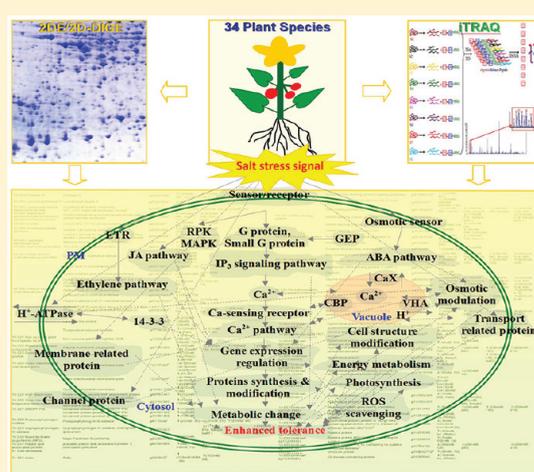


# Mechanisms of Plant Salt Response: Insights from Proteomics

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**ABSTRACT:** Soil salinity is a major abiotic stress that limits plant growth and agriculture productivity. To cope with salt stress, plants have evolved complex salt-responsive signaling and metabolic processes at the cellular, organ, and whole-plant levels. Investigation of the physiological and molecular mechanisms underlying plant salinity tolerance will provide valuable information for effective engineering strategies. Current proteomics provides a high-throughput approach to study sophisticated molecular networks in plants. In this review, we describe a salt-responsive protein database by an integrated analysis of proteomics-based studies. The database contains 2171 salt-responsive protein identities representing 561 unique proteins. These proteins have been identified from leaves, roots, shoots, seedlings, unicells, grains, hypocotyls, radicles, and panicles from 34 plant species. The identified proteins provide invaluable information toward understanding the complex and fine-tuned plant salt-tolerance mechanisms in photosynthesis, reactive oxygen species (ROS) scavenging, ion homeostasis, osmotic modulation, signaling transduction, transcription, protein synthesis/turnover, cytoskeleton dynamics, and cross-tolerance to different stress conditions.

**KEYWORDS:** plant, proteomics, salinity tolerance, molecular mechanisms



## 1. INTRODUCTION

Salinity is one of the most significant abiotic stresses and it limits the productivity and geographical distribution of plants. Approximately 20% of the earth's land mass and nearly half of all irrigated land are affected by salinity.<sup>1</sup> Salinity can cause ion imbalance, hyperosmotic stress and oxidative damage in plants. To cope with salinity stress, plants have evolved sophisticated mechanisms, including selective ion uptake/exclusion, compartmentalization of toxic ions, synthesis of compatible products, adjustment of photosynthetic and energy metabolism, accumulation of antioxidative enzymes, regulation of hormones, and modification of cell structure. Previous physiological, molecular genetics and functional genomics studies have provided some molecular and physiological knowledge of plant salt tolerance. Some important genes encoding proteins for osmolyte synthesis, ion channels, signaling factors and salt-responsive enzymes have been cloned and characterized, which revealed the fundamental functions of the genes/proteins in plants' response and adaptation to salinity.<sup>2</sup> In addition, high-throughput transcriptomics studies have provided immense data on gene expression at the mRNA level.<sup>3–10</sup> More than 194 transcripts in *Arabidopsis*, 10%

of the transcripts in salt-tolerant rice, and at least 2300 ESTs/cDNAs in some halophytes (e.g., *Thellungiella halophila*,<sup>3–6</sup> *Suaeda salsa*,<sup>7</sup> *Aeluropus litoralis*,<sup>8</sup> *Salicornia brachiata*<sup>9</sup> and *Festuca rubra* ssp. *litoralis*.<sup>10</sup>) have been shown to be significantly altered under salinity conditions. These data offer a global view of salt-responsive genes in different plants. However, because of post-transcriptional events and post-translational modifications such as phosphorylation and glycosylation, mRNA levels do not usually correlate with the expression levels of proteins, which are more directly related to signaling and metabolic processes under salt stress conditions. Thus, it is of essential importance to study salt stress responses at the protein level. As a necessary and complementary approach in the postgenomic era, proteomics technologies have been utilized in studying global protein expression. Recent analyses of salt-responsive proteomes in plants have yielded more information for understanding the

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Table 1. Summary of Publications on Plant Salinity Responsive Proteomics

no.	species	variety	tissue	salt (NaCl) treatment condition	IDs <sup>a</sup>	UPs <sup>b</sup>	ref <sup>c</sup>		
1	<i>Arabidopsis thaliana</i>	Col-0	root	150 mM, 6 h, 48 h	87	58	11		
		Col-0	root microsome	250 mM, 2 h	6	4	12		
			cell suspension	200 mM 6 h	70	42	13		
			culture						
		Col-0, 35S::atRZ-1a	seedling	100 mM	19	16	14		
	Col-0	leaf, leaf	50 mM, 150 mM, 5d	110	77	15			
			microsomal membrane						
2	<i>Oryza sativa</i>	Nipponbare	leaf	130 mM, 4d	31	17	16		
		Nipponbare, IR36, Pokkali	leaf sheath, leaf	50 mM, 100 mM,	5	4	17		
			blade, root	150 mM, 6 h, 24 h, 48 h					
		IR4630-22-2-5-1-3	leaf lamina	50 mM, 7d, 1d	10	8	18		
		IR651	root PM	100 mM, 14d	8	6	19		
		IR651	panicle	50 mM 7d+75 mM 5d	13	11	20		
		Nipponbare	root	150 mM, 24 h, 48 h, 72 h	12	10	21		
		Nipponbare	root apoplast	200 mM, 1 h, 3 h, 6 h	8	6	22		
		Nipponbare	root	150 mM, 12 h, 24 h	53	45	23		
		Wuyunjing 8	root plasma membrane	150 mM, 2d	34	18	24		
		Pokkali (ST), IR29 (SS)	root	50 mM 7d+100 mM 7d	3	3	25		
		Indica	root	150 mM, 2d	74	49	26		
		Nipponbare	shoot	0.5%, 2d	10	10	27		
		Shanyou 10, Liangyoupeiju	shoot	100 mM, 10d	9	9	28		
3	<i>Triticum durum</i>	Ofanto	leaf	100 mM, 2d	37	25	29		
		4	<i>Triticum aestivum</i>	SR3 (hybrid),	root	50 mm 24 h+100 mM 24 h+150 mM	86	64	30
				JN177 (parent)		24 h+200 mM 24 h			
RH8706-49, H8706-34	leaf	1%, 72 h	5	4	31				
	SR3 (hybrid),	root, leaf	200 mM, 24 h	115	77	32			
	JN177 (parent)								
		shoot mitochondrial	50 mM 1d+100 mM 1d+150 mM	8	4	33			
			1d+200 mM 7d						
5	<i>Hordeum vulgare</i>	Afzal (ST), L.527 (SS)	leaf	50 mM 12 h+100 mM 12 h+150 mM	13	13	34		
				12 h+200 mM 12 h+250 mM					
				12 h +300 mM 24 h					
		Steptoe, Morex	root	100 mM, 150 mM, 13d	22	19	35		
	DOM, OWB21, OWB73 (SS);	grain	1.5%, 2.0%, 2.5%, 10d	6	5	36			
	OWB34, OWB59,								
	REC (ST)								
	OUK305	root	200 mM, 5d	6	5	37			
6	<i>Zea mays</i>	NaEx11	shoot, root	25 mM, 9d;	14	14	38		
				25 mM 2d+50 mM 2d+75 mM 2d+100 mM 3d					
		SR12	chloroplast	25 mM, 1 h, 2 h, 4 h	18	14	39		
	SR12	root	25 mM, 1 h	18	17	40			
7	<i>Setaria italica</i>	Prasad	seedling	100 mM, 150 mM, 200 mM, 7d	29	24	41		
8	<i>Sorghum bicolor</i>	csv-17	leaf	200 mM, 96 h	21	17	42		
9	<i>Agrostis stolonifera</i>	Penncross, Penn-A4	leaf, root	2dSm <sup>-1</sup> 2d+4dSm <sup>-1</sup> 2d+2dSm <sup>-1</sup>	61	32	43		
				6d+2dSm <sup>-1</sup> 2d+8dSm <sup>-1</sup> 2d+10dSm <sup>-1</sup> 28d					
10	<i>Brassica napus</i>	Sarigol (SS), Hyola 308 (ST)	leaf	75 mM, 350 mM, 21d	40	22	44		
11	<i>Arachis hypogaea</i>	JL24	callus cell	200 mM, 12d	25	6	45		
12	<i>Glycin max</i>	Enrei	hypocotyls, root, leaves	40 mM, 7d	38	31	46		
		Enrei	hypocotyls, root	100 mM, 3d	7	7	47		
13	<i>Pisum sativum</i>		root	75 mM, 150 mM, 7d; 75 mM,	32	24	48		
				150 mM, 42d					
14	<i>Solanum lycopersicum</i>	Cervil, Levovil, Roma, SM	root	100 mM, 14d	49	36	49		
		F144 (SS), Patio (ST)	radicle, hypocotyl	120 mM, 7d	23	13	50		
		Betterboy	root, leaf	100 mM, 14d			51		

Table 1. Continued

no.	species	variety	tissue	salt (NaCl) treatment condition	IDs <sup>a</sup>	UPs <sup>b</sup>	ref <sup>c</sup>
15	<i>Solanum tuberosum</i>	Concord, Kennebec	shoot, root	90 mM, 28d	27	17	52
16	<i>Vitis vinifera</i>	Chardonnay, Cabernet Sauvignon	shoot tip	NaCl 10 mM CaCl <sub>2</sub> 1 mM 1d+NaCl 20 mM CaCl <sub>2</sub> 2 mM 5d+NaCl 55 mM CaCl <sub>2</sub> 5.5 mM 2d	28	25	53
17	<i>Nicotiana tabacum</i>	Razegui	leaf, stem, root	100 mM, 15d			54
		Petit Havana SR1	leaf apoplast	100 mM, 20d	10	5	55
		wisconsin	leaf	150 mM, 250 mM, 300 mM, 400 mM, 2d	12	7	56
18	<i>Cucumis sativus</i>		root	50 mM, 7d	27	23	57
19	<i>Beta vulgaris</i>	Evita	shoot, root, shoot PM	25 mM 1d+50 mM 1d+75 mM 1d+100 mM 1d+125 mM 3d	9	9	58
20	<i>Citrus aurantium</i>		leaf	150 mM, 16d	85	43	59
21	<i>Lathyrus sativus</i>	LP-24	leaf	500 mM, 12 h, 24 h, 36 h	44	30	60
22	<i>Populus cathayana</i>		male/female cutting	75 mM, 150 mM, 28d	72	32	61
23	<i>Bruguiera gymnorhiza</i>		main/lateral root, leaf	500 mM, 3 h, 6 h, 12 h, 24 h, 3d, 6d, 12d	3	3	62
24	<i>Thellungiella halophila</i>	Shandong	leaf, leaf microsomal membrane	50 mM, 150 mM, 5d	64	45	15
25	<i>Aster tripolium</i>		leaf	375 mM, 28d	3	3	63
26	<i>Mesembryanthemum crystallinum</i>		leaf	200 mM, 400 mM, 7d	6	3	64
27	<i>Suaeda salsa</i>		leaf	50 mM 1d+100 mM 20d; 50 mM 1d+100 mM 1d+150 mM 1d+200 mM 18d	37	26	65
28	<i>Suaeda aegyptiaca</i>		leaf	150 mM, 300 mM, 450 mM, 600 mM, 30d	26	22	66
29	<i>Salicornia europaea</i>		leaf shoot	200 mM, 800 mM, 21d 200 mM, 600 mM, 800 mM, 21d; 200 mM, 12 h, 24 h, 72 h	110	73	68
30	<i>Porteresia coarctata</i>		leaf	200 mM, 400 mM, 72 h	16	12	69
31	<i>Puccinellia tenuiflora</i>		leaf	50 mM, 150 mM, 7d	93	59	70
32	<i>Aeluropus lagopoides</i>		shoot	450 mM, 10d	88	62	71
33	<i>Physcomitrella patens</i>		gametophore	250 mM, 300 mM, 350 mM, 72 h	65	48	72
34	<i>Dunaliella salina</i>		plasma membrane cell	5 mM, 3M 5 mM, 3M	54	37	73
					57	38	74

