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Review

Phytochelatins: Advances in Tomato Research

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Abstract: Tomato (*Solanum lycopersicum*), which is considered one of the more important and widely cultivated crop members of the family Solanaceae, exhibits numerous biochemical mechanisms to alleviate the stress produced by various biotic and abiotic factors. Many researchers have found that phytochelatins (PCs) play an important role in these stress-alleviating mechanisms and, therefore, contribute significantly to the plant's coping strategies, particularly under heavy metal exposure. Ongoing research has extensively investigated tomato genotypes in plant stress research, with a particular focus on heavy metal stress. The production of PCs, synthesized from glutathione, is regulated by various factors and different stressors. Here, we aim to provide an overview of the panorama regarding the synthesis of PCs in tomato under different environmental conditions and experimental settings, as well as provide information on their broader roles in biotechnology and modulating plant tolerance and responses across diverse stress conditions and treatments within the context of tomato research.

Keywords: cadmium exposure; crops; environmental stress; heavy metals; phytochelatins; plant tolerance; Solanaceae; stress tolerance; tomato

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1. Introduction

Tomato (*Solanum lycopersicum*) is an important crop in the world economy and agriculture, thus making this species preferable for research because of its adaptability and genetic tractability. The agrifood systems provide livelihood to huge populations globally. Environmental changes and unsustainable practices pose challenges, affecting the successful implementation of resilient agricultural practices [1]. The incorporation of socio-economic aspects into tomato commercialization implies that novel and sustainable practices could enhance productivity and profitability, especially in developing regions [2]. Well-grown and commercially valuable tomato plants thus provide nutrition and benefit farmers and communities in their daily lives. Tomato is also widely recognized as a model for genetic and biochemical studies due to its well-characterized genome. Hence, there is an ongoing need for research and development on tomato cultivation.

This member of the Solanaceae family has been extensively studied for its interactions with environmental stressors, including heavy metals, which threaten plant metabolism and physiology. Ongoing research has extensively investigated biochemical, molecular, and physiological insights related to environmental stresses over the past decades in different plant species including tomato (see some of our recent investigations—Marques et al. [3–5]—and exemplary references therein). Furthermore, we previously reviewed important information about the synthesis of phytochelatins (PCs—cysteine-rich peptides

which are potentially involved in modulating tolerance and response to various environmental stressors, particularly heavy metal treatments) in plants, the particularly important role that PCs play in mediating plant tolerance to cadmium (Cd) stress, along with other important related aspects [5]. Other authors have reviewed aspects related to biosynthesis, structure, and role of PCs in metal(loid) transport and sequestration in plants [6] as well as PCs involvement in heavy metal detoxification [7]. Although heavy metal-induced yield losses in crops are not always evident, understanding tolerance and detoxification mechanisms is vital for maintaining crop quality under combined stress conditions. PCs play a crucial role in mitigating physiological damage, maintaining metal homeostasis, and modulating plant tolerance to metal stress. This emphasis on tolerance is consistent with the terminology and scope of prior studies and multiple reviews cited [5–7], which highlight the significance of PCs for stress mitigation and detoxification along with the potential development of stress-resilient plants.

Given the importance of this plant species and PCs, the aim of this review is to summarize the current panorama concerning the participation of PCs in the tomato's response to different environmental conditions and stresses. This overview, therefore, provides information on what is currently known regarding PC synthesis and research topics (Figure 1), in addition to potential strategic significance in mediating the tomato's resilience against environmental challenges, and information for future research in this field. A comprehensive literature search was conducted using the Web of Science and Scopus databases to identify, retrieve, and review relevant articles on the research topic. This approach ensured the inclusion of a wide range of high-quality, peer-reviewed studies to support the article's content.

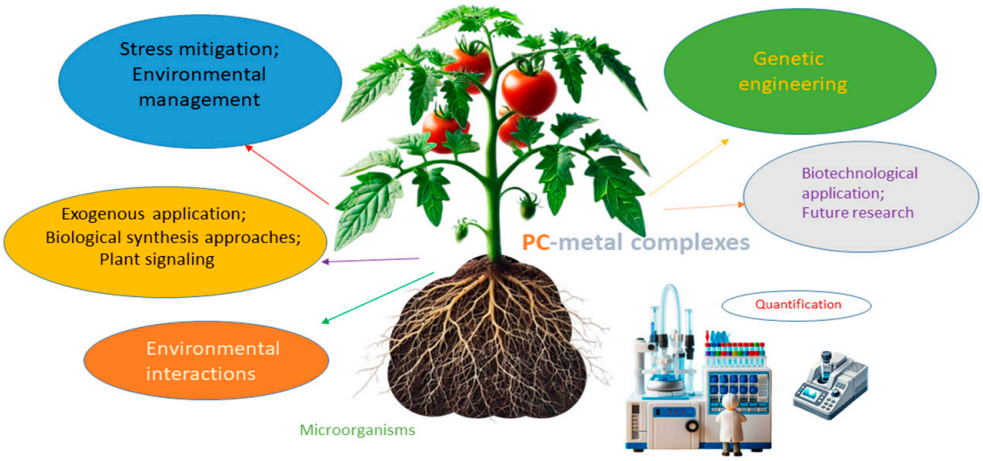

Figure 1. Overview of tomato research, highlighting multifaceted key research topics to which PCs are related.

2. Response Patterns to Cadmium Exposure with Emphasis on Phytochelatins in Tomato

A significant number of pioneering studies in this research field were primarily conducted using Cd as a stress factor. Furthermore, different observations of the Cd response have been reflected in the varying patterns of PC production under different treatment conditions, while always focusing on the regulation and role of PCs in tomato cells from either earlier or more recent studies. For example, the importance of glutathione (GSH) availability in the PC-related detoxification process in tomato cell suspension cultures has been clearly shown over 30 years ago [8,9]. In another investigation, Cd-PC complexes in roots of some tomato plants were shown to provide a protective effect against further

damage, considering that these plants exhibited higher Cd and PC levels in root tissue compared to leaf tissue [10].

