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The elevated anthocyanin level in the shaded peel of 'Anjou' pear enhances its tolerance to high temperature under high light

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ABSTRACT

Pigments, chlorophyll fluorescence, dark respiration, and the antioxidant system in the shaded peel of green 'Anjou' pear (*Pyrus communis* L.) and its bud mutation, red 'Anjou', were compared in response to high peel temperature, high light alone or in combination to determine the protective role of anthocyanins under high temperature with or without light. Under high temperature treatment alone, no difference in the maximum quantum yield of PSII (F_V/F_M) was detected between red 'Anjou' and green 'Anjou'; the superoxide dismutase activity and the glutathione pool were up-regulated in green 'Anjou' peel but remained unchanged in red 'Anjou' peel. Under high temperature coupled with high light, the F_V/F_M of green 'Anjou' peel was decreased to a lower value than that of red 'Anjou', and significant interaction was detected between temperature and light for both cultivars. Furthermore, the difference in F_V/F_M between red 'Anjou' and green 'Anjou' under high temperature coupled with high light was significantly larger than that under high light alone, indicating that this larger difference was caused by the interaction between high temperature and high light as no significant difference was detected in F_V/F_M between the two cultivars under high temperature treatment alone at any sampling point. It is concluded that the elevated anthocyanin level in the shaded peel of red 'Anjou' does not alter its thermotolerance in the dark, but makes it more tolerant of high temperature under high light.

1. Introduction

Anthocyanins play a role in mitigating photoinhibition/photodamage during leaf expansion or senescence [1–4], or under stress conditions such as high light, low temperature and strong UV-light [5–11]. The proposed mechanisms include shielding the chloroplast from excess light by absorbing blue–green light [8,12] and/or directly detoxifying active oxygen species (AOS) [13–16]. The light attenuation effect of anthocyanins has been well documented in experiments using lights with different wavelengths, i.e., anthocyanins reduce the light irradiated to chloroplast, thereby lowering the excess excitation energy pressure at the photosystems and decreasing the probability of AOS production. However, it is rather difficult to ascertain the antioxidant function of anthocyanins under high light due to the light attenuation effect of anthocyanins on AOS generation. Some studies have shown that anthocyanins could detoxify AOS directly [13,15], but the experiments were done *in vitro* and/or using the artificial radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), which may or may not reflect the role of anthocyanins in detoxifying AOS in plants *in vivo*. Indeed, the existence of anthocyanins did not increase the antioxidant capacity of anthocyanic leaves compared with their non-anthocyanic counterparts of *Quintinia serrata*, or juvenile and senescing maple (*Acer saccharum*) [4,17]. Recently, associations between the presence of anthocyanins and lower AOS levels were observed in *Pseudowintera colorata* leaves under mechanical stress [14], in *Cistus creticus*, *Photinia* × *fraseri*, *Rosa* sp., and *Ricinus communis* leaves under methyl viologen-induced oxidative stress [16], and in *Arabidopsis* mutants deficient in anthocyanin synthesis [18], but a causal relationship cannot be established without knowing the response of the antioxidant system to these manipulations. This is because the antioxidant system present in all plants, consisting of superoxide dismutase, catalase and the ascorbate-glutathione cycle, is the first defense line against the excess accumulation of AOS, and the antioxidant system could be up-regulated in parallel with anthocyanin accumulation in response to the imposed stresses.

Abbreviations: AOS, active oxygen species; APX, ascorbate peroxidase; CAT, catalase; DHA, oxidized ascorbate; DHAR, dehydroascorbate reductase; PFD, photon flux density; F_O, F_M, minimum and maximum fluorescence; F_V/F_M, maximum quantum yield of PSII; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDAR, monodehydroascorbate reductase; SOD, superoxide dismutase.

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Under high light alone or in combination with other stresses, the production of AOS such as singlet oxygen, superoxide anion and hydrogen peroxide is mainly located in chloroplast, peroxisome and mitochondria, especially in the chloroplast [19–22]. Without light, high temperature stress could induce the production of AOS in mitochondria via respiration [23,24] but cannot trigger the AOS accumulation via photorespiration or Mehler reaction in peroxisome and chloroplast. We reasoned that if anthocyanins scavenge AOS *in vivo*, they may provide protection against high temperature by scavenging the AOS produced in mitochondria, and as a result plant tissues with anthocyanins may be more tolerant of high temperature than those without anthocyanins. Whether anthocyanins alleviate the damage of high temperature to plants *in vivo* is still unknown. High temperature has been reported to cause degradation of anthocyanins in pear fruit [25] and grape berries [26]. Sunburn browning on apple, which is induced by high peel temperature under light, significantly decreases the concentration of anthocyanins in the peel [27,28]. This high temperature induced loss of anthocyanins would reduce the protective effect of anthocyanins, if any, under high temperature stress alone or in combination with other stresses such as high light. To date, the function of anthocyanins under high temperature alone or coupled with high light has not been examined in detail. Yet, high temperature coupled with high light is the main factor responsible for the occurrence of sunburn on fruit peel in summer [29–31], which significantly affects fruit finish and quality [32,33].