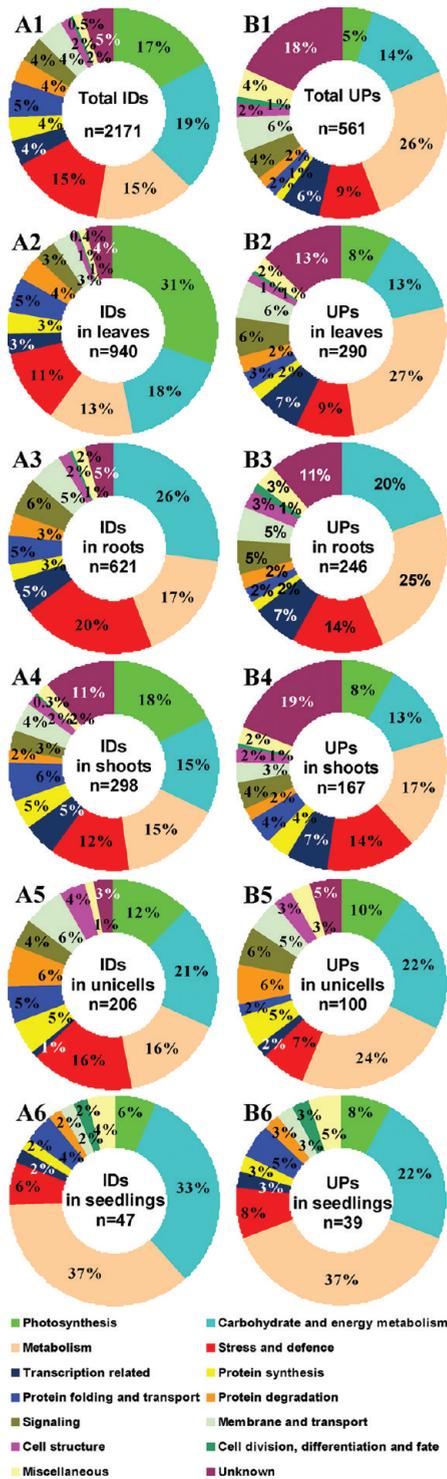
<sup>a</sup> Numerical list of salt-responsive proteins by proteomics approaches. <sup>b</sup> List of unique proteins/protein families (UPs). The UPs were defined as the proteins functional annotation as well as gi number by domain searching and similarity comparison according to the GO criteria. <sup>c</sup> List of original research articles cited in this paper. Please refer to the Reference section for details.

complex mechanisms of plant salt response and tolerance<sup>11–74</sup>. To date, more than 2171 salt-responsive proteins have been identified in shoots, leaves, roots, seedlings, radicles, hypocotyls, grains, gametophytes, and unicells from 34 plant species. These plant species include 2 model plants (*Arabidopsis thaliana*<sup>11–15</sup> and *Oryza sativa*<sup>16–28</sup>), 7 agricultural crops (*Triticum durum*,<sup>29</sup> *Triticum aestivum*,<sup>30–33</sup> *Hordeum vulgare*,<sup>34–37</sup> *Zea mays*,<sup>38–40</sup> *Setaria italica*,<sup>41</sup> *Sorghum bicolor*,<sup>42</sup> and *Agrostis stolonifera*<sup>43</sup>), 12 economic crops (*Brassica napus*,<sup>44</sup> *Arachis hypogaea*,<sup>45</sup> *Glycin max*,<sup>46,47</sup> *Pisum sativum*,<sup>48</sup> *Solanum lycopersicum*,<sup>49,50</sup> *Solanum tuberosum*,<sup>52</sup> *Vitis vinifera*,<sup>53</sup> *Nicotiana tabacum*,<sup>55,56</sup> *Cucumis sativus*,<sup>57</sup> *Beta vulgaris*,<sup>58</sup> *Citrus aurantium*,<sup>59</sup> and *Lathyrus sativus*<sup>60</sup>), 2 tree species (*Populus cathayana*<sup>61</sup> and *Bruguiera gymnorhiza*<sup>62</sup>), and 11 halophytes (*T. halophila*,<sup>15</sup> *Aster tripolium*,<sup>63</sup> *Mesembryanthemum crystallinum*,<sup>64</sup> *S. salsa*,<sup>65</sup> *Suaeda aegyptiaca*,<sup>66</sup> *Salicornia europaea*,<sup>68</sup> *Porteresia coarctata*,<sup>69</sup> *Puccinellia tenuiflora*,<sup>70</sup> *Aeluropus lagopoides*,<sup>71</sup> *Physcomitrella patens*,<sup>72</sup> and *Dunaliella salina*<sup>73,74</sup>) (Table 1). By combination of proteomics and physiological approaches, several salt adaptation strategies have been revealed from the aforementioned studies.

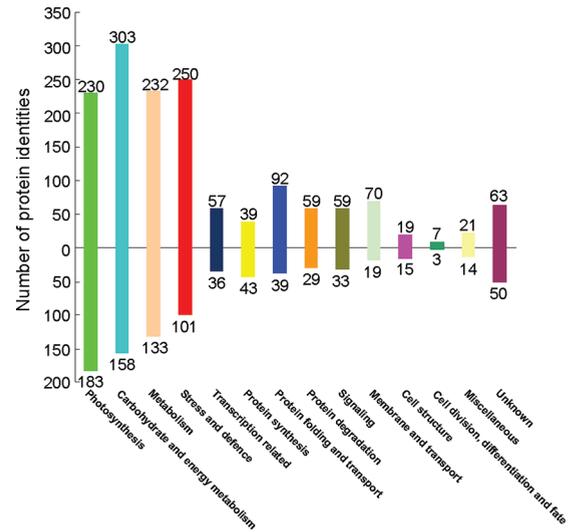
In the present review, we provide an integrated salt-responsive protein database based on these proteomics studies and summarize the mechanisms underlying plant salt response and tolerance, including changes in photosynthesis, reactive oxygen species (ROS) scavenging system, ion homeostasis, osmotic homeostasis, membrane transport, signaling transduction, transcription, protein synthesis/turnover, cytoskeleton dynamics, and crosstalks with other stresses. This generalized information lays a solid foundation necessary for further investigation of salt response/tolerance networks and ultimate rational engineering of plants for enhanced stress tolerance.

## 2. DATABASE OF SALT-RESPONSIVE PROTEINS AND THEIR CHARACTERISTICS UNDER SALINITY

Through integrated analysis of salt-responsive proteins identified in 34 plant species, we have set up a plant salinity responsive protein database (Supporting Information Table S1). The database contains information on protein names, functional categories,



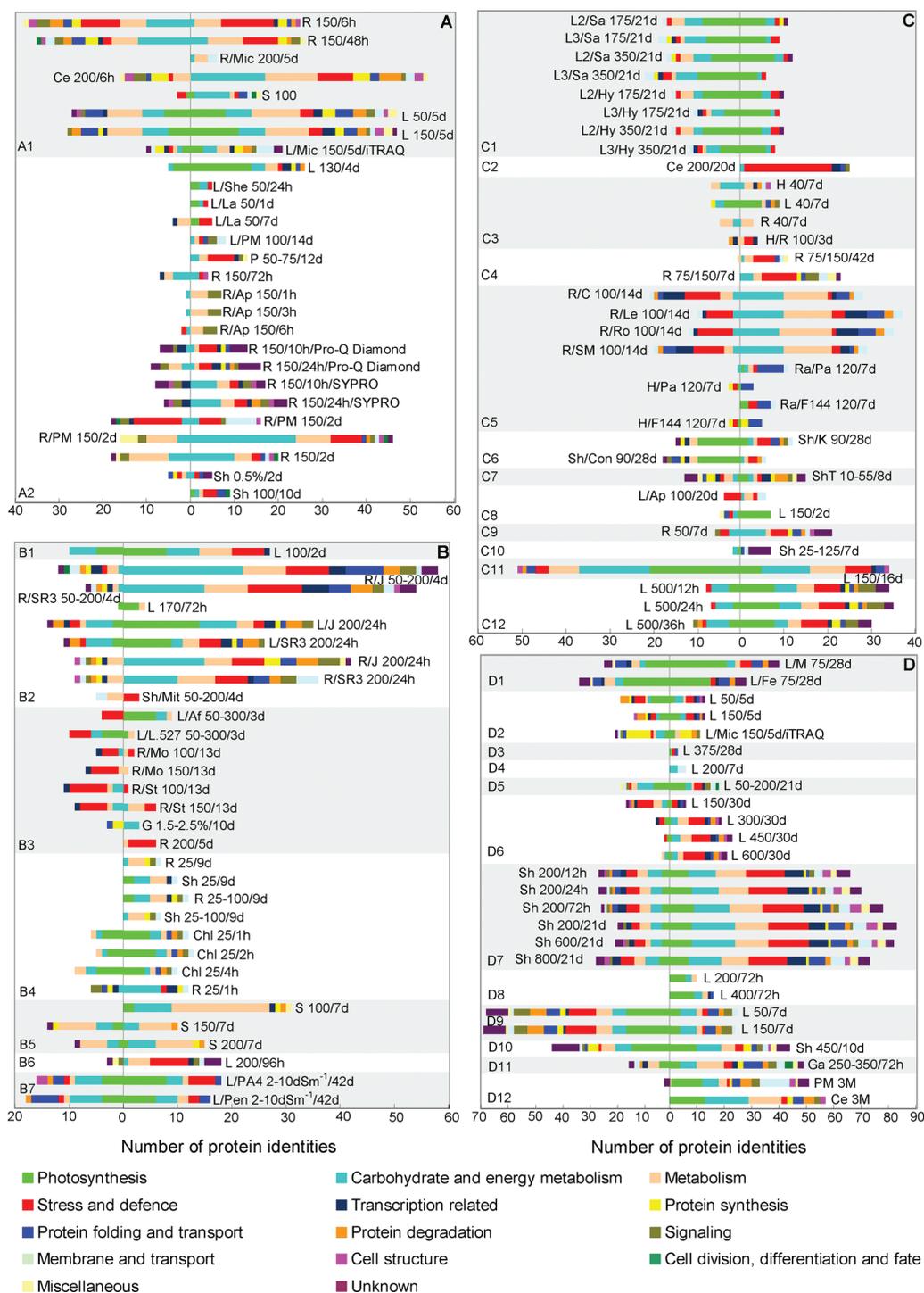
**Figure 1.** Functional categories of salinity responsive protein identities and unique proteins/protein families (UPs) in plants. The UPs are assigned based on protein sequence homology and similarity of function domains, and reclassified according to Gene Ontology, BLAST alignment and literature information. (A1–A6) Functional categories of protein identities (IDs) from salt-stressed plant species; (A1) total IDs; (A2) IDs in leaves; (A3) IDs in roots; (A4) IDs in shoots; (A5) IDs in unicells; (A6) ID in seedlings; (B1–B6) Functional categories of UPs; (B1) total UPs; (B2) UPs in leaves; (B3) UPs in roots; (B4) UPs in shoots; (B5) UPs in unicells; (B6) UPs in seedlings.



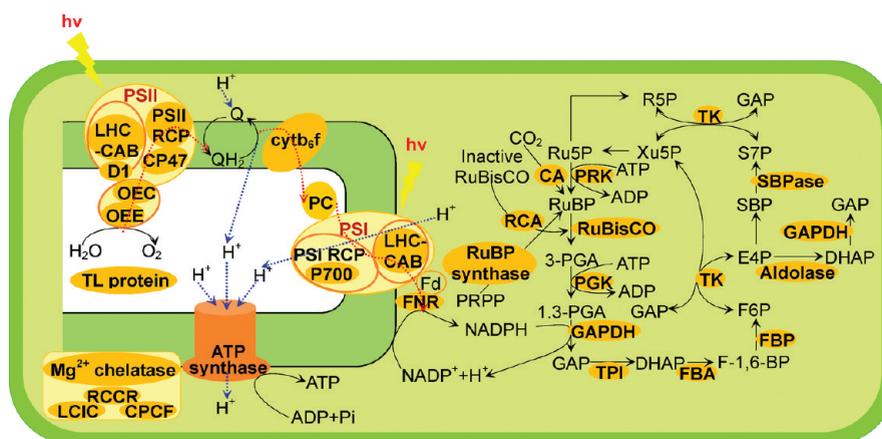
**Figure 2.** Expression patterns of salt-induced and salt-reduced protein identities in each functional category. The columns above and under the x-axis represent the numbers of salt-induced and -reduced proteins, respectively.

and dynamic patterns in response to salinity. There are 2171 salt-responsive protein identities (IDs) reported from 61 original research articles (Figure 1). These IDs are from leaves (940 IDs) (Supporting Information Table S2), roots (621 IDs) (Supporting Information Table S3), shoots (298 IDs) (Supporting Information Table S4), unicells (206 IDs) (Supporting Information Table S5), seedlings (47 IDs) (Supporting Information Table S6), grains (6 IDs), hypocotyls (31 IDs), radicles (14 IDs), and panicles (13 IDs) (Supporting Information Table S7). Some of these IDs are overlapped among various organs/tissues. On the basis of protein sequence homology and functional domain similarity, the IDs are classified into 561 unique proteins/protein families (UPs). Among the salt-responsive UPs, there are 290, 246, 167, 100, and 39 UPs identified from leaves, roots, shoots, cells, and seedlings, respectively (Figure 1, Supporting Information Table S2–S6). In addition, based on Gene Ontology, BLAST alignment and literature information, these IDs and UPs are grouped into 14 functional categories, that is, photosynthesis, carbohydrate and energy metabolism, metabolism, stress and defence, transcription, protein synthesis, protein folding and transport, protein degradation, signaling, membrane and transport, cell structure, cell division/differentiation and fate, miscellaneous, and unknown function (Figure 1). The percentages of UPs in some function categories are less than its corresponding percentages of IDs. These categories include photosynthesis (Figure 1A1/B1, A2/B2, A4/B4, A5/B5), carbohydrate and energy metabolism (Figure 1A1/B1–A4/B4, A6/B6), stress and defence (Figure 1A1/B1–A3/B3, and A5/B5), and protein synthesis (Figure 1A1/B1–A4/B4). It indicates that multiple orthologs responsible for the same functions exist in various plants, and during evolution there was conservation of molecular mechanisms underlying salt response/tolerance in different plant species.

