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L-Ascorbic Acid Improves Fruit Setting and Activates Antioxidant Enzymes in Tomato Plants (*Solanum lycopersicum* L.) Grown Under Heat Stress Conditions

Abdalla M Abdelmonaem*, Zeinab A Abdou, Hany AM Sror, Mohamed M Aboul Fotouh
Biochemistry Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: Abdalla_mohamed@agr.asu.edu.eg

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Abstract: Heat stress is an environmental problem that can negatively impact tomato productivity by reducing fruit setting and disrupting pollen development. The present study aims to understand the molecular mechanism of heat tolerance induced by L-ascorbic acid. Plants were grown in the summer of 2022 (day and night temperatures were 34.4°C and 25.2°C respectively). The plants were divided into two groups: one was sprayed with L-ascorbic acid (0.5 mM) and the other served as a control. Growth parameters, antioxidant enzymes, proline, hydrogen peroxide, and malondialdehyde levels in the leaves were determined. Additionally, a molecular docking allowed understanding the interaction between the antioxidant enzymes and L-ascorbic acid. Results indicated that L-ascorbic acid increased antioxidant enzyme activities (catalase, peroxidase, ascorbate peroxidase, polyphenol oxidase), proline content, fruit setting and growth indicators (shoot and root fresh and dry weight). It also reduced hydrogen peroxide and malondialdehyde levels in tomato leaves. Furthermore, the *in-silico* analysis revealed that L-ascorbic acid binding energies toward antioxidant enzymes were similar to those of known activators. In conclusion, foliar application of L-ascorbic acid (0.5 mM) effectively mitigated heat stress effects, activating antioxidant enzymes and eliminating reactive oxygen species, ultimately resulting in increased fruit setting.

1 Introduction

Climate changes, especially, global warming, have led to heat stress, which negatively influences tomato plant growth and productivity. Temperature plays a significant function in the development and growth of tomato plants, but high-temperature stress often poses challenges to their cultivation (Mubarok et al 2023). Heat stress can cause significant damage to tomato plants during different developmental stages, such as seed germination, and vegetative and reproductive growth (Wahid et al 2007). The tomato reproductive stage is particularly vulnerable to high-temperature stress, which negatively affects fruit setting, including pollination, fertilization, and seed formation (Kugblenu et al 2013, Driedonks et al 2016). Prolonged warming is considered a form of heat stress, which is a modest temperature increase for many

days or weeks by 2–5℃ upon ambient temperature (Wolkovich et al 2012). Heat stress causes the over-accumulation of the molecular oxygen (O_2) reactive forms, which are also called reactive oxygen species (ROS), including hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), causing oxidative damage to biological molecules like proteins, lipids, pigments, carbohydrates, and DNA, resulting in cell injuries and death (Fortunato et al 2023). The vegetative organs of the plant are less susceptible to heat stress than the reproductive organs (Ruan et al 2010, Zinn et al 2010). A complex antioxidant defense system is devolved by plants to protect their cells against oxidative damage (Lee et al 2023), including enzymatic defenses such as catalase (CAT), peroxidases (PODs), ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione S-transferase, glutathione peroxidase (GPX), and the ascorbate-glutathione pathway (AsA–GSH cycle) enzymes (monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR), which are responsible for reducing the oxidized AsA and GSH forms, and non-enzymatic defenses such as tocopherols, carotenoids, AsA, and glutathione (Foyer and Noctor 2013).

Several recent studies have been conducted to avoid fruit setting failure and increase tomato plant productivity under high-temperature stress conditions. One such study used specific extracts of *Ascophyllum nodosum* as a bio-stimulant during the pollen development stage to counteract the adverse consequences of heat stress during the critical reproductive stage, resulting in significant production benefits (Carmody et al 2020). Cultivating two tomato parthenocarpic mutants (*iaa9-3* and *iaa95*) under heat stress increased the fruit setting percentage (Mubarok et al 2023). L-Ascorbic acid is an important powerful water-soluble antioxidant vitamin in both plants and animals. It is a critical regulator of ROS in cells under stress conditions, both biotic and abiotic (Foyer and Noctor 2011). It forms one of the most effective H_2O_2 scavengers (Hasanuzzaman et al 2019). Exposing wheat plants to L-ascorbic acid increased the leaf content of L-ascorbic acid and improved antioxidant enzymes (POD, CAT, and SOD) under salinity stress (Athar et al 2009). L-Ascorbic acid treatment before heat stress at the pre-anthesis stage enhanced wheat pollen thermotolerance (Kumar et al 2014). L-Ascorbic acid plays a crucial role as a signaling molecule in enhancing tomato seedling thermotolerance by increasing the levels of endogenous content of L-ascorbic acid, proline, and the pigments of photosynthesis in the leaves and up-regulating heat shock proteins (HSPs) (Alayafi 2020).

Although previous studies have demonstrated that L-ascorbic acid can improve heat tolerance in many crops, including tomatoes, the mechanism by which L-ascorbic acid induces this tolerance is still unclear. Moreover, there is no available information about the effect of L-ascorbic acid on the fruit setting physiology in tomato plants. Therefore, the aim of this work was to evaluate the effect of L-ascorbic acid foliar application on enhancing the thermotolerance of tomato plants and the fruit set percentage. Biochemical and computational approaches were also used to examine the impact of L-ascorbic acid interaction with antioxidant enzymes in mitigating the damages associated with heat stress.

2 Materials and Methods

2.1 Plant samples and planting conditions

Tomato seeds of the commercial variety *Solanum lycopersicum* L. 023 were obtained from Takii company in Kyoto, Japan and were purchased from a local market. These seeds were cultivated at the Faculty of Agriculture, Ain Shams University, Egypt in June 2022. Healthy tomato seedlings were transplanted into plastic bags containing 30 kg of washed sand. During the experimental period, the seedlings were watered daily with Hoagland solution (Hoagland and Arnon 1950). The plastic bags were randomly allocated into two groups. The first group was sprayed with L-ascorbic acid (0.5 mM) daily for 7 days as recommended by Alayafi (2020), starting 15 days after transplantation. The second group was left untreated and used as a control. **Fig 1** shows the day and night temperature and humidity during the plant growth period.

2.2 Determination of vegetative and generative growth parameters

2.2.1 Fresh and dry weight

Growth parameters were measured as reported by Jahan et al (2019). Shoot and root fresh weight were measured using an electric balance. For dry weight measurements, shoot and root samples were dried at 80°C in an oven (72 h).

