



Proteins Susceptible to Methionine Oxidation and Methionine Sulfoxide Reductases in Crops

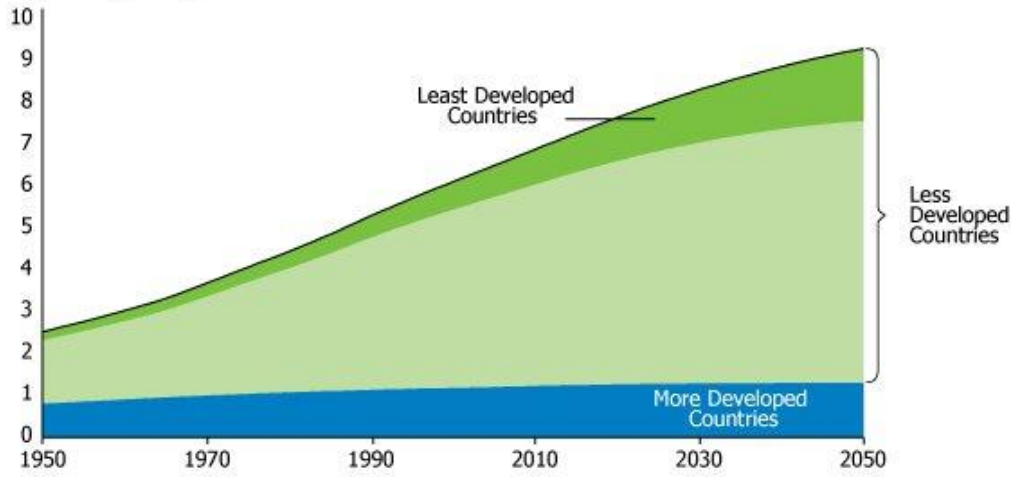


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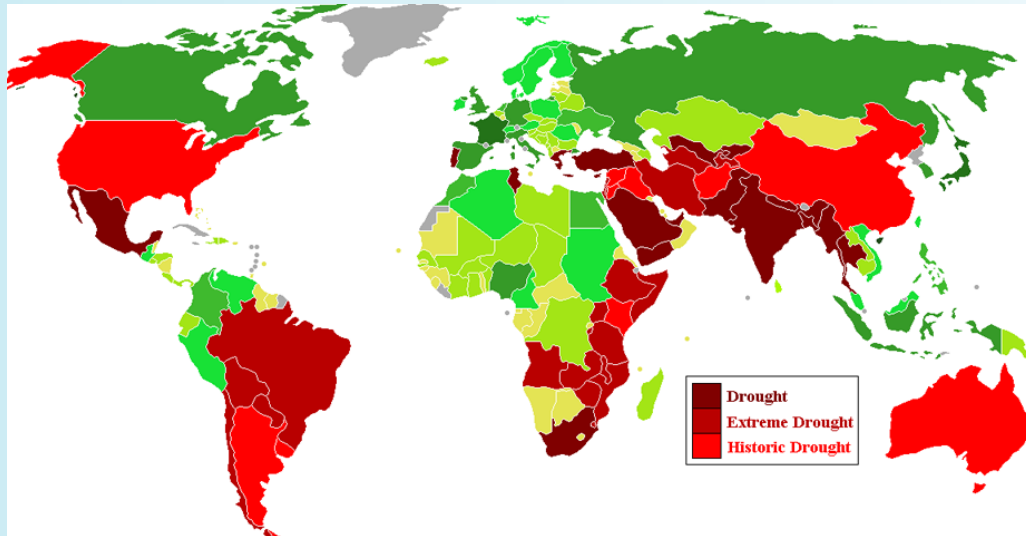


May 2015, Korea University, Seoul, Korea

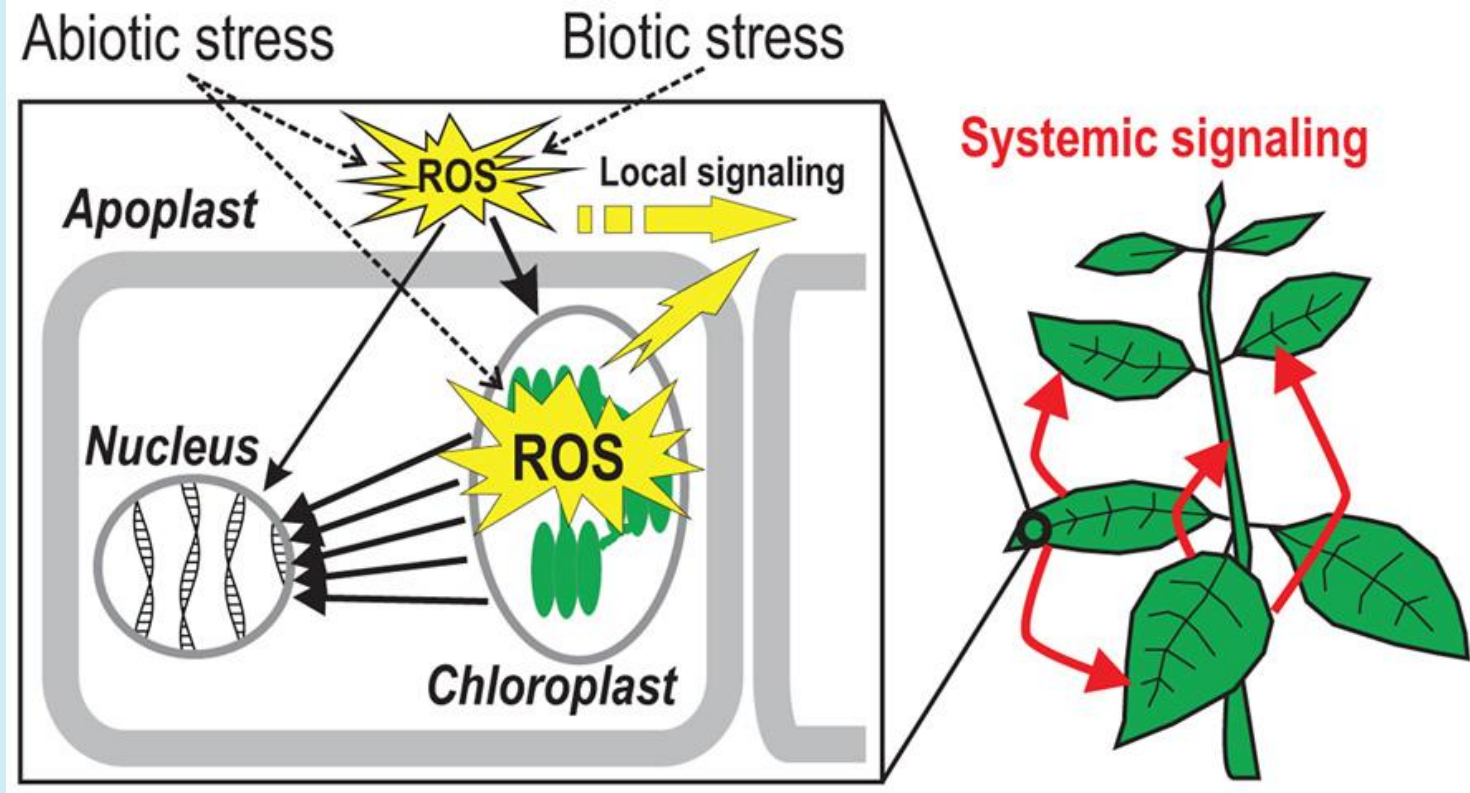
Population (billions)



Source: United Nations Population Division, *World Population Prospects: The 2010 Revision, medium variant* (2011).



Plants are constantly exposed to various stresses



Background & Hypotheses

Biotic stresses

NADPH oxidases
Peroxidases

Abiotic stresses

Oxygenases
NADPH dehydrogenases

(Annu. Rev. Plant Biol. 2004. 55:373–99)

ROS
(O_2^- , H_2O_2)

Activation of
defense pathways

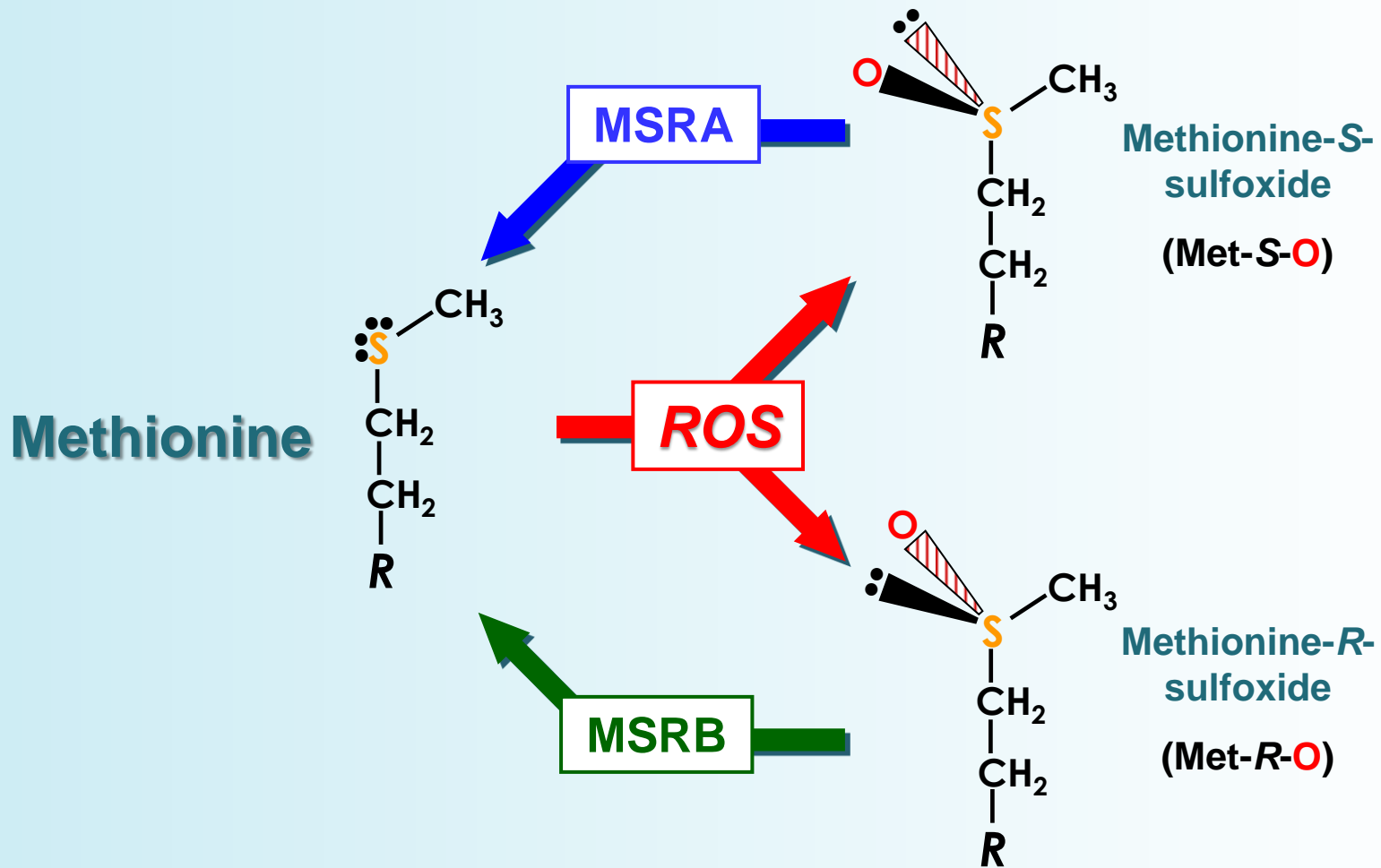
Protein
damage via
oxidation of
Met residues

