**Identification of thioredoxin targets in guard cell enriched epidermal peels using
cysTMT proteomics**

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Biological significance

Redox homeostasis is tightly regulated for proper cellular activities. Specific protein-protein interactions between redox active molecules such as thioredoxin (Trx) and target proteins constitute the basis for redox-regulated biological processes. The use of cystTMT quantitative proteomics for studying Trx reactions enabled identification of potential Trx targets that provide important insights into the redox regulation in guard cells, a specialized plant cell type responsible for sensing of environmental signals, gas exchange and plant productivity.

ABSTRACT

Thioredoxins (Trx) play central roles in cellular redox regulation. Although hundreds of Trx targets have been identified using different approaches, the capture of targets in a quantitative and efficient manner is challenging. Here we report a high-throughput method using cysteine reactive tandem mass tag (cysTMT) labeling followed by liquid chromatography (LC)-mass spectrometry (MS) to screen for Trx targets. Compared to

existing methods, this approach allows for i) three replicates of pairwise comparison in a single LC-MS run to reduce run-to-run variation; ii) efficient enrichment of cysteine-containing peptides that requires low protein input; iii) accurate quantification of the cysteine redox status and localization of the Trx targeted cysteine residues. Application of this method in guard cell-enriched epidermal peels from *Brassica napus* revealed 80 Trx *h* targets involved in a broad range of processes, including photosynthesis, stress response, metabolism and cell signaling. The adaption of this protocol in other systems will greatly improve our understanding of the Trx function in regulating cellular redox homeostasis.

Key words: cystTMT, Epidermal peels, Guard cells, LC-MS/MS, Redox proteomics, Thioredoxin targets

Cysteine modifications mediated by thioredoxin (Trx) represent an important mechanism for rapid control of cellular activities in plants, such as photosynthesis [1], seed germination [2] and pollination [3]. Different Trx isoforms in various cellular compartments have been described in plants [4, 5], and there are at least 20 *Trx* genes in the model plant *Arabidopsis thaliana* [6]. Due to the number of Trx isoforms and diverse interacting partners, the plant Trx systems are complex. For instance, reversible reduction of oxidized proteins is carried out by a specific Trx paired with a specific NADPH-

dependent Trx reductase (TrxR) [4]. Knowledge of Trx target proteins is fundamental for understanding Trx-controlled redox-regulated physiological processes.

Two main approaches, one based on the covalent binding between a Trx mutant and target proteins, and the other based on specific thiol labeling, have been developed to identify Trx targets [4, 7-11]. The first method uses a Trx mutant with the second cysteine of the conserved CxxC active site replaced by serine or alanine to trap potential targets via affinity binding from cell lysates. Eluted proteins are then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [11, 12] or by two-dimensional gel electrophoresis (2-DE) [11, 13], followed by identification using mass spectrometry (MS). The sites of cysteine modifications and the quantitation of the redox status, however, are missing from such an approach. In the second approach, fluorescent thiol group-specific probes, such as monobromobimane (mBBBr) [1] or cyanine 5 maleimide (Cy5m) [14], are used to label free sulfhydryl groups (-SH). Then the probe-labeled proteins are resolved in 2-DE to reveal potential Trx targets through image analysis. Disadvantages of this method include low sensitivity and low labeling efficiency, as well as the limitations of 2-DE techniques. A non gel-based thiol labeling workflow, i.e., isotope-coded affinity tag (ICAT) has also been applied in Trx targets identification, providing both the sites of the redox-sensitive cysteines and the quantitation of the redox status of individual peptides [7]. However, there are only two tags (i.e., heavy and light), limiting the number of samples that can be analyzed simultaneously. These traditional approaches have been employed to identify Trx targets in plants, and a total of over 500 potential targets have been discovered in various plant species and tissues [4].

