

**UNDERSTANDING OF HISTONE H₃ PHOSPHORYLATION AND
ACETYLATION ON ENHANCEMENT OF THE SOYBEAN
TOLERANCE TO SALINITY VIA PROTEOMIC PROFILE**

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The abundant dynamics of the available histone PTMs were screened inside the soybean plant under salinity stress and we demonstrated that in comparison with the control sample, H3 lysine acetylation (Lys Ac), H3 lysine 14 acetylation (H3K14Ac), H3 Serine 10 phosphorylation (H3S10Phos) increased under salinity stress. This indicates that H3K14Ac and H3S10Phos might play important roles in soybean adaptation to salinity stress and so they were chosen to explore the mechanisms of soybean tolerance.

To investigate the nucleic factors (proteins and genes), which may interact with the H3K14Ac, H3K9Ac and H3S10Phos, the precipitated proteins obtained from a ChIP assay were further separated by SDS-PAGE and identified by MALDI-TOF/ TOF. Different proteins could be identified among all three PTMs. Results showed that H3S10Phos could recruit PHD finger family protein, which was previously found to interact with GNAT (a histone acetyltransferase for H3K14), Elongin A and ISWI. Acetylation of either H3K9 or H3K14 is capable of modulating the expression of salinity inducible genes. In addition, H3S10Phos might also contribute much to chromatin remodeling and transcription, while H3K14Ac could regulate the gene translation by recruiting translation elongation factor Ts.

A scheme is proposed to illustrate the functional roles of H3 N-terminal PTMs, GmGST and GmAPX in salinity stress-induced oxidative response in soybean plants. Initially, the accumulation of cellular ROS (such as H₂O₂) could up-regulate modification of H3S10Phos and H3K14Ac, which may activate the expression of *GmGST*. Then the increased GmGST could either directly or indirectly activate downstream targets such as GmMDAR and GmAPX through the glutathione ascorbate cycle. Since GmAPX has an antioxidant effect, the accumulation of H₂O₂ could be finally reduced. On the other hand, the over-accumulated of GmAPX could transport into the nucleus and feedback to the *GmGST* expression system.

Understanding of histone H3 phosphorylation and acetylation on enhancement of the soybean tolerance to salinity via proteomic profile

PI, Erxu

Supervisor: Prof. Ngai Sai Ming



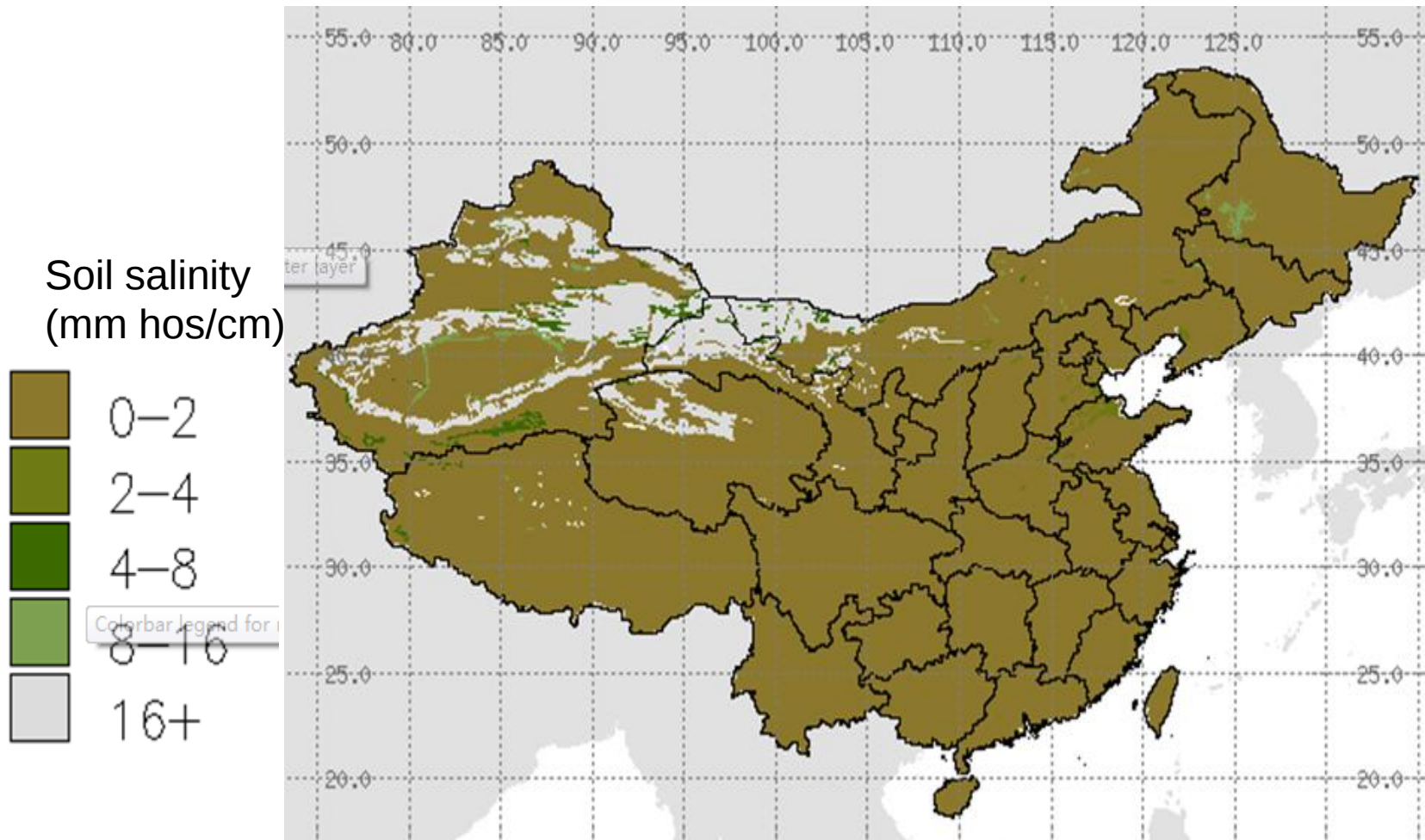
Outline

- Background
- Histone H3 N-terminal phosphorylation and acetylation
- Proteomic characterization of GmPHD5

Outline

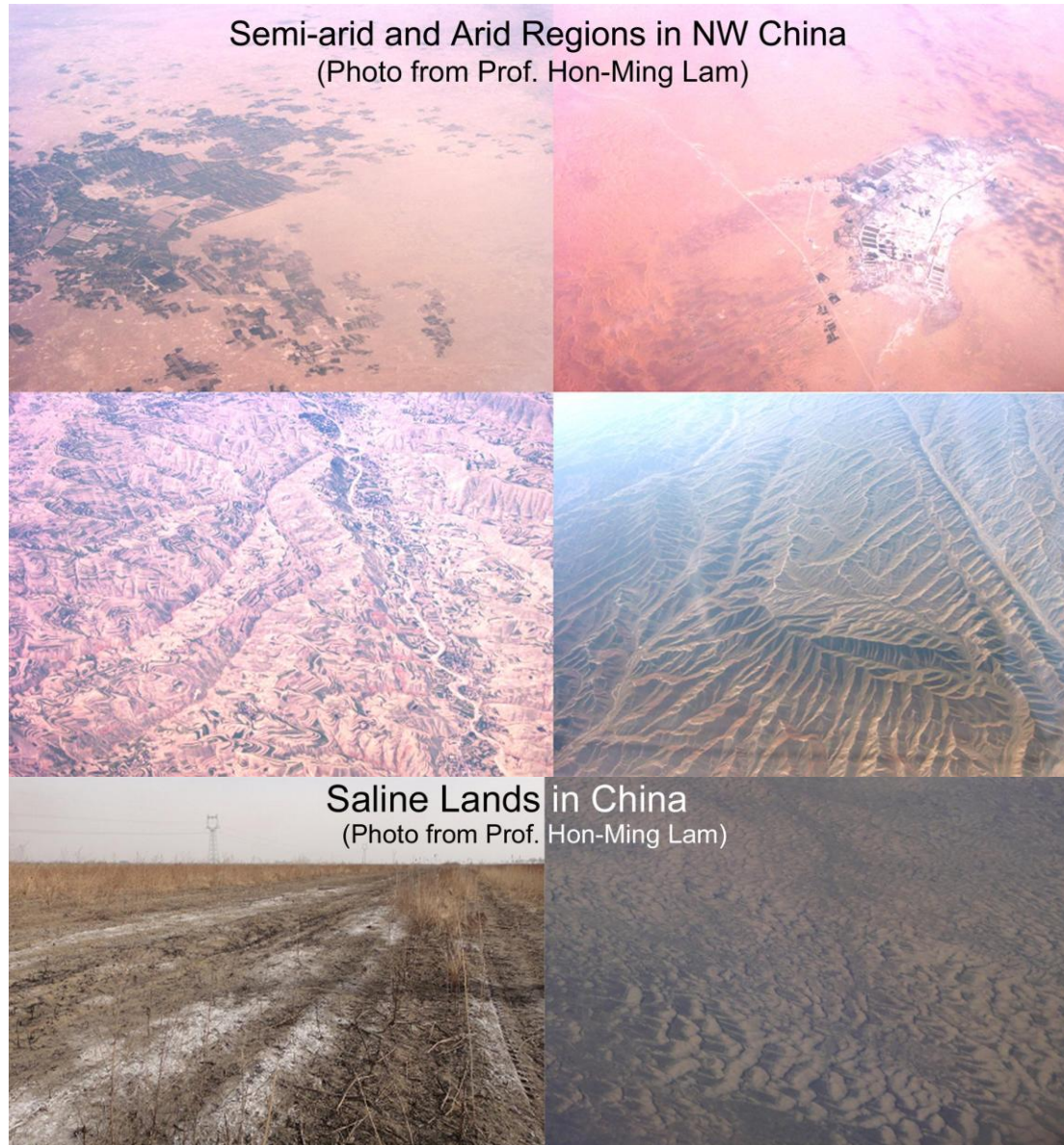
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Saline lands in the semi-arid and arid regions of China



