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Organ-Specific Proteomic Analysis of NaCl-Stressed Germinating Soybeans

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Supporting Information

ABSTRACT: A comparative proteomic approach was employed to explore proteome expression patterns in germinating soybeans under NaCl stress and NaCl−aminoguanidine treatment. The proteins were extracted from 4-day-old germinating soybean cotyledons and noncotyledons (hypocotyl and radicle) and were separated using two-dimensional polyacrylamide gel electrophoresis. A total of 63 and 72 differentially expressed proteins were confidently identified by MALDI-TOF/TOF in the noncotyledons and cotyledons, respectively. These identified proteins were divided into ten functional groups and most of them were predicted to be cytoplasmic proteins in noncotyledons. Moreover, γ-aminobutyric acid was accumulated while the major allergen (Bd 30K protein) was reduced in the germinating soybeans. The proteins involved in energy metabolism and in protein processing in endoplasmic reticulum were enriched under NaCl stress. Meanwhile, the negative effect of stress was aggravated once polyamine degradation was inhibited. Redistribution of storage proteins under stress indicated that storage proteins might not only function as seed storage reserves but also have additional roles in plant defense.

KEYWORDS: *soybean, proteomics, NaCl stress, polyamines, germination, γ-aminobutyric acid*

INTRODUCTION

Nowadays, improvement of conventional foods is desirable since functional foods are playing a positive role in disease prevention.[1] Disease control and prevention through diet is considered to be the most efficient way to improve health and bring down medical costs for the expanding global population.[2] Hence, bean sprouts, as one of the vegetables traditionally and popularly consumed in China, Korea, Japan, and Southeast Asian countries, are being targeted for designing functional foods. Functional bean sprouts contain health-promoting compounds such as γ-aminobutyric acid (GABA), phenolics, phytoalexin, and other antioxidants. Their content is greatly enhanced when sprouts are under various stimuli including biotic stress[3] and abiotic stress such as light-mediated stress, ultraviolet irradiation, hypoxia,[4] mechanical stimulation, and salt stress especially.[5,6] GABA and plant phenolics prevent certain forms of cancer and reduce the risk of cardiovascular diseases according to clinical research.[7] GABA and plant phenolics induced by salt stress in sprouts can be harnessed as a source of therapeutic and health-supporting functional ingredients, especially for diseases linked to diet and environmentally influenced diseases. Previous morphological, physiological, and genomic studies established much knowledge of metabolic process of these functional components in cellular and physiological aspects,[8] but a systematic understanding of the metabolic process of functional components and the parallel molecular processes of plant under stressful conditions, especially under salt stress, is still in its infancy.

Salt is commonly known to have negative effects on plant growth and development.[9] Polyamines (PAs) can enhance plant tolerance to various environmental stresses. Exogenous PAs raise stress tolerance of plants by stabilizing the signal transduction system, increasing cell membrane stability, maintaining cell osmotic balance, improving antioxidant enzyme system dynamics, and meliorating photosynthesis of plants.[10] The amount of PAs is increased under stressful conditions. However, it is unclear whether the enhanced endogenous PAs play a similar role to that of exogenous PAs. Additionally, research shows that GABA forms through the polyamine degradation pathway.[8] In plants, putrescine is first converted to γ-aminobutyraldehyde by diamine oxidase (DAO, EC 1.4.3.6), and then γ-aminobutyraldehyde is converted to GABA by aminoaldehyde dehydrogenase (AMADH, EC 1.2.1.19). Studies demonstrate that at least 30% of GABA accumulation is supplied by the polyamine degradation pathway under stressful conditions.[4,11] Therefore, further studies on the effect of endogenous PAs on responses to stress by high throughput analysis will provide a new understanding of the roles of endogenous PAs and the relationship between GABA and PAs. Aminoguanidine (AG) is a specific inhibitor of DAO, blocking the polyamine degradation pathway. It is convenient to study the action of endogenous PAs through inhibition of DAO activity; DAO is the rate-limiting enzyme of PA catabolism.

Proteomics is the best available molecular tool for analyzing the complete proteome in tissues or at the organelle level. It also describes the expression patterns of proteome changes in response to stressful conditions. Proteomics has been applied to research on different abiotic stresses for soybean seedlings including salt,[12,13] flooding,[14] and drought.[15] In these studies, soybean seedlings absorb nutrients from the external environ-

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ment and, especially, synthesize organic compounds via photosynthesis. However, the functional soybean sprouts should be germinated in artificial dark environment and without any external nutrients except their storage material.[3,4] Up to now, little information is available on the stress responses of soybeans germinating under dark conditions and their response at the proteome level in particular. Identification of distinct proteins has contributed to understanding of the molecular mechanism adopted by soybeans in response to stress, but the regulatory mechanisms of that operation in divergent organs are still unknown without comprehensive organ-specific proteome analyses.

Based on the aforementioned issues, in this study, proteomic responses of dark germinating soybean noncotyledons (hypocotyl and radicle) and cotyledons to NaCl stress, combined with NaCl and AG treatments, was investigated. The objective was to reveal the organ-specific defense strategy, the role of endogenous PAs, and the adaptive mechanisms of dark germinating soybeans against salt stress via identification of the differentially salt-induced endogenous PA-mediated and organ-specific proteins.

MATERIALS AND METHODS

Plant Growth Conditions. Dry soybean seeds (*Glycine max* L. cultivar *Yunhe*) were sterilized, washed, and steeped with distilled water at 30 ± 1 °C for 4 h. The soaked seeds were then placed in a bean sprouting machine to germinate in a dark incubator at 30 ± 1 °C with water containing the following additives: (A) CK, deionized water; (B) NaCl, 50 mM NaCl; (C) NaCl−AG, 50 mM NaCl and 2.5 mM AG. The culture solution was replaced every 1 day. Three independent biological experiments were performed, and 4 days after treatment, the cotyledons and noncotyledons (hypocotyl and radicle) were carefully collected and frozen in liquid nitrogen until analysis.

Malondialdehyde (MDA) Content Determination. The extent of lipid peroxidation in terms of MDA formation was measured following the method of Madhava and Sresty.[16]

GABA and Free PA (*f*PAs) Content Determination. GABA and *f*PA contents were analyzed as described by Yang et al.[11]

Protein Extraction and Two-Dimensional Polyacrylamide Gel Electrophoresis (2-DE). To minimize errors, 10 cotyledons and noncotyledons (hypocotyl and radicle) from ten germinated soybeans, respectively, were pooled for each biological repeat sample. Protein extraction was performed using trichloroacetic acid (TCA)/acetone precipitation with some modifications.[17] Briefly, sample powder was suspended in 10% (w/v) TCA in acetone containing 1 mM dithiothreitol (DTT), and held at −20 °C for 2 h. After centrifugation and rinsing, the pellets were air-dried at room temperature and dissolved at 4 °C in lysis buffer containing 7 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate, 40 mM DTT, and 2% (v/v) Pharmalyte. Insoluble residue was removed through centrifugation, and the protein content was determined according to the Bradford method.[18]

Cotyledon protein (800 μg) was applied onto a 24 cm pH 4−7 linear gradient immobilized pH gradient (IPG) strip (GE Healthcare, Buckinghamshire, UK) and was subjected to electrophoresis at 20 °C for a total of 94850 V·h. Similarly, 800 μg of noncotyledon (hypocotyl and radicle) protein was applied onto an 18 cm pH 4−7 linear gradient IPG strip and was subjected to electrophoresis for a total of 90850 V·h. Subsequently, the strips were equilibrated,[19] and the second electrophoretic dimension was performed by 12% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and was run on the Ettan Six vertical set (GE Healthcare) in electrophoretic buffer at 15 °C with a cooling device (GE Healthcare). Finally, the gels were stained with Coomassie brilliant blue (CBB) G-250.

Gel Image Analysis. The stained 2-DE gels were digitized with a gel scanner (Imagescanner III, GE Healthcare), and analyzed with PDQuest software package (Version 7.2.0, BIO-RAD, California, USA). Spots were detected, matched, and normalized on the basis of total quantity of valid spots with the parameter of percent volume according to the software guide. For each protein spot, triplicate gels were used for each sample, and the mean relative volume was used to designate the significant and differentially expressed proteins (changed by more than 1.5-fold or less than 0.66-fold and statistically significant as calculated by one-way ANOVA, $P < 0.05$).

Peptide Preparation for Mass Spectrometry Analysis. Protein spots with differential expression patterns were excised from gels, washed with double-distilled water 3 times, destained with 25 mM NH_4HCO_3 for CBB G-250 staining spots, dehydrated with 50% (v/v) acetonitrile (ACN) in 25 mM NH_4HCO_3, reduced with 10 mM DTT in 50 mM NH_4HCO_3 for 1 h at 56 °C, alkylated with 55 mM iodoacetamide in 50 mM NH_4HCO_3 for 1 h at 25 °C, dried twice with 100% ACN, and digested overnight at 37 °C with sequencing grade modified trypsin (Promega, Madison, WI, USA) in 50 mM NH_4HCO_3. The peptides were extracted twice by washing the gel pieces with 0.1% trifluoroacetic acid (TFA) in 67% ACN. Extracts were pooled and lyophilized.

Protein Identification by Nano-Liquid Chromatography−Tandem Mass Spectrometry and Database Search. The lyophilized tryptic peptides were resuspended in 5 mg/mL α-cyano-4-hydroxy-trans-cinnamic acid containing 0.1% trifluoroacetic acid (TFA) and 50% ACN.[20] MALDI-TOF-TOF MS analyses were conducted using an ABI 4800 Plus MALDI-TOF-TOF analyzer (Applied Biosystems, Foster City, CA, USA). Both the MS and MS/MS data were integrated and search was performed using the GPS Explorer software V3.6 (Applied Biosystems, USA) over the NCBI database with the help of the MASCOT search engine V3.5 (Matrix Science Ltd., London, U.K.). The following parameters were used in the search: NCBInr Viridiplantae (Green Plants) database; trypsin as the digestion enzyme; one missed cleavage allowed; cysteine carbamidomethylation as a fixed modification and methionine oxidization as a variable modification; no fixed modifications; 50 ppm for peptide ion tolerance; 0.5 Da for fragment ion tolerance. Only significant hits, as defined by the MASCOT probability analysis ($P < 0.05$) or individual ion score > 49, were accepted.

Identified proteins were annotated to their biological function according to KEGG (http://www.kegg.jp/kegg/pathway.html) and literature. Information on these proteins was obtained from the Universal Protein Resource (http://www.uniprot.org/). Moreover, WOLF PSORT prediction (http://wolfpsort.org/) was used to predict subcellular localization of all identified proteins.

RESULTS

Growth Performance, Sprout Length, and MDA Content in Germinating Soybeans. To evaluate the effect of NaCl stress on germinating soybeans, the growth performance, sprout length, and MDA content in germinating soybeans were measured. Under NaCl stress, the sprouts were significantly shorter ($P < 0.05$) than those of the control at the same germination time, but there was no significant difference ($P > 0.05$) compared with those under NaCl−AG treatment (Figure 1A). The MDA content increased significantly ($P < 0.05$) after 4 days in both organs of the NaCl-stressed and NaCl−AG treated sprouts (Figure 1B). Figure 1C shows that the growth and development of untreated sprouts were better than those of sprouts under NaCl stress and NaCl−AG treatments. The growth was inhibited and membrane integrity was lost after 4 days in both the NaCl-stressed and the NaCl−AG-treated sprouts.

GABA and *f*PA Contents in Germinating Soybeans. The GABA and *f*PA contents in the cotyledons and noncotyledons (hypocotyl and radicle) of 4-day germinating soybeans under NaCl stress and NaCl−AG treatment were analyzed. After germinating for 4 days under NaCl stress,

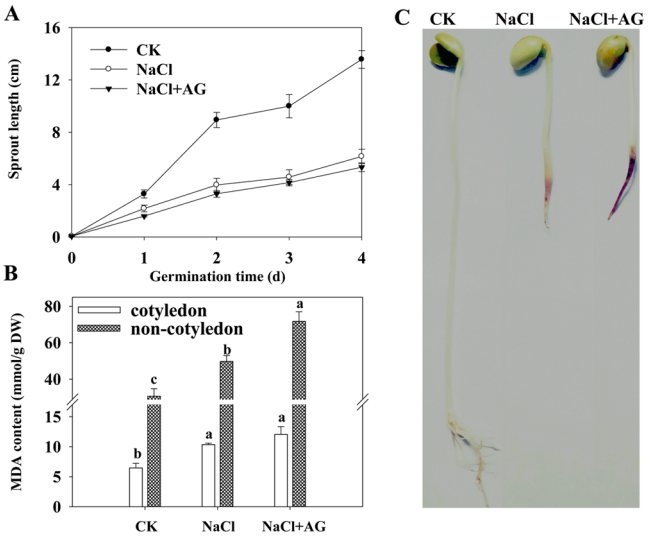


Figure 1. Effect of NaCl stress and NaCl−AG treatment on germinating soybean growth performance, sprout length, and MDA content. Noncotyledons: hypocotyl and radicle. Sprout length (A) and MDA content (B) of germinating soybeans were observed at 0, 1, 2, 3, and 4 days. The photograph of whole germinating soybeans was taken 4 days after treatment (C). Values are the mean ± SE (error bars). The lower case letters reflected the significance of differences in MDA content at germination for 4 days using the Duncan's test ($P < 0.05$).

GABA content was 1.54- and 1.70-fold higher than the control in the cotyledons and noncotyledons, respectively (Figure 2A). The GABA content in the noncotyledons of 4-day germinating soybeans treated with NaCl combined AG was not significantly ($P > 0.05$) different from that of the soybeans under NaCl stress, while the GABA content in cotyledons was significantly ($P < 0.05$) lower than in those under NaCl stress (Figure 2A). The *f*PA content decreased significantly in both organs of the 4-day germinating soybeans under NaCl stress compared with the control (Figure 2B). After treatment with AG, the DAO activity, as the rate limiting enzyme of PA catabolism, was completely inhibited (Supplementary Figure 1, Supporting Information), and the content of *f*PAs in 4-day germinating sprouts were all significantly ($P < 0.05$) higher than those of the sprouts under NaCl stress (Figure 2B).

Identification of Differentially Expressed Proteins Induced in Noncotyledons of 4-Day Germinating Soybeans under NaCl Stress and NaCl−AG Treatment. Comparative proteomic approach was used to determine the changes in protein abundance in noncotyledons of germinating soybeans exposed to NaCl stress and NaCl−AG treatment. The extracted proteins in the noncotyledons of 4-day germinating soybeans were separated using 2-DE and isoelectric focusing (IEF) on 18 cm linear IPG gels stained with CBB (Figure 3A). Three independent experiments were performed as biological replicates (Supplementary Figure 2, Supporting Information). The relative intensities of all the spots were analyzed using the PDQuest software. In total, more than 950 protein spots in germinating soybean noncotyledons were reproducibly detected on the 2-DE gels. A total of 67 proteins showed a less than 0.66-fold or more than 1.5-fold difference ($P < 0.05$) in relative volume in noncotyledons under different treatments. These proteins were excised from the gels for MALDI-TOF/TOF mass spectrometry. Finally, 63 proteins displayed in Table 1 were confidently identified according to the NCBI database (Supplementary Figure 3 and Supplementary Table 1, Supporting Information).

In the NaCl-treated group, 62 differentially expressed proteins were discovered compared with CK, including 37 (59.7%) up-regulated and 25 (40.3%) down-regulated (Figures 4A and 5A). These proteins were divided into 10 functional classes according to KEGG and the literature (Table 1, Figure 5A). Nine identified proteins were annotated as uncharacterized proteins, and six of them were searched for their homologues using BLAST (http://www.ncbi.nih.gov/BLAST/). At the amino acid level, the six proteins showed more than 65% positives, indicating that they might have similar functions (Table 2). All of the energy-related and synthesis-related proteins and most of the disease/defense-related noncotyledon proteins increased significantly under NaCl treatment, but all the transporter-related, signal transduction-related, and secondary metabolism-related and most uncharacterized noncotyledon proteins decreased under NaCl treatment (Figures 4A and 5A). The subcellular localization information of these identified proteins was also analyzed using WOLF PSORT prediction, which revealed that 36 (58%) differentially expressed proteins identified were predicted to be cytoplasmic proteins. All of the endoplasmic reticulum proteins, vacuole proteins, and mitochondrial proteins showed an increased abundance while half of the cytoplasmic proteins and chloroplast proteins showed a decreased abundance under NaCl treatment (Figure 5C).

A total of 51 noncotyledon proteins showed differential responses in the NaCl−AG treated group compared with the group under NaCl stress. Among these identified proteins, 22 were up-regulated proteins and 29 were down-regulated proteins (Figures 4A and 5B). For disease/defense-related

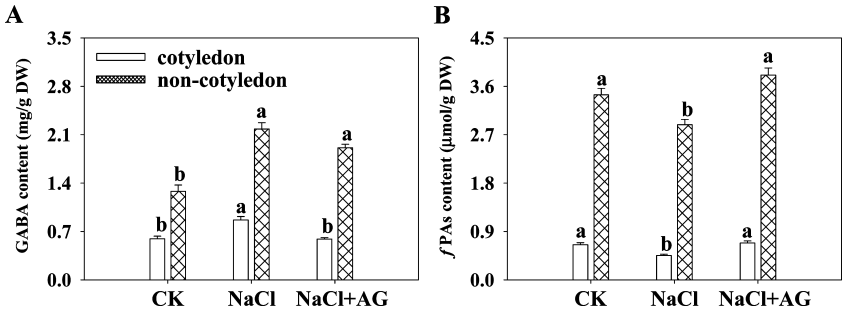


Figure 2. Changes in GABA and *f*PA content in 4-day germinating soybeans. The GABA content (A) and *f*PA content (B) in cotyledons and noncotyledons of germinating soybeans were determined. Noncotyledons: hypocotyl and radicle. Values are the mean ± SE (error bars). Data with the same letters were not significantly different compared with control as was determined using the Duncan's test ($P < 0.05$).

