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JIP-test parameters to study apple peel photosystem II behavior under high solar radiation stress during fruit development

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Abstract

Changes in apple (Malus domestica Bork.) peel PSII photochemical process during fruit development under high solar radiation field conditions were analysed by the JIP-test to increase understanding on energy dissipation and susceptibility to photooxidative damage. Fruits growing exposed (E), non-exposed (NE) or suddenly exposed (SU) to high solar radiation were evaluated at three developmental stages. Chlorophyll content and chlorophyll a fluorescence transient (OJIP), were affected by high solar radiation and development. Minimum fluorescence, maximum fluorescence, maximum quantum yield of photochemistry, and specific fluxes per cross section decreased in E and SU fruits. JIP parameters were a sensitive indicator of high solar radiation stress in apple peel. Peroxidative damage was observed in E fruits at each stage of development and in SU fruits at early and mid-stages. By delineating the photochemical events induced by solar radiation in apple fruits at different developmental stages, our findings might help to increase understanding on susceptibility of apple to photooxidation damage depending on the light environment and developmental stage they are exposed to.

Additional key words: fruit sunburn; high light stress; PSII photochemistry.

Introduction

In fruiting vegetables and fruit crops, high solar radiation stress may lead to photooxidative damage resulting in a physiological disorder known as sunburn (Racskó and Schrader 2012). This physiological disorder is frequently found in apple fruits growing under full and direct high solar radiation. Shaded apple peel may also be susceptible to photooxidative damage when sudden exposure to full sunlight occurs (Ma and Cheng 2004, Li and Cheng 2008). This may happen during hand thinning or summer pruning, and even in harvested fruits exposed to sun radiation during transport (Schrader et al. 2003).

Several changes in pigment composition take place as the apple fruit develops. As new pigments (e.g., anthocyanin) are synthetized, apple peel chloroplasts become vacuolated and gradually lose their photosynthetic function (Blanke and Lenz 1989, Li and Cheng 2008). Excessive sun radiation also causes significant changes in pigment composition in apple fruits (Racskó 2010). A decline in chlorophyll (Chl) a and b and in anthocyanin was reported in ‘Fuji’ apple peel as photooxidation and sunburn severity increased (Felicetti and Schrader 2008).

Photosystem II (PSII) photochemistry and energy flux are altered under environmental stress (Yang et al. 2012). This leads to impaired photosynthesis and subsequent...
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oxidative damage by generation of reactive oxygen species (ROS). ROS are generated during normal cell metabolism, but high amounts can result in oxidative stress and lead to lipid peroxidation and oxidative destruction of the cell (Mittler 2002).

Chl a fluorescence transient measurement allows investigations of various PSII photochemistry parameters that may be altered during environmental stress, such as absorption energy flux, trapped energy flux, electron transport flux, dissipated energy flux, and reduction of end electron acceptors (Strasser et al. 2004, Jiang et al. 2008, Yang et al. 2012). PSII photochemistry investigated using direct time-resolved fluorescence shows a polyphasic rise known as the OJIP transient (Strasser et al. 1995, 2004). The O-step reflects the minimum fluorescence (F_o) when all primary quinone electron acceptors (Q_a) are oxidized. The P-step is the peak or maximum fluorescence (F_p) and corresponds to the state when all Q_a are reduced. The rise from the O- to the J-step reflects a reduction of Q_b and is associated with the primary photochemical reactions of PSII. The intermediate I-step and the final P-step reflect the existence of fast and slow reducing plastoquinone (PQ) centres as well as different redox states of the reaction centre (RC) complex (Strasser et al. 1995). The analysis of the OJIP transient, derived from complex mathematical models, is known as the ‘JIP-test’ (Strasser et al. 1995, 2004). This test uses OJIP curve inflection points for calculating parameters that describe the photochemical activity of samples (Živčák et al. 2014).

It has been widely proved that the OJIP transient is a sensitive and reliable method for the detection and quantification of several environmental plant stresses like drought (Li and Ma 2012, Mishra et al. 2012, Brestič and Živčák 2013), heat (Chen et al. 2008, 2009; Chen and Cheng 2009), mineral (Hermans et al. 2004, Yang et al. 2012), and high light stress (Chen et al. 2008, Kalaji et al. 2012, Živčák et al. 2014). To our knowledge, only a few studies have applied this technique under high solar radiation field conditions to investigate PSII responses leading to apple peel photooxidative damage. Some studies carried out under laboratory controlled conditions indicate that high light coupled with high temperature damage PSII complexes at both the donor and acceptor sides in apple peel (Chen et al. 2008). They also show that partitioning of absorbed light energy differs between the shaded and sun-exposed sides of apple fruits when they are exposed to high light irradiance (Chen et al. 2012).