(from <http://prismmap.nacse.org/forages/>)

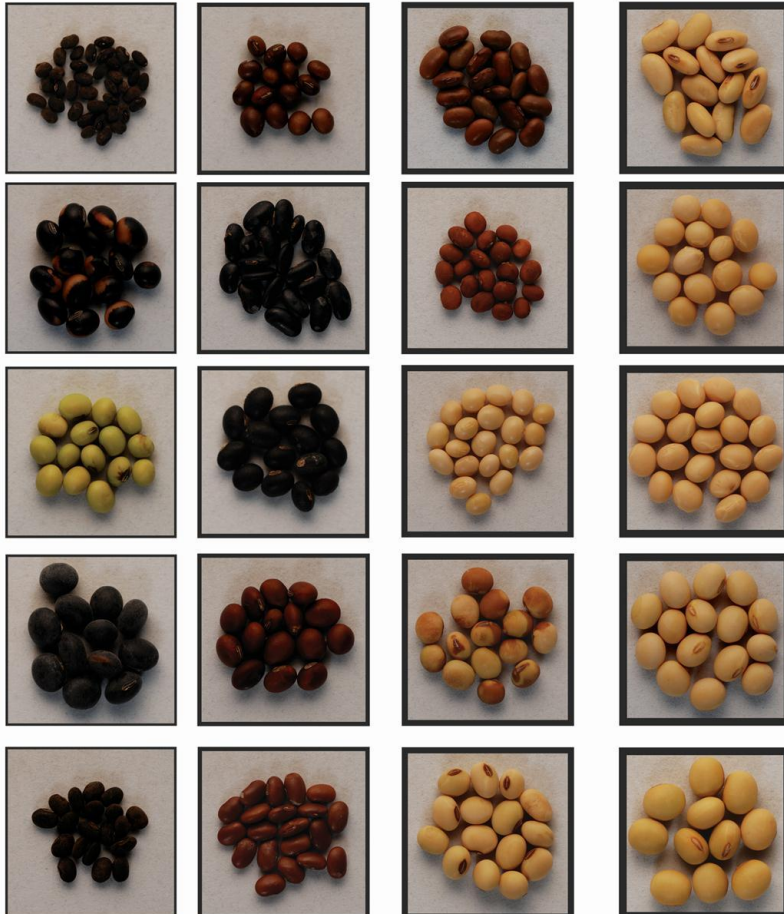
Saline lands in the semi-arid and arid regions of China



Soybean genome diversity

Biodiversity of Soybean

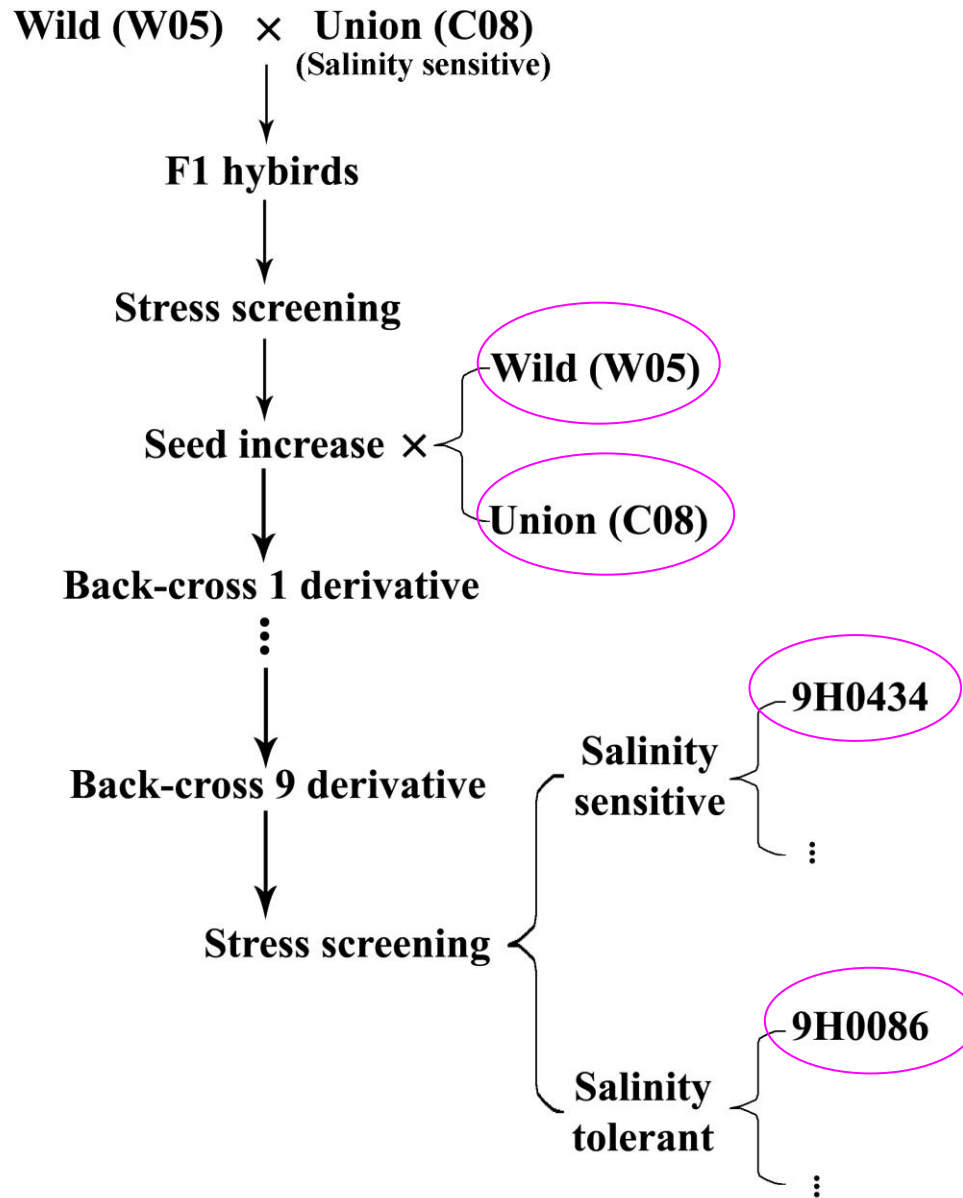
(Photo from Prof. Hon-Ming Lam)



Accession	Description	Genome coverage	Mean depth
W01	Wild; Beijing area, PRC	0.928114	4.985707
W02	Wild; Liaoning, PRC	0.945107	4.722718
W03	Wild; Inner Mongolia, PRC	0.95598	5.784117
W04	Wild; Henan, PRC	0.927056	4.375092
W05	Wild; Henan, PRC	0.951029	7.894194
W06	Wild; Heilongjiang, PRC	0.744567	1.634187
W07	Wild; Liaoning, PRC	0.907823	5.628903
W08	Wild; Heilongjiang, PRC	0.94428	5.558055
W09	Wild; Liaoning, PRC	0.816146	2.17832
W10	Wild; Heilongjiang, PRC	0.941307	4.742872
W11	Wild; Shanxi, PRC	0.941344	5.285625
W12	Wild; Anhui, PRC	0.954091	7.462448
W13	Wild; Inner Mongolia, PRC	0.95922	7.986427
W14	Wild; Inner Mongolia, PRC	0.825547	2.218275
W15	Wild; Henan, PRC	0.946831	5.250471
W16	Wild; Heilongjiang, PRC	0.92242	4.423702
W17	Wild; Liaoning, PRC	0.799289	1.993777
C01	Advanced bred line; popularized in Shandong, PRC	0.919422	4.43853
C02	Advanced bred line; popularized in Liaoning, PRC	0.951359	5.28649
C08	Advanced bred line; popularized in USA	0.977795	7.851686
C12	Advanced bred line; popularized in Shanxi, PRC	0.935454	4.811598
C14	Advanced bred line; popularized in Brazil	0.945589	5.222104
C16	Japan neutron-mutated line adapted to Taiwan	0.953999	5.624765
C17	Landrace; Sichuan, PRC	0.926871	4.653919
C19	Landrace; Jilin, PRC	0.94893	5.524911
C24	Advanced bred line; popularized in Jiangxi, PRC	0.939643	4.783565
C27	Advanced bred line; popularized in Hebei, PRC	0.948785	5.621014
C30	Advanced bred line; popularized in Henan, PRC	0.949428	5.731478
C33	Advanced bred line; popularized in Heilongjiang, PRC	0.92603	4.242454
C34	Landrace; Guangxi, PRC	0.941575	4.76983
C35	Landrace; Guangdong, PRC	0.931602	4.780462