Protein repair by
methionine sulfoxide
reductases

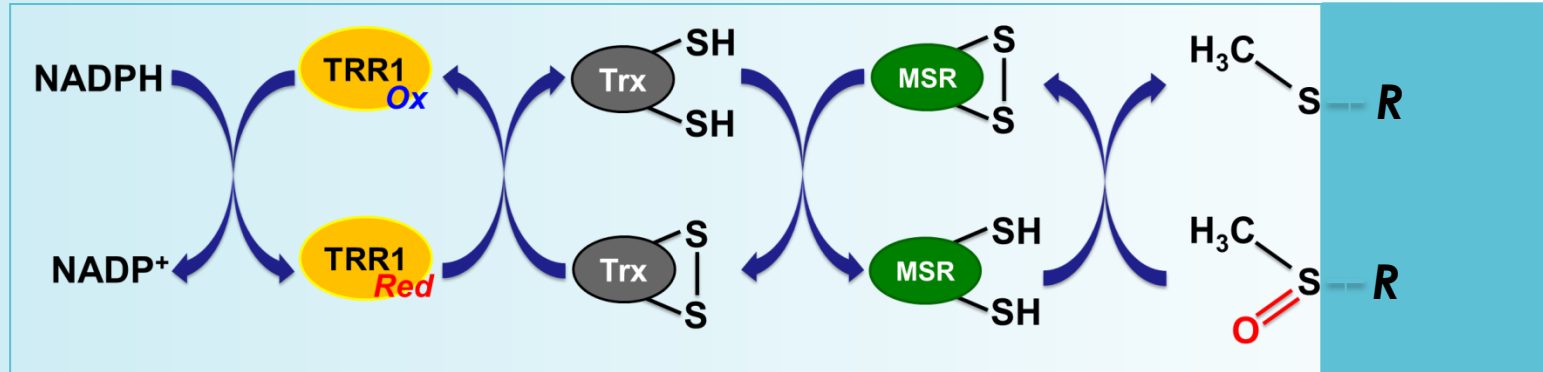
What are the most effective Msrs?

What are the targets of Met
oxidation and repair?

Oxidation and Reduction of Methionine



Msr Regeneration *In Vivo* & *In Vitro*



MSRs are regenerated by the thioredoxin system (TRR and Trx)

Genes encoding oxidases upregulated by drought in soybean leaves

Genes	Descriptions	Fold up
glyma01g42840	moderately similar to ATGPX6 (GLUTATHIONE PEROXIDASE 6)	29.51
glyma06g15030	moderately similar to peroxidase, putative	17.63
glyma01g35620	highly similar to AT4G19380 alcohol oxidase-related	14.81
glyma11g02630	moderately similar to ATGPX6 (GLUTATHIONE PEROXIDASE 6);	13.05
glyma01g37490	FAD linked oxidase family protein	9.10
glyma12g01390	highly similar to ATCKX6/ATCKX7 (CYTOKININ OXIDASE 6)	9.31
glyma18g14620	highly similar to ATPAO2 (POLYAMINE OXIDASE 2)	7.88
glyma04g14800	highly similar to AOX1A (alternative oxidase 1A)	7.36
glyma09g35210	highly similar to AT4G19380 alcohol oxidase-related	4.33
glyma11g05960	highly similar to AT5G67030 zeaxanthin epoxidase	4.33
glyma14g04950	nearly identical to AAO3 AAO3 (ABSCISIC ALDEHYDE OXIDASE 3)	4.26
glyma08g41570	highly similar to ATPAO2 (POLYAMINE OXIDASE 2)	3.35
glyma08g06810	highly similar to XF1 (SQUALENE EPOXIDASE 1); oxidoreductase	2.98
glyma05g34490	moderately similar to ATGPX3 ATGPX3 (GLUTATHIONE PEROXIDASE 3)	2.65
glyma20g38000	nearly identical to ATRBOHB (RESPIRATORY BURST OXIDASE HOMOLOG B)	2.30
glyma02g02240	moderately similar to APAO/ATPAO1 (POLYAMINE OXIDASE 1)	2.27
glyma13g32600	highly similar to XF1 (SQUALENE EPOXIDASE 1)	2.16
glyma01g25830	highly similar to glyoxal oxidase-related	2.14

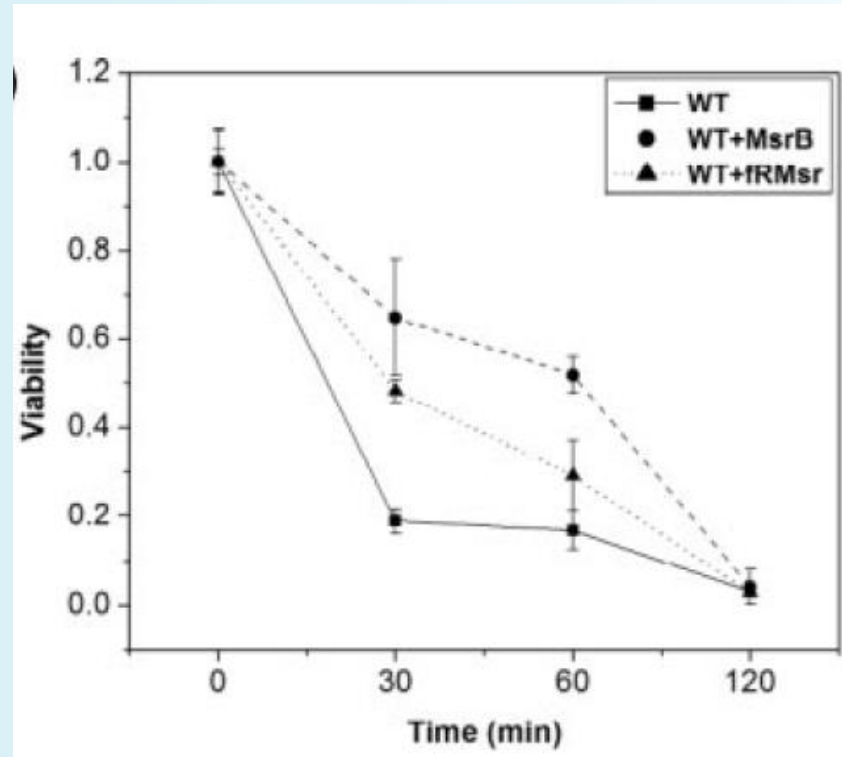
Methionine sulfoxide reductase: Occurrence

Table 1. Number of MSRA and MSRB Genes in Various Photosynthetic and Non-Photosynthetic Organisms.