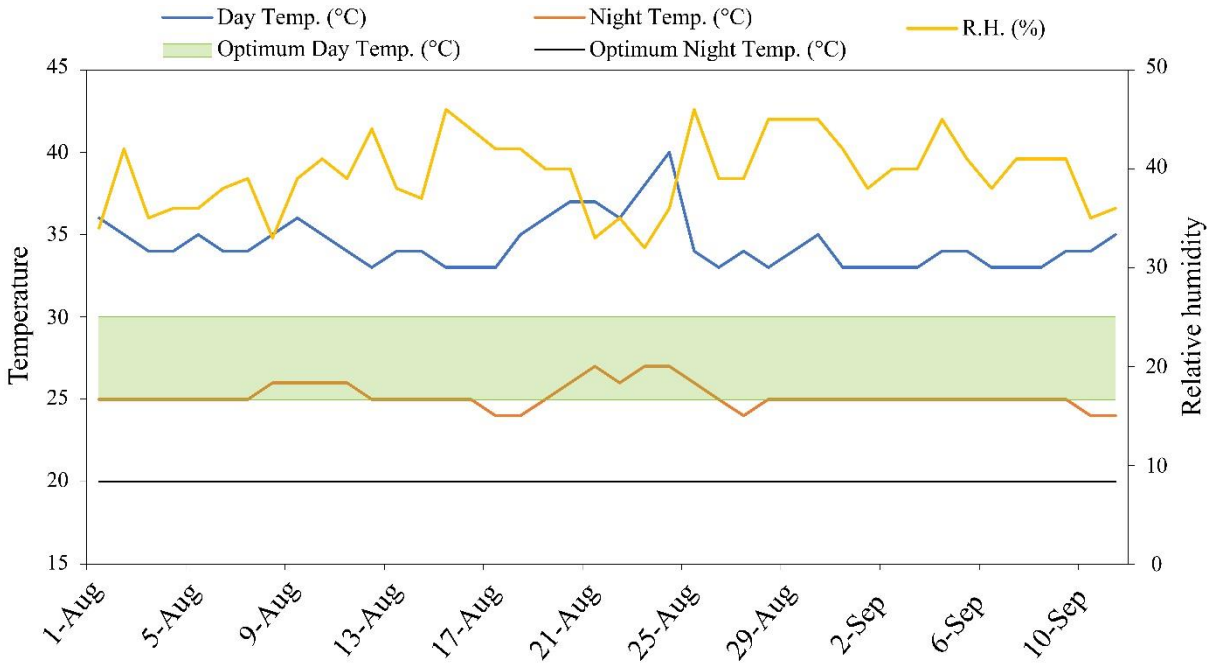


Fig 1. Environmental weather conditions during the experimental period

2.2.2 Fruit setting percentage

The fruit-setting percentage was measured according to Vijayakumar et al (2021). The total number of flowers and fruits for the first and second clusters was recorded and the following equation was used:

Fruit setting (%) = (Number of fruits / Number of flowers) × 100

2.3 Stress markers

2.3.1 Hydrogen peroxide (H_2O_2)

The leaf content of H_2O_2 was assayed as mentioned by Velikova et al (2000).

2.3.2 Malondialdehyde (MDA)

The MDA leaf content was determined as a lipid peroxidation end-product as displayed by Heath and Packer (1968).

2.3.3 Proline

The content of proline was quantified *via* the colorimetric ninhydrin method according to Troll and Lindsley (1955) and modified by Peters et al (1997).

2.4 Antioxidant enzyme assays

2.4.1 Extraction

Antioxidant enzymes were extracted according to Parvin et al (2020) with slight modifications. Leaves (0.5 g) were grinded in 4 mL precooled K-phosphate buffer (50 mM; pH 7.0) containing 1 mM AsA, 100 mM KCl, polyvinylpyrrolidone (PVP; 1%, w/v) and glycerol (10 %, w/v). The centrifugation was performed at 4 °C at 6,000 rpm for 20 min. The supernatant was utilized to determine CAT, POD, APX and PPO enzymes as well as the protein content.

2.4.2 CAT (EC: 1.11.1.6) assay

CAT activity (Unit.mg^{-1} protein) was assayed as described by Hasanuzzaman et al (2011).

2.4.3 POD (EC: 1.11.1.7) assay

POD activity (Unit.mg^{-1} protein) was measured as described by Hammerschmidt et al (1982).

2.4.4 APX (EC: 1.11.1.11) assay

APX activity (Unit.mg^{-1} protein) was determined as described by Nakano and Asada (1981).

2.4.5 PPO (EC: 1.14.18.1) assay

PPO activity (Unit.mg^{-1} protein) was determined as described by Oktay et al (1995).

The protein content was quantified *via* the assay of Bradford (1976).

2.5 Molecular Docking

2.5.1 Target Protein Preparation

Catalase 3D structure was obtained using homology modeling. *Solanum lycopersicum* (Tomato) catalase isozyme 2 (UniProt ID: Q9XHH3) was chosen as a target molecule from the UniProt database **(**UniProt Consortium **2019)** using filtration options (active site availability and transcription level protein existence). Afterward, the chosen target amino acid sequence was copied from the UniProt database and pasted into the SWISS-MODEL online server (Waterhouse et al 2018) to develop a highly reliable model from a suitable template with adequate Quaternary structure quality estimate (QSQE), Global model quality estimation (GMQE), sequence coverage, seq. identity, seq. similarity and x-ray diffraction resolution. Based on GMQE and QMEAN (Qualitative Model Energy Analysis) values (Benkert et al 2011), the most reliable model was selected. *Bacillus pumilus* catalase (SMTL ID: 4qol.1) was chosen as the most suitable template to build the modeled protein. SWISS-MODEL automatically produced calculations. However, further structural quality checking of the *Solanum lycopersicum* catalase (*Sl*CAT) model was evaluated *via* the Ramachandran plot and its plot statistics, which were generated using the PROCHECK online server evaluation (https://saves.mbi.ucla.edu/). Soybean (*Glycine max*) peroxidase (SBP) 3D structure (PDB ID: 1FHF) (Henriksen et al 2001), was used as a plant peroxidase. For polyphenol oxidase, *Solanum lycopersicum* polyphenol oxidase holo-structure (*Sl*PPO) (PDB ID: 6HQI) (Kampatsikas et al 2019), was attained from the PDB.

After obtaining the target proteins' 3D structure, water and solvent molecules were deleted if they were found, polar hydrogen atoms were added, kollman charges were calculated and the structures of the target proteins were saved as pdbqt file formats by AutoDockTools-1.5.7 (The Scripps Research Institute, San Diego, California, USA).

2.5.2 Ligand preparation

ChemDraw 16.0.1.4 was used to draw ligands and to export the structures to Chem3D 16.0.1.4, which was used to calculate ligands' energy minimization by MM2 and to obtain the structures in pdb file formats. AutoDockTools-1.5.7. was used to save the ligand structures in pdbqt file formats after the pre-docking preparations (such as gasteiger charges calculations and torsion roots detection) were done.

2.5.3 Docking

The molecular interactions were determined between the selected active sites of target proteins and the ligands (Modeled *Sl*CAT with L-ascorbic acid and metformin; SBP with L-ascorbic acid and Guaiacol; *Sl*PPO with L-ascorbic acid, Dopamine and Phloretin) *via* AutoDock Vina 1.2.0 (The Scripps Research Institute, San Diego, California, USA) (Eberhardt et al 2021).

Vina_split command was utilized for separating each ligand's conformers. The highest affinity conformers were chosen and analyzed using Discovery Studio Visualizer v21.1.0.20298 software (Dassault Systems Biovia Corp., San Diego, California, USA). For visualization of the interactions between the enzymes and the ligands, Discovery Studio Visualizer was used.

2.6 Statistical Analysis

Our recorded data were statistically analyzed *via* SPSS 20.0.0 (IBM-SPSS Corp., Armonk, New York, USA). Independent samples *t*-test at $P \leq 0.05$ was utilized for the analysis with three replicates except for fruit setting percentage, which was five replicates. Graphs were made using Microsoft Excel 365 and Adobe Illustrator 25.2.3 (Adobe Inc. Corp., San Jose, California, USA).

3 Results and discussion

3.1 Fresh and dry weight

When tomato plants are exposed to high temperatures, they tend to experience stress. However, applying 0.5 mM L-ascorbic acid to the tomato plants can help reduce this stress. As a result, the shoots and roots' fresh and dry weights of the tomato plants increased significantly, as shown in **Fig 2**. Treatment with 0.5 mM L-ascorbic acid caused a 177.78% increase in fresh weight and a 185.42% increase in dry weight of the tomato shoots, while the tomato roots saw an increase of 74.73% in fresh weight and 106.67% in dry weight

when compared to untreated plants. This increase in plant growth could be attributed to the influence of L-ascorbic acid on plant growth as a key regulator and its potent antioxidant activity (Celi et al 2023). L-Ascorbic acid participates in the cell's transition from the S cellular phase to the G1 phase (Arrigoni 1994) and contributes to plant cell expansion and division as a coenzyme for prolyl hydroxylase, which hydroxylates proline amino acids found in the cell wall glycoproteins containing hydroxyproline (Smirnoff and Wheeler 2000). Similar results were observed in safflower plants under drought stress conditions, where L-ascorbic acid increased osmoprotection and regulated the antioxidant defense system (Farooq et al 2020). In fenugreek plants, L-ascorbic acid acts as a growth regulator and chelator, increasing plant growth and biomass under heavy metal stress (Fatima et al 2020).