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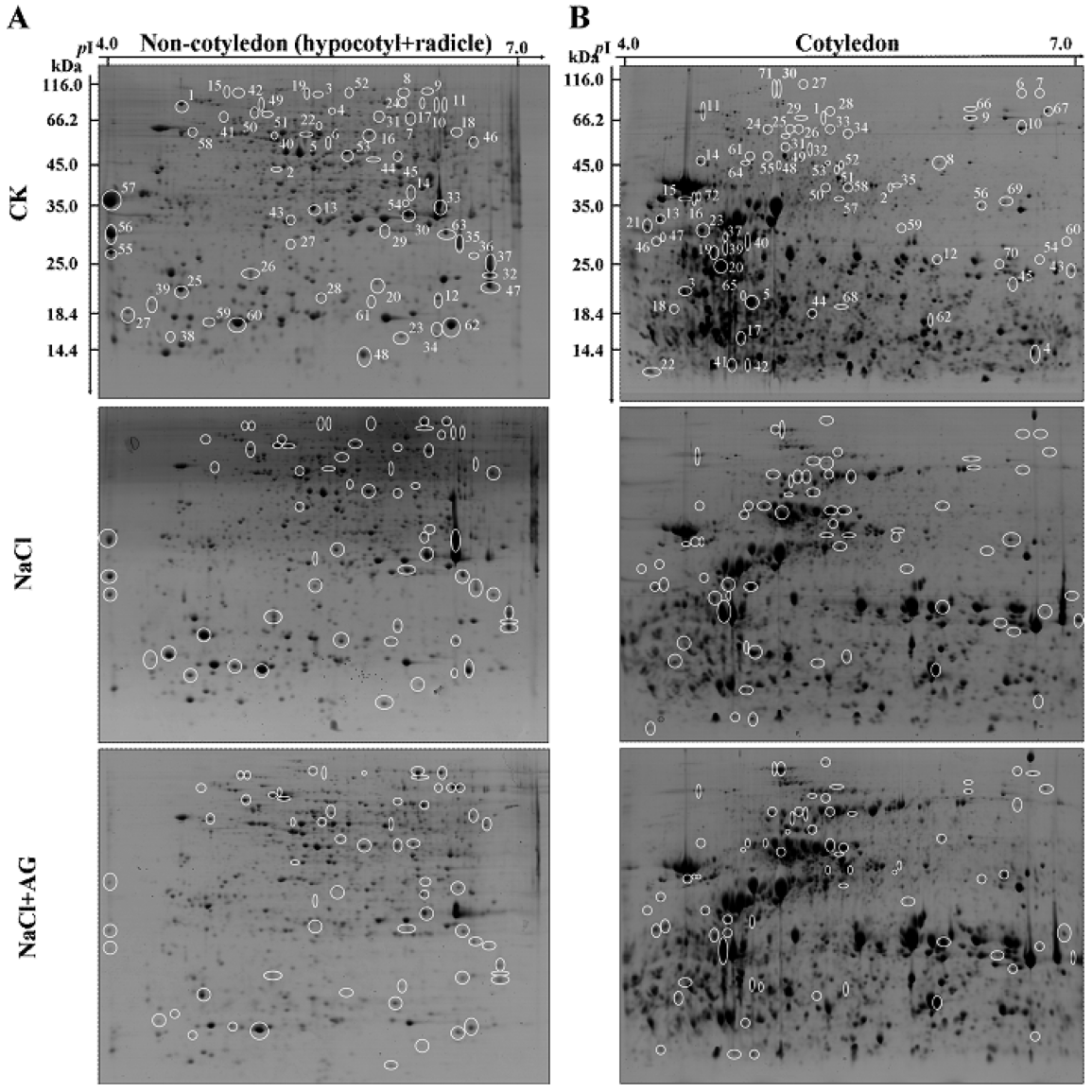


Figure 3. 2-DE pattern of 4-day germinating soybean noncotyledons and cotyledons proteins under NaCl stress or NaCl−AG treatment. Proteins extracted from noncotyledons (A) or cotyledons (B) were separated by 2-DE as described in the Materials and Methods and visualized with CBB staining. Noncotyledons: hypocotyl and radicle. Protein spot numbers are same as those shown in Tables 1 and 3.

proteins, the number of proteins that increased or decreased was equal. In addition, the majority of metabolism-related, energy-related, and protein synthesis-related proteins showed an increased abundance. However, the majority of the proteins involved in secondary metabolism, protein destination/storage, and metabolism showed a decreased abundance (Figures 4A and 5B). Most of the increased noncotyledon proteins were predicted to localize in the chloroplast, vacuole, and endoplasmic reticulum, while the cytoplasmic proteins and mitochondrial proteins were nearly equally increased or decreased (Figure 5D).

Identification of Differentially Expressed Proteins Induced in Cotyledons of 4-Day Germinating Soybeans under NaCl Stress and NaCl−AG Treatment. To analyze the extracted proteins in 4-day germinating soybean cotyledons, 24 cm linear IPG gels were used. Approximately 1150 protein spots were observed in all three biological replicates of stained gels (Supplementary Figure 4, Supporting Information). Among them, 72 protein spots that showed a more than 1.5-fold difference ($P < 0.05$) in abundance were successfully identified (Table 3; Supplementary Figure 4 and Supplementary Table 2, Supporting Information).

For the NaCl-treated group, a total of 72 proteins (45 up-regulated proteins and 27 down-regulated proteins) were significantly altered compared with CK (Figures 4B and 5E). Of these proteins, 45 (62.5%) were involved in the seed storage proteins (SSPs), and the remainder were divided into six functional classes, metabolism, energy, cell growth/division, proteolysis, transporters, and disease/defense (Table 3, Figure 5E). Most of the SSPs and metabolism-related proteins showed an increased abundance, while the energy-related, proteolysis-related, and disease/defense-related proteins showed a decreased abundance under NaCl stress. In total, different from the noncotyledon proteins, 55.5% of identified cotyledon proteins were predicted to localize in cytoplasm, and most of them were up-regulated under NaCl treatment (Figure 5G).

In cotyledons, 29 up-regulated and 13 down-regulated proteins were identified in response to NaCl−AG treatment

Table 1. Differentially Expressed Germinating Soybean Noncotyledon Proteins under NaCl Stress or NaCl Combined with AG Treatment after 4 Days

no.[a]	homolog protein[b]	accession no.[c]	plant species	tmr[d] (kda)	tpi[e]	score[f]	M.P.[g]	SC,[h] %	local[i]
			Metabolism[j]						
1	profilin-2	gil351721630	*Glycine max*	14.20	4.69	205	3	29	Cyto
2	glutamine synthetase PR-2	gil356520651	*Glycine max*	39.29	5.32	165	5	19	Chlo
3	phospholipase D α 1	gil356519347	*Glycine max*	91.94	5.48	219	3	6	Cysk
4	β-amylase	gil169913	*Glycine max*	53.98	5.31	312	3	9	Cyto
5	aminoaldehyde dehydrogenase	gil443294716	*Glycine max*	54.79	5.16	395	1	2	Chlo
6	5-methyltetrahydropteroyltriglutamate–homocysteine methyltransferase	gil356571744	*Glycine max*	84.39	5.97	425	5	7	Cyto
7	lipoxygenase-2	gil126402	*Glycine max*	97.41	6.08	57	1	1	Cyto
8	linoleate 9*S*-lipoxygenase-4	gil585418	*Glycine max*	96.64	5.71	528	10	13	Cyto
9	methionine synthase	gil351724907	*Glycine max*	84.40	5.93	432	3	8	Cyto
10	glutamine synthetase precursor	gil5733730	*Juglans nigra*	48.09	8.34	202	4	10	Chlo
11	UDP-*N*-acetylglucosamine pyrophosphorylase	gil356512205	*Glycine max*	54.69	5.88	54	3	6	Cyto
12	nucleoside diphosphate kinase	gil26245395	*Glycine max*	16.40	6.91	366	4	24	Cyto
			Energy[j]						
13	ATP synthase 24 kDa subunit	gil356512762	*Glycine max*	27.51	6.09	339	4	13	Mito
14	V-type proton ATPase subunit E	gil356528948	*Glycine max*	26.98	5.87	435	7	31	Mito
15	Os01g0654500	gil115438939	*Oryza sativa Japonica*	45.35	6.34	338	7	12	Cyto
16	NADP-dependent malic enzyme	gil356495891	*Glycine max*	65.32	5.83	274	5	12	Cyto
17	NADP-dependent malic enzyme	gil356495891	*Glycine max*	65.32	5.83	307	5	9	Cyto
18	ATPase subunit 1	gil372450254	*Millettia pinnata*	55.60	6.23	660	8	23	Mito
			Cell Growth/Division[j]						
19	cell division cycle protein 48	gil356543494	*Glycine max*	90.53	5.18	440	7	15	Cyto
20	expansin-like B1	gil356563389	*Glycine max*	28.16	6.30	609	8	32	Chlo
21	embryonic protein DC-8	gil356533407	*Glycine max*	48.77	6.12	780	10	25	Nucl
			Protein Synthesis[j]						
22	eukaryotic initiation factor 4A-15	gil356530401	*Glycine max*	47.11	5.46	494	8	25	Cysk
23	eukaryotic translation initiation factor 5A3	gil351721220	*Glycine max*	17.86	5.60	462	7	42	Cyto
24	elongation factor 2	gil356525774	*Glycine max*	94.95	5.80	71	2	3	Cyto
			Protein Destination and Storage[j]						
25	chain A, soybean trypsin inhibitor	gil3318877	*Glycine max*	20.31	4.61	862	9	58	Cyto
26	RuBisCO-associated protein	gil356505863	*Glycine max*	32.76	4.98	486	8	29	Cyto
27	Mcp20	gil18143656	*Matricaria chamomilla*	22.94	5.39	595	7	44	Chlo
28	peptidyl-prolyl isomerase FKBP1	gil357445945	*Matricaria chamomilla*	12.26	7.77	150	5	23	Cyto
29	31kD glycoprotein	gil226866	*Glycine max*	29.80	6.72	660	9	34	Cyto
30	31kD glycoprotein	gil226866	*Glycine max*	29.80	6.72	651	8	34	Cyto
31	subtilisin-like protease	gil356558946	*Glycine max*	82.53	6.24	619	9	17	Cyto
32	peptidyl-prolyl cis−trans isomerase CYP20−2	gil356576933	*Glycine max*	22.93	9.10	100	2	18	Chlo
			Transporters[j]						
33	coatomer subunit β'-2	gil356556044	*Glycine max*	35.38	5.77	586	10	33	Cyto
34	nuclear transport factor 2	gil356512387	*Glycine max*	13.72	5.86	358	4	45	Cyto
35	C nascent polypeptide-associated complex subunit α	gil356509137	*Glycine max*	23.72	4.43	134	3	19	Cyto
			Signal Transduction[j]						
36	auxin-binding protein ABP19a	gil356519747	*Glycine max*	22.00	6.95	73	2	12	Cyto
37	auxin-binding protein ABP19a	gil358248706	*Glycine max*	22.04	6.95	103	2	12	Cyto
			Disease/Defense[j]						
38	pathogenesis-related protein 1	gil356554880	*Glycine max*	17.99	5.14	378	5	45	Vacu
39	pathogenesis-related protein 1	gil356549447	*Glycine max*	19.32	4.54	538	7	69	Vacu
40	nucleoredoxin 1	gil356526803	*Glycine max*	64.97	4.86	885	8	19	Cyto
41	endoplasmin	gil356553371	*Glycine max*	93.62	4.83	74	3	4	Nucl
42	heat shock cognate protein 80	gil356552478	*Glycine max*	80.42	4.98	653	9	16	Cyto
43	ferritin light chain precursor	gil170078	*Glycine max*	28.15	5.73	344	6	18	Mito
44	MnSOD	gil351726054	*Glycine max*	26.69	8.56	317	6	29	Cyto
45	selenium-binding protein 1	gil356568342	*Glycine max*	53.79	5.67	665	9	26	Cyto
46	peroxidase precursor	gil4204759	*Glycine max*	39.23	6.85	444	6	19	Cyto
47	PR-5b protein precursor	gil351727321	*Glycine max*	26.81	6.26	250	4	27	Chlo
48	Hsc70	gil336539018	*Glycine max*	71.43	5.11	657	7	15	Cyto

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Table 1. continued

no.[a]	homolog protein[b]	accession no.[c]	plant species	tmr[d] (kda)	tpi[e]	score[f]	M.P.[g]	SC,[h] %	local[i]
			Disease/Defense[j]						
49	Hsc70	gil356505100	*Glycine max*	71.82	5.05	611	8	13	Chlo
50	luminal-binding protein 5	gil356523657	*Glycine max*	73.66	5.08	446	4	8	E.R.
51	endoplasmic reticulum HSC70	gil2642238	*Glycine max*	73.82	5.15	576	5	10	E.R.
			Secondary Metabolism[j]						
52	polyphenol oxidase A1	gil356519570	*Glycine max*	67.41	5.70	510	7	12	Chlo
53	caffeoyl-CoA *O*-methyltransferase	gil356499753	*Glycine max*	28.15	5.46	143	2	11	Cyto
54	isoflavone reductase	gil356518030	*Glycine max*	43.31	6.10	593	3	21	Cyto
			Uncharacterized Protein[j]						
55	uncharacterized protein LOC100783793	gil359806210	*Glycine max*	18.78	3.97	185	1	13	Chlo
56	uncharacterized protein LOC100788703	gil356522047	*Glycine max*	14.56	4.05	165	2	36	Chlo
57	uncharacterized protein LOC100801797	gil358248520	*Glycine max*	13.07	4.00	164	2	41	Chlo
58	uncharacterized protein At5g39570	gil356550983	*Glycine max*	40.86	4.66	512	6	20	Nucl
59	uncharacterized protein LOC100527097	gil351726232	*Glycine max*	16.70	4.80	116	3	24	Cyto
60	uncharacterized protein LOC100783267	gil359807353	*Glycine max*	17.15	5.10	180	3	19	Cyto
61	uncharacterized protein LOC100820359	gil356507945	*Glycine max*	19.00	5.92	368	4	40	Nucl
62	uncharacterized protein LOC100500241	gil351722771	*Glycine max*	11.25	4.95	168	4	43	Cyto
63	uncharacterized protein LOC100500685	gil351727803	*Glycine max*	21.65	6.43	406	5	30	Cyto

[a]Spot number; spot numbering corresponds to the 2-DE gel in Figure 3. [b]Homolog protein name and species obtained via the MASCOT software from the NCBInr database. [c]Accession number according to the NCBInr database. [d]Theoretical molecular weight. [e]Theoretical isoelectric point. [f]MOWSE score probability for the entire protein. [g]Number of query matched peptides. [h]Sequence coverage. [i]Localization category using local classification: Chlo, chloroplast; Mito, mitochondria; Nucl, nuclear; Cyto, cytoplasm; Cysk, cytoplasm skeleton; E.R., endoplasmic reticulum; Vacu, vacuole. [j]Functional classification.

compared with NaCl stress (Figures 4B and 5F). Similarly, the majority of these cotyledon proteins were SSPs. Moreover, all of the metabolism-related and energy-related proteins were up-regulated ones, and most of SSPs were down-regulated ones (Figure 5F). The chloroplast proteins and vacuole proteins were equally increased or decreased while most proteins that localized in cytoplasm, nucleus, and mitochondria increased (Figure 5H).

Proteins Commonly Responsive to Stress in the Noncotyledons and Cotyledons. In the present study, five differentially expressed proteins responsive to NaCl stress were identified in both noncotyledons and cotyledons compared with CK (Tables 1 and 3; Supplementary Figure 6A, Supporting Information). Expansin-like B1 (E20 and C12), coatomer subunit β'-2 (E33 and C69), and endoplasmin (E41 and C71) all showed an increased abundance in noncotyledons and cotyledons under NaCl stress compared with CK. In addition, the embryonic protein DC-8 (E21, C9, and C10) and the soybean trypsin inhibitor (E25 and C20) showed an increased abundance in the noncotyledons but decreased in the cotyledons. Comparison of NaCl treatment with NaCl−AG treatment indicates that coatomer subunit β'-2 (E33 and C69) and endoplasmin (E41 and C71) increased in noncotyledons but decreased in cotyledons (Figure 4B).

