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Effect of High Solar Radiation Stress on Endogenous Polyamine Content in Six Apple Cultivars (*Malus domestica* Borkh)

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ABSTRACT

Polyamines are chemical polycations that play a crucial role in plants for their response to stress conditions. This study aimed to quantify the levels of free polyamines (putrescine, spermidine, and spermine) and to examine their relationship with oxidative metabolism in the skin of apple fruits (*Malus domestica* Borkh) that exhibited symptoms of high solar radiation stress. The study focused on fruit from the cultivars 'Gala,' 'Red Delicious,' 'Fuji,' 'Cripp's Pink,' 'Golden Delicious,' and 'Granny Smith,' comparing fruit with mildly sunburned skin (Sb-1) to those with healthy skin (Sb-0), all collected at commercial harvest time. The results indicated that high solar radiation stress led to an increase in polyamine content in Sb-1 fruit skin, with cultivar-specific variations. Notably, only the Sb-1 fruit of the 'Red Delicious' cultivar showed an increase in putrescine. Spermidine levels increased in Sb-1 across all cultivars except 'Golden Delicious,' while spermine levels significantly increased in the 'Fuji,' 'Cripp's Pink,' and 'Granny Smith' cultivars. Despite the observed increases in polyamine content, phenolic content, and antioxidant capacity in Sb-1, these changes did not prevent increased lipid peroxidation, alterations in maturity indices, or the manifestation of sunburn symptoms in the affected tissue. These findings contribute to a deeper understanding of the role of polyamines in the response to high solar radiation stress, particularly in apple tissues.

Introduction

Polyamines (PAs) are low molecular weight aliphatic compounds with polycationic characteristics, present across various organisms, including plants. They play a crucial role in regulating numerous physiological processes associated with growth, development, and responses to both abiotic and biotic stresses. In plant cells, the most abundant PAs include 1,4-diaminobutane, commonly known as putrescine (PUT), which serves as a precursor for the triamine 1,8-diamino-4-ozaoctane, or spermidine (SPD), and the tetramine 1,12-diamino-4,9-diazadodecane, or spermine (SPM), along with its structural isomer thermospermine (Pal et al., 2015).

The primary biosynthetic pathway of PAs begins with the decarboxylation of ornithine to produce PUT. An alternative pathway, unique to plants,

starts with the decarboxylation of arginine via arginine decarboxylase (ADC). From PUT, SPD and SPM are synthesized through the addition of aminopropyl groups, a reaction facilitated by the enzymes spermidine synthase (SPDS) and spermine synthase (SPMS). These aminopropyl groups are derived from methionine, which is first converted to S-adenosylmethionine (SAM) and subsequently decarboxylated in a reaction catalyzed by S-adenosylmethionine decarboxylase (SAMDC) (Thomas et al., 2020).

PAs can exist in both soluble and insoluble forms. Soluble PAs may be free or covalently conjugated to low molecular weight compounds such as phenols, whereas insoluble forms are often covalently bound to lipids, proteins, or cell wall polysaccharides (Alcazar et al., 2020). Numerous studies have demonstrated that PAs accumulate under various abiotic stress conditions, including drought, salinity, extreme temperatures, UV radiation, heavy metals, and ozone exposure (Chen et al., 2019; Jadhav et al., 2019). PAs are critical in facilitating plant adaptation to stress through complex interactions with other growth regulators and signaling molecules. The pathways that regulate PA levels share common substrates with other molecules involved in stress responses, such as nitric oxide, H_2O_2, γ-aminobutyric acid, ethylene, and proline. Consequently, it is challenging to disentangle the specific contributions of PAs from those of other molecules in the context of stress responses (Napieraj et al., 2023).

Putrescine (PUT), spermidine (SPD), and spermine (SPM) have been identified as key polyamines (PAs) involved in the development of various apple plant organs (Huo et al., 2020; Shelp et al., 2018). However, their roles under environmental stress conditions within apple cultivation remain largely unexplored. High solar radiation and elevated temperatures are known to cause sun damage, or sunburn, to apple fruit. Apple production areas are often characterized by intense radiation, low cloud cover, high temperatures, and significant evaporation, all of which contribute to a heightened risk of sun damage (Racskó and Schrader, 2012). Apple cultivars exhibit varying degrees of susceptibility to sunburn, potentially due to genotypic, physiological, morphological, and metabolic differences, particularly in their antioxidant capacities. For example, studies have shown that 'Granny Smith' and 'Jonagold' are highly susceptible to sunburn, while cultivars such as 'Fuji,' 'Golden Delicious,' 'Braeburn,' 'Boskoop,' and 'Red Delicious' exhibit moderate susceptibility. In contrast, 'Cripps Pink,' 'Idared,' and 'Topaz' are less susceptible (Morales-Quintana et al., 2020).

Sunburn in apple fruit is associated with chlorophyll degradation in the epidermis, disruption of cell compartmentalization, and an imbalance between the intracellular antioxidant system and the production of reactive oxygen species (ROS). Depending on the severity, sunburn can manifest as sub-lethal or mild, resulting in yellow or brown spots on the fruit skin. Increased intensity or prolonged exposure to sunlight can lead to necrosis of the epidermal tissue. When high solar radiation is combined with elevated temperatures, the plant's energy dissipation mechanisms can be overwhelmed, leading to enhanced ROS production and, consequently, oxidative stress (Munné-Bosch and Vincent, 2019). ROS can induce lipid peroxidation, protein denaturation, and DNA mutations. To counteract these harmful effects, plants have evolved a wide array of antioxidant compounds, which are closely linked to their ability to withstand such stress conditions (Calzadilla et al., 2014).

The interaction between PAs, ROS, and antioxidants represents one of the most complex physiological and biochemical mechanisms in plants, particularly under stress conditions (Minocha et al., 2014; Pottosin et al., 2014). However, information on PA metabolism in apple plants, and its relationship with abiotic stress conditions and ROS, is limited. For instance, studies on apple seedlings exposed to UV-C radiation reported an increase in PA levels (Kondo et al., 2011). Under salt stress, the apple ADC gene, MdADC, was isolated, and its expression was shown to be upregulated. Nevertheless, the role of ADC activity, MdADC expression, and its product PUT in the salt stress response of apple, a perennial crop, remains unclear (Liu et al., 2006).

Currently, there is no information on the relationship between oxidative stress caused by high solar radiation and PA content in apple fruit skin. It is plausible that high solar radiation and the resulting oxidative stress may trigger a metabolic response in the apple skin, leading to increased PA levels, similar to other abiotic stress conditions. Therefore, the aim of the present study was to determine the variations in free PA content and their relationship with oxidative metabolism in the skin of six apple cultivars exhibiting sunburn symptoms.

Materials and Methods

Plant materials

Apple (*Malus domestica* Borkh) fruits from the following cultivars were used: 'Gala' (GL), 'Red Delicious' (RD), 'Fuji' (FJ), and 'Cripp's Pink' (CP), with red or bicolored skin, and 'Golden Delicious' (GD) and 'Granny Smith' (GS), with yellow or green skin. The fruits were collected from a conventional orchard in the Upper Valley of Rio Negro, Patagonia, Argentina (39°01'S, 67°44'W; 244 masl) during the commercial harvest of the 2019/2020 growing season. Each cultivar was harvested at the official commercial harvest time: GL at 127 d after full flowering (DAFB), RD at 146 DAFB, FJ at 177 DAFB, CP at 193 DAFB, GD at 145 DAFB, and GS at 172 DAFB. The ripening stages suitable for commencing the official commercial harvest for the six cultivars are presented in Table 1.

