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FOCUS ARTICLE

Comparative phosphoproteomic analysis of tomato genotypes with contrasting cadmium tolerance

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Abstract

Key message **A first insight into the effects of cadmium exposure on the phosphoproteome of tomato plants by performing a comparative analysis of tomato genotypes with contrasting cadmium tolerance.**

Cadmium (Cd) is a heavy metal that is considered a major environmental pollutant and a potential threat to human health, ranking among the priority metals that are of public health significance. Cd is known to affect metabolic processes in living organisms, including both animals and plants, with no essential function in most eukaryotes and extreme toxicity to biological systems in part due to high solubility in physiological conditions (Marques et al. 2021). Contamination of agricultural areas by Cd has increased considerably in several countries through anthropogenic practices (e.g., production and application of fertilizers and pesticides) over the last few decades, posing a potential risk to human health through crop plants.

Due to its high phytoaccumulation index, low soil adsorption coefficient, and high soil–plant mobility, Cd can easily enter food webs (Shahid et al. 2016). Even at low concentrations in soil or water and even after short-term exposure, Cd exposure can rapidly induce multiple physiological effects during plant development (Piotto et al. 2018). Most studies to date that have focused on Cd-induced plant responses have utilized non-crop species, creating a need for a focus on Cd-exposed crop plants. Understanding the mechanisms of plant uptake, response, and tolerance to Cd stress in crop species is important to develop efficient strategies for mitigating the impacts of Cd contamination on crop yield and food safety, including through the generation of Cd-tolerant and Cd-excluding crop cultivars.

Tomato (*Solanum lycopersicum*) is an economically important crop species and one of the most cultivated and consumed vegetables worldwide. This species also serves as a model organism for fleshy-fruited plants, and thus has been used to study plant Cd response and tolerance (Piotto et al. 2018). Studies using tomato as a model include investigations into the effects of Cd on plant nutritional status and antioxidant capacity (Borges et al. 2019a), plant Cd accumulation and differential expression in the root proteome under short-term Cd exposure (Borges et al. 2019b), and explicit characterization of Cd-induced transgenerational effects (Nogueira et al. 2021). In several of these studies, the tomato genotypes Pusa Ruby (PR) and Calabash Rouge (CR) have been characterized as Cd-tolerant and Cd-sensitive, respectively, when compared to other tomato cultivars (Borges et al. 2019a, b; Piotto et al. 2018).

Investigating the dynamics of cell signaling in response to Cd exposure is crucial to understand the molecular mechanisms by which plants cope with Cd stress (Marques et al. 2019). Protein phosphorylation is one of the most widespread and important post-translational modifications that regulates signal transduction. Phosphoproteomic

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technologies have been successfully employed to investigate abiotic stress-induced phosphorylation events. However, our knowledge of changes in phospho-regulation during the plant Cd stress response is still very limited, even though several proteomic studies have identified key proteins involved in both the Cd stress response and indicative of Cd tolerance, such as glutathione biosynthesis-related proteins (reviewed by Marques et al. 2021). In the present work, we aim to provide a first insight into the effects of Cd exposure on the phosphoproteome of tomato plants. To achieve this goal, we here perform a comparative analysis of tomato genotypes with contrasting Cd tolerance by employing a liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based high-throughput label-free quantitative approach, by which thousands of phosphopeptides can be identified and quantified.

20-day-old PR and CR tomato seedlings were grown in a hydroponic system according to the growth conditions described in Borges et al. (2019b), in either control (Cd-free) or Cd-containing (35 µM $CdCl_2$) hydroponic solution. The use of 35 µM was based on the work of Piotto et al. (2018), which indicated this concentration as suitable for examining and screening tomato lines for tolerance/sensitivity to Cd toxicity after short-term exposure.