When plants are under salinity conditions, most of the salt-responsive proteins (1501 IDs) are induced, and only 856 protein IDs show decreased expression. The increased proteins represent the majority in each functional category (Figure 2). This suggests that in general, plants respond to salinity and/or exhibit salt tolerance through increasing their protein expression and metabolic activities. This is consistent with results of previous studies in which overexpression of salt-responsive genes in transgenic



**Figure 3.** Expression patterns of salinity responsive protein identities from different plant species and stress conditions in each functional category. The columns in the left of the y-axis are the salt-reduced proteins, and the columns in the right of the y-axis are the salt-induced proteins. The letters and numbers marked on the columns represent the plant organs/tissues, NaCl concentration (mM) and treatment times (days or hours), respectively. (A) model plants; (A1) *Arabidopsis thaliana*; (A2) *Oryza sativa*; (B) agricultural plants; (B1) *Triticum durum*; (B2) *Triticum aestivum*; (B3) *Hordeum vulgare*; (B4) *Zea mays*; (B5) *Setaria italica*; (B6) *Sorghum bicolor*; (B7) *Agrostis stolonifera*; (C) economic plants; (C1) *Brassica napus*; (C2) *Arachis hypogaea*; (C3) *Glycin max*; (C4) *Pisum sativum*; (C5) *Solanum lycopersicum*; (C6) *Solanum tuberosum*; (C7) *Vitis vinifera*; (C8) *Nicotiana tabacum*; (C9) *Cucumis sativus*; (C10) *Beta vulgaris*; (C11) *Citrus aurantium*; (C12) *Lathyrus sativus*; (D) trees and halophytes; (D1) *Populus cathayana*; (D2) *Thellungiella halophila*; (D3) *Aster tripolium*; (D4) *Mesembryanthemum crystallinum*; (D5) *Suaeda salsa*; (D6) *Suaeda aegyptiaca*; (D7) *Salicornia europaea*; (D8) *Porteresia coarctata*; (D9) *Puccinillia tenuiflora*; (D10) *Aeluropus lagopoides*; (D11) *Physcomitrella patens*; (D12) *Dunaliella salina*. Af, Afzal; Ap, apoplast; C, Cervil; Ce, cell; Chl, chloroplast; Con, Concord; d, day; Fe, female; G, grain; Ga, gametophore; h, hour; H, hypocotyl; Hy, Hyola308; iTRAQ, isobaric tags for relative and absolute quantitation; J, Jinan 177; K, Kennebec; L, leaf; L2, leaf2; L3, leaf3; La, lamina; Le, Levovill; M, male; Mic, microsomal fraction; Mit, mitochondrial; Mo, Morex; P, panicle; Pa, Patio; PA4, Penn-A4; Pen, Penncross; PM, plasma membrane; Pro-Q diamond, a phosphoprotein specific fluorescent stain; R, root; Ra, radical; Ro, Roma; S, seedling; Sa, Sarigol; Sh, shoot; She, sheath; ShT, shoot tip; SR3, Shanrong No.3; St, Steptoe; SYPRO, SYPRO Ruby, a fluorescent total-protein stain.



**Figure 4.** Schematic presentation of salinity responsive proteins involved in the photosynthesis in plants. The red-dashed lines show the electron transfer, and the blue-dashed lines indicate the transport of protons. ADP, adenosine diphosphate; ATP, adenosine triphosphate; CA: carbonic anhydrase; CAB, chlorophyll a/b-binding protein; CP47, photosystem II chlorophyll-binding protein 47; CPCF, chloroplast post-illumination chlorophyll fluorescence increasing protein; cytb<sub>6</sub>f, cytochrome b<sub>6</sub>f; DHAP, dihydroxyacetone phosphate; E4P, erythrose-4-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; F6P, fructose 6-phosphate; FBA: fructose-bisphosphate aldolase; FBP: fructose-bisphosphatase; Fd, ferredoxin; FNR, ferredoxin-NADP(H) oxidoreductase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GAP, glycerate 3-phosphate; hv, light energy; LCIC, low-CO<sub>2</sub> inducible protein; LHC, light-harvesting complex; NADP<sup>+</sup>/NADPH, nicotinamide adenine dinucleotide phosphate; OEC, oxygen evolving complex; OEE, oxygen evolving enhancer protein; P700, photosystem I P700 chlorophyll a apoprotein; PC, plastocyanin; PGA, 3-phosphoglycerate; PGK, phosphoglycerate kinase; Pi, inorganic phosphate; PRK, phosphoribulokinase; PRPP, protoporphyrin; PS I, photosystem I; PS II, photosystem II; Q, quinone; QH<sub>2</sub>, reduced quinone; R5P, ribose-5-phosphate; RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; S7P, sedoheptulose-7-phosphate; SBP, sedoheptulose-1,7-bisphosphate; SBPase, sedoheptulose-1,7-bisphosphatase; TK, transketolase; TL, thylakoid lumen; TPI, triose-phosphate isomerase; Xu5P, xylulose-5-phosphate.

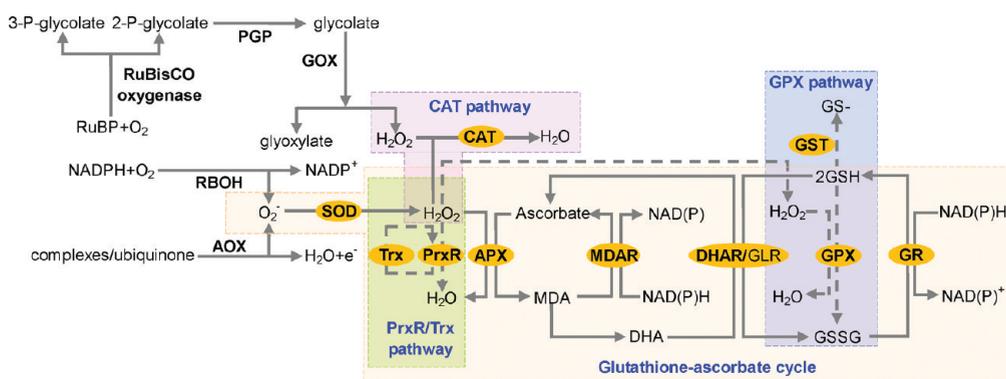
plants leads to enhanced salt tolerance.<sup>75–77</sup> However, when inspected carefully, the salt-responsive proteins exhibit diverse expression patterns in different plant groups (especially glycophytes and halophytes) and under different salinity conditions (e.g., salt concentration and treatment time) (Figure 3). For example, most of photosynthesis-related proteins are induced in *A. thaliana*,<sup>15</sup> *O. sativa*,<sup>16–18</sup> *T. durum*,<sup>29</sup> *T. aestivum*,<sup>31,32</sup> *A. stolonifera*,<sup>43</sup> *G. max*,<sup>46</sup> *V. vinifera*,<sup>53</sup> *N. tabacum*,<sup>56</sup> *L. sativus*,<sup>60</sup> *P. cathayana*,<sup>61</sup> *S. europaea*,<sup>68</sup> *P. coarctata*,<sup>69</sup> and *D. salina*.<sup>73,74</sup> However, in salt-tolerant species (e.g., *T. halophila*,<sup>15</sup> *S. salsa*,<sup>65</sup> *S. aegyptiaca*,<sup>66</sup> and *P. tenuiflora*<sup>70</sup>), more photosynthetic proteins are reduced in expression under salt stress. Similarly, in some species (*A. thaliana*,<sup>12–15</sup> *O. sativa*,<sup>16–20,23,26,28</sup> *T. aestivum*,<sup>30,32</sup> *H. vulgare*,<sup>34,36</sup> *Z. mays*,<sup>38,40</sup> *S. italica*,<sup>41</sup> *S. bicolor*,<sup>42</sup> *A. stolonifera*,<sup>43</sup> *A. hypogaea*,<sup>45</sup> *P. sativum*,<sup>48</sup> *S. lycopersicum*,<sup>49</sup> *S. tuberosum*,<sup>52</sup> *C. sativus*,<sup>57</sup> *M. crystallinum*,<sup>64</sup> *S. europaea*,<sup>68</sup> *P. coarctata*,<sup>69</sup> *A. lagopoides*,<sup>71</sup> *P. patens*,<sup>72</sup> and *D. salina*<sup>73,74</sup>), proteins involved in carbohydrate and energy metabolism are induced by salinity but reduced in halophyte species such as *S. salsa*.<sup>65</sup>

In comparison with glycophytes, the protein expression patterns in halophytes imply specific salt-responsive metabolisms. Proteomic studies identified 622 salt-responsive IDs in dicotyledonous halophytes (e.g., *T. halophila*,<sup>15</sup> *M. crystallinum*,<sup>64</sup> *S. salsa*,<sup>65</sup> *S. aegyptiaca*,<sup>66</sup> *S. europaea*<sup>68</sup> and *A. tripolium*<sup>63</sup>), monocotyledonous halophytes (*P. tenuiflora*,<sup>70</sup> *A. lagopoides*<sup>71</sup> and *P. coarctata*<sup>69</sup>), as well as salt-tolerant tree (*B. gymnorhiza*<sup>62</sup>), moss (*P. patens*<sup>72</sup>), and algae (*D. salina*<sup>73,74</sup>) (Figure 3D). The majority of the salt-responsive proteins are involved in photosynthesis, energy metabolism, ROS scavenging, and ion homeostasis (Figure 3D). This makes halophytes highly efficient in photosynthetic and energy metabolism, ion exclusion/compartimentalization, compatible product synthesis, induction of antioxidative enzymes and hormones, as well as modification of cell structure. The specific proteins and/or protein expression patterns in different halophyte groups/species also reflect evolution of different salt tolerance mechanisms.

In monocotyledonous halophytes *A. lagopoides*,<sup>71</sup> the increased metabolism-/defense- related proteins, decreased photosynthetic proteins, as well as induced amino acids and reduced tricarballic acid cycle (TCA cycle)-related metabolites, suggest that the enhancement of energy formation, amino acid biosynthesis, C4 photosynthetic pathway, and detoxification are the main strategies for salt tolerance. Furthermore, the reduction of photosynthesis also took place in monocotyledonous *P. tenuiflora* under salt treatment<sup>70</sup> due to down-regulation of the light-harvesting complex (LHC) and Calvin cycle enzymes. Proteomics investigation has also revealed that *P. tenuiflora* plants developed diverse reactive oxygen species (ROS) scavenging mechanisms to cope with moderate salinity, including enhancement of the photorespiration pathway and thermal dissipation, synthesis of the low-molecular-weight antioxidant  $\alpha$ -tocopherol, and accumulation of compatible solutes. In strong contrast to glycophytes, certain high salinity conditions sometimes exert no adverse effect on CO<sub>2</sub> assimilation in some halophytes, such as *S. salsa*.<sup>65</sup> In single cell salt-tolerant algae, *D. salina*,<sup>73,74</sup> a unique high salinity responsive mechanism has been developed. *D. salina* can tolerate high salinity through marked enhancement of photosynthetic CO<sub>2</sub> assimilation, and synthesis of glycerol and osmolytes in the cells.<sup>73,74</sup> In addition, some novel information has been discovered in dicotyledonous halophyte *M. crystallinum* using a targeted quantitative proteomics approach.<sup>64</sup> In salt-treated *M. crystallinum* tonoplast, the membrane association of glycolytic enzymes (aldolase and enolase) can interact with the vacuolar H<sup>+</sup>-ATPase to stimulate its activity. This results in enhanced V-ATPase mediated ion transport under salinity stress.