Little is known about apple peel PSII photochemistry (OJIP transient) evolution during fruit development either adapted or not to high solar radiation under field conditions. The aim of this study was to investigate changes in apple peel photochemical processes during fruit development under natural high solar radiation on field conditions.

Materials and methods

Plant material and field conditions during sampling:
Ten twenty-year-old apple trees (Malus domestica Borkh.) cv. Red Delicious, grown in the Alto Valle of Rio Negro, North Patagonia, Argentina (39°04’0.8”S, 67°43’22.8”W), were selected during the 2012–2013 growing season. Spacing among trees was 2.5 × 4.0 m, and they received standard horticultural practices and disease and pest control. At 65, 110, and 140 d after full bloom (DAFB) fruits from these trees, growing either exposed (E) or not exposed (NE) to sun were picked at peak sunlight radiation (between 12:00 and 14:00 h). At 10:00 h on the same day, NE fruits (not adapted to high sun radiation) were selected and suddenly exposed (SU) to sun without being detached from the plant and kept under this condition during three hours before they were picked. Environmental conditions and average fruit size at the different sampling times are shown below. All physiological measurements were performed on the peel of the selected fruits using 4–5 replicates per treatment.

<table>
<thead>
<tr>
<th>Environmental conditions</th>
<th>Days after full bloom</th>
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<tbody>
<tr>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Maximum solar radiation</td>
<td>780</td>
</tr>
<tr>
<td>Average air temperature</td>
<td>34.8</td>
</tr>
<tr>
<td>PAR at E condition</td>
<td>1,780</td>
</tr>
<tr>
<td>PAR at NE condition</td>
<td>752</td>
</tr>
</tbody>
</table>

Chl determination: Three peel discs (1 cm in diameter and 1 mm thick) were extracted from each fruit and incubated with 2 ml of dimethyl-sulfoxide at 65°C during 2 h for Chl extraction. Absorbance was measured using a spectrophotometer (Beckman Coulter DU 800) at 665.1 and 649.1 nm. Chl a and Chl b were calculated following Wellburn (1994). Chl a ([μg ml⁻¹] = 12.47 × A665.1 – 3.62 × A649.1), Chl b ([μg ml⁻¹] = 25.06 × A649.1 – 6.50 × A665.1).

Anthocyanin determination: Anthocyanin extraction and analysis were carried out as described by Giusti et al. (2005). Anthocyanins in fruit peel were extracted with a solution of HCl:H₂O:MeOH (0.5:20:79.5, by volume). Absorbance was measured using a spectrophotometer (Beckman Coulter DU 800) and expressed as mg of cyanidin-3-glucoside.

Chl a fluorescence (OJIP) transient was measured with a portable fluorimeter (Pocket PEA, Hansatech Instruments Ltd., Norfolk, UK) according to Strasser et al. (1995). Photon flux density was set at 3,500 μmol(photon) m⁻² s⁻¹ during 1 s. Data were sampled at 10-μs intervals for the first 300 μs, and then acquisition rates were lower as the kinetics of the fluorescence signal slowed. All measurements were made on peel discs 1 cm in diameter and 0.5 cm thick, dark-adapted for 30 min at room temperature.

