Scientia Horticulturae 192 (2015) 244–249

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Salinity induced fruit hypodermis thickening alters the texture of tomato (*Solanum lycopersicum* Mill) fruits

Miriam Silva Ruiz[a,b], Hagai Yasuor[b], Alon Ben-Gal[b], Uri Yermiyahu[b], Yehoshua Saranga[a], Rivka Elbaum[a,*]

[a] The R. H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 7610001, Israel
[b] Gilat Research Center, Agricultural Research Organization of Israel, mobile post Negev 2, 85280, Israel

ARTICLE INFO

Article history:
Received 17 January 2015
Received in revised form 25 May 2015
Accepted 1 June 2015
Available online 24 June 2015

Keywords:
Solanum lycopersicum
Salt stress
Texture
Hypodermis
Cell wall
Organoleptic assessment

ABSTRACT

Irrigation of tomato (*Solanum lycopersicum* Mill) plants with brackish (including saline) water improves fruit taste and reduces yields. Salinity additionally leads to toughening of tomato fruit skin, the causative mechanism for which is unknown. The aim of this study is to characterize the effects of salinity on tomato fruit skin texture. Tomato (cherry and full size) plants were irrigated with fresh water (control, ED = 1.01 dS m^{-1}) and saline water (up to 12.61 dS m^{-1}). Organoleptic assessment was compared to the force needed to puncture the cherry tomato fruit skin and to specific skin dry weight. The skin structure of cherry and full-size tomatoes was characterized by light microscopy. Chemical characterization of cell walls building the skin of cherry tomatoes was conducted by Raman micro-spectroscopy. Skin penetration force was found to be linearly correlated with its specific weight and with consumers' perceived thickness. Fruit histology revealed a linear correlation between thickness of the sub-epidermis and salinity of the irrigation water. The tougher tomato skin obtained under conditions of salinity is attributed to increased number of hypodermal cell layers rather than to changes in cell wall composition.

1. Introduction

Soil and water salinity present a major challenge to agriculture in arid regions (Ben-Gal et al., 2006; Tanji, 1990). Research efforts have primarily examined the effects of salinity on crop growth and yield (Kahlaoui et al., 2011; De Pascale et al., 2012). Tomato (*Solanum lycopersicum* Mill) plants exposed to high concentrations of salts in their root zone produce smaller biomass (Magán et al., 2008). In addition, total fruit yield is reduced as a result of lower fruit average weight, apparently due to reduced cell expansion during fruit development (Belda and Ho, 1993). The reduction in yields and fruit size is accompanied by increased fruit sugar and acid concentrations (Cuartero and Fernández-Muñoz, 1998), thus improving the fruit flavor.

Fruit textural and histological properties are also affected by salinity, however, not conclusively: In tomato grown in perlite under high salinity (electrical conductivity [EC] up to 8.6 dS m^{-1}) the fruit firmness was reduced, while the cuticle thickness was increased (Leonardi et al., 2004). Others found no effect (Krauss et al., 2006) or increase ((Flores et al., 2003) in fruit firmness when plants were grown hydroponically under salinity of up to 10 dS m^{-1}. Tomato skin consists of cutinized epidermis attached to two or more hypodermal cell layers of small, flattened cells, with thick pectinized walls, which cover the main portion of the pericarp (Reeve, 1970). Skin strength is governed by its ability to stretch rather than its thickness, and varies with tomato variety, fruit maturation stage (Barrett et al., 1998), and location on the fruit surface (Holt, 1970). While salinity was suggested to increase the toughness of tomato skin (Petersen et al., 1998)) the underlying structural changes in the fruit and their association to other fruit traits are yet unknown. Here we report salinity-induced changes in mechanical, chemical, organoleptic perception, and microstructural properties of tomato fruit skin.

2. Materials and methods

Experiments were conducted with five varieties of cherry tomato. To verify our results, we repeated the microscopic analysis with one variety of full size tomato.