3.2 Fruit setting percentage

The application of 0.5 mM L-ascorbic acid on tomato plants grown in heat-stress conditions significantly increased the fruit setting percentage **(Fig 3)**. Untreated heat-stressed plants recorded a lower fruit setting percentage (18.65%). However, the fruit setting in the plants treated with L-ascorbic acid increased significantly to 3.6 times compared to the control and reached 67.34% **(Fig 3)**. The reduction of fruit setting induced by heat stress is the main reason for tomato yield decline in heat stress conditions, which is a serious issue in tomato production (Mubarok et al 2023). The observed reduction of fruit setting could be attributed to male gametophyte abortion when the plants are subjected to heat stress (Alsamir et al 2021). Heat stress inhibits pollen generation due to the accumulation of ROS, including hydroxyl radical ($HO^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which trigger lipid peroxidation and the oxidation of proteins, nucleic acids and sugars, eventually resulting in the cell death of pollen and anther (Djanaguiraman et al 2011, De Storme and Geelen 2014). Under abiotic stresses, exogenous L-ascorbic acid treatment scavenges ROS in the plant, protecting the cell membrane's stability (Hasanuzzaman et al 2019). In this investigation, the higher fruit-setting percentage of L-ascorbic acid-treated plants compared to the control plants could result from the ability of L-ascorbic acid to scavenge ROS, which highly accumulated under high temperature and caused the failure of pollen germination and subsequent fruit-setting reduction.

3.3 Stress markers (hydrogen peroxide, malondialdehyde and proline)

Treatment with L-ascorbic acid (0.5 mM) significantly reduced the accumulation of H_2O_2 and MDA concentration in tomato plants grown under heat-stress conditions, as shown in **Fig 4**. The reduction in H_2O_2 level was approximately 57% in the treated plants relative to the control **(Fig 4A)**, while the MDA concentration decreased by 44% **(Fig 4B)**. Although the observed changes in proline content in tomato leaves after L-Ascorbic acid treatment were insignificant **(Fig 4C)**, the reduction in hydrogen peroxide concentration could be due to the ability of L-ascorbic acid to scavenge the prooxidant and activate antioxidant enzymes, which mitigated the damage to cellular membranes and lipid peroxidation, causing low concentrations of MDA. These results were similar to those reported by Alayafi (2020) in which tomato seedlings were exposed to L-ascorbic acid.

3.4 Antioxidant enzymes

The application of L-ascorbic acid (0.5 mM) as a foliar spray on tomato plants under heat stress has been found to significantly increase the activities of CAT, POD, APX and PPO enzymes. The results, presented in **Fig 5**, indicate that the treated plants showed about 66.67% increase in CAT activity, 88.33% increase in POD activity, 162.5% increase in APX activity and 224.88% increase in PPO activity compared to the control plants. Heat stress can cause direct damage to plant cells and tissues, which may include protein denaturation, aggregation and raised membrane lipids fluidity (Gupta et al 2013). In addition, it can also cause indirect damage by causing inactivation of chloroplast and mitochondria enzymes, protein degradation, protein synthesis inhibition and loss of membrane integrity (Wahid 2007). L-Ascorbic acid plays a significant function in protecting plants from these direct and indirect heat stress injuries by reducing the oxidation of lipids and proteins, which is known to occur under various abiotic stresses (Naz et al 2016). The current work suggests that the elevated activities of antioxidant enzymes in the L-ascorbic acid-treated plants, compared to the control group, could be attributed to the ability of L-ascorbic acid to protect antioxidant enzyme structures from oxidative damage. Similar results have been reported in cotton plants and fenugreek plants, where the application of L-ascorbic acid under heat stress and heavy metal stress conditions, respectively, improved the activities of antioxidant enzymes (Kamal et al 2017, Fatima et al 2020).

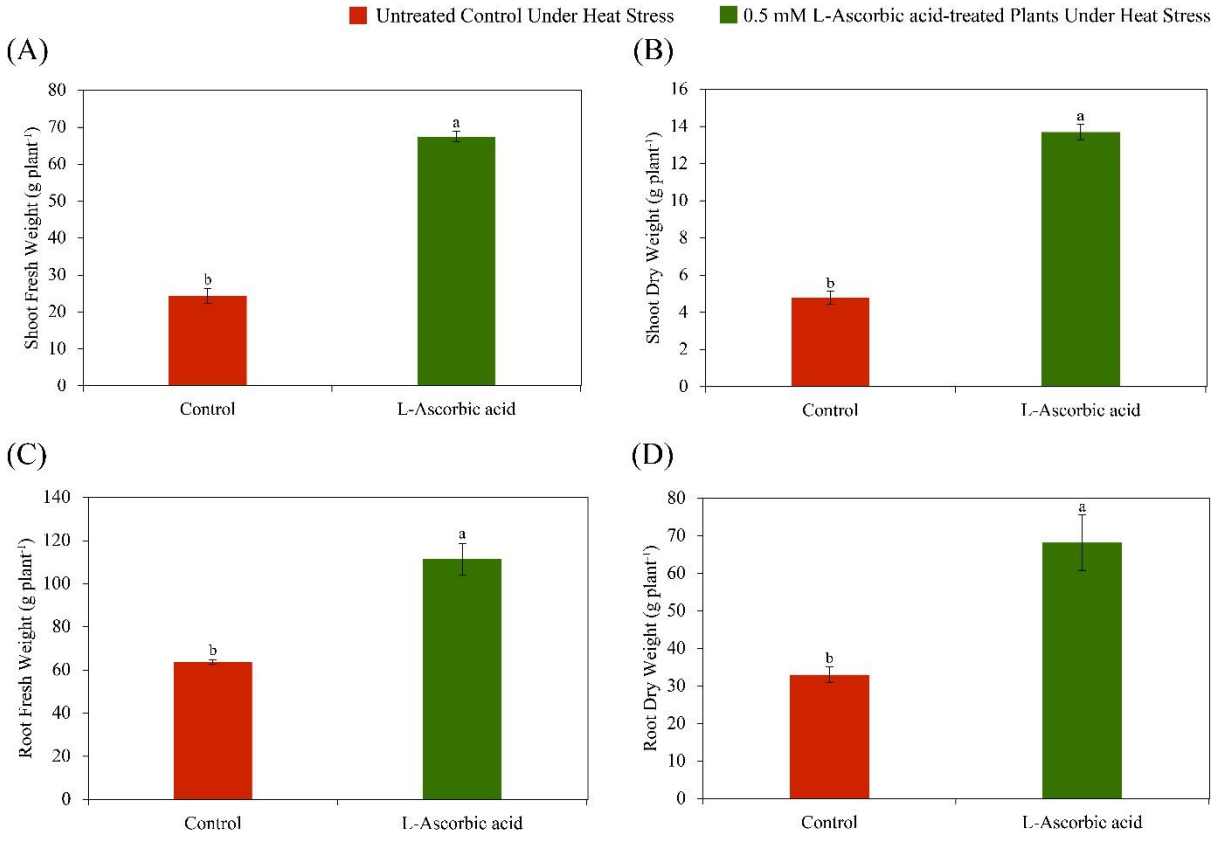


Fig 2. Effect of L-ascorbic acid (0.5 mM) exogenous application on vegetative growth parameters of tomato plants grown under heat stress conditions (A) shoot fresh weight (g plant^{-1}), (B) shoot dry weight (g plant^{-1}), (C) root fresh weight (g plant^{-1}) and (D) root dry weight (g plant^{-1}). Data were plotted as mean ± standard error (SE) (n=3). Different small letters on the bars refer to statistical differences between the control and the L-ascorbic acid-treated plants using the t-test at $P \leq 0.05$