The OJIP transient was analysed according to the JIP-
Membrane peroxidative damage was determined by quantification of thiobarbituric acid (TBA) reactive species produced during lipid peroxidation as described by Hodges et al. (1999). Apple peel samples (200 mg of fresh mass) were macerated in liquid nitrogen and homogenized in 0.1% (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 × g for 5 min. Aliquots of the supernatant (500 μl) were added to 2 ml of reaction medium (TBA+) consisting of TBA 0.1% and TCA 20% and incubated at 95°C for 30 min. The reaction was stopped by rapid cooling on an ice bath and samples were subsequently centrifuged at 10,000 × g for 10 min at 4°C. Readings were determined using a spectrophotometer (Beckman Coulter DU 800) at 440 (to eliminate interference from sucrose), 532, and 600 nm. TBA forms red complexes with low molecular mass aldehydes such as malondialdehyde (MDA), a byproduct of the peroxidation process. To eliminate interference from anthocyanin, aliquots of the same supernatant (500 μl) were added to 2 ml of TCA 20% (TBA−) and processed along with TBA+ samples by measuring absorbance at 532 and 600 nm. The concentration of MDA/TBA complexes was calculated as follows: MDA [nmol ml−1] = [{[(A_{TBA−532} – A_{TBA+532}) – (A_{TBA−600} – A_{TBA+600}) – (A_{TBA−440} – A_{TBA+440}) × e] / c} × 10; where c represents the extinction coefficient at 532 nm = 157,000 mM cm−1 (Hodges et al. 1999) and e is the molar absorption of sugar at 532 nm/molar absorption of sugars at 440 nm = 0.057142857 (Hodges et al. 1999).

Statistical analysis: Sampling was performed with five replicates per sun-exposed type and DAFB. Data analyses were carried out using one- and two-way analysis of variance (ANOVA) (InfoStat 2013, Di Rienzo et al. 2013). Means were compared using the DGC test (Di Rienzo et al. 2002) at a level of p < 0.05. JIP-test parameters, Chl content, and membrane peroxidative damage were submitted to a principal component analysis (PCA) to get a closer insight in the relation between measured parameters.

Results

Pigment content: As DAFB increased fruit peel Chl content (a, b, and total) decreased for the three sun exposure conditions (NE, E, and SU; Table 1). On NE, E, and SU fruits degradation rates during development were, respectively, 68, 88, and 60% for Chl a; 55, 63, and 57% for Chl b; and 64, 79, and 59% for total Chl. No significant difference was observed in SU fruit Chl contents between 110 and 140 DAFB. The Chl a/b ratio decreased as DAFB

<table>
<thead>
<tr>
<th>DAFB</th>
<th>Sun exposure</th>
<th>Chl a [μg cm⁻²]</th>
<th>Chl b [μg cm⁻²]</th>
<th>Chl total [μg cm⁻²]</th>
<th>Chl a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>NE</td>
<td>4.70 ± 0.14²</td>
<td>2.06 ± 0.06²</td>
<td>6.76 ± 0.20⁴</td>
<td>2.28 ± 0.03³</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>3.19 ± 0.25³</td>
<td>1.78 ± 0.09³</td>
<td>4.97 ± 0.32⁴</td>
<td>1.79 ± 0.08⁴</td>
</tr>
<tr>
<td></td>
<td>SU</td>
<td>4.20 ± 0.30⁴</td>
<td>2.05 ± 0.13³</td>
<td>6.25 ± 0.42⁵</td>
<td>2.04 ± 0.06⁶</td>
</tr>
<tr>
<td>110</td>
<td>NE</td>
<td>2.39 ± 0.12²</td>
<td>1.29 ± 0.11³</td>
<td>3.68 ± 0.22⁴</td>
<td>1.90 ± 0.15⁵</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>1.63 ± 0.12³</td>
<td>1.07 ± 0.06⁴</td>
<td>2.70 ± 0.18³</td>
<td>1.52 ± 0.06⁶</td>
</tr>
<tr>
<td></td>
<td>SU</td>
<td>1.89 ± 0.12³</td>
<td>1.03 ± 0.07⁴</td>
<td>2.92 ± 0.18³</td>
<td>1.86 ± 0.12⁵</td>
</tr>
<tr>
<td>140</td>
<td>NE</td>
<td>1.50 ± 0.19³</td>
<td>0.93 ± 0.05⁵</td>
<td>2.42 ± 0.21³</td>
<td>1.61 ± 0.17⁶</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.39 ± 0.02³</td>
<td>0.63 ± 0.08⁸</td>
<td>1.04 ± 0.07⁷</td>
<td>0.62 ± 0.09⁸</td>
</tr>
<tr>
<td></td>
<td>SU</td>
<td>1.67 ± 0.34⁶</td>
<td>0.89 ± 0.16⁶</td>
<td>2.56 ± 0.48⁸</td>
<td>1.90 ± 0.22⁸</td>
</tr>
</tbody>
</table>
increased in NE fruits between 65 and 110 DAFB and in E fruits between 110 and 140 DAFB. Solar radiation environment affected the Chl content at each stage of fruit development. NE fruits presented higher Chl concentration (a and total) and Chl a/b ratio than that of E fruits. Chl b content was affected by sun exposure at 110 and 140 DAFB. SU fruits showed 20% degradation for both Chl a and total Chl at 110 DAFB. At 65 and 140 DAFB, no differences in content were found for Chl a, b, and total Chl between SU and NE fruits (Table 1).

