Quantitative proteomic analysis of the Salmonella-lettuce interaction

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Summary

Human pathogens can internalize food crops through root and surface uptake and persist inside crop plants. The goal of the study was to elucidate the global modulation of bacteria and plant protein expression after Salmonella internalizes lettuce. A quantitative proteomic approach was used to analyse the protein expression of Salmonella enterica serovar Infantis and lettuce cultivar Green Salad Bowl 24 h after infiltrating S. Infantis into lettuce leaves. Among the 50 differentially expressed proteins identified by comparing internalized S. Infantis against S. Infantis grown in Luria Broth, proteins involved in glycolysis were down-regulated, while one protein involved in ascorbate uptake was up-regulated. Stress response proteins, especially antioxidant proteins, were up-regulated. The modulation in protein expression suggested that internalized S. Infantis might utilize ascorbate as a carbon source and require multiple stress response proteins to cope with stresses encountered in plants. On the other hand, among the 20 differentially expressed lettuce proteins, proteins involved in defense response to bacteria were up-regulated. Moreover, the secreted effector PipB2 of S. Infantis and R proteins of lettuce were induced after bacterial internalization into lettuce leaves, indicating human pathogen S. Infantis triggered the defense mechanisms of lettuce, which normally responds to plant pathogens.

Introduction

Outbreaks of diseases associated with contamination of fresh produce by human pathogens have increased in the past decades (Lynch et al., 2009; Schikora et al., 2012). Better practice during postharvest processing or the use of a terminal control such as disinfection could reduce the load of microorganisms on the surfaces of fresh produce. However, concerns are raised over food crops contaminated with human pathogens that get internalized in plants during field production, because washing or disinfection may not be effective to remove the internalized bacteria (Wei et al., 1995; Weissinger et al., 2000).

Human pathogens can internalize into plants through root or leaf uptake. Salmonella enterica serovars Cubana and Dublin can accumulate inside hydroponically grown alfalfa and lettuce, respectively, through root uptake both at the level of 4 log CFU/g fresh weight (Dong et al., 2003; Klerks et al., 2007). Internalization of human pathogens can also occur through root uptake when the pathogens are introduced by contaminated soil or irrigation water (Wachtel et al., 2002; Hora et al., 2005; Klerks et al., 2007). A recent study on leaf uptake shows that spray irrigation with contaminated water can lead to the internalization of Escherichia coli O157:H7 into spinach leaves (Erickson et al., 2010).

The fate of human pathogens inside plants is determined by their interaction with plants. Schikora and colleagues (2008) found that Salmonella enterica serovar Typhimurium infiltrated into Arabidopsis leaves multiplied within the first 2 days after infiltration and remained viable for at least 4 days. Escherichia coli O157:H7 could survive inside spinach leaves for up to 14 days after spray inoculation (Erickson et al., 2010). In contrast, internalized S. Newport could not be detected in basil leaves 22 h after...
introducing the bacteria by placing cut petiole in a bacteria suspension (Gorbatschov et al., 2013). Despite the important findings reported in these studies, it is still poorly understood how internalized human pathogens adjust their metabolism to survive inside plants.

In recent years, mRNA-based transcriptomic approaches have been used to examine the gene expression of human pathogens living in and on plants. After spray-inoculated on lettuce leaf surface for 1–3 days, E. coli K-12 and O157:H7 up-regulated genes associated with starvation and curli production (Fink et al., 2012). Similarly, E. coli K-12 cells that were attached to and internalized inside the lettuce root up-regulated genes involved in attachment, stress responses and protein synthesis (Hou et al., 2012). After 15–30 min of exposure to lettuce leaf lysates, E. coli O157:H7 up-regulated its flagellar machinery, fimbrial, type III secretion system (T3SS) (a virulent factor) and stress response (especially oxidative stress) genes (Kyle et al., 2010). Collectively, these observations suggest that human pathogens encounter stresses in plants and the temporal changes in the expression of certain genes depend on the location of the bacteria (i.e. outside or inside the plant root or leaf). Despite the important information gained from these transcriptomic studies, it should be noted that the expression levels of mRNA and proteins are not directly proportional and transcriptomics cannot detect post-translational modifications on proteins (Abbott, 1999).