* Corresponding author. Fax: +972 8 9489899.
E-mail address: rivka.elbaum@mail.huji.ac.il (R. Elbaum).

http://dx.doi.org/10.1016/j.scienta.2015.06.002
0304-4238/

2.1. Cherry tomatoes

2.1.1. Growth

Five cherry tomato cultivars, dcr-522 (AB Seeds, Israel), 1335 and 46008 (Hazera Genetics, Israel), 2206 and 2204 (Gadot Agro, Israel), were grafted on cv. Register (4402) rootstocks. Four-week-old tomato plants were transplanted on August 3 2010 into sandy soil in a net house at the Ramat Negev R&D experimental station in Southern Israel (30°59′N; 34°42′E). Each plant was split to two stems. Tomato plants were irrigated with either control (EC = 1.9 dS m^{-1}) or saline water (EC = 5.0 dS m^{-1}). The experiment was arranged in a factorial design composed of two salinity levels, five cultivars, with five replicates. Each bed was irrigated with randomly assigned EC, and cultivars were randomly arranged along the bed. Each 2 m wide bed consisted of one row with 25 cm spaced plants, at a density of 20,000 plants ha^{-1}. Each plot was 6 m long and contained 24 plants. Irrigation water of the designated EC levels was obtained by mixing local brackish ground water (EC 4.37 dS m^{-1}, 31.8 mM Cl^-, 5.27 mM Ca^{2+}, 2.47 mM Mg^{2+}, 4.1 mM SO_4^{2-}–S, 0.12 mM B^{3+}, 28.6 mM Na^+) with desalinated water originating from a regional desalination plant (EC 0.24 dS m^{-1}, 0.57 mM Cl^-, 0.85 mM Ca^{2+}, 0.04 mM Mg^{2+}, 4.85 μM SO_4^{2-}–S, 1.18 μM B^{3+}, 0.87 mM Na^+) and nutrients from commercial liquid fertilizers (Fertilizers & Chemicals Ltd., Haifa, Israel). Fertilizer 6-6-6 (N-P_2O_5-K_2O) was used during the first month, and 4-2-6 during the remaining growth period. After the first month, both irrigation solutions contained 6.9 mM N, 0.6 mM P, and 2.5 mM K and microelements. Red mature fruits were harvested weekly at the research station. Our analyses were conducted on a subsample of the harvests taken place on April and May 2011. The subsamples were composed of four fruit clusters comprising a minimum of 25 fruits from each field plot (specific to cultivar and salinity level in five replicates).

2.1.2. Skin penetration force

The force required for skin rupture was measured with a digital force Gauge (DFG550, Chatillon, USA) by manually punching each fruit at about its equatorial line, and recording the maximum force of resistance to puncture. The maximum force of resistance to puncture each tomato was measured in four fruits per sample.

2.1.3. Skin specific weight

The specific skin dry weight was measured by removing an area of 1.38 cm^2 from ten tomatoes per sample. The fruit pieces were boiled in water for 30 sec at 98 °C. The skins were manually separated, and the remaining pulp was removed by gentle scrubbing. The isolated skins were dried at 60 °C for two days, and weighed.

2.1.4. Organoleptic assay.

An organoleptic survey was carried out by volunteers in a room lit in red light. We followed recommendations from Meilgaard et al., (2007) regarding: (a) the order, coding, and number of samples given to each volunteer; (b) time given to volunteers to become oriented inside the room; and (c) assurance that the volunteers understood the test protocols. Each volunteer tasted red-ripe fruits from each treatment and cultivar and graded their sweetness (1 for "not sweet", 2 for "medium sweetness", and 3 for "sweet"), and skin thickness (1 for "thick", 2 for "medium thickness", and 3 for "thin"). Assessment of the fruit sweetness is reported in the Supplementary material, Table S1.