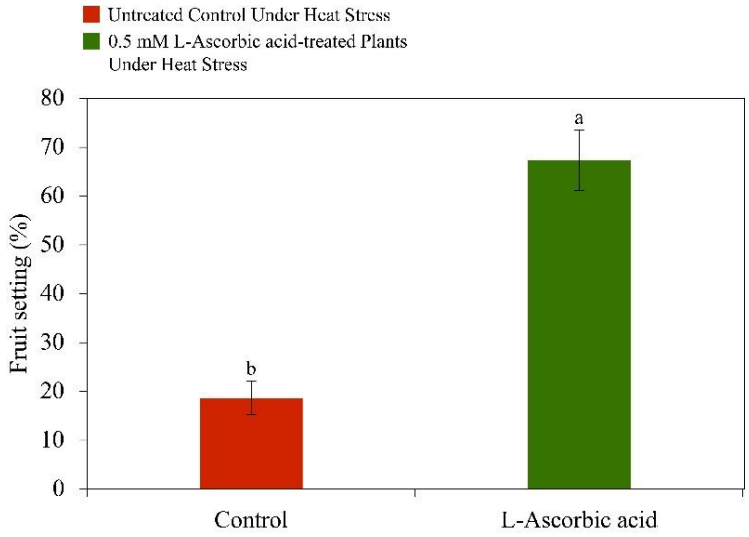


Fig 3. Influence of L-ascorbic acid (0.5 mM) on fruit setting percentage of tomato plants under heat stress. Data were plotted as mean ± standard error (SE) (n=5). Different small letters on the bars refer to statistical differences between the control and the L-ascorbic acid-treated plants using the t-test at $P \leq 0.05$

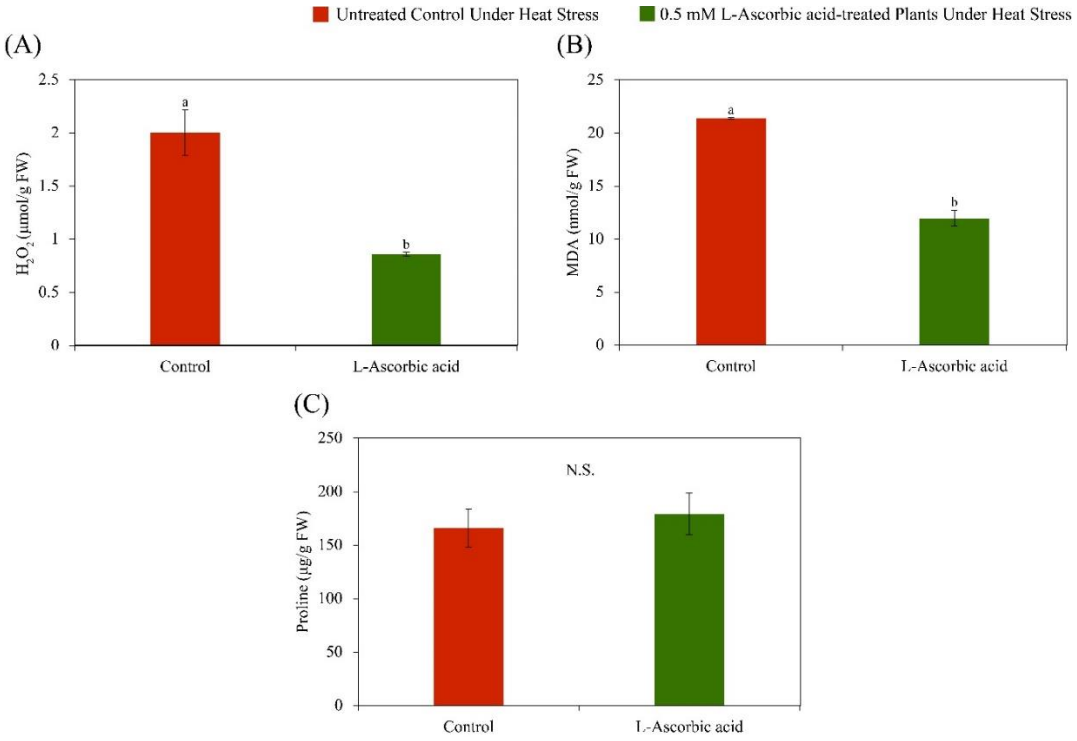


Fig 4. Influence of L-ascorbic acid (0.5 mM) on (A) hydrogen peroxide (µmol/g FW), (B) malondialdehyde (MDA) (nmol/g FW) and (C) proline (µg/g FW) in leaves of tomato plants under heat stress. Data were plotted as mean ± standard error (SE) (n=3). Different small letters on the bars refer to statistical differences between the control and the L-ascorbic acid-treated plants using the t-test at $P \leq 0.05$, but N.S. indicates differences that aren't statistically significant

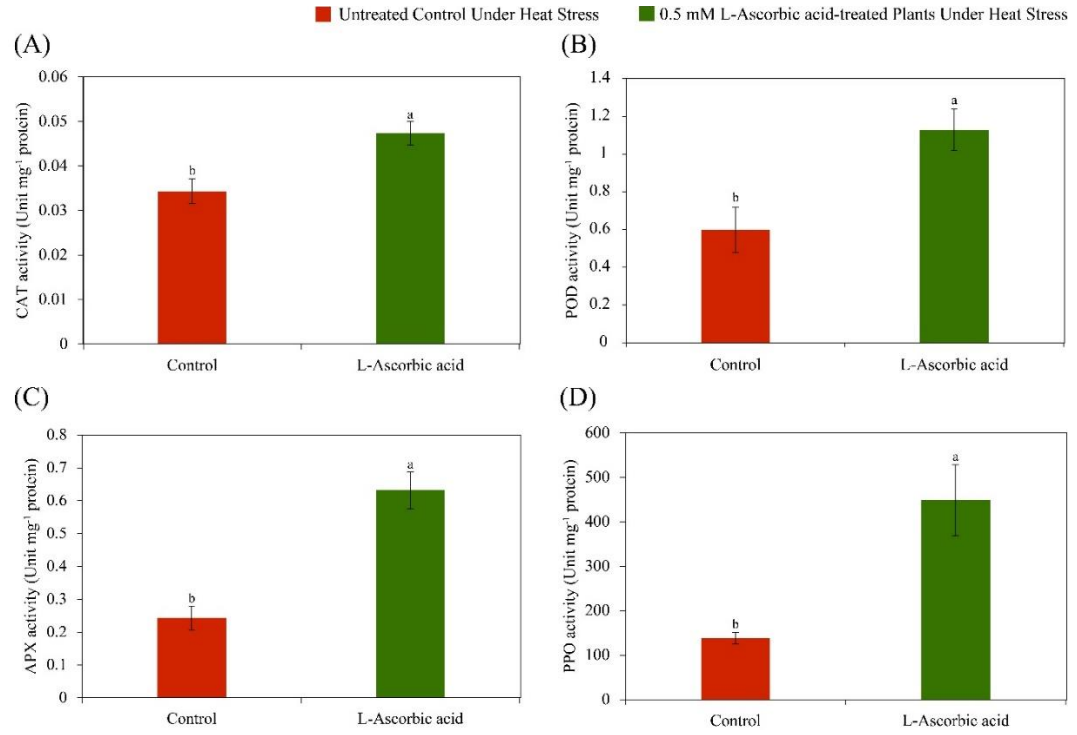


Fig 5. Influence of L-ascorbic acid (0.5 mM) on (A) catalase (CAT) activity (Unit mg^{-1} protein), (B) peroxidase (POD) activity (Unit mg^{-1} protein), (C) ascorbate peroxidase (APX) activity (Unit mg^{-1} protein) and (D) polyphenol oxidase (PPO) activity (Unit mg^{-1} protein) in leaves of tomato plants under heat stress. Data were plotted as mean ± standard error (SE) (n=3). Different small letters on the bars refer to statistical differences between the control and the L-ascorbic acid-treated plants using the t-test at $P \leq 0.05$