Anthocyanins were measured in E and NE fruits at 140 DAFB. At this late stage of development, NE fruits showed 8.9 mg of cyanidin-3-glucoside per 100 g of fresh mass, 30% higher than that of E fruits.

Chl a fluorescence transient and JIP test: Chl a fluorescence (OJIP) transients showed a typical polyphasic rise in E, NE, and SU fruits (Fig. 1). Solar radiation exposure and fruit development affected Chl a fluorescence intensity with an important reduction at the P-step, and a slight increase at the O-step (Fig. 1A–C). Differences in variable fluorescence are visualised by F₀ normalization, plotted as Fₐ/Fₐ (Fig. 1D–F). NE fruits showed the highest variable fluorescence during fruit development in comparison to E and SU fruits. The higher reduction in Fₐ/Fₐ was observed at 110 DAFB, mainly in SU fruits with respect to NE fruits (Fig. 1E).

In OJIP transients, Fₐ and Fₐ were affected by solar radiation and fruit development. At 65 DAFB, Fₐ and Fₐ were higher in E than that in NE and SU fruits. At 110 DAFB, both Fₐ and Fₐ were higher in NE than that in E fruits, but no differences were found between solar radiation exposure at 140 DAFB (Fig. 2A, C).

Variable fluorescence (Vₐ) was higher in E and SU than that in NE fruits at all stages, except at 65 DAFB, where SU was greater than that of NE (Fig. 2B). E and NE fruits showed a similar Fₐ/Fₐ at 65 DAFB (Fig. 2D). However, at 110 and 140 DAFB, Fₐ/Fₐ in E fruits decreased by 22 and 32%, respectively, compared to NE fruits. Fₐ/Fₐ in SU fruits was 32, 45, and 41% lower at 65, 110, and 140 DAFB, respectively, when compared to NE fruits (Fig. 2D). The area under the OJIP curve, indicating the reduced plastoquinone pool (Area), decreased during the fruit development (Fig. 2E). At 65 DAFB, E fruits showed the largest Area, with a reduction of 67 and 84% observed at 110 and 140 DAFB, respectively. Meanwhile, in NE fruits, Area was reduced by 66% at 140 DAFB; at this time point, E, NE, and SU fruits showed the similar Area (Fig. 2E). The time to reach Fₐ (t₈₅) was similar for all exposure conditions at 65 DAFB. In E and NE fruits, t₈₅ increased at 110 DAFB, and in E and SU fruits at 140 DAFB.

For both E and NE fruits, phenomenological fluxes per excited cross section were reduced as DAFB increased (Fig. 3). Density of active reaction centres per cross section (RC/CSₐ) was also reduced as DAFB increased (Fig. 3). These reductions, when analysed in relative values to 65 DAFB, were faster in E than that in NE fruits. Relative to NE fruits, the effects of sun radiation varied according to the morphological developmental stage (Fig. 4). At 65 DAFB, RC/CSₐ, and all analysed phenomenological fluxes were higher in E than that in SU and NE fruits (Fig. 4A). SU fruits showed an increase in dissipated energy (ΔL⁰/CSₐ) and a reduction in both trapped energy (TRₐ/CSₐ) and electron transport flux (ETₐ/CSₐ) (Fig. 4A). At 110 DAFB, phenomenological fluxes were reduced by sun exposure despite the higher RC/CSₐ observed in E fruits (Fig. 4B). At each morphological developmental stage, an increase in ΔL⁰/CSₐ was observed in SU fruits (Fig. 4A–C).