PC biosynthesis has been found to be differentially regulated by Cd concentration and different treatments. Lower concentrations resulted in higher PC levels, while at high doses of Cd, PC accumulation was less effective, resulting in growth inhibition. At high Cd concentrations, PC synthesis in tomato roots decreased by 54%, accompanied by disrupted redox balance and heightened oxidative stress. Endogenous hydrogen sulfide (H_2S) also regulated PC biosynthesis, as elevated H_2S levels were accompanied by enhanced PC synthesis, thereby alleviating Cd toxicity in tomato, even under low dose of Cd treatment [11]. In another study, the exogenous application of selenium (Se) increased PC contents in leaves of Cd-accumulating cherry tomato cultivars (particularly in the cultivar with higher Cd accumulation), which, in turn, potentially facilitated Cd chelation [12].

A key way to investigate stress response on this research field is with the use of tomato counterparts with different levels of tolerance/resistance to the stress. PC production and Cd tolerance may vary widely among studies in tomato, demonstrating a range of adaptive responses that need to be characterized. Cd-binding PCs have been shown to be synthesized in tomato cell lines rapidly after Cd exposure, particularly in the tolerant ones, where PCs complex with Cd, forming Cd-PC complexes that sequester the Cd and lessen the toxic effects [13]. Some authors observed that in Cd-resistant tomato cells grown in 6 mM $CdCl_2$, enzyme activity was found to be higher compared to unselected cells [14]. Tolerance patterns are also closely linked to the availability of GSH, as seen in studies where Cd-tolerant tomato cell suspension cultures maintained higher levels of GSH, which supports continuous PC synthesis [8]. Also, cells lines showing higher γ-glutamylcysteine synthetase (GCS) activity have increased Cd tolerance as they are able to continue GSH production and, therefore, PC synthesis [13].

It is interesting to note that the time course of PC accumulation indicated that PCs in tomato cell suspension cultures are initially synthesized in greater quantities than the amount of Cd available, which implies a preventative detoxification mechanism [14]. As Cd becomes compartmentalized or excreted, the surplus PCs were degraded, further suggesting the flux nature of Cd tolerance in tomato cells [15]. In the most Cd-tolerant tomato cell lines, as much as 90% of Cd was complexed with Cd-PC, and there was a tendency for higher molecular weight PCs to accumulate, once again indicating an efficient detoxification mechanism [16].

Research has also shown that, in some experimental settings, Cd tolerance in tomato cell lines is not lost even after extended periods of no exposure to Cd, indicating a stable adaptive response linked to the synthesis and accumulation of PCs [17]. Other investigations have found that, compared to other plant species, such as cereals, tomato roots seem to be less efficient in Cd accumulation and PC complex formation, which illustrates the diversity of tolerance mechanisms among different plant taxa [18].

Such an elegant and effective model has shown different Cd tolerance patterns among contrasting tomato genotypes [3–5], and much of the research has focused on the synthesis and function of PCs in these responses (refer to examples listed in the next sections). Furthermore, Cd mitigation and associated PC levels are not always linked to changes in both Cd tolerance and accumulation. For example, Hasan et al. [19] observed GSH-induced increases in PC contents alongside enhanced Cd tolerance, despite no reported differences in Cd accumulation patterns in tomato tissues. In addition, insights into signaling and transcriptional regulation under Cd exposure along with PC quantification in tomato tissues were also provided [19]. In another investigation, the supplementation with melatonin induced increases in PC levels, which was accompanied by reduced Cd content in leaves of tomato plants, indicating the melatonin-related effect on Cd transport [20].

As previously shown and reviewed by different research groups [5,21,22], PC synthesis is closely related to the differential regulation of Cd accumulation and tolerance in different plant species. Even so, such insights have not been fully elucidated within the context of tomato research. Thus, despite such pioneering insights and others produced over the years, it is evident that more studies using contrasting tomato genotypes for Cd tolerance in parallel with the evaluation of Cd accumulation and transport parameters are necessary. The acquisition of insights related to PC synthesis using such approaches is essential to dissect the differences between Cd response, accumulation and tolerance patterns in this research field.

3. Tomato Response to Different Stresses

PCs are known to be more than just a component of the response to Cd stress in tomato plants, rather, they are a key component in the response to different environmental conditions that tomato plants experience, as discussed in this and following sections.

In response to Cd stress, some tomato plants exhibited higher levels of PCs, which are activated by metal ions such as Cd, Ag, copper (Cu), and others, where the enzyme responsible for PC synthesis, PC synthase (PCS), played a pivotal role in detoxification in a tomato organ-specific manner, reaching peak activity at pH 8.0 and 35 °C, and is also activated in response to various heavy metals in tomato cell cultures [14].

Tomato plants exhibit metal-specific tolerance and response, as observed in their responses to Cd and Cu stress. Investigations comparing the effects of Cd and Cu in tomato suspension-cultured cells [23] and tomato plant tissues [24] indicated that Cd was shown to induce PC synthesis, whereas Cu did not induce detectable PC synthesis [23,24]. Cu stress did not result in the synthesis of detectable cytoplasmic Cu-binding peptides; instead, Cu was primarily retained in the tomato cell walls [23]. On the other hand, subsequent investigation using tomato tissues revealed that, in contrast to Cu treatment alone, Cu combined with nitric oxide (NO) was associated with increased levels of PCs in both tomato roots and leaves, along with suggested mitigation of biotoxicity and oxidative damage [25]. Furthermore, potential mechanisms related to Cu stress alleviation in tomato have been found [26]. This indicates that, regarding tomato Cu research, such contrasts in experimental conditions and the use of exogenous molecules as well as integrated mitigation mechanisms may have a notable effect and should be considered in the modulation of PC synthesis. In addition, the sequestration of Cd by PCs in tomato seedlings may ameliorate its toxic effects, potentially accounting for the relatively lower lipid peroxidation observed in Cd-treated roots compared to Cu-treated roots [24]. The variation in Cd and Cu toxicity in tomato may thus be due to the peculiar properties of these metals to stimulate PC synthesis. In contrast to Cd, excessive cobalt (Co) was not suggested to induce PC synthesis in tomato, according to some authors [27], and once again indicating that the detoxification pathways differ significantly depending on the metal.

