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Evidence for a Leaf-to-Fruit Signal Regulating the Antioxidant System in Tomato Fruits Under Stress: Involvement of ABA, Ascorbate Redox State, H_2O_2 and NO

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Abstract

In plants, environmental stresses trigger reactive oxygen species (ROS) overproduction, causing oxidative damage but also generating systemic signals that prime defense responses in distal organs. How such signals are transmitted from leaves to fruits is a key question for crop stress tolerance and quality. Here, tomato plants were subjected to mercury chloride (5 ppm $HgCl_2$) for 24 hours to induce a selective arrest of water flux and to evaluate oxidative parameters and antioxidant defense in leaves, fruit peduncles and fruits. Mercury treatment induced oxidative damage — evidenced by increased H2O2 and MDA accumulation — in leaves and fruit peduncles, while fruits remained unaffected. Nevertheless, antioxidant enzyme activities (SOD, CAT, APX, DHAR and MDHAR) and transcript levels of SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2 and SlMDHAR were upregulated in fruits, despite the absence of local oxidative stress, suggesting a systemic leaf-to-fruit signal priming antioxidant defenses. To test this hypothesis, detached tomato fruits were treated with four putative stress signals: abscisic acid (ABA), exogenous H_2O_2, nitric oxide (NO, via sodium nitroprusside, SNP) and varying ascorbate (AsA) redox states. Oxidative parameters (H_2O_2 and MDA), ascorbate levels, and antioxidant enzyme activities and transcript levels were monitored at 4, 8 and 24 h after treatment. The results indicate that ABA, AsA redox state, H_2O_2 and NO each modulate antioxidant enzyme activities and gene expression in tomato fruits. Notably, while ABA, H_2O_2 and AsA redox state generally induced antioxidant enzyme activities, NO acted primarily as a direct ROS scavenger, suppressing enzymatic induction, an effect reversed by the NO scavenger cPTIO. Frequent dissociation between activity and transcript levels indicates post-transcriptional regulation. These findings support a model in which ABA, H_2O_2 act as interconnected systemic signals co-ordinating antioxidant defense in fruits under leaf stress.

Keywords: Antioxidant Enzymes, Ascorbate, Stress Signalling, Oxidative Stress, Solanum Lycopersicum L

1. Introduction

Under a variety of environmental stresses, including drought, salinity, heavy metals, heat, cold, mineral deficiency, fungal infection and insect damage, the generation of reactive oxygen species (ROS) increases, leading to oxidative stress [1]. Elevated ROS, including singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2), can damage essential membrane lipids, proteins and nucleic acids [2]. To counteract this, plants possess a multi-layered antioxidant system comprising enzymatic components — superoxide dismutase (SOD), catalase (CAT) and enzymes of the ascorbate–glutathione cycle: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) — as well as non-enzymatic antioxidants including α-tocopherol, carotenoids, glutathione and ascorbate [3,4]. The ability to perceive stress stimuli, generate and transmit appropriate signals, and initiate appropriate responses is central to plant survival under adverse conditions [5,6]. ROS not only act as toxic by-products but also as key regulatory signals that connect stress perception with the activation of defense networks and the establishment of stress

resilience [7]. Among the key signalling molecules identified, abscisic acid (ABA), the redox state of ascorbate, H_2O_2 and nitric oxide (NO) have been shown to mediate antioxidant responses to both biotic and abiotic stresses [8-10].

Beyond their role in cellular damage, ROS function as ubiquitous signal molecules in higher plants. Produced by NADPH oxidase homologs (RBOHs) at the apoplast, ROS can propagate rapidly from cell to cell as a 'ROS wave', transmitting systemic signals from stressed local tissues to distal organs within minutes [7,11]. The leucine-rich-repeat receptor-like kinase HPCA1 (H_2-INDUCED Ca^{2+} INCREASES 1) has been identified as a central ROS receptor required for cell-to-cell propagation of such ROS waves and systemic acclimation to stress [12]. These systemic ROS signals initiate preemptive defense responses [9,13,14]. H_2O_2 directly regulates the expression of numerous defense-related genes, including those encoding antioxidant enzymes, modulators of ROS production, and signalling proteins such as kinases, phosphatases and transcription factors [14-17]. ROS signalling in higher plants is integrated with other signalling pathways, forming interconnected networks [18,19]. Among plant hormones positioned downstream of ROS signals, abscisic acid (ABA) is a key regulator of adaptive responses to environmental stresses and various developmental processes [20]. Stress-induced upregulation of antioxidant enzyme activity can involve both ABA-dependent and ABA-independent pathways [21]. A recent mechanistic insight revealed that the thiol peroxidase PRXIIB can directly sense H_2O_2 and interact with the ABA-related protein phosphatase ABI2, illustrating the tight molecular integration between ROS and ABA signalling at the protein level [22]. Moreover, ABA has been proposed to induce secondary signalling molecules — including H_2O_2 and NO — which in turn modulate antioxidant enzyme activities [8,9,23,24].

Antioxidants are not passive ROS scavengers; they function as key redox signalling compounds. Low-molecular-weight antioxidants such as ascorbate serve as information-rich redox buffers that interact with numerous cellular components and influence gene expression in response to biotic and abiotic stresses [25]. The ascorbate redox state (the ratio of reduced AsA to total ascorbate) determines the lifetime and specificity of H_2O_2 signals, suggesting that AsA redox buffering occupies a central position in stress signal transduction [26]. Furthermore, AsA redox state has been shown to control gene expression through epigenetic mechanisms, including DNA methylation and histone modifications, linking redox signalling to chromatin-level regulation of stress responses [27]. Nitric oxide (NO), a reactive nitrogen species, acts as a signal molecule mediating responses to both biotic and abiotic stresses [28,29]. Recent studies indicate that NO plays a protective role against oxidative stress by directly quenching ROS and by modulating the expression of defense-related genes [27,30]. At the molecular level, NO exerts many of its effects through S-nitrosylation of target proteins: notably, S-nitrosylation of RBOHD suppresses apoplastic ROS production while enhancing APX activity, thereby fine-tuning the balance between ROS-mediated signalling and oxidative damage [30,31].

In a previous study, we demonstrated that $HgCl_2$ treatment rapidly induces water deficit specifically in leaves and fruit peduncles, while fruits remain unaffected [32]. Despite this, fruit antioxidant defenses were upregulated, suggesting the existence of a leaf-generated systemic signal that alerts fruit cells to incoming stress. In the present work, we investigated the roles of ABA, ascorbate redox state, H_2O_2 and NO as putative carriers of this signal by applying them exogenously to detached tomato fruits and monitoring changes in oxidative parameters and antioxidant enzyme activities and gene expression at 4, 8 and 24 hours post-treatment.

2. Materials and Methods

2.1. Plant Material and Treatments

Tomato (*Solanum lycopersicum L., cv. 'Micro-tom'*) seeds were germinated in boxes filled with peat. At the emergence of the first true leaf, seedlings were transplanted into 4 L plastic containers filled with a peat–vermiculite mixture (1:1, v/v). Plants were grown in a growth chamber maintained at 60 ± 5% relative humidity, with day/night temperatures of 25/20°C and a 16/8 h light/dark photoperiod under 300 µmol m-2 s-1 fluorescent light. Plants were irrigated twice weekly with a nutrient solution containing: 8 µM $MnCl_2$, 0.5 µM $CuSO_4 \cdot 5H_2O$, 1.4 µM ZnSO4, 46 µM H3BO3, 0.25 µM $Na_2MoO_4 \cdot 2H_2O$, 4.1 mM KNO_3, 3.4 mM $Ca(NO_3)2 \cdot 4H_2O$, 0.9 mM K_2SO_4, 1 mM $MgSO_4 \cdot 7H_2O$ and 1.5 mM KH_2PO_4, with Fe-EDDHA chelate (0.6%) as the iron source [33]. On the remaining days, plants were watered with distilled water. Flowers were tagged at anthesis to determine fruit age. Plants were divided into two groups: control plants (C) irrigated daily with distilled water, and treated plants (S) irrigated with 5 ppm $HgCl_2$ per liter of growth substrate. Leaves and mature-green fruits were harvested from five plants per group at 24 h post-treatment, immediately frozen in liquid nitrogen, ground to a fine powder and stored at −80°C.