When energy fluxes were analysed per active RC, absorption flux (ABS/RC) and dissipated flux (ΔL⁰/RC) were greater in SU than that in E and NE fruits at all stages. The only exception was at 140 DAFB, when
ABS/RC and DI/RC in E fruits reached levels similar to those measured in SU fruits. In contrast, energy fluxes per active RC in NE fruits were not affected during development. Quantum yield for electron transport ($\phi_{E0}$) had similar behaviour as $F_v/F_m$; it was lower in SU than that in NE fruits at all morphological developmental stages and remained constant in NE fruits throughout the development. However, the efficiency with which an electron is transferred from the reduced intersystem electron acceptors to PSI ($\delta_{R0}$) was highest in SU fruits at all developmental stages, and in E fruits at 110 and 140 DAFB. In turn, both the probability that a trapped exciton moves an electron beyond $Q_a$ ($\Psi_{E0}$) and the performance index (PI$_{abs}$) were affected by sun radiation at each stage of development (Fig 2F). Compared to NE fruits, PI$_{abs}$ in SU fruits was 69, 91, and 77% lower at 65, 110, and 140 DAFB, respectively, while in E fruits, PI$_{abs}$ decreased by 69 and 76% at 110 and 140 DAFB, respectively (Fig. 2F).

Membrane peroxidation: Compared to NE, peel membrane peroxidation was 129, 75, and 229% higher in E fruits at 65, 110, and 140 DAFB, respectively, and 64 and 88% higher in SU fruits at 65 and 110 DAFB, respectively. Membrane peroxidation levels remained constant in E and NE fruits during development, and no differences were found between NE and SU fruits at 140 DAFB (Fig. 5).

**Principal component analysis:** We used multiparametric analysis to evaluate solar radiation exposure and fruit development effects in fruit peel in order to identify relations between JIP-test parameters, Chl content, and membrane peroxidative damage. Principal component analysis is an exploratory technique that concentrates the information of measured variables in new complex variables not correlated with each other. These new variables, called Principal Components (PC), are linear combinations of the original variables and explain the maximum variation of parameters. The first Principal Component (PC1, Fig. 6) determined about 64% of total changes in apple fruit peel, and is mainly explained by $F_v/F_m$, PI$_{abs}$, $\phi_{E0}$, and DI/RC, ABS/RC, and $\delta_{R0}$. The second component (PC2) reflected 16% of changes and is sensitive to MDA, $F_o$, $F_m$, and Area. Chl concentration is more correlated to parameters like $F_o$, $F_m$, and Area, and negatively correlated to $t_{Fm}$. While MDA is more correlated to $V_J$ and negatively correlated to ET$_0$/RC and $\Psi_{E0}$. The sample distribution within PC1/PC2 biplot could be positioned into two relatively good separated clusters (Fig. 6). The first cluster, characterized by higher values of PSII functionality, includes NE fruits at all stages.

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**Fig. 2.** OJIP transient parameter analysis for dark-adapted Red Delicious apple fruits exposed (E), non-exposed (NE), and suddenly exposed (SU) to sun light at 65, 110, and 140 d after full bloom (DAFB). (A) Minimum fluorescence ($F_0$). (B) Relative variable fluorescence at the J-step ($V_J$). (C) Maximum fluorescence ($F_m$). (D) Maximum quantum yield of primary photochemistry ($F_v/F_m$). (E) Reduced plastoquinone pool size (Area). (F) Performance index on absorption basis (PI$_{abs}$). Data are means ± SE ($n$ = 5). Values followed by the same letter are not significantly different at $p$≤0.05.

**Fig. 3.** Phenomenological fluxes per excited cross section in (A) sun-exposed (E) and (B) non-exposed (NE) Red delicious apple fruits. Radar plot of amount of active PSII RCs per cross section (RC/CS$_0$), absorption flux per CS (ABS$_0$/CS$_0$), dissipated energy flux per CS (DI$_0$/CS$_0$), trapped energy flux per CS (TR$_0$/CS$_0$), electron transport flux per CS (ET$_0$/CS$_0$), and reduction of end acceptors at PSI electron acceptor side per CS (RE$_0$/CS$_0$) at 65, 110, and 140 d after full bloom (DAFB). All values are relative to 65 DAFB within each condition.
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of development and E fruits at 65 DAFB. While the second cluster, characterized by lower PSII functionality, includes AE fruits at all stages of development and E fruits at 110 and 140 DAFB.

Discussion

In line with previous investigations in Gala (Li and Cheng 2008) and Golden apple fruits (Chen et al. 2012), our findings indicate that Red Delicious apple peel Chl contents decrease during fruit development (Table 1). This trend paralleled the decrease in photosynthetic capacity as fruits become older (Li and Cheng 2008).

