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Softening processes after ripening are a major factor contributing to the perishability of fleshy fruits and, together with mechanical damage, represents the onset of physiological decay. Softening involves multiple co-ordinated events leading to modifications of the cell wall architecture. Several studies described that UV-B radiation positively affects both the nutraceutical and aesthetical qualities of fruits. However, very few studies investigated the effect of UV-B irradiation on the activity of cell wall-related enzymes. This research aimed to study how different UV-B treatments (10 min and 60 min) affect the activity of cell wall-modifying enzymes (pectin methylesterase, polygalacturonase and β-galactosidase) together with the expression of some of their isoforms up to 36 h after UV-B treatment of peach (cv. Fairtime, melting phenotype) fruits. Results revealed that UV-B radiation did not affect the soluble solid content and the titratable acidity, two important parameters influencing consumers choice and taste. On the contrary, UV-B was effective in reducing the loss of firmness 24 h after the 60 min irradiation. Generally, a lower activity of the hydrolytic enzymes compared to untreated fruits was observed, regardless of the UV-B dose. However, gene expression did not reflect the corresponding enzymatic activity. Based on these results, UV-B irradiation might be a successful tool in reducing the loss of firmness of peach fruit in post-harvest, thus improving their quality and shelf-life.

Introduction

Fruit and vegetables represent essential components of the Mediterranean diet, especially thanks to their high levels of micronutrients (e.g. vitamins and minerals), nutraceuticals (e.g. phenolics, carotenoids), fibres, etc. However, high percentages of plant-based food are wasted yearly due to their low shelf-life. Quality of fruit after harvest is influenced by internal factors, like genotype and stress-related responses, and external factors, such as adverse environmental conditions.[1] Many methods have been implemented in order to postpone the decay of fruit and vegetables, including both thermal (cold storage) and non-thermal treatments such as controlled/modified atmosphere packaging, ionizing radiation, edible coatings, ultrasounds, high hydrostatic pressure, and dense phase carbon dioxide.[1–8] The softening process, one of the main causes of perishability in fruits, is due to degradation of cell wall structure and components, which leads to several consequences such as loss of cell- and tissue-turgor and water content.[9] Such biochemical changes are finely regulated by a complex of coordinated enzymatic reactions, which are responsible of weakening the cell wall structure especially by degrading the pectic polysaccharides and breaking down the links between cell wall polymers, such as cellulose, xyloglucan and glucomannan. Among the most important lytic enzymes, a key role is played by pectin methylesterases (PMEs), polygalacturonases (PGs) and galactosidases (GALs).

PMEs belong to a large multigene family, which counts around 66 putative isoforms in Arabidopsis.[10] PMEs are involved in demethylesterification of homogalacturonan, a α-D-galacturonic acids-based polysaccharidic polymer, contributing as component of the middle lamella to the adhesion of cells.[11,12] The product of such enzymatic activity represents in turn the substrate for further cell wall-modifying enzymes.[13] Endopolygalacturonases (endo-PGs) are enzymes involved in hydrolysis of α-(1–4) galacturonan bonds, and their upregulation is correlated with an accelerated softening process.[14] Other glycoside hydrolytic enzymes, β-galactosidase (β-GAL), fulfil their function by hydrolysing β-D-galactosidic residues. Its presence is widely distributed within the plant kingdom, and its activity has been associated with increase of galactose levels and loss of firmness in several fruits.[15–18]

Peach (*Prunus persica* L.), a climacteric and soft-fleshed fruit, is widely consumed worldwide, and its richness in antioxidant compounds makes peach a valuable healthy food. However, peach producers are challenged by its high perishability, especially due to fast ripening and softening processes. Peach cultivars can be classified as melting flesh (MF) or non-melting flesh (NMF) phenotypes, according to their differential modifications in terms of

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softening during the ripening process. The main cell wall modifying enzyme responsible for such classification is endo-PG, whose high activity was found in fully ripe MF peaches, while very low levels were detected in fruit of NMF- cultivars.[19–21] More recently, it has been found that the difference in endo-PG activity between MF and NMF cultivars is due to a transcriptional regulation of its isoforms, which are highly expressed in fully ripe MF peach fruit, while are lowly expressed in fully ripe NMF or unripe MF fruit.[22] Generally, it has been found that the expression of several PME, endo-PG and β-GAL isoforms is influenced by many abiotic factors, such as cold,[23,24] heat,[25,26] and ethylene [27] treatments.

UV application has been found to induce biochemical and molecular changes in post-harvest fruits. Depending on the species and cultivar, post-harvest UV-B radiation affects positively the quality of fruits, especially by enhancing the antioxidant activity and phenolic content.[28–30] Also UV-C irradiation has been found to correlate with increased firmness by lowering the activity of cell wall-modifying enzymes in tomato fruit.[31,32] Literature about post-harvest UV-B application for extending shelf-life of peaches is scarce. To date, only one work [33] investigated the effect of UV-B exposure on peach fruit, reporting that the irradiation significantly lowers the softening process in MF "Suncrest" peach cultivar. However, UV-B treatment was conducted continuously for 36 h,[33] which would be enormously expensive from an applicative point of view. For this reason, the present work aimed to investigate the effect of shorter UV-B treatments, 10 and 60 min, on the activity of several cell wall-modifying enzymes, namely PMEs, PGs and β-GALs during a recovery time up to 36 h after the UV-B exposure, and on the expression of their corresponding genes. Moreover, since a positive effect of UV-B in delaying softening was observed only in the MF type (cv "Suncrest" [33]), a different MF cultivar ("Fairtime") was used in the present study to support the relationship between the melting trait of peach flesh and UV-B responses.

