African Journal of Agricultural Research Vol. 7(6), pp. 902-917, 12 February, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1980
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Construction of a proteomic reference map for analysis of development of etiolated rice seedlings

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Accepted 23 January, 2012

When rice seedlings grow in darkness, they will be yellow in color and show typical etiolated characters such as elongation of hypocotyls and stem, rolling up of young leaves around the stem tip. To better understand the development of etiolated rice seedlings at the molecule level, a proteomic reference map of etiolated rice seedlings was established by combining Two-Dimensional Gel Electrophoresis (2-DE) and matrix-assisted laser desorption/ionization–time-of-flight-mass spectrometry (MALDI-TOF-MS). In this study, a total of 1170 protein spots were detected. 200 protein spots were picked for MALDI-TOF-MS analysis, and 162 proteins were chosen for further analysis and construction of the proteomic reference map. As expected, most of the chosen proteins were metabolism and energy metabolism-associated, suggesting that the early stages of development of etiolated rice seedlings involved in complex metabolic activities. We further discussed the potential functions of these proteins in the development of etiolated rice seedlings. Our data also showed that a large number of proteins had not yet been annotated in rice.

Key words: Etiolated rice seedlings, proteomic, two-dimensional gel electrophoresis, matrix-assisted laser desorption/ionization–time-of-flight-mass spectrometry.

INTRODUCTION

Rice is a very important crop that provides food for more than half of the world population especially in South-East Asia. In addition, rice is also a model plant with a small genome of 430 Mb (Somerville and Somerville, 1999), and is commonly used for genomic analysis and genetic engineering. Proteomics was established for proteins study particularly for their structures and functions (Anderson and Anderson, 1998) and is widely applied to the study of various cells and organisms at the molecular level (Komatsu et al., 2004). Before proteome was proposed by Australia scientist, Wilkins and Williams in 1994, Akira had applied 2-DE to study the proteins in rice leaf, chloroplast, stem, root and other tissues (Tsugita et al., 1994). During the past few years, considerable proteomic studies have been carried out in various tissues, organelles and subcellular compartments. The rice proteome database, containing the reference maps and protein information from a wide variety of rice tissues and subcellular compartments was established in 2004 (Komatsu et al., 2004). Along with the genome sequencing of two rice subspecies, *indica* and *japonic*a (Goff et al., 2002; Yu et al., 2002), the rice proteome database will greatly contribute to the study of rice and other cereals at the molecule level. Plant growth is influenced by a number of environmental factors such as light, temperature, oxygen, carbon dioxide and soil nutrients. Light is an essential environmental factors for plant growth and development.

Without light, a number of light-dependent physiological processes that a plant must proceeds will be blocked and

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Abbreviations: 2-DE, Two-dimensional gel electrophoresis; **IEF,** isoelectric focusing; **MALDI-TOF-MS,** matrix-assisted laser desorption/ionization–time-of-flight-mass spectrometry (MALDI-TOF-MS); **PMF,** peptide mass fingerprinting; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

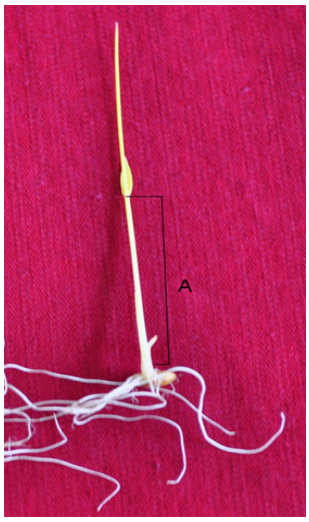

Figure 1. The etiolated rice seedlings that grew in darkness for 10 days at 28°C. A shows the elongated stem.

the plant would not be able to survive. When rice seedlings grow in darkness, they will show etiolation phenotype including yellowish color, pronounced apical hooks, elongated hypocotyls and incompetent chloroplasts (Chory et al., 1996; Clouse, 2001) and be called etiolated rice seedlings. Komatsu et al. (1999) had made global proteomics differences between green and etiolated rice shoots. However, due to various reasons, only 15 proteins had been identified in his analysis, provided very limited information for the development of etiolated rice shoots. Yang et al. (2007) also investigated the proteins differently expressed in de-etiolated rice seedlings upon exposure to light. However, both of them focused on comparative proteomics analysis rather than the whole picture of protein expression patterns of etiolated rice seedlings. Since proteins are the major players in most processes of living cells, knowledge of the proteome of etiolated rice seedlings will be beneficial to elucidate the molecule mechanism of the formation of the etiolation phenotype. In order to better understand the development and growth of etiolated rice seedlings at the molecule level, and discover the characteristics of the proteome of etiolated rice seedlings, we decided to construct a reference proteome map of etiolated rice seedlings.

