Journal of Plant Physiology 171 (2014) 1706–1712

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Physiology

Individual phenolic response and peroxidase activity in peel of differently sun-exposed apples in the period favorable for sunburn occurrence

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ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 13 August 2014
Accepted 14 August 2014
Available online 23 August 2014

Keywords:
Individual phenols
Dihydrochalcone
HPLC–MS
Peroxidase
Sunburn

ABSTRACT

Extreme weather events like high solar radiation can cause stress in apple fruits (*Malus domestica* Borkh.). The aim of the study was to make a screening of individual phenols and peroxidase activity in apple peel as a response to sunburn and different sun-exposures in the period when weather conditions are suitable for sunburn occurrence. Apple fruits of 'Golden Delicious' and 'Braeburn' were sampled. Fruit temperature and color were measured prior HPLC–MS^2 and peroxidase activity analyses. Sunburned peel was darker and more yellow-red in comparison to healthy peel, which appeared yellow-green. Fruit temperature, total as well as individual flavonols and dihydrochalcones, total hydroxycinnamics and perixodase activity were highest in sunburned peel in comparison with healthy sun-exposed peel, furthermore both were different than shaded sides of both fruits and peel of apples inside the tree crown; moreover in sunburned peel dihydrochalcones were determined for the first time. Chlorogenic acid was up to 2.5 times higher, 3-hydroxy-phloretin-2′-*O*-xyloglucoside was up to 10 times higher and quercetin-3-galactoside was up to 33 times higher in sunburned peel, comparing to shaded sided peels. Flavanols did not show a distinct pattern. A deeper insight in phenolic response against environmental stress caused by high solar radiation and high air temperatures has been made.

Introduction

Increasingly frequent extreme weather events and longer periods of high solar radiation and high temperatures cause stress in apple fruits eventually resulting in sunburn. Three different types of sunburn have been defined for apple fruit: sunburn necrosis, sunburn browning (Schrader et al., 2001) and photo-oxidative sunburn (Felicetti and Schrader, 2008b). Sunburn necrosis causes a necrotic spot on the sun-exposed side of the fruit and is caused by thermal death of epidermal and subepidermal cells, due to very high temperatures of $52 \pm 1\,^{\circ}C$. Sunburn browning results in a yellow, bronze or brown spot on the sun-exposed side of the fruit, and is caused by a combination of high fruit temperatures (46–49 °C), and high solar radiation (Schrader et al., 2001). Photo-oxidative sunburn is caused by sudden exposure of shaded apples to sunlight, and it results in photobleaching and eventually necrosis (Felicetti and Schrader, 2008b). The most prevalent and costly of the three is sunburn browning (Felicetti and Schrader, 2009). The thresholds for fruit surface temperature (FST), photosynthetically active radiation (PAR) and ultraviolet radiation (UV), which damage photosystem II (PSII) and potentially lead to sunburn, is hard to define, due to different responses of cultivars and their fruit maturity. Acclimation also varies between years (Glenn and Yuri, 2013).

Apple peel exposed to excessive solar radiation undergoes a number of changes, among them a decrease in chlorophyll (in anthocyanin free fruits) and the accumulation of additional amounts of carotenoids and flavonols (Merzlyak et al., 2002, 2005; Solovchenko and Schmitz-Eiberger, 2003). Felicetti and Schrader (2008a) discovered that as the severity of sunburn increases in 'Fuji' apples, anthocyanin concentrations decreased and quercetin glycoside, chlorogenic acid and epicatechin increased. Chen et al. (2008) concluded that high temperatures coupled with high levels of sun radiation result in photo-oxidative damage on the sun-exposed apple peel by causing photoinhibition to PSII complexes. Up-regulation of xanthophyll cycle and the antioxidant system in response to the photo-oxidative stress was also confirmed. Additionally, an increase in polyphenoloxidase (PPO) and peroxidase (POX) activity has been observed (Hao and Huang, 2004).

There are several researches about sunburn in apples, well summarized in the recent article of Racsko and Schrader (2012). There is

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http://dx.doi.org/10.1016/j.jplph.2014.08.010
0176-1617/

however less information about individual phenolic compounds in differently sun-exposed and sunburned apple peels. With development of more detailed analyses, such as mass spectrometry, deeper insight in plant response to differently sun-exposed and sunburned apple peel is enabled. A thorough study was made on apples during the summer in the time suitable for sunburn occurrence: air and fruit temperature was measured, measurements of solar radiation, fruit color, phenolic content and peroxidase activity of sunburned and non-sunburned apples out- and inside the tree crown (sun-exposed and shaded side of apples), were made. The study was performed on two cultivars 'Braeburn' and 'Golden Delicious'. The aim of the study was to make a screening of individual phenols and peroxidase activity in apple peel as a response to sunburn and different sun-exposure in the period when weather conditions are suitable for sunburn occurrence. These results will help gain a better understanding of physiological response to high radiation and temperature through phenols and peroxidase activity in apple fruits.