93 To identify Trx targets quantitatively in a more efficient manner, we implemented a
94 new approach to differentially label cysteine residues with cysTMT (Figure 1). The
95 cysTMT reagents contain six tandem mass tags that react specifically with sulfhydryl
96 groups and have been used to quantify modification on cysteine residues [15]. We
97 applied this method using guard cell enriched epidermal peels from *Brassica napus*, in
98 which redox regulation plays essential roles in cellular signaling and metabolism [16-19].
99 Briefly, total protein samples from cell lysates were oxidized by air to induce disulfide
100 bond formation [20]. Exposure of the samples to air overnight effectively oxidized the
101 proteins, as the contents of free thiols measured by 5,5-dithio-bis-(2-nitrobenzoic acid)
102 (DTNB) dropped significantly after the overnight reaction (Supplemental Figure S1). The
103 DTNB results of the oxidized samples with and without IAM blocking were not
104 statistically different, indicating that air oxidation of free thiols was complete
105 (Supplemental Figure S1). Activated (reduced) Trx *h* was then incubated with the
106 oxidized samples to reduce thiol groups of target proteins, whereas the introduction of
107 Trx had no effect on non-target proteins. To confirm the activity of Trx *h*, both oxidized
108 and reduced Trx *h* were incubated with the pre-oxidized samples. The results showed that
109 only the reduced Trx *h* was able to reduce the oxidized thiols, giving rise to free thiols
110 and thus a significant increase in the DTNB reading (Supplemental Figure S1). The
111 newly formed -SH were immediately and irreversibly alkylated with iodoacetamide
112 (IAM). The alkylation efficiency is approximately 97% based on the
113 carbamidomethylated cysteine peptides and total cysteine containing peptides identified
114 from an aliquot of the samples. After acetone precipitation overnight and brief
115 lyophilization of the protein pellets, the remaining disulfide bonds were then reduced by

the strong reducing agent Tris (2-carboxyethyl) phosphine (TCEP), which doesn't interfere with the downstream cystTMT reagents. All the available thiols were labeled with cystTMT tags: 126, 127 and 128 for the control groups without Trx *h* treatment, and 129, 130 and 131 for the three replicates with Trx *h*. All the samples were combined and separated by SDS-PAGE. The gel lane was cut into different sections, which were digested with trypsin. The cystTMT labeled peptides were enriched by incubation with an immobilized anti-TMT antibody resin for 2 h at room temperature. Peptides were eluted with 50% acetonitrile and 0.4% trifluoroacetic acid for LC-MS/MS analysis. Peptide samples were analyzed using a Q ExactiveTM Orbitrap mass spectrometer, and the raw data were searched using Proteome Discoverer (see Supplemental Materials and Methods for details).

In this method, the quantitative values, i.e., peak intensities of the cystTMT tags were used to determine the redox status of cysteines, thus indicating the putative Trx targets (Figure 1). For the non-target proteins, the same levels of cystTMT labeling with Trx treatment (129, 130, and 131) and without (126, 127, and 128) were expected. Thus, the respective ratios of 129, 130, and 131 to 126, 127, and 128 should theoretically be close to 1. Because cysteines in target proteins were reduced by Trx and then irreversibly blocked by IAM, less labeling of these cysteines compared to those of the control groups occurred. The ratios between control and Trx-treated samples of the Trx-targeted cysteines, therefore, are greater than one (Figure 1). Statistically, a ratio greater than 1.22 is sufficient to define a Trx reduction event in previous experiments [4]. On the other hand, the ratio for Trx itself is expected to be less than one, as a result of the catalytic motif being oxidized.

Our workflow resulted in identification of 767 non-redundant peptides with cysTMT tags at 1% false discovery rate (FDR) (Supplemental Table 1). Compared to the 199 peptides identified from the ICAT labeling experiment [7], the dramatic increase in cysteine-containing peptides may be attributed to the enrichment effect of six-plex cysTMT, which allows for the identification of low-abundance proteins. The number reported here is also consistent with the low occurrence of cysteines in plant proteome. For instance, cysteine only represents 2% of the amino acid residues in the proteome of the model plant *Arabidopsis* [4]. In addition, we also identified 1366 high confident proteins in the guard cell-enriched samples (cysTMT enriched and non-enriched samples) (Supplemental Table 2), which is comparable to previous guard cell proteome profiling studies [19, 21].