Chromium (Cr) stress was also related to the response in some tomato-related investigations. 5-Aminolevulinic acid (5-ALA) application increased GSH and PC levels in leaves of Cr-treated tomato seedlings, thus enhancing Cr tolerance, in parallel with additional oxidative stress tolerance mechanisms [28]. Citric acid-induced Cr tolerance is also associated with enhanced PC synthesis and hydrogen sulfide (H_2S) production, suggesting that these compounds act synergistically in metal detoxification, with parallel modulation of Cr accumulation in root cell walls and sequester in leaf vacuoles [29].

Tomato plants also employ PCs to mitigate arsenic (As) as the addition of H_2S and silicon together increases PC production and decreases As concentrations in roots and shoots [30], even though the exact relationship between PCs and such As accumulation pattern is worthy of further elucidation. Regarding lithium (Li) in tomato plants, biochar

and steel slag amendments alleviated Li-induced oxidative stress along with modulation of PC levels [31]. Some findings also highlighted the differential expression and activity of PCS genes in response to various heavy metal stressors in tomato including As e.g., [32,33], which will be addressed in greater detail in the forthcoming section on PCS gene expression and activity.

PCs have a significant role and associations in multifaceted ways in tomato plants under various environmental conditions in a varying investigation contexts, including studies focused on biological synthesis approaches, various exogenous applications and/or signaling molecules. For example, some authors produced Cd sulfide (CdS) nanoparticles with quantum dot properties from tomato tissues, and after extracting Cd from the root biomass, about 69% of Cd was found to be associated with PCs [34]. The use of selenium nanoparticles (SeNPs) increased thiol compound levels, including PC synthesis, for enhanced tomato Cd tolerance [35]. In tomato, other compounds such as melatonin is also known to increase PC production and H+-ATPase activity (which was associated with mechanisms to overcome Cr-induced toxicity [36]), and Cd detoxification and associated redox balance [20]. Also, transglutaminase (TGase) activity and transcript level were shown to be induced by Cd stress, which in turn increased PC production related to polyamine (PA) regulation and NO signaling, both of which contributed to increased Cd detoxification [37].

Some of the tomato-based experiments did not explicitly measure PCs but instead might calculate them from the GSH levels or GSH alone. An in vitro investigation covering insights into excess boron (B) mitigation in tomato calli showed elevated protein thiols and GSH levels, even though these were alleviated by phenolic acids (PAs), which might imply a modulation in PC production [38]. Selenium-induced changes in GSH and non-protein thiol content in edible spinach and ground tomato, although not measured directly as PCs, suggest a thiol component in detoxification modulation, which depends on the form and dose of selenium as well as on the organ and plant species [39]. These findings indicate that GSH and related compounds serve as a potential marker of the activity of PC in stress-exposed tomato plants.

Cr-stressed tomato roots were also investigated, where the exogenous application of a NO donor promoted PC accumulation in addition to parameters related to Cr mitigation (e.g., decreased Cr accumulation) [40]. Other tomato-based investigations showed that NO signaling is closely related to PC production with respect to As stress [33] and Cr toxicity in parallel with the additional use of calcium (Ca) and sulfur (S) [41], which in turn further enhanced the defense mechanisms in tomato, even though further research is needed to elucidate the exact relationship between PC production and Cr accumulation in tomato tissues.

Melatonin has been shown to work synergistically with H_2S towards As mitigation [42] and NO signaling under Al stress [43] in parallel with the regulation of PC synthesis in tomato. This implies that PCs have a more general function in detoxifying regarding different environmental toxics, contributing to the preservation of cellular redox homeostasis along with the alleviation of stress-related injury.

In Table 1, summarized information is presented regarding this research topic across various experimental settings.

Table 1. Examples of studies covering different experimental conditions and providing direct or indirect evidence for PC synthesis in tomato.

Tomato-Related Information	Growth Medium	Age of Plants Used for Stress Exposure	Stress or Environmental Treatment Information	Stress Duration	Methodology for Quantification of PCs	Plant Organs Where PCs Were Quantified	Phytochelatin Synthase (PCS) Gene Expression or Activity	Non-Enzymatic Antioxidants	Enzymatic Antioxidants	Oxidative Stress Parameters	Exemplary Findings	References
Lycopersicon esculentum	Glass vials containing Hoagland's solution	Seeds/caryopses, age unspecified	Cr(VI) at 5 mg/L and 10 mg/L	7 days	HPLC with DTNB post-column derivatization, 412 nm detection	Roots, leaves (no PCs detected)	NM	GSH	NM	Indirectly via ultrastructural changes	Inhibited root/shoot growth; electron-opaque Cr precipitates in leaves	[44]
Solanum lycopersicum L., variety Azad T-2	Soil amended with CC and SS	25-day-old plants	LiCl at 20 mg/kg, combined CBC and SS	Duration unspecified, biochemical analysis after 25 days	PCs measured using TAST content	Roots and shoots	PCS expression not measured, PC synthesis inferred from TAST	GSH, AsA, proline, soluble sugar, phenolics	SOD, CAT, APX, GR activities enhanced by CBC and SS	H_2O_2, $O_2^{\bullet -}$, MDA, EL, elevated by Li stress	Li stress increased PCs by 406%, mitigated by CBC and SS (49% in roots, 54% in shoots)	[31]
Solanum lycopersicum L. cv. 'SC 2121'	Hoagland's nutrient solution under controlled conditions	10-day-old seedlings	As (50 μM, $Na_2HAsO_4 \cdot 7H_2O$, with NaHS (0.2 mM) and Si (2.0 mM)	10 days	PCs quantified as the difference between NPTs and GSH	Leaf tissue	PCS expression not measured; PC synthesis inferred from non-protein thiol content	GSH), AsA); elevated GSSG and reduced GSH/GSSG ratio	SOD, CAT, GR, MDHAR, DHAR; As increased SOD and GR but reduced others	H_2O_2, MDA, LOX activity, EL; elevated under arsenic stress	PC synthesis increased 6.1-fold under arsenic; NaHS and Si enhanced PCs and GSH	[30]
Solanum lycopersicum L. cv. 'SC 2121'	Hydroponic system with nutrient solution (Hoagland and Arnon, 1950)	7-day-old seedlings	Cr stress (50 μM $K_2Cr_2O_7$) after 3-day pre-treatment with 100 μM citric acid	10 days, following a 3-day pre-treatment with citric acid	PCs quantified along with GSH and GSSG; specific PC isoforms not mentioned	Roots and leaves	PCS activity not measured; PCs linked to chromium detoxification	Proline, GSH, AsA	APX, GR, MDHAR, DHAR (AsA-GSH cycle enzymes)	H_2O_2, MDA, and EL	Citric acid and H_2S increased PC synthesis, reducing Cr toxicity	[29]
Solanum lycopersicum L. cv. 'Çiko F1'	Peat and garden soil mixture (1:1), enriched with N, P, K, and B	3-week-old plants	Heavy metal stress (Cu, Cd, Pb at 10, 20, 50 ppm)	Applied 3 times at 2-day intervals; leaves harvested after 2 weeks	*PCS1* gene expression via RT-qPCR; specific PC isoforms not mentioned	Leaves	PCS1 expression significantly upregulated by heavy metal stress	Proline	NM	NM	PCS1 expression and proline content induced by Pb and Cd; PCs chelated metals	[32]
Lycopersicon esculentum Mill. cv. 'Ibiza F1'	Nutrient solution	10-day-old seedlings	Cd ($CdCl_2$) and Cu ($CuSO_4$) at 1–50 μM	7 days	SH-containing peptides measured by gel filtration chromatography	Roots	NM	SH-rich peptides (e.g., GSH)	NM	TBARS; LOX	PCs induced by cadmium in roots; copper did not induce PCs and caused more oxidative damage	[24]

