

**COMPARATIVE METABOLOMIC ANALYSIS OF SEEDLING LEAVES FROM
TEN DIFFERENT SALT-TOLERANT SOYBEAN VARIETIES UNDER NORMAL
AND SALT STRESS CONDITIONS**

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In this study, we aimed to establish a fast and powerful tool for discriminating the salt-tolerance ability of different soybeans on the basis of a metabolomic profile. Ten soybean varieties with different salt-tolerance properties were selected for this study, including two parental soybean lines (one salt-tolerant wild soybean: W05; and one salt-sensitive cultivated soybean: C08) and eight hybrid offspring soybean lines (four salt-tolerant soybeans: 9H0086, 9H0124, 9H0391 and 9H0736; and four salt-sensitive soybeans: 9H0380, 9H0400, 9H0434 and 9H0590), which were produced by the cross-breeding of the two parental lines. They were cultured in 1×Hoagland nutrient solution in a greenhouse with natural light. In the salt-treated group, soybeans were treated with 0.6% NaCl for 48 hours when they produce 3-4 trifoliolate leaves. In the control group, soybeans were cultivated under normal conditions. Total small molecule extracts of seedling leaves were profiled by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-Fourier transform mass spectrometry (LC-FT/MS). Finally, we found that the metabonome of soybean seedling leaves was mainly dominated by 139 metabolites, including carboxylic acids, sugars, amino acids, fatty acids, alcohols, isoflavonoids, flavonoids, amines, phospholipids and pigments. The salt-tolerant soybean W05 contained higher amounts of disaccharides, sugar alcohols, acetylated amino acids and unsaturated fatty acids than salt-sensitive soybean C08, but with lower quantities of monosaccharides, carboxylic acids and succinylated amino acids. Based on the above metabolomic variations between W05 and C08, the unique salt-tolerance abilities of the other eight soybean lines (9H0086, 9H0124, 9H0391, 9H0736, 9H0380, 9H0400, 9H0434 and 9H0590) were successfully determined. Further investigations demonstrated that the ability of soybean to tolerate salt was mainly based on the synthesis of compatible solutes, induction of ROS scavengers, induction of plant hormones and cell membrane modifications. This study was the first attempt to demonstrate that the metabolomic technology might provide a fast and powerful tool to discriminate the salt-tolerance characters of different soybeans.



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High-throughput metabolic analysis of soybean seedling leaves under salinity stress

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Comparative metabolomic analysis of seedling leaves from ten different salt-tolerant soybean varieties under normal and salt stress conditions

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School of Life Sciences

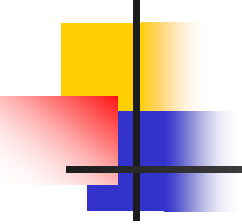
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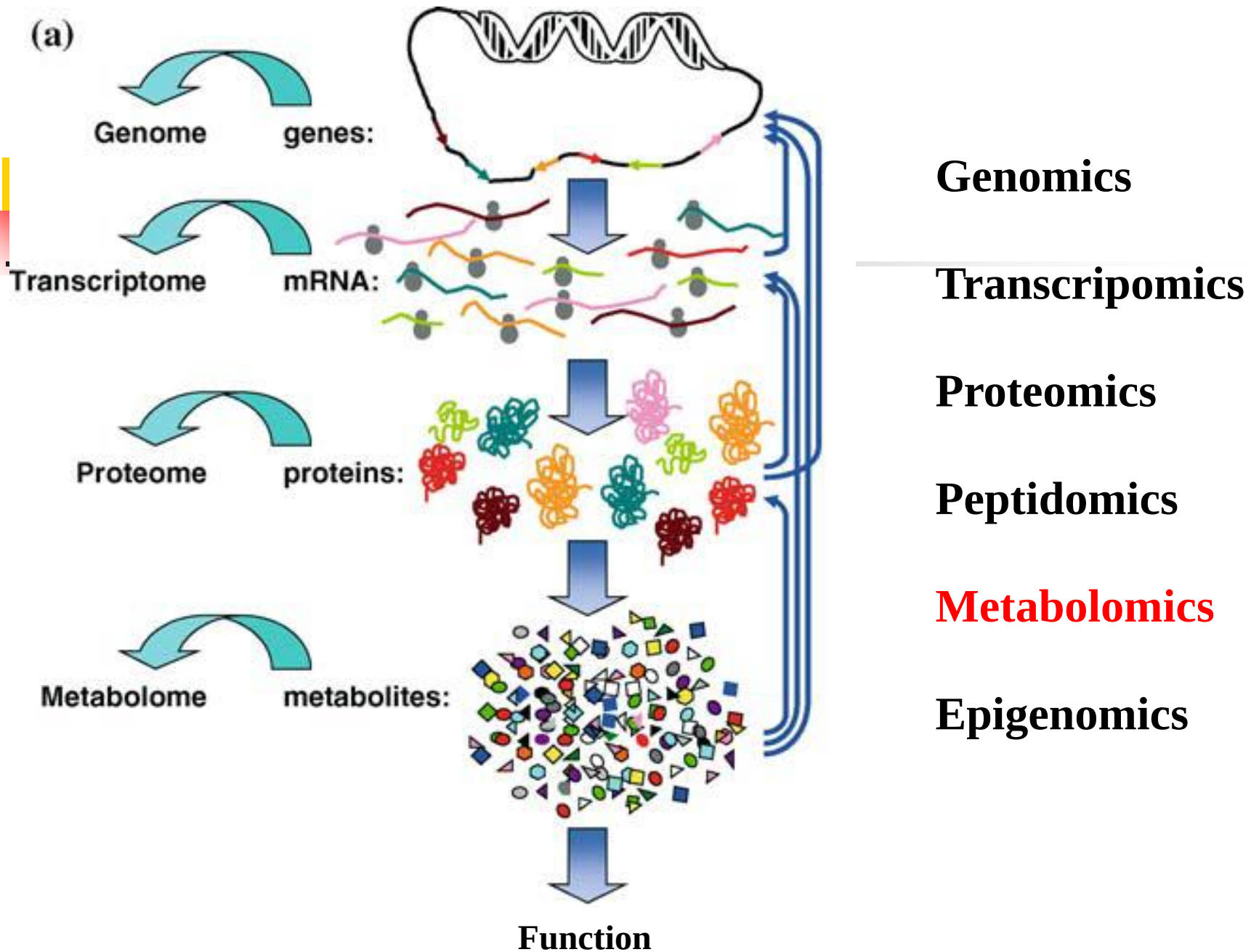
The Chinese University of Hong Kong