3.5 Molecular Docking

3.5.1 Molecular structure of antioxidants enzymes

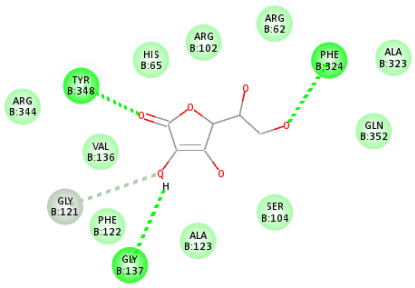
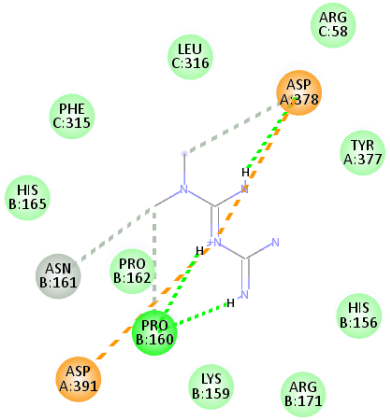
In order to investigate how L-ascorbic acid protects antioxidant enzymes and prevents heat stress damage in tomato plants, we conducted molecular *in silico* studies. Homology modeling was performed to obtain the 3D structure of *Sl*CAT using *Bacillus pumilus* catalase. SBP and *Sl*PPO were obtained from the Protein Data Bank.

3.5.2 Molecular Docking Analysis

Using AutoDock Vina 1.2.0, the interactions between the selected active sites of target proteins and their ligands were determined. To validate the molecular docking between L-ascorbic acid and *Sl*CAT, SBP and *Sl*PPO, molecular docking was performed using metformin for *Sl*CAT, guaiacol for SBP and both dopamine and phloretin for *Sl*PPO. To ensure the accuracy of the AutoDock Vina method, we compared the interactions of the known ligand for each enzyme with the selected active site to the interactions of the tested ligand (L-ascorbic acid). To validate the results of the AutoDock Vina method, we docked the known ligands for each enzyme with the active site and compared their interaction with L-ascorbic acid.

The results of molecular docking between the ligands (L-Ascorbic acid and Metformin) and the modeled *Sl*CAT are presented in **Table 1**. The table shows the binding energy, type of interactions, interacted residues, bond length and 2D interactions between the ligands and the modeled *Sl*CAT. The close binding energy (-6.4 Kcal/mol) of L-ascorbic acid to the known CAT activator (Metformin: -5.9 Kcal/mol) is also shown in **Table 1**. The 3D interactions of L-ascorbic acid and Metformin with *Sl*CAT can be visualized in **Fig 6**.

Table 1. Interactions between modeled *Solanum lycopersicum* catalase (*Sl*CAT) and its activators (L-ascorbic acid and the known activator, metformin)

Ligands	2D Interactions	Type of Bonds (Interaction)	Interacted residues	Bond Length (A°)	Binding Energy (Kcal/mol)
L-Ascorbic acid		Hydrogen bonds	Phe B:324	2.29	-6.4
			Tyr B:348	1.97	
			Gly B:137	3.04	
		Carbon hydrogen bond	Gly B:121	3.6	
Metformin		Attractive charge	Asp A:378	5.45	-5.9
			Asp A:391	4.26	
		Hydrogen bonds	Pro B:160 (2)	1.9	
				2.29	
			Asp A:378	2.39	
		Carbon hydrogen bonds	Asp A:378	3.6	
			Pro B:160	3.36	
			Asn B:161	3.8	

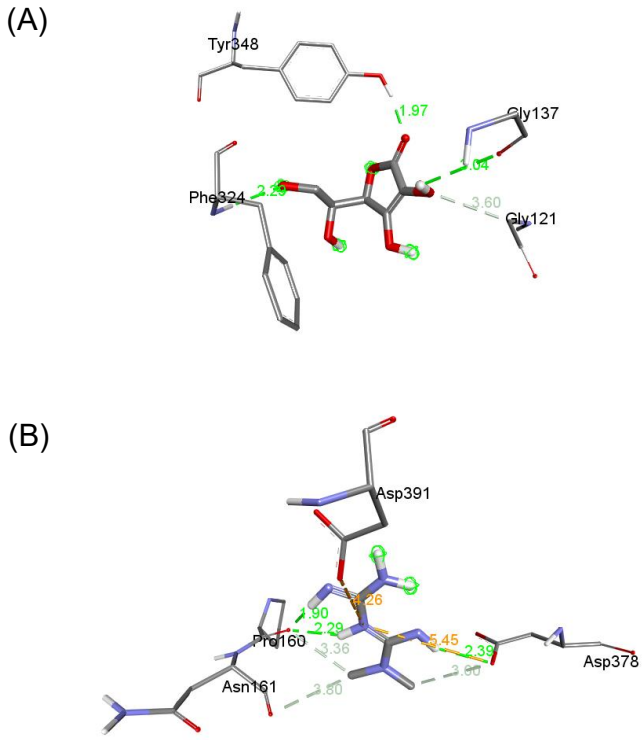


Fig 6. 3D interactions of (A) L-ascorbic acid and (B) metformin with the modeled *Solanum lycopersicum* Catalase (*Sl*CAT). Green dashes refer to hydrogen bonds, gray dashes refer to carbon-hydrogen bonds and attractive charges interactions in orange dashes. Ligands in the thicker gray sticks and the enzyme residues in the thinner gray sticks. Numbers in the dashes refer to the bond length in A°

Metformin activates CAT by interacting directly with its active site *via* hydrogen bonds and does not affect the catalase expression level. The active site of *Sl*CAT (His65 and Asn138) and the binding site (Tyr348) were obtained from the alignment of the template with its substrate. In contrast, L-ascorbic acid interacts with different residues on chain B, forming hydrogen bonds with Phe324, Tyr348, Gly137 and carbon hydrogen bond with Gly121 and also van der Waals forces with His65, Arg102, Arg62, Ala323, Gln352, Ser104, Ala123, Phe122, Val136 and Arg344 **(Table 1)**. On the other hand, Metformin interacts with Asp378 and Asp391 (chain A residues), forming attractive charges interaction and three hydrogen bonds, two with Pro160 (chain B residue) and one with Asp378 (chain A residue). Additionally, Metformin forms carbon-hydrogen bonds with Asp378 (chain A residue), Pro160 and Asn161 (chain B residues) and van der Waals forces with Tyr377 (chain A residue), His156, Arg171, Lys159, Pro162, His165 (chain B residues), Phe315, Leu316, Arg58 (chain C residues) **(Table 1)**. Although L-ascorbic acid and Metformin interact with different residues, the binding energies (L-ascorbic acid: -6.4 Kcal/mol; Metformin: -5.9 Kcal/mol) are highly close. Therefore, L-ascorbic acid has a higher affinity towards the modeled *Sl*CAT active site and is potentially capable of directly activating it.

Molecular docking analysis (**Table 2**) shows the type of interactions, interacted residues, bond length, binding energy and 2D interactions between the SBP and two ligands: L-ascorbic acid and Guaiacol. The molecular docking analysis showed that L-ascorbic acid and guaiacol have -5.2 Kcal/mol and -4.9 Kcal/mol binding energies, respectively. This indicates that L-ascorbic acid has a higher affinity to the active site of SBP than Guaiacol, which is a known peroxidase substrate.