(Lam HM., et al., Nature Genetics, 2010, 42(12): 1053-61)

Soybean genome diversity



(Lam HM., et al., Nature Genetics, 2010, 42(12): 1053-61)

Outline

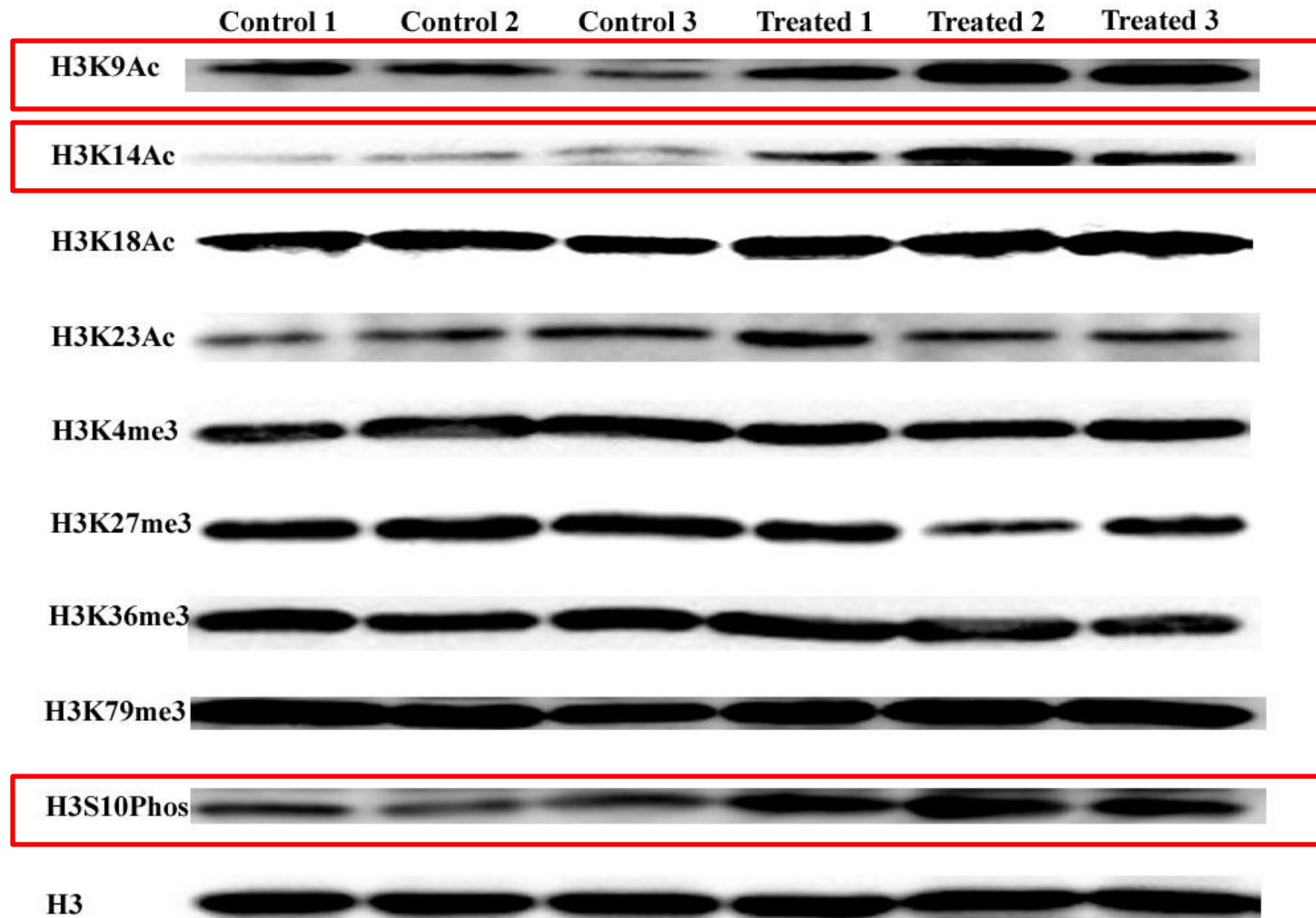
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Dynamic patterns of histone H3 N-terminal PTMs are similar between soybean parent and its offspring

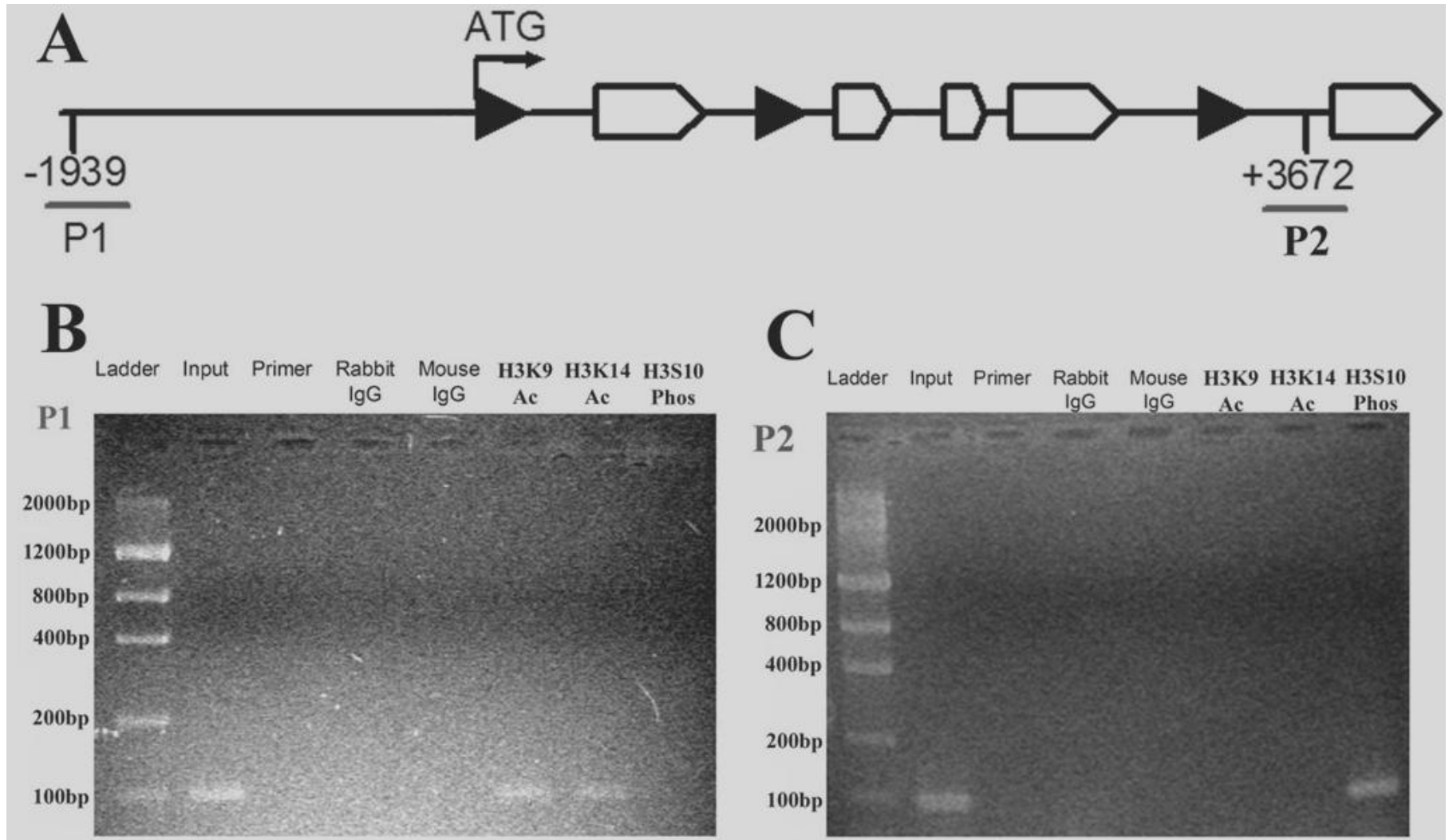
(Unpublished data)



(Note: Control1 and Treated 1 sample came from a parent soybean species; Control 2 and Treated 2 samples came from one offspring species, while Control 3 and Treated 3 samples came from another offspring.