Table 1. *Cont.*

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Solanum lycopersicum L. var. 2270	Hoagland solution	30-day-old seedlings	As (10 mg/L) alone or with SNP (100 µM, NO donor) and cPTIO (200 µM, NO scavenger)	15 days	Quantified as NPTs; GSH subtracted	Roots and leaves	Gene expression of PCS was evaluated and upregulated by SNP under As stress	GSH, AsA, phenolic compounds	CAT, APX, SOD, and GR enzymes	MDA, H_2O_2, and key antioxidant enzyme activities (e.g.,CAT, APX, SOD, GR)	SNP alleviated As stress by boosting antioxidant activities, PC synthesis, and reducing As translocation to shoots via immobilization in roots	[33]
Solanum lycopersicum var. Super 2270	Solution with macronutrients and micronutrients at pH 4.0	21-day-old seedlings; stress lasted for an additional 14 days	Al (148 µM) with Mel (150 µM) and cPTIO (100 µM,)	14 days	RP-HPLC	Roots and leaves	NM	GSH	SOD, CAT, GR, and APX enzymes	MDA, H_2O_2, $O_2\bullet^-$, EL	Mel alleviated Al stress by boosting antioxidants, increasing GSH and PCs, and limiting Al translocation to leaves via immobilization in rotor	[43]
Solanum lycopersicum L.	Hoagland's solution supplemented with As and H_2S	20-day-old seedlings	As (10 mg/L) with Mel (150 µM) and H_2S (0.2 mM)	14 days	Subtracting GSH from total NPTs	Roots and leaves	*PCS* upregulated by Meland H_2S	Glutathione (GSH), phytochelatins (PCs), polyphenols, and anthocyanins	SOD, CAT, POD, and APX enzymes	$O_2\bullet^-$, H_2O_2, MDA, and ABTS levels	Meland H_2S alleviated As stress by boosting antioxidants, increasing GSH and PCs, and reducing As translocation to shoots via root immobilization	[42]
Solanum lycopersicum L. Mill., cv. BL-1076	Nutrient solution applied in Petri plates	10-day-old seedlings	25 µM $K_2Cr_2O_7$ with Mel (30 µM) and HT, (1 mM)	48 h in the dark	NPTs subtracted from GSH content	Roots	NM	AsA-GSHcycle, GSH	SOD, POX, CAT, APX, and GR enzymes	H_2O_2, $O_2\bullet^-$, TBARS, and EL	Mel increased PCs, K content, and H^+-ATPase activity, alleviating Cr toxicity; modulation of Cys and H_2S biosynthesis was crucial	[36]
Solanum lycopersicum L. var. Damini-131	Hydroponically grown in half-strength Hoagland nutrient medium	15-day-old seedlings	25 µM $K_2Cr_2O_7$ with 50 µM SNP and 200 µM cPTIO to explore NO's role	7 days	NPTs subtracted from GSH	Roots	Not assessed	GSH	APX, MDHAR, DHAR, and GR, AsA -GSH cycle	ROS accumulation, MDA, MSI, and histochemical ROS visualization	NO (via SNP) increased PCs and NPTS, reducing Cr(VI) toxicity	[40]