### 3. PHOTOSYNTHESIS

Salinity perturbs plant water uptake and biosynthesis of abscisic acid (ABA) in leaves,<sup>78</sup> leading to quick response in stomatal conductance. It also disrupts the osmotic, ionic and nutrient balances in plants. This affects photosynthetic electron



**Figure 5.** Schematic presentation of the salinity responsive proteins/enzymes in ROS scavenging system in plants. 2-P-glycolate, 2-phosphoglycolate; 3-P-glycolate, 3-phosphoglycolate; AOX, alternative oxidase; APX, ascorbate peroxidase; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GLR, glutaredoxin; GOX, glycolate oxidase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; NAD<sup>+</sup>/NADH, nicotinamide adenine dinucleotide; NADP<sup>+</sup>/NADPH, nicotinamide adenine dinucleotide phosphate; PGP, phosphoglycolate phosphatase; PrxR, peroxiredoxin; RBOH, respiratory burst oxidase homologue (NADPH oxidase); RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; SOD, superoxide dismutase; Trx, thioredoxin.

transport and the activities of enzymes for carbon fixation.<sup>2,79</sup> Previous studies have focused on these physiological changes and characterized several photosynthesis-related genes (e.g., the genes encoding chlorophyll a/b binding proteins (CAB), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and RuBisCO activase (RCA)) involved in salinity tolerance.<sup>3,4,7</sup> Proteomics results have greatly enhanced our understanding of the photosynthetic processes underlying salinity response and tolerance. A total of 367 photosynthesis-related IDs in 27 plant species are regulated by salinity, representing 26 UPs (Supporting Information Table S1). These salt-responsive proteins are involved in regulation of light reaction, CO<sub>2</sub> assimilation, and other photosynthesis-related processes. Among them, twelve light reaction-related UPs are affected by salinity (Figure 4). They function in light-harvesting, proton gradient formation, electron transfer, and energy production.

In the processes of light reaction, light is captured by LHC to split water by an oxygen evolving complex (OEC). The OEC functions in light-induced oxidation of water to produce molecular oxygen, reduce plastoquinone, and generate a transmembrane proton gradient. The LHC chlorophyll a/b-binding protein (LHC-CAB),<sup>15,39,43,44,59,63,65,70–73</sup> OEC,<sup>15–17,29,31,32,39,44,50,60,66,68,69,71</sup> and oxygen evolving enhancer protein (OEE)<sup>15,17,28,32,34,39,43,44,46,52,56,60,61,70,71</sup> are responsive to salinity and cause changes in the activity of photosystem II (PSII) in coping with salt stress. PSII reaction center protein (gj21537121),<sup>53</sup> PS II D1 protein<sup>71</sup> and PS II chlorophyll-binding protein 47 (CP47 protein)<sup>15,69,73</sup> are also affected by salt stress. Interestingly, CP47 in *D. salina*,<sup>73</sup> *O. sativa*<sup>69</sup> and *T. halophila*,<sup>15</sup> as well as a 33 kDa OEC protein in *A. thaliana*,<sup>15</sup> *O. sativa*,<sup>16,17</sup> *P. coarctata*,<sup>69</sup> *L. sativus*,<sup>60</sup> *S. lycopersicum*<sup>50</sup> and *S. europaea*,<sup>68</sup> are all increased under salt stress conditions. These changes will protect reaction center proteins including D1 protein from stromal protease digestion to ensure optimal PSII function.<sup>80</sup> Moreover, the electrons released from PSII are transferred to PSI via Cytochrome *b<sub>6</sub>f* complex. Proteomics results showed that the abundances of Cytochrome *b<sub>6</sub>f* complex in *T. halophila*,<sup>15</sup> *Z. mays*,<sup>39</sup> *A. lagopoides*,<sup>71</sup> *A. stolonifera*<sup>43</sup> and *A. thaliana*,<sup>15</sup> as well as PSI reaction center protein in *S. italica*,<sup>41</sup> *H. vulgare*,<sup>34</sup> *A. stolonifera*,<sup>43</sup> *P. coarctata*,<sup>69</sup>

and *S. europaea*,<sup>68</sup> are affected by salt stress. The changes in abundance may modulate electron transfer efficiency and trans-membrane electrochemical proton gradients, thereby affecting ATP synthesis and NADPH formation. In the salt-stressed plants, multiple isoforms of chloroplast ATP synthases<sup>15,16,18,29,31,39,44,46,61,65,70–74</sup> and ferredoxin NAD(P)(H) oxidoreductases (FNR)<sup>15,29,32,39,43,44,58,59,65,70,74</sup> are found to be regulated by salinity. These results imply that the adjustment of ATP synthesis and thermal dissipation take place in plants under salinity.

In addition to light reaction changes, the expression of fourteen Calvin cycle related enzymes has been affected by salinity (Figure 4). Among them, nine were Calvin cycle-specific enzymes, including carbonic anhydrase (CA),<sup>15,29,32,43,52,59,65,70,73,74</sup> ribulose-1,5-bisphosphate (RuBP) synthetase,<sup>70</sup> ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) large subunit (LSU),<sup>15,16,29,32,38,43,44,46,50,53,56,59–61,65,68–72,74</sup> RuBisCO small subunit (SSU),<sup>15,16,29,31,38,43,44,46,52,56,60,66,70–72</sup> RCA,<sup>15,16,18,29,43,44,46,59,61,65,68–71,74</sup> RuBisCO binding protein (RBP),<sup>14,16,29,46,61</sup> fructose biphosphatase (FBP),<sup>16,65,72</sup> sedoheptulose-1,7 biphosphatase (SBPase),<sup>34,53,59,65,66,70,74</sup> and phosphoribulokinase (PRK).<sup>15,29,34,44,59,70,74</sup> CO<sub>2</sub> is dissolved and kept in chloroplasts through CA activity, and then fixed by RuBisCO to produce 3-phosphoglycerates (PGA). During this process, RBP and RCA function as chaperones to maintain the complex assembly and RuBisCO activity. FBP, SBPase, PRK, and the other six enzymes (phosphoglycerate kinase (PGK),<sup>15,18,23,29,30,32,40,43,49,59,60,71</sup> glyceraldehyde-3-phosphate dehydrogenase (GAPDH),<sup>11,14,15,23,26,29,30,32,38,43,46,50,57,59,60,65,68,71–74</sup> triose-phosphate isomerase (TPI),<sup>11,20,21,23,24,29,30,32,34,40,44,46,59,68,71,72,74</sup> fructose-bisphosphate aldolase (FBA),<sup>11,13,15–17,24,26,29,30,34,38,43,44,46,57,59–62,64,68,71</sup> transketolase (TK),<sup>11,16,26,46,49,59,70,71</sup> and aldolase<sup>23,32,43</sup>) catalyze the rest of the carbohydrate assimilation steps. Here we choose to classify these six enzymes in the category of carbohydrate and energy metabolism because they are also involved in other carbon metabolism (Figure 4, Supporting Information Table S1). Most of these CO<sub>2</sub> assimilation-related enzymes display diverse changes in different plant species under salt stress. This implies that the photosynthesis machinery is sensitive to salt stress and the CO<sub>2</sub>-assimilating pathways in different plants have evolved divergent mechanisms in response to salinity.

Proteomics studies have also revealed other photosynthesis-related proteins affected by salt stress. In 3 M NaCl treated *D. salina*, low-CO<sub>2</sub> inducible protein (LCIC) is induced.<sup>73</sup> LCIC contributes to the carbon-concentrating mechanism as a component of the inorganic carbon transport system in the plasma membrane, and plays an important role in salt tolerance of the algae *D. salina*. In contrast, two thylakoid lumen (TL) proteins, TL18.3 and TL19, are decreased in *T. halophila*<sup>15</sup> and *A. thaliana*,<sup>14</sup> respectively. TL18.3 functions in the regulation of D1 protein turnover and the assembly of PS II monomers into dimers.<sup>81</sup> TL19, a member of PS I subunit III, is involved in the oxidation of plastocyanin in the electron transport chain.<sup>82</sup> Their reduced abundance under salinity suggests that the thylakoids have been damaged because of salt stress. Furthermore, a red chlorophyll catabolite reductase (RCCR) is induced in NaCl-treated *C. aurantium*.<sup>59</sup> RCCR catalyzes the conversion of an intermediary red chloroplast catabolite (RCC) into primary fluorescent catabolites (pFCCs) during chloroplast breakdown.<sup>83,84</sup> Its absence causes leaf cell death as a result of the accumulation of RCC, which leads to the production of singlet oxygen.<sup>85</sup> The increased abundance of RCCR is needed for the detoxification of chlorophyll catabolites and thus plays an important role in salinity tolerance.

#### 4. ROS SCAVENGING SYSTEM

Salt stress causes over-reduction of electron transport chain in mitochondria and chloroplasts, photorespiration, fatty acid oxidation, and various detoxification reactions, cell wall peroxidases, germin-like oxalate oxidases and amine oxidases in the apoplast.<sup>86,87</sup> These processes often accompany rapid increases of reactive oxygen species (ROS), including superoxide radicals ( $\cdot\text{O}_2^-$ ), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals ( $\cdot\text{OH}$ ), which can perturb cellular redox homeostasis and result in oxidative damage to many cellular components and structures.<sup>79,88,89</sup> ROS scavenging system needs to be activated to alleviate such oxidative damages for enhanced salt tolerance.<sup>89</sup> Proteomic studies have revealed 184 protein IDs (representing 12 UPs) as ROS scavenging-related proteins, most of which (143 IDs) induced by salinity in 24 plant species (Figure 5, Supporting Information Table S1). The proteins are involved in superoxide dismutation, glutathione-ascorbate cycle, catalase (CAT) pathway, peroxiredoxin/thioredoxin (PrxR/Trx) pathway, and glutathione peroxidase (GPX) pathway (Figure 5). As a key enzyme of scavenging ROS, superoxide dismutases (SOD) are usually induced by salinity to enhance the timely dismutation of superoxide into oxygen and H<sub>2</sub>O<sub>2</sub>, which is subsequently removed through different pathways.

##### Glutathione–Ascorbate Cycle

It is one of the most important antioxidant protection systems for removing H<sub>2</sub>O<sub>2</sub> generated in cytosol, mitochondria, chloroplast and peroxisomes.<sup>86,87</sup> In this cycle, H<sub>2</sub>O<sub>2</sub> is reduced to water by ascorbate peroxidase (APX) using ascorbate (AsA) as the electron donor. The oxidized AsA (monodehydroascorbate, MDA) is still a radical, which can be converted into dehydroascorbate (DHA) spontaneously or by monodehydroascorbate reductase (MDAR). DHA is then reduced to AsA by dehydroascorbate reductase (DHAR) at the expense of glutathione (GSH), yielding oxidized glutathione (GSSG). Finally, GSSG is reduced by glutathione reductase (GR) using NADPH as electron donor. Five enzymes, APX,<sup>11,15,20,25,29,30,35,43,49,50,57,59,61,63,65,66,68,70</sup> MDAR,<sup>11,59,68,70</sup> DHAR,<sup>15,20,28,30,32,37,59,66</sup> GPX<sup>11,66,68,70</sup> and GR,<sup>26</sup> are found in salt stress proteomics studies and they showed

different expression patterns. In salt treated *O. sativa*,<sup>20,25,26,28</sup> APX, DHAR, MDAR and GR are all induced by salinity. However, the expression of APX in *C. sativus*<sup>57</sup> and MDAR in *A. thaliana*<sup>11</sup> are reduced.