In this study, we successfully constructed a comprehensive reference map using 2-DE and MALDI-TOF-MS techniques. Besides those proteins reported, a large number of proteins with unknown functions were identified and their potential roles in rice development were also discussed.

MATERIALS AND METHODS

Oryza sativa L. *indica* cultivar *Kasalath* was used in this study. Rice seeds were soaked in water for one day, then germinated and grown in darkness for 10 days at 28°C. The seedlings were collected and frozen in liquid nitrogen and stored at -80°C before use. The protein extraction was performed according to Carpentier et al. (2005) with some modifications. 1 g of frozen samples were finely powdered in liquid nitrogen using a pestle and mortar, and homogenized extensively in 4 ml of ice-cold extraction buffer (20 mM Tris-HCl, pH 7.5, 250 mM sucrose, 10 mM EDTA, 1 mM PMSF, 2% β-mercaptoethanol, 1% v/v Triton X-100) and finally centrifuged at 30,000 × g for 20 min at 4°C. An equal volume of Tris-HCl pH 7.5-saturated phenol was added in the supernatant that was collected after centrifugation. After centrifugation (30,000 × g for 20 min at 4°C), the phenol phase was collected and precipitated overnight with three volumes of 100 mM ammonium acetate in methanol at –20°C. After centrifugation, the pellet was washed three times with ice-cold acetone. At last, the pellet was vacuum-dried and dissolved in 200 ml lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 0.2% Bio-Lyte, 1% DTT). Protein concentration was determined by the Bradford method, BSA was used as the standard (Bradford, 1976). IPG strip (17 cm, pH 4 to 7) was rehydrated 12 h with 400 µl isoelectric focusing (IEF) electrophoresis buffer containing 1200 µg proteins.

The first-dimensional IEF was carried out at 17°C with current limit of 50 mA, applying the following program: a linear increase from 0 to 250 V over 30 min, 1000 V for 5 h, 10,000 V for 5 h, and then held at 10,000 V for a total of 60 kVh. After IEF, the strip was reduced with 5 ml equilibration solution (6 M urea, 20% glycerol, 2% SDS, 375 mM Tris- HCl, pH 8.8) containing 1% w/v DTT for 15 min and alkylated with 2.5% w/v iodoacetamide in 5 ml equilibration buffer for 15 min. The strip was then transferred to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for second dimension electrophoresis with 75 V for 45 min, 200 V for 5 h. Protein spots in SDS-PAGE were stained by CBB R-250 (40% methanol, 10% acetic acid and 0.1% w/v CBB R-250). The electrophoretic pattern of the 2-DE gel was analyzed using PDQuest software, version 7.0 (Bio-Rad Laboratories), using a semiautomatic method, with manual correction and editing of the incorrect spot features created by automatic analysis (Xu et al., 2008). The protein spots were excised from the SDS-PAGE and were treated as follows: the gel pieces were sonicated with purified water for 10 min and destained with 25 mM ammonium bicarbonate in 50% acetonitrile by sonication.

The colourless gel pieces were dehydrated with 25 mM ammonium bicarbonate, 50 and 100% acetonitrile separately and then rehydrated in 25 mM ammonium bicarbonate with 20 µg/ml trypsin overnight at 37°C for digestion. After digestion, the peptides were mixed with saturated a-cyano-4-hydroxycinnamic acid in 50% v/v acetonitrile containing 1% trifluoroacetic acid (Liang et al., 2007). The tryptic peptides were analyzed by MAL-DI-TOF mass spectrometer (Bruker, Ultraflex tof/tof). External calibration was performed with standard peptides. The matrix and the autolytic peaks of trypsin served as internal standards for mass calibration. The peptide mass fingerprinting (PMF) was searched against rice data in the NCBI database using MASCOT software (Matrix Science, London). *O. sativa* was chosen for the taxonomic category and the searching parameters were set according to Kerim et al. (2005).