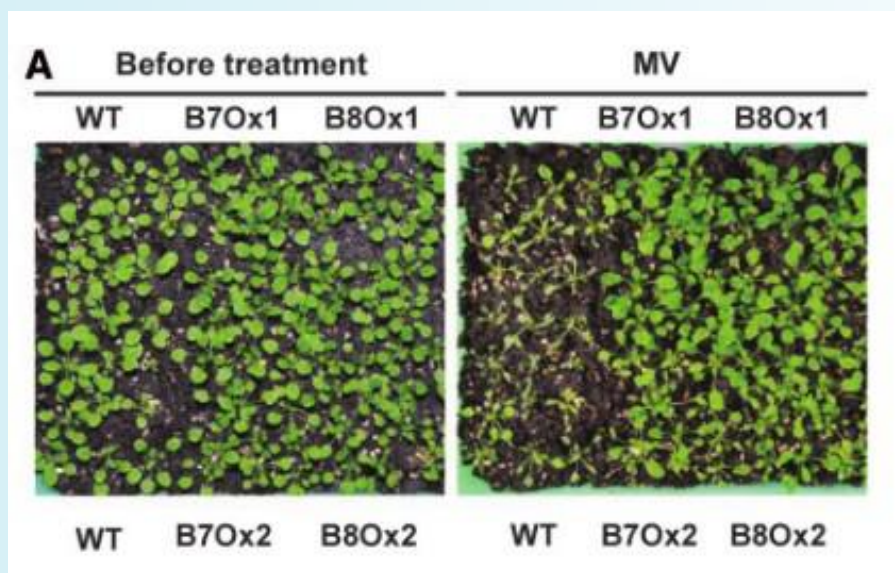
Kingdom	Organism	MSRA		MSRB		
		Sec	Cys	Sec	1-Cys	2-Cys
Photosynthetic eukaryotes	<i>Arabidopsis thaliana</i>	–	5*	–	1	8
	<i>Populus trichocarpa</i>	–	5	–	1	3
	<i>Vitis vinifera</i>	–	3	–	1	2
	<i>Oryza sativa</i>	–	4	–	1*	2
	<i>Physcomitrella patens</i>	–	5	–	1	2
	<i>Chlamydomonas reinhardtii</i>	1	4	–	1	2
	<i>Ostreococcus lucimarinus</i>	1	2	–	2	1
	<i>Ostreococcus tauri</i>	1	3	–	2	1
Photosynthetic prokaryotes	<i>Anabaena</i> sp. PCC 7120	–	2	–	1	–
	<i>Synechocystis</i> sp. PCC 6803	–	2	–	1	–
	<i>Synechococcus</i> sp. CC9311	–	2	–	2	–
Non-photosynthetic eukaryotes	<i>Homo sapiens</i>	–	1*	1	2**	–
	<i>Drosophila melanogaster</i>	–	1*	–	–	1*
	<i>Saccharomyces cerevisiae</i>	–	1	–	–	1
Non-photosynthetic prokaryote	<i>Escherichia coli</i>	–	1	–	–	1

Asterisk (*) indicates possible alternative splicing for one gene. Sec and Cys indicate genes encoding selenocysteine-containing and cysteine-containing enzymes, respectively.

Over-expression of MsrB & fMsr in yeast conferred oxidative-stress tolerant



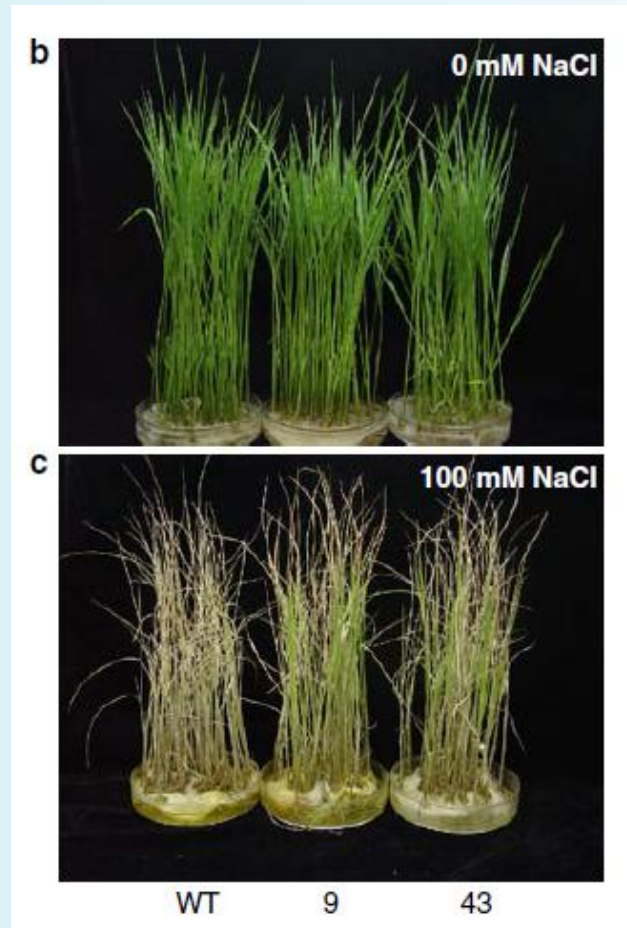
Over-expression of *AtMsrB7* & *AtMrsB8* conferred tolerant to methyl viologen in *Arabidopsis*



B7Ox: transgenic *Arabidopsis* overexpressing *MsrB7*
B8Ox: transgenic *Arabidopsis* overexpressing *MsrB8*
WT cây *Arabidopsis* chủng dại

(Li et al., 2012, *Plant Cell Physiol.* 53(10): 1707–1719)

Over-expression of *OsMsrA* in rice confers salt-tolerant to the plants



OsMSRA4.1

Guo *et al.*, 2009, *Planta*, **230**:227-238

**Soybean
Methionine Sulfoxide
Reductases**

GmMSRs: domain features and location predictions

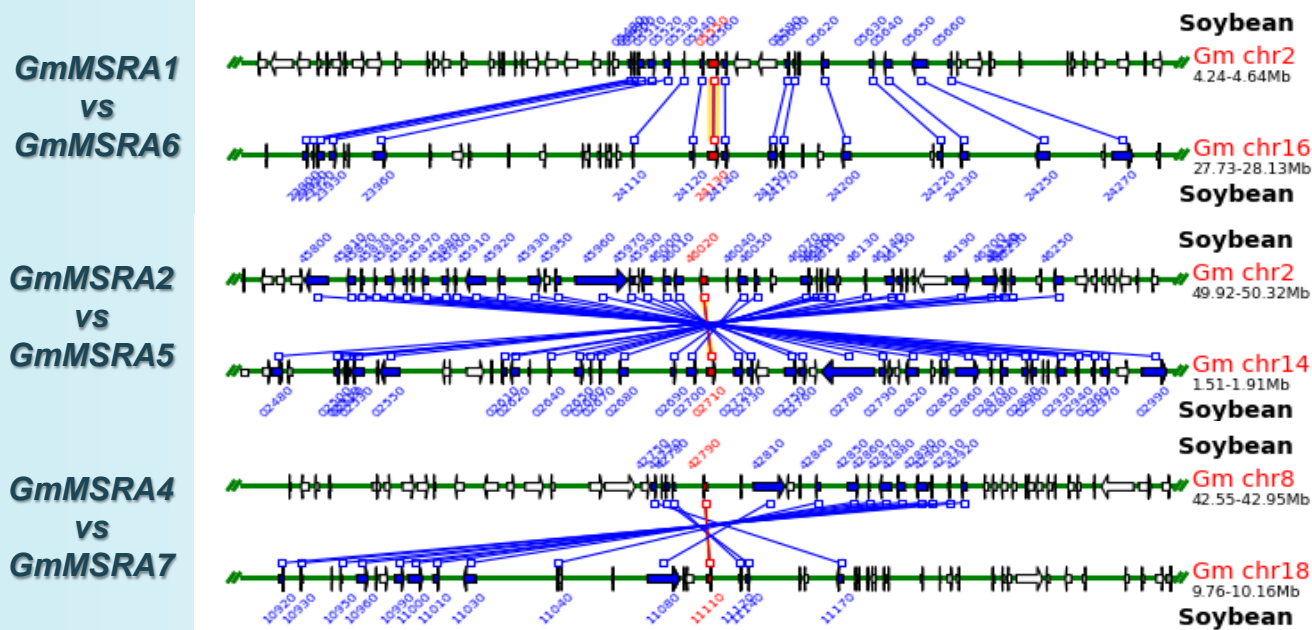
Names	Gene ID	E values*	Active site	cTP**	mTP	SP	Other	Location	Reliability	TPlength
GmMSRA1	Glyma02g05550	1.7E-46		0.008	0.177	0.758	0.234	S	3	28
GmMSRA2	Glyma02g46020	4.0E-63	[108]	0.972	0.012	0.029	0.025	C	1	77
GmMSRA3	Glyma04g36480	4.8E-58	[36]	0.127	0.109	0.14	0.909	_	2	-
GmMSRA4	Glyma08g42790	1.2E-60	[45]	0.117	0.313	0.032	0.515	_	4	-
GmMSRA5	Glyma14g02710	3.0E-63	[108]	0.964	0.015	0.017	0.02	C	1	77
GmMSRA6	Glyma16g24130	3.0E-46		0.006	0.086	0.924	0.048	S	1	28
GmMSRA7	Glyma18g11110	2.0E-60	[45]	0.109	0.472	0.023	0.356	M	5	56

Names	Gene ID	E values*	Active site	cTP**	mTP	SP	Other	Location	Reliability	TPlength
GmMSRB1	Glyma08g25610	1.0E-48	[190]	0.869	0.061	0.02	0.195	C	2	45
GmMSRB2	Glyma13g28320	6.0E-52	[121]	0.133	0.083	0.11	0.854	_	2	-
GmMSRB3	Glyma13g32680	1.1E-51	[189]	0.971	0.088	0.01	0.038	C	1	67
GmMSRB4	Glyma15g06650	1.1E-51	[189]	0.956	0.102	0.01	0.056	C	1	67
GmMSRB5	Glyma15g10750	1.2E-52	[121]	0.132	0.071	0.1	0.871	_	2	-