In this study, we compared green 'Anjou' fruit with its bud mutation, red 'Anjou', in response to high light, high temperature alone or in combination to determine whether anthocyanins have any protective function under high temperature alone or coupled with high light. The shaded peel of red 'Anjou' vs green 'Anjou' pear was chosen as the experimental system for the following reasons. First, red 'Anjou' and green 'Anjou' are near isogenic, if not isogenic. Fruit characteristics are essentially the same except skin color. Although the genetic basis of the mutation from green 'Anjou' to red 'Anjou' remains to be characterized, it is most likely that MYB transcriptional factor is responsible for the mutation based on the work on apple [34–36]. Second, the red color exists on the entire peel of red 'Anjou', including the shaded peel. Since the shaded peel has been acclimated to the low light environment during fruit development and has not been exposed to high light and high temperature in contrast to the sun-exposed peel, the shaded peel is expected to be more susceptible to high light/temperature stress, which would make the protective function of anthocyanins, if any, more readily detectable.

2. Materials and methods

2.1. Plant materials and treatments

Six-year-old green 'Anjou' pear (*Pyrus communis* L.) and its bud mutation, 'Columbia red Anjou' trees on Old Home × Farmingdale 97 rootstock were used in this study. The trees were grown at a spacing of 3.6 m × 4.8 m in North–South rows at the Mid-Columbia Research and Extension Center of Oregon State University in Hood River, OR, USA. They were about 3.8 m tall with a central leader. Each cultivar was replicated 5 times with 3 trees in each replicate in a randomized complete block design. All the trees received standard horticultural practices and disease and pest control.

On July 25, 2007, approximately 107 days after bloom, well-exposed fruit with clear sun-exposed and shaded sides from the west side of the tree canopy were selected, gently detached, wrapped in wet paper towel and dark-adapted overnight at 22 °C. Fruit fresh weight and diameter were 99.7 ± 4.3 g fruit^{-1} and 51.5 ± 0.7 mm for green 'Anjou', 112.4 ± 3.8 g fruit^{-1} and 52.5 ± 0.6 mm for red 'Anjou', respectively.

Peel discs (4 cm^2 each, 3 mm thick) were taken from the shaded side of each fruit after overnight dark-adaptation, and the discs were put onto four layers of wet cheesecloth in a stainless steel water jacket, the temperature of which was controlled by a NESLAB RTE-10 refrigerated water bath (Thermo Electron Corp., Newington, NH, USA). In our preliminary experiments, detached fruit wrapped in wet paper towel and peel discs (4 cm^2 each, 3 mm thick) kept on wet cheese cloth were found to maintain their photosynthesis and chlorophyll fluorescence properties unchanged for at least 48 h and 8 h, respectively (unpublished data). The photon flux density (PFD) at the surface of the fruit peel discs was provided by a 1000 W HID lamp (Energy Technics Horticulture Lighting, York, PA, USA) at 1000 μmol m^{-2} s^{-1}, with a water-circulating heat filter (made of plexiglass) placed in between the peel discs and the lamp. Peel discs were treated with peel temperature of 31.0 °C in the dark, high light (PFD of 1000 μmol m^{-2} s^{-1}) at peel temperature of 31.0 °C, high peel temperature of 42 °C in the dark, or high light coupled with high temperature (PFD of 1000 μmol m^{-2} s^{-1} at peel temperature of 42.0 °C) for 0, 20, 40 or 60 min. Each treatment was replicated 5 times, corresponding to the 5 replicates in the field. For each replicate of any given treatment, four groups of peel discs were used, which allowed for sampling at 4 different time points as described above. At each sampling point, peel discs were dark-adapted for one hour at room temperature before chlorophyll a fluorescence transients were measured.

At the end sampling point (after 60 min stress treatments), peel discs (1.2 cm^2 each, 1.0 mm thick) were taken quickly from the treated 3 mm thick discs with a razor blade. These discs were frozen in liquid nitrogen and stored at −80 °C until analysis of pigments, antioxidant enzymes, and antioxidant metabolites.

2.2. Chlorophyll a fluorescence measurements

Chlorophyll a fluorescence was measured via a Handy-PEA fluorometer (Hansatech Instruments Limited, Norfolk, UK) on 2 peel discs per replicate for any given treatment at each sampling time. The main reason that we used Handy-PEA was due to its red light source (peaked at 550 nm), which is not absorbed by anthocyanins, thereby excluding any possible interference of anthocyanins on fluorescence measurements [37]. Saturating light pulse of 3000 μmol m^{-2} s^{-1} was provided by an array of three LEDs. Maximum quantum yield of PSII (F_V/F_M) was calculated as: $F_V/F_M = (F_M - F_O)/F_M$, where F_O and F_M were the dark adapted minimum and maximum fluorescence.

2.3. Dark respiration measurements

Dark respiration of peel discs was measured at the end sampling point (60 min) with a ChloroLab2 liquid-phase oxygen electrode system (Hansatech Instruments Limited, Norfolk, UK) in the dark. One peel disc (0.4 cm^2 in size, 1.0 mm thick) was taken from the treated 3 mm thick discs after being dark adapted at room temperature for 1 h, immediately placed into the reaction chamber using a custom-made holder, which was inserted through the hole in the centre originally designed for adding inhibitors to the chamber. The chamber was filled with 1.5 ml of distilled water, and oxygen uptake was measured at 25 °C for 10 min. Two peel discs were measured per replicate for any given treatment.

2.4. Pigment extraction and analysis

Chlorophylls and carotenoids in peel tissues were extracted with 80% acetone, and anthocyanins were extracted with a mixture