Table 1. Recommended ripening stages for the onset of the official commercial harvesting season for the six cultivars in the Upper Rio Negro Valley, Patagonia, Argentina.

Cultivar	Day after full bloom (DAFB)	Firmness (N)	Total soluble solids (°Brix)	Tritatable acidity (%)	Starch index (Scale 1-6)
GL	120	76,5 – 83,3	11 - 12	0,35 – 0,50	2-3
RD	139	73,5 – 83,3	≥ 10	0,30 – 0,40	≥ 1
FJ	170	63,7 – 68,6	12 – 14	0,40 - 0,50	2-3
CP	186	73,5 – 83,3	13 - 15	0,70 – 0,75	2-3
GD	138	63,7 – 73,5	≥ 11	0,50 – 0,70	≥ 2
GS	165	63,7 – 73,5	≥ 10	0,80 – 0,85	≥ 2

GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. Adapted from (Benítez et al., 2005).

For each cultivar, 50 fruits were selected with visible signs of mild sunburn on the fruit skin (Sb-1), according to a classification by Shrader et al. (2008). This treatment group had a yellow or slightly discolored area, with no brown or necrotic area (Fig. 1). The treatment group opposite to Sb-1 had fruits with visually healthy tissues (Sb-0). Physicochemical and biochemical determinations were carried out on Sb-0 and Sb-1 separately.

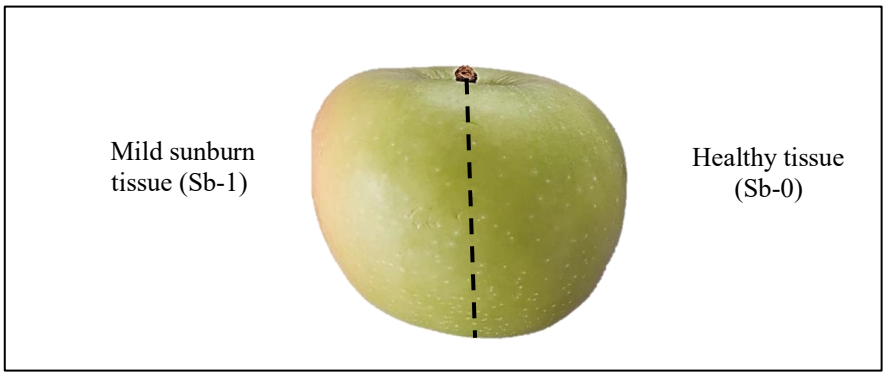

Fig. 1. A typical apple fruit with sunburn grade 1 on the exposed side (Sb-1) and no sunburn on the unexposed side (Sb-0).

Maturity indices

Maturity indices were independently assessed for 20 fruits in both Sb-0 and Sb-1 groups. Flesh firmness was measured using an Effegi penetrometer (FT 327, Alfonsine, Italy) with an 11 mm tip, and the results were expressed in Newtons (N). Middle slices of the apples were treated with Lugol's solution (0.33% iodine + 0.66% potassium iodide) to calculate the starch index, which was rated on a scale from 1 to 6, representing the least-to-most-ripe. Total soluble solids and titratable acidity were measured in the juice extracted from the pulp tissue immediately beneath the peel, excluding the peel itself. Total soluble solids were determined using a hand-held refractometer, with results expressed in degrees Brix (°Brix). Titratable acidity was measured by titration to an endpoint of pH 8.1 using 0.1 N NaOH, and the results were reported as a

percentage of malic acid equivalent (Mitcham et al., 1996).

Biochemical analysis

For each cultivar, six apple fruits were sampled. Skin samples (1 mm thick) corresponded to the Sb-0 and Sb-1 areas, which were collected separately.

Total chlorophyll

The chlorophyll content of the fruit skin was determined through extraction with dimethyl sulfoxide (DMSO) and quantified using spectrophotometry, following the method of Wellburn (1994). Three skin discs, each with a diameter of 10 mm, were sampled using a punch. The discs were weighed and placed in a test tube containing 3 mL of DMSO. The samples were then incubated in an oven at 65 °C for 2 h. After cooling for 15 min, the absorbance was measured at 665.1 nm for chlorophyll a and 649.1 nm for chlorophyll b using a UV-Vis DU-80 Beckman Coulter spectrophotometer (Germany). The results were expressed as µg of total chlorophyll g^{-1} FW.

Malondialdehyde content (MDA)

MDA content in apple skin were assessed by measuring thiobarbituric acid reactive substances (TBARs) through the 2-thiobarbituric acid (TBA) reaction (Spera et al., 2023b). Data was expressed as nmol MDA g^{-1} FW.

Methanolic extract profiling

A skin sample of 0.35 g was homogenised with 2 mL methanol/HCl 32% v/v-distilled water (159/1/40, v/v/v). The mixture was shaken at 150 rpm for 2 h at room temperature and centrifuged at 15,000 g for 20 min. The supernatant was collected and used for the DPPH free radical scavenging assay and the determination of total phenolic content.

DPPH-free radical scavenging assay

The antioxidant capacity (free radical scavenging activity) was assessed using the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) assay method, with minor modifications based on the procedure of Brand-Williams et al. (1995). A DPPH solution was prepared by dissolving 4.1 mg of DPPH in 100 mL of methanol, achieving an absorbance of 1.1 at 515 nm. For the assay, 40 µL of the metabolic extracts were mixed with 120 µL of distilled water, and 20 µL of this mixture was then combined with 780 µL of methanolic DPPH to create the test sample. The blank sample consisted of 20 µL of double-distilled water mixed with 780 µL of methanolic DPPH. Both the test and blank samples were incubated at 4 ºC for 30 min in the dark, followed by absorbance measurements at 515 nm. The radical scavenging activity of the test samples, expressed as a percentage inhibition of DPPH, was calculated using the following formula:

$$DPPH\ percentage\ inhibition = [\frac{(A_A - A_B)}{A_A}]\ x\ 100$$

where A_A and A_B represent the absorbance values of the blank and test samples, respectively.

Total phenolic content

The total polyphenol content was determined following the protocol of Emmons et al. (1999), with modifications. The methanolic extract was diluted 1:3 with double-distilled water. Ten microliters of the diluted sample were mixed with 780 µL of double-distilled water and 50 µL of Folin-Ciocalteu reagent (Anedra) and allowed to stand in the dark for 8 min. Subsequently, 150 µL of 20% w/v sodium carbonate solution (Anedra) was added, and the reaction mixture was incubated in the dark at room temperature for 2 h. The absorbance was then measured at 760 nm. Each sample was assayed in duplicate. A calibration curve was established using various concentrations of anhydrous gallic acid (5 g L^{-1}, Biopack). The total polyphenol content was expressed as mg of gallic acid per 100 g of fresh tissue.

Free polyamines determination

Free polyamines (PAs) were extracted from 0.25 g of fresh peel tissue using 0.2 N perchloric acid and derivatized with dansyl chloride. The samples were analyzed via high-performance liquid chromatography (HPLC) coupled with fluorescence detection, following the method of Campestre et al. (2011). PA separation was performed using an HPLC system (Waters 1525) equipped with Luna C18 (2) reverse-phase columns (Phenomenex) and detected with a spectrofluorometer (Waters 2475) at excitation and emission wavelengths of 415 nm and 510 nm, respectively. The solvent flow rate was set at 1.5 mL min^{-1} with the following elution gradient: 0-4.5 min with an acetonitrile-water solution (70:30), 4.5-9 min with pure acetonitrile, and 9-15 min with an acetonitrile-water solution (70:30). Peak areas were integrated and normalized to heptanediamine, and the results were interpolated using a polyamine standards

calibration curve. The free polyamine content was expressed in nmol g^{-1} FW.