Table 1. *Cont.*

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Solanum lycopersicum L. var. NS 585	Hydroponically using a nutrient solution	21-day-old seedlings	Cr(VI) exposure with Ca and S, and SNP for mitigation	21 days	NPTs subtracted from GSH	Leaf and root tissues	NM	Carotenoids, proline, GSH	SOD, CAT, and GST	$O_2^{\bullet -}$, H_2O_2, MDA	Ca and S alleviated Cr(VI) toxicity by increasing PCs and GST; NO-related stress mitigation was observed	[41]
Solanum lycopersicum L., cv. Improved Maofen 802F1	Improved Hoagland nutrient solution	5–6 true leaves	Cu stress with 50 μM $CuCl_2$; SNP (NO donor), Hb (inhibitor), BSO (scavenger)	1, 3, 6, 24, and 48 h	TAST and TG measurement	Roots and leaves	NM	GSH	Enzymes involved in GSH synthesis	NM	Increased GSH and PCs for Cu detoxification; NO enhanced activities of GSH metabolism-related enzymes	[25]
Solanum lycopersicum L. cv SC 2121	Hoagland's nutrient solution	13-day-old tomato seedlings	Cr(VI) stress with 50 μM $K_2Cr_2O_7$; 5-ALA treatment	Cr treatment: 14 days; 5-ALA treatment: 3 days before Cr	Enzymatic assay method)	Roots, Leaves	NM	GSH, AsA, DHA, Proline	GST, GR, APX, MDHAR, DHAR	H_2O_2, MDA, EL	5-ALAenhanced PCs and GSH levels; Foliar application of 5-ALA prior to Cr treatment improved plant growth and	[28]
Lycopersicon peruvianum L.	Murashige and Skoog medium (cell suspension cultures)	1.5 days after the end of the logarithmic growth phase	Cu, Cd, Zn, Pb	Short-term: 10 min to 1 week; Long-term: up to 2 weeks	HPLC/ICP-MS system for PCs; Isoforms: PC_2, desGly-PC_2	Roots, Leaves	NM	NM	NM	NM	Cu and Cd complexation; transient process in detoxification	[45]
Solanum lycopersicum cv. Grosse Lisse	Gamborg's B5 liquid medium with 3% sucrose, pH 5.8	21-day-old cultures (hairy roots)	100 μM $CdSO_4$	Harvested after 4, 7, and 9 days of Cd exposure	Anion-exchange chromatography and gel filtration for Cd-PC complexes	Hairy roots	NM	GSH	NM	Cd and inorganic sulphide content in biomass	PCs form Cd-PC complexes; Hairy roots produce CdS nanoparticles with quantum dot properties	[34]
Solanum lycopersicum L. cv. Ailsa Craig	Hoagland's nutrient solution (hydroponics), nutrient soil (soil experiments)	Three-week-old tomato seedlings	100 μM Cd in hydroponics, 0.65 mg/Kg Cd in soil	Up to 10 days	HPLC for quantification of PC_2 and PC_3; PC_4 in SeNP-treated plants	Shoots and roots	*PCS1* and *PCS2* gene expression evaluated, but SeNPs had no additional effect	GSH, cysteine	NM	EL, photosynthesis rate)	SeNPs enhanced Cd tolerance by increasing thiol compound synthesis, reducing Cd in shoots, and improving Su/Se accumulation	[35]

Table 1. *Cont.*

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Solanum lycopersicum L. cv. Five Star	Hoagland nutrient solution	Tomato seeds sterilised and grown in sand; seedlings harvested after 20 days for hydroponic experiments	Cd: 5 μM (low dose) and 25 μM (high dose); hypotaurine (HT, 100 μM) also used	7 days	Subtracting the amount of GSHfrom NPT	Roots	NM	GS, AsA, e, cysteine	APX, DHAR, MDHAR, GR	$O_2\bullet^-$, H_2O_2, protein carbonylation, MSI	Cd at low dose led to differential antioxidant responses and increased PC levels in roots, with higher Cd doses resulting in oxidative stress; Addition of HT resulted in decreased PC levels	[11]
Lycopersicon esculentum Mill. cv. 63/5 F1	Hydroponically grown	After 10 d on control medium	CdC_2—1, 5, 10, 25, and 50 μM; 100 μM	7 days	Subtracting the amount of GSH from NPT	Roots; leaves	NM	GSH	NM	TBARS	Cd-induced increase in PC levels, especially in roots	[10]
Lycopersicon esculentum Mill. cv VFNT-Cherry	Cell suspension cultures	Cultures were initiated with an inoculum of 20 mg cells (fresh weight) per mL of medium	GSH, BSO, and $CdCl_2$	3 or 4 days after inoculation	HPLC (PC_2 to PC_4 quantification)	Cell suspension cultures	NM	GSH, cysteine	NNM	NM	Differential PC production in response to Cd exposure in cell cultures	[8]
Solanum lycopersicum' cerasiforme—cv. Hanluzhe (HLZ), cv. Lvfeicui (LFC)	Hydroponic conditions with nutrient solution (pH adjusted to 6.5)	Plants used after development of four true leaves	Cd (50 μM) and Se (2.5 μM) exposure	15 days	Enzyme immunoassay (ELISA kit)	Leaves	Increased PCSase activity after Cd exposure (LFC plants)	GSH, AsA, DHA	SOD, POD, CAT, GPX, APX, MDHAR, DHAR, $O_2\bullet^-$	H_2O_2, MDA, EL	Se application increased PC levels; Se mitigated Cd-induced oxidative stress	[12]
Lycopersicon esculentum, Money Maker variety	AB2 solid medium + Zn-enriched AB2 liquid medium	40–45-day-old calluses	100 μM CuCl or a sugar mixture (glucose:mannose 1:1 ratio)	24 h	LC/MS/MS	Stem cells cultured from tomato leaves	RT-PCR analysis of *PCS* gene expression	Phenolic acids, flavonoids, including rutin, coumaric acid, protocatechuic acid, chlorogenic acid	M,	ROS production using CM-DCFDA dye, metal chelating activity, comet assay for DNA damage	CuCl or sugar mixture induced high antioxidant and metal-chelating activities, reduced oxidative stress and DNA damage, and stimulated collagen synthesis	[46]
Solanum lycopersicum L. cv. Ailsa Craig	Hydroponic system using Hoagland's nutrient solution	Four-leaf stage	25 and 100 μM Cd (CdCl2), Mel at concentrations of 25, 50, 100, 250, and 500 μM	14 days	HPLC (PC_2, PC_3, and PC_4)	Leaves and roots	Gene expression of *SlPCS*	GSH	SOD, CAT, APX, G-POD, GR	H_2O_2, MDA, EL	Melatonin enhanced antioxidant enzyme activities and PC (PC_2, PC_3, and PC_4) synthesis, improving Cd tolerance	[20]