Materials and methods

Plant material and UV-B treatment

Organic peach fruits (*Prunus persica* L., cv Fairtime, MF phenotype) from a biological supermarket were used in this study. Each fruit was accurately checked not to have any superficial injury, and only homogeneous peaches in color and shape were used. Five peach fruit were sampled as soon as they were carried to the laboratory of the Department of Applied Genetics and Cell Biology at the University of Natural Resources and Life Sciences, Vienna (Austria), and they were considered time 0 (T_0). Remaining peaches were randomly divided in three groups: one with control fruits (unirradiated, CTR), one with peaches treated with 10 min UV-B exposure (UVB-10), and one with peaches treated with 60 min UV-B exposure (UVB-60). The UV-B irradiation was performed in climatic chambers at 24 °C, equipped with both UV-B and white light tubes (Philips F17TL741 17W T8 Fluorescent Tube Cool White Light - RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands). UV-B tubes (Philips Ultraviolet-B narrowband, TL 20W/01 – RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands) provided an irradiance of 2.31 W m^{-2} at fruit height, reaching 10.70 W m^{-2} of total irradiance considering also the white light. Chambers for unirradiated CTR fruits were equipped only with white light tubes. Flesh pieces taken from the UV-B-irradiate pole of five fruits were sampled after 6 h, 12 h, 24 h, 36 h from the end of UV-B exposure, dipped in liquid nitrogen and lyophilized. CTR samples were collected at same times as UV irradiated samples. Since the flesh from each fruit was sampled and analyzed individually, it represents a biological replicate.

Determination of fruit quality traits

Firmness of peach flesh was assessed using a penetrometer with a 5 mm probe on a flat surface by peeling two opposite regions on the equatorial side of each fruit. Flesh firmness (FF) was expressed in Newtons (N). Total soluble solid content (SSC) was measured using a digital refractometer on the same regions where FF was evaluated. SSC values were expressed as ° Brix. Finally, titratable acidity (TA) was obtained by titrating 10 mL of juice with 0.1 N NaOH to an endpoint of pH 8.0 by using a pH-meter. Since the predominant organic acid in peach fruit is malic acid,[34] TA measures were expressed as g of malic acid/100 mL of juice.

RNA isolation and cDNA synthesis

Freeze-dried material was used for RNA extraction with the LiCl/CTAB method,[35] with slight modification. Briefly, 3 mL of pre-heated RNA extraction buffer (2% [w/v] hexadecyltrimethylammonium bromide, CTAB; 2% [w/v] polyvinylpyrrolidone (average molecular weight 40000), PVP; 100 mM Tris/HCl pH 8.0; 25 mM EDTA; 2 M NaCl; 0.5 g/L spermidine and 2.7% [v/v] 2-mercaptoethanol) were added to 50 mg of fine-powdered sample. The suspension was incubated at 65 °C for 5 min, then 3 mL of ice-cold chloroform:isoamylalcohol (24:1) were added and shaken well. The sample was centrifuged (4250 g for 20 min at 4 ° C) and the supernatant with nucleic acids was extracted again with ice-cold chloroform:isoamylalcohol (24:1) followed by another centrifugation step. 10 M LiCl at 4 °C was added to allow the selective precipitation of RNA, and the samples were incubated overnight. RNA pellet was isolated through centrifugation (12000 g for 1 h at 4 °C) and rinsed with cold 75% EtOH. RNA samples were finally rehydrated in 30 µL RNAse free water and stored at -80 °C. The concentration of each RNA sample was measured using Qubit RNA HS Assay Kit (Invitrogen) and WPA Biowave spectrophotometer (Biochrom Ltd.,Cambridge, England) and their integrity was evaluated by agarose gel electrophoresis. Contaminant genomic DNA was removed by enzymatic digestion using RQ1 DNAse (Promega) according to the manufacturer's instructions. RNA was then reverse-transcribed into cDNA (400 ng per sample) using iScript cDNA synthesis kit (BioRad, Hercules, CA, USA) according to the manufacturer's protocol. The resulting cDNA was used for quantitative real-time polymerase chain reaction (RT-qPCR).