Four biological replicates of fresh leaves were sampled from treatment and control groups 4 days after Cd treatment onset and immediately frozen in liquid nitrogen and subsequently stored at − 80 °C until further freeze-drying. The choice of Cd exposure for 4 days was based on the work of Borges et al. (2019b), which performed the first quantitative proteomic analysis of the PR and CR genotypes. This period is sufficient for plants to recognize the stress, but short enough to avoid severe damage to the sensitive genotype. Protein extraction from the freeze-dried leaves, phosphopeptide preparation, LC–MS/MS data acquisition, and data analysis were performed as described in Methods S1. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al. 2019) partner repository with the dataset identifier PXD024021.

In total, 6348 phosphopeptides were identified and quantified. Significant differences were found in the phosphorylation status of 360 protein groups between the genotypes under Cd treatment (Table S1). Meanwhile, 127 protein groups showed differential phosphorylation between genotypes under the control (Cd-free) condition (Table S2). This finding indicates that the Cd treatment induced an increased contrasting phosphorylation status between genotypes, compared to the Cd-free condition.

Contrasting phosphorylation between genotypes was observed for certain classes of Cd tolerance-related proteins only after Cd exposure (e.g., ATP-binding cassette (ABC) transporters), while other protein groups experienced a large increase in the number of proteins with contrasting phosphorylation under the Cd treatment condition compared to the control one (including heat shock proteins (HSPs)) (Table 1). Phytochelatin (PC)-dependent vacuolar Cd sequestration (i.e., the sequestration of PC-Cd complexes from the cytosol into vacuoles) has been shown to be mediated by ABC transporters (Marques et al. 2021). In tomato plants, PC biosynthesis plays a role in mitigation of Cd toxicity (reviewed by Marques et al. 2021). The results obtained in the present study may imply that ABC transporter-modulated action of PCs is involved in the contrasting Cd tolerance of the tomato genotypes. HSPs are known to play crucial roles in protecting plants against abiotic stresses, including heavy metal stresses. HSPs act as molecular chaperones, preventing protein aggregation, assisting with the accurate folding of both nascent proteins and misfolded proteins accumulated under stress, and promoting selective degradation of misfolded or denatured proteins (reviewed by Hasan et al. 2017). ABC transporters and HSPs have also been reported to be differentially phosphorylated in Cd-exposed rice plants (Zhong et al. 2017), indicating their importance for Cd response across diverse crop plants.

Among proteins known to be involved in plant hormone signaling, certain ethylene signaling-related proteins and multiple auxin repressed/dormancy-associated proteins were found to be contrasting in phosphorylation between the tomato genotypes under Cd exposure (Table 1). This finding aligns with previous observations that changes in auxin and ethylene regulation occur during plant Cd stress responses, including in tomato (reviewed by Marques et al. 2019). In addition, both kinases and phosphatases were differentially phosphorylated by Cd stress in the present study (Table 1), including some mitogen-activated protein kinases (MAPKs) and one protein phosphatase belonging to the PP2C (type 2c protein phosphatase) family. The differential phosphorylation of MAPKs under Cd stress has also been previously noted in rice plants (Zhong et al. 2017), along with the finding that the phosphorylation of some PP2Cs likely promotes binding to abscisic acid (ABA) receptors, facilitating the role of the ABA central signaling complex under Cd stress. This consilience of evidence indicates that this mechanism may be at work in PR tomato as well.

Multiple families of transcription factors (TFs) were found to be differentially phosphorylated by comparing the tomato genotypes under Cd exposure including zinc finger, WRKY (proteins containing the WRKY domain), and basic Leucine Zipper (bZIP) TFs (Table 1). These families of TFs may, therefore, be involved in Cd tolerance in PR tomato, as some of these families of TFs have been previously reported to experience increased phosphorylation in rice seedlings (Zhong et al. 2017), including the zinc finger C3H12/Os01g0917400 and the bZIPs Os01g0174000 and Os03g0239400. The overexpression of some members of

Table 1 Key contrasting phosphorylated proteins between the leaves of Cd-tolerant tomato genotype Pusa Ruby (PR) and Cd-sensitive tomato genotype Calabash Rouge (CR) under Cd stress or control conditions