Statistical analysis

Data analysis was performed by InfoStat software v. 2018 (Di Rienzo et al., 2018). Data were analyzed by one-way ANOVA and were delineated by several statistical indications: NS no significance, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Cultivar groupings were made according to PA contents as obtained from cluster analysis while corresponding to a dendrogram using the average chaining method and Euclidean distance. Cophenetic correlation functioned as a guide to select the most appropriate grouping method.

Results

Maturity indices

Table 2 presents variations in maturity indices between the sunburned surface area (Sb-1) and the healthy surface area (Sb-0) on fruits of the six apple cultivars. Flesh firmness was significantly higher in the Sb-1 area compared to the Sb-0 area across all cultivars. Specifically, the Sb-1 area in GL and CP exhibited an increase of 15.5 N compared to Sb-0, while GS showed a difference of 13.4 N. For GD, FJ, and RD, the differences were 7.2 N, 6.6 N, and 5.3 N, respectively. Soluble solids content also increased in the Sb-1 area relative to Sb-0 in most cultivars, with the exception of GL and RD, where the increase was not statistically significant. Titratable acidity did not differ significantly between the damage levels across the cultivars. Starch degradation was higher in the Sb-1 area than in Sb-0 only for CP. Regardless of the damage level, starch degradation indices were approximately 6 in GL, RD, CP, and GD, indicating a more advanced maturity stage compared to GS and FJ, which had starch degradation values close to 2 and 3.5, respectively.

Oxidative metabolism

The total chlorophyll content in healthy fruit skin varied among the cultivars studied, influencing the changes caused by high solar radiation (Fig. 2). In the red-skinned cultivars GL and FJ, significant increases were observed in the chlorophyll content (61 and 70%, respectively) in the Sb-1 tissue compared to the Sb-0 tissue. However, RD and CP did not show statistically significant differences in chlorophyll content across the different sunburn levels. In contrast to the red and/or bicolor pigmented cultivars, the Sb-1 tissue in GD and GS had significantly lower total chlorophyll content compared to the Sb-0 tissue, with decreases of 54% in GS and 74% in GD.

Table 2. Maturity indices of fruits with sunburn symptoms side (Sb-1), and healthy side (Sb-0).

Cultivar	Sunburn degree	Firmness (N)	Total soluble solids (° Brix)	Tritatable acidity (%)	Starch index (1-6)
GL	Sb-0	79.0 ± 4.56	13.2 ± 0.36	0.36 ± 0.02	5.30 ± 0.16
	Sb-1	94.6 ± 4.67	14.4 ± 0.48	0.34 ± 0.03	5.70 ± 0.15
		*	*NS*	*NS*	*NS*
RD	Sb-0	64.6 ± 1.51	14.3 ± 0.51	0.27 ± 0.02	4.60 ± 0.60
	Sb-1	69.9 ± 1.69	15.4 ± 0.70	0.26 ± 0.01	5.40 ± 0.40
		*	*NS*	*NS*	*NS*
FJ	Sb-0	69.2 ± 1.33	13.2 ± 0.09	0.36 ± 0.01	3.17 ± 0.31
	Sb-1	75.8 ± 1.33	13.8 ± 0.17	0.33 ± 0.01	3.67 ± 0.33
		**	*	*NS*	*NS*
CP	Sb-0	82.2 ± 1.14	13.9 ± 0.22	0.77 ± 0.07	4.76 ± 0.23
	Sb-1	97.7 ± 2.43	16.0 ± 0.21	0.77 ± 0.05	5.52 ± 0.21
		***	***	*NS*	*
GD	Sb-0	68.8 ± 0.64	15.1 ± 0.49	0.57 ± 0.02	5.17 ± 0.22
	Sb-1	76.0 ± 1.98	16.5 ± 0.37	0.55 ± 0.02	5.83 ± 0.17
		**	*	*NS*	*NS*
GS	Sb-0	77.0 ± 1.29	10.6 ± 0.14	0.89 ± 0.01	1.80 ± 0.22
	Sb-1	90.4 ± 1.74	12.1 ± 0.22	0.81 ± 0.04	2.35 ± 0.21
		***	***	*NS*	*NS*

GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

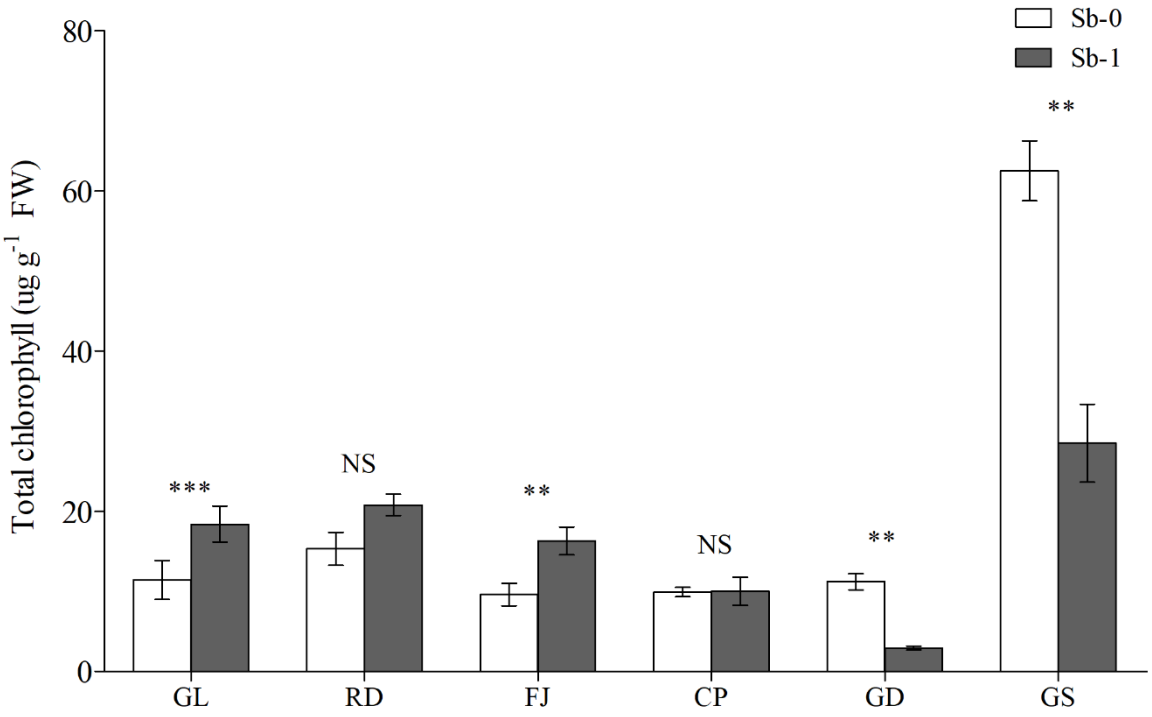

Fig. 2. Total chlorophyll content in sunburned apple peel (Sb-1), regarding healthy peel (Sb-0). GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. Data are mean values ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Sunburned tissues increased membrane lipid peroxidation in most cultivars, except CP (Fig. 3). In GL, CP and GD, there were no statistically significant differences between Sb-0 and Sb-1. However, in RD, FJ and GS, membrane lipid peroxidation in Sb-1 was significantly higher than in Sb-0 by 52, 57, and 36%, respectively.