Different from transcriptomics, proteomics directly studies the ultimate products of gene expression. Although the advantages of using proteomics to study bacterial adaptation to plant-associated environments has been recognized (Knief et al., 2011), the application of proteomics in studying the interaction between human pathogens and plants has been limited. The objective of this study was to investigate the proteomic responses of Salmonella after internalizing lettuce leaf and the proteomic responses of lettuce leaf to internalized Salmonella. Two-dimensional nanoliquid chromatography-tandem mass spectrometry (2D nano LC-MS/MS) approach was utilized for the quantitative shotgun proteomic analysis. Two comparisons were made: global proteome profiles of internalized Salmonella versus Salmonella grown in Luria Broth (LB), and proteome profiles of lettuce leaf containing internalized Salmonella versus lettuce leaf without internalized Salmonella.

Results and discussion

Quantitative proteomic analysis

Leaves of 5-week old leafy lettuce (Lactuca sativa) cultivar Green Salad Bowl were inoculated by Salmonella enterica serovar Infantis (S. Infantis) suspension using syringe infiltration (Katagiri et al., 2002). Because previous experiences with syringe infiltration showed only a fraction of the cells in a bacterial suspension could end up in leaves, 300 µl of 10^10 CFU/ml bacterial culture (stationary phase S. Infantis grown in Luria Broth), which was washed and resuspended in sterile water, was infiltrated to ensure sufficient internalized S. Infantis cells to elicit significant proteomic response. Control plants were infiltrated with the same amount of sterile water. Two biological replicates were included in each group (i.e. treatment and control groups). Lettuce leaves were harvested 24 h after infiltration, and bacteria on the leaf surface were removed by sonication and vortexing for 4 times. Bacterial and plant proteins in lettuce leaf samples containing internalized S. Infantis were separated (details in Supporting Information). In addition, bacterial protein was extracted from stationary phase S. Infantis grown in LB and plant proteins from lettuce leaf without internalized S. Infantis. Protein digestion and 2D LC-MS/MS analysis were performed as previously described in the literature (Nandakumar et al., 2011; Li et al., 2012). The acquired MS/MS spectra from the bacterial protein samples were searched against the S. Typhimurium 14028S database (5323 sequences), and those from the lettuce protein samples against both the Lactuca sativa expressed sequence tag (EST) database (128172 sequences) and a custom-made database including Lactuca sativa protein sequences (1506 entries) on NCBI (Cho et al., 2009). The criteria for protein identification included the detection of at least one unique peptide per protein and a protein probability score of ≥90%. Relative quantitation of proteins was done by using the label-free method of spectral counting (Liu et al., 2004) with the normalized spectral counts for each protein. Proteins having ≥2-fold change in abundance (P ≤ 0.05) were considered as differentially expressed. More details about the methods can be found in Supporting Information.

Protein expression profile of S. Infantis

The protein expression profile was compared between the S. Infantis internalized in lettuce leaves (i.e. 24 h after infiltration) and the stationary-phase S. Infantis grown in LB medium (i.e. immediately before infiltration). A total of 541 proteins were detected, and 50 proteins were differentially expressed (≥2-fold and P < 0.05), among which 34 proteins were up-regulated and 16 were down-regulated (Table 1).