Materials and methods

Plant material

The experiment was carried out in the summer of 2013 at University experimental orchard in Ljubljana (lat. 46°20 N, long. 14°28 E). Apple fruits were collected from 12-year-old 'Braeburn' and 'Golden Delicious' trees, grafted on M9 rootstock and grown according to the system of integrated production. The rows were orientated from northeast to southwest. Three different types of apples were collected from the middle of the canopy height: sunburned (sunburn browning according to classification of Schrader et al., 2001), healthy outside as well as healthy inside the canopy. For each, 24 fruits from 12 apple trees were collected. Immediately after sampling, the apples were peeled (about 2 mm thick): sun-exposed and shaded side separately, therefore there were five different treatments: sunburn (SB); shade side of sunburned apple (SB-Sh); healthy sun-exposed side (HS); healthy shade side of apple (H-Sh) and inside the crown (SH). Peels were immersed in liquid nitrogen and stored at −80 °C until further use.

Measurements in the orchard

The sampling was made on 8th of August at 1 pm, the hottest day in the summer; with maximum temperature of 40.2 °C (average temperature for 1st ten days of August was 27.5 °C). Air temperature, apple fruit temperature, total radiation and PAR were measured in the orchard just before sampling. Air temperature was measured with automatic weather station situated in the faculty experimental field. Apple fruit temperature was measured twice (in the morning and just before sampling at 1 pm) with portable digital waterproof thermometer P 300 (Dostmann electronic GMBH, Germany), where the probe was inserted about 6 mm into the fruit. Ten apple fruits were used each time for the temperature measurements, wherein different apple fruits were used in the morning and at 1 pm (due to destructive method). The apples used at 1 pm, were also used in later analysis, but the punctured part of the skin was excluded. Total radiation and PAR were measured using Li 1000 Datalogger (LI-COR, USA). The total radiation and PAR were measured 10 times one meter above the ground, wherein the sensors were parallel to the ground; the measurements of radiation above the fruits were made above the sunburn (where the sensor was placed directly above the sunburn), opposite sunburn (where the sensors were facing inward the tree crown) and inside the crown (the sensors were parallel to the ground).

Color measurements

The color of ten apple fruits with sunburn, ten healthy apple fruits and ten apple fruits within the tree crown for each cultivar was measured. The measurements of sun-exposed and shaded sides were made on the same fruit. The peel color was measured using a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan). Parameter L and h° values were recorded. Parameter L^* values correspond to a dark–bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). Hue angle (h°) is derived from a^* and b^*, h° corresponds to the basic tint of color (Lancaster et al., 1997). Hue angle is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).

Extraction and determination of individual phenolic compounds

The extraction of fruit samples was done as described by Mikulic-Petkovsek et al. (2010) with some modifications. The peels collected from samples were ground in the mortar and 0.5 g of sample was extracted with 6 mL of methanol (Sigma–Aldrich) containing 3% (v/v) formic acid (Fluka) and 1% 2.6-di-tert-butyl-4-methylphenol (BHT) (Sigma–Aldrich) (w/v) in a cooled ultrasonic bath for 1 h. After 10 min centrifugation at 12,857 × g, the supernatant was filtered through a Chromafil AO-20/25 polyamide filter (Macherey-Nagel, Düren, Germany) into a vial. The vials were placed in Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA). The detection was made with a diode array detector at 280 and 350 nm. At 280 nm the hydroxycinnamic acids, dihydrochalcones and flavanols and at 350 nm flavonols were detected. For the separation of phenolic compounds Phenomenex (Torrance, CA) HPLC column C18 (150 × 4.6 mm, Gemini 3μ) with attached Phenomenex security guard column was used. The injection volume for extracted samples was 10 μL, and the flow rate maintained at 0.6 mL min^{-1}. The column temperature was 25 °C. The elution solvents were aqueous 0.1% formic acid (A) and 0.1% formic acid in acetonitrile (B) (Fluka). Samples were eluted according to the linear gradient from 5 to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30 to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions (Wang et al., 2002).

All phenolic compounds were identified by an HPLC-Finnigan MS detector and an LCQ Deca XP MAX (Thermo Finigan, San Jose, CA) instrument with electrospray interface (ESI) operating in negative ion mode. The analyses were carried out using full scan data-dependent MS^2 scanning from m/z 110 to 1300. Column and chromatographic conditions were identical to those used for the HPLC-DAD analyses. The injection volume was 10 μL and the flow rate maintained at 0.6 mL min^{-1}. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, respectively; the source voltage was 3 kV for negative ionization and normalized collision energy was between 20 and 35%. Spectral data were elaborated using the Excalibur software (Thermo Scientific).

Comparing retention times and the spectra, adding the standard solution to the sample and fragmentation were used for identification of compounds. Quantification was achieved according to the concentrations of corresponding external standard. For the quantification of phenolic compounds the following standards were used: chlorogenic acid and quercetin-3-*O*-rutinoside (rutin) from Sigma–Aldrich Chemie, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, procyanidin B1 and B2, (−)-epicatechin, *p*-coumaric acid, caffeic acid and phloridzin dihydrate from Fluka Chemie (Buch, Switzerland), quercetin-3-*O*-xyloside and quercetin-3-*O*-arabinofuranoside from Apin