Research Article

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Cell wall metabolism of peaches and nectarines treated with UV-B radiation: a biochemical and molecular approach

Claudia Scattino,[a] Noemi Negrini,[b] Silvia Morgutti,[b] Maurizio Cocucci,[b] Carlos H Crisosto,[c] Pietro Tonutti,[d] Antonella Castagna[a]* and Annamaria Ranieri[a]

Abstract

BACKGROUND: Ultra-violet B (UV-B) radiation has been shown to improve, at least in selected genotypes, both the health-promoting potential and the aesthetic properties of tomato and peach fruits during their post-harvest period. The effects of post-harvest UV-B treatment on the cell-wall metabolism of peaches and nectarines (*Prunus persica* L. Batsch) were assessed in this study. Three cultivars, Suncrest (melting flesh, MF) and Babygold 7 (non-melting flesh, NMF) peaches and Big Top (slow melting, SM) nectarine, differing in the characteristics of textural changes and softening during ripening, were analysed.

RESULTS: The effects of UV-B differ in relation to the cultivar considered. In MF 'Suncrest' fruit, UV-B treatment significantly reduced the loss of flesh firmness despite the slight increase in the presence and activity of endo-polygalacturonase. The activity of exo-polygalacturonase increased as well, while endo-1,4-β-D-glucanase/β-D-glucosidase, β-galactosidase and pectin methylesterase were substantially unaffected by the treatment. The UV-B-induced reduction of flesh softening was paralleled by the inhibition of *PpExp* gene transcription and expansin protein accumulation. The UV-B treatment did not induce differences in flesh firmness between control and UV-B-treated NMF 'Babygold 7' and SM 'Big Top' fruit.

CONCLUSION: Based on these results, post-harvest UV-B treatment may be considered a promising tool to improve shelf-life and quality of peach fruit.

Keywords: peach; UV-B treatment; flesh softening; Endo-PG; expansins; post-harvest

INTRODUCTION

During fruit storage, the evolution and changes of quality parameters are affected by both intrinsic (genotype) and extrinsic (environment) factors. Many physical treatments are currently used to maintain the post-harvest quality of fruits and vegetables, such as modified atmosphere packaging, heat treatment, storage at low temperature, irradiation with ionising and non-ionising radiations, etc.[1–3] Treatments with ultra-violet (UV) radiation were demonstrated to be effective in delaying fruit ripening and senescence and in reducing the incidence of post-harvest spoilage. UV-C is effective in increasing the antioxidant capacity of strawberries in the post-harvest period, in controlling the yellowing of broccoli florets, and reducing the incidence of chilling injury in stored peaches and peppers.[4,5] Treatments with low and ambient UV-B radiation (less harmful for the user than UV-C) inhibit the yellowing of stored green fruits and vegetables and increase the antioxidant content of the produce.[6,7] Recently, it has been shown that post-harvest UV-B treatments can improve, at least in selected genotypes, both the health-promoting potential and the aesthetic properties of tomato and peach fruits by increasing anthocyanin contents.[8,9] UV wavelengths may also affect other ripening-related parameters, such as firmness, which, in strawberries, is affected by changes in the UV wavelengths transmitted by polythene films in protected cultivation.[10] Similarly, tomato firmness was influenced by post-harvest UV-B treatments, either in a positive[11] or in a negative way.[8] However, information on the effects of post-harvest UV treatments on fruit cell wall metabolism is scarce and limited to the use of UV-C radiation.[12,13]

Peaches and nectarines (*Prunus persica* L. Batsch) are soft-fleshed drupes with a limited post-harvest life. Fruit softening during

* *Correspondence to: Antonella Castagna, Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy. E-mail: antonella.castagna@unipi.it*

a *Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy*

b *Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy, University of Milan, Via Celoria 2, Milan, Italy*

c *Department of Plant Sciences, University of California at Davis, One Shields Avenue, Davis, CA, 95616, USA*

d *Institute of Life Sciences, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy*

ripening is the major phenomenon that contributes to the perishability of fleshy fruit, together with mechanical damage, onset of physiological disorders and decay. The softening process involves multiple co-ordinated events leading to several modifications of the cell wall architecture; transpirational loss of water and cell turgor is also involved.[14–16]

A major structural change is the degradation of polyuronides operated by a number of degrading enzymes including polygalacturonases (PGs), pectin methylesterases, glycosidases and galactosidases. In the complex process of cell-wall dismantling, a central role is widely acknowledged to endo-acting polygalacturonases (Endo-PGs).[17] In particular, in ripening peach fruit, Endo-PGs play a key role in determining the melting-related phenotype.[18–20] Cooperatively to those enzymes, expansins (Exp) contribute to cell wall disassembly with a non-enzymatic mechanism.[21] Expansins are able to loosen the cell wall by disrupting non-covalent linkages at the cellulose/hemicellulose interface, relaxing the constraint to turgor-driven cell expansion.[22–24] The involvement of these (and other) enzymes has been ascertained also in ripening peach fruit by means of both molecular and biochemical studies.[16,19,25–27]

According to the characteristics of textural changes and softening during ripening, peach fruit is essentially classified as melting flesh (MF) and non-melting flesh (NMF). MF peach phenotype is characterised by a rapid loss of flesh firmness (melting) in the last ripening stage in correspondence to the peak of ethylene production.[19,28] The NMF phenotype softens slowly but never melts despite high ethylene production and shows a firm texture even when the fruit is fully ripe.[29,30] NMF fruit, traditionally grown for canning purposes, often shows a limited development of the red coloration and aroma.[31] Peculiar softening traits characterise slow melting (SM) fruit, such as the 'Big Top' nectarines that retain flesh firmness on the tree for a long time, allowing full development of organoleptic quality.[20]