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Solanum lycopersicum L. cv. Ailsa Craig	Hydroponic system using Hoagland's nutrient solution	Four-leaf stage	100 µM Cd ($CdCl_2$), GSH applied exogenously every five days, BSO used to inhibit GSH biosynthesis	14 days	HPLC (PC_2, PC_3)	Leaves and roots	Gene expression of *PCS*	GSH	SOD, CAT, APX, GR	H_2O_2, MDA, EL	GSH mitigated Cd-induced oxidative stress by enhancing the antioxidant system, increasing PC synthesis, and promoting Cd sequestration into vacuoles and cell walls	[19]
Solanum lycopersicum L. cv. Ailsa Craig (WT)	Peat and vermiculite mixture (2:1 ratio), controlled greenhouse	Seedlings after second true leaves fully expanded	100 µM Cd, O-phen (TGase inhibitor), Putrescine (PAs donor), SNP (NO donor)	15 days	HPLC (PC_2, PC_3, PC_4	Leaves	NM	GSH	PAs, NO	EL, chlorophyll content, Fv/Fm ratio, Cd content	TGase overexpression increased PC2, PC3, and PC4 levels, enhanced Cd binding to cell walls, and reduced Cd transport to shoots	[37]
Lycopersicon esculentum Mill., cv. 'Palace'	Murashige-Skoog medium	Suspension cells were subcultured	Exposure to 10–200 µM $CdSO_4$ e	3 days	HPLC (PC_2-PC_4 isoforms)	Suspension-cultured cells from root tissues	PCS activity; tomato cells showed high activity	GSH, AsA	NM	NM	tomato cells produced PCs and showed substantial Cd tolerance	[47]

The following abbreviations are used in the table: **5-ALA**: 5-aminolevulinic acid; **ABTS**: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), marker of antioxidant capacity; **Amf**: arbuscular mycorrhizal fungus; **APX**: ascorbate peroxidase; **As**: arsenic; **AsA**: ascorbate; **B**: boron; **BSO**: buthionine sulfoximine (GSH synthesis inhibitor); **Ca**: calcium; **CAT**: catalase; **CBC**: coconut biochar; **Cd**: cadmium; **$CdCl_2$**: cadmium chloride; **cPTIO**: 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (NO scavenger); **Cr**: chromium; **Cr(VI)**: hexavalent chromium; **Cu**: copper; $CuSO_4$: copper sulfate; **DHA**: dehydroascorbate; **DHAR**: dehydroascorbate reductase; **DTNB**: 5,5′-dithiobis(2-nitrobenzoic acid); **EL**: electrolyte leakage; **GPX**: glutathione peroxidase; **GR**: glutathione reductase; **GSH**: reduced glutathione; **GSSG**: oxidized glutathione; **GST**: glutathione-S-transferase; **Hb**: hemoglobin (inhibitor of NO); **HPLC**: high-performance liquid chromatography; **HT**: hypotaurine (a scavenger of H_2S); **H_2O_2**: hydrogen peroxide; **K**: potassium; **$K_2Cr_2O_7$**: potassium dichromate; **LC/MS/MS**: liquid chromatography coupled to tandem mass spectrometry; **Li**: lithium; **LiCl**: lithium chloride; **LOX**: lipoxygenase; **MDA**: malondialdehyde; **MDHAR**: monodehydroascorbate reductase; **Mel**: melatonin; **MSI**: membrane stability index; **N**: nitrogen; **$Na_2HAsO_4 \cdot 7H_2O$**: sodium arsenate; **NaHS**: sodium hydrosulfide; **NM**: not mentioned; **NO**: nitric oxide; **Non-protein thiols**: compounds containing sulfhydryl (-SH) groups not bound to proteins; **$O_2\bullet^-$**: superoxide radical; **P**: phosphorus; **PAs**: polyamines; **Pb**: lead; **PCS**: phytochelatin synthase; **PCS1**: gene encoding phytochelatin synthase 1; **PCs**: phytochelatins; **ppm**: parts per million; **RP-HPLC**: reverse-phase high-performance liquid chromatography; **RT-qPCR**: reverse transcription quantitative polymerase chain reaction; **S**: sulfur; **Se**: selenium; **SeNPs**: selenium nanoparticles; **Si**: silicon; **SNP**: sodium nitroprusside (NO donor); **SOD**: superoxide dismutase; **SS**: steel slag; **TAST**: total acid-soluble thiol; **TBARS**: thiobarbituric acid-reactive substances; **TG**: total glutathione; **TGases**: transglutaminases.

4. Genetically Engineered Plants: PC Synthesis Within the Tomato Research Context

Genetic investigations including gene overexpression, gene silencing, and/or mutants has led to a greater understanding of how to modulate stress tolerance and response in genetically engineered plants, including tomato. Ahammed et al. [48] showed that overexpression of *SlRING1*, a RING E3 ubiquitin ligase, greatly enhanced Cd tolerance in tomato transgenic plants by upregulating transcript levels of key antioxidant enzymes and PCS, leading to a reduction in oxidative stress and Cd accumulation in aerial parts and root tissues. Other investigations such as this (see Table 2) clearly demonstrates that genetic engineering techniques have the ability to improve Cd tolerance through the manipulation of specific genes that influence PC production, metal transport, and antioxidative defense within the tomato research context. However, more research is definitely needed to sort out and decipher the upstream and downstream events related to PC synthesis regulation to obtain a complete picture of the regulatory networks in tomato.

Table 2. Examples of studies covering genetic engineering approaches and modulation of PC synthesis in tomato plants.