Condition	Protein description		Protein accession	Sequence (phosphopeptide)	log2 ratio
Cd exposure	ABC transporter	ABC transporter family protein	Solyc01g006720.3.1	SSPMNGDVAGGGGG PETLSR	− 1186
		ABC transporter family protein	Solyc01g006720.3.1	LTGASPGR	− 1379
		Pleiotropic drug resistance ABC transporter	Solyc05g053600.3.1	GNSSNSIFSR	− ∞
		ABC transporter family protein	Solyc11g069710.2.1	LNSGSLPSPPLPD-GAVITR	− ∞
		Drug resistance transporter-like ABC domain protein	Solyc12g019630.1.1	NASIRETKTIK	∞
	Chaperone	BAG family molecular chaperone regulator 8, chloroplastic	Solyc02g088660.3.1	ESEDEEEEEEDQQSPR	− 1153
	Heat shock protein	Heat shock protein 70; Heat shock protein 70	Solyc01g103450.3.1;Solyc 11g020040.2.1	AVVTVPAYFNDSQR	∞
		DnaJ heat shock N-terminal domain-containing protein	Solyc01g109890.3.1	DSSLRSENTDM-VDSGPSSN	∞
		DnaJ heat shock N-terminal domain-containing protein	Solyc01g109890.3.1	SENTDMVDSGPSSN	1432
		Heat shock protein 70 family protein	Solyc02g080470.3.1	SFATDSER	− 2135
		Heat shock 70 kDa protein	Solyc07g043560.3.1	EQTASEAEKPSADENS-DHDEL	∞
	Kinase	Protein serine/threonine kinase	Solyc01g007120.3.1	LIEGELENHSDSDDEG-TADEEDEEDINNTNVK	∞
		Nucleoside diphosphate kinase	Solyc01g089970.3.1	IIGATNPLESAAGTIR	∞
		MAP kinase 12	Solyc02g031860.3.1	SDDSAASQFIMAHAY-SEGSQQIIESVNK	∞
		Protein kinase domain-containing protein; Protein kinase domain-containing protein	Solyc02g031860.3.1; Solyc02g031900.2.1	NWSFFQK	∞
		Protein kinase domain-containing protein; Protein kinase domain-containing protein	Solyc02g031860.3.1; Solyc02g031900.2.1	RNTLVTGGVR	∞
		Non-specific serine/threonine-protein kinase	Solyc02g072540.3.1	SVFDIEDNNSDGEGP-SNR	∞
		Serine/threonine-protein kinase ATM	Solyc02g086750.2.1	LSVSIPESDEVGNQQD-NAGPLSR	− 1657
		LRR receptor-like kinase	Solyc03g006030.3.1	DSQQADSLTMATTER	− ∞
		Protein kinase	Solyc03g111670.3.1	PAGAVTGAAAE-AGTSSSK	− 2358
		Pantothenate kinase 2	Solyc03g112910.3.1	SFDELLELSQR	1042
		Receptor protein kinase CLAVATA1, putative	Solyc04g076990.3.1	ISGTTSPMLGK	∞
		LOW QUALITY: BRI1 kinase inhibitor 1	Solyc04g079110.1.1	GAYSAPVSMK	− ∞
		Mitogen-activated protein kinase 9	Solyc04g080730.3.1	DQFMTEYVVTR	− ∞

Table 1 (continued)