Mature-green fruits from additional control plants were harvested between 09:00 and 10:00 h. The pedicel of each fruit was inserted into Murashige and Skoog basal medium (Sigma, M5519) supplemented with 4% sucrose, ensuring good contact between the pedicel and the medium. Cultures were maintained under 300 µmol m^{-2} s^{-1} cool-white fluorescent light at 25°C. The following treatments were applied to the medium: H_2O_2 at 250, 500 or 1000 µM; ABA at 0.1 mM; SNP (nitric oxide donor) at 0.5 mM; SNP (0.5 mM) + cPTIO (1 mM) as a NO scavenger combination; or defined AsA redox states of 0.5 (50 µM AsA + 50 µM DHA), 0.75 (75 µM AsA + 25 µM DHA) or 1.0 (100 µM AsA, no DHA). Fruits were collected at 4, 8 and 24 h after treatment initiation, immediately frozen in liquid nitrogen, ground and stored at −80°C.

2.2. Leaf and Fruit Water Parameters

Pre-dawn leaf water potential (LΨw) was measured using a pressure chamber [34]. Fruit water potential (FΨw) was determined using a PSYPRO™ water potential system with C-52 chambers (Wescor Inc., Logan, UT, USA), and fruit osmotic potential (FΨO) was measured with a VAPRO vapour pressure osmometer (Wescor). Fresh weight (FW) was recorded immediately after harvest; dry weight (DW) was obtained after drying at 70°C for 72 h. Water

content was calculated as: WC (%) = [(FW − DW) / FW] × 100.

2.3. Determination of H_2O_2 and MDA Contents

H_2O_2 content was determined using the optimized potassium iodide colorimetric microplate assay described by Junglee *et al*. (2014) [35]. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) as a thiobarbituric acid (TBA) reactive substance according to Murshed *et al*. (2008a), with absorbance read at 532 nm (non-specific absorption at 600 nm subtracted) and the MDATBA complex quantified using an extinction coefficient of 155 mM^{-1} cm^{-1} [33].

2.4. Determination of Ascorbate and Dehydroascorbate Contents

Total ascorbate (AsA + DHA) and reduced AsA were measured according to Kampfenkel *et al*. (1995), adapted for microplates by Murshed *et al*. [33,36]. Briefly, frozen powder (0.5 g) was extracted in cold 6% (w/v) TCA and centrifuged at 16,000 × g at 4°C for 15 min. Total ascorbate was determined after DTT reduction followed by a ferric chloride/2,2-bipyridyl colorimetric reaction (absorbance at 525 nm); reduced AsA was measured in parallel without the reduction step. DHA was calculated as the difference between total ascorbate and AsA and quantified against an L-ascorbic acid standard curve.

2.5. Antioxidant Enzyme Assays

2.5.1. Protein Extraction

Proteins were extracted according to Murshed *et al.* (2008b) [37]. Frozen fruit powder (300 mg) was homogenized in 1 mL of 50 mM MES/KOH buffer (pH 6.0) containing 40 mM KCl, 2 mM $CaCl_2$ and 1 mM AsA. Extracts were centrifuged at 16,000 × g at 4°C for 15 min and supernatants were used immediately for enzyme assays. Protein concentration was determined by the Bradford method.

2.5.2. Enzyme Assays

All enzyme activities were measured in 200 µL kinetic reactions at 25°C using a microplate reader. APX, DHAR and MDHAR activities were measured as described by Murshed *et al*. (2008b) [37]. SOD activity was measured by the photochemical NBT reduction method adapted from Dhindsa et al. (1981), using a commercial SOD standard curve and 96-well microplate format; absorbance was read at 560 nm [38]. CAT activity was assayed according to Aebi (1984) in 50 mM phosphate buffer (pH 7.0) with 15 mM H_2O_2, by monitoring the decrease in absorbance at 240 nm (extinction coefficient: 43.6 M^{-1} cm^{-1}) [58,59].

2.6. RNA Extraction, cDNA Synthesis and Quantitative RT-PCR

Total RNA was isolated from 100 mg frozen fruit powder using Tri Reagent (MRC, Molecular Research Center) according to the manufacturer's instructions [60]. RNA was quantified spectrophotometrically and integrity was verified on 1% agarose gels; only samples with A260/A280 ratios between 1.8–2.0 and A260/A230 ratios ≥ 2.0, and showing intact 28S/18S rRNA bands on gel, were used for downstream analysis. First-strand cDNA was synthesized from 4 µg total RNA using the MasterScript™ RT-PCR System (5 PRIME), with initial template denaturation at 65°C for 5 min, reverse transcription at 42°C for 60 min using oligo-d(T) primers, and inactivation at 85°C for 5 min. Quantitative PCR was performed using RealMasterMix SYBR ROX (5 PRIME) on a Mastercycler ep Realplex (Eppendorf). PCR efficiency was validated for each primer pair using a five-point serial dilution of cDNA, and only primers with efficiencies between 90–110% were used [61,62]. Relative transcript abundance of target genes was normalized to *SlActin* expression. Specific primer pairs for *SlActin, SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2* and *SlMDHAR* were designed from GenBank sequences using DNAMAN software (Table 1). Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method [39,63]. Three independent RNA extractions were performed per treatment, each analyzed in duplicate. Amplicons were purified (QIAquick PCR Purification Kit, QIAGEN), sequenced (Genome Express) to confirm specificity, and deposited in GenBank (accession numbers in Table 1).

PCR fragment	Encoded protein	Primer sequence (5' → 3')	Size (bp)	Accession number
SlAPXt	Thylakoid-bound ascorbate peroxidase	Sense : TTCACCCAATGACTTCCCT Antisense: TATCATTTAGTCCCATTCTGT	699	FJ532352
SlAPXcyto	Cytosolic ascorbate peroxidase	Sense : GTTGAAGGTCGCTTGCCG Antisense: CCAAGGTATGGGCACCAG	118	FJ532353
SLDHAR1	Dehydroascorbate reductase	Sense : TGCCTCTGTGGGCTCGAA Antisense: ACCACCCTGCGATGACGT	335	FJ532354
SlDHAR2	Dehydroascorbate reductase	Sense : ACAACTCCTAACAAGCTCGG Antisense: GTCCAAGCGACAAATCAGCA	430	FJ532355
SlMDHAR	Monodehydroascorbate reductase	Sense : GGAGAAGTTTCGTTGCTGCT Antisense: TGAGCAGCTTTCCTGAATTGT	371	FJ544908
SlActin	Actin	Sense : ATGACTCAAATCATGTTTGAG Antisense: TACCTTAATCTTCATGCTGCT	633	FJ532351

Table 1: Primer sequences used for quantitative RT-PCR analysis of antioxidant-related genes in tomato (Solanum lycopersicum L.) mature green fruits. For each gene, the encoded protein, sense and antisense primer sequences (5′ → 3′), amplicon size (bp) and GenBank accession number are given. SlActin was used as the reference gene for normalization of transcript abundance.

2.7. Statistical Analysis

All experiments were conducted with three independent biological replicates, and results are expressed as means ± standard error (SE). Statistical significance was assessed by Kruskal-Wallis followed by post-hoc Dunn's test (Benferroni's correction method) at $p < 0.05$, using the R statistical software [40,64]. For the detached-fruit experiment, each biological replicate consisted of fruits pooled from five independent plants per treatment.