The active site of the SBP comprises of His42, Arg38, Pro139 and the haem group, where His42 and Arg38 are the catalytic residues. The molecular docking results reveal that L-ascorbic acid and Guaiacol interact with the SBP target protein through various binding interactions. L-ascorbic acid interacts with the SBP target protein through hydrogen bonds formation with Arg38, Ser73, Arg175, Leu68 (2), Hem350, carbon hydrogen bond interactions with Pro141 and van der Waals forces with His42, Pro139, Pro69, Ala140 and Thr178. Similarly, Guaiacol interacts with the SBP

target protein through two hydrogen bonds with Arg38, Pi-alkyl interaction with His42, Hem350 (3), Pro69, Pro141 and van der Waals forces with Pro139, Ser73, Ala140 and Arg175. The 3D interactions of L-ascorbic acid and guaiacol with SBP are illustrated in **Fig 7**. Interestingly, the higher affinity of L-ascorbic acid to bind to the active site of SBP than its substrate (Guaiacol) suggests that L-ascorbic acid could activate SBP directly.

In the current investigation, the molecular docking of three ligands (L-Ascorbic acid, dopamine and phloretin) with *Sl*PPO was investigated, with a focus on residues, bond length and 2D interactions. The results obtained from the molecular docking are illustrated in **Table 3** and indicate that L-ascorbic acid has a -6.1 Kcal/mol binding energy, which is higher than the dopamine binding energy (-5.7 Kcal/mol) but lower than the phloretin binding energy (-8.4 Kcal/mol). The 3D interactions of the three ligands with *Sl*PPO are illustrated in **Fig 8**.

Table 2. Interactions between the soybean peroxidase (SBP) and its activators (L-ascorbic acid and the known substrate, guaiacol)

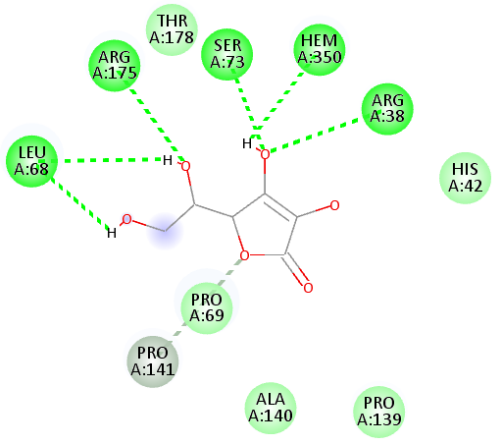
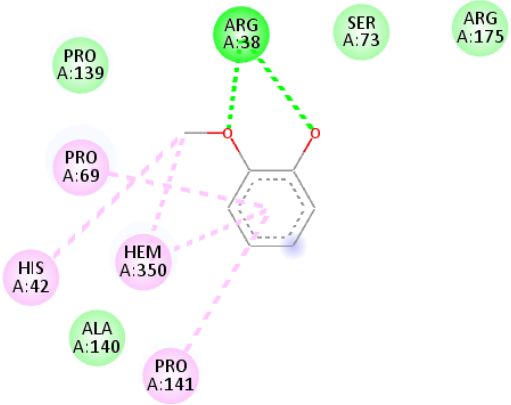
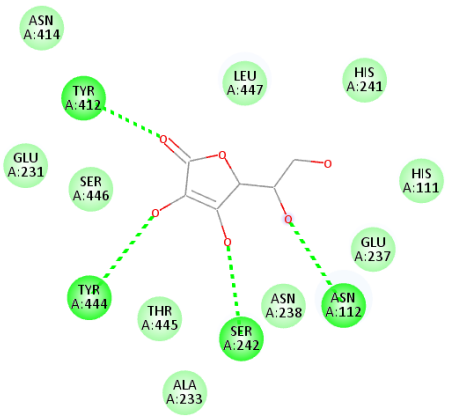
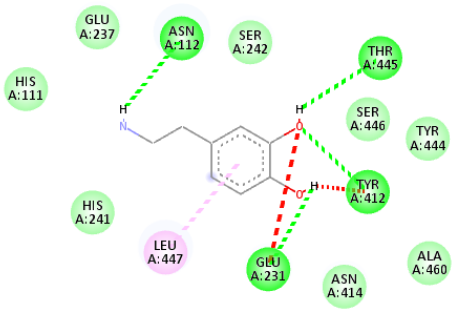
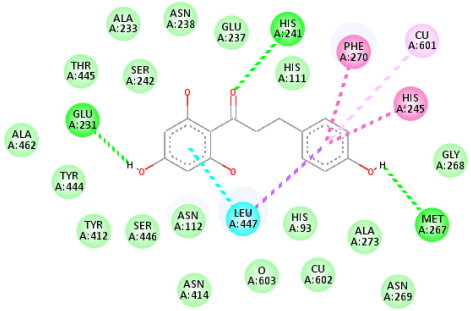
Ligands	2D Interactions	Type of Bonds (Interaction)	Interacted residues	Bond Length (A°)	Binding Energy (Kcal/mol)
L-Ascorbic acid		Hydrogen bonds	Arg38	2.28	-5.2
			Ser73	1.86	
			Arg175	2.54	
			Leu68 (2)	3.08	
				2.24	
			Hem350	2.23	
		Carbon hydrogen bond	Pro141	3.53	
Guaiacol		Hydrogen bonds	Arg38 (2)	2.18	-4.9
				2.06	
		Pi-Alkyl	His42	4.62	
			Hem350 (3)	4.41	
				4.53	
				4.42	
			Pro69	4.76	
			Pro141	5.17	
					

Table 3. Interactions between *Solanum lycopersicum* polyphenol oxidase (*Sl*PPO) and its ligands (L-ascorbic acid, dopamine and phloretin)

Ligands	2D Interactions	Type of Bonds (Interaction)	Interacted residues	Bond Length (A°)	Binding Energy (Kcal/mol)
L-Ascorbic acid		Hydrogen bonds	Asn112	2.47	-6.1
			Ser242	2.24	
			Tyr412	2.24	
			Tyr444	2.71	
Dopamine		Hydrogen bonds	Asn112	2.28	-5.7
			Tyr412	2.63	
			Thr445	2.8	
			Glu231	2.58	
		Pi-Alkyl	Leu447	4.42	
		Unfavorable Donor-Donor	Tyr412	1.04	
		Unfavorable Acceptor-Acceptor	Glu231	2.99	
Phloretin		Hydrogen bonds	His241	2.98	-8.4
			Met267	2.97	
			Glu231	1.97	
		Pi-Donor hydrogen bond	Leu447	2.84	
		Pi-sigma	Leu447	3.73	
		Pi-Pi T-shaped	His245	4.73	
			Phe270	4.63	
		Pi-Alkyl	Leu447	4.59	
			Cu601	5.33	
					

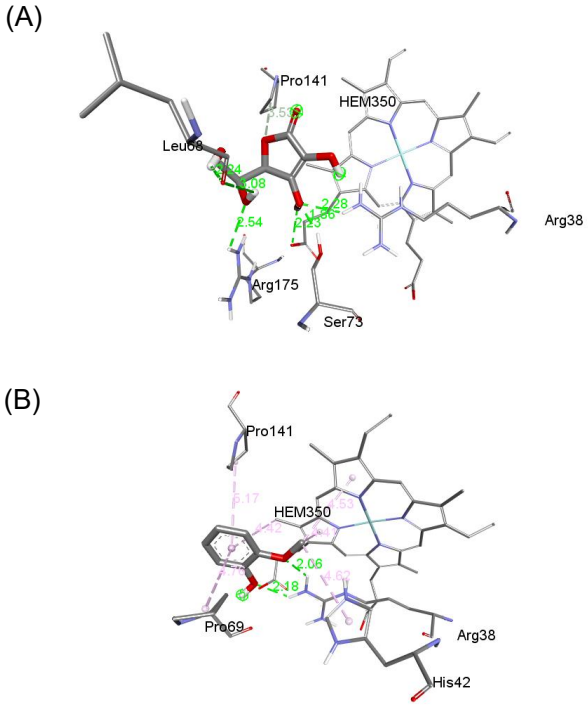


Fig 7. 3D interactions of (A) L-ascorbic acid and (B) guaiacol with the soybean peroxidase (SBP). The green dashes refer to the hydrogen bonds. The gray dash refers to the carbon-hydrogen bond. The pink dashes refer to pi-alkyl interaction. Ligands in the thicker gray sticks and the enzyme residues in the thinner gray sticks. Numbers in the dashes refer to the bond length in A°