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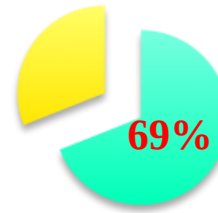


Outline

- Introduction of metabolomics and soybean
- Objectives, why we did this study?
- Metabolic profiling of soybean leaves
- Summary



Soybean



Protein



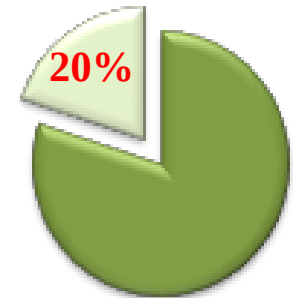
Oil



The soybean (U.S.) or soya bean (UK) (Glycine max) is a member of family Fabaceae and the world foremost provider of protein and oil.



Salinity stress



Pictures were downloaded from the internet, only for the research communication.

Salt-sensitive vs. Salt-tolerance



Glycine max



Glycine soja



Jackson (*G. max*)

JWS156-1 (*G. soja*)

Soybean genome diversity

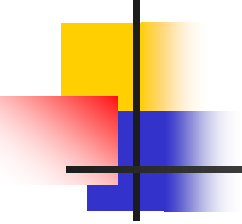
Biodiversity of Soybean

(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)



Objectives

To screen the soybean seedling leaf metabolome



Understanding the metabolic differences between salt-sensitive and salt-tolerant soybeans

To investigate the altered metabolites in soybean under salinity stress



Identification of plant stress tolerance elements and the underlying adaptive mechanisms



1×Hoagland nutrient solution



3-4 trifoliolate leaves

0.6% NaCl



48 hours

Leaves collection



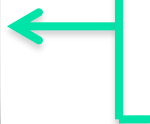
Frozen in liquid N2



Homogenization

MeOH/H2O 80/20 (v/v)

Plant material



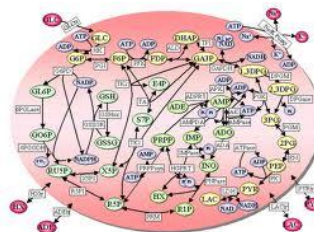
Supernatants



LC-MS analysis

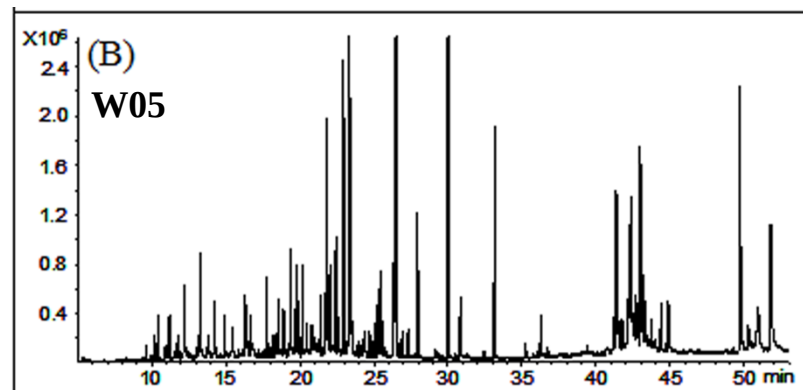
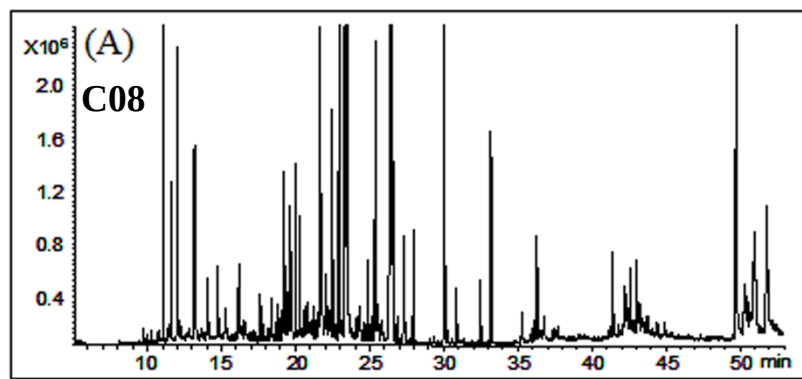