*E-values for PF01625 (PMSR) or PF01641 (SelR) domains

**Predicted by TargetP (<http://www.cbs.dtu.dk/services/TargetP/>), C; chloroplast, M; mitochondria, S; secreted pathway

Segmental duplication in *GmMSRAs*

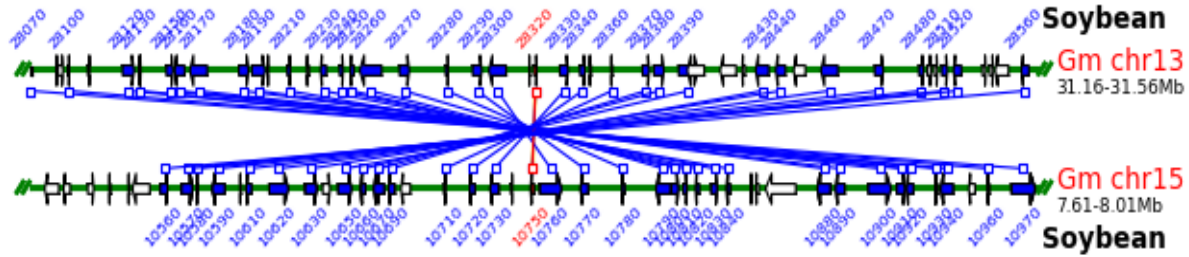


K_a and K_s of the *GmMSRA* duplicated pairs

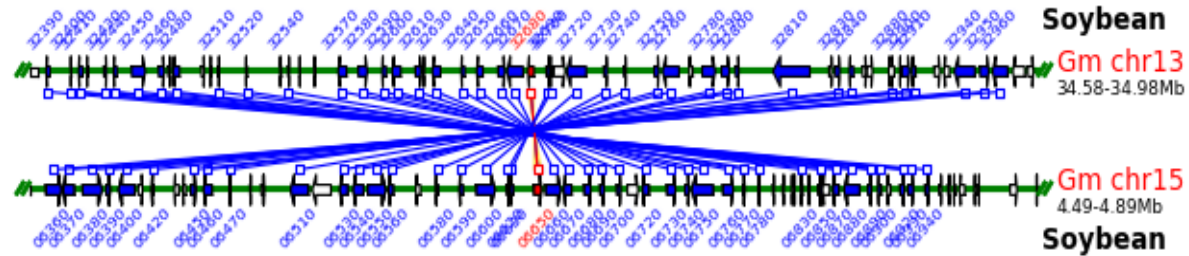
	K_a	K_s	Identity (cds)
<i>GmMSRA1</i> vs <i>GmMSRA6</i>	0.02	0.18	93%
<i>GmMSRA2</i> vs <i>GmMSRA5</i>	0.02	0.11	96%
<i>GmMSRA4</i> vs <i>GmMSRA7</i>	0.02	0.08	97%

Segmental duplication in *GmMSRBs*

***GmMSRB2*
vs
*GmMSRB5***



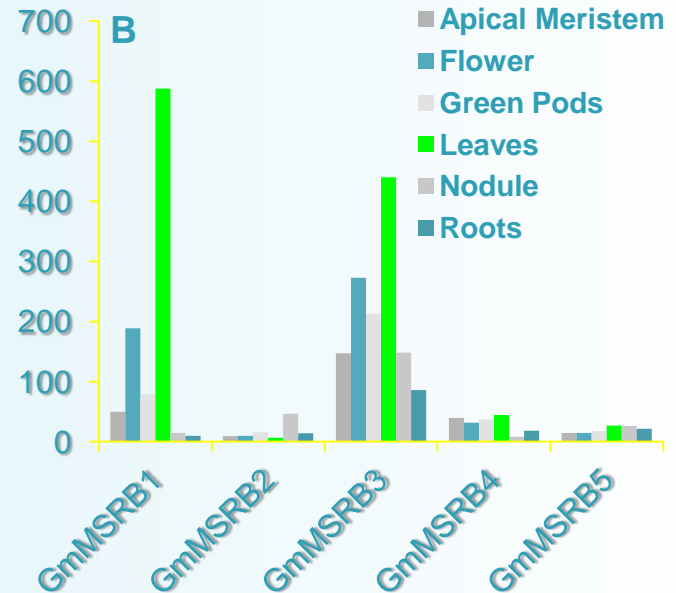
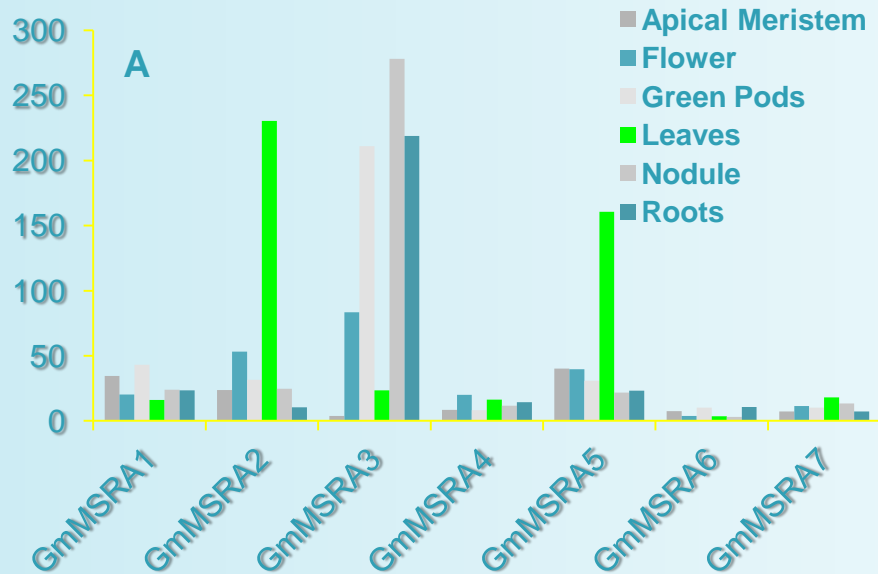
***GmMSRB3*
vs
*GmMSRB4***



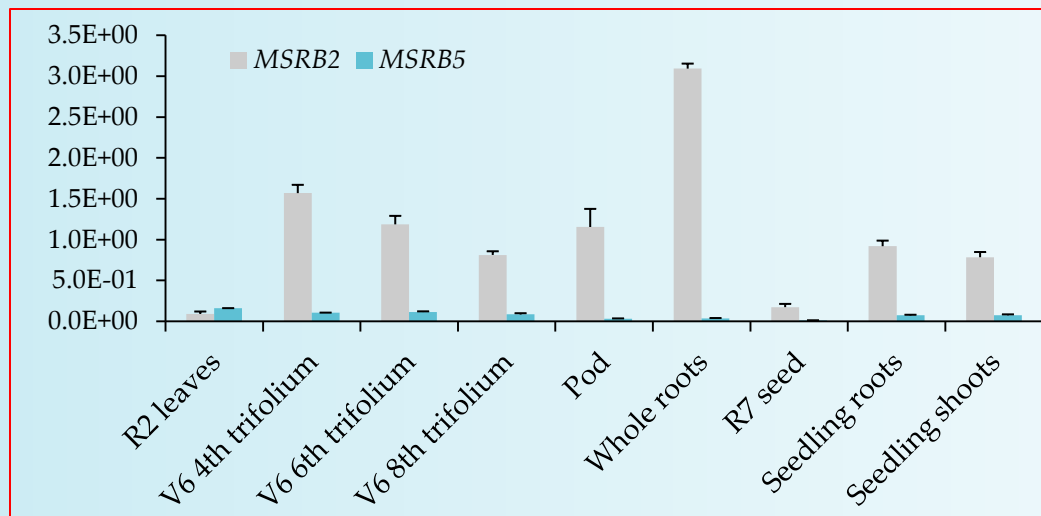
K_a and K_s of the *GmMSRB* duplicated pairs

	K_a	K_s	Identity (cds)
<i>GmMSRB2</i> vs <i>GmMSRB5</i>	0.02	0.09	96%
<i>GmMRSB3</i> vs <i>GmMSRB4</i>	0.05	0.14	92%

Expression of *GmMSRAs* (A) and *GmMSRBs* (B) in various tissues of soybean plants

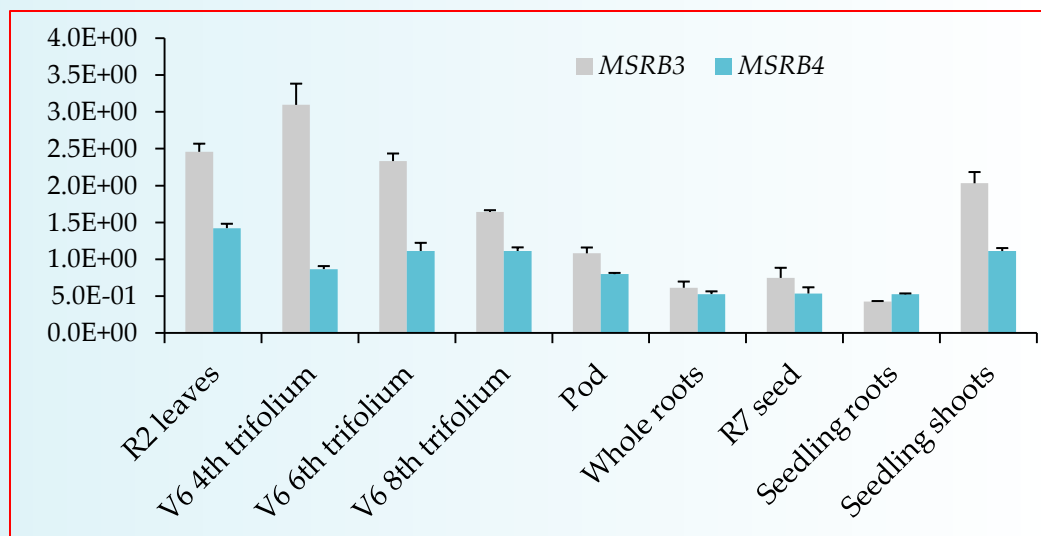


Expression diversion among duplicated *GmMSRBs*

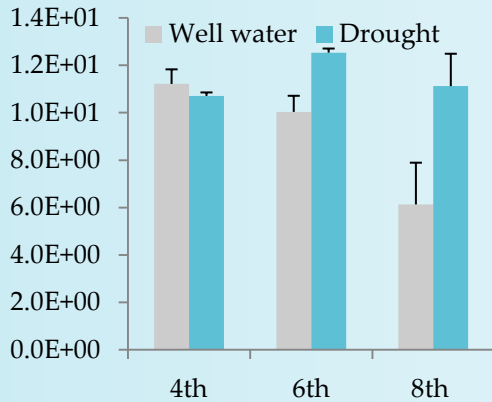


K_a and K_s of the *GmMSRB* duplicated pairs

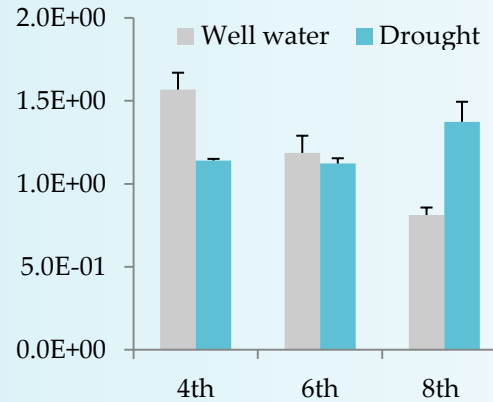
	K_a	K_s	Identity (cds)
<i>GmMSRB2</i> vs <i>GmMSRB5</i>	0.02	0.09	96%
<i>GmMSRB3</i> vs <i>GmMSRB4</i>	0.05	0.14	92%