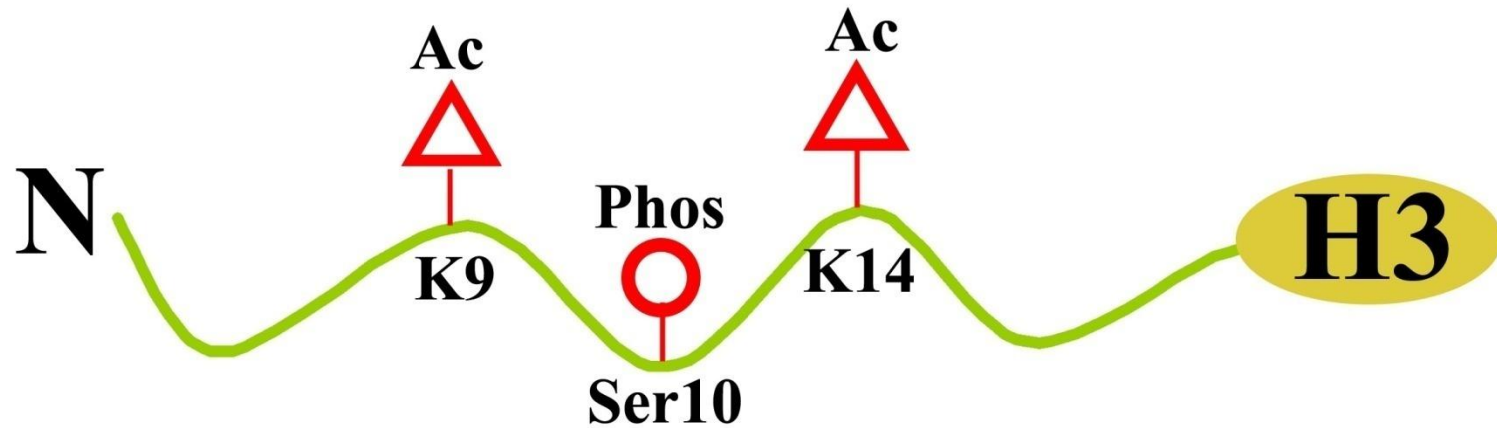
Antibody	Accession Number	Protein Name	MW	pI	Pep count	Coverage (%)	Score
Anti- H3K9Ac	gi 134194	Stress-induced protein SAM22	16762	4.69	4	17.72	111
Anti- H3K9Ac	gi 238478566	DNAJ heat shock N-terminal domain-containing protein [Arabidopsis thaliana]	34377.6	6.36	6	26.64	40
Anti- H3K9Ac	gi 255637984	Similar to disease resistance- responsive, dirigent domain- containing protein	20871	7.92	8	24.47	205
Anti- H3K9Ac	gi 397482	Heat shock protein 70 cognate [Arabidopsis thaliana]	71327	6.33	3	13.67	330
Anti-H3S10P	gi 95928518	SMC (Structural Maintenance of Chromosomes) protein-like [Desulfuromonas acetoxidans DSM 684]	106154	6.36	17	19.27	91
Anti-H3S10P	gi 255571843	Eukaryotic translation initiation factor 3f, eif3f, putative [Ricinus	31463	8.85	4	11.15	99
Anti-H3S10P	gi 30680213	PHD finger family protein	91071.3	6	11	16.87	55
Anti-H3S10P	Glyma18g16060.1	Similar to serine/threonine-protein kinase	44235.9	9.34	8	39.11	63
Anti- H3K14Ac	Glyma11g07790.1	Similar to CDK5 regulatory subunit-associated protein [Nicotiana tabacum]	56756.7	6.22	9	23.29	50
Anti-	gi 132270	Rubber elongation factor protein	14713	8.73	8	33.33	156
Anti- H3K14Ac	gi 82582811	Heat shock protein 90 [Triticum aestivum]	75626	5.85	3	1.06	103

Histone H3 PTMs locate on promoter and ORF regions of *GmGST* (ChIP part II)



(Wu, T*, Pi E*, et al., BMC Plant Biol. 2011, 11(1):178)

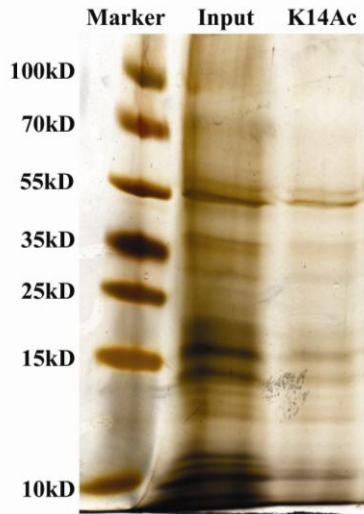
PTM-peptides library (H3 N-terminal residue 1-20)



Residue 1-20: ARTKQTAR**K**STGG**K**APRKQL

	K9Ac	Ser10Phos	K14Ac
Control			
H3K9ac	+		
H3Ser10phos		+	
H3K14ac			+
H3K9acSer10phos	+	+	
H3K9acK14ac	+		+
H3Ser10phosK14ac		+	+
H3Ser10phosK14ac	+	+	+