GC-MS analysis

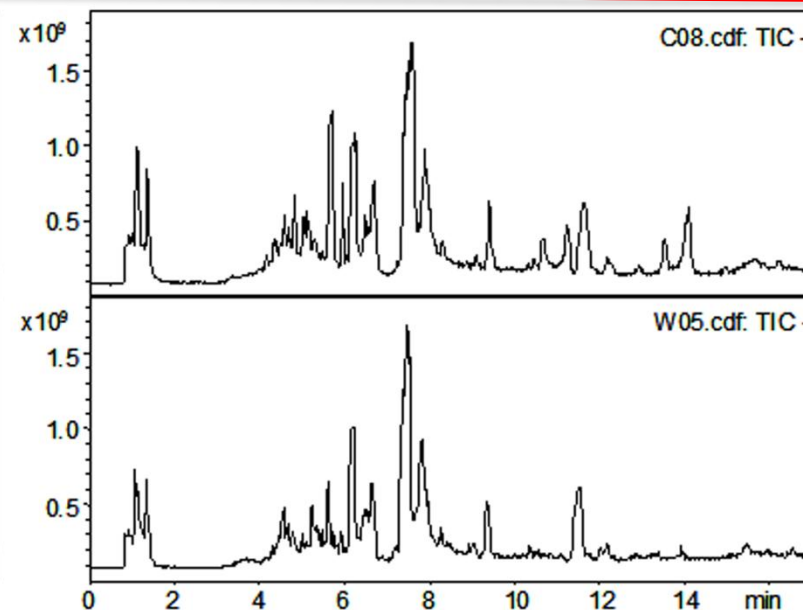
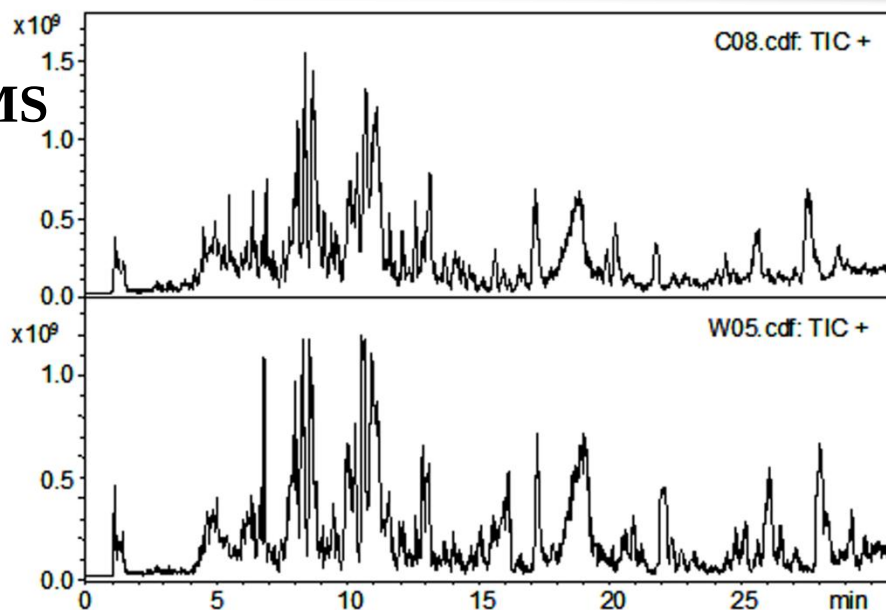


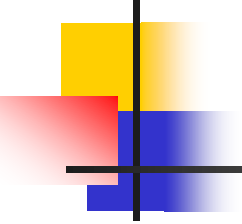
Metabolic profiling of C08 and W05

GC-MS



LC-MS





Metabonome of soybean seedling leaves

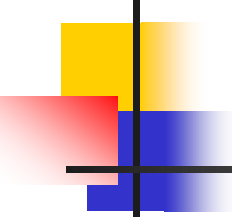
The metabonome of soybean seedling leaves was mainly dominated by 139 metabolites, including:

carboxylic acids, sugars, amino acids, fatty acids, alcohols, isoflavonoids, flavonoids, amines, phospholipids and pigments.

139 metabolites

Carboxylic acids		Amino acids		Sugars and phosphates		Alcohols		Fatty acids	
1	Abscisic acid	21	Alanine ^a	45	1-Kestose	63	C16 Sphinganine	83	Arachidic Acid
2	Acetic acid ^a	22	Asparagine	46	Fructose	64	C16 Sphingosine	84	Arachidonic acid
3	Biotin	23	Glutathione	47	Fructose-6-phosphate	65	C17 Sphinganine	85	Dodecanoic acid ^a
4	Butyric acid ^a	24	Glutathione disulfide	48	Galactose ^a	66	Cholesterol	86	Lignoceric acid
5	Caffeic Acid	25	Glycine ^a	49	Glucose ^b	67	Glycerol ^a	87	Linoleic acid
6	Chlorogenic Acid	26	Homoarginine	50	Glucose-6-phosphate	68	Inositol ^a	88	Linolenic Acid ^b
7	Cinnamic acid ^a	27	Homocystine	51	Lactose	69	Kushenol A	89	Myristic acid
8	Citric acid	28	N-Acetyl-glutamine	52	Lactose 6-phosphate	70	Kushenol B	90	Octadecanoic acid ^b
9	Ferulic acid ^a	29	N-Acetyl-lysine	53	Maltose ^a	71	Lactitol	91	Oleic acid
10	Fumaric acid ^a	30	N-Acetyl-methionine	54	Methylglucoside	72	Maltitol	92	Palmitic acid ^b
11	Galactonic acid ^a	31	N-Acetyl-tryptophan	55	Rhamnose	73	Mannitol	93	Palmitoleic acid
12	Geniposidic acid	32	N-Acetyl-tyrosine	56	Ribose ^a	74	Mannitol-1-phosphate		
13	Isocitric acid	33	N-Glycosyl-asparagine	57	Sedoheptulose	75	Phytosphingosine		
14	Jasmonic Acid	34	N-Linoleoyl-glycine	58	Sorbopyranose ^a	76	Phytosphingosine-1-phosphate		
15	Malic acid ^a	35	N-Oleoyl-serine	59	Sucrose	77	Sitosterol		
16	Malonic acid ^a	36	N-Palmitoyl-glycine	60	Sucrose-6-phosphate	78	Sphinganine		
17	Pantothenic Acid	37	N-Succinyl-citrulline	61	Xylose ^a	79	Sphinganine-1-phosphate		
18	Propanoic acid ^a	38	N-Succinyl-ornithine	62	Xylulose ^a	80	Sphingosine		
19	Ribonic acid ^a	39	Phenylalanine ^a			81	Sphingosine-1-phosphate		
20	Tartaric acid	40	Serine ^a			82	Xylitol ^a		
		41	Threonine ^a						
		42	Tryptophan						
		43	Tyrosine						
		44	Valine ^a						