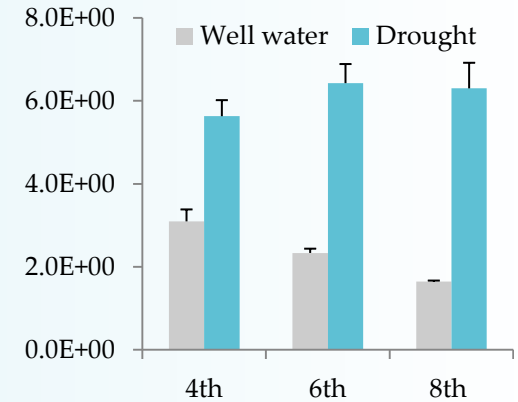
Expression of *GmMsrBs* is induced under drought



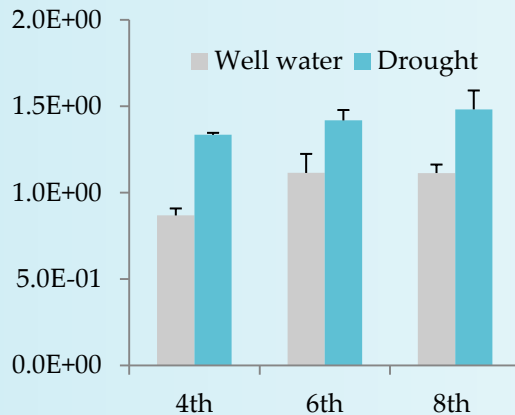
GmMSRB1



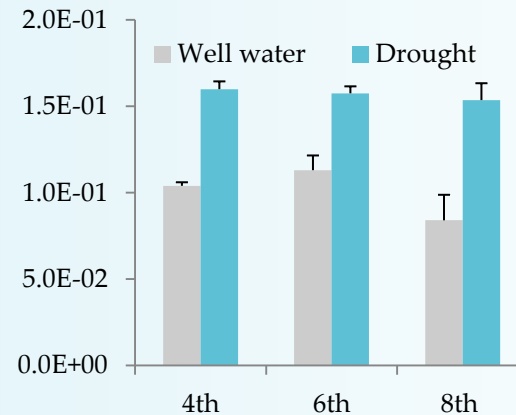
GmMSRB2



GmMSRB3



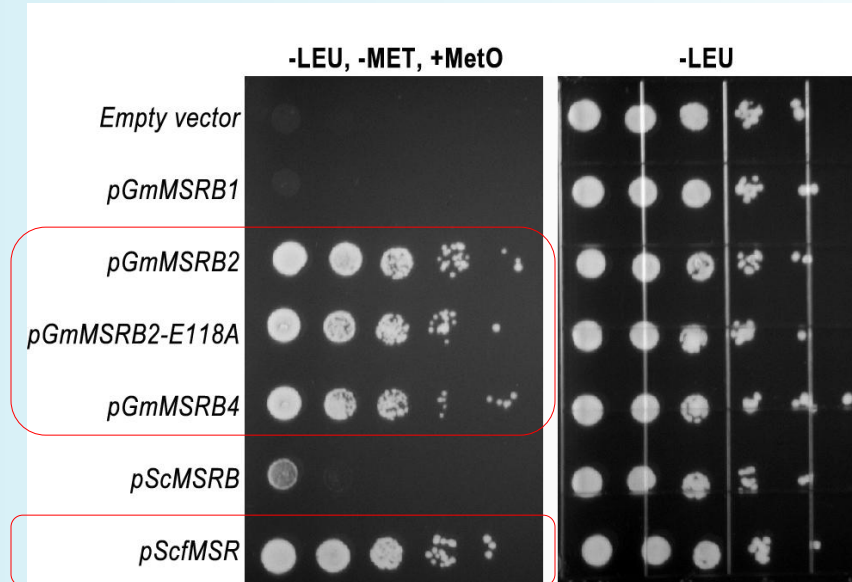
GmMSRB4



GmMSRB5

Soybean trifolia leaves at normal and drought conditions

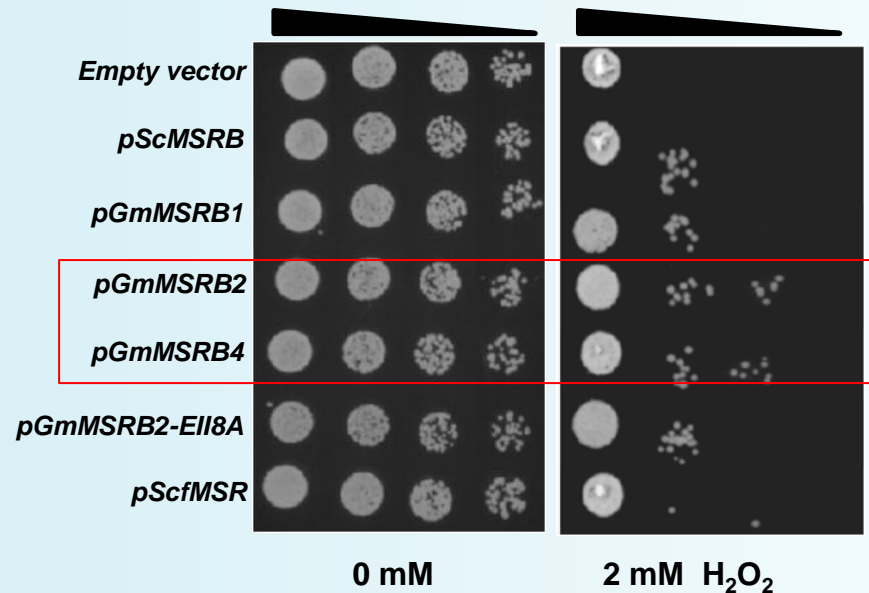
COMPLEMENTATION OF *GmMSRBs* ON MetO MEDIA IN MSR-TRIPLE YEAST MUTANT



Empty vector, p425-GPD; *pGmMSRB1*, soybean MSRB1 with signal peptide removed; *pGmMSRB2*, soybean MSRB2; *pGmMSRB2-E118A*, soybean MSRB2 E118A mutant; *pGmMSRB4*, soybean MSRB4 with signal peptide removed; *pScMSRB*, yeast MSRB; *pScfMSR*, yeast free methionine sulfoxide reductase

GmMSRB2 and GmMSRB4 can reduce free MetO at similar extent as that of fMSR from yeast

OVEREXPRESSION OF *GmMSRBs* PROTECTS AGAINST OXIDATIVE STRESS IN MSR-TRIPLE MUTANT YEAST



Empty vector, p425-GPD; *pGmMSRB1*, soybean MSR1 with signal peptide removed; *pGmMSRB2*, soybean MSR2; *pGmMSRB2-E118A*, soybean MSR2 E118A mutant; *pGmMSRB4*, soybean MSR4 with signal peptide removed; *pScMSRB*, yeast MSR; *pScfMSR*, yeast free methionine sulfoxide reductase