Known Trx targets represent a small fraction of a plant proteome [4]. Thus, the *in vitro* Trx reaction should not alter the redox status of majority of the proteins, giving rise to a average ratio of all available cysteine residues labeled by cysTMT between the control and the Trx-treated groups to be close to 1. Our data showed an average ratio of 1.04 (Supplemental Table 1), indicating that the labeling strategy introduced no bias and is appropriate for Trx target identification. Using criteria of both fold-change and statistical analysis (Supplemental Materials and Methods), 80 putative Trx *h* targets were identified for the first time in guard cell enriched samples and classified based on biological functions (Table 1 and Supplemental Figure S2). Interestingly, a broad range of Trx targets were identified here, with ~ 60% (46 out of 80) being previously reported in other cells or tissues, and ~40% (34 out of 80) being newly identified targets (Supplemental Table 1).

Enzymes controlling redox homeostasis are well-known Trx targets [11, 22, 23]. For example, 2-Cys peroxiredoxin functions in the NAD(P)H-Trx system in both cytosol and plastid [24]. Ferredoxins have also been identified as putative Trx *h* targets, in agreement with previous reports [7, 10]. However, ferredoxin functions predominantly in chloroplasts with Trx isoforms *f*, *m*, *x*, and *y* [22]. Thus, the interaction with Trx *h* (predominantly cytosol, mitochondria, and endoplasmic reticulum) might not represent *in planta* situation. The loss of Trx subcellular specificity also occurs in other *in vitro* assays such as the capture method using Trx affinity columns [11]. Isolation of a single cellular compartment for specified Trx interactions has been performed to address the issue of specificity [24].

Both large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase, phosphoribulokinase, and fructose-bisphosphate aldolase 1 were identified as Trx *h* targets in guard cell-enriched epidermal peels (Table 1). This is in line with studies in other plant systems [8, 25, 26]. In addition, subunit psaK of photosystem I reaction center was identified as a Trx *h* target [27]. Similarly, we identified photosystem I subunit VII, i.e., psaC. Furthermore, a novel protein containing 2Fe-2S iron-sulfur cluster binding domain was also identified here. These findings are in agreement with the notion that photosynthesis is highly redox-regulated and that this process is actively involved in stomatal function [28].

Functional classification of the 80 putative Trx *h* target proteins revealed that 31 participate in energy and metabolic processes. Significantly, 18 of those targets have been previously identified (Supplemental Table 1) [2, 7, 8, 12, 22-25, 29, 30]. Amino acid metabolism has been demonstrated to be regulated by Trx; in particular,

aminotransferases are reduced by Trx [8, 22, 30]. Interestingly, we identified a γ -glutamylcysteine synthetase, which catalyzes the first step in the biosynthesis of the cellular antioxidant glutathione (GSH). In addition, cysteine synthetase was also reported to be Trx target [12]. This finding of Trx regulation of γ -glutamylcysteine synthetase and thus GSH biosynthesis is new. It will be intriguing to further investigate this new Trx interaction and its physiological significance in maintaining cellular GSH levels.

Another major group of the Trx *h* targets participates in protein synthesis, folding, transport and degradation. A substantial number of chaperonin, isomerase, and lipid transfer proteins were repetitively identified as targets [2, 7, 14, 22, 30-32], highlighting the key role of Trx in protein turnover and transport between cellular compartments. Notably, aspartic protease in guard cell 1 (ASPG1) was identified with a reduced-to-oxidized ratio of 2.4, indicating extensive reduction by Trx. ASPG1 functions in abscisic acid (ABA) mediated drought response, and overexpression of *ASPG1* reduced water loss in *Arabidopsis* [33]. Reactive oxygen species (ROS) burst and redox regulation of key kinases represent important signaling events in ABA signal transduction [17-19]. However, the role of Trx in this process remained elusive. The identification of ASPG1 as the potential Trx target may open the door to fill this knowledge gap.