Nucleic factors recognizing histone PTMs

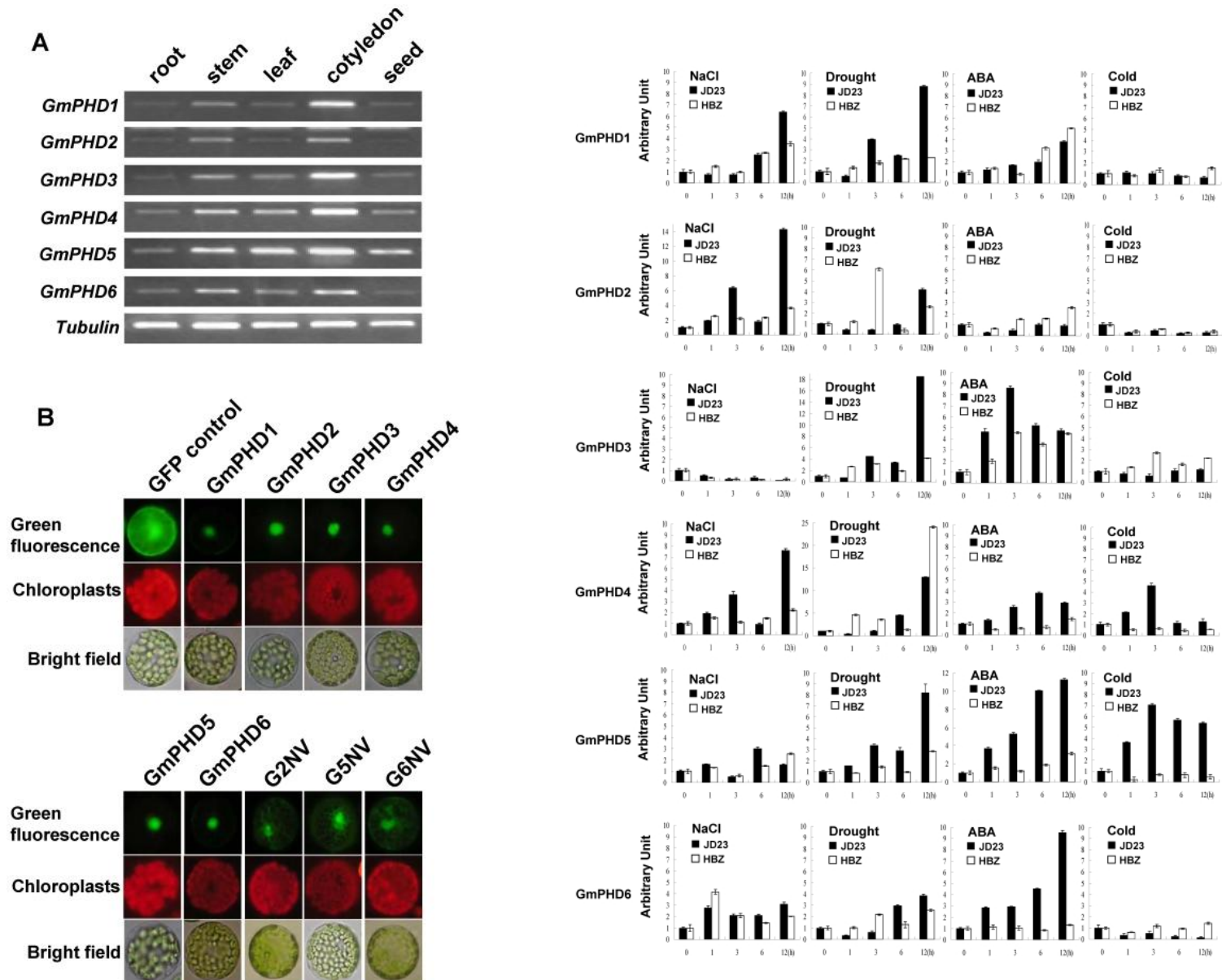


Accession No.	Protein Name	Beads	H3	K9	S10	K14	K9S10	K9K14	S10K14	K9S10K14
gi 310561	ascorbate peroxidase [Glycine max]				✓		✓		✓	
gi 971558	peroxidase 1A [Medicago sativa]					✓				
gi 122022	RecName: Full-Histone H2B.1					✓				
gi 1125104	seed coat peroxidase isozyme [Glycine max]			✓				✓		✓
gi 296142019	putative glycine-rich RNA-binding protein 1 [Malus prunifolia]			✓		✓			✓	
gi 132270	Rubber elongation factor protein			✓	✓	✓	✓		✓	✓
gi 255637875	unknown [Glycine max]				✓	✓				
gi 563329	histone 2B					✓				
gi 5726567	glycine-rich RNA-binding protein [Glycine max]			✓		✓			✓	
gi 255637984	unknown [Glycine max]				✓	✓			✓	
gi 255639497	unknown [Glycine max]				✓		✓			✓
gi 159488448	histone H2B variant [Chlamydomonas reinhardtii]					✓				
gi 5002342	peroxidase 1 precursor [Phaseolus vulgaris]			✓	✓		✓	✓	✓	✓
gi 28476864	hypersensitive-induced reaction protein 2 [Hordeum vulgare subsp. vulgare]				✓					
gi 255647273	unknown [Glycine max]				✓		✓			
gi 316937084	SGF14h [Glycine max]				✓	✓	✓	✓	✓	
gi 255641583	unknown [Glycine max]				✓	✓				
gi 2879818	14-3-3 protein [Solanum tuberosum]				✓	✓				
gi 224293	histone H4					✓			✓	
gi 224142253	histone H4 [Populus trichocarpa]					✓			✓	
gi 302833425	histone H2B [Volvox carteri f. nagariensis]					✓				
gi 255641611	unknown [Glycine max]					✓				

Outline

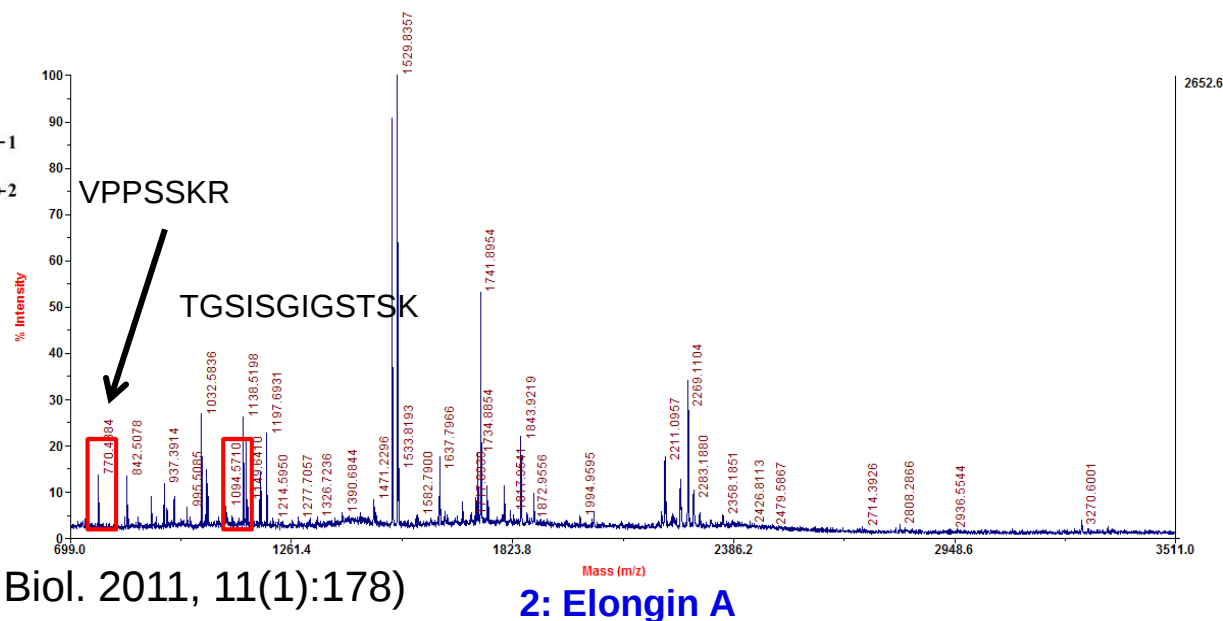
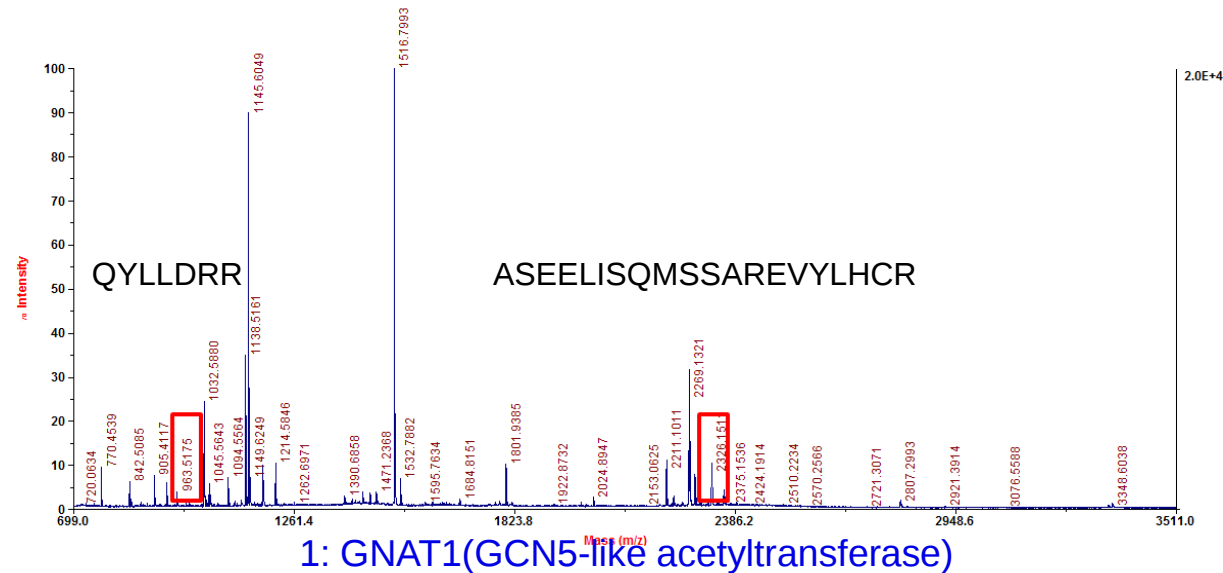
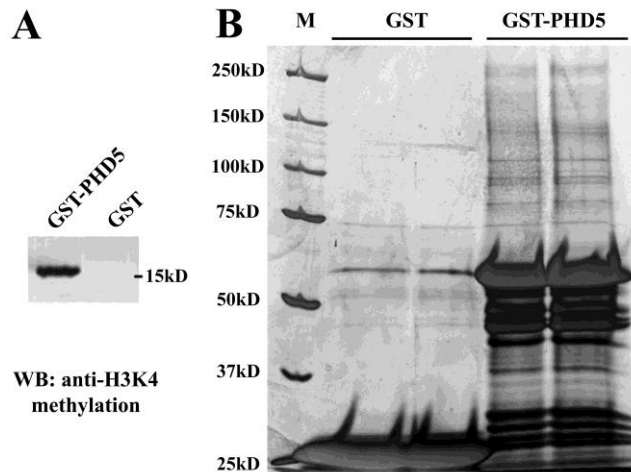
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Soybean GmPHD-type transcription regulators improve stress tolerance