##### CAT Pathway

H<sub>2</sub>O<sub>2</sub> also can be reduced to H<sub>2</sub>O in CAT pathway mainly localized in peroxisomes. Proteomics studies have shown that CAT levels are increased in *O. sativa*<sup>16,26</sup> but decreased in *C. aurantium*,<sup>59</sup> *C. sativus*<sup>57</sup> and *H. vulgare*<sup>35</sup> under salt conditions. In addition, a peroxisomal biogenesis factor 11 (PEX11) is induced in *S. europaea* under 200–600 mM salt.<sup>68</sup> The increased PEX11 expression will help to keep the peroxisome integrity and stimulate its proliferation. This is important for the CAT pathway activity,<sup>90</sup> as well as for lipid catabolism, photorespiration, and hormone biosynthesis in plants under salt stress.<sup>91</sup>

##### PrxR/Trx Pathway

This pathway is a central antioxidant defense system in plants. PrxRs constitute a multigenic family involved in ROS metabolism.<sup>92</sup> It employs a thiol-based catalytic mechanism to reduce H<sub>2</sub>O<sub>2</sub> and is regenerated using Trxs as electron donors.<sup>93</sup> Proteomics results have revealed that PrxRs<sup>13–15,29,32,34,40,57,60,66,68,70–72</sup> and Trxs,<sup>15,22,49,60,70</sup> the two key proteins in this pathway, are affected by salinity. PrxRs are increased in *A. thaliana*,<sup>13,14</sup> *C. sativus*,<sup>57</sup> *P. patens*,<sup>72</sup> and *Z. mays*<sup>40</sup> under salt conditions. However, they exhibit different changes in salt stressed *S. aegyptiaca*<sup>68</sup> and *T. halophila*.<sup>15</sup>

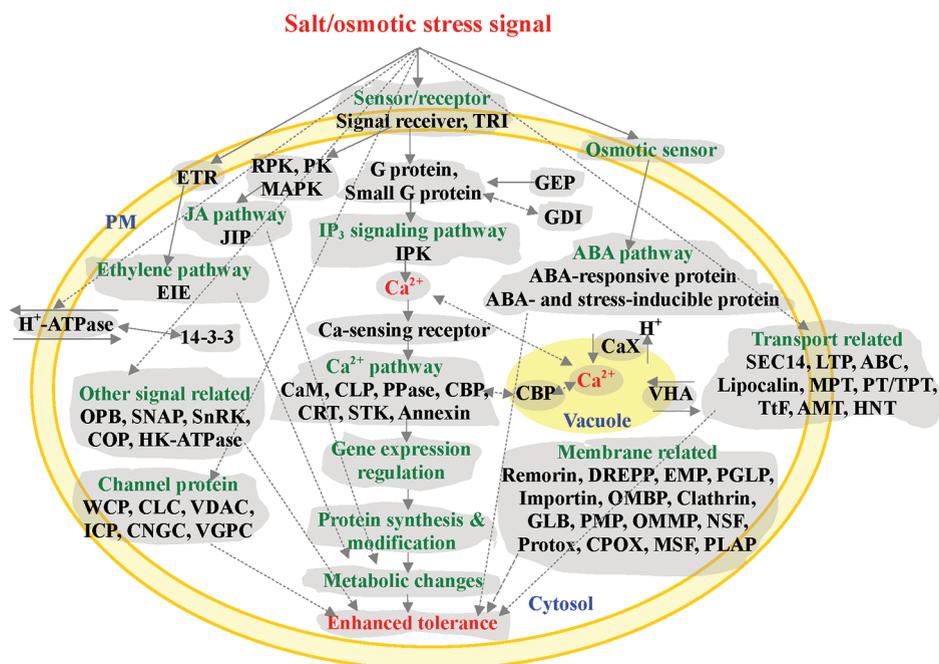
##### GPX Pathway

This pathway is generally considered to be a major enzymatic defense system against oxidative membrane damage.<sup>94</sup> GPX can reduce H<sub>2</sub>O<sub>2</sub> to the corresponding hydroxyl compounds using GSH and/or other reducing equivalents. In *S. europaea*, GPX is induced under salt conditions.<sup>68</sup> However, in *S. aegyptiaca*<sup>66</sup> and *A. thaliana*,<sup>11</sup> GPXs are reduced under gentle salt stress but increased under severe salt stress conditions. In addition, glutathione S-transferases (GSTs) have GPX activity and can use GSH to reduce organic hydroperoxides of fatty acids and nucleic acids to the corresponding monohydroxy-alcohols.<sup>95,96</sup> In the proteomic literatures, most of the GSTs are increased in salt-stressed plants,<sup>11,13–15,23,28,30,32,34,35,37,42,43,59–61,68,70,71,73</sup> except in *A. thaliana*,<sup>11,14</sup> *C. aurantium*,<sup>59</sup> *H. vulgare*,<sup>34,35</sup> and *P. tenuiflora*.<sup>70</sup> In most cases, GSTs play a pivotal role in preventing the degradation of organic hydroperoxides to cytotoxic aldehyde derivatives, and therefore, they protect plants from oxidative damage under salt stress.<sup>97</sup>

Peroxidases (PODs), which are also important in ROS scavenging, have shown increased levels in all the salt-stressed plants except *N. tabacum*,<sup>55</sup> *O. sativa*<sup>22,24</sup> and *S. lycopersicum*.<sup>49</sup> In addition, germin like proteins (GLPs) possess both oxalate oxidase activity<sup>98</sup> and SOD activity.<sup>99</sup> The increased abundance of GLPs in *N. tabacum*<sup>55</sup> may provide another ROS scavenging pathway under salt stress conditions.

#### 5. OSMOTIC HOMEOSTASIS

Salts in the soil can cause physiological water deficit and osmotic stress. To maintain osmotic homeostasis, plants accumulate osmolytes, such as proline, soluble sugars and glycine betaine (GB). GB is a major osmolyte for stabilizing protein quaternary structure and highly ordered membrane state, as well as reducing lipid peroxidation during salinity stress.<sup>1,100,101</sup> Exogenous GB can improve *S. lycopersicum* salt tolerance by regulating the expression of proteins related to photosynthesis,



**Figure 6.** Graphic depiction of identified signaling pathways elements and membrane and transport related proteins under salinity. 14-3-3, 14-3-3 protein; ABA, abscisic acid; ABC, ATP-binding cassette (ABC) transporter protein; AMT, ammonium transporter; CaM, calmodulin; CaX, H<sup>+</sup>/Ca<sup>2+</sup> antiporter; CBP, calcium-binding protein; CLC, chloride channel protein; CLP, calcineurin-like phosphoesterase; CNGC, cyclic nucleotide-gated ion channel; COP, constitutive photomorphogenic homologue; CPOX, coproporphyrinogen III oxidase; CRT, calreticulin; DREPP, developmentally regulated plasma membrane polypeptides; EIE, ethylene-induced esterase; EMP, endomembrane protein; ETR, ethylene receptor; G protein, guanine nucleotide-binding protein; GDI, guanosine diphosphate (GDP) dissociation inhibitor protein; GEP, guanine nucleotide-exchange protein; GLB, nonvascular plant hemoglobin GLB; HK-ATPase, histidine kinase-like ATPase; HNT, high affinity nitrate transporter; ICP, ion channel protein; IPK, inositol 1,3,4-triphosphate 5/6-kinase; JA, jasmonate acid; JIP, jasmonate-inducible protein; LTP, lipid transfer protein; MAPK, mitogen-activated protein kinase; MPT, mitochondrial phosphate translocator; MSF, major facilitator superfamily; NSF, N-ethyl-maleimide-sensitive factor attachment protein; OMBP, outer membrane biogenesis related protein; OMMP, outer mitochondrial membrane porin; OPB, Octicosapeptide/Phox/Bem1p; PGLP, peptidoglycan-associated lipoprotein; PK, protein kinase; PLAP, plastid lipid associated protein; PM, plasma membrane; PMP, plasma membrane polypeptide; PPase, protein phosphatase; Protox, protoporphyrinogen oxidase; PT/TPT, phosphate/triose-phosphate translocator; RPK, receptor protein kinase; SEC14, phosphatidylinositol/phosphatidylcholine transfer protein; SNAP, synaptosome-associated protein; SnRK, sucrose nonfermenting related kinase; STK, serine/threonine kinase; TRI, transforming growth factor (TGF)-beta receptor-interacting protein; Tf, transferrin; VDAC, voltage-dependent anion channel protein; VGPC, voltage-gated potassium channel; VHA, vacuolar H<sup>+</sup>-ATPase; WCP, water channel protein.

energy metabolism, detoxification, transcription, translation and protein folding.<sup>50</sup> Proteomics studies have shown that GB synthesis-associated choline monooxygenases (CMOs) are induced in salt treated *S. salsa*,<sup>65</sup> *S. europaea*,<sup>68</sup> and *S. aegyptiaca*.<sup>66</sup> In addition, late embryogenesis abundant (LEA) proteins usually involved in salt tolerance, function to protect the steady structure of proteins, membranes and cells.<sup>100</sup> In salt-treated *O. sativa*<sup>26</sup> and *G. max*,<sup>47</sup> osmotic stress induces the expression of LEA proteins in roots and hypocotyls. Several other LEA type proteins, such as cold-regulated proteins and cold-responsive group-3 LEA/RAB-related COR proteins, are all increased in *S. europaea*<sup>68</sup> and *T. aestivum*<sup>29</sup> under stress conditions. Another osmotic regulation-related protein is osmotin. In *A. thaliana* roots,<sup>11</sup> *S. tuberosum* shoots<sup>52</sup> and *B. gymnorhiza*,<sup>62</sup> osmotin abundance is increased under salinity. Moreover, an ABA-/salt-responsive 40-kDa protein, Osr40c1s, is also increased in response to salt stress in *O. sativa* panicles,<sup>20</sup> *A. lagopoides*,<sup>71</sup> and *A. stolonifera*.<sup>43</sup> Osr40c1 consists of a duplicated domain of 151 amino acids that can form amphiphilic  $\alpha$ -helical structures that associate with membrane proteins for salt tolerance.<sup>102</sup>

## 6. SALT STRESS SIGNAL TRANSDUCTION

Salt stress signaling consists of ionic signaling, osmotic signaling, detoxification signaling, and signaling to coordinate cell division and

expansion.<sup>103</sup> The signal transduction for salt tolerance is a hot topic, and several salt-responsive signaling pathways, such as salt overly sensitive (SOS) signaling pathway, ABA signaling pathway, Ca<sup>2+</sup> signal transduction pathway, protein kinase pathway, phospholipid pathway, ethylene signaling pathway, and jasmonate acid (JA)-induced signaling pathway,<sup>103–107</sup> have been predicted. Recently, proteomics studies have identified 85 IDs (24 UPs) as signal transduction-related proteins in response to salt stress (Figure 6, Supporting Information Table S1).

### G-Protein-coupled Receptors

Under salinity conditions, some stress signals (e.g., ions, ROS and ethylene) are perceived by their receptors/sensors and are transduced through kinase-mediated protein phosphorylation and/or G-proteins to regulate the corresponding signaling and metabolic pathways. Two types of receptors, the ethylene receptor (ETR) and a transforming growth factor (TGF)-beta receptor-interacting protein, are found to be induced in salt treated *T. aestivum*<sup>32</sup> and *D. salina*.<sup>74</sup> In addition, some of G-proteins/small G-proteins and three isoforms of receptor protein kinase (RPK) identified from *T. aestivum*,<sup>32</sup> *D. salina*,<sup>73</sup> *A. thaliana*<sup>15</sup> and rice<sup>20,22</sup> are stimulated by salinity conditions. This suggests that corresponding signaling pathways (e.g., ethylene and ABA signaling pathways) may be involved in salt response.<sup>104</sup> In addition, the reduced levels of a signal receiver and G-proteins/small G-proteins in wheat,<sup>32</sup> *Arabidopsis*<sup>11,13</sup> and

rice,<sup>23</sup> as well as two abundance-changed guanine nucleotide-exchange proteins (GEP) involved in small GTPase activation in *V. vinifera*<sup>53</sup> and rice,<sup>23</sup> suggest that G-protein-coupled receptors are dynamically regulated to cope with salinity.

### Phospholipid Signaling Pathway

Phospholipid signaling systems are typically grouped according to the phospholipases that catalyze the formation of lipids and other second messengers, such as diacylglycerol and calcium.<sup>103</sup> Salt stress has been shown to increase the levels of inositol 1,3,4-triphosphate 5/6-kinases in *A. thaliana*<sup>13</sup> and *S. europaea*.<sup>68</sup> The kinase is at a critical branching point in the biosynthetic pathway of inositol phosphates, and has also been shown to be involved in phosphorylation of several transcription factors.<sup>108</sup> However, its direct connection with salinity is unclear.