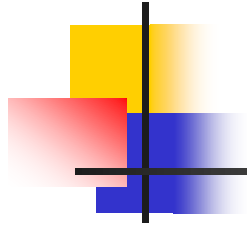
^a 26 metabolites were monitored by GC-MS. ^b 4 metabolites were detected by both GC-MS and LC-MS systems. The other 109 metabolites with no notation were monitored by LC-MS



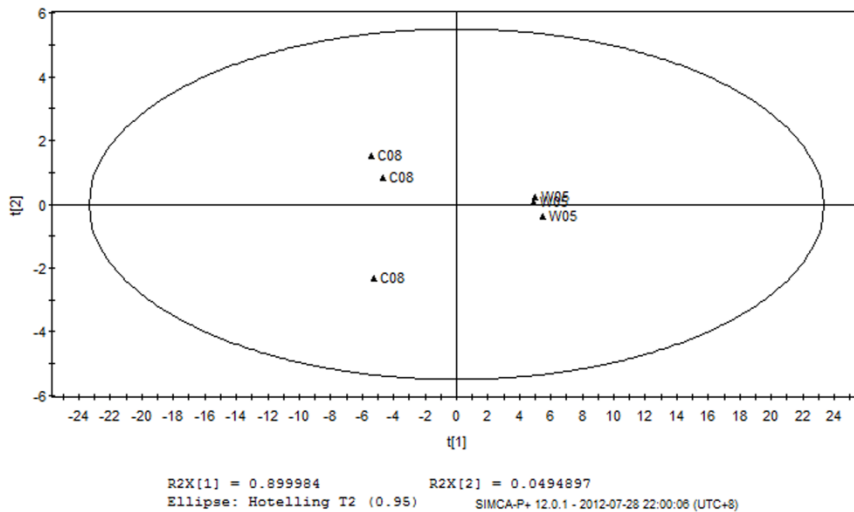
Isoflavonoids		Flavonoids		Amines		Pigments		Phospholipids	
94	Daidzein	105	Isorhamnetin	119	Adenosine	125	Carotene (α/β)	128	PC(12:0/16:0)
95	Daidzin	106	Isorhamnetin-3-(6"-acetylglucoside)	120	Methylguanosine	126	Cyanidin	129	PC(16:0/12:0)
96	Formononetin	107	Isorhamnetin-3-glucoside	121	Uracil	127	Cyanidin-3-(6"-acetylglucoside)	130	PC(14:0/18:2(9Z,12Z))
97	Genistein	108	Kaemferol	122	Uric acid			131	PC(18:2(9Z,12Z)/14:0)
98	Genistin	109	Kaempferol-3-(6"-acetylglucoside)	123	Uridine			132	PE(12:0/18:0)
99	Glycitein	110	Kaempferol-3-glucoside	124	Xanthosine			133	PE(18:0/12:0)
100	Glycitin	111	Luteolin					134	PE(14:0/18:3(6Z,9Z,12Z))
101	Malonyldaidzin	112	Luteolin-7-glucoside					135	PE(14:0/18:3(9Z,12Z,15Z))
102	Malonylgenistin	113	Naringenin					136	PE(18:3(6Z,9Z,12Z)/14:0)
103	Malonylglycitin	114	Naringin					137	PE(18:3(9Z,12Z,15Z)/14:0)
104	Ononin	115	Quercetin					138	PE(16:0/18:1(9Z))
		116	Quercetin-7-(6"-acetylglucoside)					139	PE(18:1(9Z)/16:0)
		117	Quercimeritrin						
		118	Quercitrin						

^a 26 metabolites were monitored by GC-MS. ^b 4 metabolites were detected by both GC-MS and LC-MS systems. The other 109 metabolites with no notation were monitored by LC-MS

Variation in the whole metabolome

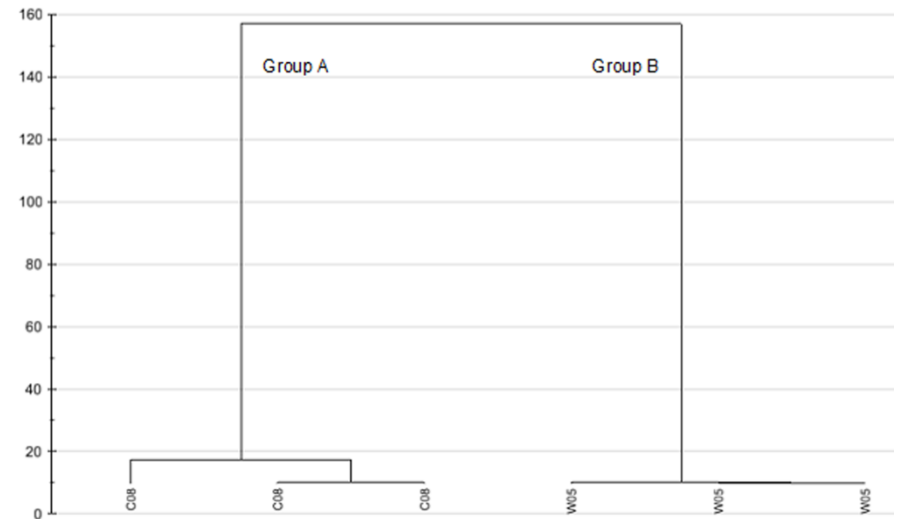


C08 W05



Principal component analysis (PCA)

