Analysis of Soybean Embryonic Axis Proteins by Two-Dimensional Gel Electrophoresis and Mass Spectrometry

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Abstract: A proteomic approach based on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) for protein separation and subsequent mass spectrometry (MS) for protein identification was applied to establish a proteomic reference map for the soybean embryonic axis. Proteins were extracted from dissected embryonic axes and separated in the first dimension using a pH range from 4-7. A total of 401 protein spots were isolated, digested with trypsin, and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We identified 335 protein spots by searching National Center for Biotechnology Information (NCBI) non redundant databases using the Mascot search engine and found a total of 200 unique proteins. Gene Ontology (GO) analysis was employed to understand the molecular processes in which the identified embryonic axes proteins are involved. The majority of proteins play a functional role in catalytic activity (42.9%) and binding (39.3%), followed by nutrient reservoir activity (5.3%), structural molecular activity (4.0%), antioxidant activity (3.2%), transporter activity (2.4%), enzyme regulator activity (1.2%), molecular transducer activity (0.8%), and transcription regulator activity (0.8%). Our 2D-profiling of soybean axis proteins has established for the first time a baseline proteome on which to investigate and compare factors affecting soybean embryonic development and the interaction of beneficial and pathogenic soilborne organisms during seed germination.

Keywords: Soybean, embryo, proteomics, two-dimensional gel electrophoresis, LC-MS/MS.

INTRODUCTION

Soybean, *Glycine max* (*G. max*), provides an inexpensive source of protein for human food and the animal industry, and is also valuable for oil production. Soybean seeds are attached to the pod by the hilum, through which the seeds receive nutrients during their growth and development. The seeds, which are covered by the seed coat, are composed of two large cotyledons in which storage proteins are synthesized for use during germination, and an embryonic axis that develops into the adult plant [1]. Soybean cotyledons contain ~20% oil and ~ 40% protein, which supply the needs of the young plant during emergence and for about 7-10 days after emergence. The embryonic axis consists of the epicotyl, hypocotyl, and radical. During seed germination, embryo axes are most vulnerable to environmental stresses in the field and are the immediate target for colonization by both beneficial and pathogenic soilborne and seed inoculated microbes [2-5].

Proteomics is an efficient method for examining alterations in protein profiles caused by various stress stimuli. Recent improvements in separation methods, instrumentation, mass accuracy and sensitivity have led scientists to adopt this method universally [6-8]. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), in which proteins are separated based on their isoelectric point (pI) in the first dimension and SDS-PAGE, in which proteins are separated by their molecular weight (MW) in the second dimension have been widely used to study cellular function in prokaryotic and eukaryotic organisms.

Variation in anti-nutritional, allergen and storage proteins in soybean seed among different genotypes has been investigated using 2D-PAGE followed by spot identification with LC-MS/MS [9, 10]. Although a number of proteomic studies on different organs and organelles of soybean and other crops have been published [11-14], information on soybean axis proteomics is lacking. Molecular investigations on symbiotic as well as pathogenic interactions on emerging soybean seedlings necessitate a baseline proteomic map of embryonic axis. In the present study, we describe the extraction, separation, and identification of the proteins of the soybean embryonic axes. The Mascot search engine was used to search NCBInr databases for protein identification. The identified proteins were clustered into cohesive groups based on their biochemical functions.

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MATERIALS AND METHODS

Protein Extraction from Embryonic Axes

We utilized the phenol solubilization method of Hurkman and Tanaka [15] to extract embryonic axis proteins. Soybean cultivar Williams 82 seeds were imbibed in water for 10 min. The embryonic axes were dissected from the imbibed seeds with forceps. Two grams of embryonic axis tissue was ground in liquid nitrogen to a fine powder in a mortar and pestle. The powder was extracted with 6 ml of extraction buffer [0.9 M sucrose, 100 mM Tris-Cl, pH 8.8, 0.4 % β-mercaptoethanol, 10 µL of protease inhibitor solution (Sigma P-9599)]. The slurry was chilled on ice and an equal volume of Tris-equilibrated phenol was added. The sample was mixed vigorously for 30 min at 22° C followed immediately by centrifugation at 4000xg for 20 min at 15° C in a swinging-bucket rotor. The upper phenolic phase was removed and added to ten volumes of freshly prepared 100% methanol with 0.1 M ammonium acetate (chilled to -80° C). Protein precipitation progressed for 1 h at -80° C and was followed by centrifugation at 6000xg for 10 min at 4° C. The supernatant was discarded and the protein pellet was resuspended vigorously in 100% methanol with 0.1 M ammonium acetate and 10 mM DTT (chilled to -20° C). Following centrifugation as above, the protein pellet was allowed to air dry to near dryness and solubilized in 2D sample buffer (7 M urea, 2 M thiourea, 1% CHAPS, 2% C7BzO) with vortexing for further analysis [12].

2D-PAGE Analysis

An IPGPhor apparatus (GE Healthcare, Piscataway, NJ) was used for IEF with immobilized pH gradient (IPG) strips (pH 3.0 - 10.0, linear gradient, 13 cm). The IPG strips were rehydrated 12 hrs with 250µL rehydration buffer (8M urea, 2% CHAPS, 0.5% pharmalyte, 0.002% bromophenol blue) containing 350 µg protein. The voltage settings for IEF was 500 V for 1 hr, 1000 V for 1 hr, 5000 V for 1 hr, and 8000 V to a total 46.86 kVh. Following electrophoresis, the protein in the strips was denatured with equilibration buffer (50mM Tris-HCl pH 8.8, 6M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, 1% DTT) and then incubated with the same buffer containing 2.5% iodoacetamide instead of DTT for 30 min at room temperature. The second dimension electrophoresis was performed on a 12.5% gel using a Hoefer SE 600 Ruby electrophoresis unit (GE Healthcare, Piscataway, NJ). After completion of electrophoresis the gels were immediately removed from the cassette and soaked in 250 mL of 5:4:1 (methanol: water: acetic acid) for 40 min. This solution was removed and gels were then stained with Colloidal Coomassie G-250 for overnight. After destaining with ddH20 for several hours, gels were scanned using an HPScanjet 5470c. Embryonic axis proteins resolved by 2D PAGE were excised with a 1.5 mm Spot Picker (The Gel Company, San Francisco, CA, USA).

In-Gel Digestion & Mass Spectrometry

Protein digestion was performed as described previously [12]. Spots were excised from the stained gel and digested with porcine trypsin. The resulting peptides were analyzed with a LTQ Orbitrap XL hybrid ion trap, Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Initial peptide separation was achieved by reverse phase chromatography on a 100 x 0.18 mm BioBasic-18 column using a 30 minute gradient from 5-40% ACN in 0.1% formic acid at a flow rate of 3µl/min. The instrument's operation was in data dependent mode along with a duty cycle that acquired MS/MS spectra of the five most abundant ions in the linear ion trap, determined by a high resolution survey scan (r = 30,000 @ m/z 400) over the range of 400-1600 m/z. Real time calibration through the lock mass option allowed for a maintained high mass accuracy of the survey scans. The lock mass used for calibration was the polydimethylcyclosiloxane ion $(Si(CH_3)_2O)_6$ (protonated m/z 445.120025) generated during electrospray process from ambient air [16]. Application of dynamic exclusion allowed for prevention of continuous analysis of repeat ions. Two or more acquisitions of MS/MS spectra from a given ion subsequently placed the parent mass on the exclusion list for 3 minutes. The electrospray voltage was set to 3.5 kV and 10 units of sheath gas assisted with desolvation. The temperature for the capillary transfer tube was set to 200°C. To trigger an MS/MS spectrum, a minimum ion count of 5,000 was set. The software used to prepare searchable DATA files for the MS/MS data was Mascot Distiller ver. 2.3.0.0 (www.matrixscience.com).

Protein identification was performed using the Mascot search engine (http://www.matrixscience.com), which uses a probability based scoring system. NCBI non-redundant databases were selected as the primary databases to be searched. The parameters for database searches with MS/MS spectra were set as before [12]. Positive identifications of proteins by MS/MS analysis required a minimum of two unique

peptides, with at least one peptide having a significant ion score.

RESULTS AND DISCUSSION

Separation of Soybean Embryonic Axis Proteins

We employed a phenol solubilization method to extract embryonic axis proteins according to Hurkman and Tanaka [15]. Due to distinct advantages of 2D gel separation and subsequent protein identification over multidimensional protein identification technology (MudPIT) [17], we followed 2-D PAGE technique for soybean embryonic protein resolution. Three biological replicates of protein extracts were separated by 2D-PAGE. The gels were stained with colloidal Coomassie Blue (CBB) and inspected to ensure reproducibility of experiments, after which a reference gel was chosen. We chose this staining method because it is MS compatible and allows reproducible protein detection. Initial analyses were performed with immobilized pH gradient (IPG) strips with a pH range of 3 to 10 (data not shown). We observed that the region from pH 4 to 7 was a highly dense area on the proteome map of the soybean embryo; therefore, we used pH 4 to 7 IPG strips to improve spot resolution and identification (Figure **1**). A representative 2D-PAGE protein pattern of soybean embryo axis is presented in Figure **1**. Four hundred and one spots were excised from the 2D-PAGE gels and digested with trypsin. The tryptic digests were purified and then analyzed by LC-MS/MS. The dynamic range of some proteins such as storage proteins is very large and can mask clear detection of other proteins [18]. However, we were able to identify a