Condition	Protein description		Protein accession	Sequence (phosphopeptide)	log2 ratio
		Protein kinase-like protein	Solyc05g009540.3.1	SPLQGGQIGDVLAS-GAGGLGQGTPK	∞
		Kinase family protein	Solyc06g068985.1.1	LTDVSSPEK	− 1460
		Diacylglycerol kinase	Solyc07g006580.3.1	GSPELTTTDLSTSR	2276
		MAP kinase kinase kinase 57	Solyc07g055130.3.1	AVSLPSSPHEFR	− 1178
		MAP kinase kinase kinase 57	Solyc07g055130.3.1	NVSDFHHDDPEISR	− ∞
		LOW QUALITY: Serine/threonine-kinase pakA-like protein	Solyc07g062730.1.1	LSLSESSNR	∞
		Mitogen-activated protein kinase 2; Mitogen-activated protein kinase 1	Solyc08g014420.3.1; Solyc12g019460.2.1	VTSETDFMTEYVVTR	− 1231
		LRR receptor-like kinase	Solyc08g081940.3.1	EEATPLPDTQSDSQ	− 1006
		Kinase family protein	Solyc09g090200.3.1	LSGALLSDGRPAPPR	− ∞
		MAP kinase kinase kinase 75	Solyc10g055720.2.1	SVFPLPAFGSSPN-LEALALEASK	1428
		Calcium-dependent protein kinase family protein	Solyc10g074570.2.1	NSLNLSMR	− ∞
		Kinase family protein	Solyc10g077020.2.1	SASPEIVEVHEES-FRLEPDK	∞
		LOW QUALITY: Calcium/calmodulin protein kinase	Solyc11g006900.1.1	GSTNVSEPGSPK	∞
		Receptor-like protein kinase	Solyc12g008500.2.1	LSGEIPQLVSSR	∞
		Integrin-linked protein kinase family	Solyc12g019410.2.1	SPSIENAER	1498
	Kinase/phosphatase	CAI-1 autoinducer sensor kinase/phosphatase cqsS isoform 1	Solyc02g080150.2.1	SADPALVEEDASSGSGE-DINMLDGHNK	∞
	Phosphatase	Inositol-1,4,5-triphosphate-5-phosphatase 2	Solyc00g009110.3.1	SFNTYSSFK	− 1212
		Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	Solyc01g107750.3.1	SPTSPTDEHVDGTST-SPTSLFGTFTK	− ∞
		RNA polymerase II C-terminal domain phosphatase-like protein	Solyc02g078550.3.1	VQPHGWFPAEEEVSPR	− ∞
		Protein phosphatase 2C-like	Solyc04g074190.3.1	ILSNSSNLGR	∞
		RNA polymerase II C-terminal domain phosphatase-like 2	Solyc09g014440.3.1	SPGIFQESDASR	− 1017
		Phosphoinositide phosphatase family protein	Solyc11g022380.2.1	IGSNNEILNSLIK	∞

Table 1 (continued)

