

10.1071/FP14274\_AC

© CSIRO 2015

Supplementary Material: *Functional Plant Biology*, 2015, 42(6), 527–542.

## Supplementary Material

### **Comparative proteomic and physiological characterisation of two closely related rice genotypes with contrasting responses to salt stress**

*Seyed Abdollah Hosseini*<sup>A</sup>, *Javad Gharechahi*<sup>B</sup>, *Manzar Heidari*<sup>A</sup>, *Parisa Koobaz*<sup>A</sup>, *Shapour Abdollahi*<sup>A</sup>, *Mehdi Mirzaei*<sup>C</sup>, *Babak Nakhoda*<sup>A,E</sup> and *Ghasem Hosseini Salekdeh*<sup>D,E</sup>

<sup>A</sup>Department of Molecular Physiology, Agricultural Biotechnology Research Institute of Iran, PO Box 31535-1897, Karaj 3135933151, Iran.

<sup>B</sup>Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, PO Box 14359-44711, Tehran 2155625143, Iran.

<sup>C</sup>Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia.

<sup>D</sup>Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, PO Box 31535-1897, Karaj 3135933151, Iran.

<sup>E</sup>Corresponding authors. Emails: [h\\_salekdeh@abrii.ac.ir](mailto:h_salekdeh@abrii.ac.ir); [b.nakhoda@abrii.ac.ir](mailto:b.nakhoda@abrii.ac.ir)

**Table S1. The sequence of primers used for qRT-PCR validation of transcript levels of some of the candidate root and leaf SRPs**

Spot ID	Gene name	Sequence of forward primer (5'–3')	Sequence of reverse primer (5'–3')
3R	Thioredoxin H1	ATCGTGAAGCACGTCGGTGC	GACACCCAAGCCAAACTGACG
187R	Glutathione S-transferase	TGGTGGACTGACCTGATGGCA	AGCAACCAACTGGGCACACCT
583R	Vacuolar ATPase B subunit	CGGTCCTATTCTTTCTGTGCG	GCTAACAATTCTGGCGAATG
802R	Glutathione S-transferase	GCCTCATATCTCCCGTAACC	AGGTCAGATCCATGTTTGCG
332R	Oryzain beta chain	CTCTATCATTGGGCGTCTTC	TTGCGGACAATCCAGTAGTCT
452L	Lactoylglutathione lyase	ACCTGGTCCTGTCAAGGGAGGA	GCGATCAAGGTCACCCACACGA
486L	Phosphoglycerate kinase	GCAGTGCTTCCAATGATCCAA	CACCACAAAATCTGCGATCCA
621L	Salt stress root protein RS1	GTGTCTGGAAGACCAAGGTTT	TTGCCAATCTCCTCCTTGTTG
73L	Fiber protein Fb19	TCGCACTCGTCATCAAGAAGA	GCTAACTCATCAGGCATTTCT
456L	Putative tyrosine phosphatase	GCACCATCCCATTTCCCAAATC	ATGCGAAGATCCTCCTCTCTCT
527L	Fructose-bisphosphate aldolase, chloroplastic	CCAAGGAGGGCATGTTCTGTC	ACGACACACTGGCTACAGTGC
670L	Peroxiredoxin-2C	ACGGTTTGCTCTCCTTGCTGA	TCGCTGCCGTTCTAAGAGCT
Internal control	18S rRNA	ATGATAACTCGACGGATCGC	CTTGGATGTGGTAGCCGTTT

**Table S2. The details of number of reproducibly detected spots in root and leaf gels of salt-sensitive IR29 and salt-tolerant FL478, number of spots that showed qualitative differences, those that showed quantitative change in abundance, number of spots subjected to mass spectrometry, number of proteins with significantly high score, and finally number of qualified proteins that presented in Tables 4, S3 and S4**

	IR29		FL478	
	Leaf	Root	Leaf	Root
Protein yield (per dry weight bases)	–	–	–	–
Number of spots (mean and SD)	912	868	911	865
Qualitative differences (newly appeared/disappeared)	1	3	1	1
Quantitative differences (up/down accumulated)	45	88	27	27
Number of spot subjected to MS	39	88	19	27
Number of identified proteins with high score	34	65	16	19
Number of spot qualified after MS (spot in final table)	33	52	12	13

\*Values were calculated based on ratio between stress and control.