Metabolism. The most significant change among all differentially expressed bacterial proteins, a 37-fold increase, was seen in a putative cytoplasmic protein. The protein is determined to be an ascorbate-specific IIB component and is believed to phosphorylate ascorbate during transmembrane transport. Interestingly, ascorbate
Table 1. Proteins that were differentially expressed in *S*. *Infantis* after internalization into lettuce.

| Protein name | Uniprot Accession | Gene | Fold change | P-value | # of unique peptides |
|--------------|------------------|------|-------------|---------|---------------------|
| **Metabolism** |                  |      |             |         |                     |
| Carbon       |                  |      |             |         |                     |
| Putative PTS system, ascorbate-specific IIB component | D0ZQJ7 |       | 37.0        | <1.0E-04 | 2                   |
| Alcohol dehydrogenase | D0ZXP4 | adhP | −5.9        | <1.0E-04 | 7                   |
| Phosphorypyruvate hydratase | D0ZVP5 | eno  | −8.8        | <1.0E-04 | 7                   |
| Amino acid   |                  |      |             |         |                     |
| Tryptophan synthase subunit alpha | D0ZI5 | tppA | 20.6        | <1.0E-04 | 1                   |
| Aspartate-semialdehyde dehydrogenase | D0ZJH4 | asd  | −5.3        | <1.0E-04 | 1                   |
| Putative aspartate racemase | D0ZU78 |      | 5.0         | <1.0E-04 | 1                   |
| Nucleotide   |                  |      |             |         |                     |
| Allantoinase | D0ZP18 | allB | 3.5         | 3.4E-02  | 2                   |
| Cytidylate kinase | D0ZS15 | cmK  | 6.5         | 5.1E-04  | 1                   |
| Dihydroorotase | D0ZUK7 | pyrC | 20.5        | <1.0E-04 | 1                   |
| Lipid        |                  |      |             |         |                     |
| Acyl carrier protein | D0ZUP3 | acpP | 2.0         | <1.0E-04 | 3                   |
| Adenosylcobinamide kinase | D0ZMB8 | cobU | 4.0         | 1.7E-02  | 1                   |
| **Protein synthesis** |                  |      |             |         |                     |
| 50S Ribosomal protein L13 | D0ZY47 | rplM | 4.5         | 8.8E-03  | 2                   |
| Transcriptional regulator | D0ZRT74 |      | 10.5        | <1.0E-04 | 1                   |
| tRNA-DNA-dihydrouridine synthase C | D0ZNJ3 | yohl | 6.0         | 1.1E-04  | 2                   |
| DNA binding protein | D0ZU24 | stpA | 4.0         | 1.7E-02  | 1                   |
| 23S rRNA 5-methyluridine methyltransferase | D0ZVQ3 | rlmD | 4.0         | 1.7E-02  | 1                   |
| 30S Ribosomal subunit S22 | D0ZXP1 | rpsV | −5.7        | 1.9E-02  | 3                   |
| **Pathogen-associated molecular patterns (PAMPs)** |                  |      |             |         |                     |
| Flagellin    | D0ZLB5 | flfC | −6.0        | 1.2E-02  | 4                   |
| Elongation factor Tu | D0ZIM1 | tuf_1 | −2.1        | 3.2E-03  | 9                   |
| Lipid A biosynthesis lauroyl acyltransferase | D0ZUJB |      | 5.8         | <1.0E-04 | 1                   |
| **Stress response** |                  |      |             |         |                     |
| Superoxide dismutase | D0ZWV7 | sodB | 7.0         | 2.4E-04  | 1                   |