DISCUSSION

In the present study, the GABA content was markedly higher in both organs in the NaCl-treated germinating soybeans than in the untreated ones (Figure 2A), which confirmed that soybeans could be good materials for producing functional sprouts. Consistent with previous reports,[11] the DAO activity was completely suppressed by AG, and the GABA content decreased (Figure 2). Additionally, identical negative effects such as inhibited growth of germinating soybeans and destroyed integrity of membrane under NaCl treatment were detected, since soybeans were considered as salt-sensitive plants (Figure 1). In response to NaCl stress, the identified proteins associated with metabolism were involved in amino acid, starch, fatty acid, nitrogen, and nucleotide metabolism pathways. In plant, GABA can be formed through the GABA shunt and the polyamine degradation pathway.[6,11] On the one hand, compared with the control, the abundance of the glutamine synthetase PR-2 (spot E2) and the glutamine synthetase precursor (spot E10) were much lower under NaCl treatment (Figure 6). The glutamine synthetase (GS) catalyzed the assimilation of ammonium to glutamine using glutamate as its substrate. Hence, down-regulated GS implied a higher glutamate content, which was further converted to GABA through the GABA shunt. On the other hand, higher abundance of methionine synthase (MS, spot E9), 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (spot E6), and aminoaldehyde dehydrogenase (spot E5) were observed in the NaCl-treated soybeans (Figure 6). MS and homocysteine methyltransferase, which are involved in the polyamine biosynthesis pathway, catalyze the transfer of a methyl group, resulting in the formation of methionine. Subsequently, the decreased *f*PA content was converted to GABA by the polyamine degradation pathway, since aminoaldehyde dehydrogenase, identified as the indispensable enzyme of polyamine degradation pathway was up-regulated under NaCl stress. The decreased abundance of GS and increased abundance of MS have also been documented on soybeans under other stresses.[19] Compared with NaCl treatment, application of AG decreased GS, and the three enzymes referred to above, which were involved in polyamine biosynthesis, further increased. These facts suggested that more glutamate as substrate was converted to GABA because the polyamine degradation pathway was suppressed. The significantly increased *f*PA content in both organs would in turn promote ion homeostasis and lower the ROS levels. Moreover, the remarkable expression of methionine synthase was an effect

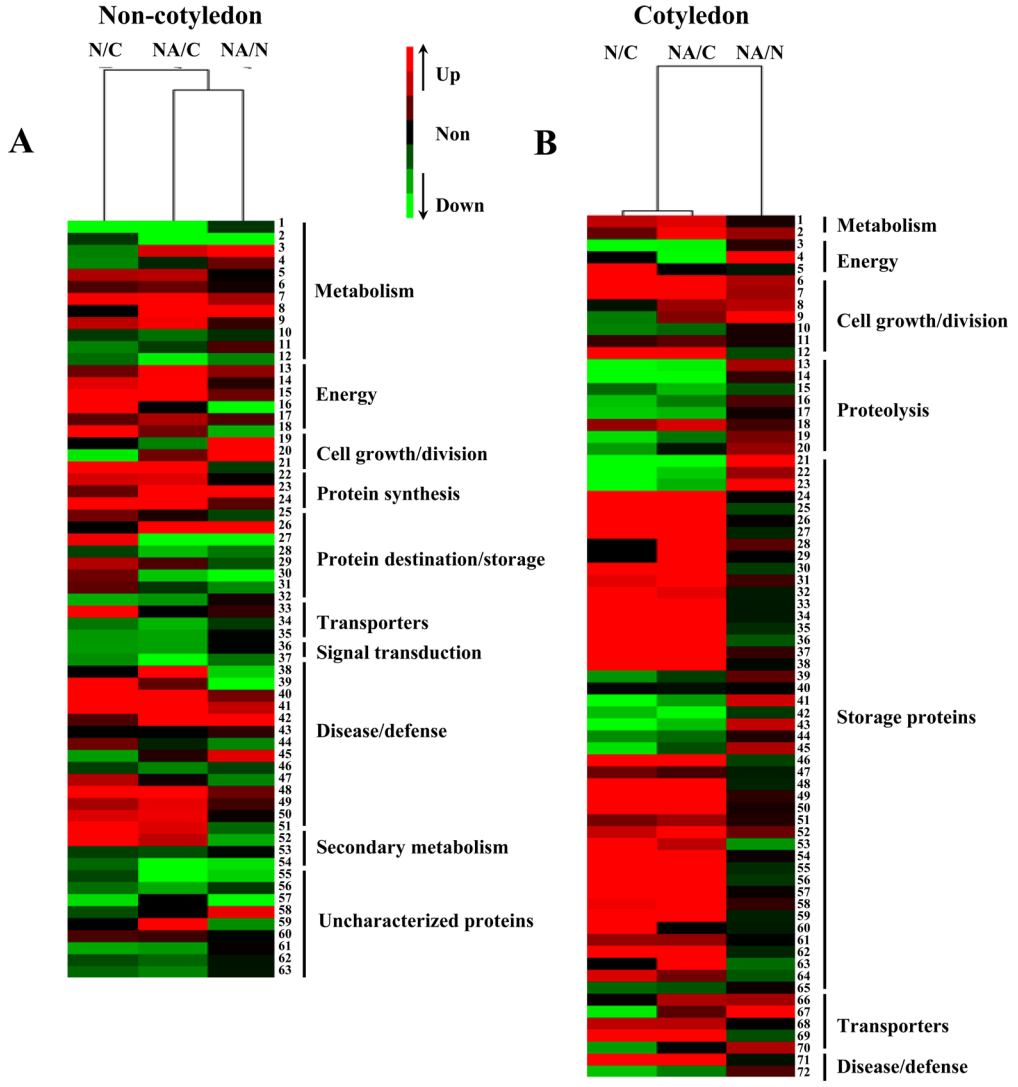


Figure 4. Proteins differentially expressed in response to NaCl stress or NaCl−AG treatment in noncotyledons and cotyledons. The expression change of noncotyledons (A) and cotyledons (B) based on the log2 transformed expression ratios of protein spots was performed using the Gene Cluster 3.0 software. The results were visualized using the JAVA TREEVIEW software. Noncotyledons: hypocotyl and radicle. C, CK; N, NaCl; NA, NaCl−AG treatment. Protein spot numbers are same as those shown in Tables 1 and 3.

of elevated activity of mechanisms that enhanced NaCl stress tolerance, since it was involved in detoxification of ROS.

In our proteome analysis, NaCl stress enhanced expression of all the identified proteins associated with energy metabolism. ATPases are membrane-bound enzymes, and they combine ATP synthesis with the transport of protons. The ATP synthase 24 kDa subunit (spot E13), the V-type proton ATPase subunit E (spot E14), the ATPase subunit 1 (spot E18), and two NADP-dependent malic enzymes (spots E16 and E17) are critical enzymes of glycolysis and the TCA cycle, which support plant growth. The V-type proton ATPase subunit E compartmentalizes Na^+ into the vacuole by generating a proton electrochemical gradient to supply a driving force utilized by the tonoplast Na^+/H^+ antiporter.[21] Studies have proven that increased expression in malate enzyme is an adaptive response to environmental stresses.[22] Up-regulated NADP-dependent malic enzyme in our soybean proteome analysis confirmed its key role in NaCl stress. Contrarily, the NADP-dependent malic enzyme (spot E16) and the ATPase subunit 1 (spot E18) decreased significantly while the other identified energy metabolism related proteins were not significantly different under NaCl−AG treatment compared with those under NaCl stress. This phenomenon suggested that NaCl stress dramatically enhanced energy metabolism in germinating soybeans while AG inhibited energy metabolism under NaCl stress.

Excess generation of reactive oxygen species (ROS), such as superoxide radical, hydroxyl radical, and hydrogen peroxide in plant cells, is an inevitable outcome of all abiotic stresses, especially NaCl stress. These ROS cause membrane damage and attack proteins and nucleic acids. Higher plants have developed an antioxidant enzymatic system to scavenge these toxic compounds. In addition, it has been proven that plant salt tolerance is positively associated with a more efficient antioxidant system. In the present study, Mn-superoxide dismutase (MnSOD, spot E44) increased significantly following its exposure to NaCl stress. This result indicated that MnSOD was the first line defense against the NaCl stress-induced ROS in germinating soybeans and suggested that the antioxidant defense mechanisms were more active in noncotyledons than in cotyledons under NaCl stress. Previous reports demonstrated that exogenous application of PAs improved the antioxidant enzyme system.[10] However, the expression of MnSOD and

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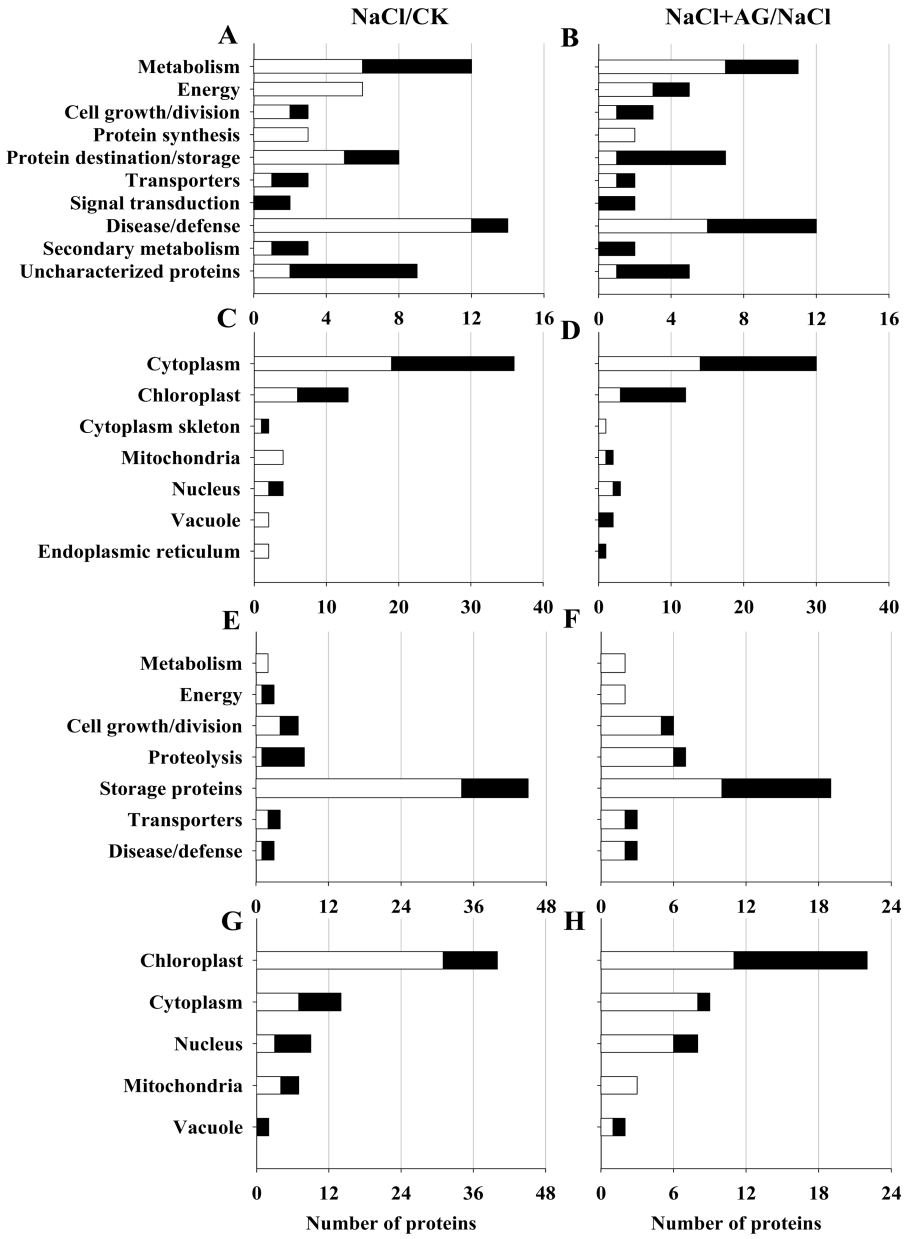


Figure 5. Classification of differentially expressed proteins in noncotyledons and cotyledons of 4-day germinating soybeans treated with NaCl or NaCl−AG. Noncotyledons: hypocotyl and radicle. The functions of differentially expressed proteins in noncotyledons (A, B), and cotyledons (E, F) treated with NaCl (A, E) and NaCl−AG (B, F) were assigned according to KEGG and literature. The localization-based classification of proteins in noncotyledons (C, D) and cotyledons (G, H) treated with NaCl (C, G) and NaCl−AG (D, H) is shown in the lower panels. Identified proteins were classified according to the subcellular localization predicted by WOLFPSORT prediction (http://wolfpsort.org). White and black columns represent increased and decreased proteins, respectively.

Table 2. Homologs of Uncharacterized Proteins in 4-Day Germinating Soybean Noncotyledons

		Homolog Protein					
no.	accession no.[a]	protein name	NCBI accession no.[b]	species	ident,[c] %	pos,[d] %	funct[e]
57	gil358248520	putative cell division protein	BAN18233.1	*Schiedea stellarioides*	64	72	cell growth/division
59	gil351726232	stress-induced protein SAM22	NP_001236038.1	*Glycine max*	92	98	disease/defense
60	gil359807353	MLP-like protein	XP_004489875.1	*Cicer arietinum*	75	88	disease/defense
61	gil356507945	glycine-rich protein 2b	XP_004505774.1	*Glycine max*	65	70	disease/defense
62	gil351722771	small ubiquitin-related modifier 1	XP_004513888.1	*Cicer arietinum*	93	95	metabolism
63	gil351727803	flavoprotein wrbA	XP_003545793.1	*Glycine max*	98	99	disease/defense

[a]The gi number of the uncharacterized proteins. [b]The accession number of homologues. [c]Identities. [d]Positives. [e]Functional classification.

POD decreased under NaCl−AG treatment. Increased endogenous PAs could not enhance the activities of ROS-scavenging enzymes to defend against the damage caused by ROS. This suggested that blocking the normal metabolic

Table 3. Differentially Expressed Germinating Soybean Cotyledon Proteins under NaCl Stress or NaCl Combined with AG Treatment after 4 Days

no.[a]	homolog protein[b]	accession no.[c]	plant species	TMr[d] (kDa)	TpI[e]	score[f]	M.P.[g]	SC,[h] %	local[i]
		Metabolism[j]							
1	UTP-glucose-1-phosphate uridylyltransferase	gil356553237	*Glycine max*	51.55	5.20	171	3	8	Cyto
2	lactoylglutathione lyase	gil356531939	*Glycine max*	33.56	6.14	211	3	14	Cyto
		Energy[j]							
3	ribulose bisphosphate carboxylase	gil224612766	*Asterella macropoda*	21.83	5.14	209	3	10	Cyto
4	RuBisCO small subunit 1	gil132086	*Glycine max*	20.28	8.87	287	6	21	Chlo
5	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	gil229464442	*Hylomecon japonica*	52.07	6.00	230	6	13	Cyto
		Cell Growth/Division[j]							
6	seed biotinylated protein 68 kDa	gil351724439	*Glycine max*	67.89	6.10	782	7	18	Mito
7	seed biotinylated protein 68 kDa	gil240254706	*Glycine max*	67.96	6.18	839	8	18	Mito
8	35 kDa seed maturation protein	gil351726750	*Glycine max*	35.32	5.96	450	8	28	Nucl
9	embryonic protein DC-8	gil356533407	*Glycine max*	48.77	6.12	575	8	22	Nucl
10	embryonic protein DC-8	gil356533407	*Glycine max*	48.77	6.12	785	10	21	Nucl
11	calreticulin-1 precursor	gil351726214	*Glycine max*	48.31	4.43	298	4	11	Mito
12	expansin-like B1	gil356563389	*Glycine max*	28.16	6.30	609	8	32	Chlo
		Proteolysis[j]							
13	Gly m Bd 30K	gil3097321	*Glycine max*	43.13	5.63	132	2	4	Nucl
14	cysteine proteinase RD21a	gil356553978	*Glycine max*	55.56	5.70	350	4	16	Chlo
15	Gly m Bd 30K	gil351721011	*Glycine max*	43.09	5.65	109	2	4	Vacu
16	Gly m Bd 30K	gil351721011	*Glycine max*	43.09	5.65	119	2	4	Vacu
17	Kunitz-type trypsin inhibitor B	gil125023	*Glycine max*	20.26	4.66	451	4	21	Cyto
18	soybean trypsin/chymotrypsin Bowman−Birk inhibitor	gil157830209	*Glycine max*	8.66	4.51	141	2	35	Chlo
19	Kunitz trypsin protease inhibitor	gil351722301	*Glycine max*	23.92	5.17	577	8	50	Cyto
20	chain A, soybean trypsin inhibitor	gil3318877	*Glycine max*	20.31	4.61	862	9	58	Cyto
		Storage Proteins[j]							
21	α′ subunit of β-conglycinin	gil9967361	*Glycine max*	65.16	5.23	334	6	12	Chlo
22	α′ subunit of β-conglycinin	gil28629838	*Glycine max*	44.99	5.62	76	2	6	Cyto
23	α′ subunit of β-conglycinin	gil9967357	*Glycine max*	63.18	4.92	317	3	8	Cyto
24	α′ subunit of β-conglycinin	gil9967361	*Glycine max*	65.16	5.23	646	8	16	Chlo
25	α′ subunit of β-conglycinin	gil9967361	*Glycine max*	65.16	5.23	852	9	22	Chlo
26	α′ subunit of β-conglycinin	gil9967361	*Glycine max*	65.16	5.23	886	9	20	Chlo
27	α′ subunit of β-conglycinin	gil9967361	*Glycine max*	65.16	5.23	712	8	20	Chlo
28	α′ subunit of β-conglycinin	gil9967361	*Glycine max*	65.16	5.23	843	9	20	Chlo
29	β-conglycinin, α chain	gil356575853	*Glycine max*	70.55	5.12	715	9	17	Chlo
30	β-conglycinin, α chain	gil121281	*Glycine max*	70.53	5.07	842	10	20	Chlo
31	β-conglycinin, α chain	gil121281	*Glycine max*	70.53	5.07	850	9	20	Chlo
32	β-conglycinin, α chain	gil121281	*Glycine max*	70.53	5.07	792	8	17	Chlo
33	α subunit of β conglycinin	gil9967357	*Glycine max*	63.18	4.92	594	8	16	Cyto
34	α subunit of β conglycinin	gil9967357	*Glycine max*	63.18	4.92	760	8	18	Cyto
35	β-conglycinin, α chain	gil121281	*Glycine max*	70.54	5.07	591	8	17	Chlo
36	β-conglycinin, α chain	gil121281	*Glycine max*	70.54	5.07	834	9	18	Chlo
37	β-conglycinin, α chain	gil121281	*Glycine max*	70.54	5.07	445	5	11	Chlo
38	soybean β-conglycinin β homotrimer complexes with *N*-acetyl-D-glucosamine	gil21465628	*Glycine max*	47.88	5.67	486	6	19	Mito
39	soybean β-conglycinin β homotrimer complexes with *N*-acetyl-D-glucosamine	gil21465628	*Glycine max*	47.88	5.67	396	5	15	Mito
40	soybean β-conglycinin β homotrimer complexes with *N*-acetyl-D-glucosamine	gil21465628	*Glycine max*	47.88	5.67	322	4	12	Mito
41	glycinin G1	gil225651	*Glycine max*	56.28	5.78	183	3	7	Chlo
42	glycinin G1	gil121276	*Glycine max*	56.30	5.89	158	3	6	Chlo
43	glycinin G1	gil121276	*Glycine max*	56.30	5.89	214	4	9	Chlo
44	mutant glycinin subunit A1aB1b	gil254029113	*Glycine max*	44.07	5.51	364	6	16	Chlo
45	glycinin A1bB2−784 precursor	gil351725539	*Glycine max*	54.83	6.01	136	1	5	Chlo
46	glycinin	gil6015515	*Glycine max*	24.35	4.46	901	7	43	Nucl
47	glycinin	gil6015515	*Glycine max*	24.35	4.46	655	4	32	Nucl
48	glycinin A1aBx	gil2225651	*Glycine max*	56.28	5.78	526	9	24	Chlo
49	glycinin A1aBx	gil2225651	*Glycine max*	56.28	5.78	293	4	16	Chlo
50	glycinin A1a subunit	gil121276	*Glycine max*	56.29	5.89	753	9	27	Chlo