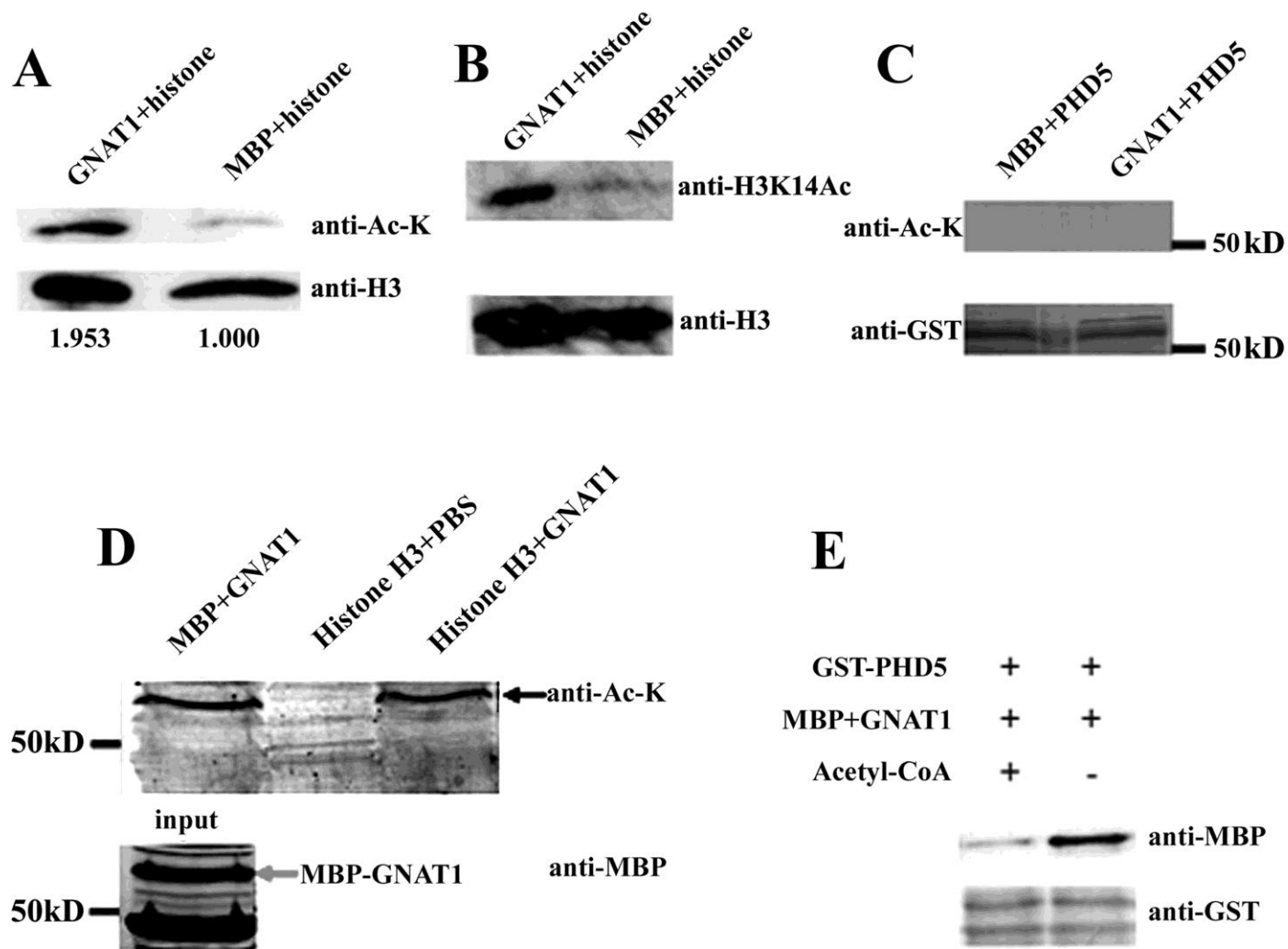
(Wei, et al., 2009. PLoS One 4(9): e7209.)

Identification of GST-GmPHD5 interaction proteins: pull down assay



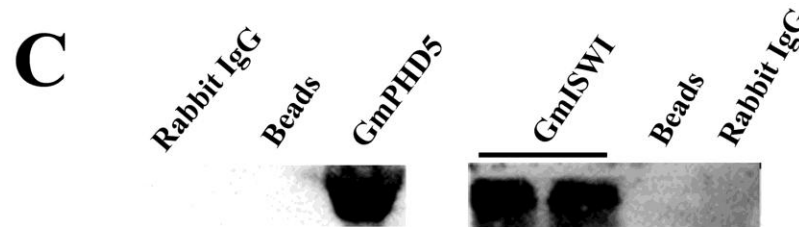
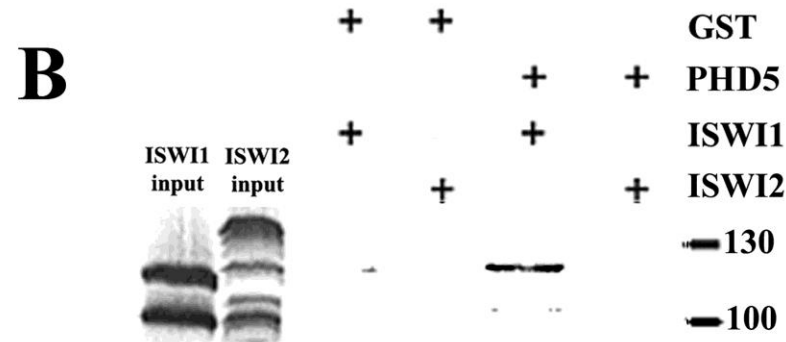
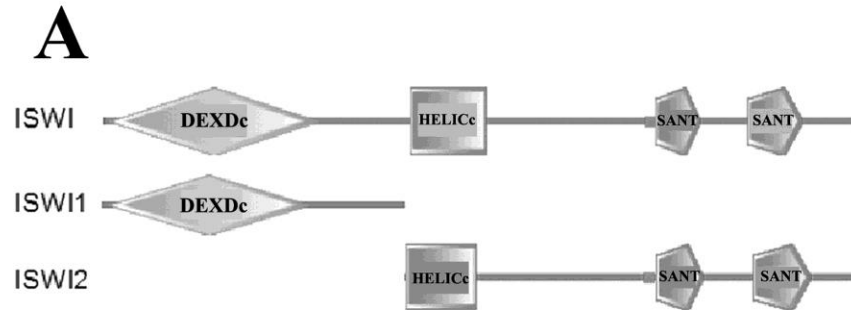
(Wu, T*, Pi E*, et al., BMC Plant Biol. 2011, 11(1):178)

GmGNAT1 is an acetyltransferase

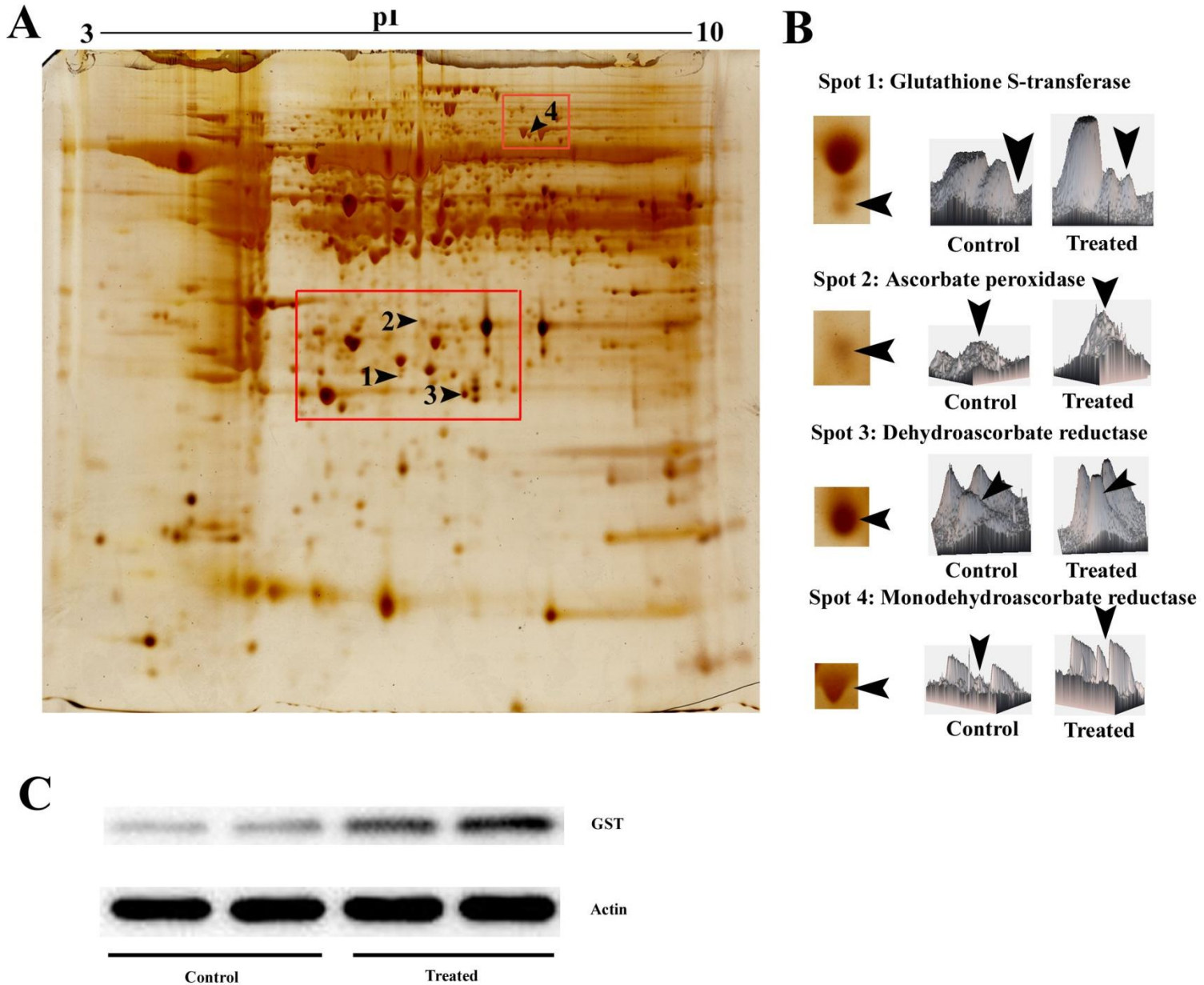


(Wu, T*, Pi E*, et al., BMC Plant Biol. 2011, 11(1):178)