| Superoxide dismutase | D0ZW6W | sodC_2 | 2.5      | 6.3E-03  | 2                   |
| Putative thiol-alkyl hydroperoxide reductase | D0ZMY2 |      | 2.0         | 2.7E-03  | 3                   |
| Bacterioferritin, iron storage and detoxification protein | D0ZIL8 | bfr  | 7.0         | 2.4E-04  | 1                   |
| NAD(P)H dehydrogenase (quinone) | D0ZTQ2 | ybaB | 3.3         | 1.2E-02  | 3                   |
| Putative intracellular proteinase | D0ZXW4 | ybaO | −25.0       | <1.0E-04 | 4                   |
| Chaperonin   | D0ZS62 | groL | −2.5        | 2.6E-02  | 1                   |
| Thioredoxin  | D0ZNP5 | trxA | −8.7        | 1.3E-04  | 7                   |
| Transcriptional regulator HU subunit alpha | D0ZQX4 | hupA | −2.5        | <1.0E-04 | 5                   |
| Hypothetical protein STM14_1832 | D0ZX6 | ydeI | −11.0       | <1.0E-04 | 3                   |
| **Cell envelope** |                  |      |             |         |                     |
| dTDP-Glucose 4,6-dehydratase | D0ZNQ1 | rffG | 5.5         | 2.2E-03  | 1                   |
| **Transport** |                  |      |             |         |                     |
| Hypothetical protein STM14_1021 | D0ZS98 | ybL  | 6.0         | 1.1E-03  | 3                   |
| Sodium/pantothenate symporter | D0ZIF6 | panF | 4.5         | 8.8E-03  | 2                   |
| Low affinity gluconate transporter | D0ZJH7 | gntU | 2.0         | 5.7E-03  | 1                   |
| Putative ABC-type multidrug transport system ATPase component | D0ZKD9 | yhiH | 3.5         | 3.4E-02  | 1                   |
| **Unknown**  |                  |      |             |         |                     |
| Hypothetical protein STM14_0531 | D0ZN35 |      | 5.5         | 2.2E-03  | 1                   |
| Phage tail component H-like protein | D0ZST4 |      | 2.2         | 1.9E-02  | 2                   |
| Putative cytoplasmic protein | D0ZX13 |      | 6.5         | <1.0E-04 | 1                   |
| Putative cytoplasmic protein | D0ZI5 | yceE | 2.0         | <1.0E-04 | 9                   |
| Hypothetical protein STM14_2884 | D0ZQJ3 | yfcC | 6.0         | 1.1E-03  | 1                   |
| Hypothetical protein STM14_3293 | D0ZTU4 | yfG  | 4.0         | 1.7E-02  | 1                   |
| Hypothetical protein STM14_4694 | D0ZW1 | yfE  | 2.4         | 1.7E-02  | 1                   |
| Putative type II restriction enzyme methylase subunit | D0ZU4 |      | 5.5         | 2.2E-03  | 1                   |
| Hypothetical protein STM14_0428 | D0ZM36 | yahO | −5.7        | 1.9E-02  | 2                   |
| Hypothetical protein STM14_0454 | D0ZM62 | psiF | −3.9        | 7.5E-04  | 4                   |
| Putative cytoplasmic protein | D0ZVJ6 |      | −14.0       | 1.0E-04  | 2                   |
| Hypothetical protein STM14_1588 | D0ZWH4 | spy  | −5.4        | 2.8E-02  | 3                   |
| Putative cytoplasmic protein | D0ZJ87 |      | −6.3        | 7.9E-03  | 1                   |
| Hypothetical protein STM14_4278 | D0ZIJ1 | yshA | −8.8        | 2.1E-04  | 2                   |
(vitamin C) is abundant in lettuce leaf [9.2 mg/100 g (USDA, 2013)], and *Salmonella* has been reported to be capable of consuming ascorbate when its preferred carbon sources are not available (Eddy and Ingram, 1953).