3. Results

3.1. Effects of Mercury Treatment

Mercury treatment induced a significant decline in pre-dawn leaf water potential (LΨw), indicating a rapid reduction in plant water status [65]. By contrast, fruit water potential (FΨw), fruit osmotic potential (FΨO) and the water content of fruits (FWC), leaves (LWC) and fruit peduncles (PWC) were unaffected (Table 2), confirming that mercury-induced water deficit was restricted to the leaf level, consistent with selective inhibition of aquaporin-mediated water flux [41,66,67]. Notably, fruit osmotic potential (FΨO; -0.77 ± 0.03 MPa) was also unchanged relative to controls (-0.72 ± 0.01 MPa), further demonstrating full preservation of the internal osmotic status of the fruit [68-70]. Furthermore, control fruits showed a remarkably high basal AsA redox state (0.89 ± 0.03), well above that of leaves (0.53 ± 0.03) and fruit peduncles (0.34 ± 0.02) (Table 4), indicating that fruits maintain a strongly reduced ascorbate pool under normal conditions that may contribute to their inherent resistance to oxidative stress. Oxidative stress markers were clearly induced in leaves and fruit peduncles but not in fruits. H_2O_2 content increased significantly in leaves and fruit peduncles of mercury-treated plants but remained unchanged in fruits; MDA content followed the same pattern, increasing in fruit peduncles while remaining unaffected in leaves and fruits (Table 3). AsA concentration and AsA redox state both increased in leaves and fruit peduncles — reflecting activation of antioxidant responses — but were unaltered in fruits [71,72]. DHA showed a reciprocal pattern: decreasing in leaves and increasing in fruit peduncles, consistent with enhanced DHA recycling under oxidative stress, while remaining unchanged in fruits (Table 4).

Strikingly, despite the complete absence of oxidative stress in fruits, the activities of all antioxidant enzymes measured — SOD, CAT, DHAR and MDHAR — and the transcript levels of *SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2 and SlMDHAR* were significantly increased by mercury treatment (Table 5) [73,74]. The magnitude of these inductions was remarkable for an organ with no detectable local oxidative stress: CAT activity increased 4.3-fold, MDHAR activity 5.3-fold, and transcript levels of *SlAPXcyto and SlMDHAR* reached 4.5- and 4.8-fold above controls, respectively. APX activity alone remained unchanged, possibly reflecting a different regulatory threshold [75]. The upregulation of fruit antioxidant defenses in the absence of any detectable local oxidative stress strongly suggests that a systemic signal originating from stressed leaves reached the fruits and primed their antioxidant machinery [76-80].

	C	S
$L\Psi_w$	- 0.34 ± 0.02 [a]	- 0.63 ± 0.03 [b]
$F\Psi_w$	- 0.45 ± 0.04 [a]	- 0.48 ± 0.10 [a]
$F\Psi_O$	- 0.72 ± 0.01 [a]	- 0.77 ± 0.03 [a]
FWC	94 ± 0.5 [a]	94 ± 0.5 [a]
LWC	91 ± 0.4 [a]	92 ± 0.6 [a]
PWC	86 ± 5.0 [a]	86 ± 0.8 [a]

Table 2: Pre-dawn leaf water potential (LΨw; MPa), fruit water potential (FΨw; MPa), fruit osmotic potential (FΨO; MPa) and water content of fruits (FWC; %), leaves (LWC; %) and fruit peduncles (PWC; %) in control plants (C) and plants treated with 5 ppm HgCl2 for 24 h. Values are means ± SE of five independent biological replicates. Different letters within rows indicate significant differences (Kruskal-Wallis test, $p < 0.05$).

	Leaves		Fruit peduncles		Fruits	
	H_2O_2	MDA	H_2O_2	MDA	H_2O_2	MDA
C	849.36 ± 17.64 [a]	34.04 ± 0.19 [a]	361.19 ± 31.26 [a]	7.87 ± 0.19 [a]	26.77 ± 1.24 [a]	14.74 ± 5.24 [a]
S	1081.70 ± 107.62 [b]	33.36 ± 0.53 [a]	985.18 ± 69.89 [b]	15.82 ± 0.53 [b]	24.41 ± 1.06 [a]	15.13 ± 6.62 [a]

Table 3: Hydrogen peroxide (H2O2; nmol g−1 FW) and malondialdehyde (MDA; nmol g−1 FW) contents in leaves, fruit peduncles and fruits of control plants (C) and plants treated with 5 ppm HgCl2 for 24 h. Values are means ± SE of five independent biological replicates. Different letters within columns indicate significant differences (Kruskal-Wallis test, p < 0.05).

	Leaves			Fruit peduncles			Fruits		
	AsA	DHA	Redox state	AsA	DHA	Redox state	AsA	DHA	Redox state
C	27.50 ± 1.65 [a]	24.41 ± 1.13 [a]	0.53 ± 0.03 [a]	7.10 ± 0.41 [a]	13.78 ± 0.52 [a]	0.34 ± 0.02 [a]	21.07 ± 1.33 [a]	2.63 ± 0.85 [a]	0.89 ± 0.03 [a]
S	41.60 ± 1.51 [b]	13.96 ± 1.22 [b]	0.75 ± 0.03 [b]	12.27 ± 1.03 [b]	18.29 ± 1.12 [b]	0.43 ± 0.03 [b]	20.40 ± 1.76 [a]	2.61± 0.39 [a]	0.89 ± 0.03 [a]

Table 4: Ascorbate (AsA) and dehydroascorbate (DHA) concentrations (nmol g−1 FW) and ascorbate redox state (AsA / [AsA + DHA]) in leaves, fruit peduncles and fruits of control plants (C) and plants treated with 5 ppm $HgCl_2$ for 24 h. Values are means ± SE of five independent biological replicates. Different letters within columns indicate significant differences (Kruskal-Wallis test, p < 0.05).

	SOD	CAT	APX	*SlAPXcyto*	*SlAPXt*	DHAR	*SlDHAR1*	*SlDHAR2*	MDHAR	*SlMDHAR*
C	1,00 ± 0.19 [a]	1,00 ± 0.18 [a]	1,00 ± 0.20 [a]	1,00 ± 0.18 [a]	1,00 ± 0.19 [a]	1,00 ± 0.17 [a]	1,00 ± 0.15 [a]	1,00 ± 0.18 [a]	1,00 ± 0.19 [a]	1,00 ± 0.20 [a]
S	1,72 ± 0.21 [b]	4,33 ± 0.52 [b]	1,21 ± 0.16 [a]	4,47 ± 0.49 [b]	1,79 ± 0.22 [b]	1,72 ± 0.18 [b]	3,17 ± 0.40 [b]	2,73 ± 0.33 [b]	5,31 ± 0.62 [b]	4,75± 0.51 [b]

Table 5: Activities of antioxidant enzymes (SOD, CAT, APX, DHAR and MDHAR) and relative transcript levels of genes encoding ascorbate–glutathione cycle enzymes (*SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2 and SlMDHAR*) in fruits of control plants (C) and plants treated with 5 ppm HgC_{12} for 24 h. All values are expressed relative to the mean value of control fruits (set to 1). Transcript levels were normalized to *SlActin* expression and quantified by the $2^{-\Delta\Delta Ct}$ method. Values are means ± SE of six replicates (three independent RNA extractions, each analyzed in duplicate). Different letters within columns indicate significant differences (Kruskal-Wallis test, p < 0.05).