Tomato Cultivar/Genotype	Growth Medium	Age at Stress Exposure	Stress Treatment	Stress Duration	Non-Enzymatic Antioxidants	Enzymatic Antioxidants	Phytochelatin Quantification Method	Plant Organ for PC Quantification	Examples of Physiological Findings	Genetic Engineering Approach	References
S. lycopersicum cv Ailsa Craig	Hoagland's nutrient solution	Three-leaf stage	100 µM $CdCl_2$	10 days	GSH	CAT, MDHAR, DHAR, GSH	*PCS* gene expression	Roots and shoots	Overexpression of *SlRING1* enhanced Cd tolerance by reducing Cd accumulation, increasing antioxidant enzyme expression, and upregulating PCS for increased phytochelatin production, resulting in reduced oxidative damage.	Overexpression of the *SlRING1* gene through transgenic technology	[48]
S. lycopersicum cv Ailsa Craig	Vermiculite and perlite (50:50), later Hoagland's solution	Four-leaf stage	100 µM Cd, 100 µM melatonin (foliar spray every 5 days)	15 days	GSH	SOD, CAT, APX, G-POD	HPLC	Roots and leaves	Melatonin improved Cd tolerance by increasing sulfur uptake, enhancing PC biosynthesis, and improving antioxidant activity. These factors led to greater Cd chelation, reduced Cd translocation, better growth, and less oxidative damage.	VIGS to silence the *COMT* gene and *COMT*-overexpressing plants	[49]
S. lycopersicum cv Hezuo 903	Hoagland's nutrient solution	Four-leaf stage	100 µmol L^{-1} $CdCl_2$, Se-Cys, Na_2SeO_3, or Na_2SeO_4 applied 3 days before Cd	15 days	Mel, Glutathione (GSH)	NM	HPLC (derivatization with monobromobimane)	Leaves	Selenium and melatonin significantly enhanced Cd tolerance. Se-induced Cd tolerance was associated with an increase in melatonin, GSH, and PCs, leading to reduced Cd uptake and minimized oxidative damage.	VIGS silence the *TDC* gene	[50]
S. lycopersicum cv Ailsa Craig	Hoagland's nutrient solution	Four-leaf stage	100 µM $CdCl_2$, 100 µmol L^{-1} melatonin	15 days	Melatonin	Enhanced antioxidant enzyme activity, specific enzymes not quantified	HPLC (derivatization with monobromobimane)	Leaves	Melatonin alleviated Cd-induced oxidative stress by enhancing PCs, improving nutrient homeostasis, and maintaining redox balance	VIGS to silence the *COMT* and *PCS* genes	[51]

The following abbreviations are used in the table: **APX**: ascorbate peroxidase, an antioxidant enzyme; **CAT**: catalase, an antioxidant enzyme; **Cd**: cadmium; **$CdCl_2$**: cadmium chloride; **COMT**: caffeic acid O-methyltransferase, an enzyme related to lignin biosynthesis; **cv**: cultivar (a cultivated plant variety); **DHAR**: dehydroascorbate reductase, an antioxidant enzyme; **G-POD**: guaiacol peroxidase, an antioxidant enzyme; **GSH**: reduced glutathione, a non-enzymatic antioxidant; **GSH1**: gene encoding glutathione synthesis; **HPLC**: high-performance liquid chromatography; **MDHAR**: monodehydroascorbate reductase, an antioxidant enzyme; **Na_2SeO_3**: sodium selenite; **Na_2SeO_4**: sodium selenate; **NM**: not mentioned; **PCS**: phytochelatin synthase, an enzyme involved in phytochelatin production; ***S. lycopersicum***: *Solanum lycopersicum* (scientific name for tomato); **Se-Cys**: selenocysteine, a bioavailable form of selenium; **SOD**: superoxide dismutase, an antioxidant enzyme; **TDC**: tryptophan decarboxylase, an enzyme involved in melatonin synthesis; **VIGS**: virus-induced gene silencing.

5. Future Research on Phytochelatins in Tomato

In addition to the future directions presented in the previous sections, the following sections provide additional information and suggestions for future research on PCs in tomato plants; in particular, these encompass research topics that we believe need to be explored further. They intend to fuse these suggestions into one robust roadmap for developing insights into PC-related mechanisms in tomato plants. Such perhaps obvious needed studies are derived from our knowledge on the research done in tomato but also with other crops.

5.1. Increasing Insights into Phytochelatin Functionality in Tomato

The studies mentioned above emphasize aspects concerning the involvement of PCs in mediating stress mitigation actions in tomato plants. Adopting approaches that have been effective in studying PCs in other plant species [as previously by our research group [5] and other authors [6,7], but are yet to be fully explored in tomato, could significantly advance the understanding of PC-mediated stress mitigation in this important crop. Additionally, the influence of signaling regulation on PC biosynthesis in tomato requires more exploration, as it has been studied more extensively in other plant species [5–7]. Investigating similar signaling interactions in tomatoes could unravel novel regulatory mechanisms.

Information regarding the temporal dynamics of PC synthesis owing to short-term and long-term exposure, tissue- and organ-specific modulation of PC synthesis, transcriptional insights, plant signaling, increasing insights into the qualitative PC quantification and related PC isoforms, from early growth tomato stages and up to the fruiting stage, has been provided by several authors (Table 1) and still needs to be investigated further. The advent of exogenous applications, nanoparticles, and other novel approaches to further PC-mediated metal detoxification and Cd tolerance in tomato plants, coupled with the quantification of different PC isoforms, has been emphasized in tomato (see cited articles in Table 1). Future work on the optimization of the exploration of exogenous agents, assessing possible synergistic actions among several agents, and the long-term assessment of their effects on PC dynamics in different tomato genotypes might be the focus of the research. Thus, the current research may address the role of PCs in ensuring metal homeostasis throughout the plant life cycle, offering significant hope for food safety.

5.2. Further Biotechnological Potential of Tomato Phytochelatins

In addition to their function in plant detoxification, the biotechnological potentials of PCs have not been fully investigated, especially linking the field of agricultural research and general biotechnological engineering. The cytoprotective effects of tomato stem cell extracts enriched with PCs have been demonstrated to protect human skin cells from heavy metal-induced damage by suppressing collagenase expression and preserving DNA integrity [46]. Using this detoxification ability, future research may aim at improving crop tolerance and safety, and the agricultural products with biotechnological applications for environmental and human health.

5.3. Interplay Between Phytochelatin Synthesis, Other Potential Mechanisms, and Multiomics Approaches

Important enzymatic and non-enzymatic antioxidant responses (Table 1) and heat shock proteins-related insights [52], along with PC quantification (Table 1), have been observed that further supports that these molecules have an integrated role in stress responses. Thus, other potential mechanisms, together with those related to PCs, should be further explored in future research.