The majority of Trx *h* targets in stress and defense, signaling, cell cycle, and cell structure are novel Trx substrates. Guard cells are rich in cell wall pectin and polysaccharides that contribute to the stomatal movement [34, 35]. Pectin acetyltransferase catalyzes the interaction between acetylated polysaccharides and cellulose [36], a key process governing the cell structure. Redox control in plant growth and development has been widely recognized [37]. Yet such regulatory mechanism in cell wall structure,

especially in guard cells, has not been reported. The identified Trx *h* target proteins may reveal new aspects of redox regulation in guard cell functions.

A great portion of the substrates revealed by this study were previously functionally characterized as Trx targets, suggesting the reliability of this cystTMT method in Trx target identification. Additionally, previous studies using traditional methods usually identified 10-50 targets in one experiment [4]. The number identified in our study has been greatly increased, suggesting utility of this new multiplex technology. Moreover, the established Trx targets could contain one or two cysteine residues, forming intra- or inter-molecular disulfide bonds [4, 38]. Thus, the presence of cysteine residue(s) could provide more credibility for a putative Trx target. In our method, Trx targeted cysteines were precisely localized in each target protein, due to the enrichment and identification of cystTMT labeled peptides. However, further investigation using biochemical and genetic tools is needed to determine the potential reaction mechanisms. For instance, analysis of target reduction upon Trx treatment and *in vivo* protein-protein interaction will improve our understanding of Trx-target mediated biological processes. It should be noted that a little over 10% of the identified cystTMT peptides exhibited ratio changes opposite to expected Trx-targets (Supplemental Table 1). These peptides and their associated proteins are deemed not to be Trx-targets. Although the reason is not known, we postulate that these proteins might be subjected to oxidization due to possible association with Trx-targets and/or irreversible oxidation might have taken place under control conditions. In addition, technical issues of incomplete alkylation due to protein structure might also contribute to false negatives. As mentioned before, an obvious challenge of *in vitro* Trx target analysis is the disruption of subcellular compartments, which may lead to

unambiguity of identification of *bona fide* targets. Although Trx *h* is not solely localized in the cytoplasm [39] and its targets were found in the chloroplasts [7, 10, 27], the biological relevance of these findings deserve further investigation.

In summary, we showed the suitability of cysTMT in identification and multiplex quantification of cysteine modifications in discovering potential Trx targets. The six-plex cysTMT represents an isobaric mass tag system that specifically labels cysteines, providing unambiguous localization of cysteine modification and a convenient way to enrich cysteine-containing peptides. The reporter ions allow for quantitation of six samples in a single experiment. We also note that there are other applications of cysTMT in redox proteomics such as cysteine redox status monitoring [40] and mapping of S-nitrosylation sites [15]. Therefore, more applications of cysTMT in redox proteomics can be expected in the future.

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Table 1. Thioredoxin *h* (Trx *h*) targets identified from *B. napus* guard cell-enriched epidermal peels using cysTMT LC-MS/MS.

FIGURE LEGENDS

Figure 1. Scheme for identification of Trx *h* targets using cysTMT labeling and tandem MS. The ordered chemical reactions for a Trx target in the control (upper panel) and Trx *h* treated (lower panel) samples are presented. Protein extracted from *B. napus* epidermal peels was pre-oxidized by incubation with air (O₂) overnight [20]. Recombinant Trx *h* was activated by incubation with DTT for 30 min at room temperature, followed by binding to the nickel column and being washed thoroughly to remove DTT. Oxidized protein extract was incubated with or without Trx *h* for 1 h at room temperature. IAM was then added to the mixture at a final concentration of 100 mM for protein alkylation to block free thiol groups. The oxidized cysteines were reduced and labeled with cysTMT tags, followed by LC-MS/MS. The MS spectra represents the theoretical distribution of the cysTMT tags at the low m/z region, where the intensities of 126,127 and 128 from the control group are higher than those of 129,130 and 131 from the Trx treated groups. The insert indicates the opposite trend for Trx *h* itself.