RESULTS

The rice seedlings that grew in darkness for 10 days showed etiolated pattern: yellowish color and elongation of the stem (Figure 1). The elongation character enables the seedlings grow through the soil as soon as possible when the seeds are buried. Preparation of high-quality proteins is a critical step for good 2-DE patterns and is

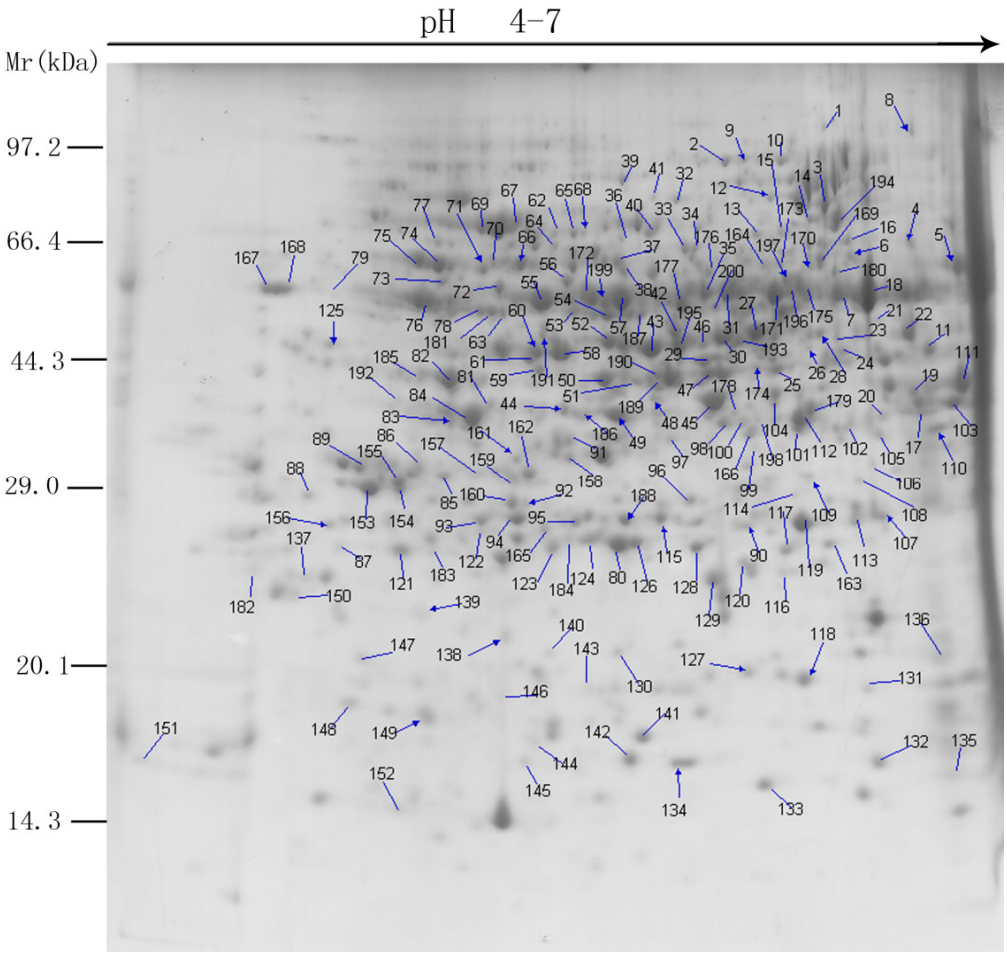

Figure 2. The proteomic reference map of 10 days old etiolated rice seedlings. Proteins (1200 µg) were separated on a 4 to 7 linear pH gradient in the first dimension and stained with coomassie brilliant blue R-250. Lines indicate the proteins that were identified and further chosen for the reference map while arrows indicate the proteins that failed to be identified or not meet the criteria.

absolutely essential for the downstream proteomic analysis. In this study, we choose the phenol based extraction method. In the map, the protein spots were well-resolved and distributed evenly in both horizontal and vertical dimensions. After CBB R-250 staining and PDQuest software analysis, a total of 1170 protein spots were detected. According to the 2-DE map of the etiolated rice seedlings, 200 spots (Figure 2) were excised for the MALDI-TOF-MS analysis. In order to confirm the results of the identified proteins, MOWSE score (higher than 60) and sequence coverage (higher than 15%) were used as criteria. Among the 200 proteins spots, we could not get the PMF data of 13 spots. Besides, 25 spots showed low scores and sequence coverage matches. This result may be attributed to two reasons: the protein spots were too faint to be analyzed by MALDI-TOF-MS and the spots contained more than one protein (Yang et al., 2006). The MOWSE scores of several proteins that identified were less than 60, but matched for high sequence coverage and be considered to meet the criteria in this study. As a result, a total of 162 proteins were chosen for the proteomics map (Table 1).