Real time quantitative PCR (RT-qPCR)

PpPME1, *PpPME2*, *Ppβ-GAL* and *PpPG* transcript levels were quantified using RT-qPCR, and their abundance was normalized using *Actin* gene, which was found to be unaffected by the UV-B treatment. Gene sequence accession numbers at NCBI GenBank were AB231903 for *PpPME1*, X95991 for *PpPME2*, AY874412 for *Ppβ-GAL*, XM007213822 for *PpPG* and KP690196 for *Actin*. Due to the

occurrence of paralogs, NCBI BLASTn analysis and CLUSTALW multi-alignments (https://www.genome.jp/tools-bin/clustalw) were carried out among homologous sequences from *Prunus persica* Genome (https://www.rosaceae.org/species/prunus_persica/genome_v1.0) in order to design specific primer pairs (Table 1). Before RT-qPCR experiments, the specificity of amplified PCR products was checked by sequencing. 10 ng of cDNA were used for each RT-qPCR reaction, in a reaction mix with 250 nM primers and 1× Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocol. PCR runs were conducted in a StepOne real-time PCR System (Applied Biosystems, Foster City, CA, USA) with the following thermal cycling conditions: hold 95 °C, 20 s; 40 cycles 95 °C, 3 s; 60 °C, 30 s. Primer specificity and quality were assessed by checking the presence of a single peak in the melt curve at the end of amplification reaction. The PCR efficiency for each primers pair (Table 1) was calculated with a linear regression analysis of serial dilutions of cDNA (from 100 ng to 0.01 ng). The PCR amplification was conducted in three biological replicates, each of them analysed in triplicate. For each PCR run, the amplification of *Actin* gene was also included in the plate and results were normalized with respect to the *Actin* reference gene copy number. All RT-qPCR results showed are means from five independent biological replicates and for each independent biological replicate, the relative transcript amount was calculated as the mean of three technical replicates, calculating SE values in relative quantification.[36]

Extraction and assay of cell wall degrading enzymes

Extraction and activity assay of pectin methylesterase (PME, EC 3.1.1.11), polygalacturonase (PG, EC 3.2.1.15) and β-galactosidase (β-GAL, EC 3.2.1.23) were conducted according to Manganaris et al.[37] with few modifications. Since our aim was to evaluate the UV-B-induced changes in activity of cell wall-related enzymes during shelf-life, the enzymatic activities were not tested for the 6 h recovery time point.

PME were extracted from 0.1 g of lyophilized material and homogenized in 1.5 M NaCl containing 10% PVPP and 2 µL mL^{-1} PMSF (solubilized in 50 mg/mL DMSO). The suspension was stirred for 10 min at 4 °C and centrifuged (15000 x g, 45 min at 4 °C). After adjusting the pH to 7.5 with NaOH, the resulting supernatant was used to measure the PME activity.[38] PME activity was assessed in a mixture containing 500 µL of 0.5% (w/v) pectin (Pectin from citrus peel, Galacturonic acid ≥74.0% on dried basis, from Sigma-Aldrich), 37.5 µL of 0.01% (w/v) bromothymol blue in 3 mM phosphate buffer (pH 7.5), 100 µL ddH_2O and 200 µL of enzymatic extract. 200 µL of double-distilled water were added instead of the enzymatic extract for the blank. The changes in absorbance at 620 nm were recorded. The results were expressed as mmol min^{-1} mg^{-1} of protein.

PG and β-GAL were extracted homogenizing 0.1 g of lyophilized material in 1.5 M buffer containing 50 mM sodium acetate, 1 M NaCl, 10% PVPP and 2 µL mL^{-1} PMSF (solubilized in 50 mg mL^{-1} DMSO), pH 5.0. The homogenate was stirred for 1 h at 4 °C and centrifuged (11500 x g, 30 min at 4 °C). The supernatant was dialyzed overnight against 50 mM sodium acetate (pH 5.0) without NaCl and collected in a new tube for PG and β-GAL activity assays.

PG activity was measured in a reaction mixture containing 600 µL of 50 mM sodium acetate buffer, 0.2% (w/v) polygalacturonic acid (pH 4.5) and 400 µL of enzymatic extract. 400 µL of double-distilled water were added instead of the enzymatic extract for the blank. The mixture was incubated at 30 °C to allow the enzymatic reaction to occur. Every 30 min an aliquot (100 µL) was taken and the reaction was stopped adding 1 mL of borate buffer containing 0.62% boric acid and 0.2% NaOH, and 1% 2-cyanoacetamide, as described in the 2-cyanoacetamide assay.[39] The mixture was mixed well and incubated in boiling water for 10 min. Once the samples cooled down, the absorbance was read with a spectrophotometer at 276 nm using quartz cuvettes. Results were expressed as Δ Abs min^{-1} mg^{-1} of protein.

β-GAL activity was determined in a reaction mixture containing 600 µL of 50 mM sodium acetate buffer pH 5.0 (pH 4.5), 10 mM p-nitrophenyl- β -D-galacto-pyranoside and 400 µL of enzymatic extract. 400 µL of double-distilled water were added instead of the enzymatic extract for the blank. The mixture was incubated at 30 °C for up to 4 h, and every hour an aliquot (25 µL) was taken and mixed with 600 µL of 0.4 M sodium carbonate. After shaking, the absorbance of the samples was read at 400 nm and results were expressed as nmol min^{-1} mg^{-1} of protein.

The protein content for each enzymatic extract was determined using bovine serum albumin (BSA) as standard (BioRad Protein Assay; Bio-Rad Laboratories, Segrate, Italy).