Figure 2. Annotated mass spectrum of a representative cysteine-containing peptide showing differential redox status from control and treated samples. The MS/MS ions used to identified the peptide were labeled, and the intensity of individual cysTMT peak for quantification was inserted in the upper-left corner. This peptide was used to identify the Photosystem I reaction center subunit N as a putative Trx *h* target.

SUPPORTING INFORMATION

Supplemental Figure S1. Thx *h* reduces air-oxidized proteins from *B. napus* epidermal guard cells. Data from DNTB assay of free thiols under different treatments are presented as means with standard errors of three biological replicates. Inserted p values indicate whether the differences among the groups are statistically significant using t-test.

Supplemental Figure S2. Classification of the 80 putative Trx *h* targets into molecular functions.

Supplemental Table 1. Trx *h* targeted cysteine residues identified in guard cell enriched epidermal peels from *B. napus*.

Supplemental Table 2. Proteome of guard cell-enriched epidermal peels from *B. napus*.

Supplemental Materials and Methods

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Table 1. Thioredoxin *h* targets identified from *B. napus* guard cell-enriched epidermal peels using cystMT LC-MS/MS. Lowercases c and m in the peptide sequences represent cystMT labeled cysteines and oxidized methionine, respectively.

Accession	Protein Name	Peptides containing Trx <i>h</i> targeted cysteines	Fold change
Redox Homeostasis (8)			
119952	Ferredoxin, root R-B2	AGAcSTcAGQIVK	1.5038
3023740	Ferredoxin	AGAcSScAGK	1.2916
296514476	2-Cys peroxiredoxin	TLQALQYVQENPDEVcPAGWKPGEK	1.5688
75336180	Peroxiredoxin Q, chloroplastic	GKPVVVYFYPADETPGcTK	1.2992
297816586	Peroxiredoxin-2E, chloroplastic	TILFAVPGAFTPTcSQK	1.5676
79320786	Thioredoxin-dependent peroxidase 1	VILFGVPGAFTPTcSmK	1.2575
255552726	Ferredoxin-thioredoxin reductase, chloroplast	SGTYFcVDK	1.8156
297809773	Thioredoxin M2, chloroplastic-like isoform X2	ADGPVLVDFWAPWcGPcK	1.3889
Photosynthesis (9)			
52354317	AT3G27370, containing 2Fe-2S iron-sulfur cluster binding domain	DcGPmVLDALIK	1.5295
1352767	Ribulose biphosphate carboxylase large chain	WSPELSSAcEVWK	1.2671
266891	Ribulose biphosphate carboxylase small chain	QVQcISFIA YKPPSFTG	1.5856
113201045	Photosystem I subunit VII	TEDcVGcK	1.5317
		AcPTDVLEmIPWDGcK	1.4872
		cESAcPTDFLSVR	1.4280
		IYDTcIGcTQcVR	1.4074
		TEDcVGcKR	1.2986
115778	Chlorophyll a-b binding protein 1, chloroplastic	ELEVIHcR	1.3319
		NRELEVIHcR	1.2311
297819782	Photosystem II subunit O-2	FcFEPTSFTVK	1.4521
1709825	Photosystem I reaction center subunit N	AFTVQFGScK	1.8025
23197622	Phosphoribulokinase	YFSPVYLFDEGSTISWIPcGR	1.3024
312281829	Fructose-bisphosphate aldolase 1, chloroplastic, putative	ALQNTcLK	1.7186
		GILAMDESNATcGK	1.3228
Energy (13)			
6598624	Ubiquinol-cytochrome c reductase, putative	cVKPLLEYQAcVK	1.5866
120659	Glyceraldehyde-3-phosphate dehydrogenase A	cSDVSSTIDSSLTmVmGDDmVK	1.4456
120675	Glyceraldehyde-3-phosphate dehydrogenase	SDLNIVSNAScTTNcLAPLAK	1.8287
270381252	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	VPTPNVSVVDLTcR	1.7431
145329204	Triose-phosphate isomerase	AAYALSEGLGVIAcIGEK	1.3390