		4h		8h		24h	
		H_2O_2	MDA	H_2O_2	MDA	H_2O_2	MDA
ABA	C	26.44 ± 1.23	10.1 ± 1.2 [a]	20.96 ± 1.02	12.6 ± 1.3 [a]	16.1 ± 1.05 [a]	10.96 ± 1.03
	Treated	30.8 ± 1.15 [b]	10.1 ± 1.0 [a]	30.95 ± 2.03	12.8 ± 1.2 [a]	13.5 ± 1.0 [b]	5.44 ± 0.5 [b]
ARS	C	26.44 ± 1.23	12.03 ± 1.43	20.81 ± 1.14	8.98 ± 0.63 [a]	16.30 ± 1.45	10.36 ± 0.85
	0.5	25.48 ± 1.70	11.48 ± 1.12	21.21 ± 1.23	8.09 ± 0.84 [a]	18.32 ± 1.62	9.65 ± 0.94 [a]
	0.75	20.13 ± 1.42	11.77 ± 1.34	16.13 ± 1.16	9.60 ± 0.81 [a]	12.98 ± 1.41	10.37 ± 1.04
	1	1.57 ± 0.6 [c]	12.41 ± 1.45	6.21 ± 0.42 [c]	10.40 ± 1.10	3.82 ± 0.9 [c]	9.41 ± 0.85 [a]
H_2O_2	C	23.41 ± 1.7 [a]	11.87 ± 1.3 [a]	19.88 ± 1.2 [a]	9.15 ± 0.4 [a]	15.99 ± 1.5 [a]	10.06 ± 0.6 [a]
	250 µM	25.18 ± 1.5 [a]	9.50 ± 0.8 [b]	31.08 ± 2.4 [b]	8.10 ± 0.5 [a]	17.91 ± 1.7 [a]	6.05 ± 0.5 [b]
	500 µM	31.67 ± 1.6 [b]	7.84 ± 0.4 [c]	39.21 ± 2.5 [c]	10.25 ± 1.2 [a]	21.86 ± 1.5 [b]	5.66 ± 0.4 [b]
	1000 µM	34.74 ± 1.1 [c]	7.67 ± 0.6 [c]	44.97 ± 3.5 [c]	9.16 ± 0.9 [a]	26.81 ± 1.3 [c]	6.16 ± 0.5 [b]
NO	C	25.04 ± 2.4 [a]	10.11 ± 1.0 [a]	21.20 ± 2.3 [a]	11.63 ± 0.8 [a]	16.87 ± 1.3 [a]	10.93 ± 0.6 [a]
	SNP	15.57 ± 1.3 [b]	10.47 ± 0.5 [a]	7.99 ± 0.7 [b]	8.87 ± 0.4 [b]	11.71 ± 1.3 [b]	8.80 ± 0.8 [a]
	SNP+PTIO	30.20 ± 1.4 [c]	10.45 ± 0.3 [a]	42.65 ± 0.3 [c]	13.05 ± 0.8 [a]	28.11 ± 0.8 [c]	11.55 ± 0.7 [a]

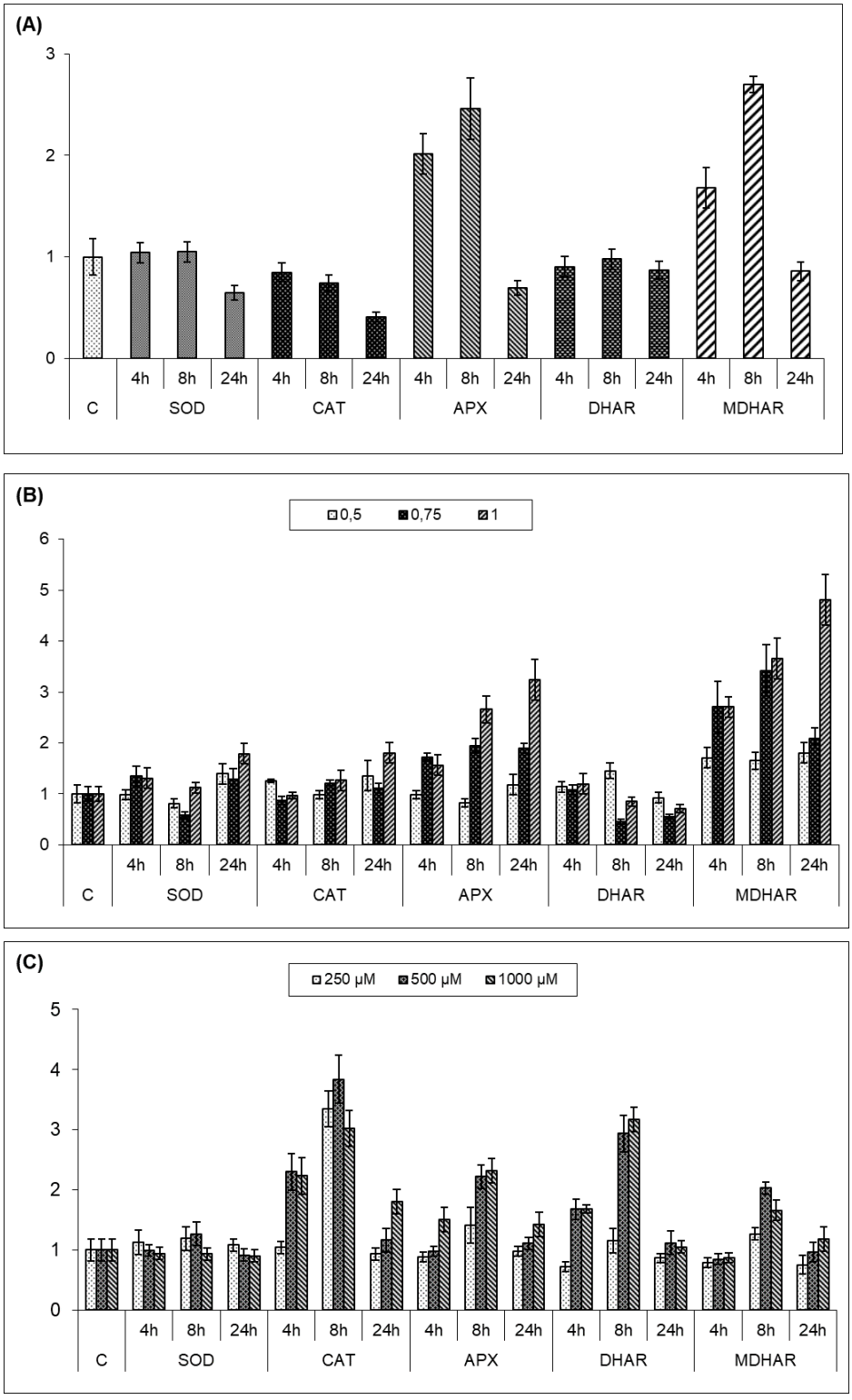
Table 6: Hydrogen peroxide (H_2O_2; nmol g−1 FW) and malondialdehyde (MDA; nmol g−1 FW) contents in control fruits (C) and fruits treated for 4, 8 and 24 h with: 0.1 mM ABA; defined ascorbate redox states (AsA/[AsA+DHA] = 0.5, 0.75 or 1.0; ARS); 250, 500 or 1000 µM H_2O_2; 0.5 mM SNP (nitric oxide donor); or 0.5 mM SNP + 1 mM cPTIO (NO scavenger). Values are means ± SE of five independent biological replicates. Different letters within columns indicate significant differences (Kruskal-Wallis test, $p < 0.05$).