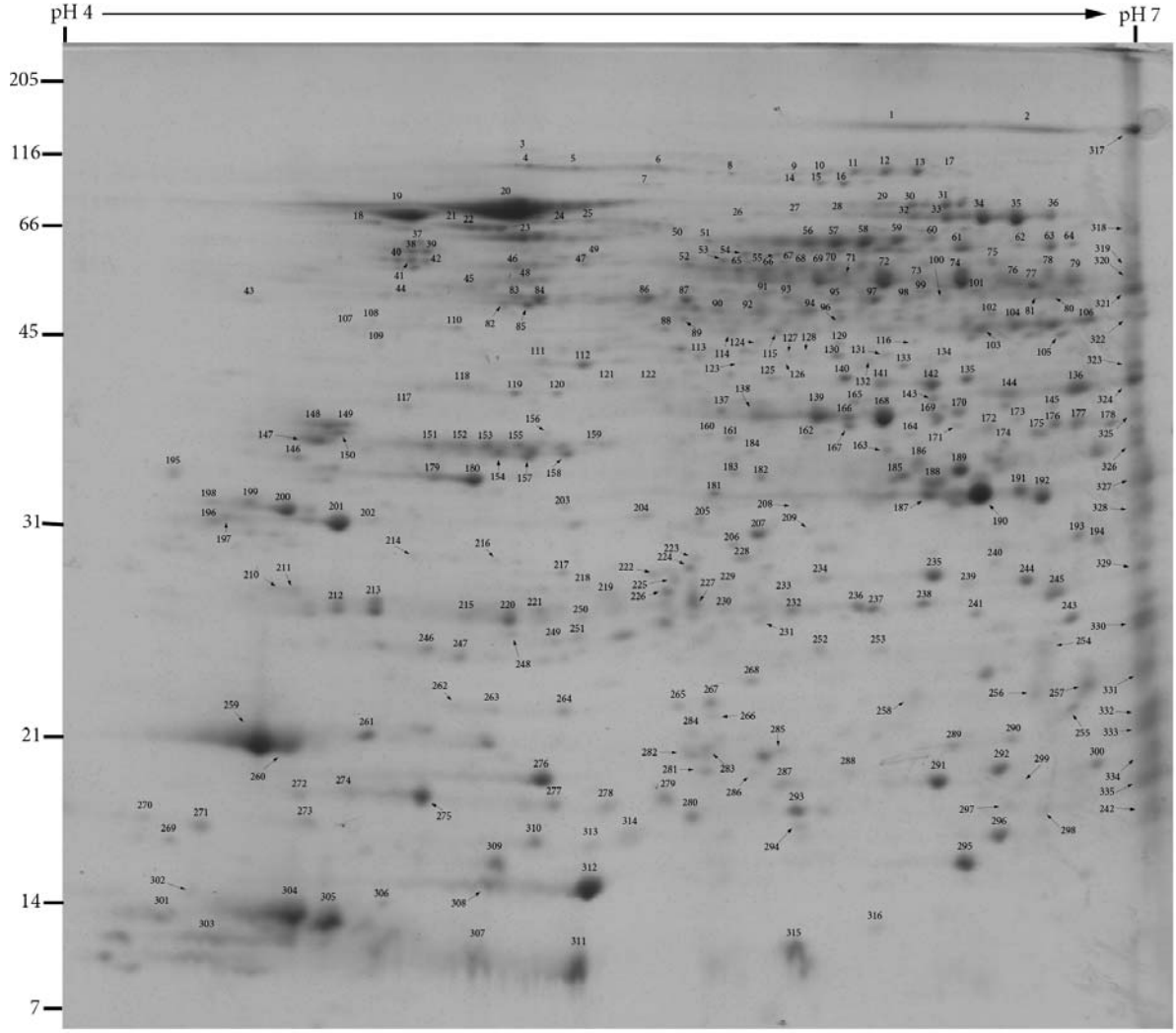

Figure 1: 2-D proteomic reference map of soybean embryonic axes. Proteins (350 µg) were separated in gradients of pH 4–7 for the first dimension, and resolved in the second dimension using 12.5% SDS-PAGE. The resulting gels were stained with Colloidal Coomassie Blue G-250. The protein spots were excised and identified with LC-MS/MS. Detailed information on the protein information was listed in Table **1**.

Table 1: Proteins Identified from Soybean Embryonic Axes by LC-MS/MS

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
1	seed maturation protein PM39 [*Glycine max*]	5.69 / 46665	259	4	10	gi|5802248		
2	seed maturation protein PM39 [*Glycine max*]	5.69 / 46665	839	12	34	gi|5802248		
3	PREDICTED: hypothetical protein [*Vitis vinifera*]	5.16 / 85382	133	3	3	gi|225442661		
4	Transitional endoplasmic reticulum ATPase, putative [*Ricinus communis*]	5.32 / 90084	901	13	18	gi|255540583		
5	Poly [ADP-ribose] polymerase 3 [*Glycine max*]	5.41 / 92771	518	9	11	gi|75213086		
6	Poly [ADP-ribose] polymerase 3 [*Glycine max*]	5.41 / 92771	706	13	17	gi|75213086		
7	ATP-dependent Clp protease ATP-binding subunit clpA homolog CD4A, chloroplastic [*Solanum lycopersicum*]	6.24 / 102892	601	9	12	gi|399212		
8	Aconitate hydratase [*Cucurbita maxima*]	5.74 / 98570	200	4	4	gi|1351856		
9	aconitase [*Arabidopsis thaliana*]	5.98 / 101439	94	2	2	gi|599625		
10	Aconitate hydratase, cytoplasmic [*Cucurbita maxima*]	5.74 / 98570	188	4	4	gi|1351856		
11	eukaryotic translation elongation factor, putative [*Ricinus communis*]	5.87 / 95026	403	9	9	gi|255544686		
12	eukaryotic translation elongation factor, putative [*Ricinus communis*]	5.87 / 94981	496	8	12	gi|255565836		
13	eukaryotic translation elongation factor, putative [*Ricinus communis*]	5.87 / 94091	630	11	15	gi|255565836		
14	embryo-specific urease [*Glycine max*]	5.68 / 90841	89	2	2	gi|32170829		
15	embryo-specific urease [*Glycine max*]	5.68 / 90841	332	6	6	gi|32170829		
16	embryo-specific urease [*Glycine max*]	5.68 / 90841	294	5	6	gi|32170829		
17	cytosolic aconitase [*Nicotiana tabacum*]	5.88 / 98692	145	2	4	gi|11066033		
18*	Heat shock 70 protein [*Spinacia oleracea*]	5.42 / 74472	440	7	13	O50036	0	88
19	alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	1056	27	32	gi|9967357		
20	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	812	14	22	gi|9967361		
21*	Heat shock protein 70 [*Gossypium hirsutum*]	5.17 / 71589	963	15	31	A5C0Z3	0	100
22	70 kDa heat shock cognate protein 1 [*Vigna radiata*]	5.11 / 71371	1025	16	29	gi|45331281		
23	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	1014	21	30	gi|9967361		
24	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	512	9	17	gi|9967361		
25	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	208	3	6	gi|9967361		
26*	Eukaryotic translation initiation factor 3 subunit, putative [*Ricinus communis*]	5.03 / 42801	176	3	7	B9RBD0	0	91
27	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	200	5	8	gi|240254706		
28	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	567	9	18	gi|240254706		

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
29	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	464	7	15	gi|240254706		
30	methionine synthase [*Glycine max*]	5.93 / 84401	681	11	18	gi|33325957		
31	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	855	14	25	gi|240254706		
32	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	1016	17	31	gi|240254706		
33	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	1420	27	43	gi|240254706		
34	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	1414	25	42	gi|240254706		
35	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	1110	21	34	gi|240254706		
36	seed biotinylated protein 68 kDa isoform [*Glycine max*]	6.18 / 67963	997	18	31	gi|240254706		
37	alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	616	11	24	gi|9967357		
38	protein disulfide isomerase [*Glycine max*]	5.13 / 58953	630	8	22	gi|171854980		
39	protein disulfide isomerase [*Glycine max*]	5.13 / 58953	568	2	21	gi|171854980		
40	protein disulfide isomerase [*Glycine max*]	5.13 / 58953	460	7	19	gi|171854980		
41	protein disulfide isomerase [*Glycine max*]	5.13 / 58953	465	8	20	gi|171854980		
42*	RuBisCO large subunit-binding protein subunit alpha, chloroplastic [*Pisum savitum*]	5.24 / 61931	774	10	20	P08926	0	85
43*	26S proteasome regulatory subunit S5A [*Cryophytum crystallinum*]	5.56 / 26141	158	3	14	O81340	1E-124	91
44	Tubulin beta-1 chain [*Zea mays*]	4.82 / 50614	303	5	11	gi|135449		
45	beta-conglycinin alpha prime subunit [*Glycine max*]	5.60 / 72739	505	8	14	gi|15425631		
46	beta-conglycinin alpha prime subunit [*Glycine max*]	5.60 / 72739	698	12	21	gi|15425631		
47	GroEL-like chaperone, ATPase [*Medicago truncatula*]	6.27 / 61473	546	7	20	gi|92882356		
48	beta-conglycinin alpha prime subunit [*Glycine max*]	5.60 / 72739	747	12	25	gi|15425631		
49	GroEL-like chaperone, ATPase [*Medicago truncatula*]	6.27 / 61473	463	8	13	gi|92882356		
50	V-H(+)-ATPase subunit A [*Glycine max*]	5.29 / 69077	230	4	6	gi|156616913		
51	V-H(+)-ATPase subunit A [*Glycine max*]	5.29 / 69077	466	8	16	gi|156616913		
52	chaperonin hsp60 [*Zea mays*]	5.68 / 61549	195	4	6	gi|22242		
53	GroEL-like chaperone, ATPase [*Medicago truncatula*]	6.27 / 61473	138	2	5	gi|92882356		
54*	Chaperonin containing t-complex protein 1, epsilon subunit, tcpe, putative [*Ricinus communis*]	5.39 / 59733	471	10	15	B9RMR9	0	93
55*	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (EC 5.4.2.1) [*Ricinus communis*]	5.40 / 61371	208	3	7	B9S1V6	0	90
56	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	379	5	19	gi|29469054		