Dopamine and phloretin have been found to interact with *Sl*PPO directly, activating it. This information was used to validate the molecular docking method and to compare their binding energy to the binding of L-ascorbic acid to *Sl*PPO. The active site of holo-*Sl*PPO consists of Phe270, His245, Ser242, His241, Glu237, His283, His93, His111, His120 and the two copper ions (CuA and CuB). L-Ascorbic acid (-6.1 Kcal/mol) formed hydrogen bonds with Asn112, Ser242, Tyr412, Tyr444 and van der Waals forces with His111, Glu237, Asn238, Ala233, Thr445, Ser446, Glu231, Asn414, Leu447 and His241. Dopamine (-5.7 Kcal/mol) formed hydrogen bonds with Asn112, Tyr412, Thr445, Glu231 and pi-alkyl with Leu447. It also formed unfavorable donor-donor with Tyr412 and unfavorable acceptor-acceptor with Glu231 and van der Waals forces with His111, Glu237, Ser242, Ser446, Tyr444, Ala460, Asn414 and His241. Phloretin (-8.4 Kcal/mol) formed hydrogen bonds with His241, Met267, Glu231 and pi-donor hydrogen bond and pi-sigma with Leu447. It also formed pi-pi T-shaped with His245, Phe270 and Pi-alkyl with Leu447, Cu601 and van der Waals forces with His111, Glu237, Asn238, Ala233, Thr445, Ser242, Ala462, Tyr444, Tyr412, Ser446, Asn112, Asn414, His93, Ala273, Asn269, Gly268, Cu602 and O603. Phloretin has a higher affinity to the *Sl*PPO active site than L-ascorbic acid, which has a higher affinity than dopamine. It is well known that L-ascorbic acid inhibits PPO directly and prevents fruit browning and it also protects the activity of potato PPO during the extraction of the enzyme. Therefore, the higher *Sl*PPO activity in L-ascorbic acid-treated plants in the current investigation may be due to the protective function of L-ascorbic acid or the upregulation of *Sl*PPO-related genes.

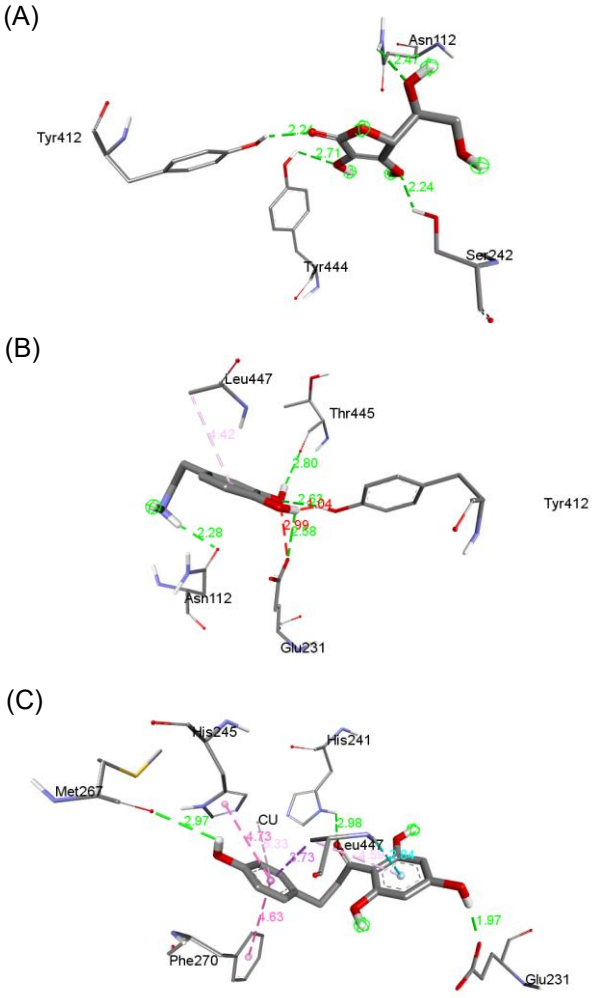


Fig 8. 3D interactions of (A) L-Ascorbic acid, (B) dopamine and (C) phloretin with the *Solanum lycopersicum* polyphenol oxidase (*Sl*PPO). The green dashes refer to the hydrogen bonds. The cyan dashes refer to the pi-donor hydrogen bond. The red dashes refer to unfavorable interactions. The other dashes refer to hydrophobic interactions. Ligands in the thicker gray sticks and the enzyme residues in the thinner gray sticks. Numbers in the dashes refer to the bond length in A°

The results of the molecular docking analysis suggest that the rise in antioxidant enzyme activities in plants treated with L-ascorbic acid may be due to direct interactions between the enzymes (CAT and POD) and L-ascorbic acid, acting as an activator. In the case of PPO, the increase in activity may be due to indirect interactions.

4 Conclusions

Tomato plants that are exposed to high temperatures undergo oxidative damage, which can limit plant growth and reduce fruit setting. However, the application of L-ascorbic acid can help mitigate the harmful effects of high-temperature stress. This is because L-ascorbic acid can increase and protect the activities of CAT, POD, APX and PPO enzymes, while also decreasing ROS and lipid peroxidation in the tomato leaves. As a result, L-ascorbic acid can promote growth and increase fruit setting. It appears that L-ascorbic acid can activate antioxidant enzymes, making it a useful treatment for mitigating high-temperature stress-induced damage to tomato plants. To achieve the best results, it is recommended to spray L-ascorbic acid (0.5 mM) during the pollen development stage when cultivating tomato plants in high-temperature conditions during the summer season. This can help improve the yield of the plants.

References

Alayafi AAM (2020) Exogenous ascorbic acid induces systemic heat stress tolerance in tomato seedlings: Transcriptional regulation mechanism. *Environmental Science and Pollution Research* 27, 19186-19199. https://doi.org/10.1007/s11356-019-06195-7

Alsamir M, Mahmood T, Trethowan R, et al (2021) An overview of heat stress in tomato (*Solanum lycopersicum* L.). *Saudi Journal of Biological Sciences* 28, 1654–1663. https://doi.org/10.1016/j.sjbs.2020.11.088

Arrigoni O (1994) Ascorbate system in plant development. *Journal of Bioenergetics and Biomembranes* 26, 407-419. https://doi.org/10.1007/BF00762782

Athar HR, Khan A, Ashraf M (2009) Inducing salt tolerance in wheat by exogenously applied ascorbic acid through different modes. *Journal of Plant Nutrition* 32, 1799-1817. https://doi.org/10.1080/01904160903242334

UniProt Consortium (2019) A worldwide hub of protein knowledge. *Nucleic Acids Research* 47, D506-D515. https://doi.org/10.1093/nar/gky1049

Benkert P, Biasini M, Schwede T (2011) Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics* 27, 343-350. https://doi.org/10.1093/bioinformatics/btq662

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Carmody N, Goñi O, Łangowski Ł, et al (2020) *Ascophyllum nodosum* extract biostimulant processing and its impact on enhancing heat stress tolerance during tomato fruit set. *Frontiers in Plant Science* 11, 807. https://doi.org/10.3389/fpls.2020.00807

Celi GEA, Gratão PL, Lanza MGDB, et al (2023) Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. *Plant Physiology and Biochemistry* 202, 107970. https://doi.org/10.1016/j.plaphy.2023.107970

De Storme N, Geelen D (2014) The impact of environmental stress on male reproductive development in plants: Biological processes and molecular mechanisms. *Plant, Cell and Environment* 37, 1-18. https://doi.org/10.1111/pce.12142