		4h			8h			24h		
		AsA	DHA	Redox state	AsA	DHA	Redox state	AsA	DHA	Redox state
ABA	C	20.5 ± 1.3 [a]	2.5 ± 0.3 [a]	0.89 ± 0.02 [a]	15.2 ± 0.6 [a]	4.4 ± 0.4 [a]	0.78 ± 0.03 [a]	9.4 ± 0.2 [a]	2.2 ± 0.2 [a]	0.81 ± 0.08 [a]
	Treated	8.2 ± 0.7 [b]	2.4 ± 0.4 [a]	0.77 ± 0.03 [b]	5.6 ± 0.4 [b]	3.5 ± 0.4 [b]	0.62 ± 0.05 [b]	2.9 ± 0.3 [b]	0.1 ± 0.0 [b]	0.97 ± 0.05 [b]
ARS	C	21.5 ± 1.1 [a]	2.6 ± 0.4 [a]	0.89 ± 0.04 [a]	14.0 ± 1.2 [a]	4.2 ± 0.0 [a]	0.77 ± 0.05 [a]	10.0 ± 0.6 [a]	2.1 ± 0.1 [a]	0.83 ± 0.06 [a]
	0.5	21.3 ± 1.9 [a]	1.4 ± 0.1 [b]	0.94 ± 0.03 [a]	14.2 ± 0.8 [a]	1.1 ± 0.2 [b]	0.93 ± 0.04 [b]	10.0 ± 1.0 [a]	1.5 ± 0.2 [b]	0.87 ± 0.4 [a]
	0.75	17.2 ± 0.7 [b]	2.5 ± 0.3 [a]	0.87 ± 0.06 [a]	19.8 ± 1.8 [b]	1.1 ± 0.1 [b]	0.95 ± 0.04 [b]	7.0 ± 0.9 [b]	0.4 ± 0.0 [c]	0.95 ± 0.03 [b]
	1	13.2 ± 0.5 [c]	3.6 ± 0.4 [c]	0.79 ± 0.02 [b]	22.6 ± 2.0 [b]	1.9 ± 0.3 [c]	0.92 ± 0.05 [b]	9.9 ± 0.7 [a]	1.7 ± 0.3 [a]	0.85 ± 0.04 [a]
H_2O_2	C	19.4 ± 1.7 [a]	2.4 ± 0.3 [a]	0.89 ± 0.02 [a]	15.4 ± 0.8 [a]	4.4 ± 0.5 [a]	0.78 ± 0.06 [a]	9.7 ± 0.8 [a]	2.1 ± 0.3 [a]	0.82 ± 0.06 [a]
	250 µM	18.9 ± 1.5 [a]	0.1 ± 0.0 [b]	0.95 ± 0.04 [a]	14.8 ± 0.6 [a]	3.1 ± 0.3 [b]	0.83 ± 0.05 [a]	10.5 ± 1.1 [a]	0.8 ± 0.1 [b]	0,93 ± 0.03 [b]
	500 µM	10.6 ± 0.6 [b]	3.3 ± 0.4 [c]	0.76 ± 0.05 [b]	30.6 ± 2.4 [b]	1.1 ± 0.2 [c]	0.97 ± 0.04 [b]	7.0 ± 0.4 [b]	0.1 ± 0.0 [c]	0.99 ± 0.04 [b]
	1000 µM	26.3 ± 1.1 [c]	1.0 ± 0.2 [d]	0.96 ± 0.05 [a]	17.6 ± 1.9 [a]	1.5 ± 0.3 [c]	0.92 ± 0.05 [b]	1.9 ± 0.1 [c]	11.1 ± 1.0 [d]	0.15 ± 0.02 [c]
NO	C	21.3 ± 1.9 [a]	2.6 ± 0.4 [a]	0.89 ± 0.05 [a]	14.0 ± 1.2 [a]	4.6 ± 0.5 [a]	0.75 ± 0.06 [a]	10.3 ± 1.3 [a]	2.3 ± 0.0 [a]	0.82 ± 0.07 [a]
	SNP	19.5 ± 1.3 [a]	7.1 ± 0.5 [b]	0.94 ± 0.04 [a]	11.0 ± 0.8 [b]	5.4 ± 0.6 [a]	0.67 ± 0.05 [a]	3.6 ± 0.3 [b]	0.8 ± 0.1 [b]	0.82 ± 0.04 [a]
	SNP+PTIO	11.2 ± 1.4 [b]	8.7 ± 0.3 [c]	0.56 ± 0.06 [b]	6.6 ± 0.8 [c]	6.8 ± 0.4 [b]	0.49 ± 0.04 [b]	6.0 ± 0.8 [c]	7.7 ± 0.8 [c]	0.44 ± 0.03 [b]

Table 7: Ascorbate (AsA) and dehydroascorbate (DHA) concentrations (mmol g−1 FW) and ascorbate redox state (AsA/[AsA+DHA]) in control fruits (C) and fruits treated for 4, 8 and 24 h with: 0.1 mM ABA; defined ascorbate redox states (AsA/[AsA+DHA] = 0.5, 0.75 or 1.0; ARS); 250, 500 or 1000 µM H_2O_2; 0.5 mM SNP (nitric oxide donor); or 0.5 mM SNP + 1 mM cPTIO (NO scavenger). Values are means ± SE of five independent biological replicates. Different letters within columns indicate significant differences (Kruskal-Wallis test, $p < 0.05$)

Antioxidant enzyme activities responded coherently to the early ABA-induced ROS elevation. APX and MDHAR activities increased at 4 and 8 h — consistent with upregulation of the ascorbate–glutathione cycle to handle rising H_2O_2 — before declining at 24 h, paralleling the decrease in H_2O_2 content. SOD and CAT were unaffected at 4 and 8 h but decreased at 24 h, while DHAR remained unchanged throughout (Figure 1A) [83]. At the transcript level, *SlAPXcyto* followed the same biphasic pattern as APX activity (increasing at 4–8 h, decreasing at 24 h), reaching a peak induction of approximately 13.5-fold at 8 h (Figure 2A). Notably, *SlAPXt* showed a near-identical induction (approximately 12.5-fold at 8 h), suggesting that ABA co-regulates both the

cytosolic and thylakoid-bound APX isoforms at the transcript level at this time point, despite their distinct subcellular localizations. *SlDHAR1, SlDHAR2* and *SlMDHAR* showed similar profiles (Figure 2A, 3A) [84]. However, *SlAPXt* and *SlDHAR1* showed divergent kinetics — *SlAPXt* decreased at 4 h before increasing at 8 h, while *SlDHAR1* increased at 4 h only — indicating that individual genes within the ascorbate–glutathione cycle are differentially regulated by ABA (Figs. 2A, 3A). The frequent discordance between enzyme activities and their corresponding transcript levels suggests that post-transcriptional mechanisms contribute significantly to ABA-mediated antioxidant regulation [85].