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
57	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	469	7	19	gi|29469054		
58	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	477	7	19	gi|29469054		
59	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	425	7	19	gi|29469054		
60	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	333	4	12	gi|29469054		
61	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	782	12	30	gi|170010		
62	NADP-dependent malic enzyme [*Triticum aestivum*]	6.51 / 71385	150	2	4	gi|162957175		
63	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	782	12	35	gi|170010		
64	malate dehydrogenase (NADP+) [*Vitis vinifera*]	6.27 / 65696	178	4	7	gi|225445108		
65	chaperonin containing t-complex protein 1, beta subunit [*Ricinus communis*]	5.54 / 56190	323	4	10	gi|255539122		
66	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	243	3	9	gi|170010		
67	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	243	3	9	gi|170010		
68	sucrose binding protein homolog S-64 [*Glycine max*]	6.32 / 56142	301	4	10	gi|6179947		
69	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	347	7	14	gi|170010		
70	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	603	11	26	gi|29469054		
71	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	229	3	9	gi|170010		
72	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	436	10	16	gi|170010		
73	51 kDa seed maturation protein [*Glycine max*]	6.65 / 51065	595	10	28	gi|414977		
74	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	506	10	18	gi|170010		
75*	Chaperonin containing t-complex protein 1 [*Ricinus communis*]	6.20 / 60929	449	7	13	B9SS36	0	95
76	51 kDa seed maturation protein [*Glycine max*]	6.65 / 51065	1119	19	37	gi|414977		
77	ATP synthase subunit alpha, mitochondrial [*Glycine max*]	6.23 / 55581	923	15	33	gi|231585		
78	51 kDa seed maturation protein [*Glycine max*]	6.65 / 51065	731	11	26	gi|414977		
79	late embryongenesis abundant protein [*Glycine max*]	6.33 / 50613	70	1	2	gi|170010		
80	Lea protein [*Glycine max*]	7.08 / 49484	1123	17	44	gi|311698		
81	Lea protein [*Glycine max*]	7.08 / 49484	430	7	17	gi|311698		
82*	UDP-glucose pyrophosphorylase [*Amorpha fruticosa*]	5.41 / 51576	570	10	21	Q8W557	0	91
83	ATP synthase beta subunit [*Triticum aestivum*]	5.56 / 59326	514	8	20	gi|525291		
84*	UDP-glucose pyrophosphorylase [*Amorpha fruticosa*]	5.41 / 51576	687	28	13	Q8W557	0	91

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
85	ATP synthase beta subunit [*Triticum aestivum*]	5.96 / 59326	962	14	31	gi|525291		
86	enolase [*Glycine max*]	5.31 / 47975	626	10	29	gi|42521309		
87	enolase [*Glycine max*]	5.31 / 47975	774	15	36	gi|42521309		
88*	Helicase, C-terminal [*Medicago truncatula*]	5.46 / 47140	770	11	34	A2Q689	0	96
89*	Argininosuccinate synthase [*Glycine max*]	6.55 / 52671	370	4	17	C6T9M0	0	100
90	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	619	12	22	gi|9967361		
91	inosine monophosphate dehydrogenase [*Glycine max*]	5.54 / 53488	170	3	5	gi|4468193		
92	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	622	11	22	gi|9967361		
93	Xylose isomerase [*Zea mays*]	5.35 / 54381	265	5	10	gi|195627736		
94	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	432	8	15	gi|9967361		
95	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	365	5	18	gi|29469054		
96*	Elongation factor 1, gamma chain; Glutathione S-transferase, C-terminal; Thioredoxin-like fold [*Medicago truncatula*]	5.77 / 47725	328	5	13	Q1SL16	0	84
97	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [*Glycine max*]	6.00 / 53033	990	16	32	gi|91214125		
98	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	601	9	27	gi|29469054		
99	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	229	3	11	gi|29469054		
100	sucrose-binding protein 2 [*Glycine max*]	6.12 / 56082	504	8	21	gi|29469054		
101	Lea protein [*Glycine max*]	7.08 / 49484	574	9	26	gi|311698		
102	6-phosphogluconate dehydrogenase [*Glycine max*]	5.55 / 56851	313	6	15	gi|2529229		
103	alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	743	15	24	gi|9967357		
104*	EBP1 [*Ammopiptanthus mongolicus*]	6.26 / 43867	332	6	19	A0EVX1	0	94
105*	Formate dehydrogenase [*Phaseolus vulgaris*]	6.28 / 43082	476	6	25	D2DWA5	0	92
106*	EBP1 [*Ammopiptanthus mongolicus*]	6.26 / 43867	238	4	12	A0EVX1	0	94
107	uv excision repair protein rad23 [*Ricinus communis*]	4.62 / 40747	106	2	4	gi|255551362		
108	beta amylase [*Triticum aestivum*]	8.60 / 31100	202	2	12	gi|32400764		
109*	uv excision repair protein rad23 [*Ricinus communis*]	4.80 / 41228	191	4	10	B9SSZ6	1E-168	74
110*	26S protease regulatory subunit 6A homolog [*Solanum lycopersicum*]	4.98 / 47627	672	11	26	P54776	0	94
111	Actin [*Glycine max*]	5.31 / 41938	301	4	15	gi|255636850		
112*	Actin [*Ananas comosus*]	5.31 / 41767	383	5	23	E9LWD6	0	94

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
113	Elongation factor Tu [*Glycine max*]	6.33 / 52590	452	7	16	gi|1169494		
114	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	596	11	21	gi|9967361		
115	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	687	13	23	gi|9967361		
116*	Fumarylacetoacetase [*Medicago truncatula*]	5.95 / 46097	186	3	12	Q2HT34	0	83
117	Spermidine synthase [*Glycine max*]	4.54 / 20713	74	1	6	gi|255634452		
118	40S ribosomal protein SA [*Glycine max*]	5.10 / 34056	231	4	15	gi|3334320		
119*	Glutamine synthetase [*Glycine max*]	5.22 / 39359	328	5	14	C6T8F0	0	100
120	Glutamine synthetase [*Glycine max*]	5.22 / 39359	235	5	10	gi|255635501		
121	protein disufide isomerase-like protein [*Glycine max*]	5.73 / 40673	126	1	9	gi|49257111		
122	protein disufide isomerase-like protein [*Glycine max*]	5.73 / 40673	161	3	9	gi|49257111		
123	Phosphoglycerate kinase [*Nicotiana tabacum*]	8.48 / 50317	376	5	18	gi|2499497		
124	Pv42p [*Phaseolus vulgaris*]	6.50 / 41627	111	2	5	gi|1113941		
125	phosphoglycerate kinase, chloroplast, putative [*Musa acuminata*]	8.74 / 50162	334	4	12	gi|102140037		
126*	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic [*Glycine max*]	5.67 / 49826	91	2	5	P45621	0	99
127*	Fumarylacetoacetase [*Medicago truncatula*]	5.95 / 46124	112	2	5	Q2HT34	0	82
128*	Transaminase mtnE, putative [EC 2.6.1.1] [*Ricinus communis*]	6.48 / 50623	116	2	6	B9S7T6	0	84
129	NADPH-specific isocitrate dehydrogenase [*Glycine max*]	6.13 / 49508	583	8	24	gi|169989		
130*	Fumarylacetoacetase [*Medicago truncatula*]	5.95 / 46097	346	8	19	Q2HT34	0	83
131	tryptophanyl-tRNA synthetase [*Arabidopsis thaliana*]	5.67 / 46010	175	2	5	gi|15229319		
132*	Transaminase mtnE, putative [EC 2.6.1.1] [*Ricinus communis*]	6.48 / 50623	204	3	10	B9S7T6	0	84
133*	Elongation factor Tu [*Glycine max*]	6.62 / 50222	792	14	33	C6T8W5	0	100
134	Aspartate aminotransferase [*Glycine max*]	7.16 / 50725	283	5	15	gi|169915		
135*	Phosphoglycerate kinase [*Glycine max*]	9.32 / 25297	496	9	45	C6T920	1E-126	100
136	glyceraldehyde-3-dehydrogenase C subunit [*Glycine max*]	6.72 / 36815	409	8	24	gi|74475508		
137*	Adenosine kinase, putative [*Ricinus communis*]	5.50 / 38020	239	4	10	B9T0A9	1E-178	89
138	35 kDa seed maturation protein [*Glycine max*]	5.96 / 35320	369	5	29	gi|4102190		
139	35 kDa seed maturation protein [*Glycine max*]	5.96 / 35320	845	18	56	gi|4102190		
140*	Phosphoglycerate kinase [*Glycine max*]	9.32 / 25297	580	10	57	C6T920	1E-126	100