The present work aimed to investigate whether post-harvest UV-B treatment was effective in slowing down the flesh softening process in peach and nectarine fruit with different flesh phenotypes (MF 'Suncrest', SM 'Big Top', and NMF 'Babygold 7'). Based on preliminary dose–response experiments which showed that a 36 h treatment induced phenolic accumulation in the two cultivars ('Suncrest' and 'Big Top') which reacted positively to UV-B,[9] while a prolonged exposure (starting from 48 h) caused visible injury (browning) on fruit surface, in the present research peaches were subjected to a 36 h post-harvest UV-B treatment. Activities of a few cell-wall degrading enzymes, as well as changes in transcript and protein levels of Endo-PGs and expansins were evaluated. Some quality-related traits [flesh firmness, soluble solids content (SSC), titratable acidity (TA), ethylene production] were measured as well.

EXPERIMENTAL

Plant material and UV-B treatment

Peach (*Prunus persica* L. Batsch) fruits of the yellow-fleshed cultivars 'Suncrest' [melting (M)] and 'Babygold 7' [non-melting (NM)] and 'Big Top' nectarine [slow-melting (SM)] were harvested in correspondence of flesh firmness values of about 60 N for MF and SM and about 30 N for NMF fruit, respectively. These flesh firmness values were chosen in order to allow fruit to achieve at least the 'ready to buy' stage at the end of the experiment, based on flesh firmness evaluations.[32]

For each variety, about 45 fruits without defects, selected for uniform size and appearance, were collected and 15 fruits, representing the t_0 sample, were immediately sampled. The remaining fruits were placed for 36 h in thermo-regulated chambers (20°C; relative humidity 85%), under a photosynthetic photon flux density of 500 mol m^{-2} s^{-1} (Powerstar HQI-BT 400 W/D; Osram, Munich, Germany), in the absence (control fruit) or in the presence (UV-B-treated fruit) of UV-B radiation. Each chamber was equipped with three UV-B lamp tubes (Philips Ultraviolet B, TL 20W-12RS; Koninklijke Philips Electronics, Eindhoven, the Netherlands), providing 1.69 W m^{-2} at fruit height. In the control chamber the UV-B lamps were shielded with benzophenone-treated polyethylene film to block the UV-B radiation.[33] To ensure uniform UV-B dose, fruits were aligned in rows parallel to the lamp tubes with their peduncle facing down, approximately 40 cm under the lamps.

At the end of the UV-B treatment, mesocarp samples from the distal part of each fruit (the part directly exposed to UV-B) were pooled, frozen in liquid nitrogen and stored at −80°C for subsequent analyses.

Determination of fruit quality traits

Fruit flesh firmness (in newtons) was measured after removing a small disc of skin from the equatorial zone of each cheek, by a digital penetrometer with an 8 mm probe (Model 53205; TR, Forlì, Italy). Total SSC (in °Brix) was measured by a digital refractometer (Model 53011; TR). Titratable acidity (TA) was determined by titration of 10 mL of juice with 0.1 mol L^{-1} NaOH to an endpoint of pH 8.2 by using an automatic Schott Gerate titrator (Model T80/20; Schott, Mainz, Germany), and expressed as meq NaOH 100 mL^{-1}.

Measurement of ethylene

Ethylene production was quantified in whole, healthy fruit individually incubated in sealed jars (1.2 L) at room temperature (22°C) for 30 min. Head-space samples (2 mL) were withdrawn with a hypodermic syringe in a gas chromatograph (HP5890; Hewlett-Packard, Menlo Park, CA, USA) equipped with a dual flame ionisation detector and stainless-steel column (150 × 0.4 cm internal diameter, packed with Hysep T). Column and detector temperatures were 70°C and 350°C, respectively. Nitrogen was used as carrier at a flow rate of 30 mL min^{-1}. Ethylene production was expressed as nL h^{-1} g^{-1} fresh weight.

Cell-wall enzyme extraction and activity assay

Exo-polygalacturonase (Exo-PG, EC 3.2.1.67), endo-1,4-β-D-glucanase/β-D-glucosidase (EGase, EC 3.2.1.4) and β-galactosidase (β-Gal, EC 3.2.1.23) were extracted according to Manganaris[34] with some modifications. Frozen samples were homogenised with half volume of 50 mmol L^{-1} sodium acetate buffer (pH 5.0), 1 mol L^{-1} NaCl and 10% polyvinylpolypyrrolidone (PVPP). The homogenate was stirred for 2 h at 4°C and centrifuged (15 000 × *g*, 30 min, 4°C). The supernatant was collected, dialysed overnight against the extraction buffer without NaCl and assayed for enzyme activities at 30°C in 1 mL of a reaction mixture containing 600 µL of 37.5 mmol L^{-1} sodium acetate buffer (pH 4.5) and the proper substrates (0.2% polygalacturonic acid for Exo-PG, 0.2% carboxymethylcellulose for EGase, and 10 mmol L^{-1} *p*-nitrophenyl-β-D-galactopyranoside for β-Gal).[35] The reactions were started by addition of the enzyme extract, and aliquots of the reaction mixture were withdrawn in the course of the subsequent 4 h.

Exo-PG activity was measured by recording the increase in absorbance at 276 nm due to the generation of reducing ends

(2-cyanoacetamide assay),[36] and expressed as μmol of galacturonic acid min^{-1} mg^{-1} protein. EGase activity, generating reducing sugars, was expressed as increase in absorbance at 276 nm (ΔAbs_{276}) min^{-1} mg^{-1} protein. For β-Gal activity, aliquots of the reaction mixture were poured into 600 μL of 0.4 mol L^{-1} Na_2CO_3 and the change in absorbance at 400 nm, due to the formation of *p*-nitrophenol, was recorded. The enzyme activity was expressed as μmol *p*-nitrophenol min^{-1} mg^{-1} protein.