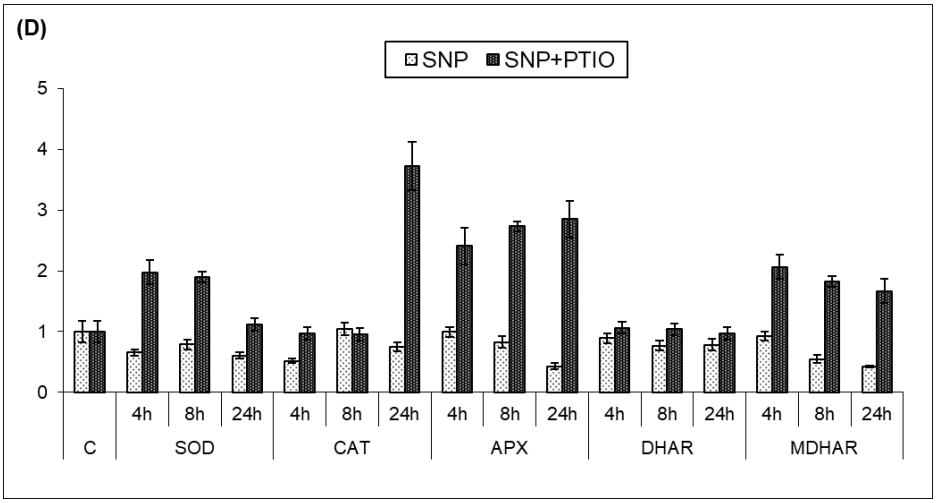
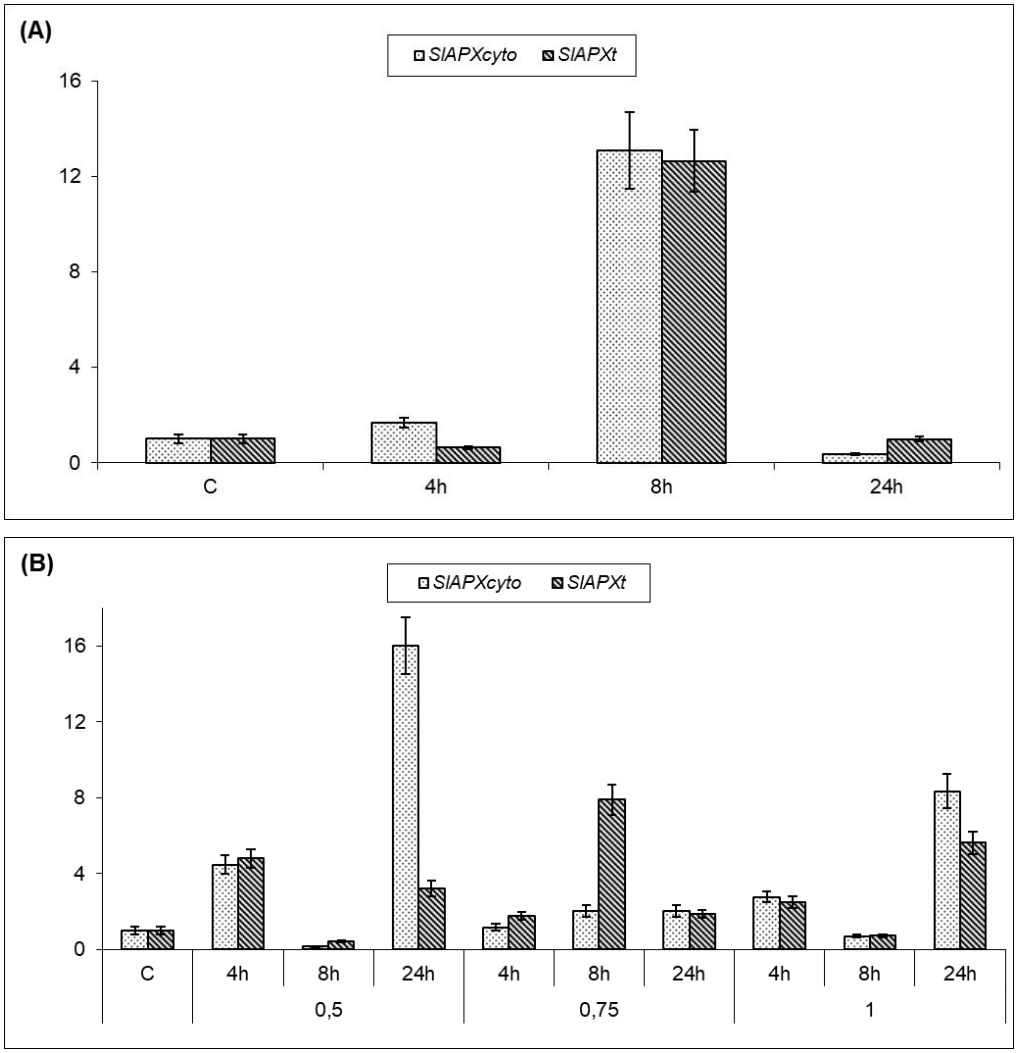


Figure 1: Activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) in control fruits (C) and fruits treated for 4, 8 and 24 h with: (A) 0.1 mM ABA; (B) defined ascorbate redox states (AsA/[AsA+DHA] = 0.5, 0.75 or 1.0); (C) 250, 500 or 1000 µM H2O2; (D) 0.5 mM SNP or 0.5 mM SNP + 1 mM cPTIO. All values are expressed relative to the mean value of control fruits (set to 1). Values are means ± SE of five independent biological replicates.



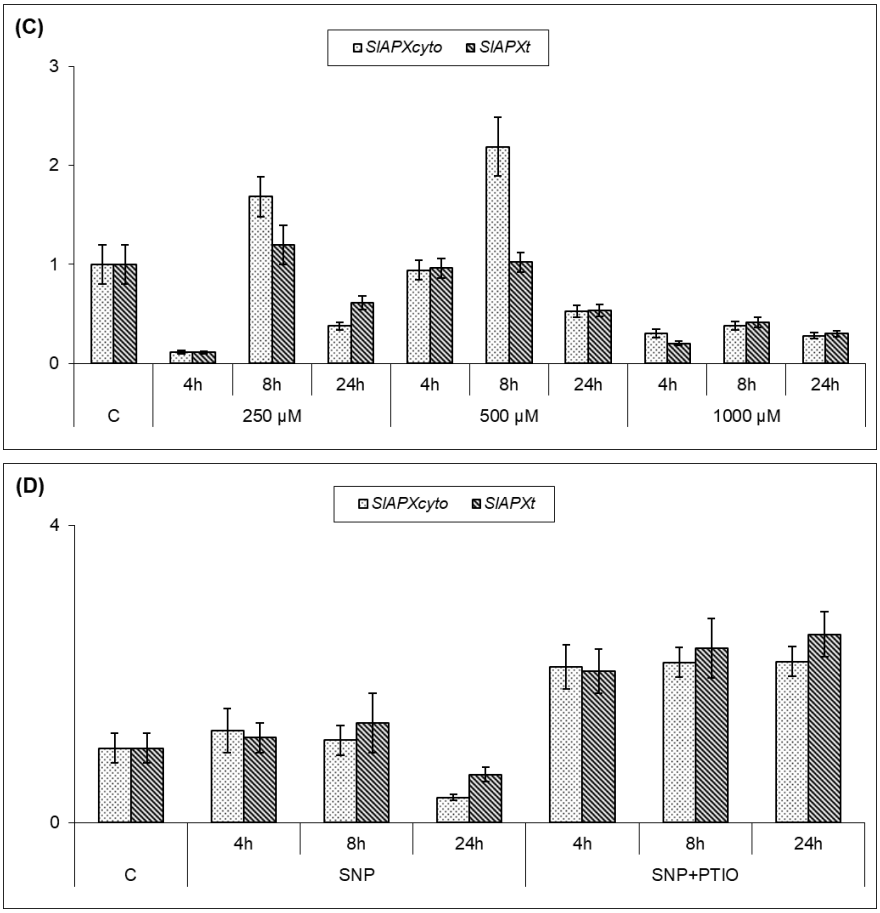


Figure 2: Relative transcript levels of SlAPXcyto and SlAPXt in control fruits (C) and fruits treated for 4, 8 and 24 h with: (A) 0.1 mM ABA; (B) defined ascorbate redox states (AsA/[AsA+DHA] = 0.5, 0.75 or 1.0); (C) 250, 500 or 1000 µM H2O2; (D) 0.5 mM SNP or 0.5 mM SNP + 1 mM cPTIO. Transcript levels were normalized to SlActin expression using the 2−ΔΔCt method and are expressed relative to the mean value of control fruits (set to 1). Values are means ± SE of six replicates (three independent RNA extractions, each analyzed in duplicate).

3.3. Effects of Ascorbate Redox State Treatments

The AsA redox state of the culture medium had a concentration-dependent effect on fruit oxidative status. H_2O_2 content was unaffected in fruits cultured at a redox state of 0.5, but decreased significantly at redox states of 0.75 and 1.0, indicating that a more reduced AsA environment promotes H_2O_2 scavenging. MDA was unaffected across all conditions (Table 6) [86]. Changes in fruit AsA and DHA concentrations were complex and time-dependent, reflecting both uptake from the medium and active metabolic regulation of the ascorbate pool (Table 7). The enzymatic response was dominated by a consistent and strong induction of MDHAR across all AsA redox state conditions (Figure 1B), suggesting that MDHAR — which regenerates AsA from monodehydroascorbate — is particularly sensitive to the apoplastic AsA redox environment [87]. APX activity was also markedly induced at redox states of 0.75 and 1.0 but not at 0.5, consistent with the observed H_2O_2 decrease in these conditions. By contrast, SOD and CAT were largely unresponsive, with only isolated changes at specific time points (Figure 1B). DHAR activity decreased at the highest AsA redox states (0.75 and 1.0), suggesting a functionally coherent adjustment of the ascorbate-recycling machinery: when the medium is highly reduced and DHA availability is low, the DHAR reaction — which regenerates AsA from DHA — becomes less necessary, and its downregulation represents an energetically efficient reallocation within the ascorbate–glutathione cycle [88-90].