In Table 1, most of the proteins matched very well between the theoretical and experimental Mrs and pIs, but several proteins showed substantial discrepancies. The substantial discrepancies might be caused by several factors such as post translational modifications, polymeric forms of proteins, proteolytic degradation of proteins, or genomic sequence which could contain segments that are spliced out of the functional protein (Song et al., 2007). The determination of the potential roles of the proteins involved in physiological pathway was performed using KEGG software (http://www.genome.jp/kegg/) (Muccilli et al., 2009). The 162 chosen proteins were classified into 12 categories by their functions according to a previous study (Bevan et al., 1998). The largest category was metabolism-associated proteins (25.31%). Proteins that were classified into unknown function (15.43%) were second in order, followed by energy metabolism (13.58%), secondary

Table 1. The proteins that were identified and chosen for the reference map.

Spot No.	Score	Accession No.	Description	Theoretical Mr(kDa)/pl	Experimental Mr(kDa)/pl	Peptides matched	SCa)	Protein categorization
1	72	gi|108862990	Putative 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	79.3/7.19	126.1/6.32	9	17	Secondary metabolism
2	162	gi|222640045	Hypothetical protein OsJ_26310	104./6.37	106.1/6.00	20	35	Unknown function
3	149	gi|115489654	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	85.0/5.93	75.2/6.33	22	39	Secondary metabolism
7	155	gi|11466795	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	53.4/6.22	57.1/6.35	20	48	Energy metabolism
10	125	gi|115450595	Aconitate hydratase 1	106.9/6.45	106.9/6.20	18	23	Energy metabolism
11	81	gi|11466793	Formate dehydrogenase	41.5/6.68	47.8/6.53	9	29	Metabolism
13	97	gi|218188979	Hypothetical protein OsI_03572	65.8/5.79	71.3/6.14	10	27	Unknown function
14	132	gi|29367609	Phenylalanine ammonia-lyase	72.9/6.11	68.5/6.30	17	32	Metabolism
15	89	gi|115460876	Similar to Alpha-L-arabinofuranosidase/beta-D-xylosidase isoenzyme	81.9/6.42	72.2/6.20	15	27	Secondary metabolism
16	68	gi|115470493	Succinate dehydrogenase (ubiquinone) flavoprotein subunit	69.5/6.61	64.2/6.37	10	21	Energy metabolism
17	148	gi|115461348	Protochlorophyllide reductase	41.4/9.62	38.6/6.51	16	43	Secondary metabolism
18	275	gi|57283874	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	53.3/6.23	56.6/6.40	25	49	Energy metabolism
19	63	gi|115434034	Similar to Isoflavone reductase homolog IRL	34.4/6.26	41.3/6.50	6	24	Secondary metabolism
20	117	gi|4884530	Beta-1,3-glucanase	35.7/7.01	35.6/6.43	12	50	Metabolism
21	57	gi|115482462	RNA-binding protein Musashi	45.6/6.24	47.8/6.42	5	18	Protein synthesis
22	68	gi|115485529	Enolase-phosphatase E1	57.5/5.61	50.6/6.48	9	20	Metabolism

Table 1. Contd.

23	75	gi|108707679	Aspartate aminotransferase	43.4/6.12	49.6/6.35	7	26	Metabolism
24	68	gi|47848479	Putative acetyl-CoA C-acyltransferase	41.4/6.15	47.4/6.36	5	28	Secondary metabolism
25	58	gi|218199984	Hypothetical protein OsI_26792	41.6/6.01	44.6/6.24	9	22	Unknown function
27	159	gi|100801534	S-adenosylmethionine synthetase	42.3/5.83	50.6/6.20	15	48	Secondary metabolism
29	131	gi|115477483	Glutamate-1-semialdehyde 2,1-aminomutase	50.4/6.48	45.7/6.05	21	49	Metabolism
30	99	gi|3024122	S-adenosylmethionine synthase 2	43.3/5.68	50.0/6.09	13	43	Secondary metabolism
31	72	gi|89280711	ATP synthase F0 subunit 1	55.5/5.85	59.9/6.12	10	25	Energy metabolism
32	62	gi|115454943	NADH dehydrogenase (ubiquinone) Fe-S protein 1	82.1/5.86	80.0/5.94	15	26	Energy metabolism
33	134	gi|222624734	Hypothetical protein OsJ_10464	62.4/5.67	67.8/5.97	16	42	Unknown function
34	66	gi|125570422	Hypothetical protein OsJ_01810	62.4/5.52	67.9/5.99	15	28	Unknown function
35	96	gi|77550703	Adenosylhomocysteinase	50.0/5.88	58.2/6.02	10	37	Metabolism
36	66	gi|115444549	V-type H+-transporting ATPase subunit A	68.8/5.37	70.0/5.72	7	18	Transporter
37	156	gi|115456165	Methylenetetrahydrofolate reductase (NADPH)	67.0/5.38	64.8/5.73	18	45	Metabolism
38	89	gi|226887786	Chain A, Ketol-Acid Reductoisomerase (Kari) in complex with Mg2+	57.6/5.46	64.8/5.71	12	22	Secondary metabolism
39	102	gi|38347158	OSJNBa0039C07.4	98.5/5.79	85.5/5.72	13	22	Unknown function
40	106	gi|115454931	Phosphoglucomutase	63.1/5.40	71.6/5.84	12	36	Metabolism
41	78	gi|115470116	Oligopeptidase B	84.7/5.77	81.1/5.85	9	17	Secondary metabolism