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
141*	Phosphoglycerate kinase [*Glycine max*]	9.32 / 25297	196	3	18	C6T920	1E-126	100
142	alcohol dehydrogenase-1CN [*Phaseolus acutifolius*]	5.88 / 41624	563	10	27	gi|452767		
143	35 kDa seed maturation protein [*Glycine max*]	5.96 / 35320	327	5	25	gi|4102190		
144	glyceraldehyde-3-dehydrogenase C subunit [*Glycine max*]	6.72 / 36815	375	7	23	gi|74475508		
145*	L-lactate dehydrogenase [EC 1.1.1.27] [*Vitis vinifera*]	7.14 / 37661	145	2	8	A5AJN7	0	100
146	glycinin [*Glycine max*]	5.21 / 64351	286	5	12	gi|18641		
147	glycinin [*Glycine max*]	5.21 / 64351	99	2	3	gi|18641		
148	glycinin [*Glycine max*]	5.21 / 64351	483	10	20	gi|18641		
149	glycinin [*Glycine max*]	5.21 / 64351	341	7	20	gi|18641		
150	glycinin [*Glycine max*]	5.21 / 64351	120	2	3	gi|18641		
151	Glycinin G2 [*Glycine max*]	5.46 / 54927	280	5	10	gi|121277		
152	Glycinin G2 [*Glycine max*]	5.46 / 54927	294	4	10	gi|121277		
153	unknown [*Glycine max*]	5.01 / 24662	341	7	24	gi|255641502		
154	Glycinin G1 [*Glycine max*]	5.89 / 56299	464	8	15	gi|121276		
155*	Fructokinase-like protein [*Cicer arietinum*]	5.00 / 18587	441	7	49	Q8LPE5	6E-80	89
156*	Epoxide hydrolase 3 [*Prunus persica*]	5.16 / 35314	376	5	26	D8L7V9	1E-140	76
157*	Glycinin G1 [Cleaved into: Glycinin A1a subunit; Glycinin Bx subunit] [*Glycine max*]	6.15 / 56099	350	6	11	P04776	0	99
158	Glycinin G1 [*Glycine max*]	5.89 / 56299	238	4	8	gi|121276		
159	Glycinin G1 [*Glycine max*]	5.89 / 56299	157	2	5	gi|121276		
160*	Nodule-enhanced malate dehydrogenase [*Glycine max*]	8.11 / 43463	450	6	20	O81278	0	92
161	isoflavone reductase homolog 2 [*Glycine max*]	5.60 / 33919	242	5	22	gi|6573171		
162	isoflavone reductase homolog 2 [*Glycine max*]	5.60 / 33919	341	5	25	gi|6573171		
163*	Aldo-keto reductase, putative (EC 1.1.1.200) [*Ricinus communis*]	5.93 / 35035	249	3	16	B9RG54	1E-158	87
164*	2-alkenal reductase[*Artemisia annua*]	5.94 / 38068	424	9	30	C0LNV1	1E-137	69
165	malate dehydrogenase [*Glycine max*]	8.23 / 36347	377	6	19	gi|5929964		
166	cytosolic malate dehydrogenase [*Glycine max*]	6.32 / 35846	305	3	20	gi|42521311		
167	cytosolic malate dehydrogenase [*Glycine max*]	6.32 / 35846	303	3	20	gi|42521311		
168*	35 kDa seed maturation protein [*Glycine max*]	5.89 / 34556	1048	22	59	Q9ZTY1	1E-170	97

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
169	malate dehydrogenase [*Glycine max*]	8.23 / 36347	529	9	26	gi|5929964		
170	35 kDa seed maturation protein [*Glycine max*]	5.96 / 35320	453	9	33	gi|4102190		
171*	Putative quinone oxidoreductase [*Fragaria ananassa*]	6.68 / 34488	416	6	27	Q941I0	1E-130	74
172*	Seed maturation protein PM34 [*Glycine max*]	7.59 / 32048	268	4	22	Q9LLQ6	1E-160	95
173*	Alcohol dehydrogenase, putative (EC 1.3.1.74) [*Ricinus communis*]	6.37 / 38841	158	3	11	B9RM02	1E-151	76
174	NAD(P)H-dependent 6'-deoxychalcone synthase [*Glycine max*]	6.32 / 35639	184	3	12	gi|112837		
175*	Annexin, putative [*Ricinus communis*]	6.18 / 25194	629	8	50	B9RJJ1	1E-103	83
176	Seed maturation protein PM34 [*Glycine max*]	6.60 / 32032	166	3	10	gi|9622153		
177*	Seed maturation protein PM34 [*Glycine max*]	7.59 / 32048	421	5	25	Q9LLQ6	1E-160	95
178*	Annexin, putative [*Ricinus communis*]	6.18 / 25194	827	11	57	B9RJJ1	1E-103	83
179	late embryogenesis abundant protein group 5 protein [*Arachis hypogaea*]	4.74 / 27410	135	3	8	gi|312599835		
180	late embryogenesis abundant protein group 5 protein [*Arachis hypogaea*]	4.74 / 27410	126	5	8	gi|312599835		
181*	Lactoylglutathione lyase [*Gossypium hirsutum*]	5.37 / 31740	174	2	15	D2D330	1E-137	82
182	lactoylglutathione lyase, putative [*Ricinus communis*]	5.21 / 33186	174	2	14	gi|255554865		
183	Cysteine synthase [*Glycine max*]	5.69 / 34362	547	8	34	gi|18252506		
184	Enoyl-ACP reductase [*Nicotiana tabacum*]	8.88 / 41925	255	3	14	gi|2204236		
185*	Maturation-associated protein [*Glycine max*]	6.07 / 23704	795	16	60	Q39876	1E-137	99
186	Maturation protein [*Glycine max*]	6.02 / 25644	628	10	58	gi|170020		
187*	Maturation-associated protein [*Glycine max*]	6.07 / 23704	864	20	62	Q39876	1E-137	99
188*	Maturation-associated protein [*Glycine max*]	6.07 / 23704	1016	24	88	Q39876	1E-137	99
189	Maturation protein [*Glycine max*]	6.02 / 25644	1235	25	90	gi|170020		
190*	Maturation-associated protein [*Glycine max*]	6.07 / 23704	1289	32	93	Q39876	1E-137	99
191	seed maturation protein PM24 [*Glycine max*]	5.14 / 26938	371	5	24	gi|6648964		
192*	Seed maturation protein PM34 [*Glycine max*]	6.38 / 32097	516	15	31	Q9LLQ6	1E-111	68
193*	Ran protein/TC4 protein-like [*Solanum tuberosum*]	6.25 / 25512	169	3	19	Q38JF9	1E-129	100
194*	Embryo-specific protein [*Oryza sativa* subsp. indica]	6.42 / 27260	266	4	20	Q9ZNS9	1E-78	60
195*	Nascent polypeptide associated complex alpha subunit, putative [*Ricinus communis*]	4.28 / 24116	158	3	15	B9S5M4	5E-87	76
196	glycinin [*Glycine max]*	5.21 / 64351	144	3	6	gi|18641		

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
197	glycinin [*Glycine max]*	5.21 / 64351	170	3	9	gi|18641		
198	seed maturation protein PM24 [*Glycine max*]	5.14 / 26938	294	4	24	gi|6648964		
199	seed maturation protein PM24 [*Glycine max*]	5.14 / 26938	371	5	24	gi|6648964		
200	seed maturation protein PM24 [*Glycine max*]	5.14 / 26938	586	10	64	gi|6648964		
201	seed maturation protein PM25 [*Glycine max*]	4.99 / 25827	738	14	59	gi|6648966		
202	seed maturation protein PM26 [*Glycine max*]	4.83 / 26201	182	5	14	gi|6648968		
203	mutant glycinin subunit A1aB1b [*Glycine max*]	5.60 / 43963	242	4	12	gi|254029115		
204	mutant glycinin subunit A1aB1b [*Glycine max*]	5.51 / 44066	106	1	5	gi|254029113		
205*	Proteasome subunit alpha type [*Glycine max*]	5.60 / 27533	480	11	38	C6TG97	1E-140	100
206*	NAD-dependent epimerase/dehydratase [*Zea mays*]	8.91 / 35877	274	4	16	B6T962	1E-117	81
207*	Putative uncharacterized protein [*Glycine max*]	5.72 / 27847	293	6	23	C6SWV3	1E-142	100
208*	Isocitrate lyase and phosphorylmutase [*Medicago truncatula*]	5.90 / 32236	340	6	25	A2Q4X2	1E-129	76
209*	Isocitrate lyase and phosphorylmutase [*Medicago truncatula*]	5.79 / 29646	118	2	12	A2Q4X2	1E-129	76
210	alpha' subunit of beta-conglycinin [*Glycine max*]	5.23 / 65160	233	3	8	gi|9967361		
211*	Proteasome subunit alpha type [*Glycine max*]	4.70 / 26151	494	8	39	C6TGU2	1E-130	100
212	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	82	1	7	gi|5733686		
213	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	92	1	7	gi|5733686		
214*	26S proteasome regulatory particle non-ATPase subunit 12 [*Camellia sinensis*]	5.03 / 31190	204	3	14	Q0GEA1	1E-130	86
215	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	207	3	19	gi|5733686		
216*	Isopentenyl pyrophosphate isomerase [*Pueraria montana* var. lobata]	5.13 / 27583	144	3	15	Q6EJD1	1E-133	97
217*	Probable ATP synthase 24 kDa subunit, mitochondrial [*Arabidopsis thaliana*]	6.40 / 27586	409	7	42	Q9SJ12	1E-89	68
218*	Proteasome subunit alpha type [*Glycine max*]	5.51 / 25562	98	2	8	C6TH59	1E-128	100
219	ferritin [*Hordeum vulgare* subsp. vulgare]	5.55 / 15892	81	1	11	gi|15788943		
220	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	488	8	42	gi|5733686		
221	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	166	2	19	gi|5733686		
222	In2-1 protein [*Glycine max*]	5.21 / 27071	105	2	10	gi|11385579		
223*	Proteasome subunit alpha type [*Glycine max*]	5.60 / 27533	330	6	24	C6TG97	1E-140	100
224*	Anthocyanin 3'-O-methyltransferase [*Glycine max*]	5.62 / 26791	350	7	23	F2YBA9	1E-93	74