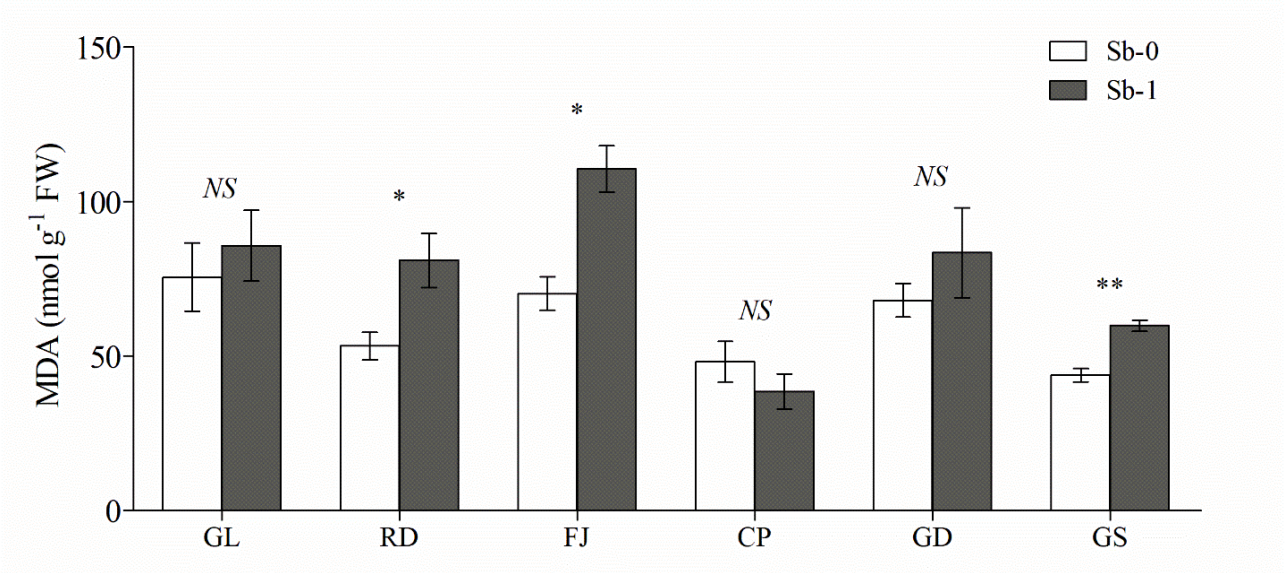

Fig. 3. MDA content in sunburned apple peel (Sb-1), regarding healthy peel (Sb-0). GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The antioxidant capacity-DPPH significantly increased in Sb-1 compared to Sb-0 in all cultivars (Fig. 4). The GL cultivar had the highest antioxidant capacity in Sb-0 (46.7%) and Sb-1 (63.4%). On the contrary, GD had the lowest value regarding this parameter in Sb-0 (16.0%), although it had a higher value in Sb-1 (28.2%). The RD cultivar showed the highest increase (113%) in antioxidant capacity.

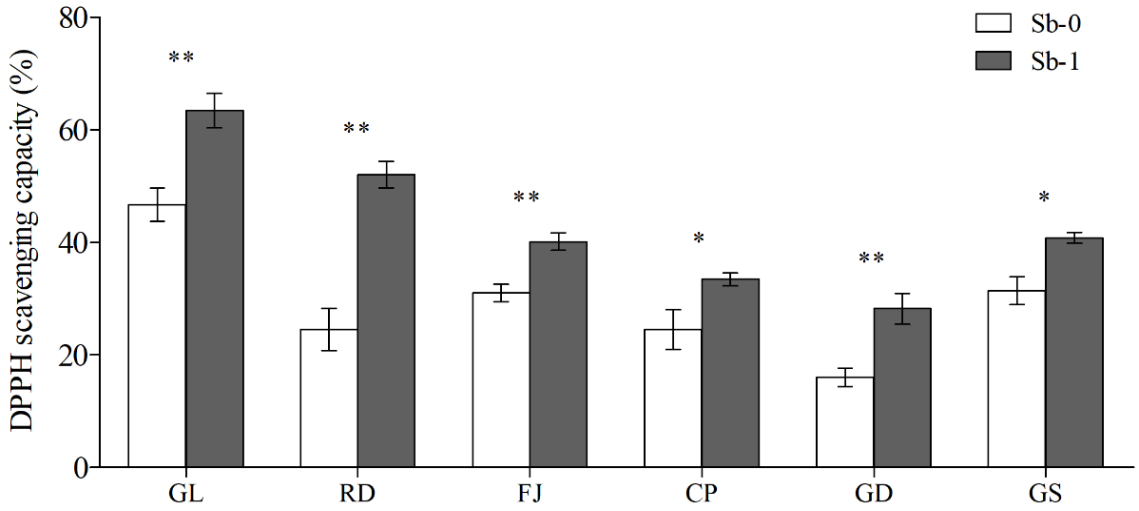

Fig. 4. Antioxidant capacity-DPPH in sunburned apple peel (Sb-1), regarding healthy peel (Sb-0). GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

All cultivars showed significant increases in total polyphenol content in Sb-1 compared to Sb-0. The GD and CP cultivars had the lowest increases (87.9 and 106.4%, respectively) (Fig. 5). On the other hand, the total polyphenol content in the Sb-1 of GS increased by 174.7% and in RD by 156.7%, while GL and FJ showed increases of 137.7% and 121.3%, respectively.

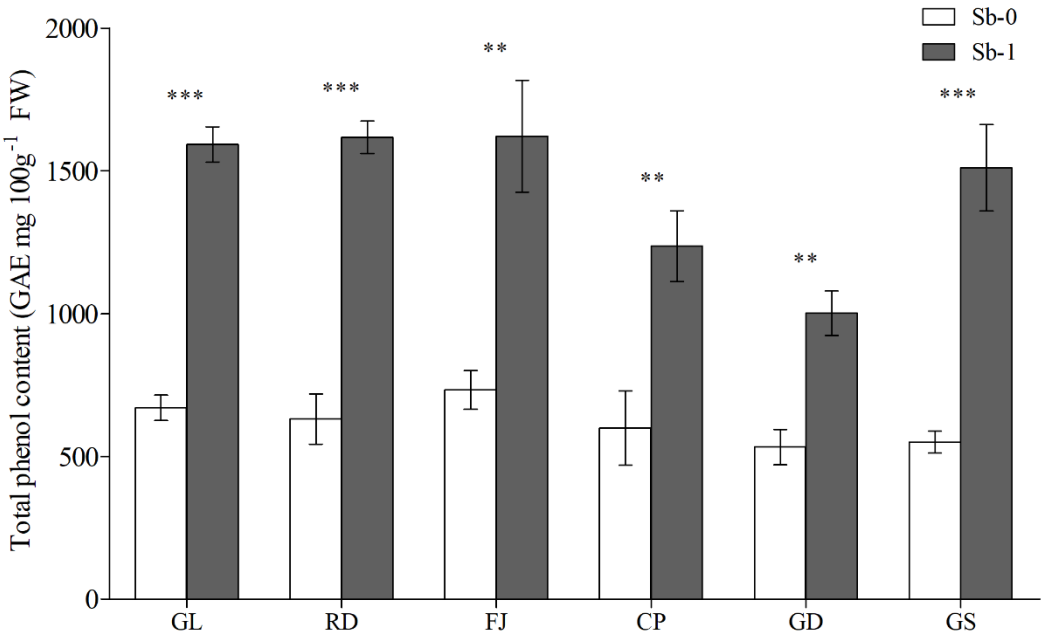

Fig. 5. Total phenol content in sunburned apple peel (Sb-1), regarding healthy peel (Sb-0). GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Free polyamines content