Djanaguiraman M, Prasad PVV, Al-Khatib K (2011) Ethylene perception inhibitor 1-MCP decreases oxidative damage of leaves through enhanced antioxidant defense mechanisms in soybean plants grown under high temperature stress. *Environmental and Experimental Botany* 71, 215-223. https://doi.org/10.1016/j.envexpbot.2010.12.006

Driedonks N, Rieu I, Vriezen WH (2016) Breeding for plant heat tolerance at vegetative and reproductive stages. *Plant Reproduction* 29, 67-79. https://doi.org/10.1007/s00497-016-0275-9

Eberhardt J, Santos-Martins D, Tillack AF, et al (2021) AutoDock Vina 1.2.0: New docking methods, expanded force field and python bindings. *Journal of Chemical Information and Modeling* 61, 3891-3898. https://doi.org/10.1021/acs.jcim.1c00203

Farooq A, Bukhari SA, Akram NA, et al (2020) Exogenously applied ascorbic acid-mediated changes in osmoprotection and oxidative defense system enhanced water stress tolerance in different cultivars of safflower (*Carthamus tinctorious* L.). *Plants* 9, 104. https://doi.org/10.3390/plants9010104

Fatima A, Farid M, Alharby HF, et al (2020) Efficacy of fenugreek plant for ascorbic acid assisted phytoextraction of copper (Cu); A detailed study of Cu induced morpho-physiological and biochemical alterations. *Chemosphere* 251, 126424. https://doi.org/10.1016/j.chemosphere.2020.126424

Fortunato S, Lasorella C, Dipierro N, et al (2023) Redox signaling in plant heat stress response. *Antioxidants* 12, 605. https://doi.org/10.3390/antiox12030605

Foyer CH, Noctor G (2011) Ascorbate and glutathione: The heart of the redox hub. *Plant Physiology* 155, 2-18. https://doi.org/10.1104/pp.110.167569

Foyer CH, Noctor G (2013) Redox signaling in plants. *Antioxidants and Redox Signaling* 18, 2087-2090. https://doi.org/10.1089/ars.2013.5278

Gupta NK, Agarwal S, Agarwal VP, et al (2013) Effect of short-term heat stress on growth, physiology and antioxidative defence system in wheat seedlings. *Acta Physiologiae Plantarum* 35, 1837-1842. https://doi.org/10.1007/s11738-013-1221-1

Hammerschmidt R, Nuckles EM, Kuc J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology* 20, 73-82. https://doi.org/10.1016/0048-4059(82)90025-X

Hasanuzzaman M, Bhuyan MHMB, Anee TI, et al (2019) Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants* 8, 384. https://doi.org/10.3390/antiox8090384

Hasanuzzaman M, Hossain MA, Fujita M (2011) Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnology Reports* 5, 353-365. https://doi.org/10.1007/s11816-011-0189-9

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125, 189-198. https://doi.org/10.1016/0003-9861(68)90654-1

Henriksen A, Mirza O, Indiani C, et al (2001) Structure of soybean seed coat peroxidase: A plant peroxidase with unusual stability and haem-apoprotein interactions. *Protein Science* 10, 108-115. https://doi.org/10.1110/ps.37301

Hoagland DR, Arnon DI (1950) The Water-Culture Method for Growing Plants Without Soil. Vol. 347, Circular California Agricultural Experiment Station, 2nd (ed), 32 pp.

Jahan MS, Shu S, Wang Y, et al (2019) Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biology* 19, 414. https://doi.org/10.1186/s12870-019-1992-7

Kamal MA, Saleem MF, Shahid M, et al (2017) Ascorbic acid triggered physiochemical transformations at different phenological stages of heat-stressed Bt cotton. *Journal of Agronomy and Crop Science* 203, 323-331. https://doi.org/10.1111/jac.12211

Kampatsikas I, Bijelic A, Rompel A (2019) Biochemical and structural characterization of tomato polyphenol oxidases provide novel insights into their substrate specificity. *Scientific Reports* 9, 4022. https://doi.org/10.1038/s41598-019-39687-0

Kugblenu YO, Danso EO, Ofori K, et al (2013) Screening tomato genotypes for adaptation to high temperature in West Africa. *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science* 63, 516-522. https://doi.org/10.1080/09064710.2013.813062

Kumar RR, Goswami S, Gadpayle KA, et al (2014) Ascorbic acid at pre-anthesis modulate the thermotolerance level of wheat (*Triticum aestivum*) pollen under heat stress. *Journal of Plant Biochemistry and Biotechnology* 23, 293-306. https://doi.org/10.1007/s13562-013-0214-x

Lee C, Harvey JT, Qin K, et al (2023) Physio-biochemical responses of grafted tomatoes differing in thermotolerance to heat stress and recovery. *Scientia Horticulturae*, 308, 111546. https://doi.org/10.1016/j.scienta.2022.111546

Mubarok S, Jadid N, Widiastuti A, et al (2023) Parthenocarpic tomato mutants, *iaa9-3* and *iaa9-5*, show plant adaptability and fruiting ability under heat-stress conditions. *Frontiers in Plant Science* 14, 1090774. https://doi.org/10.3389/fpls.2023.1090774

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in Spinach chloroplasts. *Plant and Cell Physiology* 22, 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Naz H, Akram NA, Ashraf M (2016) Impact of ascorbic acid on growth and some physiological attributes of cucumber (*Cucumis sativus*) plants under water-deficit conditions. *Pakistan Journal of Botany* 48, 877-883.

Oktay M, Küfreviolu I, Kocaçalişkan I, et al (1995) Polyphenoloxidase from Amasya Apple. *Journal of Food Science* 60, 494-496. https://doi.org/10.1111/j.1365-2621.1995.tb09810.x

Parvin K, Nahar K, Hasanuzzaman M, et al (2020) Exogenous vanillic acid enhances salt tolerance of tomato: Insight into plant antioxidant defense and glyoxalase systems. *Plant Physiology and Biochemistry* 150, 109-120. https://doi.org/10.1016/j.plaphy.2020.02.030

Peters W, Beck E, Piepenbrock M, et al (1997) Cytokinin as a negative effector of phosphoenolpyruvate carboxylase induction in *Mesembryanthemum crystallinum*. *Journal of Plant Physiology* 151, 362-367. https://doi.org/10.1016/S0176-1617(97)80266-0

Ruan YL, Jin Y, Yang YJ, et al (2010) Sugar input, metabolism and signaling mediated by invertase: Roles in development, yield potential and response to drought and heat. *Molecular Plant* 3, 942-955. https://doi.org/10.1093/mp/ssq044

Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants: Biosynthesis and function. *Critical Reviews in Plant Sciences* 19, 267-290. https://doi.org/10.1080/07352680091139231

Troll W, Lindsley J (1955) A photometric method for the determination of proline. *The Journal of Biological Chemistry* 215, 655-660. https://rb.gy/l9qln0

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science* 151, 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Vijayakumar A, Shaji S, Beena R, et al (2021) High temperature induced changes in quality and yield parameters of tomato (*Solanum lycopersicum* L.) and similarity coefficients among genotypes using SSR markers. *Heliyon* 7, e05988 https://doi.org/10.1016/j.heliyon.2021.e05988

Wahid A (2007) Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of Plant Research* 120, 219-228. https://doi.org/10.1007/s10265-006-0040-5

Wahid A, Gelani S, Ashraf M, et al (2007) Heat tolerance in plants: An overview. *Environmental and Experimental Botany* 61, 199-223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Waterhouse A, Bertoni M, Bienert S, et al (2018) SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Research* 46, W296-W303. https://doi.org/10.1093/nar/gky427

Wolkovich EM, Cook BI, Allen JM, et al (2012) Warming experiments underpredict plant phenological responses to climate change. *Nature* 485, 494-497. https://doi.org/10.1038/nature11014

Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: Uncovering the weakest links. *Journal of Experimental Botany* 61, 1959-1968. https://doi.org/10.1093/jxb/erq053