We have discussed antioxidants [53] as well as some omics approaches that are imperative in elucidating the differential protein synthesis associated with PC biosynthe-

sis [5,54]. Such approaches, including the parallel quantification of PCs in conjunction with metabolomics data, like that of Kumar et al. [55], are relevant in order to gain complete knowledge of Cd detoxification pathways. Importantly, PC biosynthesis is highly dependent on the availability of GSH and cysteine, which are both major precursors for PC synthesis [5]), thus, it is relevant to further monitor such PC biosynthesis-related molecules in further studies in addition to other potential PC biosynthesis-related molecules.

In some studies summarized in Table 1, it is noted that the measurement of enzymatic and non-enzymatic antioxidants were not consistently performed alongside PC quantification. However, without complete tracking of these antioxidant systems, the entire redox balance is not fully understood, nor is its correlation to PC synthesis during various stress conditions. Future research should incorporate a more combinatorial approach that would monitor antioxidant responses and PC production at the same time, since this would make apparent the interplay between these mechanisms and their overall role in tolerance and response in tomato.

5.4. Phytochelatin Synthase Gene Expression and Activity

Some studies have evaluated the gene expression or activity of PCS in tomato under various stress conditions. For example, some authors showed the induction of genes like *PCS1* upon different heavy metal stressors in the detoxification process in tomato leaves [32]. In another investigation, the evaluation of As stress in tomato seedlings revealed that NO induced the expression of PC-related genes in tomato seedlings, as well as reduced As translocation from roots to aerial parts, while SNP treatment enhanced *PCS* expression in response to arsenic [33]. Furthermore, *PCS1* expression in tomato was significantly upregulated by heavy metal stress, as confirmed via RT-qPCR [32], while melatonin and hydrogen sulfide treatments increased the expression of *PCS1* and *PCS2* genes [42]. In another study, Cd stress induced *PCS* expression, with melatonin further enhancing its expression in both tomato leaves and roots [20], and GSH application boosting *PCS* transcripts under the same stress [19]. However, selenium nanoparticles showed no additional effect beyond Cd stress on *PCS1* and *PCS2* expression [35]. Using Northern blot analysis, PCS expression was evaluated via *LePCS1* mRNA levels, though specific isoforms were not detailed [56]. In cherry tomato, PCS activity was enhanced under Cd stress, and selenium application further amplified these effects [12]. Lastly, RT-PCR analysis revealed increased PCS expression under Cu chloride or sugar mixture treatments [46], while PCS activity was notably significant in tomato cells compared to azuki bean cells [47]. Altogether, this information shows tomato ability to modulate tolerance across different environmental stressors at different PC regulation levels. Despite this integrated information, not all of the experiments that have analyzed the level of PCs in plant tissues have also examined the expression of PCS or its activity. Future investigations and greater attention to cross-species research might hopefully delve deeper into this dual regulation and shed some light on the molecular pathways of plant tolerance using different tomato tissues and plant organs.

5.5. Phytochelatins and Metallothioneins: Similarities and Synergies

PCs are similar to metallothioneins in that they are molecules that bind metals, and a comparison between the two is promising. A few studies have been conducted on PCs and metallothioneins in tomato plants, focusing on how they work together or independently of each other regarding detoxification mechanisms [20,33,46,56]. Such a comparison would provide promising avenues for genetic engineering or other biotechnological methods of increasing metal resistance in plants.

5.6. Microorganisms and Multi-Stress Responses in Tomato Plants

Some investigations have shown that certain microorganisms are capable of having a significant impact regarding the modulation of metal tolerance in tomato plants, as well as insights related to PCs. For example, Ahmad et al. [57] showed that the influence of a nonpathogenic fungal inducer (*Penicillium oxalicum*) against *Alternaria alternata* in tomato plants was associated with induced resistance through alterations in defense-related gene expression, including PC biosynthesis, and it worked synergistically with salicylic acid pathways. In another study, grafting tomato onto Maxifort rootstock line combined with AM inoculation enhanced Cd tolerance by inducing higher PC2 synthesis, elevated antioxidant enzyme activity, and superior nutrient status [55]. On the other hand, arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* colonization affected metal transport and detoxification in tomato, with the *LePCS1* gene, coding for PCS, not being differentially expressed under AMF colonization [54]. These studies show different or contrasting effects on PC-related parameters, in addition to the numerous environmental stress conditions shown here (Table 1). Such investigations indicate that microbial interactions and combined stress responses deserve further investigation in tomato plants as they simulate real-world conditions where plants are subjected to simultaneous biotic and abiotic environmental factors. Furthermore, this research field might be better served by examining the dissection of differences among a broader spectrum of contrasting tomato genotypes in their response and tolerance to environmental stresses.

6. Concluding Remarks

The information presented in this article underscores the relevant participation of PCs in tomato plants' response to different environmental stress factors, confirming progress in the research covering metal-PC complexes from early research [58,59] to the current scenario (Table 1). Although much has been learned about the molecular mechanisms of PC biosynthesis, additional studies are warranted to evaluate gene expression, enzymatic activity, and the integration of multiple approaches. The synergism of PCs with other physiological and molecular mechanisms provides exciting possibilities for increasing and modulating stress tolerance. Research might continue to explore the genetic and physiological responses in contrasting tomato genotypes, specifically regarding their implications for sustainable agriculture.

Our ongoing studies have pursued many analyses toward further developing physiological, biochemical, and molecular insights into Cd stress tolerance and responses within various tomato genotypes. Our laboratories continue to employ diverse methodologies, including multi-omic approaches, in our attempts to unravel the intricate mechanisms underlying different Cd tolerance parameters, including those related to PC synthesis in tomato plants [3,5,54]. In particular, we study multi-omics approaches [3,54,60] as well as the interactions of various contrasting genotype-grafting combinations in tomato, foreshadowing the potential applications of grafting, which could extend beyond basic research and find practical agricultural contexts [3,4]. It also serves as an important insight into how the application of horticultural grafting techniques could enhance the production of PCs and improve overall plant resilience. Accordingly, we plan to present our preliminary findings on the synthesis of PC in different tomato genotypes with varying Cd tolerances, adding to our understanding of grafting as a potential tool for increasing heavy metal tolerance from both basic science and applied mitigation perspectives, towards advancing applicable agricultural science and environmental stress mitigation within the tomato research context.

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