In all cultivars, an increase in total PAs was observed in sunburned tissues compared to the healthy tissues. The differences were statistically significant in cultivars RD, CP, and GS (Fig. 6). In RD, this increase in PAs of the Sb-0 surface area was 189.2 nmol g^{-1} FP, compared to the Sb-1 surface area. In CP and GS cultivars, this increase was 103.6 nmol g^{-1} FP and 111.3 nmol g^{-1} FP, respectively.

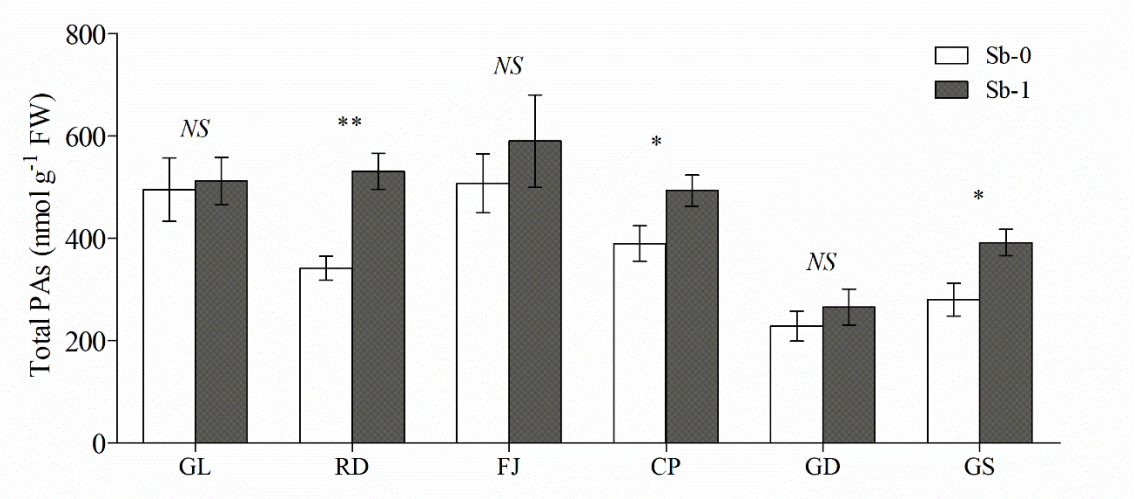

Fig. 6. Total polyamines content (PAs) in sunburned apple peel (Sb-1), compared to healthy peel (Sb-0). GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In the apple peels of the six cultivars, the PAs were PUT, SPD, and SPM. PUT was the most abundant polyamine (150-400 nmol g^{-1} FW), followed by SPD (50-160 nmol g^{-1} FW), and finally SPM (30-50 nmol g^{-1} FW), although their exact amounts depended on cultivar (Fig. 7).

No significant differences in PUT content were detected between Sb-0 and Sb-1, with the exception of the RD cultivar. In this case, a 70% increase of this metabolite was observed in tissues exposed to high solar radiation (Fig. 7A).

GL, RD, FJ, CP, and GS cultivars showed statistically significant changes in SPD concentrations in the Sb-1 surface area, with increases of 22, 39, 115, 64, and 89%, respectively, compared to the Sb-0 surface area. In GD, no significant differences occurred between the two treatment groups (Sb-0 and Sb-1) regarding the SPD concentration (Fig. 7B).

The Sb-1 surface area on the fruits of FJ, CP, and GS showed a significantly higher SPM content compared to Sb-0. However, no significant variations were observed between both tissues in GL, RD, and GD regarding the SPM content (Fig. 7C).

The ratio (SPD + SPM)/PUT (Table 3) showed statistically significant increases in the Sb-1 compared to the Sb-0 in FJ, which increased from 0.27 to 0.53, and in CP, which increased from 0.37 to 0.56.

Relative free polyamines cluster

Based on the relative contents of PUT, SPD, and SPM among the six cultivars, they were grouped using the hierarchical grouping method via average linkage. Clustering was performed independently on the Sb-0 tissues and the Sb-1 tissues (Fig. 8).

The relative content of each polyamine in the Sb-0 apple peel (Fig. 8A) allowed for the establishment of two major groups, the first consisting of RD, GS, and GD, and the second comprising FJ, GL, and CP. Groupings were made according to the results that described Sb-1 tissues (Fig. 8B), which showed modifications in group conformation compared to Sb-0 tissues. In this case, all red and/or bicolored cultivars joined in one group, whereas GD and GS were placed separately.