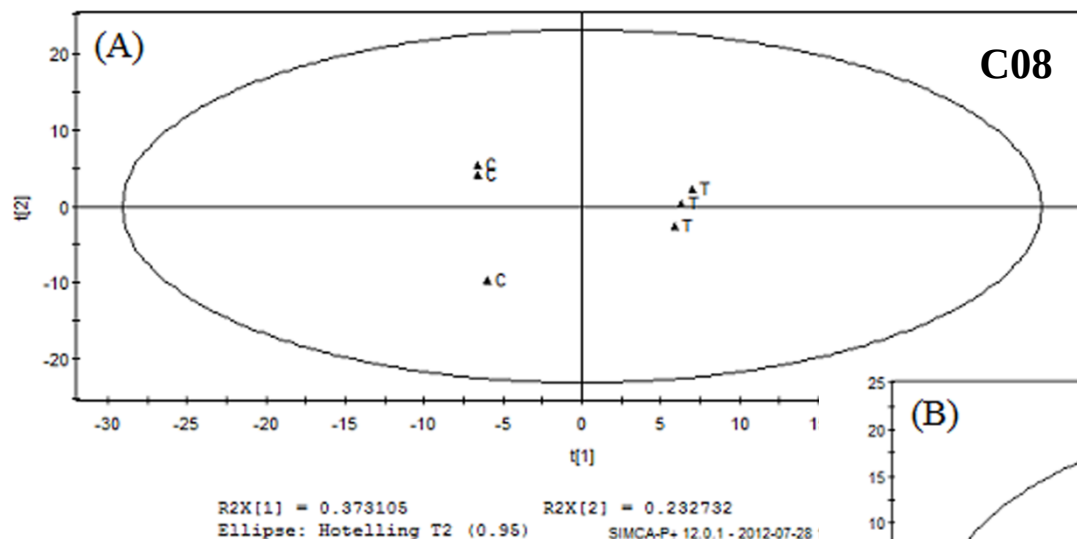
C08 W05



Hierarchical cluster analysis (HCA)