GmMSRB2 and GmMSRB4 offered better protection under redox stress in yeast

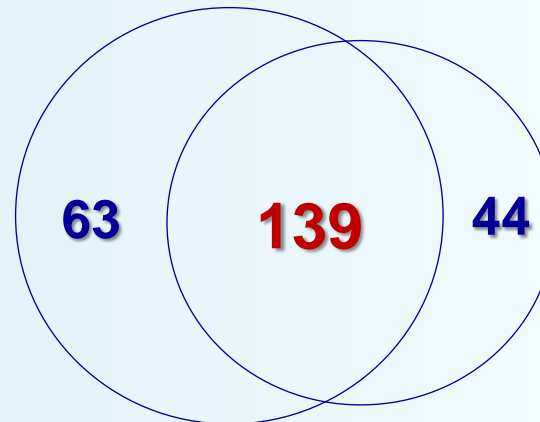
GENE EXPRESSION REGULATION BY OVEREXPRESSING *GmMSRs* IN ARABIDOPSIS

	UP*	DOWN
<i>35S:GmMSRB2-3D</i>	202	50
<i>35S:GmMSRB4-1C</i>	183	10

*Fold-change cut-off: 2.0 ; corrected p-value < 0.05

35S:GmMSRB2-3D

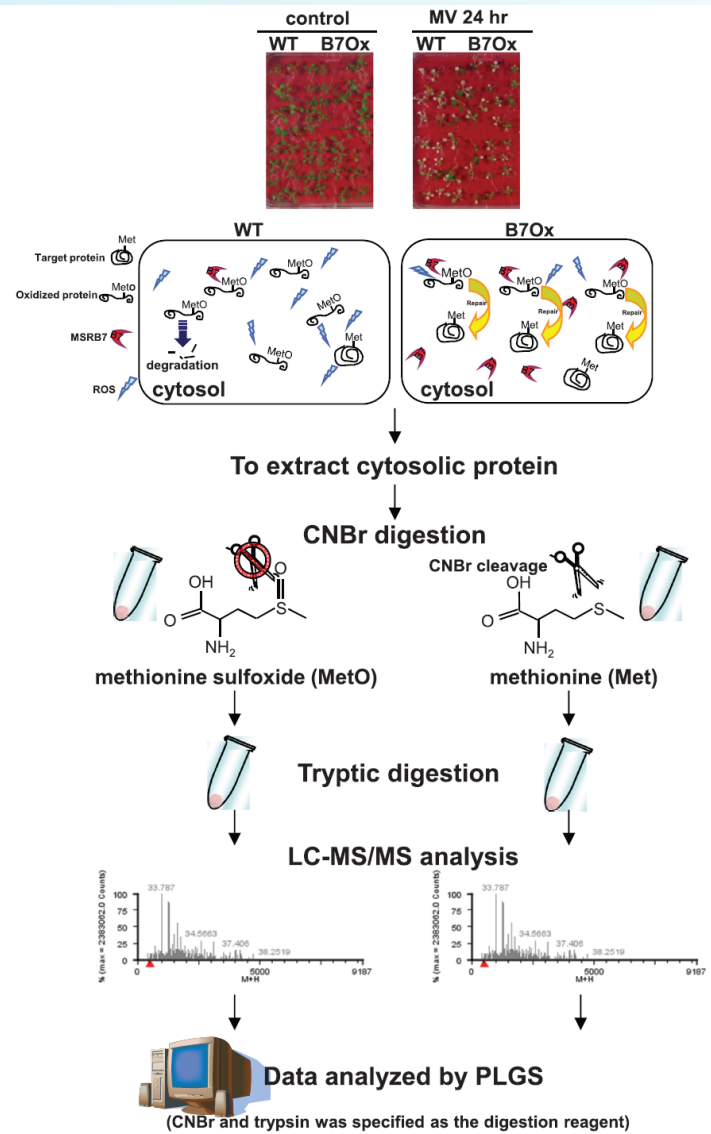
35S:GmMSRB4-1C



TOP INDUCED GENES IN 35S:*GmMSR_x* ARABIDOPSIS

TAIR10	Description	35S: <i>GmMSRB2-3</i>			35S: <i>GmMSRB4-1</i>		
		FC	Log FC	q-value	FC	Log FC	q-value
AT5G45340	Arabidopsis thaliana abscisic acid 8'-hydroxylase 3 (CYP707A3) mRNA, complete cds [NM_123902]	27.36	4.77	5.90E-03	14.98	3.91	3.48E-02
AT1G33760	Arabidopsis thaliana ethylene-responsive transcription factor ERF022 (AT1G33760) mRNA, complete cds [NM_103095]	20.48	4.36	2.90E-03	29.92	4.90	1.17E-02
AT1G72920	Arabidopsis thaliana Toll-Interleukin-Resistance domain-containing protein (AT1G72920) mRNA, complete cds [NM_105950]	17.16	4.10	3.98E-03	16.39	4.03	3.72E-03
AT5G51190	Arabidopsis thaliana ethylene-responsive transcription factor ERF105 (AT5G51190) mRNA, complete cds [NM_124498]	15.22	3.93	5.95E-03	16.59	4.05	2.24E-02
AT1G74930	Arabidopsis thaliana ethylene-responsive transcription factor ERF018 (ORA47) mRNA, complete cds [NM_106151]	12.93	3.69	5.26E-03	10.97	3.46	1.45E-02
AT5G61600	Arabidopsis thaliana ethylene-responsive transcription factor ERF104 (ERF104) mRNA, complete cds [NM_125553]	11.46	3.52	2.90E-03	12.34	3.63	6.19E-03
AT1G27730	Arabidopsis thaliana zinc finger protein STZ/ZAT10 (STZ) mRNA, complete cds [NM_102538]	11.43	3.51	5.61E-03	13.41	3.75	6.19E-03
AT3G44260	Arabidopsis thaliana putative CCR4-associated factor 1-9 (AT3G44260) mRNA, complete cds [NM_114294]	9.81	3.29	5.95E-03	8.06	3.01	1.29E-02
AT5G41740	Arabidopsis thaliana TIR-NBS-LRR class disease resistance protein (AT5G41740) mRNA, complete cds [NM_123539]	9.66	3.27	1.14E-02	7.47	2.90	1.30E-02
AT1G80840	Arabidopsis thaliana putative WRKY transcription factor 40 (WRKY40) mRNA, complete cds [NM_106732]	9.64	3.27	1.96E-02	18.82	4.23	2.10E-02
AT2G40000	Arabidopsis thaliana HS1 PRO-1 2-like protein (HSPRO2) mRNA, complete cds [NM_129558]	9.26	3.21	5.71E-03	10.87	3.44	1.61E-02
AT4G27280	Arabidopsis thaliana EF-hand, calcium binding motif-containing protein (AT4G27280) mRNA, complete cds [NM_118862]	9.14	3.19	2.90E-03	10.07	3.33	1.34E-02
AT3G09870	Arabidopsis thaliana SAUR-like auxin-responsive protein (AT3G09870) mRNA, complete cds [NM_111822]	9.11	3.19	1.92E-02	12.29	3.62	4.53E-02
AT1G72910	Arabidopsis thaliana Toll-Interleukin-Resistance domain-containing protein (AT1G72910) mRNA, complete cds [NM_105949]	8.27	3.05	2.90E-03	7.61	2.93	9.07E-03
AT4G24570	Arabidopsis thaliana dicarboxylate carrier 2 (DIC2) mRNA, complete cds [NM_118590]	7.14	2.84	2.12E-02	9.67	3.27	1.80E-02
AT1G07135	Arabidopsis thaliana glycine-rich protein (AT1G07135) mRNA, complete cds [NM_100587]	6.56	2.71	5.95E-03	6.94	2.79	9.29E-03
AT4G17490	Arabidopsis thaliana ethylene-responsive transcription factor 6 (ERF6) mRNA, complete cds [NM_117854]	5.89	2.56	1.24E-02	7.12	2.83	2.24E-02
AT1G21910	Arabidopsis thaliana ethylene-responsive transcription factor ERF012 (AT1G21910) mRNA, complete cds [NM_102039]	5.58	2.48	5.90E-03	7.65	2.94	1.38E-02
AT1G35210	Arabidopsis thaliana uncharacterized protein (AT1G35210) mRNA, complete cds [NM_103219]	5.56	2.48	1.19E-02	8.88	3.15	1.17E-02
AT2G44840	Arabidopsis thaliana ethylene-responsive transcription factor 13 (ERF13) mRNA, complete cds [NM_130048]	5.55	2.47	1.41E-02	6.63	2.73	2.75E-02
AT1G68840	Arabidopsis thaliana AP2/ERF and B3 domain-containing transcription factor RAV2 (RAV2) mRNA, complete cds [NM_105558]	5.29	2.40	6.38E-03	6.49	2.70	2.24E-02
AT3G55980	Arabidopsis thaliana zinc finger CCCH domain-containing protein 47 (SZF1) mRNA, complete cds [NM_115456]	5.06	2.34	1.12E-02	7.26	2.86	6.19E-03

What are the targets of Met
oxidation & repair?



41 potential substrates of AtMSRB7

Table 1. Potential substrates of MSRB7

Ten-day-old *B7Ox* and wild-type *A. thaliana* were treated with or without 10 μ M MV for 24 h. ND, not determined.

Accession no.	Locus	Description	Score	<i>B7Ox</i> /WT MV 0 h	<i>B7Ox</i> /WT MV 24 h	Unique
IPI00537995	At1g35720	Annexin D1	218.40	<i>B7Ox</i> 24h	<i>B7Ox</i> 0h	<i>B7Ox</i> only
IPI00535149	At4g02520	Glutathione S transferase F2	188.83	<i>B7Ox</i> 24h	<i>B7Ox</i> 0h	<i>B7Ox</i> only
IPI00525727	At4g37930	Mitochondrial, serine hydroxymethyltransferase mitochondrial	78.56	<i>B7Ox</i> 24h	<i>B7Ox</i> 0h	<i>B7Ox</i> only
IPI00523477	At5g38420	Chloroplastic, ribulose biphosphate carboxylase small chain 2B	2906.54	<i>B7Ox</i> 24h	3.03	
IPI00532772	At1g66200	Glutamine synthetase cytosolic isozyme 1	254.75	<i>B7Ox</i> 24h	1.47	
IPI00532945	At2g02930	Glutathione S-transferase F3	170.04	<i>B7Ox</i> 24h	ND	<i>B7Ox</i> 24h
IPI00520226	At4g14960	Tubulin α 6 chain	261.54	<i>B7Ox</i> 24h	ND	<i>B7Ox</i> 24h
IPI00530621	At1g19570	Dehydroascorbate reductase 1 (DHAR1)	155.99	<i>B7Ox</i> 24h	1.00	
IPI00544626	At3g01500	Chloroplastic, isoform 1 of carbonic anhydrase	756.83	<i>B7Ox</i> 24h	1.00	
IPI00534087	At5g56010	Heat-shock protein 81 3	203.99	2.38	<i>B7Ox</i> 0h	
IPI00533497	At3g09260	β -Glucosidase	102.90	1.43	<i>B7Ox</i> 0h	
IPI00544876	At3g55800	Chloroplastic, sedoheptulose 1,7 biphosphatase	111.61	1.41	<i>B7Ox</i> 0h	
IPI00539020	At1g67090	Chloroplastic, ribulose biphosphate carboxylase small chain 1A	3213.8	1.63	1.59	
IPI00521186	At5g38430	Chloroplastic, ribulose biphosphate carboxylase small chain 1B	2947.88	1.05	2.30	
IPI00656928	At4g35090	Catalase 2	108.7	0.76	1.54	
IPI00532582	At4g21280	Isoform 2 of oxygen evolving enhancer protein 3	296.93	0.75	<i>B7Ox</i> 0h	
IPI00891841	At5g38410	Similar to ribulose biphosphate carboxylase small chain 2B	2291.48	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00532125	At1g54040	Epithiospecifier protein	596.6	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00846574	At5g14740	β -Carbonic anhydrase 2	330.81	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00518163	At2g39730	Ribulose biphosphate carboxylase oxygenase activase	291.06	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00542532	At1g24020	MLP-like protein 423	245.59	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00516423	At4g25050	Acyl carrier protein 4	193.68	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00518090	At1g13440	Glyceraldehyde-3-phosphate dehydrogenase C2 (GAPC2)	190.2	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00656779	At2g21330	Fructose biphosphate aldolase	160.8	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00518620	At3g32980	Peroxidase 32	147.69	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00539116	At5g26000	Myrosinase	135.6	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00524641	At2g21170	Chloroplastic, triosephosphate isomerase	127.79	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00523903	At5g02490	Heat-shock cognate 70 kDa protein 2	121.8	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00536062	At2g47730	Glutathione S-transferase F8	117.32	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00526611	At1g56410	Early response to dehydrogenase 2 (HSP70)	116.83	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00547926	At3g18780	Actin 2	115.01	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00538349	At1g63940	Monodehydroascorbate reductase 6	103.82	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00539389	At1g16030	Heat-shock protein 70B (HSP70B)	96.9	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00539339	At3g04790	Ribose 5-phosphate isomerase related	91.58	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00545934	At5g12250	Tubulin β 6 chain	84.79	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00530539	At5g64290	Dicarboxylate transport 2	84.74	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00525001	At5g62690	Tubulin β 2 β 3 chain	82.8	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00523675	At4g23210	Isoform 2 of cysteine rich receptor-like protein kinase 13	79.72	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00518916	At5g24300	Chloroplastic amyloplastic, soluble starch synthase	78.84	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00517585	At5g52250	Transducin family protein	76.69	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h
IPI00530974	At1g52770	Phototropic responsive NPH3 family protein	76.1	ND	<i>B7Ox</i> 0h	<i>B7Ox</i> 0h