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
225*	Proteasome subunit alpha type [*Glycine max*]	5.51 / 25562	304	6	21	C6TH59	1E-128	100
226*	Ferritin [*Glycine max*]	5.44 / 28204	206	3	17	C6TM43	1E-137	100
227	allergen Gly m Bd 28K [*Glycine max*]	5.73 / 52780	176	5	6	gi|12697782		
228*	Proteasome subunit alpha type-6 [*Glycine max*]	5.83 / 27489	603	9	45	O48551	1E-138	100
229*	Triosephosphate isomerase [*Glycine max*]	6.15 / 19701	111	2	13	C6T6J1	7E-99	100
230	glutathione S-transferase GST 24 [*Glycine max*]	5.74 / 24928	120	2	10	gi|11385463		
231*	DHAR class glutathione transferase DHAR2 [*Populus trichocarpa*]	5.81 / 23530	202	5	13	D2WL74	2E-95	81
232	glutathione S-transferase GST 24 [*Glycine max*]	5.74 / 24928	104	1	10	gi|11385463		
233	eukaryotic translation initiation factor iso4E [*Phaseolus vulgaris*]	5.71 / 22922	173	3	15	gi|156153120		
234	triosephosphate isomerase [GIycine max]	5.87 / 27415	402	7	23	gi|77540216		
235	triosephosphate isomerase [*Glycine max*]	5.87 / 27415	824	14	51	gi|77540216		
236	2,4-D inducible glutathione S-transferase [*Glycine max*]	6.24 / 24759	315	4	28	gi|2920666		
237	glutathione S-transferase GST 24 [*Glycine max*]	5.74 / 24928	564	11	43	gi|11385463		
238	glutathione S-transferase GST 14 [*Glycine max*]	7.14 / 25340	279	6	26	gi|11385443		
239	cysteine proteinase inhibitor [*Glycine max*]	7.27 / 27659	200	5	16	gi|1944319		
240*	Proteasome subunit alpha type [*Glycine max*]	6.62 / 27657	297	4	29	C6TAW4	1E-138	100
241*	Chalcone--flavonone isomerase 1A [*Glycine max*]	6.23 / 23277	501	9	50	Q93XE6	1E-116	99
242*	60S ribosomal protein L14, putative [*Ricinus communis*]	10.42 / 15404	225	2	34	B9SV21	5E-59	85
243	MnSOD [*Glycine max*]	8.56 / 26690	363	3	25	gi|147945633		
244*	Cysteine proteinase inhibitor [*Glycine max*]	6.73 / 27779	520	13	43	O04720	1E-128	93
245*	1-Cys peroxiredoxin [*Medicago truncatula*]	6.44 / 24576	322	5	37	Q6E2Z6	1E-108	85
246	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	229	4	19	gi|5733686		
247	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	359	5	31	gi|5733686		
248	maturation protein pPM32 [*Glycine max*]	5.49 / 18871	79	1	7	gi|5733686		
249*	Proteasome subunit beta type [*Glycine max*]	5.30 / 25170	228	4	18	C6SVE5	1E-131	100
250*	GroES chaperonin, putative [*Ricinus communis*]	6.77 / 26640	521	8	49	C6TNA3	1E-109	80
251*	Small heat-shock protein, putative [*Ricinus communis*]	6.93 / 25694	126	2	13	B9RV59	9E-66	57
252*	Proteasome subunit beta type [*Ricinus communis*]	5.86 / 22699	424	9	43	B9RTN1	1E-104	93

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
253	Hsp22.3 [*Glycine max*]	5.88 / 22417	125	2	10	gi|710434		
254	allergen Gly m Bd 28K [*Glycine max*]	5.73 / 52780	194	5	10	gi|12697782		
255	Glycinin G1 [*Glycine max*]	5.89 / 56229	173	3	6	gi|121276		
256*	Glycinin G1 [*Glycine max*]	6.15 / 56099	158	2	6	P04776	0	99
257	Glycinin G1 [*Glycine max*]	5.89 / 56299	327	5	12	gi|121276		
258	Glycinin G1 [*Glycine max*]	5.89 / 56299	293	5	12	gi|121276		
259	trypsin inhibitor subtype A [*Glycine max*]	4.99 / 24346	573	14	46	gi|18770		
260	trypsin inhibitor subtype A [*Glycine max*]	4.99 / 24346	604	12	46	gi|18770		
261	trypsin inhibitor subtype A [*Glycine max*]	4.99 / 24346	182	3	19	gi|18770		
262*	ATP synthase d chain [*Cucumis melo* subsp. melo]	5.11 / 19944	248	4	30	E5GCK9	2E-81	86
263*	ATP synthase d chain [*Cucumis melo* subsp. melo]	5.20 / 19911	475	7	54	E5GCK9	6E-83	88
264*	Glutathione peroxidase [*Glycine max*]	6.59 / 18605	317	4	37	C6SZK3	4E-92	100
265*	Universal stress protein 1 [*Gossypium arboreum*]	5.61 / 17992	259	4	25	B0YQX0	6E-62	72
266	disease resistance response protein 1 [*Glycine max*]	6.49 / 28395	204	4	15	gi|296051287		
267*	Glutathione peroxidase [*Glycine max*]	6.59 / 18662	359	7	43	C6SZX7	4E-92	100
268*	Mitochondrial import inner membrane translocase, subunit Tim17/22 [*Medicago truncatula*]	5.91 / 18957	102	3	14	Q2HU23	7E-78	81
269*	60S acidic ribosomal protein P3, putative [*Ricinus communis*]	4.46 / 11885	191	5	36	B9SNZ6	5E-37	68
270*	60S acidic ribosomal protein P3, putative [*Ricinus communis*]	4.29 / 12313	99	1	14	B9SNZ6	2E-34	66
271	seed maturation protein PM28 [*Glycine max*]	4.66 / 9506	392	8	67	gi|4836405		
272	seed maturation protein PM22 [*Glycine max*]	5.16 / 16735	197	3	38	gi|4585271		
273*	G.max mRNA from stress-induced gene (H4) [*Glycine max*]	4.73 / 16808	214	3	21	Q43453	3E-79	92
274	seed maturation protein PM22 [*Glycine max*]	5.16 / 16735	422	9	46	gi|4585271		
275	seed maturation protein PM22 [*Glycine max*]	5.16 / 16735	680	17	58	gi|4585271		
276*	Late embryogenesis abundant protein [*Vigna radiata*]	5.53 / 12338	604	12	68	A4VBF1	2E-52	92
277*	Peroxiredoxin [*Pisum sativum*]	5.41 / 17469	264	3	36	B3GV28	1E-81	92
278	alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	659	11	23	gi|9967357		
279*	Peroxiredoxin [*Pisum sativum*]	5.41 / 17469	285	4	36	B3GV28	1E-81	92
280*	Putative uncharacterized protein [*Glycine max*]	5.73 / 14170	280	8	44	C6T0B5	4E-69	100

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
281*	Disease resistance response protein 1 [*Glycine max*]	5.18 / 20523	178	3	25	E0YNQ4	9E-55	57
282	alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	414	6	17	gi|9967357		
283	disease resistance response protein 1 [*Glycine max*]	6.49 / 28395	277	4	19	gi|296051287		
284*	Glutathione peroxidase [*Glycine max*]	8.95 / 25255	250	3	24	C6SYT7	1E-125	100
285*	CBS1 [*Hyacinthus orientalis*]	9.09 / 22993	386	6	35	Q676Z5	9E-82	81
286	seed maturation protein PM31 [*Glycine max*]	6.10 / 17907	99	2	9	gi|4838149		
287	seed maturation protein PM31 [*Glycine max*]	6.10 /19907	146	3	14	gi|4838149		
288*	17.5 kDa class I heat shock protein [*Glycine max*]	5.98 / 17518	141	2	19	P04794	4E-82	98
289*	CBS1 [*Hyacinthus orientalis*]	9.04 / 22884	541	9	40	Q676Z5	4E-84	80
290	ADP-ribosylation factor [*Glycine max*]	6.42 / 20396	287	4	28	gi|4324967		
291	seed maturation protein PM31 [*Glycine max*]	6.10 / 17907	144	4	14	gi|4838149		
292*	Heat shock protein 17.5 [*Malus domestica*]	6.33 / 17729	315	5	36	B2ZBG8	3E-67	80
293	Superoxide dismutase [Cu-Zn] 4AP [*Zea mays*]	5.64 / 15175	195	4	26	gi|134598		
294*	Em protein [*Glycine max*]	6.30 / 11134	225	3	49	P93165	1E-44	88
295*	Em protein [*Glycine max*]	6.30 / 11134	279	3	55	P93165	1E-44	88
296	Nucleoside diphosphate kinase [*Glycine max*]	6.91 / 16402	518	7	48	gi|26245395		
297*	USP-like protein [*Astragalus sinicus*]	6.73 / 18353	305	4	39	Q07A28	3E-73	84
298*	FK506-binding protein 2 [*Vicia faba*]	5.92 / 15994	217	3	24	Q41649	2E-63	85
299	ubiquitin-conjugating enzyme E2 variant 1D [*Arabidopsis thaliana*]	6.20 / 16693	121	2	13	gi|18409633		
300	heat-shock protein [*Jatropha curcas*]	6.85 / 18076	162	4	18	gi|284433776		
301*	60S acidic ribosomal protein P2, putative [*Ricinus communis*]	4.38 / 11109	196	3	38	B9SSU4	4E-37	74
302	Trypsin inhibitor subtype A [*Glycine max*]	4.99 / 24346	176	3	14	gi|18770		
303	Alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	888	15	29	gi|9967357		
304	2S albumin [*Glycine max*]	5.20 / 19018	235	4	27	gi|5902685		
305	2S albumin [*Glycine max*]	5.20 / 19018	203	4	27	gi|5902685		
306	seed maturation protein PM22 [*Glycine max*]	5.16 / 16735	265	6	31	gi|4585271		
307	napin-type 2S albumin 1 precursor [*Glycine max*]	6.00 / 18393	124	2	12	gi|4097894		
308	late embryogenesis-abundant protein [*Glycine max*]	5.52 / 11485	347	5	48	gi|1762955		

(Table 1). Continued.