The activity of each enzyme was determined in three biological replicates. For each of them, two serial dilutions of the extract were analyzed to ensure the reliability of the data.

Statistical analysis

JMP software (SAS Institute, Inc., Cary, NC) was employed to perform the statistical evaluations. Significant statistical differences in the UV-B-treated samples, both in terms of transcript abundance and enzymatic activity, were evaluated by one-way ANOVA followed by Tukey–Kramer post hoc test at the significance level $P \leq 0.05$ considering at each recovering time point (0 h, 6 h, 12 h, 24 h, 36 h) independently.

Results and discussion

Flesh firmness is the only quality trait influenced by UV-B treatment

Some of the most important fruit parameters that address the consumers' choice are the firmness, the soluble solid content (SSC) and the titratable acidity (TA). Particularly, the last two significantly affect people's perception of sweetness and sourness, thus contributes to the taste of the fruit.[40,41] On the other hand, the loss of firmness occurring during ripening and senescence of fruits drastically increases the susceptibility to mechanical damages, as well as to pathogen and pest attacks.[42]

Data about peach quality traits are reported in Table 2. At the beginning of the experiment, average flesh firmness was 25.6 N. During storage, both control and UV-B-treated peaches underwent a decrease, but UV-B-exposed fruit showed a reduced loss of firmness after 24 h from the UV-B irradiation, regardless of the UV-B dose. Control fruit underwent a decrease in firmness of 72.7% and 95% 24 h and 36 h after the beginning of the experiment, respectively.

However, at the 24 h recovery time point, both 10 min and 60 min UV-B-treated samples had a significant reduction in the softening process by 8.6% and 36.6% compared to the control, respectively. However, after 36 h from the UV-B irradiation, the firmness degree did not differ among control and UV-B exposed fruit. Thus, the higher the UV-B dose the firmer stayed the peaches. However, the duration of the UV-B-induced reduction in firmness loss was only transient, since, after 36 h, the firmness in the UV-B-treated groups was the same as the one in the control group. Regarding flesh firmness, the range limits for "ready to eat" fruits is set to 8.8 - 13.2 N for retail marketability.[43] While in our study the controls and the 10 min UV-B-exposed fruit reached such range already 24 h after the treatment, the 60 min UV-B-treated fruits displayed a slower decrease in firmness. Literature about UV-B effects on peach firmness is scanty. In accordance to our findings, Scattino et al.[33] showed a reduced softening in a melting-fleshed peach cultivar after 36 h of UV-B irradiation. However, effectiveness of short-term UV-B exposure (e.g. 60 min) in decreasing loss of peach flesh firmness has not been reported before. In mature-green tomato, UV-B (10, 20, 40 and 80 kJ m^{-2}) similarly maintained a higher level of firmness during storage at 14 °C in the dark.[44] Post-harvest UV-C radiation was also effective in delaying softening processes of fruits , such as strawberry,[45,46] tomato,[31,32,47] pineapple [48] and apple.[49]

TA values started with 0.65 g of malic acid/100 mL of juice at t0, and they did not significantly change considering both the storage time and the UV-B treatment. Regarding SSC, fruits at the beginning of the experiment had a value of 14.6 °Brix, which remained unchanged for all the recovery time points in both, the control and UV-B exposed fruits. Thus, these parameters were not affected by the two short time UV-B treatments. These results are in agreement with those of Scattino et al.[33] who reported that even longer UV-B exposures as 36 h did not change SSC and TA in different peach cultivars of different melting phenotypes ("Big Top", "Suncrest" and "Babygold 7"). Similarly, also the quality parameters of apples did not change after continuous 36 h treatment with UV-B during a 21 days recovery and storage time. Since SSC and TA are fundamental parameters contributing to the general taste of peach fruits, it is important that the UV-B exposure does not alter these quality traits, in order to avoid any changes in consumers' perception and appreciation of the fruit.

UV-B treatment negatively affects the activity of softening-related enzymes

Loss of flesh firmness is a macroscopic phenomenon occurring during ripening of fruits, and it is associated with a reduction of shelf-life due to a higher predisposition to mechanical damages and pathogen infections.[42] Firmness represents not only an organoleptic property that guides the customer's choices, but it is also a parameter that regulates the marketability of peaches and nectarines which is set by the European Union.[50] Loss of firmness is mostly associated with the activity of several hydrolytic enzymes on the cell wall, which act by breaking down the polysaccharidic polymers that constitute the architecture of plant cell wall.[13,51] The role of post-harvest UV-B radiation in reducing the loss of fruit firmness by affecting the activity of cell wall-modifying enzymes has been scarcely investigated.

After each UV-B irradiation (10 min and 60 min), the activity of three fundamental enzymes related to cell wall softening, PME, PG and β-GAL, were quantified spectrophotometrically (Figure 1).