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Table 3. continued

no.[a]	homolog protein[b]	accession no.[c]	plant species	TMr[d] (kDa)	TpI[e]	score[f]	M.P.[g]	SC,[h] %	local[i]
			Storage Proteins[j]						
51	glycinin A1a subunit	gil121276	*Glycine max*	56.29	5.89	390	5	15	Chlo
52	glycinin A1a subunit	gil121276	*Glycine max*	56.29	5.89	679	8	26	Chlo
53	glycinin A1a subunit	gil121276	*Glycine max*	56.29	5.89	670	9	24	Chlo
54	glycinin A1a subunit	gil121276	*Glycine max*	56.29	5.89	490	9	21	Chlo
55	glycinin G2 precursor	gil351725363	*Glycine max*	54.36	5.46	482	6	15	Chlo
56	glycinin G2 precursor	gil351725363	*Glycine max*	54.36	5.46	819	8	23	Chlo
57	glycinin G2 precursor	gil351725363	*Glycine max*	54.93	5.46	765	9	22	Chlo
58	glycinin G2 precursor	gil351725363	*Glycine max*	54.93	5.46	741	9	22	Chlo
59	glycinin G2 precursor	gil351725363	*Glycine max*	54.93	5.46	354	6	15	Chlo
60	glycinin A3B4 subunit	gil126144646	*Glycine max*	58.12	5.78	482	6	19	Chlo
61	glycinin G4 subunit	gil255224	*Glycine max*	64.09	5.38	695	6	16	Chlo
62	glycinin G4 subunit	gil255224	*Soybeans*	64.10	5.38	309	3	8	Chlo
63	Gy5	gil736002	*Glycine soja*	58.61	5.52	979	8	21	Chlo
64	glycinin A5A4B3 precursor	gil351734402	*Glycine max*	64.21	5.17	1019	8	23	Chlo
65	2S albumin precursor	gil351727517	*Glycine max*	19.02	5.20	149	6	13	Mito
			Transporters[j]						
66	maturation polypeptide	gil351723085	*Glycine max*	50.61	6.33	441	7	25	Nucl
67	maturation polypeptide	gil351723085	*Glycine max*	50.61	6.33	920	10	28	Nucl
68	sucrose-binding protein	gil356536206	*Glycine max*	58.35	6.08	209	4	9	Cyto
69	coatomer subunit β'-2	gil356556044	*Glycine max*	35.38	5.77	586	10	33	Cyto
			Disease/Defense[j]						
70	NAD-dependent formate dehydrogenase	gil4760553	*Oryza sativa*	41.45	6.87	244	3	11	Cyto
71	endoplasmin	gil356553371	*Glycine max*	93.62	4.83	74	3	4	Nucl
72	acidic chitinase	gil4835584	*Glycine max*	32.18	5.01	412	6	12	Chlo

[a]Spot number; spot numbering corresponds to the 2-DE gel in Figure 3. [b]Homolog protein name and species obtained via the MASCOT software from the NCBInr database. [c]Accession number according to the NCBInr database. [d]Theoretical molecular weight. [e]Theoretical isoelectric point. [f]MOWSE score probability for the entire protein. [g]Number of query matched peptides. [h]Sequence coverage. [i]Localization category using local classification: Chlo, chloroplast; Mito, mitochondria; Nucl, nuclear; Cyto, cytoplasm; Cysk, cytoplasm skeleton; E.R., endoplasmic reticulum; Vacu, vacuole. [j]Functional classification.

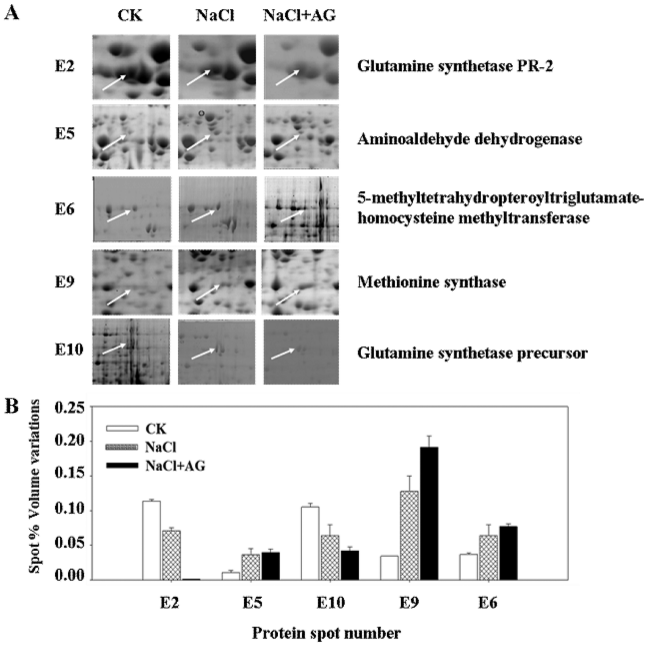


Figure 6. Differentially regulated proteins involved in GABA metabolism upon NaCl stress and NaCl−AG treatment in germinating soybeans. (A) Magnified views of differentially regulated proteins; (B) relative protein levels of differentia regulated proteins. Protein spot numbers are same as those shown in Tables 1 and 3.

processes of endogenous PAs would aggravate the negative effects of NaCl stress. It is noteworthy that nine differentially expressed proteins were associated with protein processing in endoplasmic reticulum, including four heat shock proteins (spots E42, E48, E49, and E51), two endoplasmins (spots E41 and C71), cell division cycle protein 48 (spot E19), luminal-binding protein 5 (spot E50), and calreticulin-1 precursor (spot C11). Almost all the proteins were up-regulated under NaCl stress except the cell division cycle protein 48. These results implied that promotion of protein processing in endoplasmic reticulum might be a very important strategy of germinating soybeans under NaCl stress.

Secondary metabolites play an important role in osmotic adjustment and ROS scavenging in response to environmental stressors.[23] Compared with CK, down-regulated caffeoyl-CoA-*O*-methyltransferase (CCoAOMT, spot E53) and isoflavone reductase (IFR, spot E54) and up-regulated polyphenol oxidase A1 (spot E52) in NaCl-stressed soybeans were detected in the present study. CCoAOMT plays a key role in the supply of substrates for the synthesis of lignin units.[24] Lignification of the cell wall may be one important strategy adopted by plants in response to NaCl stress. The down-regulated CCoAOMT might be linked to the reduced synthesis of monolignols, which was possibly due to stress-imposed growth suppression.[25] However, under NaCl−AG treatment, there was no significant difference in CCoAOMT abundance compared with NaCl treatment. IFR, associated with isoflavonoid biosynthesis pathways, was down-regulated under NaCl stress compared with CK and further significantly decreased under NaCl−AG

treatment. Additionally, polyphenol oxidase increased under NaCl stress but decreased after AG was added. It is well-known that isoflavonoids and polyphenol are potent antioxidants functioning as scavengers of ROS under stressful conditions. Accordingly, the accumulation of isoflavones and polyphenol could play an essential role in regulating the growth response. Taken together, these results indicated that the secondary metabolite synthesis pathways were targets for stress response, possibly altering the development of soybeans.

Auxin-binding protein ABP19a (spot E36 and E37), associated with auxin signaling pathway and mediating the action of auxins, was dramatically down-regulated under NaCl stress compared with the control. Furthermore, compared with NaCl treatment, its abundance decreased after AG was applied. It is known that auxins play a vital role in division and expansion of plant cells.[26] Therefore, the down-regulated auxin-binding protein ABP19a indicated that NaCl and NaCl−AG treatment inhibited plant growth of germinating soybeans, which was fully explained by the reduced sprout length and the inhibited growth performance.

In developing soybean seeds, the expression profiles of SSP are of a complex nature.[27] It has been displayed that SSP may not only function as seed storage reserves but also have additional roles.[28] Under stress conditions, SSP is mobilized and its composition and abundance is changed.[12,25,29,30] In the present study, 62.5% of the differentially expressed proteins (spots C21−C65) in the NaCl-stressed germinating soybean cotyledons represented various kinds of SSPs, almost all of which were assigned to β-conglycinin subunits and glycinin. β-conglycinin and glycinin are the hydrolysis products of globulin and are mobilized to provide carbon and nitrogen for seed germination and growth. GABA-enriched germinating soybeans were germinated in a dark artificial environment, so organic compounds could not be synthesized via photosynthesis. These storage materials were the major source of nutrients for the soybeans. Under NaCl stress, the growth of germinating soybeans was inhibited resulting in soybeans being unable to use SSPs for growth, and therefore they showed higher SSP abundance. However, the expression profiles of different types of subunits of β-conglycinin and glycinin were inconsistent in response to NaCl stress and NaCl−AG treatment. It remains to be seen whether these SSP changes could improve NaCl stress tolerance in germinating soybeans. Furthermore, only two up-regulated SSPs (spots E29 and E30) were detected and identified as 31 kD glycoprotein in noncotyledons. The 31 kD glycoprotein has been proven to accumulate during cell development and serve as a source of amino acids for the developing embryo. The up-regulated glutamine synthetase PR-2 (spot E1), aminoaldehyde dehydrogenase (spot E5), glutamine synthetase precursor (spot E6), methionine synthase (spot E9), and GABA content in the present study might also support the idea that 31 kD glycoprotein can be assumed from the paralleled uptake of amino acids.[25] In conclusion, these results might further indicate an additional role in plant defense of SSPs besides storage reserves.

A group of proteins such as eukaryotic initiation factor 4A-15 (elF, spot E22), eukaryotic translation initiation factor 5A3 (spot E23), and elongation factor 2 (spot E24), associated with protein biosynthesis pathways, were up-regulated by NaCl stress. Studies confirm that eukaryotic translation initiation factors play a key role in the cell division and cell death processes.[31] The elF was up-regulated under NaCl stress and further enhanced under NaCl−AG treatment. This could be caused by endogenous PAs, which were involved in the posttranslation activation of elF and increased significantly after application of AG under NaCl stress. Studies also show that protein synthesis can be enhanced by exogenous PAs.[32] However, our results indicated that increased endogenous PAs had a consistent stimulatory effect on the incorporation of amino acids into protein. The regulated expression response patterns of the three proteins indicated that the promotion of protein biosynthesis was required for germinating soybeans to respond to stress.

It is generally known that soybean seeds contain plenty of allergenic proteins, and thus their extensive usage was limited in the food industry. Gly m Bd 30K proteins (spots C13, C15, and C16) represent a major allergen because more than 65% of soybean-sensitive patients are allergic to the protein, although its content is lower than 1% in soybean seeds.[33] In the present study, Bd 30K protein showed a less than 0.42-fold or even 0.08-fold difference ($P < 0.05$) in abundance in germinating soybean cotyledons under NaCl stress or AG treatment, compared with CK.

The comparative proteomic and physiological analyses indicated that the molecular mechanisms that contributed to tolerance in germinating soybeans were complex. Based on the results of this study, the following conclusions could be drawn: (i) soybean germination under certain stressful conditions would be an effective way to accumulate functional ingredients and to decrease allergens; (ii) most of the differentially expressed proteins in soybean cotyledons were SSPs, and redistribution of SSPs is likely to play an important role in defense; (iii) down-regulated GS and up-regulated MS were responsible for accumulation of GABA and coping with NaCl stress; (iv) enhanced energy metabolism and accelerated protein processing in endoplasmic reticulum were very important strategies for germinating soybean response to NaCl stress; (v) the inhibition of polyamine degradation might further aggravate the negative effects of NaCl stress on germinating soybeans.

■ ASSOCIATED CONTENT

Supporting Information

Changes in DAO activity, 2-DE pattern from non-cotyledon and cotyledon proteins, volume variations of differentially expressed proteins in non-cotyledon and cotyledon, proteins commonly responsive to stress in the non-cotyledon and cotyledon, and peak lists for proteins identified in the non-cotyledon and cotyledon. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Figure Captions:

Supplementary Figure 1. Changes in DAO activity in cotyledons and non-cotyledons of 4-day germinating soybeans. ND: not detected. Non-cotyledons: hypocotyl and radicle.

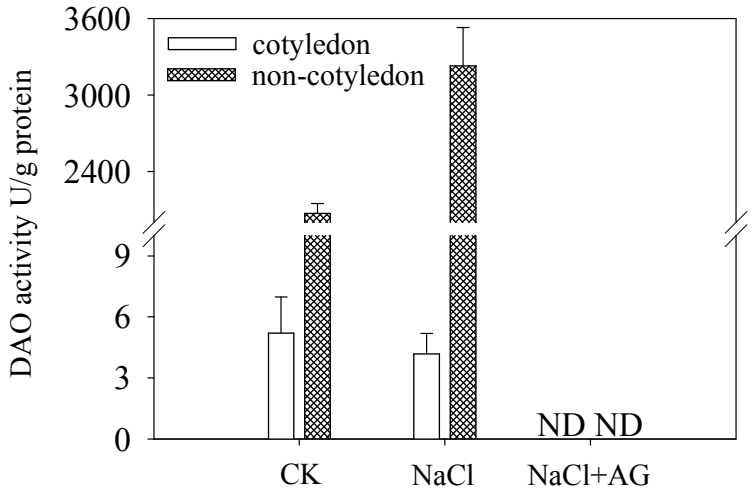
Supplementary Figure 2. 2-DE pattern from three biological replicates of 4-day germinating soybean non-cotyledon proteins under NaCl stress or NaCl-AG treatment. Non-cotyledons: hypocotyl and radicle.

Supplementary Figure 3. Volume variations of differentially expressed proteins in 4-day germinating soybean non-cotyledon. Non-cotyledons: hypocotyl and radicle. Staining intensities were quantified using PDQuest software. Results are presented as the mean±SE of relative protein intensity for gels from three biological replicates. Lower case letters reflect the significance of differences in intensity using the Duncan's test ($P < 0.05$). Spot numbers are same as those shown in Table 1.

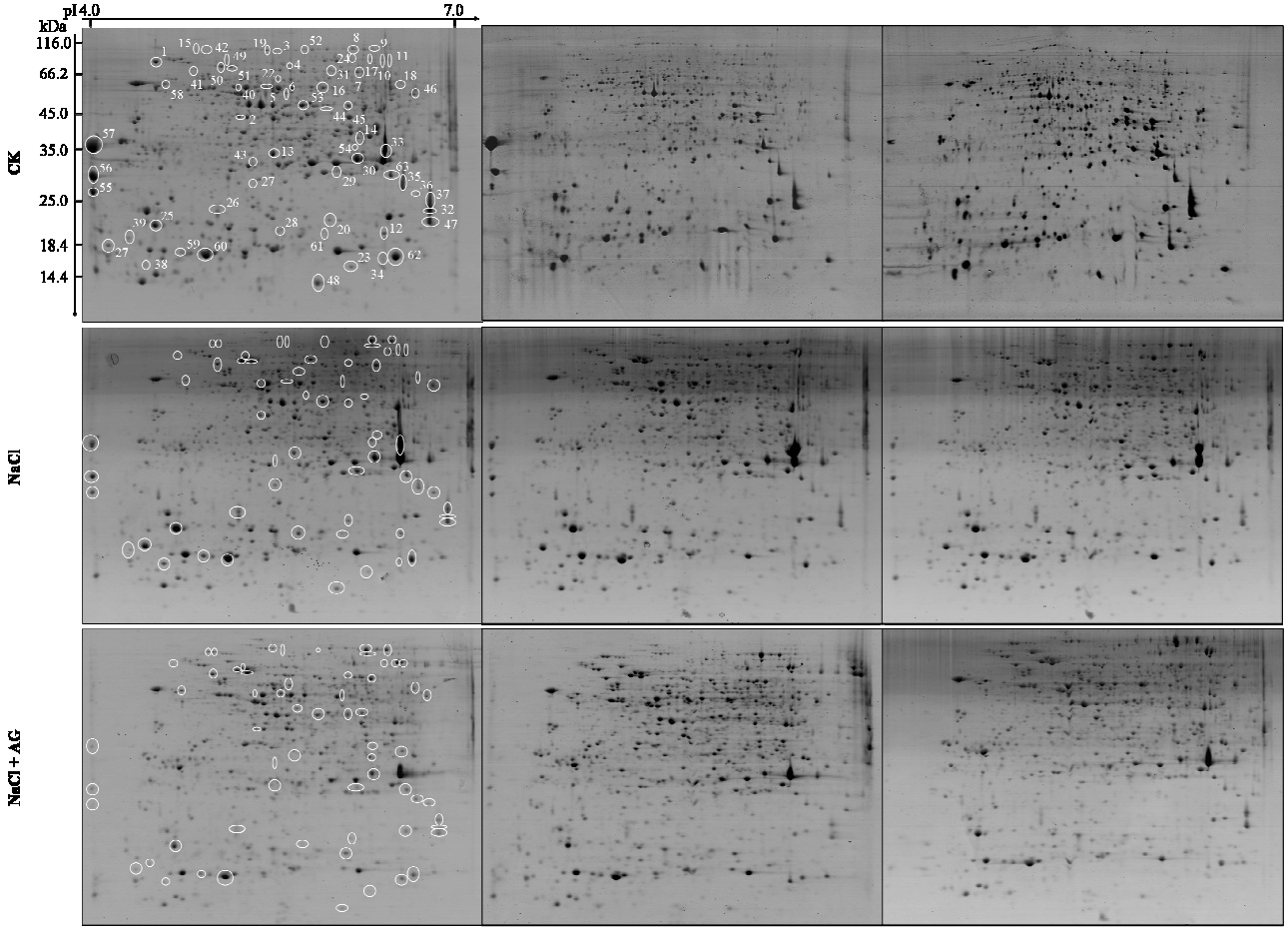
Supplementary Figure 4. 2-DE pattern from three biological replicates of 4-day germinating soybean cotyledon proteins under NaCl stress or NaCl-AG treated.