GST-GmPHD5 interact with GmISWI through its N terminus

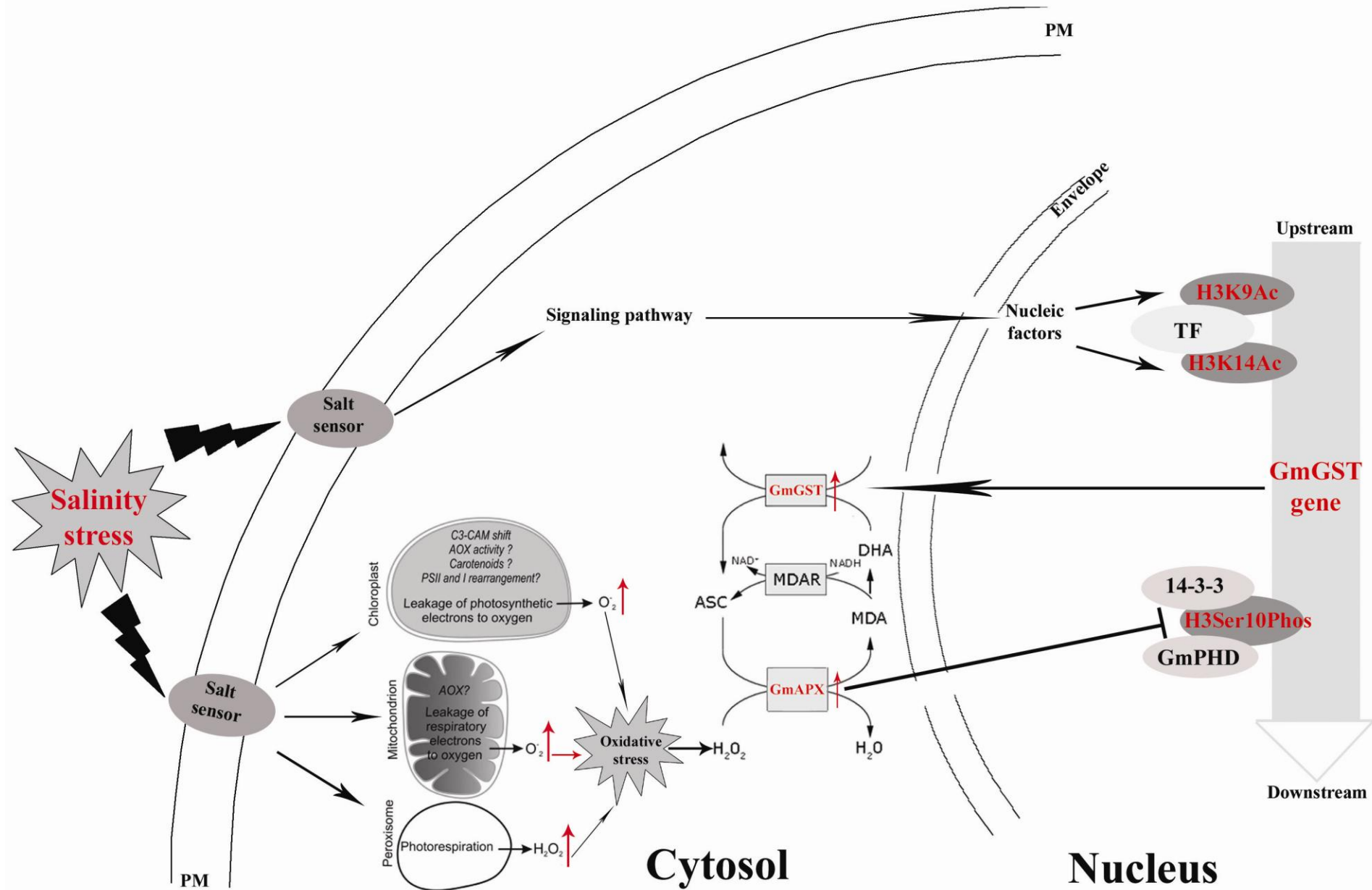


GST, APX and MADR increasing under salinity stress



(Wu, T^{*}., Pi E^{*}., et al., BMC Plant Biol. 2011, 11(1):178)

Summary and hypothesis



Thank you!

Q & A

Comparative proteomics of soybean plants upon salinity stress



Union (C08)
control



Union (C08)
treated



Wild (W05)
control

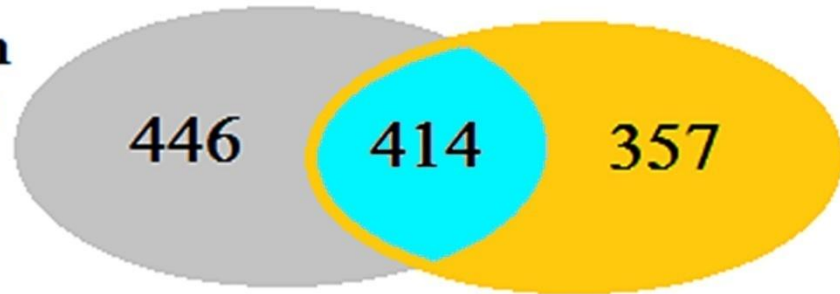


Wild (W05)
treated

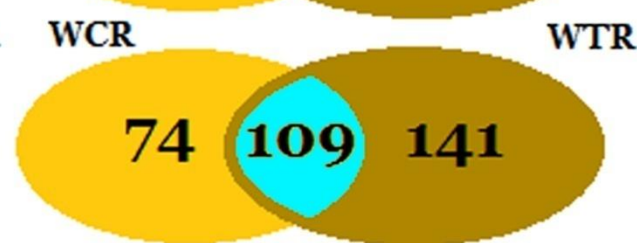
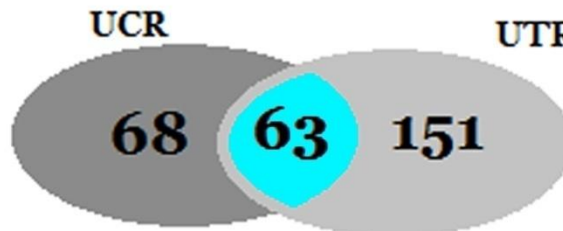
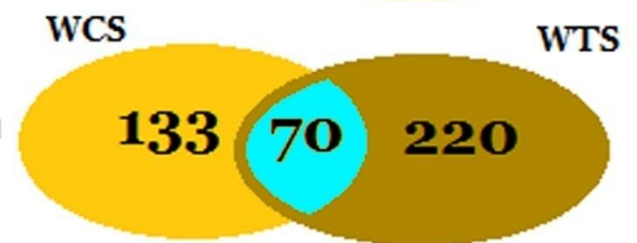
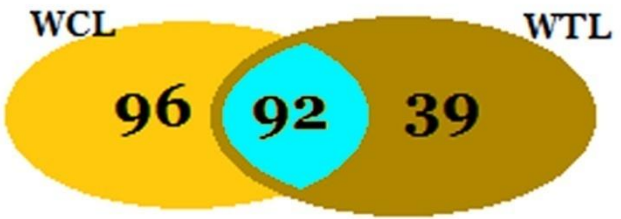
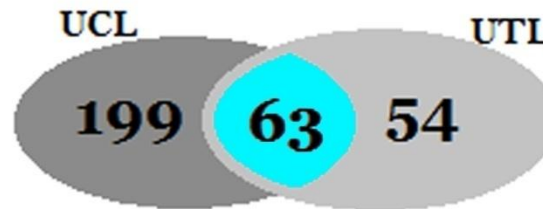