Pectin methylesterase (PME, E.C. 3.1.1.11) was extracted by homogenising frozen mesocarp in one volume of 1.5 mol L^{-1} NaCl plus 10% PVPP. The homogenate was stirred for 10 min at 4°C and centrifuged (15 000 × *g*, 30 min, 4°C). The supernatant was collected and adjusted to pH 7.5. PME activity was determined by measuring the increase in absorbance at 620 nm of a mixture containing 0.5% pectin, 0.01% bromothymol blue in 3 mmol L^{-1} phosphate buffer (pH 7.5) and an appropriate aliquot of the extract. Activity was expressed as mmol galacturonic acid min^{-1} mg^{-1} protein.

Endo-PG (E.C. 3.2.1.15) was extracted according to Morgutti *et al.*[19] The frozen samples were homogenised with liquid nitrogen in the presence of 10% PVPP and four volumes of 1 mmol L^{-1} dithiothreitol plus 2 mmol L^{-1} phenylmethylsulfonylfluoride (PMSF). After centrifugation (11 000 × *g*, 20 min, 4°C), the cell-wall-enriched pellet was washed with four volumes of the same solution and re-centrifuged. The pellet was suspended (1 mL g^{-1} initial fresh weight) in high-salt extraction buffer, pH 5.5 [40 mmol L^{-1} sodium acetate, 1.5 mol L^{-1} NaCl, 20 mmol L^{-1} β-mercaptoethanol (β-ME), 2 mmol L^{-1} PMSF], stirred overnight at 4°C and centrifuged (11 000 × *g*, 30 min, 4°C) twice to completely eliminate tissue debris. The supernatant was filtered (Amicon Ultra Centrifugal Filters Ultracel-10K – Regenerated cellulose, 10000 MWCO; Millipore, Billerica, MA, USA) with two volumes of the extraction buffer without NaCl and stored at −80°C.

Expansins were extracted homogenising frozen mesocarp with one volume of extraction buffer, pH 7.5 (100 mmol L^{-1} Tris-HCl, 5 mmol L^{-1} $MgCl_2$, 7 mmol L^{-1} β-ME, 2 mmol L^{-1} PMSF, 0.025% Triton X-100, 1 mmol L^{-1} ethylenediaminetetraacetic acid, 10% PVPP). After centrifugation (9600 × *g*, 40 min, 4°C), the pellet was washed twice with the extraction buffer, suspended in one volume of denaturing buffer, pH 6.8 [100 mmol L^{-1} Tris-HCl, 4% sodium dodecyl sulfate (SDS), 5% β-ME, 20% glycerol, 4 mmol L^{-1} PMSF] and heated (90°C, 20 min). The expansins-enriched supernatant was recovered after centrifugation (9000 × *g*, 40 min, 4°C) and stored at −80°C.[37]

The protein content was determined using bovine serum albumin as a standard (Bio-Rad Protein Assay; Bio-Rad Laboratories, Segrate, Italy).[38] For expansins-enriched extracts, proteins were quantified by using a Plus-One 2-D Quant Kit (GE Healthcare S.r.l., Milan, Italy).

Electrophoretic and western blot analysis of Endo-PG and expansins

Polygalacturonase activity was visualised by specific staining[19,39] following native-PAGE (10%) carried out in a MiniProtean apparatus (Bio-Rad Laboratories). SDS-PAGE (10%)[40] was performed in a MiniProtean apparatus, after denaturation of salt-extracted proteins in SDS sample buffer.[41] Molecular weight markers were Full-Range Rainbow Molecular Weight Markers RPN800E (GE Healthcare S.r.l.). Western blot analysis was conducted as previously described[19] using rabbit anti-Endo-PG polyclonal antibodies (Primm S.r.l., Milan, Italy) raised against a synthetic polypeptide, able to recognise an active form of the enzyme,[19,20] constructed on a conserved region of the complete sequence of a Pp-Endo-PG from ripe peach fruit (CAA54150).[19,42] Expansins were detected using a 1:1500 dilution of polyclonal antibodies against a purified form of expansin (Expansin1 from *Solanum lycopersicum*, Anti-LeExp1 antibodies),[43] acknowledged to recognise peach expansins as well.[44,45]

Gene expression

Total RNA was isolated from freeze-dried mesocarp using E.Z.N.A.® SQ Total RNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's instructions. Samples were concentrated to a volume of 20 μL using the RNA Clean & Concentrator™-5 (Zymo Research, Orange, CA, USA). RNA integrity was visually inspected on a 1% agarose gel (MOPS Buffer/Formaldehyde Protocol)[46] and quantified and assessed for purity using an Eppendorf BioPhotometer (Eppendorf, Hamburg, Germany). First-strand cDNA was synthesised from about 1 μg of total RNA with the QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany). Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using the StepOnePlus™ procedure (Applied Biosystems, Foster City, CA, USA), using SYBR® Green, in a total reaction volume of 15 μL containing 2 μL of template (diluted 1:5), 0.5 μL of reverse and forward primers (Table 1; final primer concentration 10 μmol L^{-1}) , 7.5 μL iTaq™ SYBR® Green Supermix with ROX (Bio-Rad Laboratories, Hercules, CA, USA), and 4.5 μL of RNA-free water. Conditions for the qRT-PCR assay were: 95°C for 10 min followed by 40 cycles of 95°C for 30 s and 60°C for 30 s. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method[47] using *actin* gene as the reference. Average expression levels for each RNA were determined from the highly consistent triplicate reactions, with the range of the reactions never higher than 0.5 threshold cycle (Ct). *PpEndo-PG* gene specific primers were designed on the sequences reported by Gonzales-Aguero.[48] The primer sets used for assessment of *PpExp1*, *PpExp2* and *PpExp3* gene expression were designed on the sequences reported by Pegoraro *et al.*[49] *Actin* primers were designed on the sequences reported by El-Sharkawy *et al.*[50] (Table 1).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using the NCSS 2000 (NCSS Statistical Software, Kaysville, UT, USA) software. Significant differences between UV-B treated and control fruits were calculated using at least three replicates, according to Tukey's test ($P \leq 0.05$).