### ABA Signaling Pathway

The phytohormone ABA plays a major role as an endogenous messenger in the control of plant water status and osmotic stress tolerance through guard cell regulation.<sup>103,109</sup> Many ABA-inducible genes share *cis*-regulatory elements (i.e., ABA-responsive elements, ABREs) and are involved in the regulation of plant stress responses. Interestingly, osmotic stress-responsive genes can be ABA independent, ABA dependent, or partially ABA dependent.<sup>103</sup> The salt-/osmotic-responsive genes have been considered as either “early-response genes” (typically encoding transcription factors with quick and often transient expression) or “delayed-response genes” (slowly activated by stress with sustaining expression). However, most of these genes and their expression products are not known. In proteomics studies, several ABA-related proteins in response to salt stress, such as ABA-responsive proteins (ABR17 and ABR18) and ABA/stress-inducible proteins (ASR1), are found to be increased in *P. sativum*<sup>48</sup> and *O. sativa*.<sup>25</sup> Previous studies have proved that overexpression of pea ABR17 protein in *A. thaliana* can affect diverse protein expressions, including photosynthesis-related proteins (e.g., PS I, CAB, ribose-5-phosphate isomerase, Cp29, RCA, and OEE), DNA damage repair-related protein (e.g., DRT112), enolase, and glycine-rich RNA-binding proteins.<sup>110</sup> These proteins have been shown to be associated with enhanced stress tolerance. ASR1 has also been found to be regulated by ABA and salt in tomato.<sup>111</sup> In addition, genetic analysis of ABA-deficient mutants has demonstrated the necessity of ABA signaling in stomatal control of water loss.<sup>112</sup> A desiccation-related protein is also regulated by salt in *V. vinifera*.<sup>53</sup> All of these results suggest that plants activate ABA signaling pathways to protect themselves from water deficit associated with salinity.

### JA, ethylene (ET), and Salicylic Acid (SA) Signaling Pathways

Cross-talks between ABA, JA, ET, and SA pathways are important signaling processes in plant stress responses. In general, JA and ET signaling pathways are involved in responses to wounding, abiotic stresses (e.g., drought and high salinity), and necrotrophic pathogens. SA pathway is another important signaling pathway in response to general defense responses as well as to biotrophic pathogens.<sup>104,113</sup> However, the molecular components and how they function together are not clear. Proteomics studies have revealed a salt-inducible ethylene receptor in *T. aestivum*,<sup>30,32</sup> salt-regulated mitogen-activated protein kinases (MAPKs) in *A. thaliana*<sup>15</sup> and *L. sativus*,<sup>60</sup> salt-responsive jasmonate-inducible proteins in the roots of *H. vulgare*<sup>35</sup> and *A. thaliana*,<sup>11</sup> as well as many members of pathogenesis-related protein (PR protein) family (e.g., PR1, PR5, PR10, and PR17) in *L. sativus*,<sup>60</sup> *A. hypogaea*,<sup>45</sup> *O. sativa*,<sup>22,26</sup>

*S. lycopersicum*,<sup>49</sup> *A. thaliana*,<sup>15</sup> *T. halophila*,<sup>15</sup> *H. vulgare*,<sup>37</sup> *V. vinifera*,<sup>53</sup> *P. patens*,<sup>72</sup> and *S. europaea*.<sup>68</sup> MAPKs serve as negative regulators of SA, and positive regulators of JA-activated gene expression.<sup>103</sup> MAPKs have also been postulated to be involved in the integration of SA and JA-dependent signals to evoke appropriate responses against pathogens and other stresses. In addition, some PR proteins have been proved to be affected by JA/ET or SA signaling. They include AtPR12 induced by JA/ET signaling, AtPR1 up-regulated by SA signaling,<sup>114</sup> as well as OsPR10 induced by JA/ET but suppressed by SA signaling in response to high salinity. Clearly, proteomics has begun to show powerful applications in unraveling the molecular mechanisms underlying hormone signaling in salt tolerance.

### Ca<sup>2+</sup>/Calmodulin (CaM) Signaling Pathway

Salt stress-induced Ca<sup>2+</sup>-dependent signaling network has been reported to mediate Na<sup>+</sup> homeostasis and salt resistance.<sup>106</sup> In plant cells, Ca<sup>2+</sup> is a ubiquitous second messenger involved in numerous signaling pathways. The Ca<sup>2+</sup>/calmodulin (CaM) pathways have been implicated in mediating stress responses and tolerance in plants. Many members of the Ca<sup>2+</sup> signaling pathway have been found in salt treated plant species using proteomics approaches. In maize chloroplast, a Na<sup>+</sup>-sensing element, calcium-sensing receptor, is induced at 25 mM NaCl for one hour, but decreased after four hours.<sup>39</sup> In *Arabidopsis*,<sup>11,15</sup> rice,<sup>26</sup> and *S. salsa*,<sup>65</sup> calcium-binding proteins (CBPs) are regulated by salinity. In addition, salt regulates the dynamics of calmodulin in rice,<sup>24</sup> maize<sup>40</sup> and *A. lagopoides*,<sup>71</sup> calcineurin-like phosphoesterase in wheat,<sup>32</sup> as well as calreticulin in *Arabidopsis*,<sup>11</sup> rice,<sup>26</sup> *S. tuberosum*,<sup>52</sup> *G. max*<sup>46</sup> and *D. salina*.<sup>73</sup> The changes may contribute to the modulation of intracellular Ca<sup>2+</sup> levels and induction of specific protein kinase/phosphatase systems.<sup>11</sup> Therefore, it is suggested that the regulation of Ca<sup>2+</sup> signaling network is closely related to the activation of the SOS signal transduction pathway, which regulates cellular Na<sup>+</sup>/K<sup>+</sup> homeostasis and the osmolytes accumulation.<sup>103</sup>

### 14-3-3 Proteins

The 14-3-3 group of proteins are ubiquitous and multifunctional regulators in many cellular signaling pathways. They interact with a number of signaling molecules, such as calcium-dependent protein kinase (CDPK), and MAPK. Importantly, 14-3-3 proteins act as positive regulators of plasma membrane (PM) H<sup>+</sup>-ATPase by interacting with the C terminus, which is essential for the control of ion transport and cytoplasmic pH.<sup>115</sup> 14-3-3 proteins are known to be involved in responses to diverse stresses including salinity. They can play roles in stress response at multiple levels including regulating target proteins with functions including signaling, transcription activation and defense. They also work as components of transcription factor complexes associated with ABA-induced gene expression. In proteomics studies, many members of 14-3-3 groups, such as 14-3-3 protein (gi13928452, and gi12229593),<sup>40,45</sup> 14-3-3-like protein (gi1168189, and gi7267542),<sup>30,72</sup> GF14a (XP\_48289),<sup>19</sup> GF14b (gi50924768)<sup>19</sup> and GF14 Kappa isoform (gi30698122)<sup>13</sup> are regulated by salinity conditions in *T. aestivum*,<sup>30</sup> *A. hypogaea*,<sup>45</sup> *A. thaliana*,<sup>13</sup> *O. sativa*,<sup>19,24</sup> *P. patens*<sup>72</sup> and *Z. mays*.<sup>40</sup> The results lead us to propose that the changes in 14-3-3 proteins regulate multiple pathways involved in salt stress response.

## 7. ION HOMEOSTASIS AND CROSS-MEMBRANE TRANSPORT

Under salinity conditions, high apoplastic levels of Na<sup>+</sup> and Cl<sup>-</sup> alter the aqueous and ionic thermodynamic equilibrium.

This results in hyperosmotic stress, ionic imbalance and toxicity. Therefore, plants have to reestablish cellular ion homeostasis by regulating ion uptake/exclusion and *in vivo* compartmentalization. The maintenance of ion (e.g.,  $K^+$  and  $Na^+$ ) homeostasis is a fine-tuned process that mainly relies on the proton-motive forces created by the action of  $H^+$ -ATPases, various ion channels and transporters.<sup>116</sup> Recently, phototropin and 14-3-3 proteins have been found to work cooperatively as well as independently to regulate the activity of plasma membrane  $H^+$ -ATPases and, hence, the opening and closing of the ion channels (e.g.,  $K^+$  channel).<sup>117,118</sup> Current proteomics literature has shown that  $H^+$ -ATPase,<sup>11,13,15,24,30,38,48,49,57,64,68,70,73</sup> ATP-binding cassette (ABC) transporter,<sup>30,32,72</sup> other ion channels and transporters<sup>30,32,40,58,65,68,71,72</sup> are significantly affected by salinity (Figure 6, Supporting Information Table S1).

### $H^+$ -ATPase

$H^+$ -ATPases are one of the most important enzymes required for the maintenance of ion homeostasis in plant cells. All the plasma membrane  $H^+$ -ATPases in *D. salina*,<sup>73</sup> and most of the vacuolar  $H^+$ -ATPases in glycophytes (*A. thaliana*,<sup>13</sup> *O. sativa*,<sup>24</sup> *T. aestivum*,<sup>30</sup> *Z. mays*<sup>38</sup> and *P. sativum*<sup>48</sup>) and halophytes (*S. europaea*,<sup>68</sup> *M. crystallinum*,<sup>64</sup> and *P. tenuiflora*<sup>70</sup>) are induced by certain salt treatment conditions. It has been speculated that the increased levels of  $H^+$ -ATPases create more driving force for  $Na^+$  transport by SOS1, which is essential for salt tolerance.<sup>13,119,120</sup> Vacuolar  $H^+$ -ATPases, the major  $H^+$ -pumps on the tonoplast, can generate the proton electrochemical gradient for vacuolar  $Na^+/H^+$  antiporter to compartmentalize  $Na^+$  in the vacuoles.<sup>121</sup> The increased levels and/or activities of the vacuolar  $H^+$ -ATPases are proposed to be a cost-effective strategy for  $Na^+$  sequestration and osmotic adjustment under salt stress.<sup>15</sup> In addition, the aforementioned mitochondrial-/chloroplast-located  $H^+$ -ATPase are also proposed to contribute to ion balance.<sup>13,16,31</sup>

### ABC Transporters and Other Transporters

ABC transporters are induced in *P. patens* gametophores<sup>72</sup> and *T. aestivum*<sup>30,32</sup> under salinity. The ABC transporter is in charge of transporting of stress-related secondary metabolites, such as alkaloids, terpenoids, polyphenols and quinines.<sup>122</sup> It also has been found to be induced in salt-stressed *Synechocystis*.<sup>123</sup> An ABC transporter (*AtMRPS*) mutant in *Arabidopsis* displays salt hypersensitivity.<sup>124</sup> This highlights the importance of the ABC transporter in response to salinity. In addition, a high affinity nitrate transporter (gj52789941) and an ammonium transporter (BM446979) are induced in *D. salina*.<sup>73</sup> It has been reported that nitrate uptake in *D. salina* is driven by a  $Na^+$  electrochemical gradient rather than  $H^+$  transport.<sup>125,126</sup> This suggests that *Dunaliella*, as a salt-tolerant algae, has developed a salt dependent adaptation mechanism to utilize either nitrate or ammonium as a nitrogen source.