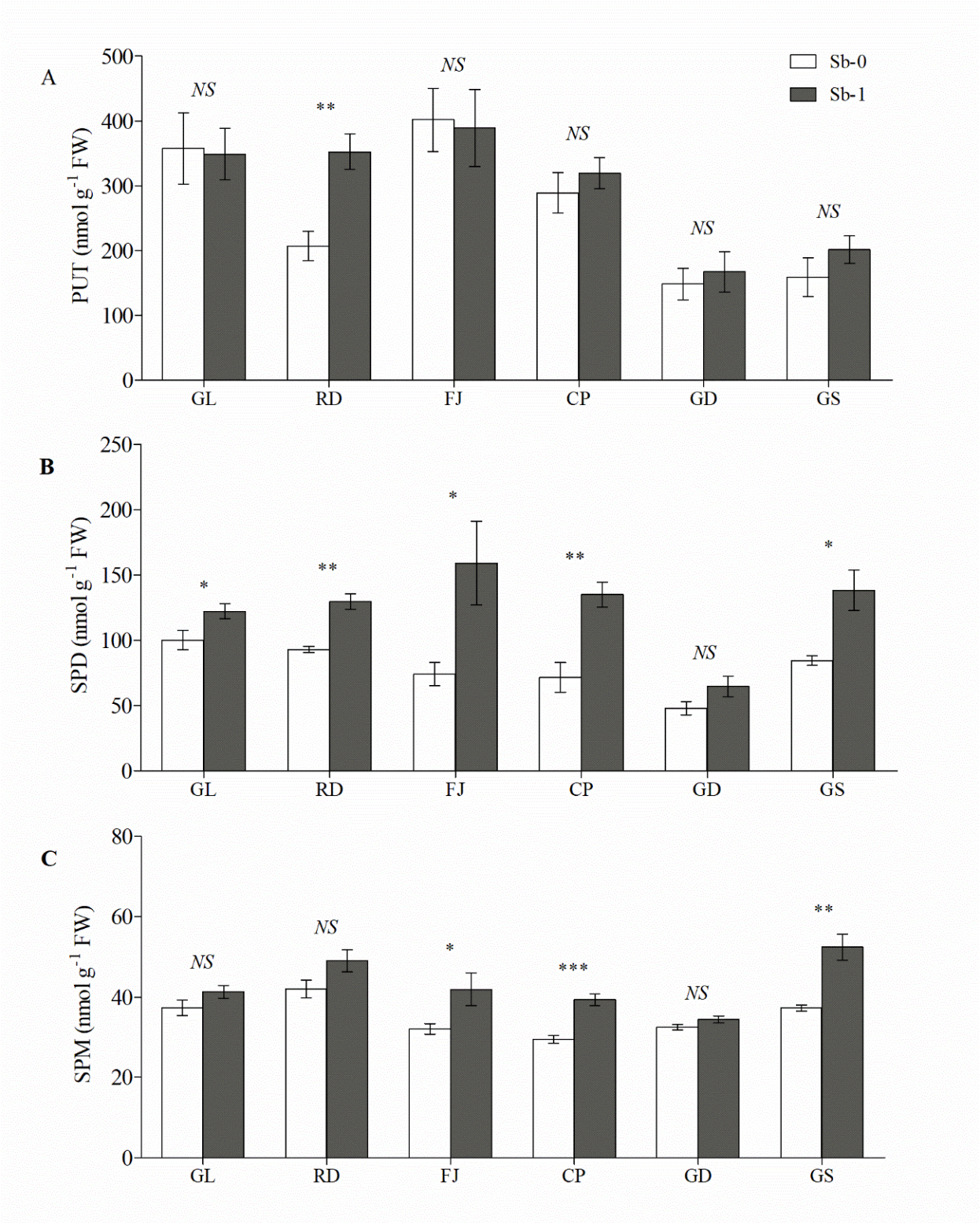

Fig. 7. Various polyamine contents in sunburned apple peel (Sb-1) and healthy peel (Sb-0). (A) Putrescine (PUT); (B) spermidine (SPD); (C) spermine (SPM). GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp's Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Polyamines ratios (SPD + SPM)/PUT in sunburn apple peel (Sb-1), regarding healthy peel (Sb-0).

Cultivar	Sunburn degree	Ratio (SPD + SPM)/PUT
GL	**Sb-0**	0.41 + 0.10
	Sb-1	0.49 + 0.09
		NS
RD	**Sb-0**	0.68 + 0.16
	Sb-1	0.51 + 0.04
		NS
FJ	**Sb-0**	0.27 + 0.04
	Sb-1	0.53 + 0.16
		**
CP	**Sb-0**	0.37 + 0.17
	Sb-1	0.56 + 0.09
		*
GD	**Sb-0**	0.58 + 0.13
	Sb-1	0.66 + 0.20
		NS
GS	**Sb-0**	0.82 + 0.22
	Sb-1	0.98 + 0,28
		NS

GL: 'Gala'; RD: 'Red Delicious'; FJ: 'Fuji'; CP: 'Cripp´s Pink'; GD: 'Golden Delicious'; GS: 'Granny Smith'; PUT: Putrescine; SPD: Spermidine; SPM: Spermine. The values presented are means ± SE (n = 6). NS, no significance level; *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

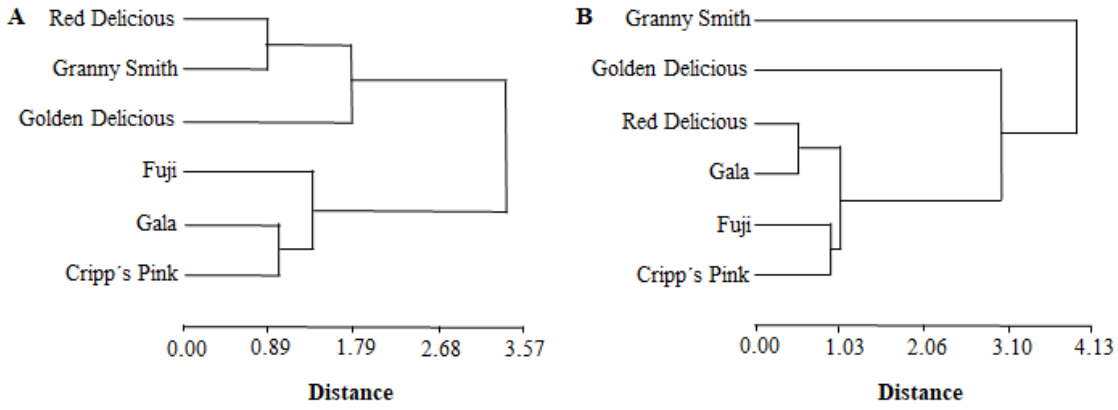

Fig. 8. Dendrogram of the cluster analysis for each apple cultivar according to the relative contents of putrescine, spermidine, and spermine using the complete linkage method and Euclidean distance. A: healthy apple peel (Sb-0), with a cophenetic correlation coefficient of 0.89, B: sunburned apple peel (Sb-1), with a cophenetic correlation coefficient of 0.97.

Discussion

Flesh firmness and total soluble solids content were the indices that most consistently reflected the impact of high solar radiation on apple fruit tissues (Table 2). The findings on flesh firmness align with previous studies on apples, which demonstrated that areas exposed to excessive solar radiation or with sunburn damage exhibited higher firmness values than healthy areas (Racskó and Schrader, 2012; Tartachnyk et al., 2012). In pome fruits, the increased flesh firmness on the sunburned side may result from a reduction in cell size, an increase in the number of cells per surface area, or a thickening of the cell wall due to alterations in cell wall structure and

composition (Gambetta et al., 2021; Racskó and Schrader, 2012; Spera et al., 2023a).

Total soluble solids content at harvest was significantly higher in the Sb-1 sector compared to Sb-0 across all six cultivars evaluated in this study (Table 2). Previous research has reported the accumulation of glucose and sorbitol in the sunburned skin of many apple cultivars (McTavish et al., 2020). The elevated sugar content in sun-exposed tissues is likely a consequence of reduced metabolism of these molecules under environmental stress. Sorbitol may also function as an antioxidant, enabling fruit to withstand environmental and oxidative stress caused by high temperature and radiation (Torres et al., 2013).

Sunburned apple fruit generally exhibit higher starch degradation compared to healthy fruit (Racskó and Schrader, 2012). Consistent with these observations, starch degradation in the present study was significantly higher in the Sb-1 sector than in Sb-0 for the GL, CP, and GS cultivars (Table 2). Variations in carbohydrate metabolism may be attributed to stress tolerance mechanisms that influence processes such as cell turgor, protection of cell membranes and proteins, and the energy required for the synthesis of protective metabolites and chemical messengers (Dong and Beckles, 2019).

In this study, no significant differences in titratable acidity were observed between the Sb-0 and Sb-1 areas in any of the cultivars studied (Table 2). Some previous studies have reported lower titratable acidity in apple fruit with mild sunburn (Racskó and Schrader, 2012). It is possible that the level of damage considered accounts for the discrepancies found in other studies, where more severe damage resulted in decreased titratable acidity. Additionally, other research has shown that the sun-exposed side of apple fruit exhibits no change in titratable acidity compared to the unexposed side at harvest (Racskó and Schrader, 2012).

The decrease in malic acid in fruit is typically associated with progressive ripening, as malic acid serves as a carbon source for various metabolic processes. Although high radiation stress generally promotes acid reduction in affected tissues, it is yet to be confirmed whether this reduction signifies accelerated ripening or, as suggested for sugars, an activation of stress-response metabolic pathways (McTavish et al., 2020). Recent studies have identified the enzyme malic NADP as a key factor in enhancing tolerance to abiotic stress. This enzyme catalyzes the oxidative decarboxylation of malate, thereby increasing levels of NADPH, which serves as a reductant for other reactions, is involved in the synthesis of defense compounds, and directly contributes to the metabolism of reactive oxygen species (ROS), reducing the damage caused by oxidative stress (Sun et al., 2019).