At the transcript level, *SlAPXcyto* and *SlAPXt* generally increased but with complex, time- and redox state-dependent patterns (Figure 2B). *SlDHAR1, SlDHAR2* and *SlMDHAR* transcripts were induced at redox states of 0.5 and 1.0 across most time points, and at redox state 0.75 mainly at 8 h (Figure 3B). One notable exception was the strong induction of *SlMDHAR* transcripts (approximately 8-fold) at redox state 0.5 after 24 h, which coincided with the highest *MDHAR* enzyme activity across this treatment — representing the single case in this dataset where *SlMDHAR* transcript and MDHAR activity showed a clear positive correlation. Beyond this, the changes in enzyme activities and transcript levels were frequently discordant across conditions — notably MDHAR activity increased while SlMDHAR transcripts showed variable

patterns — consistent with post-transcriptional regulation, possibly through direct redox modification of enzyme proteins by the altered AsA/DHA environment [42,91].

3.4. Effects of H2O2 Treatments

Exogenous H_2O_2 elevated fruit H_2O_2 content in a concentration-dependent manner in most treatment conditions, confirming uptake and accumulation, while paradoxically decreasing MDA in fruits treated for 4 and 24 h with all concentrations (Table 6). This decrease in MDA despite elevated H_2O_2 suggests that exogenous H_2O_2 primarily acts as a signal that activates antioxidant defenses rather than causing immediate oxidative damage at the concentrations tested. AsA and DHA concentrations showed concentration-dependent and time-dependent changes (Table 7). Of particular note, fruits treated with 1000 µM H_2O_2 for 24 h showed a dramatic 5-fold accumulation of DHA (11.1 ± 1.0 vs 2.1 ± 0.3 mmol g−1 FW in controls), accompanied by a collapse of the AsA redox state to 0.15 ± 0.02 — by far the lowest value recorded across the entire study (Table 7). This profound oxidation of the ascorbate pool at the highest H_2O_2 concentration and duration of treatment suggests an overload of the ascorbate-recycling capacity, consistent with the transition from signalling to damage at very high H_2O_2 concentrations [92].

Antioxidant enzyme responses were concentration-dependent. At 250 µM H_2O_2, only modest effects were observed, with MDHAR increasing at 8 h as the sole consistent response. At 500 µM H_2O_2, CAT, APX and DHAR activities all increased, peaking at 8 h and returning towards control levels at 24 h — a pattern consistent with the transient, self-limiting nature of H2O2-induced signalling. At 1000 µM, CAT and APX were induced but with a blunted profile, suggesting that very high H_2O_2 concentrations may exceed the optimal signalling window. SOD was unaffected at all concentrations (Figure 1C). The transcript response contrasted markedly with the enzyme activity response. Transcript levels of *SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2 and SlMDHAR* were predominantly suppressed, particularly at 250 and 1000 µM H_2O_2, with only transient increases at 8 h under 500 µM H_2O_2 (Figs. 2C and 3C). This pronounced dissociation between enzyme activity induction and transcript suppression strongly supports post-transcriptional control — notably redox-based post-translational activation of pre-existing enzyme proteins — as a major mechanism of the rapid H_2O_2 signalling response in fruits [42,93].

3.5. Effects of Nitric Oxide Generator and Scavenger Treatments

The opposing effects of SNP (NO donor) and SNP + cPTIO (NO donor + NO scavenger) on H_2O_2 content provided clear evidence that the observed responses were NO-dependent. SNP treatment decreased fruit H_2O_2 content, consistent with direct ROS quenching by NO, while SNP + cPTIO increased it, demonstrating that removal of NO leads to ROS accumulation (Table 6). MDA was unaffected by both treatments except for a modest decrease in SNP-treated fruits at 8 h, suggesting that NO suppresses oxidative damage even when its ROS-scavenging capacity is insufficient to prevent H_2O_2 accumulation alone. The ascorbate pool responded differently to SNP and SNP + cPTIO. AsA concentration decreased with both treatments, but DHA dynamics diverged: DHA increased transiently in SNP-treated fruits at 4 h, while in SNP + cPTIO-treated fruits DHA increased consistently at all time points. The AsA redox state was unchanged by SNP but decreased markedly with SNP + cPTIO, indicating that NO scavenging — and the resulting H_2O_2 accumulation — drives oxidation of the ascorbate pool (Table 7). Quantitatively, SNP + cPTIO reduced the AsA redox state to 0.44 ± 0.03 at 24 h (vs 0.82 ± 0.07 in controls), demonstrating that endogenous NO is required not only for enzymatic ROS scavenging but also for preserving the reduced state of the non-enzymatic antioxidant pool.

The most striking result was the inverse relationship between SNP and SNP + cPTIO on antioxidant enzyme activities and transcripts. SNP treatment generally suppressed or had no effect on enzyme activities (Figure 1D) and reduced *SlAPXcyto, SlAPXt and SlMDHAR* transcript levels at 8 and 24 h (Figs. 2D, 3D). By contrast, SNP + cPTIO strongly induced all enzyme activities and upregulated *SlAPXcyto, SlAPXt* and *SlMDHAR* transcripts at all time points, and transiently induced *SlDHAR2* at 8 h. *SlDHAR1* was unaffected by both treatments. These results indicate that endogenous NO suppresses the need for enzymatic antioxidant induction by maintaining low ROS levels through direct scavenging. When NO is removed by cPTIO, ROS levels rise and the enzymatic antioxidant response is derepressed. The fact that this derepression occurs at both the activity and transcript levels suggests a dual regulatory mechanism for NO: direct post-translational modulation of enzyme activity (e.g., via S-nitrosylation and transcriptional regulation of antioxidant gene expression [31].

4. Discussion

Environmental stresses such as drought, salinity, heavy metal exposure, mechanical injury, temperature extremes and pathogen attack promote the overproduction of ROS in plants, leading to oxidative damage of organelles, membrane systems and gene expression [43,44]. Plants counteract stress-induced ROS accumulation through a complex antioxidant system — both enzymatic (SOD, CAT, APX, MDHAR, DHAR) and non-enzymatic (AsA, GSH, α-tocopherol, carotenoids) — whose induction depends on species, developmental stage, metabolic state, and the duration and intensity of the stress [26,45]. Importantly, antioxidant enzyme activities are not solely regulated at the transcriptional level but are also subject to extensive post-translational modifications (PTMs) including S-nitrosylation, tyrosine nitration, phosphorylation and persulfidation, which can activate or inhibit enzymatic function in a rapid and reversible manner [31,46].

In the present study, $HgCl_2$ treatment — which inhibits plasma membrane aquaporins, thereby blocking water uptake— selectively induced oxidative stress in leaves and fruit peduncles without affecting fruit water status or oxidative parameters (Tables 2–4) [41]. Strikingly, despite the absence of local oxidative stress in fruits, antioxidant enzyme activities and the transcript levels of

SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2 and *SlMDHAR* were all upregulated in fruits (Table 5). This dissociation between local oxidative damage and systemic upregulation of fruit antioxidant defenses strongly supports the existence of a leaf-derived signal priming distant tissues against impending stress — a phenomenon consistent with systemic acquired acclimation (SAA), in which ROS waves propagate from locally stressed tissues to prime distal organs [7,9,47]. Such preemptive activation is part of the early defense response that may contribute to stress tolerance in non-stressed organs. It is also noteworthy that control tomato fruits maintained a basal AsA redox state of 0.89, substantially higher than that of leaves (0.53) or fruit peduncles (0.34) under the same conditions. This strongly reduced baseline ascorbate status may confer intrinsic oxidative stress resistance to the fruit, explaining why local oxidative stress markers remained undetectable even when the systemic antioxidant response was already being activated. To identify the molecular nature of this putative leaf-to-fruit signal, we applied ABA, H_2O_2, NO (via SNP) and defined AsA redox states exogenously to detached fruits. Our results indicate that each of these molecules modulates fruit antioxidant responses, but with distinct kinetics and modes of action.