RESULTS

Fruit quality traits and ethylene production

Data about fruit quality traits and ethylene production are shown in Table 2. At the beginning of the experiment (t_0), the average flesh firmness of MF 'Suncrest' and SM 'Big Top' fruits was essentially similar and remarkably high. After 36 h, flesh firmness decreased in control fruits of both cultivars, to a different extent according to MF or SM phenotype. NMF 'Babygold 7' fruit, although showing at t_0 the lowest firmness, did not undergo marked changes of this parameter after 36 h. The UV-B treatment significantly reduced flesh softening in MF fruit, whose firmness was 64% higher as compared to control, while UV-B did not affect this parameter in both SM and NMF fruits.

At t_0, the highest and the lowest SSC values were measured in SM 'Big Top' and NMF 'Babygold 7', respectively. This parameter

Table 1. Primers for cell-wall analysis

Gene	Accession	Direction	Primer sequence
Endo-PG	GSE7145	Forward	GTCATCTGGTGTCACAATC
		Reverse	ACCCTCAGTTGTTCCATC
Exp 1	16305104	Forward	AAACGTTGGTGGTGCCGGTGAT
		Reverse	TTGCTTGCCAACCAGTCCTGGA
Exp 2	29466640	Forward	TCCAGGACTGGTTGGCAAGCAA
		Reverse	TAGGACACCACTGTGCGGCCAT
Exp 3	29466642	Forward	GGGTGCATGGGAAGCAGCTCAT
		Reverse	CCATGGTGCCAGAGGCATCAGA

Table 2. Changes in flesh firmness, soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio, and ethylene production in MF 'Suncrest', SM 'Big Top' and NMF 'Babygold 7' fruits at t_0 and after 36 h in the absence (Control) or in the presence (UV-B) of UV-B treatment

Genotype	Time (h)	Treatment	Flesh firmness (N)	SSC (°Brix)	TA (meq NaOH 100 ml^{-1})	SSC/TA	Ethylene (nL $h^{-1}Ag^{-1}$ FW)
MF 'Suncrest'	0	t_0	56.9 ± 1.24^{a}	13.2 ± 0.39^{a}	18.6 ± 1.84^{a}	0.71 ± 0.04^{b}	5.15 ± 0.08^{c}
	36	Control	11.1 ± 0.48^{c}	14.7 ± 0.38^{a}	17.7 ± 0.82^{a}	0.83 ± 0.08^{b}	15.3 ± 0.89^{b}
	36	UV-B	18.1 ± 1.49^{b}	15.2 ± 0.15^{a}	10.8 ± 0.51^{b}	1.41 ± 0.06^{a}	23.0 ± 2.23^{a}
SM 'Big Top'	0	t_0	57.0 ± 2.24^{a}	15.3 ± 1.75^{a}	7.88 ± 0.51^{a}	1.94 ± 0.23^{a}	0.35 ± 0.06^{c}
	36	Control	36.0 ± 6.24^{b}	16.1 ± 1.16^{a}	9.40 ± 0.19^{a}	1.71 ± 0.15^{a}	18.1 ± 1.73^{b}
	36	UV-B	34.7 ± 4.78^{b}	14.6 ± 1.27^{a}	8.91 ± 0.20^{a}	1.64 ± 0.10^{a}	31.2 ± 3.20^{a}
NMF 'Babygold 7'	0	t_0	28.3 ± 3.95^{a}	8.58 ± 0.16^{a}	9.01 ± 1.37^{a}	0.95 ± 0.10^{b}	93.0 ± 12.0^{a}
	36	Control	31.7 ± 4.81^{a}	11.5 ± 1.93^{a}	8.26 ± 0.52^{a}	1.39 ± 0.15^{ab}	108 ± 14.0^{a}
	36	UV-B	28.1 ± 2.90^{a}	13.3 ± 1.21^{a}	7.79 ± 0.61^{a}	1.71 ± 0.11^{a}	157 ± 37.5^{a}

Values are the mean of five replicates ± SE.
Values followed by different letters indicate, within each genotype, significant differences between Control and UV-B-treated fruit according to one-way ANOVA followed by Tukey's test ($P \leq 0.05$).

did not change after post-harvest conservation or following UV-B treatment in any cultivar.

MF fruit exhibited the highest TA at t_0, whereas lower and essentially similar values were observed in SM and NMF fruits. In any cultivar, TA remained unchanged after 36 h under both control and UV-B treatment conditions, with the exception of MF fruit, where it decreased significantly after UV-B exposure (−39% compared to control).

The SSC/TA ratio of SM 'Big Top' at t_0 was about two-fold higher that of the other two cultivars. Following 36 h UV-B exposure this parameter significantly increased in MF 'Suncrest' fruit (+70% in comparison to control).

Ethylene production at t_0 was very high in NMF, lower in MF, and barely detectable in SM fruit. After 36 h, MF and, even more, SM control fruit showed a dramatic increase (about +200% and +5000%, respectively, compared to t_0) in ethylene production. Ethylene production was significantly induced by UV-B treatment in MF (+51%) and to an even greater extent (+72%) in SM fruit, as compared to the respective controls. In NMF fruit, after 36 h, ethylene production appeared essentially unchanged in controls and showed a not-significant increasing trend in UV-B treated samples.