**Table S3. The details of candidate leaf (L) SRPs identified using combined MS and MS/MS analysis**

Only those proteins that showed statistically significant differences ( $P \leq 0.01$ ) and displayed at least 1.5-fold change in abundance were accepted as candidate leaf SRPs. Proteins were categorised based on the biological functions retrieved from the Uni-prot database (<http://www.uniprot.org>)

Spot ID	Exp p//MW <sup>a</sup>	The p//MW <sup>b</sup>	Protein Identity/accession number in uni-prot <sup>c</sup>	Score/ %coverage <sup>d</sup>	Fold change <sup>e</sup>	
					IR29	FL478
<b>Antioxidant, oxidoreductase activity and stress response</b>						
8L	6.34/18	5.9/15.1	Superoxide Dismutase [Cu-Zn]/P28757	223/38	1.33	1.65
104L	5.04/17	8.1/18.5	Thioredoxin M5, chloroplastic/Q9ZP20	66/100	-1.85	-1.1
90L	5.11/15	5.1/13.1	Thioredoxin H1/Q0D840	363/66	1.8	1.09
452L	5.49/36	5.4/32.8	Lactoylglutathione lyase/Q948T6	98/23	2.71	1.87
670L	5.53/19	5.5/17.3	Peroxiredoxin-2C/Q9FR35	216/41	-3.33	1.01
810L	5.44/29	5.4/27.8	Ferritin/Q8LK80	452/34	2.38	1.57
284L	5.44/29	5.4/27.9	Ferritin/Q8LK80	491/35	2.15	1.44
621L	5.03/31	4.9/21.8	Salt stress root protein RS1/Q0JPA6	465/49	4.59	-1.13
73L	6.28/21	6.2/34.7	Fiber protein Fb19/Q2QNV3	335/15	1.35	2.29
<b>Photosynthesis</b>						
877L	6.06/32	6.3/42.5	Ribulose biphosphate carboxylase large chain/Q0ZJV9	296/25	2.54	1.65
260L	6.57/19	9/19.6	Ribulose biphosphate carboxylase small chain, chloroplastic/Q0INY7	73/22	2.13	1.54
193L	4.91/48	5.4/51.4	Ribulose biphosphate carboxylase/oxygenase activase, chloroplastic/P93431	647/34	-1.96	-2.22
72L	6.29/16	5.7/11	Ferredoxin-Thioredoxin Reductase/P80680	284/42	-1.92	3.36
11L	6.94/16	9.8/22.9	Oxygen-evolving enhancer protein 3, chloroplastic/Q0D5P8	454/36	1.22	1.59
63L	5.54/27	5.5/20	Chloroplast 23 kDa polypeptide of photosystem II/B0FFP0	414/39	-1.53	-1.2
629L	5.14/18	4.88/15.2	ATP synthase epsilon chain, chloroplastic/P0C2Z1	137/16	-2.08	1.3
761L	4.82/18	5.7/22.7	ATP synthase B chain, chloroplast/Q84PA4	236/27	2.2	-1.14
499L	4.51/13	5.6/15.5	Plastocyanin, chloroplastic/Q0DFC9	234/20	-1.61	-1.26
671L	5.61/22	7.6/25.5	Hypothetical protein/Q6ZK86	328/32	-1.61	1.33
<b>Carbohydrate and energy metabolism</b>						
456L	5.17/38	6.7/27.2	Putative tyrosine phosphatase/Q9LKK3	191/17	1.6	-1.31
486L	5.04/57	6.8/30.4	Phosphoglycerate Kinase/A2Y650	192/25	2.51	1.13
527L	5.22/40	6.4/41.9	Fructose-biphosphate aldolase, chloroplastic/Q40677	79/10	-4.54	-1.19
720L	5.5/24	5.6/21.9	Adenosine diphosphate glucose	110/15	1.43	1.59