At each stage of development NE fruits showed higher Chl contents than that of E fruits (Table 1). These results are also consistent with previous investigations, where changes in Chl content as result of photooxidative stress were reported on leaves and apple fruits exposed to increasing levels of solar radiation (Demmig-Adams 1998, Ma and Cheng 2004, Felicetti and Schrader 2009).

In addition, Chl degradation rates were higher in E than that in NE fruits during development. When NE fruits were suddenly exposed to sun (i.e., SU fruits), Chl content decreased only at 110 DAFB. As sun radiation intensity was similar during the three sampling dates, we presume that apple fruits that did not acclimate to solar radiation could be more susceptible to Chl degradation after sun exposure at mid-stages of development.

Chl a OJIP transient was affected by natural solar radiation exposure and fruit development (Fig. 1A–C). Similar results were reported on studies conducted under controlled conditions, where shaded sides of apple fruits were exposed to artificial high light (Chen et al. 2008). The differences found in variable fluorescence (\(F_0/F_\infty\)) show that sun exposure affects maximum fluorescence in E and SU fruits early during development. However, the highest reduction of the P-step produced by solar radiation was observed at 110 DAFB, when NE fruits where suddenly exposed to sun (i.e., SU fruits; Fig. 1D–F).

The transitory fluorescence intensity peaks (J- and I-steps) observed in the Chl a fluorescence transient represent consecutive kinetic bottlenecks in the electron transport chain, which lead to momentary maximum accumulations of \(Q_A^-\) (Strasser et al. 2004). In our study, the fact that \(V_I\) was enhanced in E and SU fruits (Fig. 2B) suggests that the acceptor side of PSII became more reduced under solar radiation. The final I–P phase, affected here by solar radiation and development, might reflect the rate of reduction of ferredoxin considered as a measure of

Fig. 4. Phenomenological fluxes per excited cross section at 65 (A), 110 (B), and 140 (C) d after full bloom (DAFB). Radar plot of amount of active PSII RCs per cross section (RC/CS), absorption flux per CS (ABS/CS), dissipated energy flux per CS (DI/CS), trapped energy flux per CS (TR/CS), electron transport flux per CS (ET/CS), and reduction of end acceptors at PSI electron acceptor side per CS (RE/CS) in Red Delicious apple fruits grown exposed (E), non-exposed (NE), and suddenly exposed (SU) to sunlight. All values are relative to those of NE fruits for each DAFB.

Fig. 5. Membrane peroxidation measured as malondialdehyde (MDA) concentration [nmol g\(^{-1}\)] in Red Delicious apple fruits exposed (E), non-exposed (NE), and suddenly exposed (SU) to sunlight sampled at 65, 110, and 140 d after full bloom (DAFB). Data are means ± SE \((n = 5)\). Values followed by the same letter are not significantly different at \(p \leq 0.05\).
the relative abundance of PSI with respect to PSII (Bussotti et al. 2011, Živčák et al. 2014).

Solar radiation affected Fv/Fm (Fig. 2D), indicating injury to the PSII complexes (Brešič and Živčák 2013) due to an increase in Fv and a decrease in Fm. Similar findings were reported by Chen et al. (2009, 2008) in apple (Malus domestica Bork) peel, and Kalaji et al. (2012) and Živčák et al. (2014) in barley (Hordeum vulgare L.) leaves. Fv/Fm was similar in E and NE fruits at early stages of fruit development (i.e., 65 DAFB; Fig. 2D) indicating that PSII susceptibility to solar radiation in E fruits could be developed at later developmental stages.

The reduction of the PQ pool (i.e., Area; Fig. 2E) observed in E and NE fruits at mid and end developmental stages, respectively, partially explains the decline in the ability to chemically process light. Reduction of the PQ pool and increases in tFm under high light exposure are in agreement with previous reports on leaves (Kalaji et al. 2012). In addition, the reduction in the ability to chemically process light during fruit development can be clearly appreciated from the decline in the number of active reaction centres per cross section observed at later stages (Fig. 3), which was faster in E than that in NE fruits.