C08 vs. W05

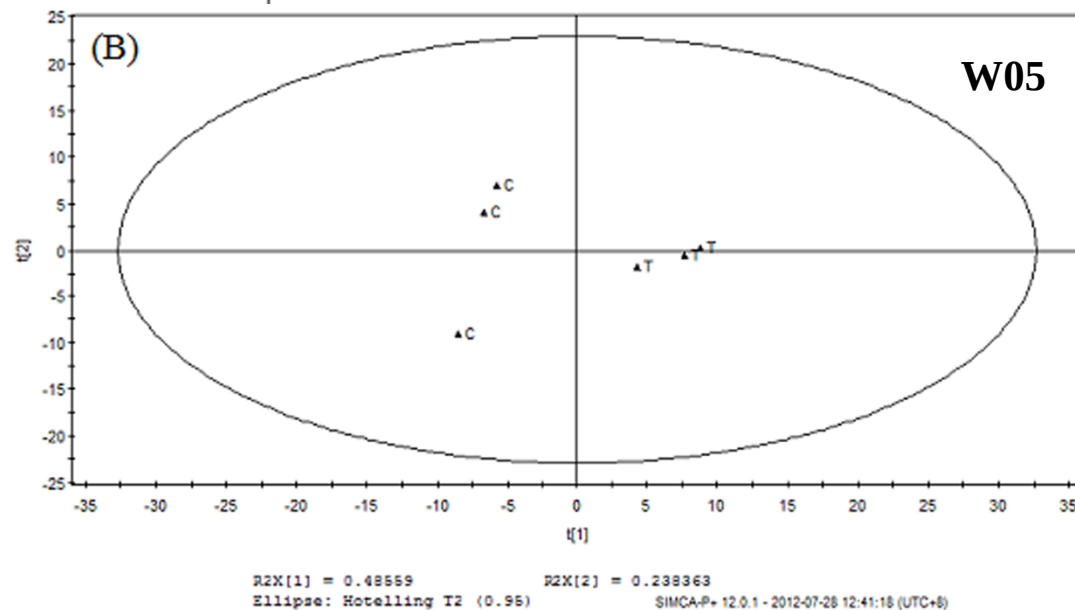
Variation in the whole metabolome



C: normal condition
T: salt stress condition

PCA

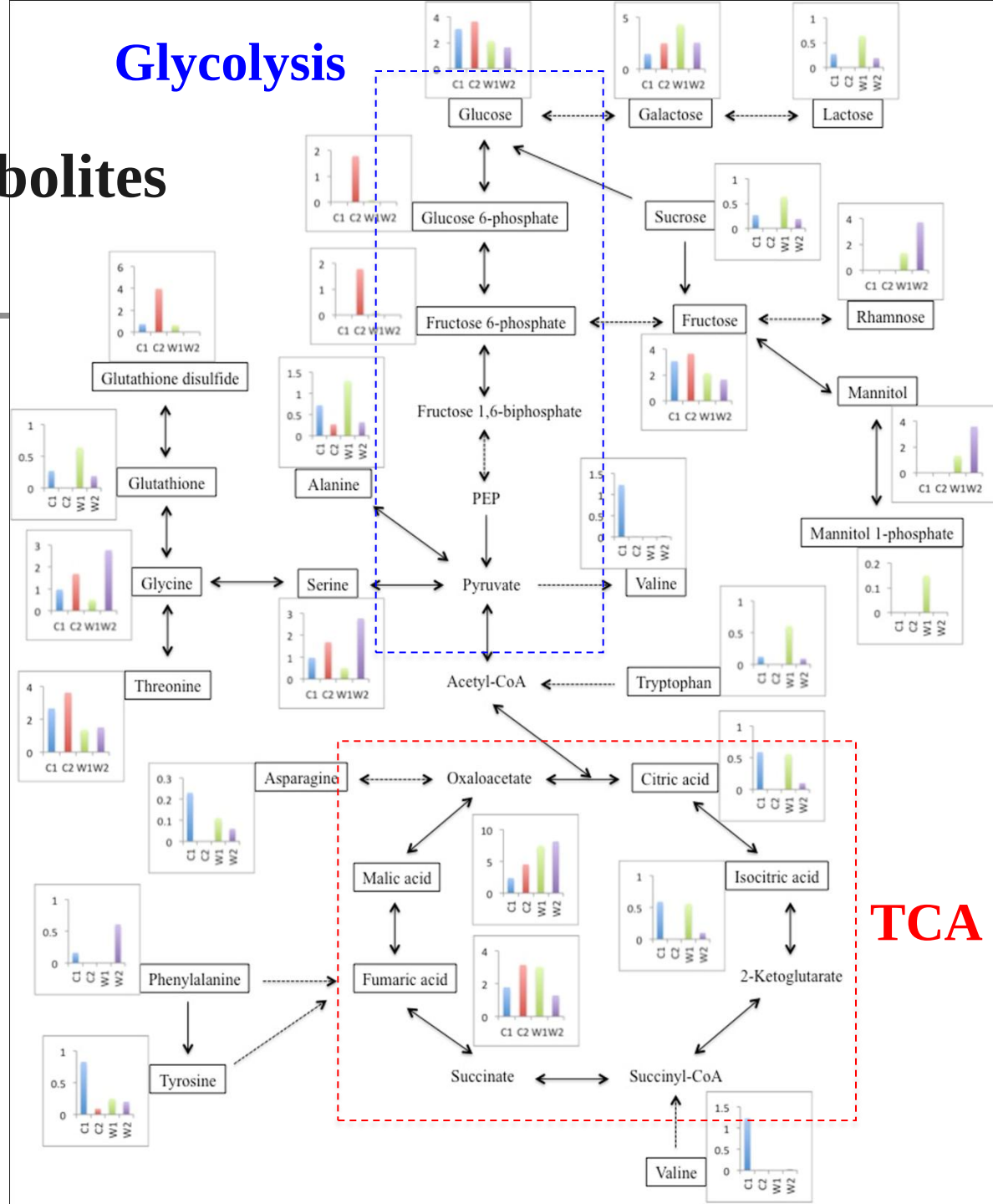
Control vs. Salinity



Altered metabolites

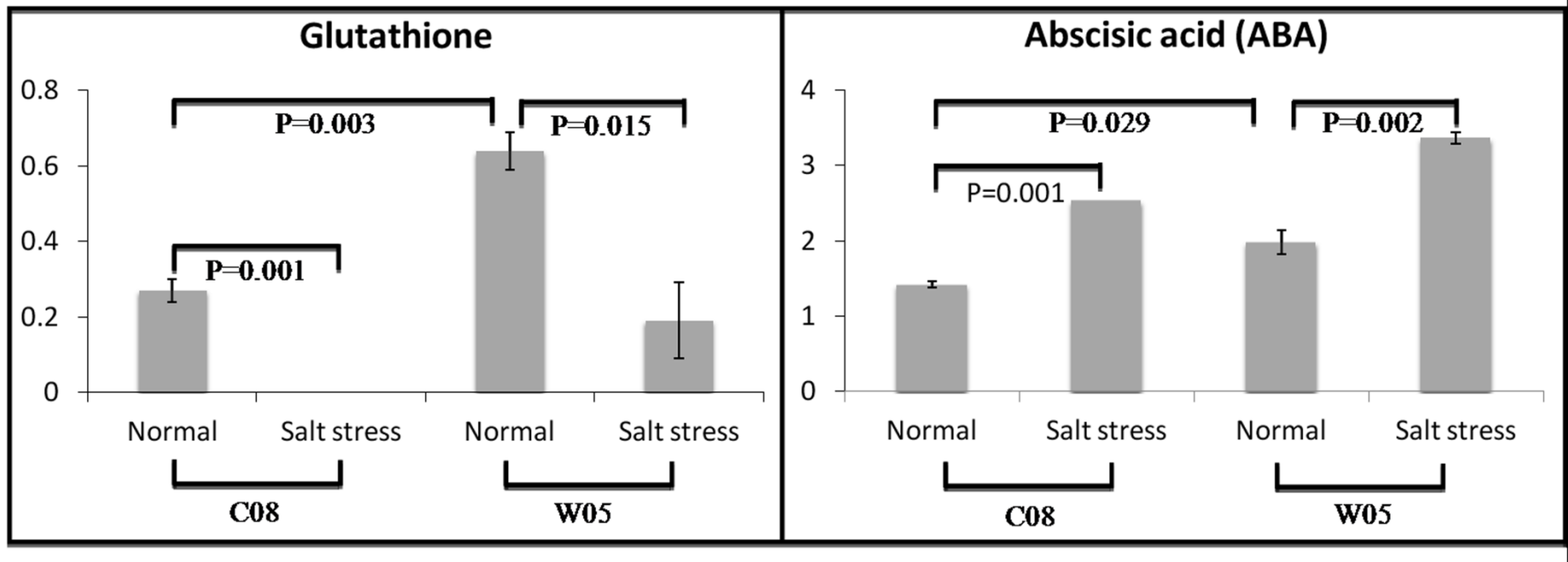
C1, C08 under normal condition;
C2, C08 under salt stress condition;
W1, W05 under normal condition;
W2, W05 under salt stress condition.