ID	Protein Identification [species]	Calc pI/Mr	MS Score	# PM	% SC	gi / Uniprot Access. No.	Blast E-Value	Blast % Identity
309	late embryogenesis-abundant protein [*Glycine max*]	5.52 / 11485	340	4	49	gi|1762955		
310	glycine-rich RNA-binding protein [*Glycine max*]	6.58 / 15894	218	3	22	gi|5726567		
311	napin-type 2S albumin 1 precursor [*Glycine max*]	6.00 / 18393	156	3	21	gi|4097894		
312	late embryogenesis-abundant protein [*Glycine max*]	5.52 / 11485	527	10	67	gi|1762955		
313*	Lactoylglutathione lyase family protein [*Arabidopsis lyrata* subsp. lyrata]	5.41 / 15536	267	5	41	D7KH42	2E-52	75
314	unknown [*Glycine max*]	5.50 / 15345	99	2	11	gi|255626071		
315	glycinin [*Glycine max*]	5.21 / 64351	62	1	2	gi|18641		
316	alpha subunit of beta conglycinin [*Glycine max*]	4.92 / 63184	997	19	33	gi|9967357		
317	P24 oleosin isoform B [*Glycine max*]	8.89 / 23378	145	3	14	gi|266689		
318	Elongation factor 1-alpha [*Solanum lycopersicum*]	9.19 / 49599	143	2	6	gi|119150		
319	Pyruvate kinase [*Glycine max*]	7.89 / 55966	322	5	11	gi|22296818		
320	51 kDa seed maturation protein [*Glycine max*]	6.65 / 51065	772	11	30	gi|414977		
321	Lea protein [*Glycine max*]	7.08 / 49484	1036	17	45	gi|311698		
322*	Putative 60S ribosomal protein L1 [*Trifolium pratense*]	10.43 / 44593	318	3	19	Q2PER2	0	87
323*	Fructose-bisphosphate aldolase [*Glycine max*]	7.12 / 38544	511	8	24	C6TMG1	0	100
324	glyceraldehyde-3-dehydrogenase C subunit [*Glycine max]*	6.72 / 36815	562	11	27	gi|74475508		
325*	Seed maturation protein PM34 [*Glycine max*]	7.59 / 32048	309	5	22	Q9LLQ6	1E-160	95
326	seed maturation protein PM34 [*Glycine max*]	6.60 / 32032	356	5	25	gi|9622153		
327	40S ribosomal S4 protein [*Glycine max*]	10.27 / 30056	420	6	29	gi|22138108		
328*	Ribosomal protein L30 [*Medicago truncatula*]	9.88 / 28154	405	9	33	Q2HVI3	1E-116	87
329*	1-Cys peroxiredoxin [*Medicago truncatula*]	6.44 / 24576	455	7	41	Q6E2Z6	1E-108	85
330	P24 oleosin isoform A [*Glycine max*]	8.01 / 23487	287	5	26	gi|1709459		
331	ribosomal protein L16 [*Arabidopsis thaliana*]	9.94 / 21003	300	4	29	gi|550544		
332*	Peptidyl-prolyl cis-trans isomerase 1 [*Glycine max*]	8.70 / 18501	233	3	24	Q8W171	6E-95	98
333	soybean seed maturation polypeptides [*Glycine max*]	9.66 / 15572	280	4	31	gi|18750		
334	seed maturation protein PM30 [*Glycine max*]	8.95 / 15145	234	5	35	gi|4838147		
335	seed maturation protein PM30 [*Glycine max*]	8.95 / 15145	234	4	35	gi|4838147		

*NCBI database search for these spots resulted in an 'unknown' or 'hypothetical' protein match. Therefore the resulting sequences were subjected to a sequence similarity search *via* BLAST under the UNIPROT database, in order to obtain proper identity of the protein. The Blast e-value and % identity are provided.
ID, spot number referred to in Figure **1**; calc pI/Mr, theoretical values for isoelectric point and molecular weight; MS Score, MOWSE Score; PM, the number of peptides matched; SC, sequence coverage by PMF using LC-MS/MS.

significant number of low abundant proteins in our embryonic tissue samples.

Identification of Embryonic Axis Proteins

Information such as assigned protein spot number, theoretical isoelectric point (pI), and molecular weight (Mr), protein identity and its original species, number of peptides matched, percentage sequence coverage, MOWSE score, database searched, and accession number of the best match for the 335 identified protein spots are listed in Table **1**. Of the 401 protein spots processed, 335 proteins were successfully identified by querying NCBInr databases using the Mascot search engine. Some of the identified proteins that had a significant score matching a hypothetical or predicted or unknown listing in NCBI were subsequently subjected to a homology search against the UniProt (www.uniprot.org) database using BLAST (Basic Local Alignment Search Tool) [19]. Taking into account the multiplicity of the spots, we identified a total of 200 unique proteins on the gel with highest probability score.

Previous reports are available on embryo proteins of buckwheat grains, tomato and other crop seeds. To our knowledge, the existing reference map of soybean embryonic axes proteins is limited. Sheoran *et al*. [20] used 2D-PAGE and compared the protein profile between embryo and endosperm of germinating tomato seeds. The authors observed 352 major protein spots in the embryos, and 369 spots in endosperm samples. They identified 75 of these spots using mass spectrometry and related their function to seed germination. Jain *et al*. [21] reported the identification of 80 protein spots in *Brassica napus* using multidimensional protein identification technology.

We found multiple spots identified as the same protein in our 2D-PAGE gels (Figure **1**). Similar results, the presence of multiple spots for single protein, have previously been reported using 2D-PAGE gels to analyze Arabidopsis seed proteins [22].

***Protein Classification of the* Identified Proteins**

To better understand the functions of the identified proteins, we have used the Gene Ontology (GO) database [23] to categorize the function of the embryonic axis proteins. MySQL was used to store and manage information from the GO databases. A total of 200 proteins were subjected to molecular functional analysis. Because the embryonic axis will be involved in rapid tissue growth upon germination, it is not surprising that a significant number of the identified proteins (42.9%) are involved in catalytic activity. About 40% of the identified proteins have some sort of binding activity, while 5.3% exhibit nutrient reservoir activity, 4.0% have structural molecule activity, 3.2% have antioxidant activity, 2.4% have transporter activity, 1.2% have enzyme regulator activity, 0.8% have molecular transducer activity, and 0.8% have transcription regulator activity (Figure **2**).

Storage Proteins

We found some seed storage proteins as abundant proteins in soybean embryonic tissues in our 2D-PAGE gels. Storage proteins are grouped into two types, β-conglycinin and glycinin, based on sedimentation rates in sucrose gradients. Beta-conglycinin, a 7S globulin, is a trimeric glycoprotein consisting of three types of non-identical but homologous polypeptide subunits: α, α′ and β, in seven different combinations with molecular weight of 180 kDa [24]. Developmental changes in the synthesis of storage proteins have been investigated in a number of legumes such as *Phaseolus vulgaris* [25], and *Pisum sativum* [26], where it was shown that storage proteins begin to accumulate after the cessation of cell division in developing cotyledons. Meinke *et al*. [1] reported that all 3 subunits of 7S protein do not appear at the same stage of development. Each subunit exhibits a characteristic pattern of accumulation during the latter stages of seed development. Twenty protein spots from our gels were successfully identified as α subunits of β-conglycinin (19, 20, 23-25, 37, 45, 46, 48, 90, 92, 94, 103, 114, 115, 210, 278, 282, 303, 316). We did not find any β -subunit of β-conglycinin even though its accumulation was clearly demonstrated in the embryonic axis by immunoblot analysis [27]. Using an immunoprecipitation technique, Meinke *et al*. [1] also reported the absence of this β -subunit in the embryonic axis of *Glycine max* cv. Provar. Alteration of β-conglycinin subunit composition in soybean seeds that had developed under elevated temperatures was observed using 2D-PAGE by Ren *et al*. [28].

Glycinin, a hexameric 11S globulin seed storage protein (360 kDa), consists of both acidic (A) and basic (B) polypeptides. Glycinin is encoded by five non-allelic genes Gy1, Gy2, Gy3, Gy4, and Gy5, which code for five precursor protein molecules G1, G2, G3, G4 and G5, respectively [29]. Based on physical properties, these five subunits are classified into two distinct major groups. Group I consists of G1 (A1aBx), G2 (A2B1a),

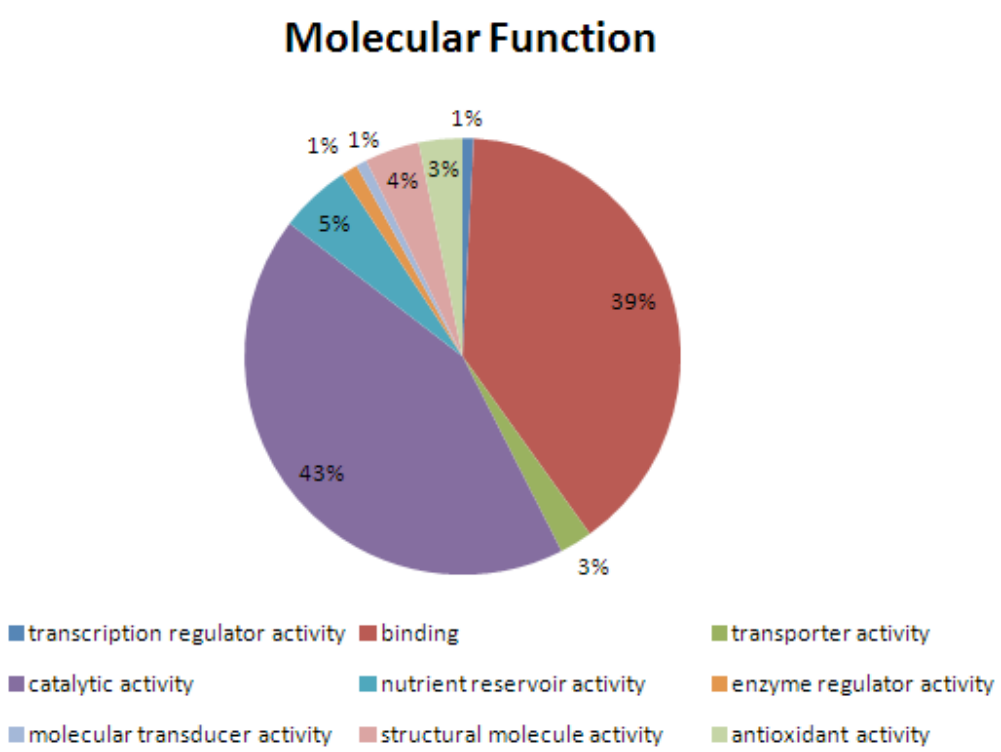

Figure 2: GO analysis of proteins identified in soybean embryonic axis. A total of 200 unique proteins were analyzed. The categories of molecular function are shown as pie charts.