			pyrophosphatase/Q8L686			
722L	5.5/26	5.7/ 21.9	Adenosine diphosphate glucose pyrophosphatase/Q8L686	209/15	-1.72	-1.56
<b>Protein biosynthesis and processing</b>						
244L	4.48/18	4.2/11.9	60S acidic ribosomal protein P3/P56724	170/19	-2.04	-1.06
316L	5.36/35	7.02/55.7	Serine carboxypeptidase 1/P37890	151/8	-1.88	-1.38
376L	5.23/85	5.2/73.6	Endosperm luminal binding protein/O24182	319/25	1.94	-1.08
18L	6.35/19	9.2/24.3	Clp amino terminal domain containing protein/Q10P51	169/22	-1.01	1.89
102L	5.14/18	5.2/14.1	Pi starvation-induced protein/A6N0L6	113/35	2.41	1.42
727L	5.09/29	7.7/26.3	Chaperonin/Q69Y99	139/11	-2.38	-1.49
838L	5.14/34	7.0/31.3	GrpE protein homolog/A2XTK3	83/11	-1.58	-1.12
110L	4.8/15	5.6/15.3	Oryzacystatin-I/P09229	109/56	1.79	1.14
<b>Amino acid biosynthesis</b>						
508L	5.15/49	5.9/46.6	Glutamine synthetase, chloroplastic/P14655	607/25	-1.69	-1.51
<b>Actin cytoskeleton</b>						
482L	5.34/50	5.2/41.7	Actin/Q8H1L9	483/28	1.81	-1.47
241L	4.7/21	4.8/16.2	Actin-depolymerizing factor 3/Q84TB6	77/38	2.27	1.32
<b>Gene transcription and mRNA processing</b>						
519L	5.57/43	7.7/41.0	Putative mRNA binding protein/Q8GTK8	213/8	-2.17	-1.25
<b>Unclassified proteins</b>						
256L	4.72/23	6.4/24.1	FHA domain containing protein/Q2QYD2	78/14	1.51	1.08
257L	4.7/23	6.4/24.1	FHA domain containing protein/Q2QYD2	264/22	1.69	1.85
208L	4.98/41	5.2/28.8	Uncharacterized protein/A2ZYH4	124/20	1.77	1.12

<sup>a</sup>Experimental (Exp) isoelectric point (pI) and molecular weight (MW) calculated based on protein migration on 2-DE gels.

<sup>b</sup>Theoretical (The) isoelectric point (pI) and molecular weight (MW) calculated based on protein sequence in the Uni-prot database.

<sup>c</sup>Protein name/accession number in the Uni-prot database.

<sup>d</sup>The MS score calculated for each target protein/ percentage of sequence coverage of the identified peptides.

<sup>e</sup>Fold change in abundance (mean percent volume of spot under stress/mean percent volume of corresponding spot under control) of each protein in the sensitive and tolerant genotypes.

**Table S4. The candidate root (R) SRPs identified using combined MS and MS/MS analysis**

Only those proteins that showed statistically significant differences ( $P \leq 0.01$ ) and displayed at least 1.5-fold change in abundance were accepted as candidate root SRPs. Proteins were categorised based on biological function retrieved from the Uni-prot database (<http://www.uniprot.org>)