Lipids, especially membrane phospholipids, are the main component of the cell membrane. Lipid synthesis and efficient transport are important to maintain cell structure homeostasis under stress conditions. Salinity and dehydration responsive lipid transfer proteins (LTPs) are up-regulated in salt stressed *N. tabacum*.<sup>55</sup> LTPs can bind a wide range of hydrophobic ligands and shuttle different lipids between liposomes and mitochondria *in vitro*.<sup>127</sup> It is suggested that LTPs may be involved in cutin transport and deposition.<sup>128</sup> Therefore, the induction of LTPs under salt stress helps to promote the increased deposition of cuticular material to leaf surface. However, in *A. thaliana* cell suspension cultures, one phospholipid transfer related protein, SEC14 is reduced under 200 mM NaCl treatment.<sup>13</sup> SEC14 is

required for trafficking from endosomes and regulates distinct trans-Golgi export pathways. In addition, salinity-induced peptidoglycan-associated lipoproteins in *D. salina* are increased to stabilize cell envelope structure by bridging the outer membrane and the peptidoglycan layer in saline solutions.<sup>73</sup> Furthermore, a chloroplast phosphate/triose-phosphate translocator and a mitochondrial phosphate translocator are increased under salt stress.<sup>15</sup>

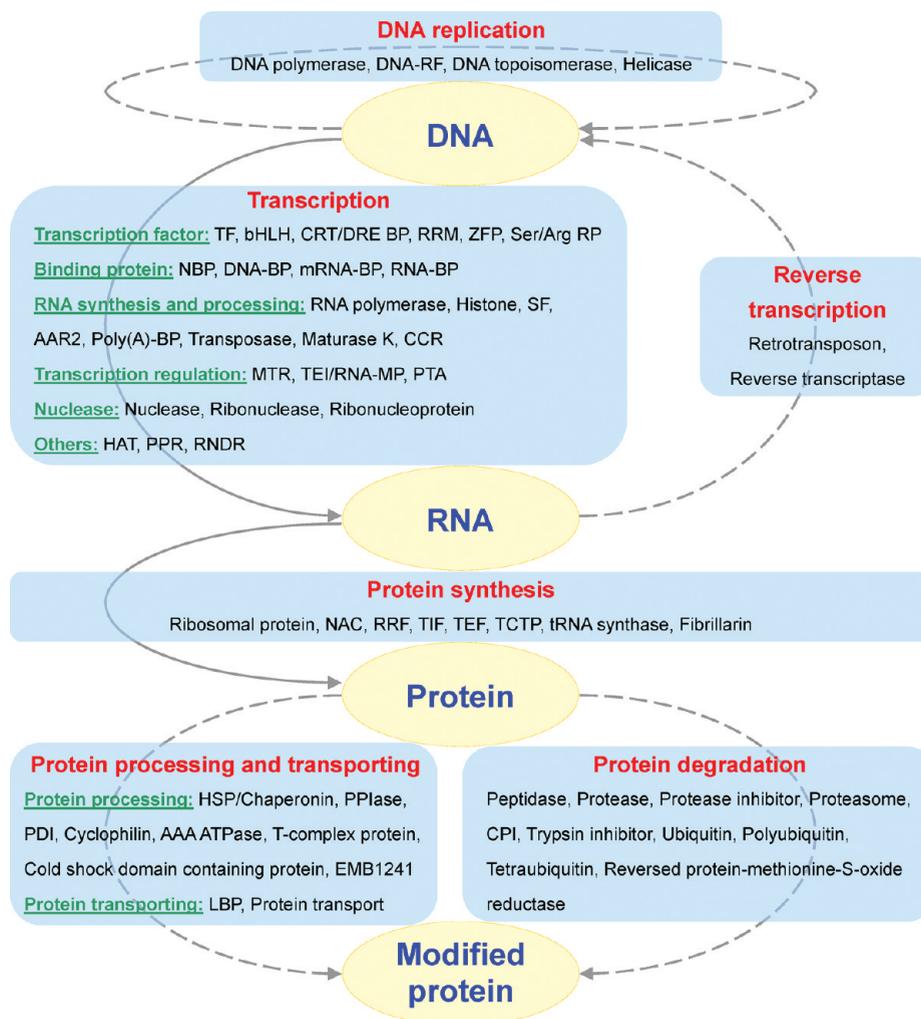
Under salinity, ion imbalance and toxicity have severe effects on metabolism. Besides  $Na^+$  ion exclusion and compartmentalization, iron uptake/binding-related proteins can help to maintain ion balance and enzyme activity.<sup>87,129</sup> Three proteins, ferritin,<sup>18,50,68</sup> iron deficiency-induced protein (IDI)<sup>35</sup> and iron deficiency-specific protein (IDS),<sup>35</sup> have abundance changes in salt-stressed plants. Ferritin, a ubiquitous multimeric iron storage protein, functions in sequestration of excess free irons and prevents formation of hydroxyl radicals through the Fenton reaction.<sup>18,130</sup> The ferritins in *S. lycopersicum*<sup>50</sup> and *O. sativa*<sup>18</sup> are all induced by certain salinity conditions. However, the IDIs and IDSs in *H. vulgare* are decreased by salt treatment,<sup>35</sup> which is helpful to avoid excessive ion uptake under salt stress.

### Ion Channel Proteins

Under salt stress, different ion channels are found to be changed in levels to maintain ion homeostasis. Voltage-gated potassium channels in *A. lagopoides*<sup>71</sup> and *T. aestivum*<sup>32</sup> are induced with the increase of salt concentrations. It is crucial for a balance of  $K^+/Na^+$  in the cells. However, a cyclic nucleotide-gated ion channel (CNGC) is reduced as a nonselective cation channel.<sup>30</sup> It is opened by the direct binding of cyclic nucleotides, cAMP and cGMP. The activity is of little voltage dependence, but is modulated by  $Ca^{2+}$ /calmodulin and phosphorylation. Interestingly, based on proteomics studies, a number of annexin isoforms are salinity-induced in *A. thaliana*,<sup>12,14,15</sup> *G. max*,<sup>46</sup> *O. sativa*,<sup>26</sup> *S. europaea*,<sup>68</sup> *S. lycopersicum*,<sup>49</sup> *S. tuberosum*,<sup>52</sup> and *T. aestivum*.<sup>32</sup> Annexin is known to function as a  $Ca^{2+}$ -permeable channel at endomembrane and plasma membrane for the formation of a ROS-stimulated passive  $Ca^{2+}$  transport pathway.<sup>131</sup> Its increase in abundance under salinity may play important roles in osmotic adjustment, and subsequently cell expansion and exocytosis.<sup>12,132</sup> In addition, a voltage-dependent anion channel protein (VDAC) is induced by salt stress in *S. europaea*,<sup>68</sup> *Z. mays*,<sup>40</sup> *B. vulgaris*,<sup>58</sup> and *A. lagopoides*.<sup>71</sup> VDAC is a barrel protein located at the outer mitochondrial membrane and is responsible for passage of small molecules (<1000 Da) into the intermembrane space. The dynamic changes in VDAC function and abundance have been found to influence mitochondrial respiration.<sup>133,134</sup> Another anion channel, chloride channel protein, is reduced in salt-treated *P. patens*.<sup>72</sup> This will limit the transport of  $Cl^-$  into the cells and increase salinity tolerance.<sup>72</sup> Concomitantly, a water channel-like protein was down-regulated by salt stress to limit water loss under salt stress.<sup>15</sup>

### Plasma Membrane and Other Membrane Associated Proteins

Some other salt-responsive proteins located at plasma membrane, nuclear membrane, chloroplast/mitochondrial membrane, and endomembrane systems are revealed by proteomics investigation (Figure 6, Supporting Information Table S1). In rice,<sup>24</sup> salt inducible and developmentally regulated plasma membrane polypeptides (DREPP PM) containing a Glu-rich site at the C terminus is proposed to be responsible for calcium binding,<sup>135</sup> and association with the  $Ca^{2+}$  signal transduction pathway under salt stress. In addition, a plant specific PM/lipid-raft protein, remorin, is associated with membrane skeletons.<sup>136</sup> The salt-induced remorin probably contributes to



**Figure 7.** Schematic representation of the salinity responsive proteins involved in transcription and protein metabolism. AAA ATPase, ATPases associated with a wide variety of cellular activities; AAR2, a gene for splicing pre-mRNA; bHLH, basic/helix–loop–helix; BP, binding protein; CCR, cassette chromosome recombinase; CPI, cysteine protease inhibitor; CRT/DRE BP, C-repeat/dehydration responsive element binding protein; DNA-RF, DNA replication factor; EMB1241, embryo defective 1241; HAT, half-a-TPR (tetratricopeptide repeat); HSP, heat shock protein; LBP, luminal-binding protein; MTR, membrane-associated transcriptional regulator; NAC, nascent polypeptide-associated complex; NBP, nucleic acid binding protein; PDI, protein disulfide isomerase; PPIase, peptidyl-prolyl *cis-trans* isomerase; PPR, pentatricopeptide repeat-containing protein; PTA, plastid transcriptionally active; RNDR, ribonucleoside-diphosphate reductase; RRF, ribosome-recycling factor; RRM, RNA recognition motif-containing protein; Ser/Arg RP, serine/arginine rich protein; SF, splicing factor; TCTP, translationally controlled tumor protein; TEF, translation elongation factor; TEI/RNA-MP, telomerase elongation inhibitor/RNA maturation protein; TF, transcription factor; TIF, translation initiation factor; ZFP, zinc finger protein.

the stabilization of damaged PM under salt stress.<sup>19,24</sup> Furthermore, two PM associated proteins, a lipocalin-like protein in *P. patens*<sup>72</sup> and an iron-binding transferrin in *D. salina*,<sup>73</sup> are increased under salinity. They are suggested to function in desiccation protection and iron uptake, respectively. Moreover, an importin (a nuclear membrane transporter) in *T. aestivum*,<sup>30,32</sup> an outer mitochondrial membrane porin in *T. aestivum*<sup>33</sup> and *A. lagopoides*,<sup>71</sup> as well as a clathrin in *Thellungiella*<sup>15</sup> are all reduced in levels by salt treatment, but an endomembrane protein in *A. thaliana*<sup>15</sup> and a Golgi associated protein in *A. lagopoides*<sup>71</sup> are increased under salinity. This reflects the adjustment in the exchange of products between nucleus and endomembrane systems under salt stress.

## 8. TRANSCRIPTION AND PROTEIN FATES

The aforementioned signaling systems can trigger changes in transcriptional regulatory networks of *cis/trans*-elements and

transcription factors. Proteomics studies have shown that the levels of transcription factors and transcription related proteins are responsive to salt stress and play a pivotal role in salinity tolerance (Figure 7, Supporting Information Table S1). The salt-induced C-repeat/dehydration-responsive elements (CRT/DRE)-binding protein in *P. coarctata*,<sup>69</sup> transcription factor basic transcription factor 3 (BTF3) in *T. aestivum*,<sup>30,32</sup> *S. lycopersicum*<sup>49</sup> and *S. aegyptiaca*,<sup>66</sup> and basic/helix–loop–helix (bHLH) in *G. max*<sup>47</sup> and *V. vinifera*<sup>53</sup> are important regulatory components in the transcriptional networks and control diverse processes to cope with salt stress. In addition, the increased DNA polymerases in *Z. mays*,<sup>38</sup> DNA topoisomerases in *S. europaea*,<sup>68</sup> as well as helicases in *T. aestivum*<sup>30</sup> and *A. thaliana*<sup>15</sup> are supposed to enhance DNA replication, unwinding, and transcription under salinity. Furthermore, some RNA processing and splicing-related proteins, such as maturase K,<sup>40,60,68</sup> nucleic acid binding proteins,<sup>15,16,29,35,44,45,49,52,59,72</sup>

glycine-rich RNA-binding proteins,<sup>11,20,49,66</sup> and RNA splicing factors, are also influenced by salt.<sup>21,30</sup>

Protein synthesis plays an important role in abiotic stress adaptation. Proteomics studies have revealed many components of protein synthesis machinery to be altered in expression under salt stress conditions, including different ribosomal proteins,<sup>11,13,15,16,23,32,38,40,41,44,46,50,52,53,57,60,71,72</sup> translation initiation factors,<sup>11,13,15,18,30,32,68,70,72</sup> poly(A)-binding proteins,<sup>11,35</sup> translation elongation factors,<sup>13,15,32,36,50,71,74</sup> translationally controlled tumor proteins,<sup>15,36,40,48,53,70,71</sup> RNA recognition motif (RRM)-containing proteins,<sup>15,70</sup> and tRNA synthases<sup>13,15,27,32</sup> (Figure 7, Supporting Information Table S1). Salt stress generally represses protein synthesis.<sup>2</sup> However, some of above proteins are enhanced by salt treatment, indicating normal cellular processes require the maintenance of protein synthesis activities under salinity.

Under stress conditions, correct protein folding and transport are crucial for keeping normal cellular functions. Heat shock proteins (HSPs) and other molecular chaperons contribute to protein structure stabilization and subcellular localization.<sup>137</sup> Various HSPs/chaperonins,<sup>11,13–16,23,26,27,30,32,36,40,43,45,46,48–50,52,53,56,57,59–61,63,68–74</sup> luminal-binding proteins (LBP),<sup>14,15,68,72,74</sup> peptidyl-prolyl *cis*–*trans* isomerases,<sup>39,52,59,61,66</sup> protein disulfide isomerases (PDI),<sup>11,15,19,61,70</sup> T-complex proteins,<sup>15,30</sup> AAA ATPase superfamily proteins,<sup>27,61,70</sup> and cold shock domain containing proteins<sup>28,30,32</sup> are all affected by salinity (Figure 7, Supporting Information Table S1). These proteins function to maintain normal protein folding, repair and renaturation of the stress-damaged proteins. In addition, plants use proteasome pathways for the selective degradation of proteins. Under salt stress, some members of the proteasome pathways, such as ubiquitin/polyubiquitin/tetraubiquitin,<sup>43,57,73</sup> SKP1 protein,<sup>72</sup> proteasome components,<sup>11,16,30,32,53,59,60,66,68,70,72,74</sup> various proteases<sup>15,32,39,41,43,49,68,70,72–74</sup> and peptidases,<sup>11,13,15,23,26,49,70,72,74</sup> protease inhibitors,<sup>32,46,47,61</sup> and reversed protein-methionine-S-oxide reductases<sup>15</sup> exhibit changes in abundance (Figure 7, Supporting Information Table S1). In addition to the important roles in protein turnover, the ubiquitin-mediated degradation of proteins is speculated to function in the regulation of other cellular processes such as signal transduction and transcription. Thus, these salt-responsive proteins are vital for plant salt tolerance.