Glycolysis



TCA

Potential salt-tolerant mechanisms



non-enzymatic reactive oxygen
species (ROS) scavenger

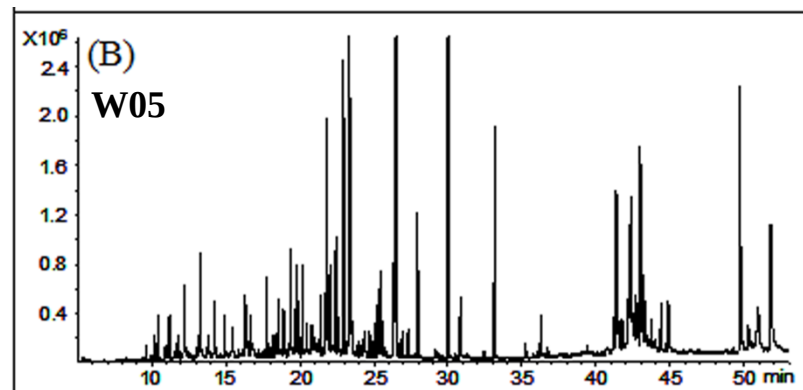
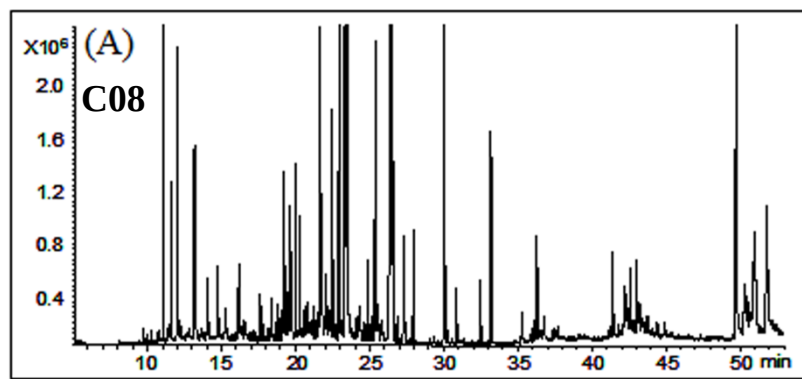
plant hormone

Eight offspring lines

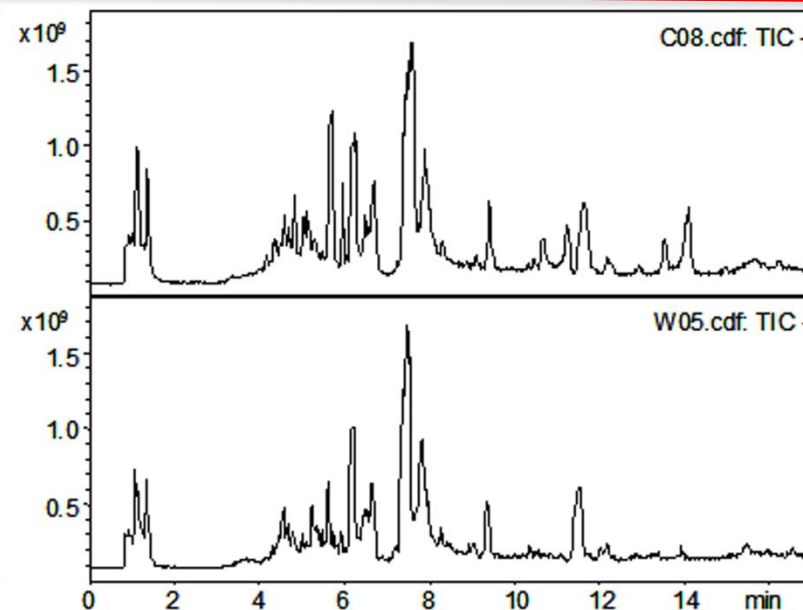
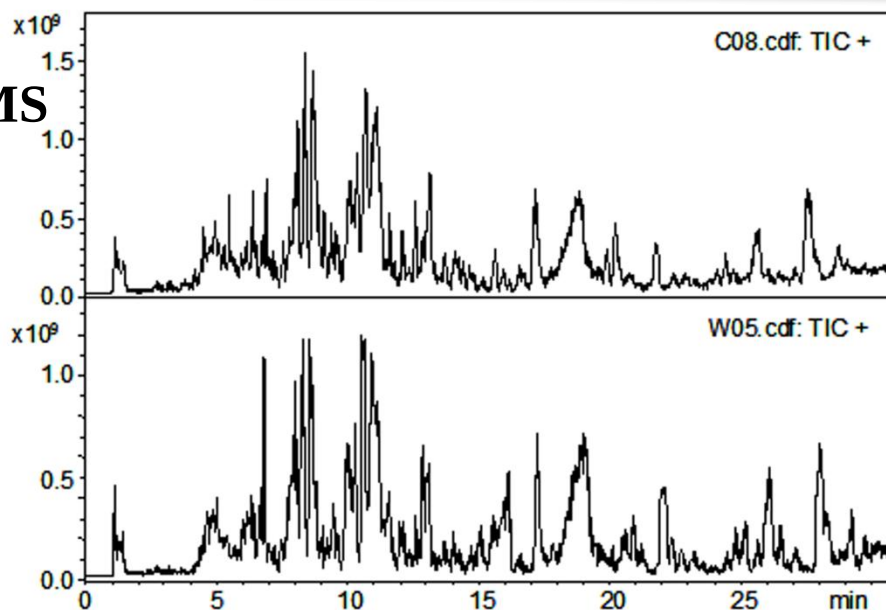