Accession	Protein Name	Peptides containing Trx <i>h</i> targeted cysteines	Fold change
222840927	3-Isopropylmalate dehydratase small subunit 3	YSIVIGGENFGcGSSR	1.9764
222849133	Triosephosphate isomerase, chloroplastic-like	IYGGSVNGGNcAELAK	1.7801
225424536	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7	cEYELV _m ER DQcAHL _L IPLNK	1.6862 1.3562
226493510	Pyruvate kinase	cENTAm _L GER	1.6895
255640681	Fructose-1,6-bisphosphatase, chloroplastic	KLDVVSNEVFSNcLR	1.2884
257350590	3-Isopropylmalate dehydratase large subunit isoform X2	VYIGScTGGK LNEPQVcVSTTNR	1.3957 1.3471
257712859	Malate dehydrogenase 1, cytoplasmic	DLAYS _c LS	1.4304
34597330	Enolase Metabolism (18)	mTAEcGDNVQIVGDDLLVTNPK	1.5856
145326672	Lactoylglutathione lyase, putative	GPTPEPLcQVMLR	1.7621
14532772	Putative methionine synthase	HETcYQIALAIKDEVEDLEK	1.5857
21593238	Haloacid dehalogenase-like hydrolase family protein	SLEALIFDcDGVILESEN _L HR	1.5856
255640791	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	AAGmTcIVTK	1.5261
2204224	Alpha-galactosidase	APLIIGcDVR	1.3260
222423482	ATP citrate lyase (ACL) family protein	TDcTmLEINPLAETSTNQLVAADAK	1.5857
222424932	GDSL-like Lipase/Acylhydrolase superfamily protein	VTNAGccGIGR	1.5146
227305852	Glycine dehydrogenase (decarboxylating) 1, mitochondrial	NAGQITcLPGQRPcR YcGYDHIDSLVDDTVPK	1.4181 1.5858
227482030	Aminomethyltransferase, mitochondrial-like	DSIIDSTVNcR	1.2961
2494843	Lactoylglutathione lyase, putative	TIQFYTEcFGmK	1.5612
295421147	Cysteine synthase, chloroplastic/chromoplastic	GcVASVAAK	1.4640
297824281	Aconitase C-terminal domain- containing protein	YSIIIGGENFGcGSSR	1.5145
297838861	Alanine-2-oxoglutarate aminotransferase 2	AmVIINPGNPTGQcLSEANLK	1.6593
32967701	S-adenosyl-L-homocystein hydrolase	GcAAAmK FDNLYGcR VAVIcGYGDVGK	1.7883 1.3956 1.2524