Table 1. Contd.

42	70	gi|115434962	Transferase family protein	46.5/5.64	48.0/5.95	11	37	Transporter
43	130	gi|75243634	IAA-amino acid hydrolase ILR1-like 3	44.0/5.44	47.8/5.83	15	47	Secondary metabolism
45	148	gi|115482534	Malate dehydrogenase	35.6/5.75	39.9/6.09	15	51	Energy metabolism
46	95	gi|115448547	Dihydropyrimidine dehydrogenase	45.7/6.29	48.7/6.01	12	27	Metabolism
47	112	gi|115474869	Flavonol 3-O-methyltransferase	40.1/5.41	43.7/6.08	15	55	Secondary metabolism
50	72	gi|115448531	Glutamine synthetase	39.4/5.51	43.1/5.64	10	41	Metabolism
51	85	gi|115463863	Hypothetical protein	39.3/5.40	42.7/5.78	9	36	Unknown function
52	93	gi|297721387	Chloroplast translational elongation factor Tu	50.6/6.19	49.1/5.66	15	37	Protein synthesis
53	75	gi|303844	Eukaryotic initiation factor 4A	47.2/5.29	54.6/5.52	9	32	Transcription
54	138	gi|115469764	Translation initiation factor 4A	47.3/5.37	53.4/5.68	12	42	Transcription
55	147	gi|56784991	Putative ATP synthase beta subunit	45.9/5.33	55.8/5.40	18	51	Energy metabolism
56	102	gi|125542883	Hypothetical protein OsI_10505	58.8/5.5	60.7/5.50	12	26	Unknown function
57	105	gi|90110845	Phosphopyruvate hydratase	48.3/5.41	58.2/5.70	13	40	Metabolism
58	83	gi|115487492	Putative eukaryotic cytoskeleton proteins actin	40.1/5.29	46.6/5.47	7	29	Cell structure
59	68	gi|115447367	Succinyl-CoA synthetase beta subunit	45.4/5.98	44.3/5.39	9	24	Metabolism
61	66	gi|19387272	Putative precursor chloroplastic glutamine synthetase	49.8/6.18	47.1/5.38	7	22	Metabolism
62	67	gi|115461066	Glutamine synthetase	47.0/5.96	71.5/5.45	10	23	Metabolism
63	101	gi|115438576	Transferase family protein	46.1/5.13	53.9/5.24	8	33	Transporter
64	116	gi|115464537	Phosphoglycerate mutase	61.0/5.25	68.5/5.45	12	31	Metabolism

Table 1. Contd.