PME, which is one of the cell wall-modifying enzymes acting upstream of the breakdown of pectic polysaccharides, was affected by both UV-B treatments. PME activity had a fluctuating trend during the recovery period, with a maximum observed at the 12 h time point (121.4 μmol min^{-1} mg^{-1} protein). However, the UV-B irradiation resulted in a downregulation of PME activity especially by reducing the fluctuations during the storage time. At the 12 h time point, the PME activity proportionally decreased with the duration of the UV-B treatments e.g. -20% and -37% in the 10 min and 60 min UV-B-exposed samples, respectively. After 24 h, however, no significance changes were detected between UV-B-irradiated and control samples, while after 36 h the unexposed samples showed a higher PME activity compared to both the UV-B-exposed ones (-12% for the 10 min-UV-B-exposed samples, and -17% for the 60 min-UV-B-exposed samples).

Although a much longer period was considered, also in banana fruit a fluctuating behavior was observed in terms of PME activity during shelf-life,[52] with a maximum activity after 15 days of storage time, and a following decrease. While studies on the effects of UV-B radiation on PME activity are scanty, studies with UV-C revealed a similar trend e.g. that an irradiation of 1.2, 3.6 or 4.8 kJ m^{-2} significantly reduced PME activity especially after 10 days of storage in fresh cut melon.[53] Similarly, in ripening tomato a 3.7 kJ m^{-2} UV-C dose was able to reduce the activity of several cell wall modifying enzymes including PME, supporting the effectiveness of UV radiation in lowering the activity of such enzymes.[32]

Like PME, also PG activity was found to be downregulated by UV-B radiation. The physiological changes of PG activity without UV-B irradiation increased by 85% only during the first 12 h, but then constantly decreased up to 36 h (-46% compared to the activity detected in the 12 h time point, reaching the same level as at the start of the experiment). PG activity was lower after both UV-B treatments compared to control samples for all the time points. Moreover, no significant differences were detected between the two UV-B treatments, except for the 12 h recovery time point, where the PG activity of the 60 min-UV-B exposed samples was 23% lower than the one of the 10 min-UV-B-exposed fruits. As stated for PME, very few published data are available concerning UV-B-driven changes of PG activity in fruits. Contrarily to what observed in this study, Scattino et al.[33] found that UV-B exposure increased PG activity in peach fruits (MF cv. Suncrest and slow-melting phenotype, SMF, cv. Big Top). However, in Scattino et al.[33] the UV-B treatment was conducted for 36 h continuously, and peaches belonged to a different cultivar. Our data are consistent with an UV-C exposure (4.1 kJ m^{-2}) that was also effective in reducing PG activity in strawberry fruits after 48 h [45] and of UV-C treatments with pineapples [48] and with fresh-cut melon.[53]

The last softening-related enzyme tested, β-GAL, showed a decrease of activity during first 12 h of recovery with no differences between the control and the UV-B irradiated. However, significant changes started to be quantifiable at longer recovery time points. Fruits exposed to UV-B radiation displayed a 39% lower activity compared to the control at the 24 h time point for both the UV-B-treated samples and 29% and 25% lower activity compared to control fruits

at the 36-h time point for the 10 min and 60 min UV-B-exposed samples, respectively. In the study conducted by Scattino et al.,[33] no variations were detected in β-GAL activity followed UV-B irradiation. However, as stated above, the experimental conditions and the peach cultivar were different from the ones used in this study indicating genotype specific effects of UV-B treatments on β-GAL activity.

Gene expression of *PpPME1*, *PpPME2*, *PpPG* and *Ppß-GAL* isoforms is independent of UV-B treatment

PpEndo-PG isoform used in this study was the one investigated by Scattino et al.,[33] which resulted to be down-regulated in the MF peach cultivar "Suncrest" after 36 h of UV-B exposure. Similarly, *PpPME1*, *PpPME2* and *Ppβ-GAL* isoforms chosen were found to be affected by many abiotic stresses, such as heat,[25] cold [24] and ethylene [27] treatments, although no data are reported about their UV-B-induced regulation. The accumulation of mRNAs encoding two pectin methylesterase isoforms (*PpPME1/*AB231903 and *PpPME2/*X95991), a polygalacturonase (*PpPG/*XM007213822) and a β-galactosidase (*Ppβ-GAL/*AY874412) isoform were examined using RT–qPCR (Figure 2).

PpPME2, the second *PME* isoform analyzed, showed a different behavior to *PpPME1*. Its expression level decreased immediately at the 6 h time point for all the samples, with the highest decrease occurring in the UVB-10 fruits. Transcript level of *PpPME2* remained significantly higher in control peaches than in UVB-10 samples for all the recovery times. Contrarily, UVB-60 fruits exhibited a peak in the 12-h recovery time point, while no differences were detected between both control and UVB-10 peaches in the other time points. Therefore at least for these two *PME* genes, profiles of transcript level were not correlated with profiles of level of enzymes activity, so observed variations of gene expression seem to be not responsible for the UV-B reduced softening of peaches.