and G3 (A1aB1b) proteins and group II contains G4 (A5A4B3) and G5 (A3B4) subunits. We observed twenty two spots of glycinin in our earlier investigation of soybean seed samples using 2D-PAGE analysis [9]. In the present work, two spots (151, 152) were identified as glycinin G2, and eight protein spots (154, 157-159, 255-258) were identified as glycinin G1. Ten other spots (146-150, 196, 197, 203, 204, 315) were also identified as glycinin. The presence of multiple spots of storage proteins has been reported in the literature [11, 12]. The presence of multiple spots with different pI and /or Mr may be due to different isoforms derived from different genes of a multigene family or due to post-translational modifications of proteins.

Two protein spots were identified as ferritin (spots 219, 226) in our 2D-PAGE. Ferritin is an iron storage protein widely distributed in plants and animals. Plant ferritin has been well characterized in legumes and is synthesized as a precursor protein with an N-terminal transit peptide responsible for its localization in plastids [30]. Parker *et al*. [31] reported that ferritin accumulates 3.5 and 5.2 fold after short and long term salt stress respectively in rice leaf lamina. We also observed two spots (spot 317, 330) of 24 kDa oleosin isoform A and B in our study. Oleosin is an oil body membrane protein and is known to exist in at least 2 isoforms in soybean [32].

Trypsin Inhibitors

Other major soybean seed proteins are the 2S proteins, which consist primarily the well-studied and characterized trypsin inhibitors [33]. Mayer and Shain [34] reported that these anti-nutritional proteinase inhibitors temporally and spatially regulate the initiation of proteolysis during germination. Soybean lines that lack either KTIs [35] or BBIs [36] have been developed and they show that these proteins are not essential for normal seed or plant growth and development.

Kunitz trypsin inhibitor (KTI) inhibits an important animal digestive enzyme, and it is abundant in mature soybean seed. KTIs have been characterized as food allergens in humans and have 32% sequence homology with a rye grass pollen allergen [37]. Becker-Ritt *et al*. [38] reported significant differences in the quantity of KTIs among different genotypes of soybeans. We reported variation of KTI expression in wild and cultivated soybean genotypes [10]. Jofuku and Goldburg [39] found that soybean KTIs are encoded by three genes, *KTI1*, *KTI2,* and *KTI3*, that are expressed during embryogenesis and in the mature plant. Four spots of the embryonic axes proteins separated by 2D-PAGE in our study (259-261, 302) were identified as trypsin inhibitor subtype A, supporting an earlier investigation by Hajduch *et al*. [40] who found four

protein spots of KTI in immature soybean seed. Vensel *et al*. [41] reported the presence of one protein spot of trypsin inhibitor in late stage wheat grain development, and its absence at an early stage.

The Bowman-Birk inhibitor (BBIs) family consists of cysteine-rich protease inhibitors with molecular masses of about 8–16 kDa, and they were discovered first in soybean seeds [42]. These protease inhibitors are double-headed, with two reactive sites on a single inhibitor molecule. Deshimaru *et al*. [43] proposed that BBIs function in the storage of sulfur amino acids during dormancy and in protection against insects and microorganism. BBIs identified in the *Fabaceae*, including soybean (*G. max*) and lima beans (*Phaseolus lunatus*), are encoded by a family of related genes [44]. We identified two spots of cysteine proteinase inhibitors (239, 244) on our gels of soybean embryonic axis proteins although Hajduck *et al*. [40] reported one spot of cysteine proteinase in immature soybean seed. Four other 2S spots (304, 305, 307, 311) in our analysis were identified as 2S-albumin.

Allergen Proteins

In addition to anti-nutritional proteins, soybeans seeds also contain three major allergens, Gly m Bd 60K, Gly m Bd 30K and Gly m Bd 28K [45]. Gly m Bd 60K consists of the α-subunit of β-conglycinin, as well as G2 subunits and acidic G1 polypeptides of glycinin [46]. Our 2D-PAGE showed all the above Gly m Bd 60K allergens in a total of thirty protein spots, (19, 20, 23-25, 37, 45, 46, 48, 90, 92, 94, 103, 114, 115, 210, 278, 282, 303, 316, 154, 157-159, 255-258, 151, 152). The second major soybean allergen, Gly m Bd 30 K or P34 protein was first identified by Kalinski *et al*. [47] from fractionated soybean oil-body membrane. Kalinski *et al*. [48] reported that P34 was encoded by more than one gene. We did not find any P34 allergen protein in our present study, although in an earlier study we reported the accumulation of P34 in wild and cultivated genotypes of mature soybean seed [49]. The third allergen, Gly m bd 28 K is a glycoprotein and has sequence similarity to members of the cupin protein family, which contains several plant allergens. Cupin domains are generally more resistant to proteolytic digestion and are stable to a number of physical and chemical denaturants [50]. Gly m Bd 28K allergen is processed into two smaller polypeptides in the soybean seed, an N-terminal polypeptide and a C-terminal polypeptide [51]. We observed the presence of two protein spots of (227, 254) of Gly m bd 28 K allergens in our 2D-PAGE analysis of embryonic axes of soybean.

Stress Related Proteins

Several types of stress related proteins were identified in soybean embryonic axes including late embryogenesis-abundant (LEA) proteins and heat shock proteins. LEA proteins belong to the dehydrin superfamily and accumulate during the latter stages of seed embryogenesis. They are denoted simply as maturation proteins [52] and slowly disappear during germination. These proteins are also found in vegetative organs, especially under stress conditions such as cold, drought, and high salinity [53]. They may be involved in binding or replacement of water under dehydration conditions. In addition, these proteins help to maintain protein and membrane structure [54]. Other in-vitro studies have shown that some LEA proteins can protect enzyme activities or interact with sugar to prevent protein aggregation [55]. Over expression of certain LEA genes in transgenic plants resulted in enhanced tolerance to abiotic stresses [56] and tolerance to drought and high salinity [57]. Liu *et al*. [58] reported that LEA proteins such as Gm PM1 and GmPM9 are metal binding proteins which may function in reducing oxidative damage induced by abiotic stress. Shih *et al*. [59] characterized two soybean LEA IV proteins, acidic (GmPM 28) and basic (GmPM1), using circular dichroism and fourier transform infrared spectrometry. They concluded that the LEA IV proteins are functional in the dry state and may stabilize desiccation-sensitive proteins and plasma membranes during dehydration.

In our study, we found LEA proteins distributed at various pH levels. Approximately twenty two LEA protein spots (61, 63, 66, 67, 69, 71, 72, 74, 79, 80, 81, 101, 179, 180, 194, 276, 294, 295, 312, 308, 309, 321) were observed in the soybean embryonic axis. We also found an additional 47 protein spots identified as seed maturation proteins (SMP) distributed widely over the 2D gel (1, 2, 73, 76, 78, 320, 138, 139, 325, 143, 168, 170, 172, 176, 177, 326, 185-192, 198-202, 212, 213, 215, 220, 221, 246-248, 333, 271, 272, 274, 275, 286, 287, 291, 306, 334, 335). In our earlier publications we have reported the variation of LEA proteins and SMP among wild and cultivated soybean seeds [60]. Hsieh *et al*. [61] found large variation of SMPs in *Glycine* species collected from Taiwan and concluded that the variation occurs in nature.

The differences in SMP spot numbers in different studies may be due to the different soybean genotypes and/or different protein extraction/detection protocols.

Pouchkina *et al*. [62] reported enhanced expression of genes encoding LEA proteins during cell dehydration.

Another type of stress related protein, the heat shock proteins (HSPs), are synthesized in response to elevated temperature and during various developmental processes, including seed maturation [63]. Hong and Vierling [64] described that HSP synthesis is developmentally regulated, being abundant in dry mature seeds and disappearing during germination. The level of HSP is ~1% of the total protein in pea and soybean [65]. HSPs function as molecular chaperones, associated with protein folding, protein translocation and degradation, assembly of oligomeric proteins, modulation of receptor activities, mRNA protection, prevention of protein denaturation, and stress-induced aggregation, and post-stress ubiquitin and chaperonin-aided repair [66]. DeRocher and Vierling [67] reported that LEAs and HSPs are involved in protective functions throughout germination in pea seeds. We found nine protein spots that were identified as HSPs in our analysis (18, 21, 22, 52, 251, 253, 288, 292, 300). Ahsan *et al*. [68] analyzed and compared HSP in soybean seedlings leaves, stem, and roots under heat stress using 2D analysis. Stupnikova *et al*. [69] demonstrated the function of pea mitochondrial HSP22 in protecting against cold or high temperature in maturing seed embryos. Additional protein spots related to stress/ pathogen/ disease/defense identified in our investigation were two spots of putative annexin (spots 175, 178), three spots of chaperonin containing t-complex protein (54, 65, 75), one spot of β-amylase (108), one spot of GroES chaperonin (250), two spots of universal stress protein (265, 297), one spot of epoxide hydrolase (156), one spot of hypothetical stress induced protein (273), four spots of peroxiredoxin (spot 245, 329, 277, 279), one spot of Pv42p (spot 124), three spots of disease resistance response protein (spot 266, 281, 283), two spots of superoxide dismutase (243, 293), two spots of CBS1(285, 289), three spots of glutathione peroxidase (264, 267, 284), and herbicide safener inducible 27-kDa In2-1 protein (spot 222). All of these proteins may protect the embryo against cold stress and oxidative stress, notably reactive oxygen species like hydrogen peroxide and superoxide radicals [70]. Vensel *et al*. [41] reported the presence of these protective proteins in wheat endosperm in the latter stages of endosperm development, and suggested that different metabolic processes can generate hydrogen peroxide during endosperm development. Scandalios *et al*. [71] reported that superoxide radicals increase dramatically in response to biotic and abiotic stresses, and also increase due to salinity [31]. Liu *et al*. [58] have recently shown that the abundance of superoxide dismutase and a disease resistance response protein were higher in the embryo than in the endosperm of physic nut (*Jatropha curcas*., L).