Supplementary Figure 5. Volume variations of differentially expressed proteins in 4-day germinating soybean cotyledons. Staining intensities were quantified using PDQuest software. Results are presented as the mean±SE of relative protein intensity for gels from three biological replicates. Lower case letters reflect the significance of differences in intensity using the Duncan's test ($P<0.05$). Spot numbers are same as those shown in Table 3.

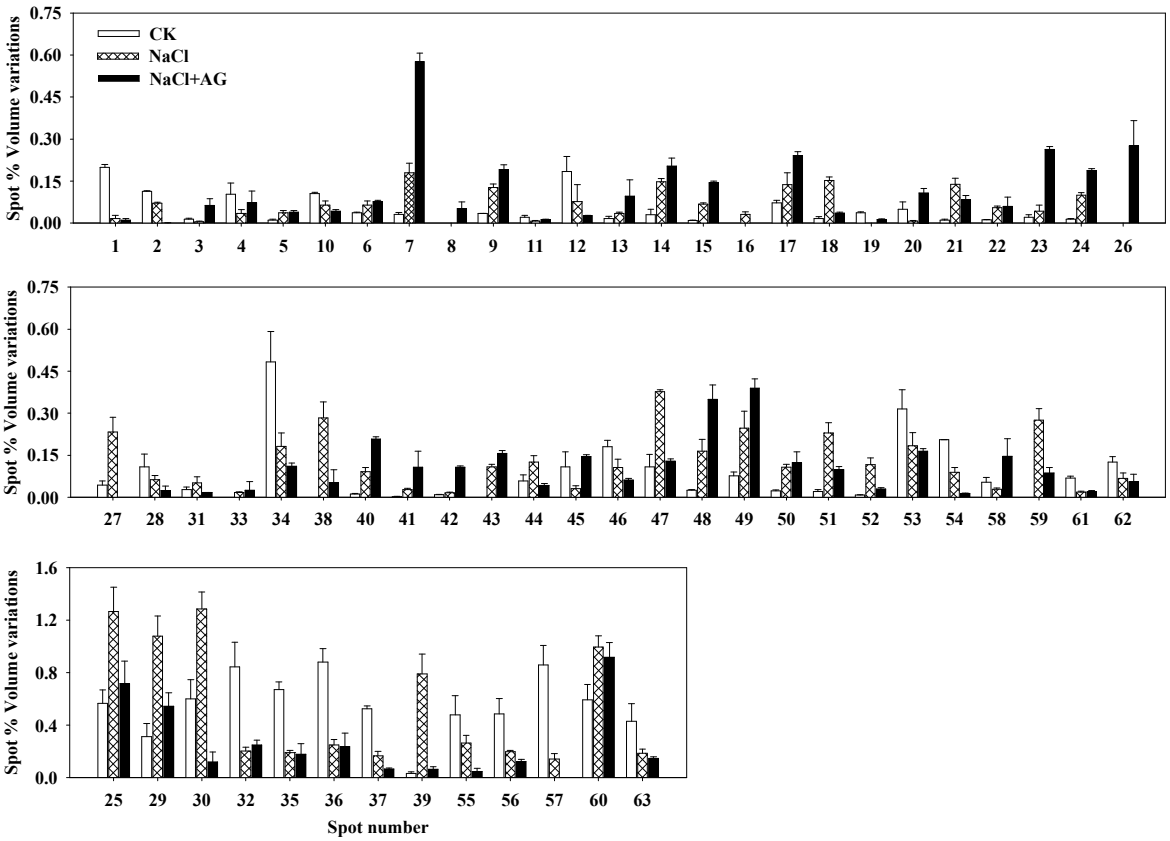
Supplementary Figure 6. Proteins commonly responsive to stress in the non-cotyledon and cotyledon of 4-day germinating soybeans. Non-cotyledon: hypocotyl and radicle. Spot numbers are same as those shown in Table 1 and Table 3.



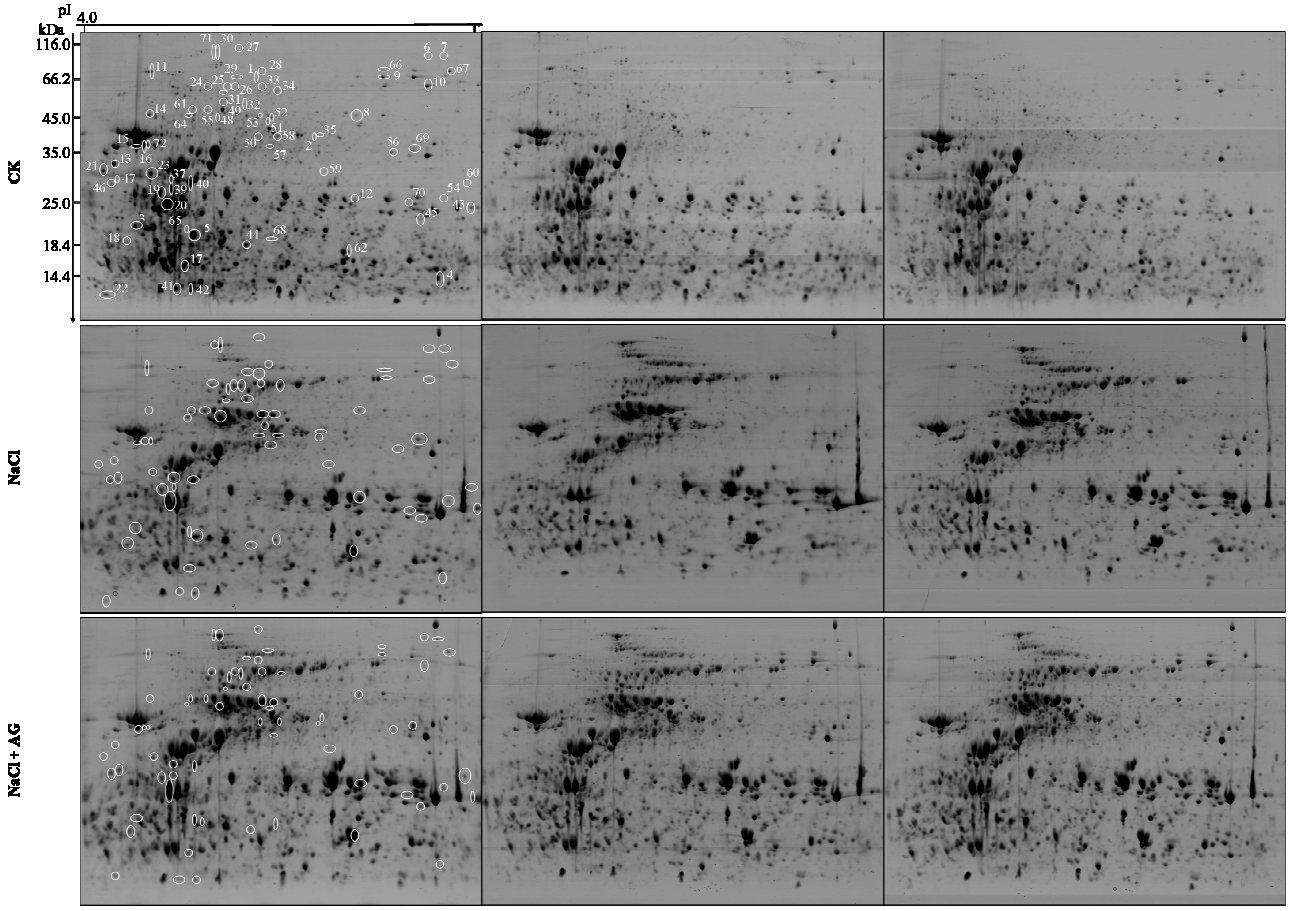
Supplementary Figure 1



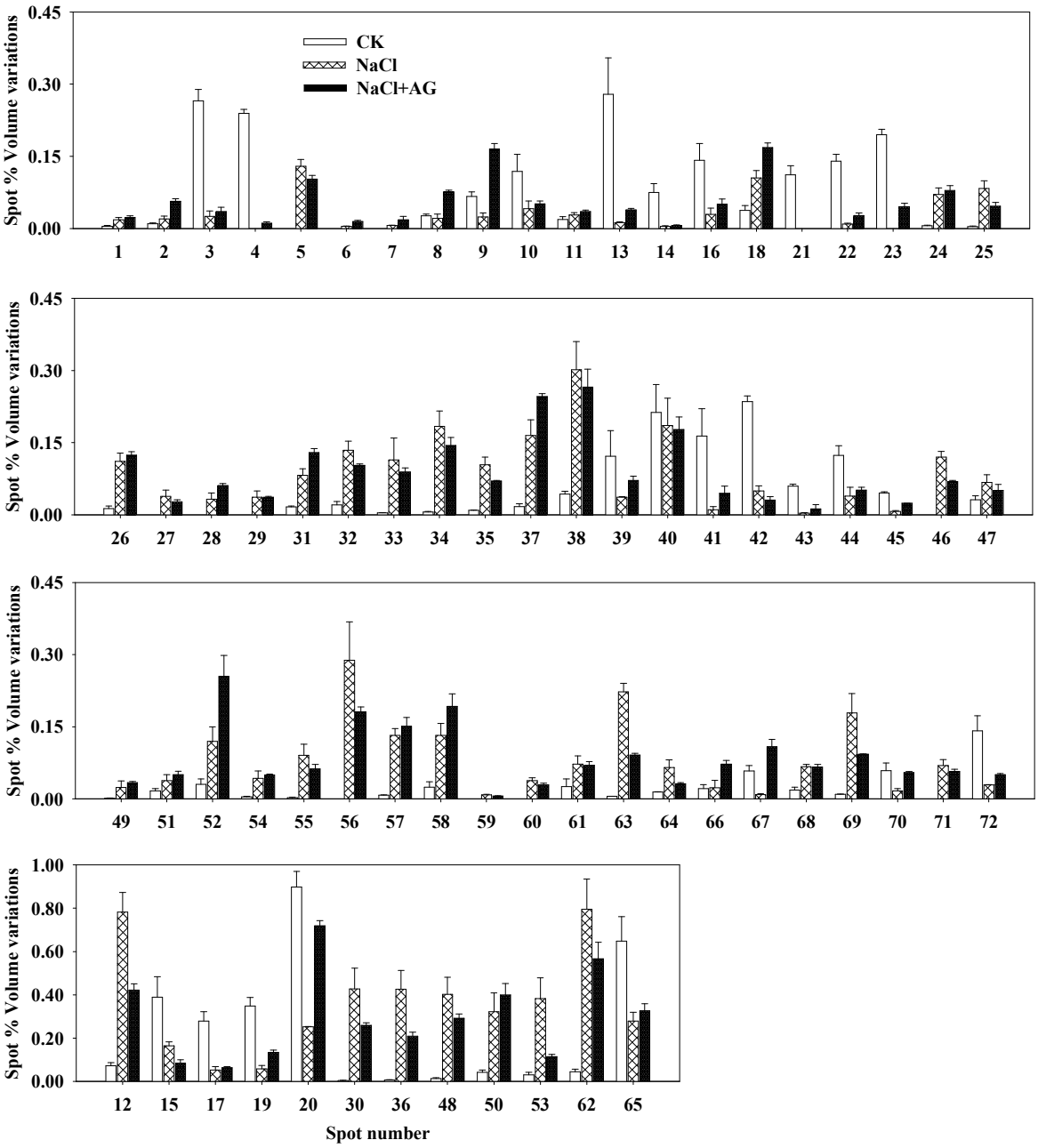
Supplementary Figure 2



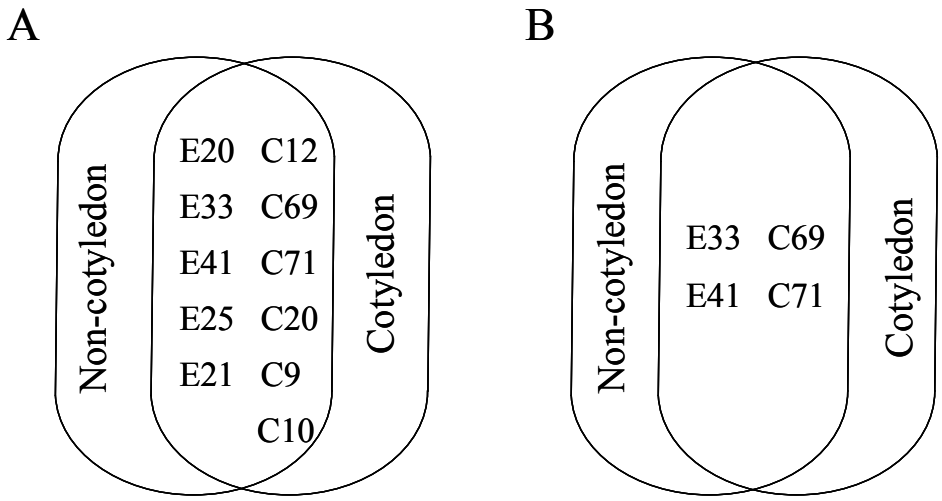
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Supplementary Table 1. Peak lists for proteins identified in the non-cotyledon (hypocotyl and radicle) of 4-day germinating soybeans.

Spot no.	Homologues protein	Observed	Peptide
1	Glutamine synthetase PR-2	1779.91	K.IIAEYIWVGGSGMDLR.S
		1795.90	K.IIAEYIWVGGSGMDLR.S + Oxidation (M)
		1827.89	K.IIAEYIWVGGSGMDLR.S + Dioxidation (W); Oxidation (M)
		2963.45	K.IFSHPDVAAEEPWYGIEQEYTLLQK.D
		3016.41	K.WNYDGSSTDQAPGDDSEVILYPQAIFK.D
2	Profilin-2	1432.76	K.YMVIQGEPGAVIR.G
		1448.75	K.YMVIQGEPGAVIR.G + Oxidation (M)
		2735.30	K.TGAALIIGIYDEPMTPGQCNMVVER.L
3	Phospholipase D alpha 1	1828.94	K.VSLYQDAHVPDNFVPK.I
		1966.00	R.LEGPIAWDVLFNFEQR.W + Dioxidation (W)
		2237.00	K.KPGEYEPSEQPDPDSDYQR.A
4	Beta-amylase	1521.71	R.AGHPEWELPDDAGK.Y
		1536.85	K.YNHAITPLKPSAPK.I
		2049.98	K.VENHAAELTAGYYNLNDR.D
5	Aminoaldehyde dehydrogenase	1319.68	R.LGPIVSEGQYKK.V + Deamidated (NQ)
6	Glutamine synthetase precursor	1612.74	R.HKDHISAYGEGNER.R
		1789.86	K.HETASINTFSWGVANR.G
		1807.95	K.IIAEYIWIGGSGIDLR.S + Dioxidation (W)
7	Lipoxygenase-2	1821.85	K.HETASINTFSWGVANR.G + Dioxidation (W)
		1748.00	K.FQTLIDLSVIEILSR.H + Deamidated (NQ)
8	Linoleate 9S-lipoxygenase-4	912.44	-.MFPFGQK.G + Acetyl (Protein N-term); Oxidation (M)
		945.52	K.LLFPHYR.D
		1322.61	R.HASDEFYLGQR.D
		1342.58	K.SAWMTDEEFAR.E
		1358.56	K.SAWMTDEEFAR.E + Oxidation (M)
		1630.75	K.SWVQEYVSFYYK.S + Dioxidation (W)
		1705.82	K.LDTQAYGDHTCIIAK.E
		1891.95	R.KDPNSEKPSDFVYLPR.D
		1984.91	R.DGGDYWTSDAGPLEAFKR.F
		2019.98	K.MPYTLLYPSSEEGLTFR.G + Oxidation (M)
9	Methionine synthase	1861.87	K.SEQAHYLDWAVHAFR.I + Dioxidation (W)
		2120.05	K.ALSGHKDEAFFSGNAAALASR.K
		2833.42	K.YIPSNTFSHYDQVLDATATLGAVPPR.Y
10	5-methyltetrahydropteroyltriglutamate--h	1096.58	K.YLFAGVVDGR.N
		1525.74	K.DVAFFSANAAAQASR.K

	omocysteine methyltransferase	1827.95	K.TLTSLNGVTAYGFDLVR.G + Deamidated (NQ)
		1861.87	K.SEQAHYLDWAVHAFR.I + Dioxidation (W)
		1989.97	R.KSEQAHYLDWAVHAFR.I + Dioxidation (W)
11	UDP-N-acetylglucosamine pyrophosphorylase	1056.51	R.FIMETPYR.V
		1072.51	R.FIMETPYR.V + Oxidation (M)
		2353.17	R.SQGLPAAAIEPVPESSVSTVEER.S
12	Nucleoside diphosphate kinase	957.57	R.GLIGEIISR.L
		1274.71	K.LVTVDRPFAEK.H
		1624.87	K.IIGATNPAQSEPGTIR.G
		1752.97	R.KIIGATNPAQSEPGTIR.G
13	ATP synthase 24 kDa subunit, mitochondrial	1726.84	K.ITIDPEDPAAVSHYAK.V
		1983.98	K.EKITIDPEDPAAVSHYAK.V
		2217.11	R.GLTDDLGAEALMIDALDKVEK.D
		2744.36	K.ASLLSESQDILSTIEIETQDIPDAR.T
14	V-type proton ATPase subunit E	902.50	K.QIQQMVR.F
		993.51	K.IRQEYER.K
		1054.67	R.LKEPSVLLR.C
		1129.65	K.DLIVQCLLR.L
		1190.59	K.IVCENTLDAR.L
15	Os01g0654500	1808.87	K.ANEISVSAEEEFNIEK.L
		1870.91	K.ELLNVSHHHDDHVYR.N
		993.47	R.HAFGDQYR.A
		1378.69	K.FEAAGIWYEHR.L
		1593.82	K.SKFEAAGIWYEHR.L
		1625.81	K.SKFEAAGIWYEHR.L + Dioxidation (W)
		1704.84	K.VVNPIVEMDGDEMTR.V
		1775.00	R.NILNGTVFREPILCK.N + Deamidated (NQ)
		1797.93	K.GGETSTNSIASIFAWTR.G
		1829.91	K.GGETSTNSIASIFAWTR.G + Dioxidation (W)
16	NADP-dependent malic enzyme	1064.67	R.LPRPQNLVK.Y
		1158.72	K.RPQGLFISLK.E
		1276.73	K.GLIYPPFSNIR.E
		1642.76	K.YQAMMELQETNER.L
		2943.64	K.LALYTALGGVRPSACLPITIDVGTNNEK.L
17	NADP-dependent malic enzyme	1064.65	R.LPRPQNLVK.Y
		1158.70	K.RPQGLFISLK.E
		1541.65	K.YAESCMYSPVYR.N + Oxidation (M)
		1627.81	R.AIFASGSPFDPVEYK.G
		1642.74	K.YQAMMELQETNER.L
18	ATPase subunit 1	1026.59	K.AVDSLVPIGR.G
		1210.67	R.VVDALGVPIDGR.G
		1438.84	R.GIRPAINVGLSVSR.V
		1537.73	R.EAFPGDVFYLHSR.L
		1738.88	K.QIIVIYAAVNGFCDR.M