PME genes belong to a large multigene family [10] with highly conserved protein domains. A *PME* transcriptome survey in Arabidopsis and Poplar indicated that isoforms exhibit different and overlapping expression patterns during development and upon abiotic and biotic stresses.[10] Thus, the missing correlation between UV-B modulated *PME* expression, PME activity and firmness of the fruits might be due to functional redundancies among members of the same gene family that can show also antagonistic effects due to differences in regulatory sequences and interactions with different PME inhibitors. The interplay between PME and PME inhibitors can be a determinant of cell adhesion, cell wall porosity and elasticity, influencing firmness of fruits.[54]

Considering *PpPG* expression, both UV-B treatments did not alter their transcript abundance after 6 h from the end of treatments. *PpPG* transcript level in UVB-10 fruits slightly increased after 12 h, but afterwards decreased and at 36 h, reaching a lower level compared with the start of the experiment. However, in the UVB-60, a significant transient decrease was observed at the 12 h time point and reached after 24 h the level of the start of the experiment and then a new decrease was detected at 36 h. Control samples showed minor variations of mRNA amount during the recovery with a decrease observed only at 36h. Contrarily to what observed in this study, Scattino et al.[33] found that a 36-h UV-B treatment did not induce any change in *PpPG* transcript level in the MF "Suncrest" and SMF "Big Top" cultivars tested.

Ppβ-GAL expression dropped in first 12 h of recovery, with a decrease faster in both UV-B treatments when compared to the control. However, only slight differences were detected at the late recovery time points. Particularly, while the UVB-10 remained at a very low expression level until the end of the recovery period, both the UVB-60 and the control samples showed an upregulation at the 24 h and 36 h time points, respectively. Although, to the best of our knowledge, no previous studies investigated the effect of UV-B radiation on *Ppβ-GAL* gene expression, its regulation following other abiotic factors has been observed also in other plant species. In ethylene-treated avocado fruits, the transcript level of four *β-GAL* paralogues has been investigated, and their expression pattern differed from the control in accordance to the isoform considered.[55] Considering all the genes tested, no correspondence was found between their expression and enzymatic activities except for *PME2* after UVB-10 treatment, where both transcript and activity levels were reduced respect to control fruits. This suggests that different mechanisms of regulation occur between enzyme activity and gene expression. Similar results were observed during ripening of apple, where activity and gene expression of *PME*, *PG* and b *β-GAL* were studied in different genotypes and in response to low temperature and ethylene.[13] In contrast, during ripening in pear fruit, levels of an mRNA encoding a β-GAL coincided with the increase in the β-galactosidase III activity.[56] More recently, *β-GAL* expression correlated with activity of the enzyme during ripening but not after ethylene treatment in mango fruit.[57]

However another possible explanation for the lack of correlation between enzyme activity and gene expression might be the high number of paralogous genes encoding for PMEs, PGs and β-GALs, as reported by previous studies though on different plant species,[10,16,58] whose expression patterns could differ each other. In this study we tested the expression of one or two members of each gene family, so it is possible that genes chosen are not related to enzymatic activity, but a correlation could occur for other members of the same gene family. In fact, it is likely that UV-B radiation modifies the expression of specific members of the same gene family differently. Multiple isoforms of *β-GAL* gene from tomato and strawberry displayed varied patterns of expression during ripening.[59,60] Also in pear fruit, during ripening or ethylene treatments, beside an increase of β-GAL activity, different expression patterns were observed for different *β-GAL* genes.[61,62] In strawberry two *PG* genes showed different correlations with PG activity.[63] In addition, the presence of mRNA transcripts encoding for a specific isoform cannot be directly correlated to the resultant total enzymatic activity, due to different transcription rate.[64,65]

Finally, transcription regulation might not indicate the full story anyway. Beside possible concomitant action of several isoforms, also post-transcriptional regulatory events may be involved.

So, UV-B might modulate the activity of these proteins by mean of post-transcriptional regulations such as translation, secretion into the cell wall and eventually post-translational modifications as observed recently in tomato ripening.[66,67]

Conclusions

This study revealed that 60 min of UV-B exposure significantly reduced the loss of firmness 24 h after the irradiation in peach fruit (cv. Fairtime, melting phenotype), which might be related to a reduced activity of some important cell wall enzymes related to fruit softening such as PME, PG and β-GAL which were found to be less active in both UV-B-treated groups (10 and 60 min). Furthermore, UV-B also affects the expression of some of their isoforms, with a gene-specific pattern. The decrease in flesh melting, likely due to the reduction in the activity of several cell-wall modifying-enzymes, would be beneficial in relation to a reduced susceptibility to physical damages and biotic attacks. However, the research about UV-B effects on cell wall dismantling enzymes in fruit is at its very beginning, thus further research is needed to better understand the mechanisms involved, in order to exploit short-term post-harvest UV-B irradiation to eco-friendly extend the shelf-life of peach fruits.

Conflicts of interest

There are no conflicts to declare.

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Table 1. Primers used in this study.