Exo-polygalacturonase, endo-1,4-β-D-glucanase/β-D-glucosidase, β-galactosidase and pectin methylesterase activities

To elucidate whether UV-B exposure could affect peach cell-wall metabolism, the *in vitro* activities of a few enzymes whose role in fruit cell-wall degradation is widely acknowledged (i.e. Exo-PG, EGase, β-Gal, PME)[17] were studied. At t_0, Exo-PG activity was different in the three cultivars, being the lowest in SM 'Big Top' and the highest in NMF 'Babygold 7'. Exo-PG activity increased after 36 h in control MF and SM fruit, whereas it remained unchanged in NMF after 36 h in both control and UV-B-treated fruit. The UV-B treatment induced significant increases in MF and SM activity (+31% and +37%, respectively, compared to control conditions; Fig. 1A). EGase activity was the highest at t_0 in MF 'Suncrest' fruit and much lower in SM and NMF. After 36 h EGase activity was found to increase in control MF fruit, while UV-B treatment significantly increased EGase activity levels only in SM (+43% compared to control fruit; Fig. 1B).

β-Gal activity at t_0 was lower in MF and SM compared to NMF. The enzyme activity did not significantly change after 36 h in control samples, nor was it affected by the UV-B treatment (Fig. 1C). In addition, no effect of UV-B treatment on PME activity of the three varieties was observed (Fig. 1D).

Endo-PG and expansins proteins

The anti-PpEndo-PG antibodies reacted with a polypeptide of about 45 kDa (PpEndo-PG; Fig. 2A), consistent with the molecular mass reported for catalytically active PG forms.[51] At t_0, PpEndo-PG was not detectable in any of the three cultivars, independently of flesh firmness (ranging from 57 N in MF and SM to 28 N in NMF; Table 2). After 36 h, PpEndo-PG was more abundant in the MF (11 N flesh firmness) than in the SM (36 N flesh firmness) control fruits. In both MF and SM the levels of PpEndo-PG were slightly increased by the UV-B treatment. In NMF fruit, PpEndo-PG remained undetectable after the 36 h of post-harvest in both control and UV-B-treated fruits (Fig. 2A and C).

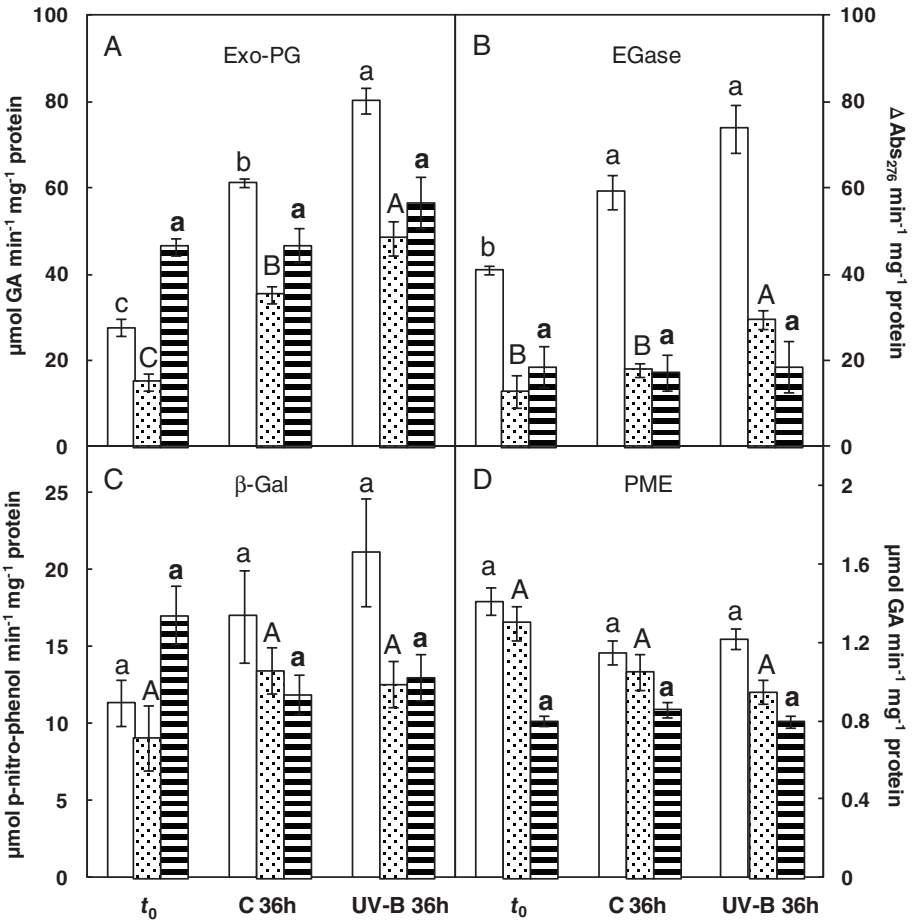

Figure 1. Enzyme activity of: (A) exo-polygalacturonase (Exo-PG), (B) endo-1,4-β-D-glucanase/β-D-glucosidase (EGase), (C) β-galactosidase (β-Gal) and (D) pectin methylesterase (PME) in the mesocarp of MF 'Suncrest' (empty bars), SM 'Big Top' (dotted bars) and NMF 'Babygold 7' (striped bars) fruits at t_0 and after 36 h of post-harvest in the absence (C 36 h) or in the presence (UV-B 36 h) of UV-B treatment. Data represent the mean of three replicates ± SE. Different letters above the bars indicate significant differences within each genotype (lower case 'Suncrest', upper case 'Big Top', bold 'Babygold 7') according to one-way ANOVA followed by Tukey's test ($P \leq 0.05$).