		1816.88	R.ITNFYTNFQVDEIGR.V
		2141.04	R.VYGLNEIQAGEMVEFASGVK.G
		2308.14	R.EVAAFAQFGSDLDAATQALLNR.G
19	Cell division cycle protein 48 homolog	1440.75	K.YQAFAQTLQQSR.G
		1786.96	R.LDQLIYIPLPDEDSR.H
		1819.88	R.HTQGFSGADITEICQR.A
		1951.97	K.GPELLTMWFGESEANVR.E + Oxidation (M)
		2197.12	R.QSAPCVLFFDELDSIATQR.G
		2505.25	K.DTHGYVGADLAALCTEAALQCIR.E
		3123.55	K.VIETDPGEYCVVAPDTEIFCEGEPLKR.E
20	Expansin-like B1	1002.54	R.VAGVSDLWR.N
		1012.43	R.AMNWYDGR.V
		1028.42	R.AMNWYDGR.V + Oxidation (M)
		1034.53	R.VAGVSDLWR.N + Dioxidation (W)
		1243.63	K.YGTVDIEYKR.V
		1343.55	R.NGAGCGTCYQVR.C + Deamidated (NQ)
		1879.90	R.NYGAVFDFPNPPSGEIR.L
		2492.04	R.ATFYGTSDGYGTPTGACGFGEYGR.A
21	Embryonic protein DC-8	901.45	K.ELEQLNR.E
		1077.43	K.GYGSHESAGGR.G
		1216.50	K.VGEYADYGSQK.A
		1232.50	K.AGEYTDYASQK.A
		1343.56	K.EPSTTTNSYSTR.E
		1377.56	K.GYGSHESAGGRGDK.V
		1499.75	K.VEESRPGAIAETLK.A
		1562.70	K.AADQIAGQTFNDVGR.F
		1834.83	R.EVIHDVDIQPDDAAAAR.E
		2965.29	K.AADQIAGQTFNDVGRFDEEEGVVNVER.R
22	Eukaryotic initiation factor 4A-15	1119.61	R.QSLRPDYIK.M
		1142.56	K.VHACVGGTSVR.E
		1360.65	M.AGLAPEGSQFDAR.Q + Acetyl (Protein N-term)
		1555.71	K.MFVLDEADEMLSR.G
		1571.70	K.MFVLDEADEMLSR.G + Oxidation (M)
		1827.94	R.GIYAYGFEKPSAIQQR.G
		2075.08	K.IQVGVFSATMPPEALEITR.K + Oxidation (M)
		2911.50	R.GIDVQQVSLVINYDLPTQPENYLHR.I
23	Eukaryotic translation initiation factor 5A3	1134.58	K.TYPQQAGTIR.K
		1262.67	K.TYPQQAGTIRK.N
		1286.51	M.SDEEHHFESK.A + Acetyl (Protein N-term)
		1407.69	K.CHFVGIDIFTAK.K
		1828.97	K.DDLKLPTDESLLTQIK.D
		2087.98	K.LEDIVPSSHNCDVPHVNR.T
		2216.08	K.KLEDIVPSSHNCDVPHVNR.T
24	Elongation factor 2	1021.54	K.FTAEELRR.I

		2220.08	R.LYMEARPLEEGLAEAIDDGK.I
25	Chain A, Soybean Trypsin Inhibitor	997.42	K.DAMDGWFR.L
		1163.64	K.GIGTIISSPYR.I
		1200.70	K.NKPLVVQFQK.L
		1230.52	R.VSDDEFNNYK.L
		1538.71	K.IGENKDAMDGWFR.L
		1554.69	K.IGENKDAMDGWFR.L + Oxidation (M)
		1762.93	R.NELDKGIGTIISSPYR.I
		3061.38	K.LVFCPQQAEDDKCGDIGISIDHDDGTR.R
		3261.57	-.DFVLDNEGNPLENGGTYYILSDITAFGGIR.A + Deamidated (NQ)
26	RuBisCO-associated protein	894.48	R.LVHSWPR.V
		926.48	R.LVHSWPR.V + Dioxidation (W)
		1280.64	K.VFVSIGGDDPFK.V
		1351.59	K.IVGEYDYYDSK.I
		1373.79	K.VSPPLFFLALNR.L
		1395.55	R.EYHHDASFMSR.C + Oxidation (M)
		1833.88	K.WDTDNVTPQLIEQFK.L
		1879.96	K.VISDTYAFIPPTWIPK.L + Dioxidation (W)
27	Mcp20	1008.48	K.NTIDGWFR.I
		1040.47	K.NTIDGWFR.I + Dioxidation (W)
		1171.69	K.GLPLIISSPFK.I
		1352.71	R.NGGTYYVLPVIR.G + Deamidated (NQ)
		2152.03	K.TETETCPLTVVQSPFEVSK.G
		2341.03	K.LVFCTSNDDSSCGDIVAPIDR.E
		2583.37	R.EGNRPLIVTHDQNHPLLVQFQK.V
28	Peptidyl-prolyl isomerase FKBP12	1151.52	K.DPGQNPFTFK.I + Deamidated (NQ)
		1716.83	K.GWDEGVIGMQIGEVAR.L
		1732.82	K.GWDEGVIGMQIGEVAR.L + Oxidation (M)
		1748.82	K.GWDEGVIGMQIGEVAR.L + Dioxidation (W)
		1764.81	K.GWDEGVIGMQIGEVAR.L + Dioxidation (W); Oxidation (M)
29	31kD glycoprotein	1022.58	R.LAVEAHNIR.A
		1142.52	K.DYINGEQFR.S + Deamidated (NQ)
		1560.76	K.TVNQQAFFYASER.E
		1893.99	R.IVGIIGDQWSDLLGDHR.G
		1925.98	R.IVGIIGDQWSDLLGDHR.G + Dioxidation (W)
		2323.20	R.IVGIIGDQWSDLLGDHRGESR.T
		2355.19	R.IVGIIGDQWSDLLGDHRGESR.T + Dioxidation (W)
		2426.15	K.TIPEECVEPTKDYINGEQFR.S + Deamidated (NQ)
		2977.51	R.EVHHNDIFIFGIDNTVLSNIPYYEK.H
30	31kD glycoprotein	1022.58	R.LAVEAHNIR.A
		1142.52	K.DYINGEQFR.S + Deamidated (NQ)
		1560.75	K.TVNQQAFFYASER.E

31	Subtilisin-like protease	1893.99	R.IVGIIGDQWSDLLGDHR.G
		1925.98	R.IVGIIGDQWSDLLGDHR.G + Dioxidation (W)
		2323.19	R.IVGIIGDQWSDLLGDHRGESR.T
		2426.14	K.TIPEECVEPTKDYINGEQFR.S + Deamidated (NQ)
		2977.50	R.EVHHNDIFIFGIDNTVLSNIPYYEK.H
		1185.60	K.TQAFEVTFSR.A
		1197.62	R.VGPTDLDIDPR.R
		1288.67	R.EFPADVILGDGR.V
		1425.76	K.LSQYPTATIEFR.G
		1733.84	K.SFSDHNLSPIPSSWK.G + Dioxidation (W)
		1746.85	K.ESNPFIHGAGHVDPNR.A
		1804.95	R.GTVIGGSPSAPQVASFSSR.G
		2029.97	K.GYESYLERPIDESQESK.S
		2409.18	R.AKLDGSESFGSIEWTDGSHVVR.S + Dioxidation (W)
32	Peptidyl-prolyl cis-trans isomerase CYP20-2	915.47	K.TPWLDQR.H
		2007.02	R.HVVFGQVLEGMDIVR.L
		1698.90	R.IVIGLYGDDVPQTAENFR.A
33	Coatomer subunit beta'-2	962.45	K.VWDYHSR.N
		994.44	K.VWDYHSR.N + Dioxidation (W)
		1023.53	K.IWDAVTYR.L
		1055.52	K.IWDAVTYR.L + Dioxidation (W)
		1519.80	K.IVEFAEHKDYIR.S
		1826.92	K.GSSQLAFGCDQGFLIVK.I
		2062.00	K.IWSLDSSAPNFTLEGHQK.G + Dioxidation (W)
		2251.25	R.SLAVHPVLPYVISASDDQVLK.L
		2385.10	-.MEQTLSFEFEHEFVQNTAR.V + Acetyl (Protein N-term)
		2401.10	-.MEQTLSFEFEHEFVQNTAR.V + Acetyl (Protein N-term); Oxidation (M)
34	Nuclear transport factor 2	1749.83	K.AFVEHYYSTFDTNR.N
		2101.05	R.NGLANLYQEGSMLTFEGQK.I + Deamidated (NQ)
		2116.05	R.NGLANLYQEGSMLTFEGQK.I + Oxidation (M)
		2773.45	K.FSQMFHLIPTPQGSYYVLNDIFR.L
35	C nascent polypeptide-associated complex subunit alpha-like protein 2	1126.70	K.LGLKPVTGVSR.V
		1541.76	K.SPNSETYVIFGEAK.I
		1963.00	K.IEDLSSQLQTQAAQQFR.M
36	Auxin-binding protein ABP19a	2713.41	K.TLKPGDIMVFPQGQLHFQVNSGNGK.A + Deamidated (NQ)
		2729.41	K.TLKPGDIMVFPQGQLHFQVNSGNGK.A + Deamidated (NQ); Oxidation (M)
37	Auxin-binding protein ABP19a	2713.40	K.TLKPGDIMVFPQGQLHFQVNSGNGK.A + Deamidated (NQ)
		2729.39	K.TLKPGDIMVFPQGQLHFQVNSGNGK.A + Deamidated (NQ); Oxidation (M)

38	pathogenesis-related protein 1	2767.33	R.SEVGVQNLAWDDTVAAFAQNYANQR.K
		2799.32	R.SEVGVQNLAWDDTVAAFAQNYANQR.K + Dioxidation (W)
		2891.31	K.VACDNGGTFITCNYAPPGNYVGQRPY.-
		2908.26	K.SNYDYNSNSCVGGECLHYTQVVWR.D
		2940.27	K.SNYDYNSNSCVGGECLHYTQVVWR.D + Dioxidation (W)
39	pathogenesis-related protein 1	930.47	R.AEVGSQSPR.Q
		2800.35	R.QTVIVPSLAWDDTVAAYAESYANQR.K + Dioxidation (W)
		3078.393	K.VTCDNGGTFITCNYDPPGNFVGERPYK.L
		191.4832	K.VTCDNGGTFITCNYDPPGNFVGERPYKL.-
		24.50328	R.KGDCQLIHSGGEYGENIAMSTGELSGTDAVK.M
		0.423312.41	K.SNYDYDSNSCVGGECLHYTQVVWANSVR.L
			K.SNYDYDSNSCVGGECLHYTQVVWANSVR.L + Dioxidation (W)
40	nucleoredoxin 1 [*Glycine max*]	1028.56	R.LDELFHVR.G
		1271.68	R.SRLDELFHVR.G
		1475.69	R.EYGVEGYPFTSAR.I
		1635.79	K.MPWLAIPFSDSDTR.S
		1753.97	K.LKHELHEHELVLTR.R
		1945.93	R.DLVSLYGADAYPFTEER.I
		2039.01	M.ADSADVTHDVVSLLSSPQR.D + Acetyl (Protein N-term)
		2502.33	R.GIPHLALLDEAGNVVTEDGVDVIR.E
41	Endoplasmin	1097.48	K.FGWSANMER.I
		1361.67	K.EQLENSPFLER.L
		1706.88	R.RVFISDEFNELLPK.Y
42	Heat shock cognate protein 80	1081.53	R.APFDLFDTR.K
		1237.62	K.RAPFDLFDTR.K
		1256.69	K.ADLVNNLGTIAR.S
		1320.65	K.HFSVEGQLEFK.A
		1527.80	K.GIVDSEDLPLNISR.E
		1541.76	K.SLTNDWEEHLAVK.H
		1845.93	R.KPEEITKEEYAAFYK.S
		2039.07	K.ITLFLKEDQLEYLEER.R
		2444.08	R.VVDSPCCLVTGEYGWTANMER.I
43	Ferritin light chain precursor	1078.58	K.ISEYVAQLR.R
		1123.61	K.LLNVHSVADR.N
		1238.57	K.GHGVWHFDQR.L
		1270.56	K.GHGVWHFDQR.L + Dioxidation (W)
		1538.85	K.SELAVPTAPQVSLAR.Q
		1666.94	K.KSELAVPTAPQVSLAR.Q
44	MnSOD	1004.55	K.NVRPDYLK.N
		1418.67	K.HHQTYITNYNK.A
		1600.76	K.FNGGGHVNHSIFWK.N + Deamidated (NQ)
		1631.76	K.FNGGGHVNHSIFWK.N + Dioxidation (W)

45	Selenium-binding protein 1	1783.98	K.RLVVETTANQDPLVTK.G
		2620.34	K.GPNLVPLIGIDVWEHAYYLQYK.N + Dioxidation (W)
		1171.75	R.FLIVPALVSGR.V
		1550.82	R.HLHVYSWPGGELR.Q
		1558.82	K.SGPGYASPLEAISGPR.E
		1568.81	K.DTGFVGSALTSNMIR.F
		1576.82	K.GFNLQHLSDGLYGR.H
		1757.95	R.QTMDLGDSGLIPLEIR.F
		1773.94	R.QTMDLGDSGLIPLEIR.F + Oxidation (M)
		2086.09	K.TQDESWSHEVAISVKPLK.V + Dioxidation (W)
		2590.27	K.INPNFFVDFGTEPDGPSLAHEMR.Y
46	Peroxidase precursor, partial	981.54	K.NAPPNLTVR.G
		1248.53	R.MHFHDCFVR.G
		1264.52	R.MHFHDCFVR.G + Oxidation (M)
		1291.66	R.DTIVATGGPFWK.V
		1982.06	K.FVHDHIHNAPSLAAALIR.M
		2347.16	K.SLVEAECPGVVSCADILTLSAR.D
47	PR-5b protein precursor	1070.44	R.TGCNFDGSGR.G K.ISCSADINGQCPGPLR.A
		1744.81	R.GQTWNLWVNPGTAMAR.I + 2 Dioxidation (W); Oxidation (M)
		1881.86	
		2898.18	R.CHDSYSYPQDDPTSTFTCPAGSNYK.V
48	Hsc70	1358.64	K.NALENYAYNMR.N
		1487.73	R.TTPSYVAFTDTER.L
		1540.78	R.ARFEELNMDLFR.K
		1556.77	R.ARFEELNMDLFR.K + Oxidation (M)
		1680.87	K.NAVVTVPAYFNDSQR.Q
		2620.27	K.GEGPAIGIDLGTTYSCVGVWQHDR.V + Dioxidation (W)
		2658.33	K.EQVFSTYSDNQPGVLIQVYEGER.A
49	Hsc70	1313.66	R.FEELNMDLFR.K
		1358.65	K.NALENYAYNMR.N
		1374.64	K.NALENYAYNMR.N + Oxidation (M)
		1473.73	R.TTPSYVGFTDTER.L
		1540.80	R.ARFEELNMDLFR.K
		1567.86	K.MREIAEAYLGSTVK.N
		1680.88	K.NAVVTVPAYFNDSQR.Q
		2642.34	K.EQVFSTYSDNQPGVLIQVFEGER.A
50	Luminal-binding protein 5	1508.76	R.ITPSWVAFTDSER.L
		1892.98	K.INDAVVTVPAYFNDAQR.Q
		2021.08	K.KINDAVVTVPAYFNDAQR.Q
		2691.33	K.SQVFTTYQDQQTTVSIQVFEGER.S
51	Endoplasmic reticulum HSC70-cognate	1508.76	R.ITPSWVAFTDSER.L
		1892.98	K.INDAVVTVPAYFNDAQR.Q
		2021.08	K.KINDAVVTVPAYFNDAQR.Q