Condition	Protein description		Protein accession	Sequence (phosphopeptide)	log2 ratio
	Phytohormones-related protein	Dormancy/auxin associated protein	Solyc01g099840.3.1	SFTEEASEDAVK	− 2044
		Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	SLSMPASPPTPGTPVT-PTNISPTVR	3323
		Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	SLSMPASPPTPGTPVT-PTNISPTVR	2697
		Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	SLSMPASPPTPGTPVT-PTNISPTVR	− 14815
		Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	SLSMPASPPTPGTPVT-PTNISPTVRK	− ∞
		Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	SLSMPASPPTPGTPVT-PTNISPTVR	− 2975
		Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	LWDDVMAGPSPDK	− 1927
		Ethylene-responsive proteinase inhibitor 1	Solyc02g090090.3.1	QSSSISQSDSVSAK	− 2006
		Ethylene signaling protein	Solyc09g007870.3.1	GVSENAQSFISDGPG-SYK	∞
		Ethylene signaling protein	Solyc09g007870.3.1	GSDYLNGQLESPSPR	− ∞
		Ethylene-responsive RNA helicase	Solyc12g044860.2.1	SDSVFGGGSNYR	− ∞
	Transcription factor	PH-response transcription factor pacC/RIM101 isoform 1	Solyc01g080510.3.1	ISPGLLLASQTSTPRLTP-PGSPPSLSASVSPSR	∞
		Zinc finger transcription factor 11	Solyc01g087170.3.1	TLTPSNLEELFSAEMNS-SPR	∞
		bZIP transcription factor	Solyc01g110480.3.1	QFSPNFGVENSK	− 1433
		Exostosin family protein; Zinc finger CCCH domain protein; Zinc finger transcription factor 35	Solyc04g064765.1.1; Solyc04g064795.1.1; Solyc04g064800.3.1	SMSPAFER	∞
		WRKY transcription factor 4	Solyc05g012770.3.1	ESSDVSLSDQR	− 2407
		Transcription factor jumonji family protein	Solyc06g008490.3.1	DVIILSDDED	− ∞
		bZIP transcription factor	Solyc06g060490.3.1	DTSGLNAENK	∞
		bZIP transcription factor	Solyc06g060490.3.1	SLSVDADFFDGLDFG-GVPTEK	∞
		Transcription factor IWS1, putative	Solyc06g066320.3.1	YGSDNEPLSP-SNAPQAEEGEEDDEIK	− 2078
		LOW QUALITY: Transcription factor, putative	Solyc06g083430.1.1	YYDTDSDEVDVSFM-DLR	− ∞
		bZIP transcription factor (DUF630 and DUF632)	Solyc09g007100.3.1	TTDHASNSGSPEITSVR	∞
		Transcription factor DP	Solyc10g078430.2.1	LPTSPPLPGILK	− ∞
		Zinc finger transcription factor 60	Solyc10g079120.2.1	TLTPSNLEDLFSAE-GSSPR	∞
		Zinc finger transcription factor 67	Solyc11g065320.2.1	SNIDNSNLQESFEHILP-DNLFASPTK	− ∞
		Transcription factor CPC	Solyc11g071220.2.1	SDSSDDDDDEFILSPK	− ∞
		Transcription factor GTE4	Solyc12g014170.2.1	FGGSPIVEANTSGDVR	∞

Table 1 (continued)

Condition	Protein description		Protein accession	Sequence (phosphopeptide)	log2 ratio
Cd-free condition (Control)	Heat shock protein	Heat shock protein 70	Solyc01g103450.3.1	SFAAEEISAQVLR	∞
	Kinase	Mitogen-activated protein kinase 13	Solyc01g080240.3.1	VAFNDTPTAIFWTDY-VATR	∞
		Leucine-rich receptor-like protein kinase family protein	Solyc02g023950.3.1	EGAVVSAQNVAAASR	∞
		Non-specific serine/threonine-protein kinase	Solyc02g072530.2.1	PASLNAFDIISFSR	∞
		Kinase	Solyc02g078780.3.1	NMSMNTSEAVLQK	− ∞
		Kinase family protein	Solyc02g083290.3.1	VQTQTGVMTAETGTYR	− ∞
		Serine/threonine-protein kinase ATM	Solyc02g086750.2.1	LSVSIPESDEVGNQQD-NAGPLSR	− ∞
		Protein kinase	Solyc02g086790.3.1	FQVTSADLSPK	− ∞
		Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase	Solyc02g087620.3.1	GSQDNLAVNK	− ∞
		LRR receptor-like kinase	Solyc03g006030.3.1	DSQQADSLTMATTER	− ∞
		Pti1-like kinase	Solyc03g116760.3.1	IPPVVDDDVLSGTEG-NADSTASEEK	− ∞
		Kinase, putative	Solyc03g118530.3.1	DFQPIVGSPDVTK	∞
		Calcium-dependent protein kinase	Solyc04g049160.3.1	TMRNTLNLGEAL-GLVESK	∞
		MAP kinase kinase kinase 75; MAP kinase kinase kinase 11	Solyc10g055720.2.1; Solyc01g111880.3.1	AQTGVMTAETGTYR	− ∞
		Integrin-linked protein kinase family	Solyc12g019410.2.1	QFSTELFR	∞
		Inositol-tetrakisphosphate 1-kinase	Solyc12g088210.2.1	GFSASSFAR	− ∞
	Kinase/phosphatase	Protein phosphatase 2C family protein; Kinase family protein	Solyc07g066260.3.1; Solyc10g005640.3.1	VNSLLSLPR	− ∞
	Phosphatase	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	Solyc01g107750.3.1	SPTSPTDEHVDGTST-SPTSLFGTFTK	− ∞
		RNA polymerase II C-terminal domain phosphatase-like protein	Solyc02g078550.3.1	VQPHGWFPAEEEVSPR	− ∞
		Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B	Solyc03g121410.3.1	GSESPGVDANGNSFD-FTTK	− ∞
		Protein phosphatase 2C family protein	Solyc04g082600.3.1	SISVEGLNLLK	− ∞
		RNA polymerase II C-terminal domain phosphatase-like 2	Solyc09g014440.3.1	SPGIFQESDASR	∞