Metabolic profiling of C08 and W05

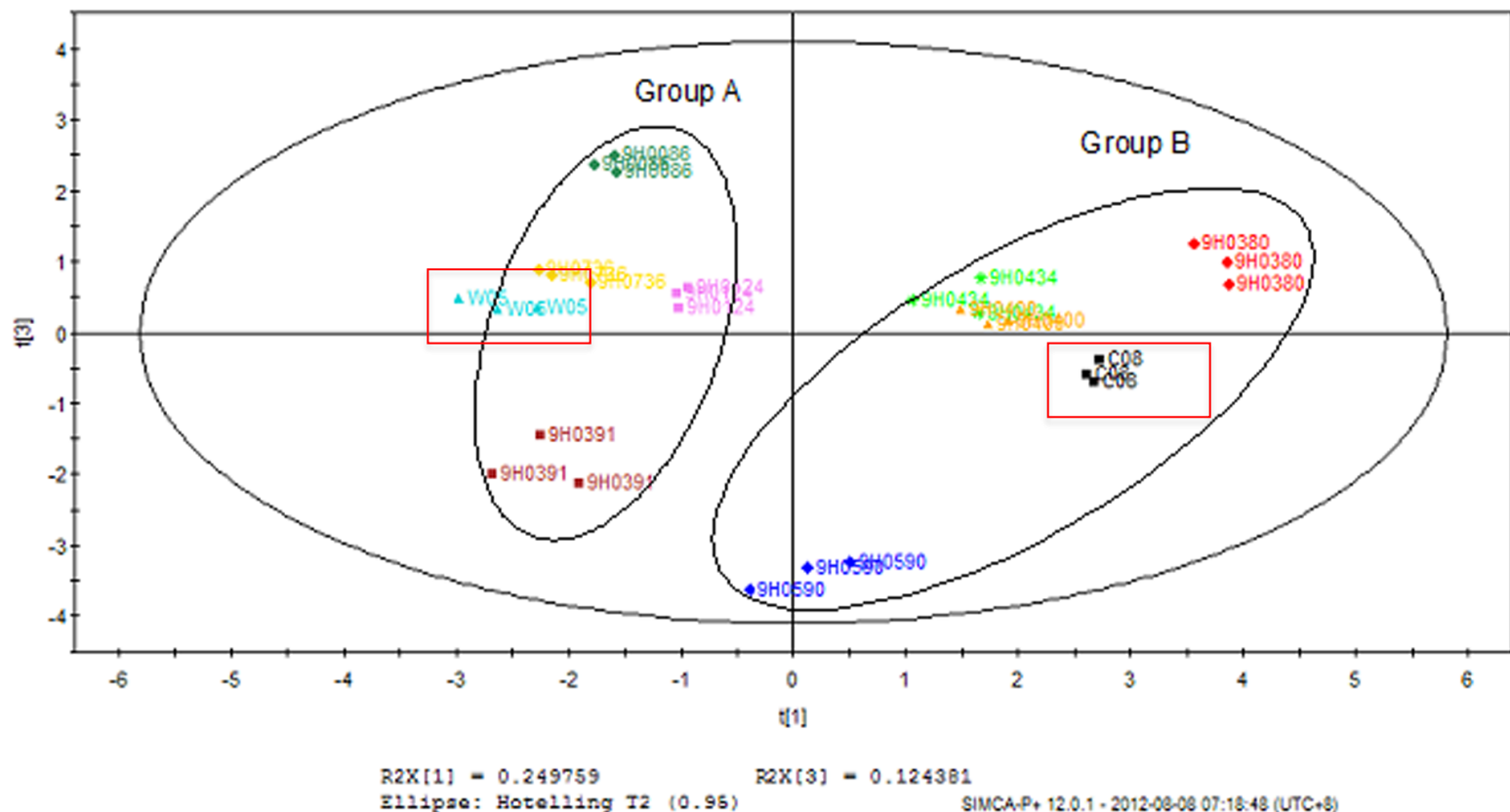
GC-MS



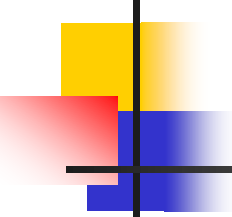
LC-MS



Cluster analysis of eight hybrid offspring soybeans



Orthogonal partial least square discriminant analysis (OPLS-DA)



Summary

- ❖ The metabolome of soybean seedling leaves is mainly dominated by 139 metabolites, including carboxylic acids, sugars, amino acids, fatty acids, alcohols, isoflavonoids, flavonoids, amines, phospholipids and pigments.
- ❖ The levels of glutathione and ABA in W05 is higher than those in C08 even under normal condition. This is possibly the main reason why W05 is more salt-tolerant than C08.
- ❖ On the basis of metabolomic analysis of the two parental soybean lines, eight hybrid offspring soybean varieties could be discriminated with OPLS-DA.

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Thank you!

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