected with data recording at 1 min intervals. The daily light cycle was set as a clear day with 14 h of darkness, 5 h of 500 μmol m^{-2} s^{-1} PAR and 2.5 h linearly increasing and decreasing radiation to simulate sunrise and sunset, respectively. The daily PAR integral was 14 mol m^{-2} d^{-1}. The minimum air temperature was 14 ± 0.8 °C and the maximum temperature reached 24 ± 1.3 °C with relative humidity of 77 ± 3% during the night and 47 ± 3% during the day. The average CO_2 concentration was 420 ± 5 μmol mol^{-1}.

Nutrient solution was provided by an automated fertigation system (HIMARCAN, Almería, Spain) applying three salinity levels: 0.4, 0.8 and 1.2 S m^{-1} by adding 15, 50 and 100 mmol L^{-1} NaCl, respectively (Table 1). Drainage solution was measured manually early in the morning three times per week using a portable EC-meter (CM35 81839, Crison, EU). The mean EC values of the drainage solutions were 0.61 ± 0.09, 1.16 ± 0.15 and 1.55 ± 0.14 S m^{-1}, respectively.

2.2. Ripening on/off the plant and maturity selection

Tomatoes were classified into maturity stages according to the OECD-standardized ripeness classes: mature green (not more than 10% of the surface shows red colour, colour index 2–3), breaker (more than 10% but less than 30% of the surface shows red colour, colour index 4–5) and pink/light red (red colour in most of the surface colour, colour index 7–8) (OECD (http://www.oecd-ilibrary.org)). Fifteen tomatoes per salinity treatment were labeled on-plant and divided in three equally sized sub-batches, one for each maturity stage. Another fifteen tomatoes per salinity treatment were harvested and also divided in three equally sized sub-batches, one for each maturity stage. The harvested fruits were stored in open trays at the bottom of the plant. All fruits were without defects and uniform in external appearance, size and shape.

2.3. Remittance VIS spectroscopy and pigments levels

A handheld photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany) was used to measure the remittance at 570 (R570), 660 (R660) and 780 (R780) nm to calculate the normalised difference vegetation index (NDVI, Eq. (1)) and the normalised anthocyanin index (NAI, Eq. (2)) (Schouten et al., 2014). These spectral reflectances are themselves ratios of the reflected over the incoming radiation in each wavelength individually; hence they take on values between 0 and 1. By design, the NDVI and NAI values thus varies between −1 and +1.

$$\mathrm{NDVI} = \frac{\mathrm{R780} - \mathrm{R660}}{\mathrm{R780} + \mathrm{R660}} \quad (1)$$