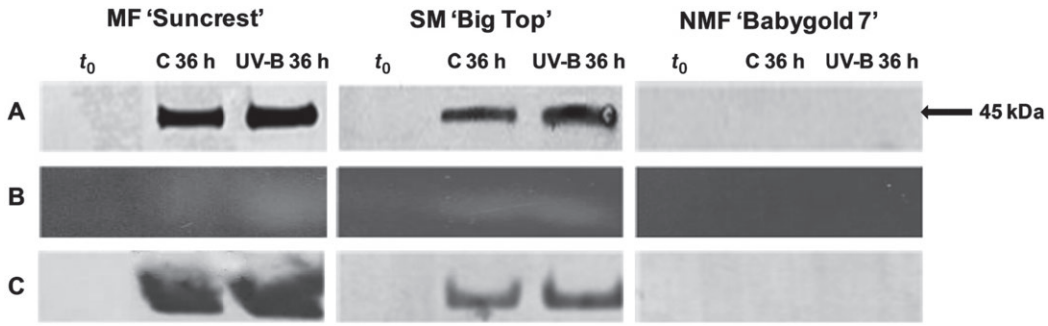

Figure 2. Levels of (A) PpEndo-PG polypeptide, (B) Endo-PG activity and (C) PpEndo-PG protein in MF 'Suncrest', SM 'Big Top' and NMF 'Babygold 7' fruit mesocarp at t_0 and after 36 h of post-harvest in the absence (C 36 h) or in the presence (UV-B 36 h) of UV-B treatment. Loading: 2 µg protein per lane for SDS-PAGE experiments, 15 µg per lane for native-PAGE experiments. The results of one experiment, representative of three, are shown.

To ascertain whether the PpEndo-PG protein level was associated with Endo-PG activity, in gel enzyme activity was monitored. At t_0 Endo-PG activity was not detectable in any cultivar. In MF 'Suncrest' gel discoloration ascribable to Endo-PG activity became apparent after 36 h in control conditions and was slightly enhanced by the UV-B treatment. A similar although less pronounced behaviour was observed in SM 'Big Top' fruit while Endo-PG activity was never detectable in NMF (Fig. 2B).

The anti-LeExp1 antibodies immunoreacted with a polypeptide band of about 27 kDa, consistent with the molecular mass reported for peach expansins.[44,45] At t_0, the expansin signal was absent or barely detectable, but after 36 h it became clearly visible in control fruit of MF, as well as, to a lesser extent, of SM. UV-B treatment lowered expansins levels in MF fruit and slightly increased them in SM, compared to the controls. In NMF, no expansins could be detected both in the absence and in the presence of UV-B treatment (Fig. 3).

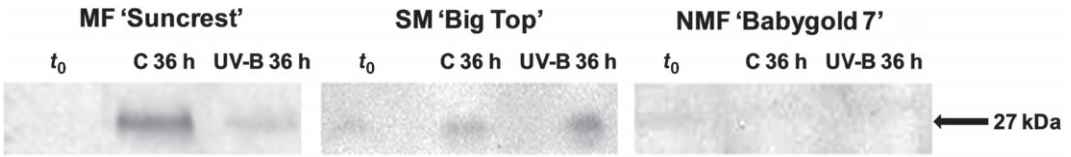

Figure 3. Levels of PpExp polypeptides in MF 'Suncrest', SM 'Big Top' and NMF 'Babygold 7' fruit mesocarp at t_0 and after 36 h of post-harvest in the absence (C 36 h) or in the presence (UV-B 36 h) of UV-B treatment. Loading: 5 µg protein per lane. The results of one experiment, representative of three, are shown.

Expression levels of a *PpEndo-PG* gene and of *PpExp* genes

In MF and SM the *PpEndo-PG* transcripts were low at t_0 and increased after 36 h in control fruit, to a greater extent in MF than in SM (about nine-fold and two-fold, respectively). In neither of the two cultivars did UV-B treatment significantly affect the transcripts levels. In NMF fruit, *PpEndo-PG* transcripts could not be detected with the primers used, at any time and in any condition.

In peach, three *expansin* genes (*PpExp1*, *PpExp2*, *PpExp3*) have been identified and described.[52] The relative expression levels of *PpExp1* and *PpExp2* remained constant after 36 h in MF and SM control fruits, whereas they significantly increased in NMF. After the UV-B treatment, *PpExp1* and *PpExp2* were significantly less transcribed than in the corresponding control, particularly in NMF. The relative expression levels of *PpExp3*, i.e. the gene proposed to be mostly involved in peach softening,[52] increased significantly during the post-harvest period in control fruit of all three cultivars. The 36-h UV-B treatment blocked the post-harvest-related increase in *PpExp3* transcript levels in MF and inhibited it in SM. In NMF fruit the *PpExp3* expression levels were dramatically lower in UV-B treated fruits than in the corresponding control, and even lower than at t_0 (Table 3).

DISCUSSION

SSC and TA are important determinants of fruit quality, affecting the consumer's perception of sweetness and sourness and the produce marketability.[53,54] Since a consumer's acceptance for peach appears to be more sensitive to the SSC/TA ratio than to the absolute SSC values,[55] the higher SSC/TA ratios induced by the UV-B treatment in MF fruit suggests the possibility of using this radiation to improve fruit quality. The observed effects of UV-B treatment seem to act in an opposite direction to UV-C rays, which are reported to reduce the SSC/TA ratio in apple fruit.[56] The opposite effect induced by UV-B and UV-C radiations on SSC/TA ratio could be attributed to the different wavelengths and, consequently, energy of these two radiations. However, since this behaviour was observed in two distinct species, i.e. apple and peach, a genotype effect cannot be excluded as well. In this context, it should be noted that in our experiment UV-B treatment affected the SSC/TA ratio only in one of the three peach cultivars, the MF 'Suncrest'.