Spot ID	Exp pI/MW <sup>a</sup>	The pI/MW <sup>b</sup>	Protein Identity/accession number in uni-prot <sup>c</sup>	Score/%coverage <sup>d</sup>	Fold change <sup>e</sup>	
					IR29	FL478
<b>Antioxidant, oxidoreductase activity and stress response</b>						
27R	5.71/19	5.7/21.3	Superoxide dismutase [Cu-Zn], chloroplastic/P93407	590/43	1.15	1.55
187R	5.99/32	5.7/23.9	Glutathione S-Transferase GSTF2/O82451	314/40	2.88	-1.49
287R	6.19/32	5.7/23.9	Glutathione S-Transferase GSTF2/O82451	143/28	1.9	-1.14
802R	6.12/30	5.6/23.9	Glutathione S-Transferase GSTF2/A6N0E3	263/32	3.08	-1.26
3R	5.12/15	5.1/13.1	Thioredoxin H1/Q0D840	159/36	-2.63	1.11
4R	5.11/16	5.1/13.1	Thioredoxin H1/Q0D840	367/54	2.08	-1.05
117R	6.02/29	5.8/23.5	Dehydroascorbate Reductase/Q65XA0	286/54	2.16	-1.23
671R	5.71/52	5.6/37.5	Peroxidase/Q8L3W2	133/24	-3.22	-1.06
574R	5.64/52	5.6/37.5	Peroxidase/Q8L3W2	72/11	-2.5	-1.56
818R	6.1/51	5.6/37.5	Peroxidase/Q8L3W2	127/11	2.05	-1.36
801R	6.16/30	5.9/24.3	Germin-like protein 8/Q6YZ97	99/11	2.35	-1.26
159R	4.64/34	5.3/30.7	Putative uncharacterized protein/Q6Z6B5	118/22	-3.7	1.53
47R	6.65/21	9.1/22.7	CBS domain-containing protein/Q9LEV3	156/33	2.76	-1.09
<b>Plastid proteins</b>						
189R	5.9/28	5.5/20.0	Chloroplast 23 kDa polypeptide of photosystem II/B0FFP0	407/39	1.78	1.44
426R	5.11/36	6.08/34.8	Putative 33 kDa oxygen evolving protein of photosystem II/Q943W1	254/29	2.33	1.93
<b>Carbohydrate and energy metabolism</b>						
356R	5.1/42	5.2/39.9	Pyruvate dehydrogenase E1 component subunit beta-1, mitochondrial/Q6Z1G7	310/21	2.01	-1.07
363R	5.26/40	5.02/35.5	Fructokinase 2/A2YQL4	477/33	-3.33	-1.47
384R	5.97/17	6.3/16.8	Nucleoside diphosphate kinase 1/Q07661	273/54	-2.08	1.52
600R	5.99/49	7.6/40.1	NAD-dependent isocitrate dehydrogenase c/Q33E21	192/12	-1.63	-1.92
652R	6.09/39	5.9/35.4	Putative NADPH-dependent mannose 6-phosphate	84/21	2.44	1.25