## 9. CYTOSKELETON AND CELL STRUCTURE

Under salinity, cytoskeleton is rapidly remodeled to allow cell size adjustment for cell turgor maintenance.<sup>13,65</sup> Proteomics studies have shown the cytoskeleton dynamics through measuring the abundance changes of basic cytoskeleton components (actin<sup>11,23,26,43,59</sup> and tubulin<sup>11,13,15,32,73,74</sup>) and other cytoskeleton-related proteins (some actin-binding proteins (ABPs),<sup>21</sup> kinesin motor,<sup>23,46,68</sup> myosin,<sup>24,32,68</sup> and xyloglucan endotransglycosylase (XET) hydrolases<sup>40</sup>) under salt stress conditions. ABPs, including actin-depolymerizing factors (ADFs),<sup>71</sup> profilins<sup>57,66,68</sup> and cyclase-associated proteins (CAPs)<sup>13</sup> can bind to actin cytoskeletons and play key roles in the remodeling. For example, ADFs modulate the dynamic organization of actin cytoskeletons by promoting filamentous actin disassembly. Profilin can bind actin monomers and cause polymerization/depolymerization of actin filaments for maintaining cell structure integrity, cell mobility, tumor cell metastasis, and growth factor signaling.<sup>138</sup> CAPs, the multifunctional ABPs, are implicated in various signal transduction pathways associated with cell growth, development, vesicle trafficking, and endocytosis.

It has been reported that cytoskeleton dynamics is connected with other physiological changes under salinity. For example, the osmotic stress regulation of actin organization correlates well with K<sup>+</sup> channel activity in guard cells.<sup>139</sup> In addition, tubulins may comigrate with P-type ATPases,<sup>140</sup> or connect with the plasma membrane for controlling cell expansion and morphology.<sup>141</sup> Furthermore, XETs are responsible for cutting and rejoining intermicrofibrillar xyloglucan chains and thus enable wall loosening required for cell expansion. Reduced expression of these enzymes is responsible for the growth inhibition under salt stress.<sup>142</sup>

## 10. CROSS-TOLERANCE TO MULTIPLE STRESSES

Plants have developed cross-tolerance mechanisms to cope with different stresses at the same time, due to various signaling and metabolic pathways are connected into networks.<sup>2</sup> For example, some biotic stress-responsive proteins/genes play important roles in salt tolerance. Microarray analysis in *A. thaliana* has revealed that from the total of 194 salt-inducible genes, only 51 genes (approximate 25%) are salinity specific, and the rest are also involved in drought and/or cold stresses.<sup>143</sup> Current proteomics results have corroborated these findings.

Lectins form a diverse group of carbohydrate-binding proteins which mainly participate in defense against predators and pathogens.<sup>144</sup> They also function in plant salt tolerance.<sup>145</sup> In proteomics studies, enhanced levels of lectins in *A. thaliana*,<sup>15</sup> *P. sativum*,<sup>48</sup> *O. sativa*,<sup>23</sup> and *S. bicolor*<sup>42</sup> are observed under certain salinity conditions. Lectin levels are also induced in rice roots and sheaths under drought conditions.<sup>146,147</sup> In addition, cytoplasmic mannose-binding lectins<sup>148–150</sup> and salt stress-induced proteins (SALT proteins) are also enhanced under salt stress.<sup>23,27</sup>

The glucosinolate-myrosinase system is generally believed to be part of the plant defense system against insects and pathogens. Glucosinolates can be degraded by myrosinases into glucose, sulfate and toxic products (e.g., nitriles, isothiocyanates, epithionitriles, and thiocyanates) against biotic factors.<sup>151,152</sup> Some studies have shown the involvement of the glucosinolate-myrosinase system in salt-responsive processes.<sup>153</sup> For instance, the expression level of myrosinase in *atRZ-1a* transgenic *A. thaliana* seedlings was lower than wild-type under 100 mM NaCl stress.<sup>14</sup> It is suggested that the decrease in the glucosinolate-myrosinase system allows more resources for cells to combat salt stress.

Other biotic stress-related proteins (e.g., elicitor peptides,<sup>47,68</sup> disease-related/resistance proteins,<sup>23,30,32,48,60,68</sup> hypersensitive-induced response proteins,<sup>19,24</sup> pathogenesis-related proteins,<sup>15,22,26,37,45,49,53,60,68,72</sup> stress inducible proteins,<sup>20,30,35</sup> and universal stress protein family proteins<sup>13,26,30,42</sup>) and abiotic stress-related proteins (e.g., cold-regulated proteins,<sup>68</sup> cold-responsive LEA/RAB-related COR proteins,<sup>29</sup> and copper homeostasis factors<sup>11</sup>) are also detected in proteomics studies to be regulated in response to salt stress.

The multifunctional glyoxalase system also contributes to salt tolerance. Plants exposed to salt stress accumulate high amounts of methylglyoxal (MG). MG is a byproduct mainly from triose phosphate in glycolysis. It is toxic to the cell by inhibition of cell proliferation,<sup>154</sup> degradation of proteins and inactivation of antioxidant defense system.<sup>155</sup> The glyoxalase system comprised of glyoxalase I (GlyI) and glyoxalase II (GlyII) catalyzes the detoxification of MG. Previous molecular and transgenic studies have shown that overexpression of GlyI and GlyII enhances plant salt tolerance.<sup>156–158</sup> Proteomics studies have found that when treated with 150 mM NaCl for 6 or 48 h,<sup>11</sup> increase of GlyI in

*O. sativa*,<sup>23,26</sup> *A. lagopoides*<sup>71</sup> and *C. aurantium*,<sup>59</sup> and decrease of GlyII in *P. tenuiflora*<sup>70</sup> and *A. thaliana* are observed. This implies that the glyoxalase system was under dynamic regulation in different plant species and under different stress conditions.

## 11. CONCLUSIONS AND PERSPECTIVES

Plant salinity response and tolerance constitute a sophisticated fine-tuned signaling and metabolic network. Previous morphological, physiological, genetic and genomic analyses have made significant discoveries of salinity-responsive genes, proteins and metabolites involved in different cellular pathways important for salt stress response and tolerance. However, systematic understanding of the molecular processes and networks is still in the beginning. Modern high throughput proteomics tools have shown utility in acquiring more detailed quantitative information on the temporal and spatial expression of proteins. By integrated analysis of current proteomics results available for 34 plant species, we found 2171 protein identities representing 561 UPs. The dynamic changes of these proteins under salinity conditions provide further invaluable information toward understanding of the underlying sophisticated cellular and molecular processes in plant salt stress response and tolerance, including photosynthesis, energy metabolism, ROS scavenging, ion/osmotic homeostasis, signaling transduction, transcription and translational regulation, and cytoskeleton dynamics. Despite the significant progress, large gaps still remain in our knowledge of transmembrane ion transport and cellular compartmentalization, sensors/receptors in signaling transduction, molecules in long-distance signaling, and metabolites in energy supply. Importantly, intracellular and intercellular molecular networks involving molecular interactions and pathway cross-talks require urgent attention and are the future focus of research in this area. Putting together the “puzzles” needs more pieces and logistics between them. The widespread applications of the advanced proteomics approaches and technologies, such as multidimensional protein fractionation, isobaric tags for relative and absolute quantitation (iTRAQ), label free quantification mass spectrometry, phosphoprotein and glycoprotein enrichment and tagging, will definitely enhance the discovery of low abundant proteins (e.g., transcriptional factors, kinases, channels and transporters) and novel regulatory mechanisms (e.g., phosphorylation) in salt stress signaling and metabolism pathways. Integration of proteomics results with findings from other large-scale “omics” and bioinformatics will facilitate the establishment of molecular networks underlying salt stress response and tolerance. This can then be used to predict and validate how diverse components generate responses and control different pathways. Such an *in silico* plant will enable prediction/modeling and rational engineering of salt stress signaling and metabolic processes toward the ultimate goal of improving plant salt tolerance for enhanced yield and bioenergy.

## ■ ASSOCIATED CONTENT

### 5 Supporting Information

The salt-responsive proteins data set. Supporting Information Table S1. Plant salt stress-responsive proteins identified by proteomics studies. Supporting Information Table S2. Salt stress-responsive proteins in leaves from different plant species identified by proteomics studies. Supporting Information Table S3. Salt stress-responsive proteins in roots from different plant

species identified by proteomics studies. Supporting Information Table S4. Salt stress-responsive proteins in shoots from different plant species identified by proteomics studies. Supporting Information Table S5. Salt stress-responsive proteins in unicells from different plant species identified by proteomics studies. Supporting Information Table S6. Salt stress-responsive proteins in seedlings from *Arabidopsis thaliana* and *Setaria italica* identified by proteomics studies. Supporting Information Table S7. Salt stress-responsive proteins in the grains, hypocotyls, radicles, and panicles from different plant species identified by proteomics studies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ■ ABBREVIATIONS

•O<sub>2</sub><sup>-</sup>, superoxide radical; •OH, hydroxyl radical; ABA, abscisic acid; ABC, ATP-binding cassette; ABP, actin-binding protein; ABR, ABA-responsive protein; ABRE, ABA-responsive element; ADF, actin-depolymerizing factor; APX, ascorbate peroxidase; AsA, ascorbate; ASR, ABA/stress-inducible protein; bHLH, basic/helix-loop-helix; BTF3, basic transcription factor 3; CA, carbonic anhydrase; CAB, chlorophyll a/b-binding protein; CaM, calmodulin; CAP, cyclase-associated protein; CAT, catalase; CBP, calcium-binding protein; CDPK, calcium-dependent protein kinase; CMO, choline monoxygenase; CNGC, cyclic nucleotide-gated ion channel; COR, cold responsive; CP47 protein, photosystem II chlorophyll-binding protein 47; CRT/DRE, C-repeat/dehydration-responsive elements; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; DREPP PM, developmentally regulated plasma membrane polypeptides; DRT, DNA damage repair-related protein; ETR, ethylene receptor; FBA, fructose-bisphosphate aldolase; FBP, fructose bisphosphatase; FNR, ferredoxin NADP(H) oxidoreductases; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GB, glycine betaine; GEP, guanine nucleotide-exchange protein; GLP, germin like protein; Gly, glyoxalase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; HSP, Heat shock protein; ID, identity; IDI, iron deficiency-induced protein; IDS, iron deficiency-specific protein; iTRAQ, isobaric tags for relative and absolute quantitation; JA, jasmonate acid; LBP, luminal-binding

protein; LCIC, low-CO<sub>2</sub> inducible protein; LEA, late embryogenesis abundant protein; LHC, light-harvesting complex; LSU, large subunit; LTP, lipid transfer protein; MAPK, mitogen-activated protein kinase; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; MG, methylglyoxal; OEC, oxygen evolving complex; OEE, oxygen evolving enhancer protein; PDI, protein disulfide isomerase; PEX11, peroxisomal biogenesis factor 11; pFCC, primary fluorescent catabolite; PGA, phosphoglycerate; PGK, phosphoglycerate kinase; PM, plasma membrane; POD, peroxidase; PR protein, pathogenesis-related protein; PRK, phosphoribulokinase; PrxR, peroxiredoxin; PS, photosystem; RBP, RuBisCO binding protein; RCA, RuBisCO activase; RCC, red chloroplast catabolite; RCCR, red chlorophyll catabolite reductase; ROS, reactive oxygen species; RPK, receptor protein kinase; RRM, RNA recognition motif; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; SA, salicylic acid; SALT proteins, salt stress-induced proteins; SBPase, sedoheptulose-1,7 bisphosphatase; SOD, superoxide dismutase; SOS, salt overly sensitive; SSU, small subunit; TGF, transforming growth factor; TK, transketolase; TL, thylakoid lumen; TPI, triose-phosphate isomerase; Trx, thioredoxin; UPs, unique protein/protein families; VDAC, voltage-dependent anion channel protein; XET, xyloglucan endotransglycosylase

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