COFRADIC TECHNIQUE

Table 1: Overview of the oxidation events identified 1 h and 3 h after the onset of high light stress

METHIONINE OXIDATION IN NUMBERS	1 h	3 h	total unique IDs
MetO peptides	365	354	507
oxidized proteins	296	294	403
proteins significant more oxidized in CAT vs WT (≥ 2 -fold)	24 (7)	29 (18)	51
proteins significant more oxidized in WT vs CAT (≥ 2 -fold)	40 (14)	18 (8)	57
protein overlap in time (with identical MetO site)	187 (175)		
MetO peptide overlap in time	206		
proteins significant more oxidized over time	17		
proteins significant less oxidized over time	3		
proteins with single oxidation event	236	247	362

COFRADIC: Combined Fractional Diagonal Chromatography

Calmodulin

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Calmodulin Oxidation and Methionine to Glutamine Substitutions Reveal Methionine Residues Critical for Functional Interaction with Ryanodine Receptor-1*

Biochemistry 2008, 47, 3085–3095

3085

Methionine Oxidation in the Calmodulin-Binding Domain of Calcineurin Disrupts
Calmodulin Binding and Calcineurin Activation[†]

Cell

**A Dynamic Pathway for
Calcium-Independent Activation
of CaMKII by Methionine Oxidation**

No calmodulin was
detected by MS
approached!??

Technical biases??

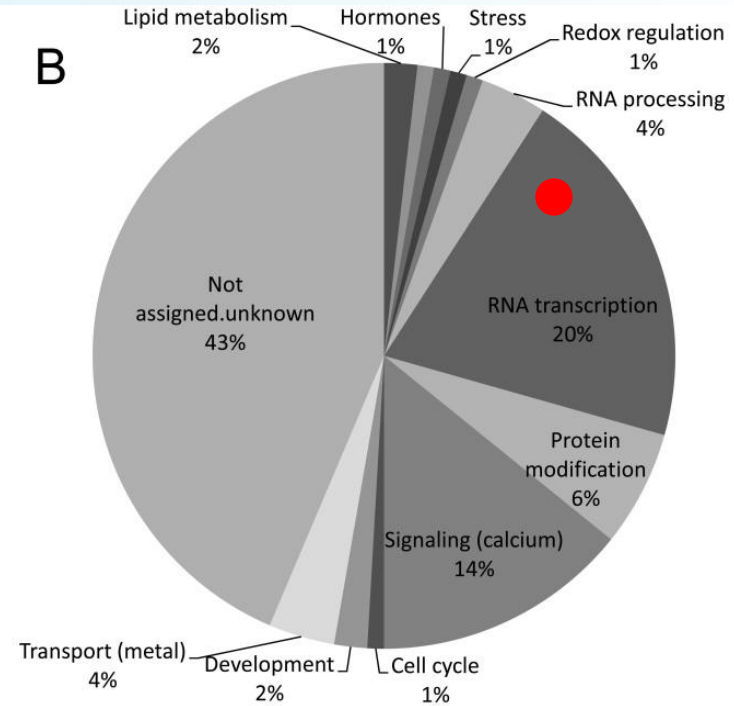
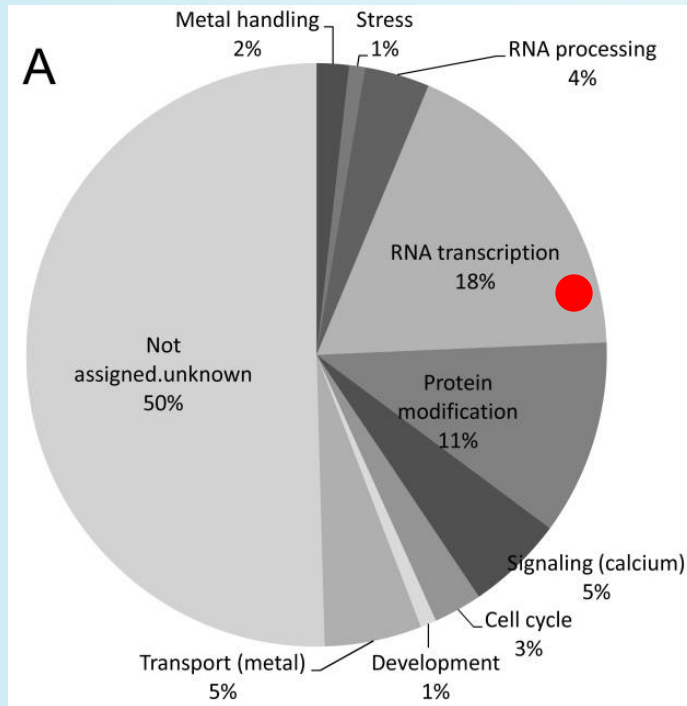
TARGETS OF METHIONINE OXIDATION & REPAIR IN PLANTS

	Met%	Met #	lengths
>AT3G05220.2 PACid:17361126	9.64	46	478
>AT1G40104.1 PACid:17350985	6.81	42	618
>AT5G39570.1 PACid:17376030	9.87	38	386
>AT1G33820.1 PACid:17350722	16.02	29	182
>AT4G16980.1 PACid:17368987	15.95	26	164
>AT3G07560.1 PACid:17361449	7.26	22	304
>AT1G67870.1 PACid:17353596	7.91	22	279
>AT3G18540.1 PACid:17362912	6.74	18	268
>AT1G64690.1 PACid:17353191	6.25	17	273
>AT4G08395.1 PACid:17367848	6.93	16	232
>AT1G47389.1 PACid:17351226	6.28	15	240
>AT5G46300.1 PACid:17376910	6.76	14	208
>AT4G35070.2 PACid:17371445	6.22	13	210
>AT5G61710.1 PACid:17378943	8.44	13	155
>AT1G33860.1 PACid:17350726	8.55	13	153
>AT1G21940.1 PACid:17349335	6.04	11	183
>AT3G49270.3 PACid:17365211	7.28	11	152
>AT1G10590.3 PACid:17347873	6.58	10	153
>AT3G11160.1 PACid:17361874	6.94	10	145
>AT5G42785.1 PACid:17376430	8.77	10	115

Approach

- Proteins with more than 6% Met content
 - Arabidopsis
 - Soybean
 - Rice
- Proteins with domains containing Met as important residues in their HMM profiles
- Proteins with Met on the surface

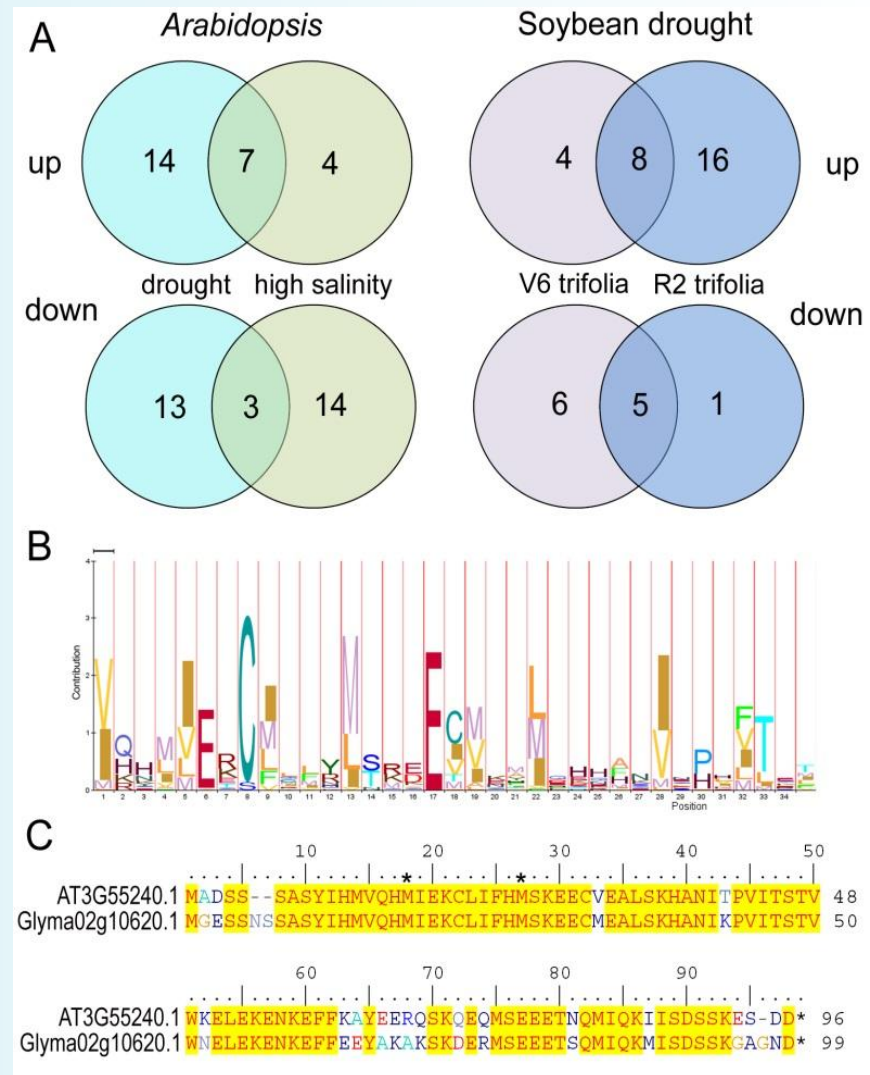
Functional categories of genes encoding MRPs in *Arabidopsis* (A) and soybean (B)