699R	-	5.7/51.0	reductase/Q6Z718 Enolase/W5FSB3	88/19	-	-
865R	5.17/40	6.3/36.7	Glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic/Q7FAH2	358/27	-2.85	-1.05
813R	5.88/30	5.5/23.6	Inorganic pyrophosphatase/Q56US1	150/18	-6.25	1.96
320R	4.96/38	4.9/37.3	Os01g0665400 protein/Q5SN59	108/11	-1.88	-1.17
<b>Protein biosynthesis and processing</b>						
248R	5.25/30	7.7/26.3	Chaperonin/Q69Y99	525/44	1.57	1.08
332R	4.76/45	5.2/49.8	Oryzain beta chain/P25777	79/9	4.47	1.22
223R	5.75/21	5.9/17.7	Eukaryotic translation initiation factor 5A-2/Q10CJ5	71/6	p	-1.12
224R	5.78/21	5.7/17.4	Eukaryotic translation initiation factor 5A/Q9ZSU2	121/42	p	-1.09
178R	5.44/21	5.6/17.5	Eukaryotic translation initiation factor 5A-2/Q2QQ48	126/33	2.51	1.48
521R	5.01/71	5.01/56.8	Protein disulfide isomerase-like 1/Q53LQ0	778/45	1.53	-1.05
<b>Amino acid biosynthesis</b>						
372R	5.44/41	6.3/41.8	Cysteine synthase/Q5JNB0	616/32	1.95	-1.12
391R	5.42/38	5.2/34.3	Cysteine synthase/Q9XEA8	457/40	1.62	-1.23
563R	6.13/48	5.9/45.8	Aspartate aminotransferase/Q84V24	293/29	1.71	1.81
578R	5.69/44	5.7/39.2	Glutamine synthetase cytosolic isozyme 1-2/P14654	336/11	2.7	1.15
840R	6.07/55	5.6/43.2	S-adenosylmethionine synthase 1/A9P822	185/20	1.73	1.05
<b>Actin cytoskeleton</b>						
469R	5.44/50	5.3/41.7	Actin-like protein/Q9M4Y2	429/23	4.2	-1.31
<b>Cell-wall biogenesis and degradation</b>						
162R	4.69/33	6.1/32.5	Putative chitinase/Q5WMX0	105/23	2.02	1.57
172R	4.51/34	6.1/32.5	Putative chitinase/Q5WMX0	250/21	-3.22	-1.11
157R	4.57/34	4.4/31.2	Putative class III chitinase/Q8S870	384/29	2.71	1.19
639R	6.16/37	7.4/35.6	Chitinase 2/Q7DNA1	96/8	-2.43	-1.63
644R	6.27/33	6.1/32.5	Putative chitinase/Q5WMX0	84/17	1.86	-1.38
268R	5.31/34	5.3/32.1	Xyloglucan endotransglycosylase/hydrolase protein 8/Q76BW5	107/20	-2.08	-1.42
259R	5.3/33	5.1/27.8	Caffeoyl-CoA 3-O-methyltransferase/Q9XGP7	540/44	1.57	1.09
61R	5.55/16	5.8/16.8	Putative uncharacterized protein/B8B6W2	79/42	-1.61	-2.32
<b>Gene transcription and mRNA processing</b>						
683R	4.61/31	4.4/22.1	NAC transcription factor/Q8RUI4	154/28	-3.84	N
866R	6.02/29	5.8/21.7	Hypothetical protein/A2WVT8	206/45	-2.04	-2.12
315R	4.73/36	6.01/61.1	Histone acetyltransferase GCN5/Q9AR19	78/26	-2.5	-1.25
69R	5.17/15	8.01/15.5	putative glycine-rich RNA-binding protein 2/O24188	504/48	-2.32	-1.75
326R	4.82/46	5.5/47.8	Putative 41 kD chloroplast nucleoid DNA binding protein/Q6Z674	192/13	-1.72	-1.05
<b>Signal transduction</b>						

5R	5.01/15	4.9/16.7	Probable calcium-binding protein CML7/Q84VG0	393/62	-1.5	1.16
143R	4.46/30	4.9/27.3	Receptor like-protein kinase/Q8S3P3	585/31	-2.56	1.13
777R	5.6/94	4.9/27.3	Receptor like-protein kinase/Q8S3P3	399/26	p	1.33
624R	5.56/36	5.06/21.3	Putative remorin 1 protein/Q5QT28	508/64	1.69	-1.12
<b>Ion compartmentalization</b>						
583R	5.17/64	5.07/54.1	Vacuolar ATPase B subunit/Q93W07	104/13	2.55	-1.14
531R	5.35/82	5.2/68.4	Putative vacuolar proton-ATPase/Q651T8	196/19	2.5	1
<b>Unclassified proteins</b>						
397R	5.37/37	5.2/35.1	Spermidine synthase 1/Q9SMB1	163/20	1.77	-1.13
705R	6.14/54	7.9/45.2	3-oxoacyl-[acyl-carrier-protein] synthase/A3B999	72/12	P	1.29
864R	5.43/42	5.2/38.6	HSR203J/Q2RAT1	125/21	1.93	-2.27
631R	5.8/38	5.6/34.2	Putative PRMC3/Q69Y17	559/50	1.69	-1.31

<sup>a</sup>Experimental (Exp) isoelectric point (pI) and molecular weight (MW) calculated based on protein migration on 2-DE gels.

<sup>b</sup>Theoretical (The) isoelectric point (pI) and molecular weight (MW) calculated based on protein sequence in the uni-prot database.

<sup>c</sup>Protein name/accession number in the uni-prot database.

<sup>d</sup>The MS score calculated for each target protein/ percentage of sequence coverage of the identified peptides.

<sup>e</sup>Fold change in abundance (mean percent volume of spot under stress/mean percent volume of corresponding spot under control) of each protein in the sensitive and tolerant genotypes.