When phenomenological fluxes per excited cross section were compared between sun exposure conditions at each developmental stage, we observed that activities at 65 DAFB were higher in E than that in NE fruits (Fig. 4A). However, these values were inverted at 110 DAFB despite the higher number of RC/CS0. This depression in phenomenological fluxes per excited cross section correlates with the lower Fv/Fm observed in E fruits at this developmental stage.

Sudden exposure to solar radiation mainly affects the PSII antenna but not the PSI reaction centre. This was reflected by the greater increase in both absorption photon flux energy (ABS/RC) and dissipated energy per active reaction centre (DI0/RC), and the unchanged trapped energy per reaction centre (TRo/RC) observed in SU compared to NE fruits. A higher absorbance flux per reaction centre (ABS/RC) value seems to indicate an increased antenna size per active reaction centre (Strasser et al. 2004, Stirbet and Govindjee 2011, Živčák et al. 2014). Photoinhibition is more accurately identified as an increase in DI0/RC and a decrease in ΨE0 rather than by a decrease in Fv/Fm (Jiang et al. 2008). Exposure to solar radiation increased DI0/RC and decreased ΨE0. This supports the view that photoinhibition occurred at 65 and 110 DAFB in SU fruits, and at 110 and 140 DAFB in E fruits. However, it was at the latest stage of development (140 DAFB) when a reduction of the activity of the PSII RC was evidenced in E fruits by a decrease in TRo/RC and ET0/RC.

Plant vitality, characterised by PIabs (Strasser et al. 2004), is a rapid and sensitive stress index used widely to compare the whole primary photochemical reaction (Chen and Cheng 2009). This index reflects the functionality of both PSII and PSI and gives information on the current state of plant performance under stress conditions (Strasser et al. 2004). Our findings show that PIabs was affected by solar radiation and developmental stages. In NE fruits, PIabs was constant throughout development (Fig. 2F) despite a concomitant decrease in photosynthetic capacity. This confirms that NE fruits were not under solar radiation stress. PIabs decreased in E fruits at mid-stages of development (i.e., 110 DAFB); while in SU fruits this phenomenon was observed at an early stage (i.e., 65 DAFB). A reduction on PIabs and an increase in DI0/RC upon exposure to solar radiation are indicative of stress due to an excess of excitation energy.

Photoinhibition of both donor and acceptor sides of PSII can increase ROS production (Racskó and Schrader 2012) leading to lipid peroxidation and membrane cell damage. Solar radiation exposure increased membrane peroxidative
damage (Fig. 5) indicating that the antioxidant system was unable to cope with the photooxidation triggered by high solar radiation (Chen et al. 2008). These results are in agreement with previous reports in apple fruits (Chen et al. 2008). Also, we found that membrane peroxidative damage was not affected by development in E and NE fruits (Fig. 5). SU fruits, in contrast, showed increased peroxidation at each stage of development; however, this increase was only significant at early and mid-developmental stages (Fig. 5) even though several parameters of PSI photochemistry such as $F_{v}/F_{m}$, flux ratios and $P_{Labs}$, among others, were affected at a late developmental stage. Red Delicious apple fruits synthesize anthocyanin during development, which we presume may play a photoprotective role at a late developmental stage. Anthocyanins photoprotective role is widely known and was previously reported in pome fruits (Merzlyak and Chivkunova 2000, Li and Cheng 2009). The fact that NE fruits at 140 DAFB showed higher anthocyanin content than that of E fruits, may contribute to explain the lower peroxidation damage in SU fruits at 140 DAFB. These observations suggest that NE fruits suddenly exposed to sun (SU) are more resistant to solar radiation stress at late, than at early or mid-developmental stages.

Decreases in Chl content, PQ pool (Area), $F_{v}/F_{m}$, and $P_{Labs}$, and increases in $D_{1s}/RC$ in fruits, which grew exposed to sunlight, suggest that apple peel PSI tolerance to high solar radiation decreases during fruit development. This study, carried out under natural high solar radiation on field conditions on fruits not detached from plants, allowed us to confirm previous studies conducted under lab-controlled conditions. Furthermore, we detected that fruits not exposed to sunlight during growth are more susceptible to photooxidative damage when they are suddenly exposed to sun at early and mid-stages of development. Of note, we used a portable fluorimeter that allowed us to confirm previous studies conducted under natural conditions on fruits not detached from plants, and the ascorbate-glutathione cycle. – Plant Sci. 228: 745-756, 2008.


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