	binding protein precursor	2053.05	R.VEIESLFDGVDFSEPLTR.A
		2089.14	K.LKEVEAVCNPIISAVYQR.S
52	Polyphenol oxidase A1, chloroplastic	932.40	K.MGYDYQR.V
		1103.52	K.SSPLYDEHR.N
		1217.59	R.SFMQQANVHR.A
		1233.58	R.SFMQQANVHR.A + Oxidation (M)
		1524.91	K.TLQLPLLLESIER.T
		1778.89	R.NSDHVNAFIDLDYKK.D
		2534.11	K.SDEWLESSFFFYDENSNYVR.V
53	Caffeoyl-CoA O-methyltransferase	1171.61	K.AGVDHKIEFR.E
		2201.20	K.ILAMDINRENYELGLPVIK.K
54	Isoflavone reductase	976.44	R.GDLYDHEK.L
		999.64	K.ILYLRPPK.N
		1429.65	R.FFPSEFGNDVDR.V
		1769.92	K.AIFNKEEDIGTYTIR.A
		1794.02	K.AQLIHNFEALGVNLVR.G
		1839.94	K.NIYSFNELVALWENK.I
		1871.93	K.NIYSFNELVALWENK.I + Dioxidation (W)
		2261.12	R.FFPSEFGNDVDRVHAVEPAK.S
55	Uncharacterized protein LOC100783793	2522.26	M.ASVEVAHQTPTTVPENETTEVIK.T + Acetyl (Protein N-term)
56	Uncharacterized protein LOC100788703	2489.23	M.ATVEVAQQTPTTVTENEATEVTK.I + Acetyl (Protein N-term)
		2817.36	K.IEETTPEQPATQVTTTEEPKEETTK.E
57	Uncharacterized protein LOC100801797	2620.29	M.ATVEVAQQTPTTVTENETTTEVTK.I + Acetyl (Protein N-term)
		2856.35	K.ETESAPVEEEKQEENKPAETVEEPK.T
58	Uncharacterized protein At5g39570	1322.62	R.VEYGYEGRPPR.Q
		1344.58	R.SEYEEKPSYGR.S
		1371.59	R.KEEPEYGSGYGR.R
		1388.58	R.KEEGEYGSGYGGR.K
		1428.61	R.KEEPEYGSGYGGR.K
		1752.72	R.KEEGEYGSGYGSGYGGR.K
59	Uncharacterized protein LOC100527097	1213.57	K.GDAQPNQDQLK.T
		2176.06	K.LTVEYQTKGDAQPNQDQLK.T
		2199.09	M.GIFTFEDEITSPVAPATLYK.A
60	Uncharacterized protein LOC100783267	877.40	K.AFPHDYK.S
		1286.65	R.HISYGEGSPLVK.S
		1381.68	K.SNADKYWQTLR.N
61	Uncharacterized protein LOC100820359	1627.78	K.AVDVTGPDESPVQGTR.G
		2033.98	R.SLALGESVEFLIDSDPEGR.T
		2163.82	R.YGGGGGGGGSCYNCGESGHFAR.D
		2476.13	K.GFGFITTDDSGEEFFVHQSSIR.S

62	Uncharacterized protein LOC100500241	1042.45	K.LMNAYCDR.Q
		1168.54	K.GQDGNEVFFR.I
		1395.71	K.VKGQDGNEVFFR.I
		2565.25	M.SGVTNNNEEDKKPTEQGAHINLK.V + Acetyl (Protein N-term)
63	Uncharacterized protein LOC100500685	1720.86	K.VYIVYYSTYGHVEK.L
		1751.96	K.LWQVPETLPEEVLAK.L
		2061.09	K.VYIVYYSTYGHVEKLAR.E
		2632.35	K.SDVPIITPNELPEADGFLFGFPTR.F
		3195.69	K.LGAPPKSDVPIITPNELPEADGFLFGFPTR.F

Supplementary Table 2. Peak lists for proteins identified in the cotyledon of 4-day germinating soybean.

Spot no.	Homologues protein	Observed	Peptide
1	UTP-glucose-1-phosphate uridylyltransferase	962.55	K.NGFISLVGR.Y
		1356.77	R.LVVEDFLPLPSK.G
		1926.95	K.ARENPENPSIELGPEFK.K
2	Lactoylglutathione lyase	1423.65	K.DPDGYAFELIQR.S
		1543.78	K.SAEVVNIVTQELGGK.I
		1684.78	K.GNAYAQVAIGTDDVYK.S
3	Ribulose bisphosphate carboxylase	1021.54	K.DTDILAAFR.V
		1393.64	R.ITYYTPDYETK.D
		2396.14	R.ITYYTPDYETKDTDILAAFR.V
4	RuBisCO small subunit 1	914.40	R.SPGYYDGR.Y
		921.50	K.EVEYLLR.K
		930.40	R.YWTMWK.L + Oxidation (M)
		962.40	R.YWTMWK.L + Dioxidation (W); Oxidation (M)
		2152.00	K.GWIPCLEFELEHGFVYR.E
		2280.09	R.KGWIPCLEFELEHGFVYR.E
5	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	930.47	R.NEGRDLAR.E
		1116.57	R.VALEACVKAR.N
		1472.66	K.EIKFEFEAMDTL.-
		1488.65	K.EIKFEFEAMDTL.- + Oxidation (M)
		1546.69	K.WSPELAAACEVWK.E
		2017.98	R.LSGGDHVHAGTVVGKLEGER.E
6	Seed biotinylated protein 68 kDa	1516.74	K.ATAVGWAAAHFSAEK.T
		1571.75	K.DTPQGSIEALQAGER.V
		2011.91	R.SAWEQISNYSDQATQGVK.E
		2039.98	K.EGTGSIVFTAIGETVSSAGEK.V
		2115.06	R.QAGGVLDAIGETIAEIAETTR.V
		2324.12	K.KPSQPQEAEERPSEGIGETVR.Q
		2373.11	K.ESDITAGKDTPQGSIEALQAGER.V
7	Seed biotinylated protein 68 kDa	981.47	R.DVGKFEMR.T
		1331.68	R.QYAQKPKPSER.N
		1516.72	K.ATAVGWAAAHFSAEK.T
		1571.74	K.DTPQGSIEALQAGER.V
		2011.89	R.SAWEQISNYSDQATQGVK.E
		2115.05	R.QAGGVLDAIGETIAEIAETTR.V
		2324.11	K.KPSQPQEAEERPSEGIGETVR.Q
		2373.11	K.ESDITAGKDTPQGSIEALQAGER.V
8	35 kDa seed maturation protein	950.47	R.QQFKEDR.A
		1274.58	K.AGEYKDYTAEK.A
		1294.64	R.LRDNTFNTWK.E
		1473.73	K.MEESRPGAVADALK.A

9	Embryonic protein DC-8	1489.71	K.MEESRPGAVADALK.A + Oxidation (M)
		1692.85	K.EAVVGKPHHEEVYAK.E
		1816.77	R.AMDGDMEEEGVLHVER.R
		2052.87	R.TNERDEGYDLHDEANFK.Q
		901.46	K.ELEQLNR.E
		1077.45	K.GYGSHESAGGR.G
		1216.52	K.VGEYADYGSQK.A
		1232.52	K.AGEYTDYASQK.A
		1377.59	K.GYGSHESAGGRGDK.V
		1499.78	K.VEESRPGAIAETLK.A
		1834.86	R.EVIHDVDIQPDDAAAAR.E
		2965.31	K.AADQIAGQTFNDVGRFDEEEGVVNVER.R
10	Embryonic protein DC-8	901.45	K.ELEQLNR.E
		950.52	K.RAVGYLSGK.T
		1104.46	K.ENVSGADEGAR.R
		1216.51	K.VGEYADYGSQK.A
		1232.50	K.AGEYTDYASQK.A
		1288.63	K.LGELKDSAADAAK.R
		1343.57	K.EPSTTTNSYSTR.E
		1471.66	K.KEPSTTTNSYSTR.E
		1532.65	K.DNAKENVSGADEGAR.R
		1834.84	R.EVIHDVDIQPDDAAAAR.E
11	Calreticulin-1 precursor	1234.66	K.YVGIELWQVK.S
		1266.65	K.YVGIELWQVK.S + Dioxidation (W)
		1997.94	K.SGTLFDNVLITDDPEYAK.Q
		2546.25	K.APLIDNPDFKDDPDLYVFPNLK.Y
12	Expansin-like B1	1002.54	R.VAGVSDLWR.N
		1012.43	R.AMNWYDGR.V
		1028.42	R.AMNWYDGR.V + Oxidation (M)
		1034.53	R.VAGVSDLWR.N + Dioxidation (W)
		1243.63	K.YGTVDIEYKR.V
		1343.55	R.NGAGCGTCYQVR.C + Deamidated (NQ)
		1879.90	R.NYGAVFDFPNPPSGEIR.L
		2492.04	R.ATFYGTSDGYGTPTGACGFGEYGR.A
13	Bd 30K	2110.93	K.NSWGEDWGEDGYIWIQR.N
		2142.91	K.NSWGEDWGEDGYIWIQR.N + Dioxidation (W)
14	Soybean Trypsin(Slash)chymotrypsin Bowman-Birk Inhibitor In Solution	1076.44	R.LNSCHSACK.S
		1960.65	-.DDESSKPCCDQCACTK.S
15	Bd 30K	2110.89	K.NSWGEDWGEDGYIWIQR.N
		2142.87	K.NSWGEDWGEDGYIWIQR.N + Dioxidation (W)
16	Bd 30K	2110.88	K.NSWGEDWGEDGYIWIQR.N

17	Kunitz-type trypsin inhibitor B	2142.86	K.NSWGEDWGEDGYIWIQR.N + Dioxidation (W)
		1059.54	R.CPLTVVQSR.N
		1163.62	K.GIGTIISSPYR.I
		1762.89	R.NELDKGIGTIISSPYR.I
		3260.45	-.DFVLDNEGNPLENGGTYYILSDITAFGGIR.A
18	Cysteine proteinase RD21a	1502.76	R.EFQLYVSGVFTGR.C
		1623.71	R.NSWGPSWGEDGYIR.L
		2788.27	R.CGTALDHGVVAVGYGTANGHDYWIVR.N
		3061.41	K.EGAVPPVKDQGGCGSCWAFSAIGAVEGINK.I
19	Kunitz trypsin protease inhibitor	1021.54	K.TCPLYVIR.D
		1571.73	R.LEGDHAYEIYSFK.F
		1626.79	K.VMAVGDDIEPYYVR.F
		1642.77	K.VMAVGDDIEPYYVR.F + Oxidation (M)
		2101.00	K.VGSGFWFVSTSGDPNDITSK.F
		2396.11	K.FCPSVPGALCAPVGTFEDADGTK.V
		3173.57	K.QGNPLEPGVGYYVWPLWADEGGLTLGQTR.N
		3205.56	K.QGNPLEPGVGYYVWPLWADEGGLTLGQTR.N + Dioxidation (W)
20	Chain A, Soybean Trypsin Inhibitor	997.42	K.DAMDGWFR.L
		1163.64	K.GIGTIISSPYR.I
		1200.70	K.NKPLVVQFQK.L
		1230.52	R.VSDDEFNNYK.L
		1538.71	K.IGENKDAMDGWFR.L
		1554.69	K.IGENKDAMDGWFR.L + Oxidation (M)
		1762.93	R.NELDKGIGTIISSPYR.I
		3061.38	K.LVFCPQQAEDDKCGDIGISIDHDDGTR.R
		3261.57	-.DFVLDNEGNPLENGGTYYILSDITAFGGIR.A + Deamidated (NQ)
21	Alpha' subunit of beta-conglycinin	986.54	R.SQQLQNLR.D
		1189.52	R.EEGQQQGEER.L
		1338.63	R.DSYNLQSGDALR.V
		1761.85	K.VLFGREEGQQQGEER.L
		1913.94	K.NILEASYDTKFEEINK.V
		2137.02	R.VPAGTTYYVVNPDNDENLR.M
22	Alpha' subunit of beta-conglycinin	986.54	R.SQQLQNLR.D
		2137.02	R.VPAGTTYYVVNPDNDENLR.M
23	Alpha' subunit of beta-conglycinin	955.51	R.SPQLQNLR.D
		1051.51	K.NPFLFGSNR.F
		1188.49	R.EEGQQQGEQR.L
		2151.96	R.VPSGTTYYVVNPDNNENLR.L
24	Alpha' subunit of beta-conglycinin	1189.48	R.EEGQQQGEER.L
		1338.59	R.DSYNLQSGDALR.V
		1383.65	R.QQQEEQPLEVR.K
		1406.68	K.TISSEDKPFNLR.S

25	Alpha' subunit of beta-conglycinin	1534.77	R.KTISSEDKPFNLR.S
		1584.83	K.LFEITPEKNPQLR.D
		1741.74	K.SQSESYFVDAQPQQK.E
		2136.95	R.VPAGTTYYVVNPDNDENLR.M
		1383.68	R.QQQEEQPLEVR.K
		1406.72	K.TISSEDKPFNLR.S
		1534.81	R.KTISSEDKPFNLR.S
		1584.87	K.LFEITPEKNPQLR.D
		1741.79	K.SQSESYFVDAQPQQK.E
		1761.84	K.VLFGREEGQQQGEER.L
		1913.93	K.NILEASYDTKFEEINK.V
		2137.01	R.VPAGTTYYVVNPDNDENLR.M
		2762.51	K.AIVVLVINEGEANIELVGIKEQQQR.Q
26	Alpha' subunit of beta-conglycinin	1383.65	R.QQQEEQPLEVR.K
		1406.69	K.TISSEDKPFNLR.S
		1534.78	R.KTISSEDKPFNLR.S
		1584.83	K.LFEITPEKNPQLR.D
		1741.75	K.SQSESYFVDAQPQQK.E
		1761.80	K.VLFGREEGQQQGEER.L
		1913.89	K.NILEASYDTKFEEINK.V
		2136.96	R.VPAGTTYYVVNPDNDENLR.M
27	Alpha' subunit of beta-conglycinin	986.51	R.SQQLQNLR.D
		1189.47	R.EEGQQQGEER.L
		1338.58	R.DSYNLQSGDALR.V
		1383.64	R.QQQEEQPLEVR.K
		1406.68	K.TISSEDKPFNLR.S
		1584.82	K.LFEITPEKNPQLR.D
		2136.93	R.VPAGTTYYVVNPDNDENLR.M
		3445.12	K.ESEEEEEDQDEDEEQDKESQESEGSESQR.E
28	Alpha' subunit of beta-conglycinin	986.53	R.SQQLQNLR.D
		1189.50	R.EEGQQQGEER.L
		1338.61	R.DSYNLQSGDALR.V
		1383.67	R.QQQEEQPLEVR.K
		1406.71	K.TISSEDKPFNLR.S
		1534.80	R.KTISSEDKPFNLR.S
		1584.85	K.LFEITPEKNPQLR.D
		2136.99	R.VPAGTTYYVVNPDNDENLR.M
		3445.21	K.ESEEEEEDQDEDEEQDKESQESEGSESQR.E
29	Beta-conglycinin, alpha chain	955.54	R.SPQLQNLR.D
		1051.53	K.NPFLFGSNR.F
		1188.53	R.EEGQQQGEQR.L
		1378.74	R.QFPFPRPPHQK.E
		1406.73	K.TISSEDKPFNLR.S
		1792.92	R.QFPFPRPPHQKEER.N

		2038.88	R.QEEEHEQREEQEWPR.K
		2152.03	R.VPSGTTYYVVNPDNNENLR.L
		2459.14	R.FESFFLSSTEAQQSYLQGFSR.N
		955.55	R.SPQLQNLR.D
		1051.54	K.NPFLFGSNR.F
		1188.54	R.EEGQQQGEQR.L
		1378.74	R.QFPFPRPPHQK.E
30	Beta-conglycinin, alpha chain	1618.86	K.FFEITPEKNPQLR.D
		1792.92	R.QFPFPRPPHQKEER.N
		2025.94	K.EQQQEQQQEEQPLEVR.K
		2038.88	R.QEEEHEQREEQEWPR.K
		2152.02	R.VPSGTTYYVVNPDNNENLR.L
		2459.13	R.FESFFLSSTEAQQSYLQGFSR.N
		1188.52	R.EEGQQQGEQR.L
		1391.86	R.LITLAIPVNKPGR.F
		1406.71	K.TISSEDKPFNLR.S
		1534.81	R.KTISSEDKPFNLR.S
31	Beta-conglycinin, alpha chain	1618.84	K.FFEITPEKNPQLR.D
		1913.92	R.NILEASYDTKFEEINK.V
		2025.93	K.EQQQEQQQEEQPLEVR.K
		2152.01	R.VPSGTTYYVVNPDNNENLR.L
		2459.13	R.FESFFLSSTEAQQSYLQGFSR.N
		1188.52	R.EEGQQQGEQR.L
		1391.87	R.LITLAIPVNKPGR.F
		1406.72	K.TISSEDKPFNLR.S
32	Beta-conglycinin, alpha chain	1534.82	R.KTISSEDKPFNLR.S
		1618.85	K.FFEITPEKNPQLR.D
		2025.94	K.EQQQEQQQEEQPLEVR.K
		2152.03	R.VPSGTTYYVVNPDNNENLR.L
		2459.14	R.FESFFLSSTEAQQSYLQGFSR.N
		955.52	R.SPQLQNLR.D
		1188.50	R.EEGQQQGEQR.L
		1391.84	R.LITLAIPVNKPGR.F
33	Alpha subunit of beta conglycinin	1406.69	K.TISSEDKPFNLR.S
		1534.78	R.KTISSEDKPFNLR.S
		1618.81	K.FFEITPEKNPQLR.D
		2025.88	K.EQQQEQQQEEQPLEVR.K
		2151.96	R.VPSGTTYYVVNPDNNENLR.L
		955.50	R.SPQLQNLR.D
		1188.47	R.EEGQQQGEQR.L
34	Alpha subunit of beta conglycinin	1406.66	K.TISSEDKPFNLR.S
		1534.75	R.KTISSEDKPFNLR.S
		1618.78	K.FFEITPEKNPQLR.D
		2025.86	K.EQQQEQQQEEQPLEVR.K