The observed protein spots of peroxiredoxin in our study may be related to embryonic axis protection against desiccation. Ostergaard *et al*. [72] reported the presence of three types of peroxiredoxin in barley seeds, that are proposed to protect the embryo and aleurone against reactive oxygen species and free radicals during seed development and desiccation. In addition, we found nine spots of biotinylated seed protein (spot 27, 28, 29, 31, 32, 33, 34, 35, 36) in our study. Biotinylated enzymes such as acetyl-CoA carboxylase, 3-methylcrotonoyl-CoA carboxylase, propionylCoA carboxylase and pyruvate carboxylase have been identified in plants. Among them, acetyl-CoA carboxylase has been well studied and shown to perform an important function during initial stages of fatty acid biosynthesis [73].

Proteins Involved in Metabolism

Several identified protein spots from our study are involved in general metabolism. Most of these are involved in amino acid metabolism and we identified them as: glutamine synthetase (spot 119, 120), tryptophanyl-tRNA synthetase (131); transaminase (spot 128, 132), ADP-ribosylation factor (spot 290), transferase (spot 134, 224, 230, 231, 232, 236, 237, 238); synthase (spot30, 77, 83, 85, 89, 117, 174, 183, 217, 262, 263); CBS1(spot 285, 289) which is an integral membrane protein involved in signaling, lyase (spot 181, 182, 208, 209); lactoylglutathione lyase (313), fumarylacetoacetate (spot 116, 127, 130) and mitochondrial import inner membrane translocase subunit (spot 268) which is involved in maintaining mitochondrial function.

Other identified proteins are involved in protein synthesis. These include: ribosomal proteins, (spot 118, 242, 269, 270, 322, 327, 301, 328, 331); NAD dependent epimerase (spot 206) which is involved in RNA binding activity; elongation factor, which belongs to a set of proteins that facilitate the events of translational elongation, (spot 11, 12, 13, 26, 318, 96, 113, 133, 233); ten spots of proteasome subunits (spots 43, 205, 211, 218, 223, 225, 228, 240, 249, 252); one spot of ubiquitin-conjugating enzyme (299); and two spots of EBP1 (spot 104, 106). Horvath *et al*. [74] reported that EBPI protein regulates organ size through cell growth and proliferation in plants and theubiquitin-proteosome system regulates cell division

and plant development by regulating different cellular signals [75]. Only one spot of ATP dependent Clp protease (spot 7), seven spots of ATPase (spots 4, 47, 49, 50, 51, 53, 88, 195), two spots of 26S protease regulatory subunit (110, 214) involved protein turnover and in energy, were found in our study. Some of the identified proteins such as spot 310 which is a glycine rich RNA binding protein, spot 193 which is identified as a Ran/TC4 protein (nuclear binding protein), spot 298 which is a FK 506 binding protein and spot 62, NADP dependent malic enzyme are considered to be housekeeping proteins.

A large proportion of embryonic axis proteins support primary metabolic processes and synthesis of different molecules such as nucleotides, amino acids, and secondary compounds. Among them, some proteins are involved in the glycolytic pathway including: nine spots of kinases (123, 125, 135, 137, 140, 141, 155, 296, 319); one spot of 2, 3-bisphosphoglycerate –independent phosphoglycerate mutase (spot 55); one spot of fructose bisphosphate aldolase (spot 323); thirteen spot of isomerases (spot 38-41, 93, 121, 122, 216, 229, 234, 235, 241, 332): and sixteen spots of dehydrogenases (spots 64, 91, 102, 105, 129, 136, 142, 144, 145, 160, 165-167, 169, 173, 324). Energy related proteins include two spots of enolase (spots 86, 87) which have previously been shown to increase during seed imbibition and play an important role in oil mobilization during seed germination and post germination [76]. Another energy related protein, ribulose -1,5-bisphosphate carboxlase/oxygenase (RUBISCO), is composed of eight large and small subunits. The large subunit has a catalytic function and the small subunit controls the activity of ribulose 1, 5-bisphosphate carboxylase [77]. Our 2D-PAGE gel showed two spots (spots 42, 97) for the large subunit of RUBISCO.

Several proteins identified in our study are involved in secondary metabolism, including reductases, which are represented by a total of 6 spots (spot 161, 162, 163, 164, 171, 184), and one spot of glutamate 1-semialdehyde2, 1-aminomutase (spot 126), which coverts glutamate semialdehyde to 5 aminoleulinic acid, a precursor for chlorophyll and heme pigments. We also found one spot of tubulin β-chain (spot 44) which may be associated with cell division and increases during wheat germination [78].

Protein spots that are involved in carbohydrate metabolism include four spots identified as aconitase (spots 8, 9, 10, 17). Using 2D-PAGE analysis, Vensel *et al*. [41] reported the presence of two spots of aconitase at early stages of wheat endosperm development that were absent in late stages of grain development. Two spots of UDP-glucose phosphorylase (spots 82, 84) were identified in our study which is similar to what Vensel *et al*. [41] found in wheat endosperm. In addition, we found two spots of poly (ADP-ribose) polymerase (spots 5, 6) and four spots of embryo specific protein (spots14, 15, 16, 194). Vensel *et al*. [41] reported only one protein spot of embryo specific protein in wheat endosperm. We found two spots of actin (spots 111, 112) in our study compared to one actin spot reported in *Jatropha curcas.L* [58]. Actin is a major cytoskeletal component involved in different cellular processes including cell division, elongation and establishment of cell polarity, gene transcription, and signal transduction [79]. There are several isoforms of actin present in plants which in wheat are expressed differentially at different stages of development [78]. Another protein we identified is sucrose binding protein (SBP), which has been demonstrated to be involved in physiological processes dependent on sucrose translocation [80]. We observed eleven protein spots of SBP (spots 56-60, 68, 70, 95, 98, 99, and 100). SBP was first identified in soybean cotyledons and is an important component of the sucrose uptake system [81]. Grimes *et al*. [80] reported this protein to be associated with the plasma membrane of several cell types engaged in sucrose transport. The accumulation of SBP was very similar among seed of the sixteen soybean genotypes investigated by Natarajan *et al*. [60]. Mooney *et al*. [11] observed multiple isoelectric species of six different SBP spots with similar masses in *G. max cv Jefferson*, and Herman *et al*. [82] detected four SBP spots in soybean seeds of the cultivar Jack. Contim *et al*. [81] reported that there are two genes encoding SBP in the soybean genome, suggesting that the various numbers of peptides/spots observed by multiple laboratories result from alternative splicing products, post-translational modification, or protein fragmentation. Other proteins observed in our 2D-PAGE analysis include UV-excision repair protein (spot 107, 109), a hypothetical protein (spot 153), two unknown proteins (spot 207, 314) and a putative uncharacterized protein (spot 280).

This information on embryonic axis proteome, together with knowledge of total seed proteome [10, 12, 60], will aide in future investigation of molecular processes and functions involved in soybean plant development and on spermospheric interactions with

beneficial and pathogenic soilborne microorganisms [5]. For example, several species of mycorrhizal fungi and the nitrogen-fixing rhizobia colonize germinating seedlings [5]. In addition, a group of free-living microorganisms, also known as as plant growth-promoting rhizobacteria (PGPR), are known to aggressively colonize roots of seedlings and exert beneficial effects on plant development [83, 84]. Conversely, soybean seeds are also attacked by a host of soilborne pathogens belonging to oomycete and fungi such as *Pythium* and *P. sojae*, *Fusarium* species and *Rhizoctonia* species causing pre- and post-emergence damping off of seedlings, root and crown rot [85]. Particularly, several *Rhizoctonia* species are reported to colonize the embryonic axes of peanut and pea seeds [2, 3]. During germination, embryonic axes have been reported to express several stress and defense related genes upon exposure to desiccation, environmental fluctuations, extreme soil conditions, herbicide and insect injuries, and colonization by beneficial and pathogenic organism [4, 86-89]. Currently, we are investigating the molecular changes occurred during infection of soybean seedlings by *R. solani*. In this context, we have recently developed both cellular and secreted proteome of *R. solani* ([90] and our unpublished data). Our proteomic data on total soybean seeds [10, 12, 60] as well as embryonic axis (this communication) will be utilized in understanding plant-pathogen interactions during the early stages of *R. solani* infection.

CONCLUSIONS

We separated soybean embryonic axis proteins using 2D-PAGE and identified 335 protein spots with LC-MS/MS. LC-MS/MS is an efficient and sensitive mass spectrometry method, which yields positive identifications from 2D-PAGE spots. The 2D-profiling of the soybean embryonic axis proteins described here has established for the first time a baseline for soybean axis proteome which will serve as an useful tool to investigate molecular events that occur during seed development, seed germination, and during invasion by beneficial and pathogenic organisms in the soybean spermosphere.

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