Accession	Protein Name	Peptides containing Trx <i>h</i> targeted cysteines	Fold change
3688156	Gamma-glutamylcysteine synthetase	YVDcTGMTFR	1.6343
4210334	2-oxoglutarate dehydrogenase, E3 subunit	GALGGTcLNVGcIPSK	1.6926
62319114	Hydroxymethylbilane synthase	AFLETLDGScR	1.7260
297821142	Carbohydrate-binding-like fold-containing protein	DSTQcAPNGYYFIPVYDK	1.5858
Protein Synthesis (3)			
1710539	60S ribosomal protein L32	TYcAEIAHNVSTK	1.3685
118488685	Elongation factor 2-like	NcDPNGPLmVYISK	1.3167
297826663	40S ribosomal protein S12	VAQLcVLAEDcNQP DYVK TLGEWAGLcK	3.3983 1.5688
Protein Folding, Transporting and Degradation (9)			
20259872	Peptidylprolyl isomerase ROC4	IYAcGELPLDA	1.3776
218197187	Chaperonin CPN60-like 2	VAGDGTTcATVLTQAILTEGcK	1.8175
257745290	Peptidyl-prolyl cis-trans isomerase	FMcQGGDFTAGNGTGGESIYGmK	1.5744
295421857	Peptidyl-prolyl cis-trans isomerase	VAcNLNDAAcK	1.3184
296081545	Protease inhibitor/seed storage/lipid transfer protein family protein	ADAEcLcEAFK	1.2729
257727803	ASPARTIC PROTEASE IN GUARD CELL 1	SLTcSAPQcASLR	2.4370
32400784	26S protease regulatory subunit 6A	AcAAQT NATFLK	2.7738
19424106	Chloroplast nucleoid DNA-binding protease, putative	ATSFSYcLVDR	1.6377
21668075	Chloroplast nucleoid DNA-binding protease, putative	VGFAPNGcS	1.4743
Membrane and Transport (8)			
310896458	Lipid transfer protein	AccSGVTSLNNmAR	1.3663
3719443	Lipid transfer protein	cLKTAASALGPNLNAGR AcGVSVFPPISTNTNcNNVK	1.3846 1.2683
21553541	Lipid transfer protein, putative	VTLCDFATGK	1.4537
4185505	Nonspecific lipid-transfer protein precursor	GGVPAPccAGVSK	1.5565
913407	Acyl-binding/lipid-transfer protein isoform III, AB/LTP III	cGV SIPYPISmSTNcDTVK GccTGV TNLNNmAR	1.3055 1.3201
156778059	Non-specific lipid-transfer protein type 1 (nsLTP1) subfamily	cLVGAANSFPSLNAAR	1.3091
913408	Acyl-binding/lipid-transfer protein isoform II, AB/LTP II	AccSGVTSLNNLAR TTPDRQQAcR cLVGAANAFPTLNAAR	1.4903 1.3952 1.2539

Accession	Protein Name	Peptides containing Trx <i>h</i> targeted cysteines	Fold change
913409	Acyl-binding/lipid-transfer protein isoform I, AB/LTP I Stress and Defense (4)	cLVGAANALPTINVAR	1.2827
26397694	Pyridoxal kinase-like protein SALT OVERLY SENSITIVE 4	SVNPNLTYVcDPVMGDEGK	1.2293
15809822	L-GALL RESPONSIVE GENE 2	TcAQDER	1.4647
297795529	Cysteine-rich repeat secretory protein 55, containing salt stress response/antifungal domain	LTPFVTLNGLVQcTR	1.2326
23321185	33 kDa secretory protein-like protein, containing salt stress response/antifungal domain Cell Divison, Differentiation and Fate (1)	EVcPNQSDAR	1.6052
1019904	Cell division cycle protein Signaling (3)	YTQGFSGADITEIcQR	1.2147
25084280	Phospholipase A2-alpha	NNDYLSQECSQK	2.1639
83032252	Ran-binding protein 1-A	FASIENcK	1.4990
297839869	Transducin family protein Cell Structure (3)	cVTAFPDQQGFLVGSIEGR	1.5858
179743760	Xyloglucan endotransglycosylase	DFDIEGcSVPGPAGcPANPK	1.6525
297794737	Pectin acetylerase	cTAAQLQTVQGYR	1.4453
		YcDGSSFTGDIEAVDPTNK	1.4063
297799230	Vegetative cell wall protein gp1- like Transcription (1)	ADYDAcNSK	1.3048
5107159	Small zinc finger-like protein	ITSScWDK	2.0728

Figure 1

Figure 1

Figure 2

Figure 2