Phosphopyruvate hydratase and alcohol dehydrogenase, two enzymes involved in glycolysis, were down-regulated 8.8- and 5.9-fold respectively. In addition, several enzymes involved in glycolysis (i.e. 6-phosphofructokinase, phosphoglycerate kinase and phosphoglycerate mutases) were detected only in the *Salmonella* grown in LB but not in the *Salmonella* grown in lettuce leaves (Fig. 1). Glycolysis starts with glucose and fructose, which are present in leaf lettuce at the levels of 0.36 g and 0.43 g per 100 g respectively (USDA, 2013).

The decrease in the abundance of multiple enzymes involved in glycolysis suggests that these monosaccharides may not be available to *Salmonella* inside lettuce leaves. Alternatively, internalized *Salmonella* may utilize less preferred but available substrates, such as ascorbate. In plants, the level of ascorbate increases under stress conditions, such as pathogen invasion (Noctor and Foyer, 1998).

**Stress response.** Stress response proteins accounted for a major class of the differentially expressed proteins (Table 1). Several proteins involved in response to oxidative stress were up-regulated. Superoxide dismutase (SodC_2, up-regulated 2.5-fold) is a periplasmic or membrane-associated protein in several gram-negative...
bacteria, and protects bacteria from extracellular reactive oxygen species (ROS) (Battistoni, 2003). Another superoxide dismutase (SodB, up-regulated 7-fold) is an intracellular protein, and removes ROS produced by aerobic metabolism (Farrant et al., 1997). Bacterioferritin, an iron storage and detoxification protein (Bfr, up-regulated 7-fold) is the major Fe storage protein in S. Typhimurium. It sequesters Fe to prevent generating highly toxic hydroxyl radical (Fe$^{3+}$ + H$_2$O$_2$ → Fe$^{3+}$ + OH$^-$ + OH$^-$) when Fe is in excess and releases Fe when exogenous Fe is limiting (Velayudhan et al., 2007). Salmonella bfr mutants appeared to be more susceptible to oxidative stress than the wild type (Velayudhan et al., 2007). Under the control of the central regulator of general stress responses RpoS (Patridge and Ferry, 2006), NAD(P)H dehydrogenase (quinone) (WraB, up-regulated 3.3-fold) is often up-regulated under stresses such as acid, salt and H$_2$O$_2$ (Pomposiello et al., 2001; Tucker et al., 2002; Cheung et al., 2003). Finally, putative thiol-alkyl hydroperoxide reductase (up-regulated 2.0-fold) is an antioxidant, which can scavenge H$_2$O$_2$ and enhance oxidative stress resistance (Hebrard et al., 2009). Because generating ROS is a universal defensive strategy employed by plants when challenged by pathogenic or beneficial bacteria (Shetty et al., 2008), it is not surprising that internalized Salmonella up-regulated multiple proteins to resist ROS.

Interestingly, about half of the stress response proteins that were differentially expressed were down-regulated in internalized Salmonella (Table 1). Chaperonin (GroL, down-regulated 2.5-fold) refolds and assembles unfolded polypeptides (Sherman and Goldberg, 1992), and is essential in cell growth and survival under heat and acid stresses (Baumann et al., 1996; Hartke et al., 1997). Genes coding for transcriptional regulator (HupA, down-regulated 2.5-fold) and putative intracellular proteinase (YhbO, down-regulated 25-fold) can increase the survival of S. Typhimurium under the exposure to artificial sea water (Haznedaroglu, 2010), and the latter can act in response to oxidative, thermal, UV and pH stresses (Abdallah et al., 2007).

Pathogen associated molecular patterns (PAMPs). PAMPs from bacteria can be recognized by host plants and can trigger plants’ basal defense responses. Known PAMPs include flagellin, lipopolysaccharide (LPS) and elongation factor Tu (EF-Tu) (Chisholm et al., 2006; Zipfel, 2008). In this study, flagellin and EF-Tu were down-regulated, while lipid A biosynthesis lauroyl acyltransferase (htrB) involved in LPS biosynthesis was up-regulated (Table 1).

Although flagellin, which are composed of flagellin, facilitate Salmonella to move toward plant roots or attach to plant leaf surface (Cooley et al., 2003; Kroupitski et al., 2009), they provide little benefit to endophytic bacteria because the endophytes are usually nonmotile upon entering plants (Hattermann and Ries, 1989; Kamoun and Kado, 1990). Studies reported that flagella mutants of E. coli O157:H7 and Salmonella could survive better in Arabidopsis and in alfalfa (Medicago sativa), respectively, than respective wild types (Inguez et al., 2005; Seo and Matthews, 2012), suggesting that the down-regulation of flagellin may increase the fitness of human pathogens in plants.