Gene	Forward (5'-3')	Reverse (3'-5')	Accession number	cDNA fragment length (bp)	PCR efficiency $E = 10^{-1/S} - 1$
PpActin	TCCAAGGGTGAGTACGATGAG	ATGACACGACACGAAAATCCAACTA	KP690196.1	120	0.99
PpPME1	CTTCCTCCTCCAGTCTCTCCA	TTGAGTACCGTCCAGCCTTGTA	AB231903.1	83	0.98
PpPME2	AGTCAACTCCACACCCACTT	TCACCATTCCACTCAAGCCA	X95991.1	120	1.04
Ppβ-GAL	GAGTACGGAGCACAGAGTAAGTT	CCCAACCCAACAGCCATATTTG	AY874412.1	83	0.99
PpPG	ATGGAACAACTGAGGGTGTGGTT	TGGATTTCAATACTTCTACAAGCAG	DQ659241	85	0.97

^{S} refers to the slope of the standard curve.

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Table 2. Flesh firmness, soluble solids content (SSC) and titratable acidity (TA) of "Fairtime" peaches in control and UV-B treated fruit before the UV-B treatment (0 h) and after 24 and 36 h from the exposure. Data represent the mean of three replicates ± SE. Different letters indicate significant differences within each recovery time point according to one-way ANOVA followed by Tukey's test ($P \leq 0.05$).

Recovery time (h)	UV-B treatment (min)	Flesh firmness (N)	SSC (° Brix)	TA (g malic acid/100 mL juice)
0	0	25.6 ±0.18	14.60±0.67	0.65±0.01
24	0	6.99 ±0.25 c	14.67±0.84	0.71±0.01
	10	9.20 ±0.36 b	16.48±1.25	0.69±0.01
	60	16.35±2.88 a	13.85±1.40	0.65±0.07
36	0	1.27 ±0.06	13.92±0.76	0.62±0.02
	10	3.31 ±0.57	15.10±1.43	0.53±0.05
	60	1.80 ±0.25	13.85±0.96	0.71±0.03

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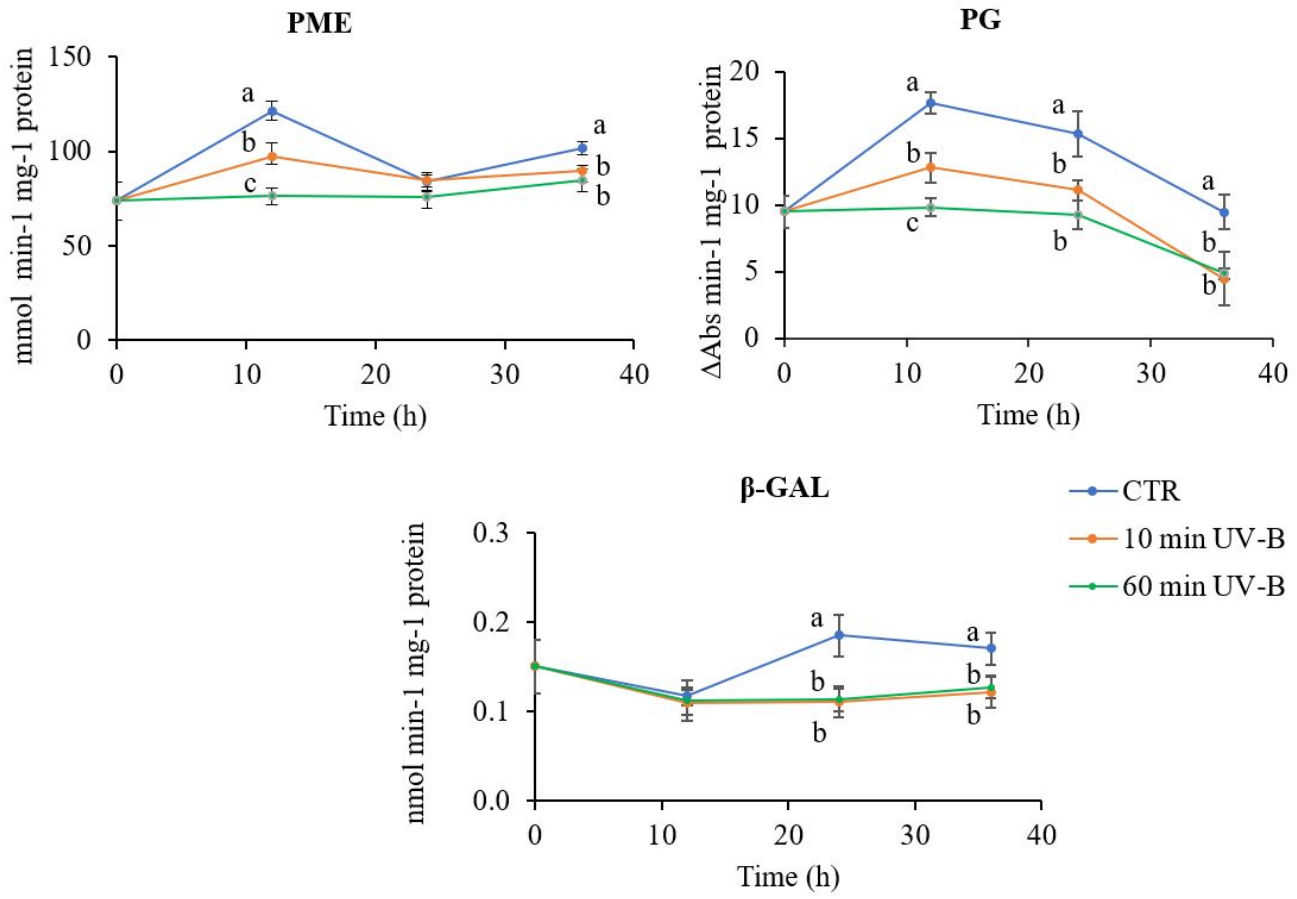