35	Beta-conglycinin, alpha chain	2151.94	R.VPSGTTYYVVNPDNNENLR.L
		2459.04	R.FESFFLSSTEAQQSYLQGFSR.N
		1079.53	R.SRDPIYSNK.L
		1183.53	R.ESYFVDAQPK.K
		1244.67	R.LQESVIVEISK.E
		1406.70	K.TISSEDKPFNLR.S
		1534.79	R.KTISSEDKPFNLR.S
		1618.82	K.FFEITPEKNPQLR.D
		2025.90	K.EQQQEQQQEEQPLEVR.K
		3372.62	R.NFLAGSQDNVISQIPSQVQELAFPGSAQAVEK.L
36	Beta-conglycinin, alpha chain	955.53	R.SPQLQNLR.D
		1188.52	R.EEGQQQGEQR.L
		1391.86	R.LITLAIPVNKPGR.F
		1406.71	K.TISSEDKPFNLR.S
		1534.80	R.KTISSEDKPFNLR.S
		1618.84	K.FFEITPEKNPQLR.D
		2025.92	K.EQQQEQQQEEQPLEVR.K
		2152.00	R.VPSGTTYYVVNPDNNENLR.L
		2459.11	R.FESFFLSSTEAQQSYLQGFSR.N
37	Beta-conglycinin, alpha chain	1183.51	R.ESYFVDAQPK.K
		1618.79	K.FFEITPEKNPQLR.D
		2025.86	K.EQQQEQQQEEQPLEVR.K
		2153.95	K.EQQQEQQQEEQPLEVRK.Y
		3372.58	R.NFLAGSQDNVISQIPSQVQELAFPGSAQAVEK.L
38	Soybean Beta-Conglycinin Beta Homotrimers Complexes With N-Acetyl-D-Glucosamine	956.51	R.SPQLENLR.D
		1064.65	K.LAIPVNKPGR.Y
		1201.59	R.VLLGEEEEQR.Q
		2181.07	R.IPAGTTYYLVNPHDHQNLK.I
		2412.22	R.VLLGEEEEQRQQEGVIVELSK.E
		2597.24	R.AILTLVNNDDRDSYNLHPGDAQR.I
39	Soybean Beta-Conglycinin Beta Homotrimers Complexes With N-Acetyl-D-Glucosamine	1361.68	R.NFLAGEKDNVVR.Q
		1396.66	R.SRNPIYSNNFGK.F
		1512.73	K.QKQEEEPLEVQR.Y
		1618.81	K.FFEITPEKNPQLR.D
		1773.82	R.QVQELAFPGSAQDVER.L
40	Soybean Beta-Conglycinin Beta Homotrimers Complexes With N-Acetyl-D-Glucosamine	1361.67	R.NFLAGEKDNVVR.Q
		1512.71	K.QKQEEEPLEVQR.Y
		1618.80	K.FFEITPEKNPQLR.D
		1773.81	R.QVQELAFPGSAQDVER.L
41	Glycinin G1	1149.55	R.VFDGELQEGR.V
		1425.84	R.VLIVPQNFVVAAR.S

42	Glycinin G1	1450.63	R.SQSDNFEYVSFK.T
		1149.54	R.VFDGELQEGR.V
		1372.67	R.ALIQVVNCNGER.V
		1450.62	R.SQSDNFEYVSFK.T
43	Glycinin G1	1030.51	R.GQSSRPQDR.H
		1979.99	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2667.21	K.GIFGMIYPGCPSTFEEPQQPQQR.G
		2683.21	K.GIFGMIYPGCPSTFEEPQQPQQR.G + Oxidation (M)
44	Mutant glycinin subunit A1aB1b	1030.49	R.GQSSRPQDR.H
		1586.75	R.FYLAGNQEQEFLK.Y
		1742.85	R.RFYLAGNQEQEFLK.Y
		1979.94	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2667.15	K.GIFGMIYPGCPSTFEEPQQPQQR.G
		2683.15	K.GIFGMIYPGCPSTFEEPQQPQQR.G + Oxidation (M)
45	Glycinin A1bB2-784 precursor	3021.35	R.IESEGGFIETWNPNNKPFQCAGVALSR.C
46	Glycinin	959.47	K.LQSPDDER.K
		1863.90	K.HFLAQSFNTNEDIAEK.L
		2234.81	R.EQDQDQDEDEDEDEDQPR.K
		2362.90	R.EQDQDQDEDEDEDEDQPRK.S
		2586.11	R.EQDEDEDEDEDKPRPSRPSQGK.R
		2804.30	K.HFLAQSFNTNEDIAEKLQSPDDER.K
		3210.23	K.WQEQQDEDEDEDEDDEDEQIPSHPPR.R
47	Glycinin	1863.89	K.HFLAQSFNTNEDIAEK.L
		2234.81	R.EQDQDQDEDEDEDEDQPR.K
		2804.30	K.HFLAQSFNTNEDIAEKLQSPDDER.K
		3210.23	K.WQEQQDEDEDEDEDDEDEQIPSHPPR.R
48	Glycinin A1aBx	1030.49	R.GQSSRPQDR.H
		1040.57	K.LNALKPDNR.I
		1417.62	K.YQQEQGGHQSQK.G
		1529.66	R.EQPQQNECQIQK.L
		1586.74	R.FYLAGNQEQEFLK.Y
		1742.85	R.RFYLAGNQEQEFLK.Y
		1979.94	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2683.15	K.GIFGMIYPGCPSTFEEPQQPQQR.G + Oxidation (M)
		2987.38	R.IESEGGLIETWNPNNKPFQCAGVALSR.C
49	Glycinin A1aBx	1742.84	R.RFYLAGNQEQEFLK.Y
		1979.94	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2667.16	K.GIFGMIYPGCPSTFEEPQQPQQR.G
		2987.36	R.IESEGGLIETWNPNNKPFQCAGVALSR.C
50	Glycinin A1a subunit	1417.62	K.YQQEQGGHQSQK.G
		1529.68	R.EQPQQNECQIQK.L
		1586.76	R.FYLAGNQEQEFLK.Y

		1742.87	R.RFYLAGNQEQEFLK.Y
		1978.98	R.RPSYTNGPQEIYIQQGK.G
		2667.20	K.GIFGMIYPGCPSTFEEPQQPQQR.G
		2683.20	K.GIFGMIYPGCPSTFEEPQQPQQR.G + Oxidation (M)
		2987.42	R.IESEGGLIETWNPNNKPFQCAGVALSR.C
		3462.60	K.GKHQQEEENEGGSILSGFTLEFLEHAFSVDK.Q
51	Glycinin A1a subunit	1030.46	R.GQSSRPQDR.H
		1417.58	K.YQQEQGGHQSQK.G
		1742.82	R.RFYLAGNQEQEFLK.Y
		1979.91	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2987.33	R.IESEGGLIETWNPNNKPFQCAGVALSR.C
		1030.48	R.GQSSRPQDR.H
		1040.56	K.LNALKPDNR.I
		1417.60	K.YQQEQGGHQSQK.G
52	Glycinin A1a subunit	1742.83	R.RFYLAGNQEQEFLK.Y
		1900.89	K.NLQGENEGEDKGAIVTVK.G
		1979.92	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2667.11	K.GIFGMIYPGCPSTFEEPQQPQQR.G
		2987.32	R.IESEGGLIETWNPNNKPFQCAGVALSR.C
		1030.46	R.GQSSRPQDR.H
		1040.55	K.LNALKPDNR.I
		1529.63	K.YQQEQGGHQSQK.G
53	Glycinin A1a subunit	1742.81	R.EQPQQNECQIQK.L
		1978.91	R.RFYLAGNQEQEFLK.Y
		2667.10	R.RPSYTNGPQEIYIQQGK.G
		2683.10	K.GIFGMIYPGCPSTFEEPQQPQQR.G + Oxidation (M)
		2987.31	R.IESEGGLIETWNPNNKPFQCAGVALSR.C
		1030.49	R.GQSSRPQDR.H
		1040.58	K.LNALKPDNR.I
		1149.55	R.VFDGELQEGR.V
		1417.63	K.YQQEQGGHQSQK.G
54	Glycinin A1a subunit	1529.68	R.EQPQQNECQIQK.L
		1742.88	R.RFYLAGNQEQEFLK.Y
		1979.98	R.RPSYTNGPQEIYIQQGK.G + Deamidated (NQ)
		2667.21	K.GIFGMIYPGCPSTFEEPQQPQQR.G
		2683.22	K.GIFGMIYPGCPSTFEEPQQPQQR.G + Oxidation (M)
		1263.61	K.EAFGVNMQIVR.N
		1279.59	K.EAFGVNMQIVR.N + Oxidation (M)
55	Glycinin G2 precursor	1494.63	K.YQQQQQGGSQSQK.G
		1503.62	R.EQAQQNECQIQK.L
		1931.84	R.NLQGENEEEDSGAIVTVK.G
		2366.02	K.QQEEENEGSNILSGFAPEFLK.E
56	Glycinin G2 precursor	1263.63	K.EAFGVNMQIVR.N
		1279.62	K.EAFGVNMQIVR.N + Oxidation (M)

		1494.66	K.YQQQQQGGSQSQK.G
		1586.74	R.FYLAGNQEQEFLK.Y
		1742.85	R.RFYLAGNQEQEFLK.Y
		2551.17	K.GKQQEEENEGSNILSGFAPEFLK.E
		3021.36	R.IESEGGFIETWNPNNKPFQCAGVALSR.C
		3192.21	R.KPQQEEDDDDEEEQPQCVETDKGCQR.Q
		1040.57	K.LNALKPDNR.I
		1263.63	K.EAFGVNMQIVR.N
		1279.62	K.EAFGVNMQIVR.N + Oxidation (M)
57	Glycinin G2 precursor	1494.66	K.YQQQQQGGSQSQK.G
		1503.65	R.EQAQQNECQIQK.L
		1586.75	R.FYLAGNQEQEFLK.Y
		1742.86	R.RFYLAGNQEQEFLK.Y
		2551.18	K.GKQQEEENEGSNILSGFAPEFLK.E
		3021.38	R.IESEGGFIETWNPNNKPFQCAGVALSR.C
		1040.55	K.LNALKPDNR.I
		1263.61	K.EAFGVNMQIVR.N
		1279.59	K.EAFGVNMQIVR.N + Oxidation (M)
58	Glycinin G2 precursor	1494.63	K.YQQQQQGGSQSQK.G
		1503.62	R.EQAQQNECQIQK.L
		1586.72	R.FYLAGNQEQEFLK.Y
		1742.82	R.RFYLAGNQEQEFLK.Y
		2551.11	K.GKQQEEENEGSNILSGFAPEFLK.E
		3021.31	R.IESEGGFIETWNPNNKPFQCAGVALSR.C
		1040.57	K.LNALKPDNR.I
		1494.65	K.YQQQQQGGSQSQK.G
59	Glycinin G2 precursor	1503.64	R.EQAQQNECQIQK.L
		1586.74	R.FYLAGNQEQEFLK.Y
		1742.84	R.RFYLAGNQEQEFLK.Y
		3021.36	R.IESEGGFIETWNPNNKPFQCAGVALSR.C
		1326.61	R.SQQQLQDSHQK.I
		2083.91	K.FNECQLNNLNALEPDHR.V
60	Glycinin A3B4 subunit	2539.14	K.GAIGFAFPGCPETFEKPQQQSSR.R
		2729.24	R.VFYLAGNPDIEHPETMQQQQQQK.S
		2745.24	R.VFYLAGNPDIEHPETMQQQQQQK.S + Oxidation (M)
		2955.35	R.VESEGGLIETWNSQHPELQCAGVTVSK.R
		959.43	K.LQSPDDER.K
		1863.82	K.HFLAQSFNTNEDIAEK.L
61	Glycinin G4 subunit	2234.72	R.EQDQDQDEDEDEDEDQPR.K
		2289.93	K.QGQHQQEEEEEGGSVLSGFSK.H
		2418.01	R.KQGQHQQEEEEEGGSVLSGFSK.H
		3210.10	K.WQEQQDEDEDEDEDDEDEQIPSHPPR.R
62	Glycinin G4 subunit	1501.70	R.NGLHLPSYSPYPR.M + Deamidated (NQ)
		2049.89	K.LNECQLNNLNALEPDHR.V

63	Gy5	2151.97	R.VESEGGLIQTWNSQHPELK.C
		1851.82	K.HFLAQSFNTNEDTAEK.L
		2121.00	K.HFLAQSFNTNEDTAEKLR.S
		2159.96	K.QGQHQQQEEEGGSVLSGFSK.H
		2288.05	R.KQGQHQQQEEEGGSVLSGFSK.H
		2539.15	K.GAIGFAFPGCPETFEKPQQQSSR.R
		2729.25	R.VFYLAGNPDIEHPETMQQQQQQK.S
		2745.26	R.VFYLAGNPDIEHPETMQQQQQQK.S + Oxidation (M)
		3043.13	K.WQEQEDEDEDEDEEYEQTPSYPPR.R
64	Glycinin A5A4B3 precursor	959.43	K.LQSPDDER.K
		1863.82	K.HFLAQSFNTNEDIAEK.L
		2234.73	R.EQDQDQDEDEDEDEDQPR.K
		2289.94	K.QGQHQQEEEEEGGSVLSGFSK.H
		2418.03	R.KQGQHQQEEEEEGGSVLSGFSK.H
		2586.03	R.EQDEDEDEDEDKPRPSRPSQGK.R
		2771.20	R.VFYLAGNPDIEYPETMQQQQQQK.S + Oxidation (M)
		3210.12	K.WQEQQDEDEDEDEDDEDEQIPSHPPR.R
65	2S albumin precursor	1185.44	K.CCTEMSELR.S
		1201.43	K.CCTEMSELR.S + Oxidation (M)
		1220.58	K.ELINLATMCR.F
		1236.57	K.ELINLATMCR.F + Oxidation (M)
		1497.61	K.CCTEMSELRSPK.C
		1513.60	K.CCTEMSELRSPK.C + Oxidation (M)
66	Maturation polypeptide	1230.52	K.VGEYADYASQK.A
		1246.52	K.GGEYKDYSAEK.A
		1320.53	K.MGEYKDYAAEK.A + Oxidation (M)
		1499.76	K.VEESRPGAIAETLK.A
		1562.70	K.ENTKENVSGAGETAR.R
		2836.26	K.AADQIAGQTFNDVGRFDEEGVVNVER.R
		3258.40	K.EATNNAYSNTEVIHDVNIQPDDVSATGEVR.D
67	Maturation polypeptide	1090.48	K.ENVSGAGETAR.R
		1178.52	K.DRTAETTDAAK.Q
		1230.51	K.VGEYADYASQK.A
		1292.57	R.FDEEGVVNVER.R
		1320.52	K.MGEYKDYAAEK.A + Oxidation (M)
		1499.76	K.VEESRPGAIAETLK.A
		1562.70	K.ENTKENVSGAGETAR.R
		1721.67	K.THDIYDSATDNNNNK.T
		2836.24	K.AADQIAGQTFNDVGRFDEEGVVNVER.R
		3258.39	K.EATNNAYSNTEVIHDVNIQPDDVSATGEVR.D
68	Sucrose-binding protein	914.47	R.AHTFVSPR.H
		1142.50	K.FEEFFGPGGR.D
		1361.67	R.AVLGLVSESETEK.I
		1724.81	K.LFDQQNEGSIFAISR.E

69	Coatomer subunit beta'-2	962.45	K.VWDYHSR.N
		994.44	K.VWDYHSR.N + Dioxidation (W)
		1023.53	K.IWDAVTYR.L
		1055.52	K.IWDAVTYR.L + Dioxidation (W)
		1519.80	K.IVEFAEHKDYIR.S
		1826.92	K.GSSQLAFGCDQGFLIVK.I
		2062.00	K.IWSLDSSAPNFTLEGHQK.G + Dioxidation (W)
		2251.25	R.SLAVHPVLPYVISASDDQVLK.L
		2385.10	-.MEQTLSFEFEHEFVQNTAR.V + Acetyl (Protein N-term)
		2401.10	-.MEQTLSFEFEHEFVQNTAR.V + Acetyl (Protein N-term); Oxidation (M)
70	Nad-dependent formate dehydrogenase	955.57	K.GVIIVNNAR.G
		1758.90	K.CDVIVINTPLTEKTR.G
		2370.12	R.YMPNHAMTPHISGTTIDAQLR.Y + Oxidation (M)
71	Endoplasmin homolog	1097.48	K.FGWSANMER.I
		1361.67	K.EQLENSPFLER.L
		1706.88	R.RVFISDEFNELLPK.Y
72	Acidic chitinase	1009.50	R.ALNSFSSQR.K
		1095.53	K.YGGVMLWNR.F
		1127.51	K.YGGVMLWNR.F + Dioxidation (W)
		1143.51	K.YGGVMLWNR.F + Dioxidation (W); Oxidation (M)
		2039.02	K.VYLSAAPQCIIPDAHLDR.A
		2167.11	K.VYLSAAPQCIIPDAHLDR.A