Table 1 (continued)

Condition	Protein description		Protein accession	Sequence (phosphopeptide)	log2 ratio
	Phytohormones-related protein	Auxin repressed/dormancy-associated protein	Solyc02g077880.3.1	SLSMPASPPTPGTPVT-PTNISPTVRK	− ∞
		1 Auxin repressed/dormancy-associated protein	Solyc02g077880.3	LWDDVMAGPSPDK	− 2304
		Ethylene-responsive RNA helicase	Solyc12g044860.2.1	SDSVFGGGSNYR	− ∞
	Transcription factor	Zinc finger transcription factor 31	Solyc04g008550.3.1	LPSRSPSPTNGTDMR	− ∞
		bZIP transcription factor	Solyc06g060490.3.1	SLSVDADFFDGLDFG-GVPTEK	∞
		bZIP transcription factor	Solyc07g062710.3.1	NEPGEDDSDNQSPVTSK	− ∞
		bZIP transcription factor (DUF630 and DUF632)	Solyc09g007100.3.1	TTDHASNSGSPEITSVR	∞
		Zinc finger transcription factor 69	Solyc11g069340.2.1	SLSESSADDT-TEPATQLK	− ∞

For log2 ratio, positive numbers indicate phosphopeptides increased in the tolerant (PR) plants compared with sensitive (CR) ones (permutation-based FDR = 0.01) or phosphopeptides exclusively detected (∞) in the tolerant plants. Negative numbers indicate phosphopeptides reduced in the tolerant (PR) plants compared with sensitive (CR) ones (permutation-based FDR = 0.01) or phosphopeptides exclusively detected (− ∞) in the sensitive plants

these TFs families has been shown to enhance Cd tolerance in different plant species (reviewed by Marques et al. 2019).

In summary, the first large-scale differential phosphoproteome analysis of Cd-tolerant and Cd-sensitive tomato plants under Cd treatment revealed a substantial number of Cd-responsive phosphoproteins that are potentially involved in Cd signaling and Cd stress tolerance in tomato. Based on the context provided by previous research into plant Cd responses, results found here indicate that tomato initiates multiple phosphorylation strategies in response to Cd exposure including changes in metal phytochelation, protection from protein misfolding and aggregation, and shifts in stress hormones. This focus article provides a starting point to integrate phosphoproteomics with data on other metabolic and physiological processes, such as those previously assessed using the PR and CR genotypes, which together may form the mechanistic basis of Cd tolerance in tomato. Moving toward more comprehensive examination of plant stress-response systems through the incorporation of phosphoproteomics will permit more targeted development of new Cd-tolerant tomato cultivars. Future work should consider strategic phosphoproteomic contrasts of a wider range of tomato genotypes which vary in both Cd tolerance and Cd accumulation, as well as determination of the significance of the key proteins including TFs identified in this article, which together will provide a fuller picture of the physiological, biochemical, and molecular responses that underly Cd tolerance in tomato.

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Author contribution statement DNM, HN, and RAA conceived the study. DNM, MLN, KDBP, and FAP designed the hydroponic system trial and sample collection. DNM and MLN performed the hydroponic system trial and sample collection. DNM, SCS, AH, and HN conducted the phosphoproteomic analysis. DNM wrote the manuscript. All authors read, worked on the article, and approved the submitted version.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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