Type III secretion system (T3SS). In addition to the differentially expressed proteins reported in Table 1, secreted effector protein (PipB2) was detected in internalized Salmonella but not in LB-grown Salmonella. PipB2 can be secreted via T3SS-2, which is often expressed after Salmonella has entered an epithelial cell or a macrophage. T3SS-1 enables bacterial invasion of epithelial cells, and T3SS-2 enhances bacterial survival and replication in epithelial cells (Waterman and Holden, 2003). A recent study demonstrated that Salmonella could suppress the immune system of Arabidopsis plants using T3SS-1 and T3SS-2 (Schikora et al., 2011).

Protein expression profile of lettuce

Two databases were used to identify lettuce proteins: expressed sequence tag (EST) sequences of Lactuca sativa from CGPDB and a custom-built database comprising Lettuce protein sequences available in NCBI (Cho et al., 2009). A total of 289 lettuce proteins were identified using the EST database with 174 and 189 proteins detected in lettuce without and with internalized Salmonella, respectively. Because lettuce sequences in the EST database are not annotated, the sequence hits from the EST database were blasted against the proteins of Arabidopsis thaliana for functional information (Cho et al., 2009). Among the lettuce proteins that are homologous to A. thaliana proteins, 17 proteins were up-regulated and 3 were down-regulated (Table 2). Using the custom-built lettuce protein database, among the 163 proteins identified, 25 proteins were detected only in lettuce with internalized Salmonella but not in control lettuce (Supporting Information, Table S1).

Several lettuce proteins were up-regulated in response to S. Infantis internalization (Table 2). Pyruvate dehydrogenase E1 subunit beta-1 (up-regulated 7.5-fold) is considered a PAMP-responsive protein. It increased in abundance when Arabidopsis was challenged by a hrpA mutant of Pseudomonas syringae, which could only activate plant basal defense (Jones et al., 2006; Jones and Dangl, 2006). 2-cys Peroxiredoxin (up-regulated 10-fold) may play a role in defense-related redox signaling,
because it can reduce reactive nitrogen peroxides generated during incompatible interactions during which the host resists to bacteria and no disease develops (Jones et al., 2004). Superoxide dismutase [Cu-Zn] 1 was up-regulated 4.3 fold, possibly as a self-protective antioxidant response to the plant ROS induced by internalized Salmonella (Jagadeeswaran et al., 2009). Ferredoxin–NADP reductase, which was up-regulated 2.7-fold following Salmonella internalization, plays a key role in regulating the relative amounts of cyclic and non-cyclic electron flow to meet plant demand for ATP and reducing power (Hanke et al., 2005; Lintala et al., 2007). Its involvement in defense response to bacteria has been inferred from computational annotation and expression patterns (Jones et al., 2006; Jones and Dangl, 2006; Heyndrickx and Vandepoele, 2012).

Using the custom-built lettuce protein database, several predicted resistance proteins (RGC1C, RGC2, RGC2C, RGC2K and NBS-LRR resistance-like protein 4T) and a putative ethylene receptor ETR1 (Supporting Information, Table S1) were detected in only lettuce containing internalized S. Infantis. This suggests Salmonella might have induced the expression of resistance proteins (R proteins), and ethylene might be involved in its regulation. It is known that R proteins can recognize specific effectors secreted by pathogens, leading to the hypersensitive response that prevents the pathogens from growing or spreading inside infected plants (Jones and Dangl, 2006). Ethylene alone with salicylic acid and jasmonic acid are the three plant hormones involving signaling and regulating R proteins (Jones and Dangl, 2006).

A few studies investigated plant responses to human pathogens using transcriptomics. Plant pathogenicity-related genes PR1, PR4, PR5 and DAD1 were induced in lettuce leaf 2 days after S. Dublin entered plants through a hydroponic growing medium (Klerks et al., 2007). The PR genes encode pathogenicity-related proteins, which can be induced as part of systemic acquired resistance (Durrant and Dong, 2004). The expression of PR1 in alfalfa and Arabidopsis was up-regulated by the internalization of S. Typhimurium (Iniguez et al., 2005; Schikora et al., 2008), likely resulting from sensing the T3SS-1 effectors of Salmonella (Iniguez et al., 2005). In this study, several R proteins were also induced by Salmonella, and the secreted effector PipB2 (T3SS-2 effectors) was concurrently detected in internalized S. Infantis.