Traditionally, variations in maturity indices in sunburned tissues have been associated with advanced fruit ripening and changes in cell wall structure. However, recent research suggests that the activation of defense metabolism and the development of tissue tolerance to excess solar radiation may be responsible for these observed variations (Dong and Beckles, 2019; Sun et al., 2019). The effect of solar radiation on chlorophyll content in the skin varied according to the cultivar and its characteristic surface color. In GD and GS cultivars, a significant decrease in total chlorophyll content was observed in Sb-1 tissue compared to Sb-0 (Fig. 2). Most previous studies have reported a decrease in chlorophyll content in the skin of sunburned apple fruit (Hernández et al., 2014; Munné-Bosch and Vincent, 2019; Racskó and Schrader, 2012; Tartachnyk et al., 2012). Chlorophyll loss is a general symptom of oxidative stress induced by high solar radiation on the fruit surface, primarily due to its degradation by ROS attack. This degradation can also function as a photoprotection mechanism, reducing light absorption by chloroplasts (Munné-Bosch and Vincent, 2019).

The surface color of the cultivar may influence the impact of high solar radiation on photosynthetic pigments (Hengari et al., 2014). In this study, the RD and CP cultivars showed no variation in chlorophyll content, whereas the GL and FJ cultivars exhibited higher chlorophyll content in the Sb-1 tissue compared to Sb-0 (Fig. 2). Anthocyanins, pigments present in red and bicolored apple cultivars, play a protective role in shielding chloroplasts from chlorophyll degradation. These pigments accumulate in the vacuoles of epidermal and mesophyll cells, acting as efficient light attenuators that indirectly prevent photooxidative stress. Specifically, they serve as natural biochemical filters for ultraviolet and blue light wavelengths, making them effective photoprotective compounds (Munné-Bosch and Vincent, 2019). The higher chlorophyll content observed in the sunburned side of the red fruits in this study may help maintain sufficient photosynthesis levels when a smaller fraction of photosynthetically active radiation reaches the chloroplasts due to the screening effect of anthocyanins (Merzlyak et al., 2002). Additionally, the specific climatic conditions of different seasons can influence the chlorophyll and anthocyanin content of fruit skin, as well as the changes that occur when the fruit is exposed

to high solar radiation and temperature during development (Waite et al., 2023).

Sun damage in fruit triggers the overproduction of reactive oxygen species (ROS), leading to peroxidation of cell membranes, loss of selective permeability, and metabolic imbalance (Feng et al., 2022). In the RD, FJ, and GS cultivars, a statistically significant increase in malondialdehyde (MDA) concentration was observed in Sb-1 tissue compared to Sb-0 (Fig. 3). While the GL and GD cultivars showed a trend towards increased lipid peroxidation in the Sb-1 area, this increase was not statistically significant (Fig. 3). The rise in lipid peroxides is a direct consequence of oxidative stress, which affects fruits displaying sunburn symptoms (Colavita, 2022; Munné-Bosch and Vincent, 2019). The increase in lipid peroxidation in sunburned fruit skin indicates that the ROS processing system could not effectively counteract the photooxidative damage induced by high solar radiation and temperature, which affects various biomolecules, including membrane lipids (d'Alessandro et al., 2020).

The ROS processing system in fruits includes the cycle-dependent excess energy dissipation system of xanthophylls and other antioxidants such as carotenoids, tocopherols, ascorbate, glutathione, flavonoids, polyamines (PAs), and various enzymatic antioxidants (Jadhav et al., 2019). In this study, antioxidant-DPPH activity in healthy tissue varied among the different cultivars studied (Fig. 4). The DPPH antioxidant activity can differ across apple cultivars and fruit regions. High solar radiation significantly increased the antioxidant capacity in Sb-1 tissue in all cultivars (Fig. 4), with the highest increases observed in RD and GD. These findings align with previous studies on apple cultivars, where an increase in antioxidant DPPH levels was noted in fruit with mild sun damage (Racskó and Schrader, 2012).

Among the most significant antioxidant compounds in apples are polyphenols, which are found in concentrations up to three times higher in the peel than in the flesh (Drogoudi et al., 2008). Our results are consistent with reports indicating that polyphenol content varies between apple cultivars. Despite these variations, polyphenol levels consistently increased in Sb-1 tissues compared to Sb-0 (Fig. 5), suggesting an enhancement of antioxidant defenses against stress caused by high solar radiation in apple fruit (Olivares-Soto et al., 2020). Phenolic compounds are crucial secondary metabolites that play vital physiological roles throughout the plant life cycle, and their ability to scavenge ROS in plant tissues under various stress conditions has been well documented (Wang et al., 2015).

PUT, SPD, and SPM have been identified as the primary polyamines (PAs) present in various organs of apple plants (Huo et al., 2020). Consistent with these findings, the free PAs detected in the skin of the six apple cultivars analyzed in this study were PUT, SPD, and SPM (Fig. 7). In all cases, PUT (Fig. 7A) was the most abundant polyamine, followed by SPD (Fig. 7B) and SPM (Fig. 7C). Exposure to high solar radiation stress led to variations in the free PAs content of apple peel. The observed increase in free PAs content in Sb-1 tissues, particularly in the RD, CP, and GS cultivars (Fig. 6), aligns with studies conducted on other species under abiotic stress conditions, such as flooding, water deficit, salinity, extreme temperatures, and heavy metal exposure, where PAs accumulation has been documented. Our findings suggest that high solar radiation stress is another condition that promotes increased free PAs content in apple peel. Generally, an increase in PAs content is associated with enhanced tolerance to stressful conditions (Alcazar et al., 2020; Chen et al., 2019; Shelp et al., 2018; Thomas et al., 2020).

The protective role of PAs under stressful conditions is linked to their chemical structure. PAs are polycationic and protonated at physiological pH, which enables them to bind to biomolecules such as nucleic acids, proteins, phospholipids of cell membranes, and other anionic cellular components, thereby stabilizing their function (Setia and Setia, 2018). In chloroplasts, PAs protect the thylakoid membrane from the harmful effects of stress and help maintain photosynthetic function (Calzadilla et al., 2014). A significant body of research indicates that stress tolerance can be enhanced through the application of exogenous PAs or by genetically manipulating endogenous PA levels in transgenic plants. However, the precise molecular mechanisms underlying the protective effects of PAs against stress remain largely unknown. Recent advances in molecular and genetic techniques have provided evidence suggesting that PAs may act as signaling molecules. For instance, alterations in endogenous PA levels have been shown to trigger transcriptional changes similar to those activated by stress. Furthermore, several studies suggest a reciprocal relationship between PAs and abscisic acid (ABA) biosynthesis, where PAs stimulate ABA production and vice versa.

PAs have also been implicated in physiological responses such as stomatal opening, mediated by the production of H_2O_2 through PA oxidation, as well as interactions with nitric oxide (NO)

signaling. In this context, PAs may work synergistically with ROS and NO to promote ABA responses in protective cells. Additionally, a possible connection between PAs, calcium ions (Ca^{2+}), and stress responses has been proposed, suggesting that a coordinated protective mechanism involving PAs, Ca^{2+}, ABA, H_2O_2, and NO helps plants adapt to climatic stress (Alcázar et al., 2020).