Flesh firmness is an indicator of the ripening stage and a reliable predictor of fruit behaviour during the shelf life, being related to fruit susceptibility to mechanical damage and microorganism spoilage.[57] Flesh firmness ranges are given in the literature to define the requirements for peach harvest ('ready to buy' fruit, 26.5–35.3 N) and retail marketability ('ready to eat' fruit, 8.8–13.2 N).[58] The European Union rules set the maximum firmness for commercial harvest of peaches at 63.7 N.[59] Fruit of MF and SM showed, at harvest, very high values of flesh firmness, close to the threshold set by the European Union. The quick decrease in this parameter observed in MF control fruit and the less rapid decrease detected in SM were consistent with the widely acknowledged behaviour of these fruit types.[20,60] Differently from MF, in NMF peach the 'melting' stage is absent.[42,61] Despite NMF fruit showed lower flesh firmness than MF at harvest, this parameter was maintained essentially constant after 36 h, consistent with evidence on the narrower changes in flesh firmness of NMF fruit compared to MF.[30,62] A similar behaviour was observed in the NMF cultivar Oro A.[63] Reports are available on the effects of UV-C radiation in contrasting flesh firmness decrease during shelf life in apple[64] and tomato;[12,13] in this last species the effect was accompanied by the inhibition of the synthesis/expression of cell wall-degrading enzymes.[13] Conflicting results have been reported on the effects of UV-B on fruit firmness. In tomato cultivar Zhenfen 202, post-harvest UV-B treatment allowed the maintenance of a significantly higher flesh firmness,[11] whereas in the cultivar Money Maker and in the '*high pigment-1*' mutant softening was enhanced by UV-B treatment.[8] These opposite effects were probably due to different UV-B doses or modalities in addition to possible, still unknown, different genotype-related anatomical features (e.g. skin morphology and characteristics) and/or biochemical/physiological mechanisms. In the present study, however, the UV-B treatment conditions were homogeneous for all the three peach cultivars, suggesting that the different UV-B effects (significant inhibition of flesh softening in MF fruit and no effect in SM and NMF ones) might be linked to a genotype-dependent response.

Ethylene is involved in the trigger and regulation of the ripening process in climacteric fruits, including peach, as well as in the plant response to several stress signals.[65] According to literature, NMF fruit evolved higher amounts of ethylene than MF and even more than SM.[20,29,66,67] UV-B radiation, in the conditions adopted in the present study, generally induced enhanced ethylene production, probably acting as a stress factor. This hormone has been suggested to be involved in the signalling pathway of UV-B, which induced enhanced ethylene production in green tissues of oat, tobacco, tomato, pear and *Arabidopsis thaliana*.[68] Consistently, in tomato fruits grown under UV-B deprivation, a marked decrease in ethylene production has been described.[69]

Dismantling of the cell-wall architecture, due to changes in expression/activity of cell-wall localised enzymes acting on specific (mainly polysaccharide) components of this structure, is an important determinant of texture changes during ripening of fleshy fruits[26,51,65] and is often controlled by ethylene.[70] Polygalacturonases and pectin methylesterase are considered as the primary degrading enzymes involved in the softening process. Their action is accompanied by other hydrolytic enzymes such as EGase and β-Gal.[13,17] It is widely accepted that PGs (exo- and endo-acting) play a key role in peach flesh softening, their different presence/activity determining the MF/SM/NMF fruit phenotype.[18–20,42]

Our results concerning PG activity appear somewhat conflicting with this widely accepted knowledge and, as discussed in detail afterwards, an involvement of expansins in explaining this behaviour is hypothesised. In UV-B-irradiated MF fruit,

Table 3. Changes in the expression levels of a *PpEndo-PG* gene and of three *PpExp* genes in MF 'Suncrest', SM 'Big Top' and NMF 'Babygold 7' fruits at t_0 and after 36 h in the absence (Control) or in the presence (UV-B) of UV-B treatment, as determined by qRT-PCR

			Relative gene expression ($2^{-\Delta\Delta Ct}$)			
Genotype	Time (h)	Treatment	*PpEndo-PG*	*PpExp1*	*PpExp2*	*PpExp3*
MF 'Suncrest'	0	t_0	1.08 ± 0.02^{b}	1.42 ± 0.02^{a}	1.26 ± 0.02^{a}	1.14 ± 0.02^{b}
	36	Control	11.6 ± 0.13^{a}	1.48 ± 0.02^{a}	1.26 ± 0.02^{a}	1.96 ± 0.05^{a}
	36	UV-B	11.2 ± 0.10^{a}	1.12 ± 0.01^{b}	1.02 ± 0.01^{b}	1.13 ± 0.02^{b}
SM 'Big Top'	0	t_0	1.50 ± 0.09^{b}	3.96 ± 0.22^{a}	5.21 ± 0.31^{a}	1.32 ± 0.03^{c}
	36	Control	3.54 ± 0.10^{a}	3.62 ± 0.05^{a}	5.50 ± 0.08^{a}	3.03 ± 0.06^{a}
	36	UV-B	3.45 ± 0.17^{a}	1.10 ± 0.03^{b}	1.74 ± 0.10^{b}	1.96 ± 0.02^{b}
NMF 'Babygold 7'	0	t_0	ND	3.58 ± 0.06^{b}	3.12 ± 0.10^{b}	5.87 ± 0.24^{b}
	36	Control	ND	6.34 ± 0.13^{a}	6.99 ± 0.13^{a}	9.64 ± 0.22^{a}
	36	UV-B	ND	1.17 ± 0.04^{c}	1.29 ± 0.05^{c}	1.46 ± 0.09^{c}