ABA is a well-established secondary messenger integrating stress signals and mediating antioxidant defenses [21,48]. ABA activates NADPH oxidases at the plasma membrane, driving H_2O_2 production, which in turn reinforces the ABA signal through a positive feedback loop [20,49,50]. Recent work has further shown that this ABA–ROS relay operates at the protein level: ABA promotes the oxidation of PP2C phosphatases such as ABI2 via H_2O_2-sensing thiol peroxidases, thereby amplifying SnRK2-mediated stress signalling [22]. At the same time, ABA can induce NO production, and ABA and NO engage in extensive crosstalk to co-regulate antioxidant enzyme activities and stress responses [8]. In our study, ABA treatment increased H_2O_2 content early (4 and 8 h) and induced APX and MDHAR activities as well as the transcripts of *SlAPXcyto, SlDHAR2* and *SlMDHAR,* consistent with an ABA– H_2O_2 relay activating ascorbate–glutathione cycle enzymes. The subsequent decrease in H_2O_2 content, enzyme activities and transcript levels at 24 h likely reflects homeostatic restoration once the initial defense response had been mounted, and is consistent with the transient nature of ABA-induced ROS bursts described in other systems [20,94].

Ascorbate does not function solely as an antioxidant; its redox state provides essential information on cellular redox homeostasis and determines the lifetime and specificity of H_2O_2 signals [25,26]. The AsA/DHA ratio thus controls the amplitude of H_2O_2-dependent signalling by modulating the rate of H_2O_2 removal by APX. In our experiments, culturing fruits in media with elevated AsA redox states (0.75 and 1.0) decreased fruit H2O2 content and markedly increased APX and MDHAR activities, consistent with accelerated AsA turnover by these enzymes. Altered AsA redox states also modified the expression of *SlAPXcyto*, *SlAPXt*, *SlDHAR1, SlDHAR2* and *SlMDHAR*, but the changes in enzyme activities and transcript levels were not consistently correlated, indicating control at the post-transcriptional level. This could involve redox-dependent phosphorylation, translation regulation, or direct oxidative PTMs of the enzymes themselves [42,46,52]. Indeed, recent work has demonstrated that multiple antioxidant enzymes including APX, MDHAR, DHAR and CAT are targets of reversible thiol-based modifications that act as molecular switches to adjust their activity independently of gene expression [46]. The observed decrease in DHAR activity at elevated AsA redox states (0.75 and 1.0) is mechanistically coherent: DHAR catalyzes the reduction of DHA back to AsA, and when DHA availability is low — as in a highly reduced medium — the DHAR reaction becomes substrate-limited. This downregulation therefore represents a substrate-driven adjustment of the cycle rather than active repression, illustrating how the ascorbate–glutathione cycle self-regulates in response to the redox state of its own substrates.

H_2O_2 is a well-established intracellular and potentially systemic signalling molecule in plants, implicated in responses to excess light, pathogen attack and physical damage [52-54]. H_2O_2 acts at low concentrations as a signalling molecule that switches on antioxidant defenses, while at high concentrations it drives oxidative damage — a concentration-dependent duality that is central to its role as a systemic stress signal [7]. In our study, exogenous H_2O_2 induced APX and CAT activities — the primary H_2O_2-scavenging enzymes — and reduced MDA accumulation, confirming a protective antioxidant response. The response was concentration-dependent: 500 µM H_2O_2 was the most effective inducer of both enzyme activities and ascorbate–glutathione cycle transcripts, whereas 1000 µM tended to suppress transcript levels, consistent with the idea that very high ROS concentrations shift the cellular response from defense to damage. Crucially, this transition is corroborated by the ascorbate pool data: at 1000 µM H_2O_2 for 24 h, DHA accumulated to 11.1 mmol g−1 FW — a 5-fold increase relative to controls — and the AsA redox state collapsed to 0.15. This exhaustion of the ascorbate pool provides direct biochemical evidence that 1000 µM H_2O_2 applied for 24 h overloads the ascorbate-recycling capacity, exceeding the optimal signalling window described for this molecule [7]. The transient nature of enzyme induction — returning to control levels by 24 h — may reflect feedback inhibition once H_2O_2 levels had been brought back under control, and is consistent with the known transient character of H_2O_2-triggered acclimation responses [9]. The frequent discordance between enzyme activity and transcript levels across all H_2O_2 treatments again points to substantial post-translational control of antioxidant enzyme activity [46].

NO has a dual function in stress responses: it can directly quench ROS — notably by reacting with superoxide to form peroxynitrite — and can modulate antioxidant gene expression through downstream signalling cascades involving MAPK and cGMP [30,55]. A key molecular mechanism is S-nitrosylation of RBOHD, which suppresses apoplastic ROS production and simultaneously enhances APX activity, thereby fine-tuning the ROS/NO balance [31]. In our study, SNP treatment decreased fruit H_2O_2 content and generally suppressed or did not alter antioxidant enzyme activities and transcript levels, consistent with NO acting primarily as a direct ROS scavenger, thereby reducing the need for

enzymatic antioxidant induction [56,57]. Conversely, when NO was scavenged with cPTIO, H_2O_2 content increased and antioxidant enzyme activities and transcript levels were induced, providing strong evidence that endogenous NO limits ROS accumulation and suppresses enzymatic antioxidant induction. Beyond its role in controlling enzymatic defenses, our data also reveal that NO is critical for maintaining the reduced state of the non-enzymatic antioxidant pool: scavenging of NO with cPTIO caused the AsA redox state to fall to 0.44 at 24 h, compared to 0.82 in controls, demonstrating that endogenous NO protects the ascorbate pool from oxidation independently of its effects on enzyme activities. This dual protective role — preserving both enzymatic and non-enzymatic antioxidant capacity — positions NO as a particularly versatile guardian of fruit redox homeostasis. These findings highlight a tight NO–ROS interplay in which NO acts as a homeostatic buffer, preventing unnecessary antioxidant enzyme induction when ROS levels are kept in check. This mechanism may be of particular relevance in fruits, where NO also plays roles in ripening regulation, and where fine control of the redox balance is needed to coordinate quality and stress tolerance [8,30].

Taken together, our results support an integrative model in which mercury-induced oxidative stress in leaves generates multiple mobile signals — ABA, H_2O_2, NO and changes in AsA redox state — that are relayed to fruits and collectively prime their antioxidant machinery prior to the onset of local oxidative damage. These signals are not independent but form an interconnected network: ABA promotes RBOH-dependent H_2O_2 production, which can in turn trigger NO biosynthesis; NO feeds back to modulate both H_2O_2 levels (via S-nitrosylation of RBOHD) and ABA signalling; and AsA redox state buffers H_2O_2 availability via APX-mediated scavenging [8,20]. Across all four signal types, we observed frequent dissociation between enzyme activity and transcript levels, strongly suggesting that post-translational mechanisms — including redox-based PTMs — represent a major layer of antioxidant regulation in tomato fruits [46]. Unravelling the precise hierarchy of these interactions and their organ-specific regulation in tomato remains a challenging but essential goal for understanding how plants coordinate systemic stress responses and for developing strategies to improve fruit quality and stress resilience in the context of climate change.

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