Several studies have linked increased PA levels to greater tissue firmness in plants. Under stress conditions, not only is PA biosynthesis altered, but changes in their catabolism also occur. The degradation of PAs by enzymes such as diamine oxidase (DAO) and polyamine oxidase (PAO) produces H_2O_2, which plays a role in cell wall maturation and lignification during development (Cona et al., 2006; Angelini et al., 2010). In this study, the variations in PA content observed in Sb-1 tissues may indicate heightened activity of overall PA metabolism, including their catabolic enzymes, leading to increased H_2O_2 production. It is possible that the higher level of firmness found in Sb-1 tissue is partly due to greater lignification of the cell wall in this area of the fruit, facilitated by the effects of H_2O_2.

There is limited information on the relative levels of each polyamine detected in different apple cultivars. In this study, cluster analysis based on the proportions of PUT, SPD, and SPM in various tissues identified two distinct groups, starting with Sb-0 tissue (Fig. 8A). The first group, comprising RD, GD, and GS, is characterized by 50-65% PUT, 20-30% SPD, and less than 15% SPM. The second group, which includes GL, FJ, and CP, shows a higher relative PUT content (over 70%), with 15-20% SPD and less than 8% SPM.

Exposure to high solar radiation stress altered these groupings (Fig. 8B). A new cluster emerged, consisting of RD, GL, FJ, and CP, characterized by PUT concentrations of 65-70%, SPD levels between 25-30%, and SPM below 10%. The yellow-skinned (GD) and green-skinned (GS) cultivars were grouped separately, distinguished by a lower proportion of PUT (50-65%), and higher levels of SPD (25-35%) and SPM (more than 10%). This study reveals that the impact of high irradiance stress on polyamine levels varies among the cultivars examined.

Research in rice has shown that cultivars of the same species can differ in their sensitivity to stress and exhibit distinct patterns of PA variation under such conditions (Jadhav et al., 2019). In our study, RD was the only cultivar that significantly increased PUT content in Sb-1 compared to Sb-0 (Fig. 7A). Several types of plant stress can induce an increase in PUT, which is often attributed to the decarboxylation of arginine via the enzyme ADC. The accumulation of PUT under various stress conditions (e.g., salinity, drought, cold) in different species is primarily due to increased ADC activity, both at the transcriptional and enzymatic levels (Thomas et al., 2020).

All tested cultivars, except for GD, exhibited an increase in SPD content in Sb-1 tissue compared to Sb-0 (Fig. 7B). Plants that are tolerant to heat stress and UV-C radiation often respond by increasing their total SPD and SPM reserves (Shao et al., 2015). Although the exact mechanisms of PA action under stress conditions are not fully understood, evidence from both plant and animal systems suggests that PAs, in addition to their biophysical effects, interact with protein kinases and transcription factors. The differential effects of SPM on phosphatidylinositol 3-kinase and phosphatidylinositol 5-kinase suggest a potential role of PAs in phospholipid-based signaling pathways (Setia and Setia, 2018).

In this study, SPM content significantly increased in longer-cycle cultivars such as FJ, CP, and GS. Previous research (Maiale et al., 2004; Zapata et al., 2004) has suggested that extended exposure to stressful conditions may allow for the utilization of PUT in the biosynthesis of higher PAs in these cultivars. The differential increase of various PAs could explain the varied responses of cultivars to abiotic stress conditions, particularly to high solar radiation stress. The increase in the (SPD + SPM)/PUT ratio is often used as a critical indicator of stress tolerance (Zapata et al., 2004). In our study, cultivars GL, FJ, CP, GD, and GS showed an increase in the (SPD + SPM)/PUT ratio in Sb-1 tissues compared to Sb-0, though this increase was not statistically significant in all cases (Table 3). These findings suggest that the (SPD + SPM)/PUT ratio could serve as a useful metric for characterizing the response of different apple cultivars' skin to high solar radiation stress.

When the balance between ROS production and the ROS-processing system is disrupted, oxidative stress occurs, causing damage to plant DNA, proteins, and lipids (Jadhav et al., 2019). Polyamines (PAs) play a multifaceted role in mitigating oxidative stress in plants. On one hand, they enhance the activity of various antioxidant enzymes, thereby effectively regulating oxidative stress induced by environmental factors (Minocha et al., 2014). Additionally, PAs are involved in signal transduction processes (Pál et al., 2015).

In this study, high solar radiation was found to increase the antioxidant capacity in the Sb-1 tissues of all cultivars (Fig. 4), which could be partially attributed to the elevated levels of PAs

and phenolic compounds detected in these tissues. It is also possible that other antioxidant metabolites, not evaluated in this study, may have contributed to this increased capacity. Except for the GD cultivar, SPD and SPM were the PAs that significantly increased in Sb-1 compared to Sb-0 in the apple skin tissues examined (Figs. 7B and C).

The protective role of PAs against oxidative stress has been extensively studied, particularly focusing on SPM (Fariduddin et al., 2013). For example, studies on cucumber seedlings exposed to hypoxic stress with exogenous SPD application showed significant increases in both SPD and endogenous SPM. This was accompanied by enhanced antioxidant enzyme activity, improved ROS scavenging capacity, and reduced membrane lipid peroxidation, ultimately leading to greater tolerance to hypoxic stress (Chen et al., 2019). Similarly, in tomato plants genetically modified to overexpress SAMDC under high-temperature stress, SPD and SPM accumulation was promoted, alongside increased synthesis of antioxidant enzymes, which mitigated oxidative degradation of membrane lipids (Jadhav et al., 2019). Furthermore, overexpression of the ODC gene in tomato plants resulted in higher concentrations of PAs and elevated levels of antioxidant metabolites such as lycopene and ascorbic acid in the fruit (Serrano et al., 2016).

It is noteworthy that PAs can also contribute to ROS production, as their catabolism generates oxidants such as H2O2 (Minocha et al., 2014). This molecule can act as a signaling molecule, participating in signal transduction under stress conditions. There is ample evidence that PAs are involved in maintaining redox homeostasis in plant tissues through this dual role in managing oxidative stress (Chen et al., 2019).

However, in our study, the increased total antioxidant capacity and PA content, particularly in Sb-1 tissues, were insufficient to prevent symptoms of mild sunburn and elevated lipid peroxidation. This is evident in cultivars RD and GS, where significant increases in total PAs (Fig. 6) and antioxidant capacity (Fig. 4) were recorded. Nevertheless, MDA levels also rose (Fig. 3). Despite this, the higher PA content may have played a role in the antioxidant response, helping to protect the fruit during oxidative stress and preventing more severe sunburn symptoms.

Conclusions

High levels of solar radiation stress induced significant metabolic and physiological modifications in the skin of apple fruits, leading to the visual manifestations of sunburn symptoms. These alterations include increased levels of free polyamines, phenolic compounds, antioxidant capacity, as well as changes in maturity indices and photosynthetic pigments. The response to high solar radiation stress, particularly in terms of polyamine levels and diversity, varied among cultivars, suggesting that these responses were strongly influenced by cultivar-specific traits. This research demonstrated that while the accumulation of free polyamines in apple skin under high irradiation conditions does occur, it is insufficient to prevent oxidative stress, resulting in increased lipid peroxidation and the subsequent development of sunburn symptoms.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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