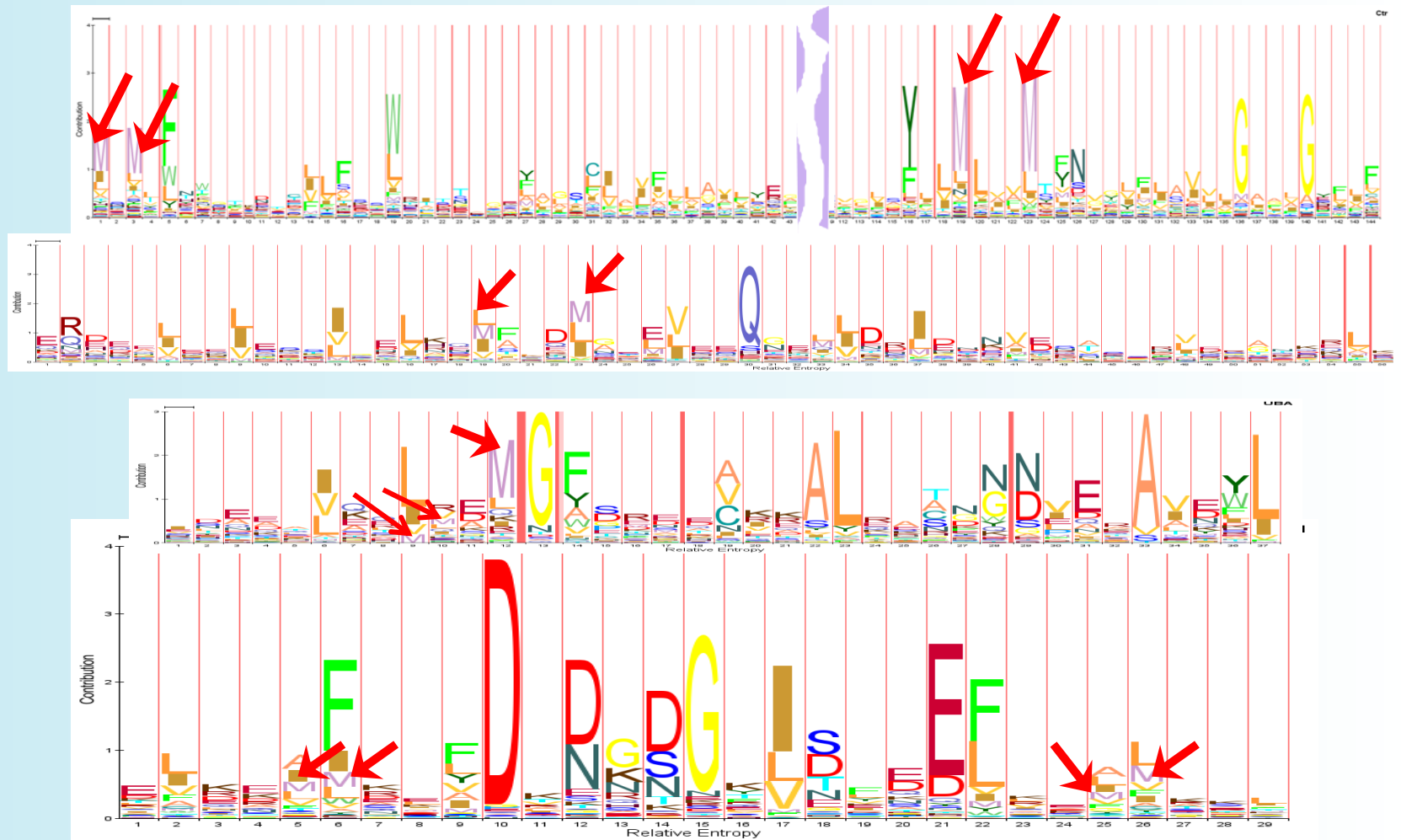
Venn diagram analyses of the expression of MRP-coding genes in *Arabidopsis* and soybean under abiotic stresses (A), and

HMM profile of *Arabidopsis* and soybean homologs share common responsiveness to drought (B) and

their sequence alignment (C).



HMM profiles that contain methionine residues at high occurrence



Genes encoding AtMRPs whose expression levels were responsive to both drought and high salinity

No	Locus IDs	Met (%)	Length (a.a.)	Drought vs untreated ¹		Salinity vs untreated ²		Gene descriptions
				Fold change ³	q-value	Fold change ³	q-value	
1	AT1G32560	6.02	134	● 135.33	0.002	3.31	0.005	LEA group 1 domain-containing protein
2	AT1G33860	8.55	153	2.37	0.092	2.16	0.003	unknown protein
3	AT3G55240	6.12	95	● -60.29	0.007	-26.88	0.001	Overexpression leads to Pseudo-Etiolation in Light phenotype
4	AT3G59900	6.20	130	10.70	0.011	-2.57	0.015	(ARGOS); unknown protein [AT3G59900.1]
5	AT3G62090	6.38	346	● 64.56	0.020	2.28	0.002	PHYTOCHROME INTERACTING FACTOR 3-LIKE 2
6	AT4G12334	6.25	113	-9.79	0.003	-3.04	0.005	Pseudogene of cytochrome P450 family protein
7	AT4G33467	8.91	102	● 337.51	0.002	6.16	0.023	unknown protein [AT4G33467.1]
8	AT4G34590	6.33	159	8.26	0.004	3.27	0.002	GBF6 (<i>A. thaliana</i> BASIC LEUCINE-ZIPPER 11)
9	AT5G42325	6.03	233	2.70	0.028	2.45	0.049	Transcription elongation factor-related
10	AT5G67390	7.43	176	-4.17	0.015	-4.15	0.001	Similar to unknown proteins (TAIR:AT1G69360.1)

Genes encoding GmMRPs whose expression responsive to drought stress in V6 and R2 leaves

No	Glyma ID	Met (%)	Length (a.a.)	V6 trifolia		R2 trifolia		Gene descriptions	Arabidopsis homologs
				Fold change	q-value	Fold change	q-value		
1	Glyma01g15910	8.08	100	3.63	0.045	4.96	0.042	no original description	
2	Glyma01g15930	6.56	458	● -20.34	0.007	-3.87	0.015	UNE10; transcription factor	AT4G00050
3	Glyma02g10620	7.22	98	● -44.63	0.007	-4.04	0.053	Overexpression leads to Pseudo-Etiolation in Light	AT3G55240
4	Glyma03g32740	6.04	481	-2.19	0.007	-2.02	0.030	PIF1, PIL5; transcription factor	AT2G20180
5	Glyma04g37040	7.91	140	● 15.03	0.012	40.08	0.005	Calmodulin-binding protein CML38	AT1G76650
6	Glyma06g39910	10.34	117	3.12	0.067	4.14	0.013	Calcium-binding EF hand family protein	AT4G27280
7	Glyma10g30380	7.43	149	7.53	0.013	5.27	0.026	CALMODULIN 5; calcium ion binding	AT2G27030
8	Glyma15g05510	7.37	96	2.93	0.025	2.41	0.023	no original description	
9	Glyma16g02510	7.26	125	2.05	0.028	4.63	0.023	Calcium-binding protein, putative	AT2G46600
10	Glyma19g43580	6.7	210	-2.01	0.160	2.42	0.078	GIF, GIF1, AN3 (ANGUSITFOLIA3)	AT5G28640
11	Glyma20g00780	6.69	285	-3.03	0.046	-2.36	0.027	Contains homeodomain (InterPro:IPR009057)	AT1G10820
12	Glyma20g22280	6.59	426	2.25	0.046	2.99	0.056	PIF3, POC1, PAP3, transcription factor	AT1G09530
13	Glyma20g36730	7.89	153	3.06	0.042	2.29	0.129	CALMODULIN 5; calcium ion binding	AT2G27030

Conclusions & On-going experiments

1. 121 and 213 genes encoding methionine-rich proteins in Arabidopsis and soybean, respectively.
2. Expression data mining indicated 57 and 35 genes encoding MRP in Arabidopsis and soybean, respectively, responsive to either drought or high salinity stresses
3. HMM profiles of stress-responsive MRP contain several conserved Met residues.
4. Among 121 AtMRPs, 21 were predicted to localized in chloroplast, while 9 were targeted to mitochondria, the two cellular organelles with higher ROS levels

On-goings

1. Collect Arabidopsis with perturbed expression of gene encoding MRP
2. Characterize their phenotype under normal and stress condition
3. Measurement of stress-related metabolites

Funding and team