### Concluding remarks

In summary, the global modulation of protein expression revealed S. Infantis may utilize alternative carbon sources such as ascorbate upon internalization because the preferred substrate/carbon sources were not available inside lettuce leaves. In the meanwhile, S. Infantis produced multiple stress response proteins to cope with the stresses encountered inside plants. On the other hand, proteins involved in lettuce’s defense response to bacterium were up-regulated, such as pyruvate dehydrogenase, 2-cys peroxiredoxin and ferredoxin–NADP reductase. Interestingly, the secreted effector PipB2 of S. Infantis and R proteins of lettuce were concurrently induced during the interaction between Salmonella and lettuce.

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**Table 2.** Proteins that were differentially expressed in lettuce after internalization of S. infantis.

| Protein name | Uniprot Accession | Gene | Fold change | P-value | # of unique peptides |
|--------------|-------------------|------|-------------|---------|---------------------|
| Pyruvate dehydrogenase E1 subunit beta-1 | Q38799 | PDH2 | 7.5 | <1.0E-04 | 2 |
| Triosephosphate isomerase | P48491 | CTIMC | 2.3 | 1.9E-02 | 2 |
| Fructose-bisphosphate aldolase 1 | F41GL7 | FBA1 | −7.2 | 5.8E-03 | 1 |
| 2-cys Peroxiredoxin | Q96291 | BAS1 | 10.0 | 5.5E-04 | 1 |
| Actin 4 | P53497 | ACT12 | 3.5 | 2.4E-02 | 1 |
| Nucleoside diphosphate kinase | F39207 | NDK1 | 2.9 | 2.8E-02 | 1 |
| Ribulose bisphosphate carboxylase/oxygenase activase | F4IVZ7 | RCA | −4.8 | 4.1E-02 | 2 |
| Selenoprotein, Rdx type | Q8W1E5 | ATSG8640 | 4.0 | 3.8E-03 | 1 |
| Superoxide dismutase [Cu-Zn] 1 | P24704 | CSD1 | 4.3 | 1.2E-02 | 1 |
| calmodulin 5 | P59220 | CAM7 | 5.7 | 2.4E-02 | 1 |
| Plasma membrane-associated cation-binding protein 1 | Q96262 | PCAP1 | 3.3 | 3.0E-02 | 1 |
| Oxygen-evolving enhancer protein 1–2 | Q41932 | PSBO2 | 2.9 | <1.0E-04 | 3 |
| Oxygen-evolving enhancer protein 1–2 | Q9S841 | PSBO2 | 3.5 | 2.4E-02 | 2 |
| Two-component response regulator-like APRR2 | Q6LA43 | APRR2 | 2.3 | 4.5E-03 | 1 |
| 30S Ribosomal protein S31, chloroplastic | O80439 | RPS31 | 3.9 | 2.3E-03 | 1 |
| Ferredoxin-NADP reductase, leaf-type isozyme 2 | Q8W493 | LFNR2 | 2.7 | 6.8E-04 | 2 |
| 40S Ribosomal protein S8-2 | Q9FIF3 | RPS8B | 6.0 | 5.5E-04 | 1 |
| photosystem I reaction center subunit 2-2 | Q9S714 | PSAE2 | 4.0 | 1.2E-02 | 2 |
| 50S Ribosomal protein L12-1, chloroplastic | P36210 | RPL12A | 4.1 | 4.0E-02 | 1 |
| Purple acid phosphatase 13 | Q9SIV9 | PAP10 | −4.8 | <1.0E-04 | 1 |

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Conflict of interest
None declared.

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