Values are the mean of three replicates ± SE.
Values followed by different letters indicate, within each genotype, significant differences between Control and UV-B-treated fruit according to one-way ANOVA followed by Tukey's test ($P \leq 0.05$).
ND, not detected.

the significantly diminished loss of firmness is accompanied by increased activity of both Exo-PG and Endo-PG and substantial invariance of EGase, β-Gal and PME. A slight discrepancy between PG activity and changes in flesh firmness was detectable also in SM fruit, where lack of UV-B effect on firmness was accompanied by increased activities of Exo-PG and, even if to a slight extent, Endo-PG. It is interesting to note that NMF fruit showed the highest Exo-PG activity at harvest, consistent with data of the literature.[71,72] In control conditions, the changes in Endo-PG activity in MF and SM fruits after 36 h of post-harvest were accompanied by changes in the levels of a PpEndo-PG protein and the corresponding *PpEndo-PG* gene transcripts.

In NMF fruit, the absence of the PpEndo-PG protein was accompanied by the complete lack of expression of *PpEndo-PG* gene, consistent with literature reports.[42,73] A different behaviour has been instead reported for another NMF peach cultivar, Oro A, where the accumulation of *PpEndo-PG* transcripts accompanied by very low protein levels suggested transcriptional regulation of PpEndo-PG synthesis.[19] The UV-B treatment had different effects on PpEndo-PG levels and activities, which increased in both MF and SM fruits, and *PpEndo-PG* gene expression, which was unaltered, compared to the related controls. The stability of *PpEndo-PG* mRNAs seems therefore unaffected by UV-B, whereas it may be speculated that the higher protein levels can be due to lower protein degradation under UV-B radiation by presently unknown mechanism(s).

Expansins are also involved in the ripening-associated fruit softening, being detected in several ripe fruits concomitant with the expression of the related *expansin* genes. In particular, *LeExp1* gene transcription increases during tomato ripening;[74] peach *PpExp1* and *PpExp2* are constitutively expressed in post-harvest in both MF and stony hard (SH) fruit, this latter being a very firm and crispy flesh type that never melts,[30,70] whereas the expression of *PpExp3* appears involved in the regulation of fruit softening.[52] Consistently, in the present work, *PpExp1* and *PpExp2* gene expression remained unchanged in control MF fruit after 36 h, whereas *PpExp3* transcript levels increased significantly, in parallel to the appearance of expansin protein and increased flesh softening. A similar trend was observed in SM fruit.

In NMF control fruit, the increased expression of all *PpExp* genes after 36 h, accompanied by the absence of the related proteins, is consistent with observations in NMF cultivar Oro A,[75] suggesting that post-transcriptional mechanisms may be involved in the regulation of expansin levels. Further studies are necessary, however, to clarify this point.

In the ripening process, *expansin* genes expression and protein accumulation are differently sensitive to ethylene, depending on the non-climacteric or climacteric nature of the fruit. In strawberry, a non-climacteric fruit, the α-*expansin* gene *FaExp2* is insensitive to ethylene, while in tomato (climacteric) the expression of the α-*expansin* gene *LeExp1* is up-regulated by endogenous and exogenous ethylene,[76] and in SH peach, which does not spontaneously produce ethylene,[20] expression of *PpExp3* occurs only upon ethylene treatment.[52] In the present work, although ethylene production was enhanced upon UV-B treatment, *PpExp* gene expression was generally inhibited. This result, together with the generally observed decrease of *PpExp* transcripts in UV-B-treated compared to freshly harvested fruit, may be tentatively explained by hypothesising a specific inhibitory effect, through so far unknown mechanism(s), of UV-B not only on gene transcription but also on transcript stability. The latter hypothesis may also explain the observed discrepancy between *PpExp* genes expression levels and presence of PpExp protein, particularly evident in NMF fruit.

On the basis of the acknowledged involvement of *PpExp3* in fruit softening, its diminished expression, paralleled by the effect on related protein, may at least partially account for the decrease in fruit softening observed in UV-B-treated MF fruit. This is consistent with results obtained in tomato, where diminished expression of a ripening-regulated expansin, *LeExp1*, reduces fruit softening,[21] and suppression of *LeExp1* increases fruit firmness.[21,37]

Expansins were detected in peach already at 70 N flesh firmness (Autumn Red cultivar).[45] Hayama *et al.*[77] reported the presence of expansins at about 45 N (SH 'Yumyeong') and 30 N (MF 'Akatsuki'). With the same anti-LeExp1 antibodies used by these authors, we could not detect expansins in MF at 57 N, but observed a slight immunoreaction signal in SM at the same flesh firmness. We hypothesise that this result is due to cultivar-related specificities in the regulation of expansin synthesis, possibly involving post-transcriptional events, as suggested by the described discrepancy between *PpExp* transcriptional activity and expansin levels.

CONCLUSIONS

Taken as a whole, our results seem to indicate, at least preliminarily, that post-harvest treatments with UV-B radiation may represent a promising non-chemical tool to improve the shelf life of peach commodities, with particular regard to MF fruit, by slowing down flesh softening. A slighter effect on the organoleptic properties of the flesh cannot be excluded, as suggested by increase in the SSC/TA ratio.

Further investigation is needed to ascertain whether our results, observed in a single MF cultivar, can be generalised to other cultivars with the same flesh texture properties.

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