2.1.5. Section preparation

A sample of ~0.5 cm^3, including epidermis and pulp tissues, was cut from each fruit midway between the pedicel and blossom end. Samples were immediately fixed in a 4% para-formaldehyde solution in phosphate buffered saline (PBS) by infiltration under vacuum for 30 min. Samples were shaken at 65 rpm for an hour at room temperature and transferred to a PBS solution at 4 °C until further analysis. Samples were rinsed three times with double distilled water, transferred into 50% polyethylene glycol (PEG) in water, and dried in an oven at 60 °C for 3 days. After evaporation of most of the water, the PEG was replaced with fresh 100% liquid PEG at 60 °C, and kept in the oven for another 24 h. The vials were then stored at 4 °C. The tomato cubes were exposed and sectioned with microtome (Leica RM2265, Leica Microsystems GmbH).

2.1.6. Microscopic analysis

Sections of 10 μm were stained with methylene blue (1:10 w/w in water) for 10 min, rinsed with double distilled water, and observed immediately under a light microscope. Images were taken from three sections of three cherry tomatoes, and four sections of four full size clustered-fruit tomatoes, representing salinity treatments of each cultivar. We measured the thickness of the hypodermal cell layer using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2014).

2.1.7. Raman microscopy

Sections of 30 μm were rinsed with double distilled water, and analyzed in a Raman micro-spectrometer (inVia Reflex, Renishaw, Wooten-under-edge, UK) equipped with a green Nd:YAG laser excitation source (532 nm, 45 mW max) under a X63 water immersion Leica objective lens. The grating was 1800 lines mm^{-1}. Scattered light was detected with a Peltier-cooled CCD detector with spectral resolution ~2 cm^{-1}. Spectra were recorded with the accumulation of 10 scans, 0.2 sec exposure time, and 10% laser power in the wavenumber region of 275–2016 cm^{-1}. Baseline adjustment, smoothing, normalizing to similar integrated signal, and peak picking were performed with the instrument control software (Renishaw WiRE 3.3). Spectra averages were calculated in Microsoft Excel.

2.2. Full size tomatoes – growth and analysis

Full size tomato plants (*L. Esculentum* Mill. cv. Ikram) grafted on cv. Register (4402) rootstock were grown in 20 L containers in a greenhouse at the Gilat Research Center of the Agricultural Research Organization (31°20′N; E34°41′E). Seedlings were planted in sandy soil on October 9 2011. Each container was irrigated daily with a leaching fraction (drainage/irrigation) of ~0.3. Irrigation solutions were based on desalinated water (EC 0.05 dS m^{-1}) supplemented with nutrient levels of 1.7 mM Ca^{2+}, 0.75 mM Mg^{2+}, 1.0 mM SO_4^{2-}–S, 0.03 mM B^{3+}. Commercial liquid fertilizer 6-6-6 was used during the first month, and 5-3-8 (N-P_2O_5-K_2O) was used during the rest of the growing period (Fertilizers & Chemicals Ltd., Haifa, Israel). After the first month, the irrigation solutions contained 5.7 mM N, 0.6 mM P, and 2.7 mM K and micronutrients. Eight salinity levels were obtained by adding NaCl to the irrigation solutions at concentrations of 0, 5, 10, 20, 30, 50, 80 and 110 mM, resulting in electrical conductivity levels of 1.01, 1.50, 2.20, 3.40, 4.65, 6.79, 9.93, and 12.61 dS m^{-1}, respectively. Treatments were arranged in a randomized block design, each block contained two containers of each treatment. Fruits were collected on December 29 2012 from representative plants within each treatment, and prepared (Section 2.1.5) for microscopic analysis (Section 2.1.6).

2.3. Statistical analyses

Physical, organoleptic and chemical fruit properties were analyzed by two-way ANOVA or best-fit linear regression with JMP 10.0 software (SAS Institute Inc., USA). Default significance levels were set at $\alpha = 0.05$.