FTICR-MS

**Union
(Co8)**



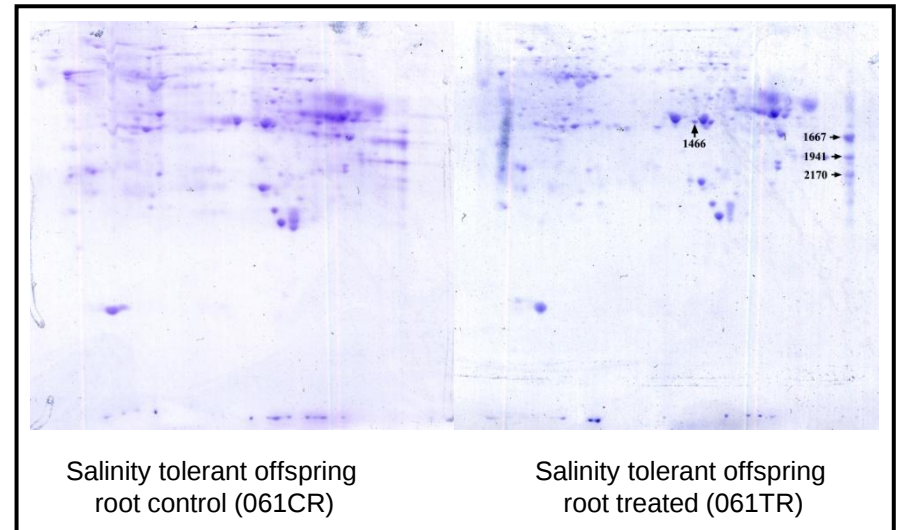
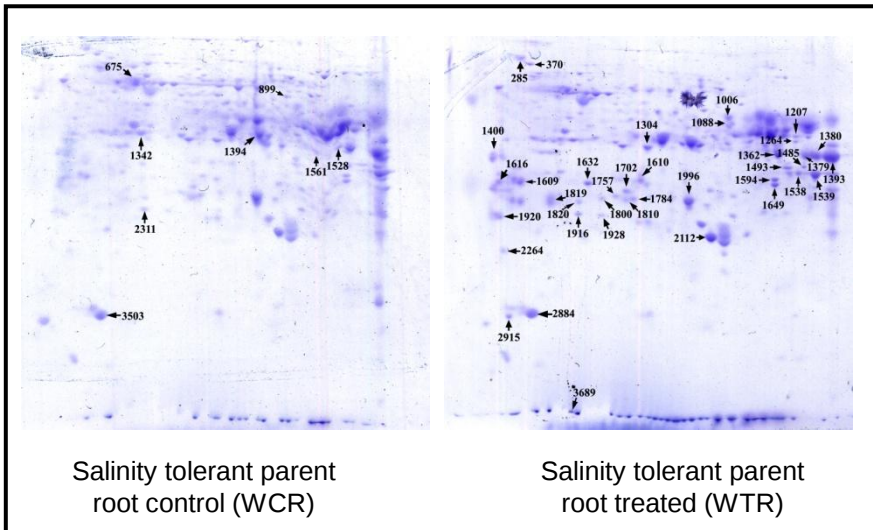
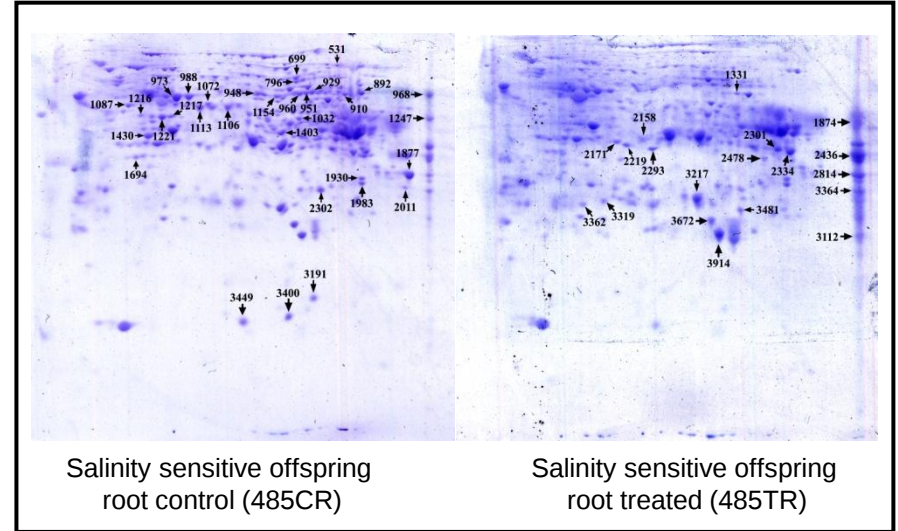
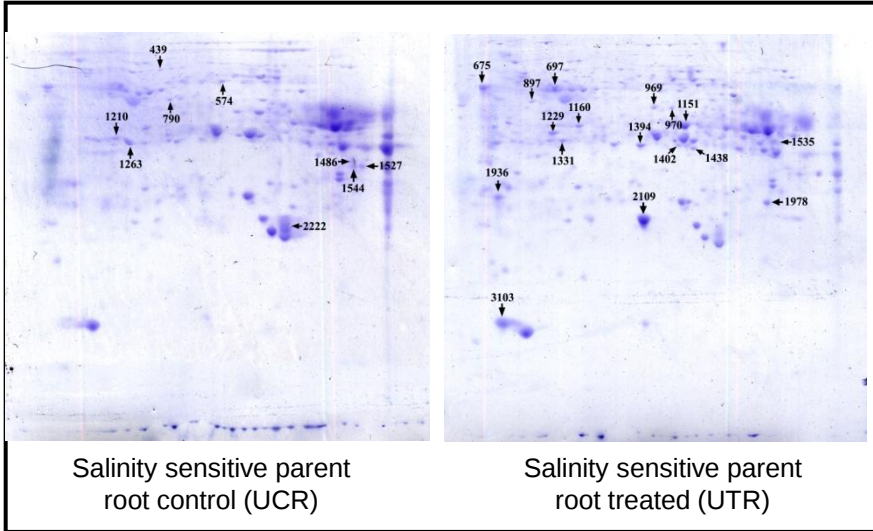
**Wild
(W05)**



Up regulated proteins associated with redox regulation or ROS signaling (Total 17 proteins)

Accession No.	Protein name	MW	Pep count	Coverage (%)	Score	Ratio	Functional classfition
gi 1168195	14-3-3-like protein	29237	2	4.23	74	1.6	Signaling pathway
gi 164459312	Extracellular Ca ²⁺ sensing receptor	41882	4	6.78	82	2.3	Signaling pathway
gi 310561	Ascorbate peroxidase	27035	7	16.40	187	2.2	Redox regulation
gi 1336082	Ascorbate peroxidase 2	27123	5	13.60	107	3.4	Redox regulation
gi 399942	Stromal 70 kDa heat shock-related protein	75469	6	9.92	148	8.4	Redox regulation
gi 20559	Hsp70 (AA 6 - 651)	70738	8	17.34	208	4.5	Redox regulation
gi 397482	Heat shock protein 70 cognate	71327	8	17.20	179	8.4	Redox regulation
gi 45331285	70 kDa heat shock cognate protein 3	71357	6	13.54	228	3.2	Redox regulation
gi 562006	PsHSP71.2	71122	8	17.29	239	3.6	Redox regulation
gi 15240737	Peroxidase 73	35905	3	10.03	83	4.5	Redox regulation
gi 225434984	Similar to HSC70-1 (heat shock cognate 70 kDa protein 1)	71190	18	30.86	441	11.6	Redox regulation
...	...	...	...	...	...	...	...

Insight of soybean response to salinity stress (based on proteomics)



Differentially expressed proteins under the salinity stress

(Total 130 different expressed proteins)

Spots No.	Accession No.	Protein names	MW/pI	Pep. counted	Pr. score	CI%
Wild (W05)						
2915	lyma09g04530	ABA-responsive protein ABR17	16522.5/4.68	8	156	100
1088	lyma12g32160	peroxidase 3	35644.1/7.12	11	134	100
1810	lyma12g07780	L-ascorbate peroxidase 2	27108.8/5.65	15	262	100
1616	lyma04g37120	elongation factor 1-delta-like	24972.7/4.42	8	190	100
1380	lyma05g22180	peroxidase 73-like	35475/9.03	12	245	100
370	lyma16g00410	70 kDa heat shock-related protein	73709.4/5.2	19	230	100
1006	lyma12g32160	peroxidase 3	35644.1/7.12	9	214	100
1393	lyma05g22180	peroxidase 73-like	35475/9.03	8	226	100
3503	lyma17g03350	stress-induced protein SAM22	16746.6/4.93	13	325	100
899	lyma08g02100	monodehydroascorbate reductase	52130.1/8.36	12	169	100
1561	lyma09g02800	ferredoxin NADP oxidoreductase	42241.3/8.38	15	223	100
...	...	...	...	...	...	...
9H0086 (061)						
1667	lyma05g22180	peroxidase 73-like	35475/9.03	12	237	100
2170	lyma03g05480	disease resistance response protein 206-like	22015.6/9.88	6	92	100
...	...	...	...	...	...	...
Union (C08)						
3103	lyma07g37250	stress-induced protein SAM22	16761.5/4.69	13	460	100
969	lyma09g01270	fumarylacetoacetase-like	40512.1/6.49	12	144	100
1151	lyma06g12780	alcohol dehydrogenase 1-like	36891.4/5.77	16	357	100
1438	lyma11g07490	isoflavone reductase homolog A622-like	33978.9/6.12	12	187	100
2109	lyma17g15690	expansin-like B1-like	27650.4/6.3	7	229	100
1263	lyma13g41960	fructokinase-2-like	35375.4/5.29	17	272	100
...	...	...	...	...	...	...
9H0434 (485)						
2436	lyma05g22180	peroxidase 73-like	35475/9.03	10	229	100
3364	lyma03g05480	disease resistance response protein 206-like	22015.6/9.88	7	122	100
3112	lyma03g38630	germin-like protein 1	22832.2/9.06	5	180	100
1331	lyma13g41960	fructokinase-2-like	35375.4/5.29	16	372	100
1334	lyma11g08920	isocitrate dehydrogenase	39315.3/6.47	11	140	100
...	...	...	...	...	...	...