Figure 1. Enzyme activity of pectin methylesterase (PME), polygalacturonase (PG) and β-galactosidase (β-GAL) in the flesh of MF 'Fairtime' peach cultivar in control samples and UV-B-treated (10 min or 60 min) samples. Data represent the mean of three replicates ± SE. Different letters indicate significant differences within each recovery time point according to one-way ANOVA followed by Tukey's test ($P \leq 0.05$).

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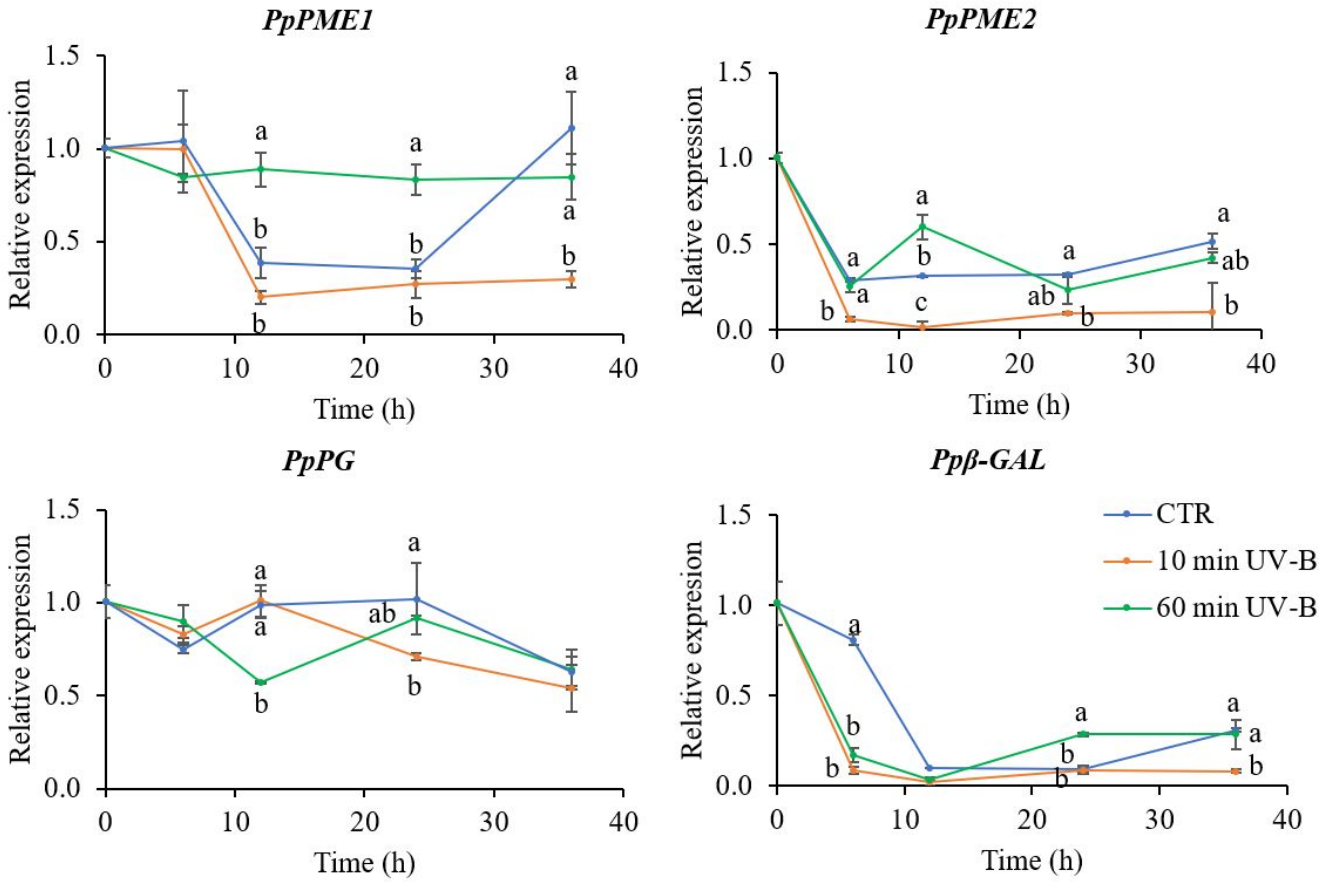

Figure 2. Effect of UV-B exposure (10 min or 60 min) on transcript abundance of some isoforms encoding pectin methylesterase (*PpPME1* and *PpPME2*), polygalacturonase (*PpPG*) and β-galactosidase (*β-GAL*) in peach flesh. Data are mean ± SE of five biological replicates. Different letters correspond to statistically significant differences according to one-way ANOVA followed by Tukey–Kramer post hoc test ($P \leq 0.05$).