65	124	gi|115448989	Hypothetical protein	73.1/5.49	71.4/5.53	19	34	Unknown function
67	112	gi|2267006	Endosperm lumenal binding protein	73.7/5.30	69.1/5.30	17	29	Unclear classification
69	130	gi|115486793	Heat shock 70 kDa protein	71.5/5.10	66.4/5.10	19	30	Transporter
70	130	gi|125578088	Hypothetical protein OsJ_34853	69.8/5.33	61.3/5.25	19	31	Unknown function
72	184	gi|115468606	V-type H+-transporting ATPase subunit B	54.1/5.07	57.9/5.23	24	55	Transporter
73	59	gi|115466004	Os06g0114000	64.3/5.60	58.7/5.03	7	20	Unknown function
74	117	gi|115484585	Protein disulfide-isomerase A1	57.0/5.01	60.9/5.02	14	38	Protein fate
75	98	gi|115488160	Similar to RuBisCO subunit binding-protein alpha subunit, chloroplast precursor	61.2/5.12	61.6/4.98	10	31	Energy metabolism
76	188	gi|1076737	Tubulin, beta 4	50.8/4.75	55.9/4.98	18	53	Cell structure
77	78	gi|115478158	Protein phosphatase 2	66.5/4.92	64.0/5.02	11	29	Signal transduction
78	74	gi|108710846	Tubulin alpha-1 chain	46.5/4.89	54.3/5.15	9	31	Cell structure
79	64	gi|115448077	Putative protein transporter	43.8/4.66	57.1/4.74	10	19	Transporter
80	130	gi|115434516	triosephosphate isomerase	27.3/5.38	25.8/5.71	14	73	Metabolism
81	109	gi|297598102	Fructokinase	34.9/5.07	42.9/5.05	10	38	Metabolism
82	87	gi|125540364	Hypothetical protein OsI_08139	34.6/5.02	43.1/5.10	15	59	Unknown function
84	132	gi|115474481	Putative fructokinase	35.9/5.02	37.9/5.12	19	55	Metabolism
85	92	gi|46805452	Putative inorganic pyrophosphatase	31.8/5.80	31.0/5.04	10	41	Metabolism
86	66	gi|6856560	Dimethyllallyl pyrophosphate isomerase	29.2/5.19	32.6/5.00	7	36	Metabolism
87	73	gi|115436436	SalT gene product	15.2/5.00	25.7/4.75	5	61	Disease and defense

Table 1. Contd.

88	67	gi|115486269	20S proteasome subunit alpha	26.1/4.7	29.0/4.67	6	44	Protein fate
89	103	gi|115458806	Similar to 14-3-3-like protein GF14-6	30.0/4.76	32.0/4.80	13	48	Signal transduction
91	75	gi|115435022	Similar to Acid phosphatase	33.2/6.33	35.2/5.50	8	30	Signal transduction
93	125	gi|115456227	Chalcone isomerase	24.0/5.15	25.5/4.92	11	69	Secondary metabolism
94	117	gi|115474285	L-ascorbate peroxidase	27.2/5.21	27.4/5.50	10	54	Disease and defense
95	138	gi|115440977	Triosephosphate isomerase	27.5/5.58	27.3/5.53	12	54	Metabolism
96	77	gi|115483066	Hypothetical protein	26.5/5.68	28.7/6.00	10	51	Unknown function
97	138	gi|51090388	Putative PrMC3	34.5/5.61	35.2/5.92	17	59	Unclear classification
98	112	gi|115461739	Hypothetical protein	38.5/5.81	37.2/6.15	11	52	Unknown function
99	104	gi|115456922	Similar to Arginine/serine-rich splicing factor 1 variant 2	28.8/9.94	33.3/5.92	12	41	Transcription
100	75	gi|115446799	Hypothetical protein	34.1/5.85	35.9/6.20	10	57	Unknown function
101	77	gi|41053022	Putative NADPH-dependent mannose 6-phosphate reductase	35.7/5.88	35.9/6.28	12	35	Metabolism
102	79	gi|115448263	Thioredoxin reductase	35.0/6.19	36.7/6.36	9	48	Metabolism
103	146	gi|115452789	Ricin B-related lectin domain containing protein	39.3/6.30	39.8/6.59	13	56	Unclear classification
104	72	gi|75252730	Full = Photosystem II stability/assembly factor HCF13	45.5/9.02	39.5/6.24	9	34	Energy metabolism
105	138	gi|115467154	Similar to Annexin p33	36.0/6.21	35.5/6.43	21	57	Signal transduction
106	98	gi|297607768	Similar to short-chain dehydrogenase/reductase	31.2/6.79	32.4/6.41	10	62	Metabolism

Table 1. Contd.

108	64	gi|115456265	NAD-dependent epimerase/dehydratase	28.0/6.34	30.6/6.38	6	40	Metabolism
111	169	gi|115459078	Glyceraldehyde 3-phosphate dehydrogenase	36.9/6.34	44.3/6.70	17	61	Energy metabolism
112	90	gi|115439261	Guanine nucleotide-binding protein beta subunit-like protein	36.7/5.97	39.4/629	10	42	Unclear classification
113	67	gi|297601669	Similar to ripening-associated protein	26.7/6.05	27.5/6.38	8	49	Unclear classification
114	63	gi|13249140	Glucanase	34.8/5.92	29.4/6.28	9	35	Metabolism
116	71	gi|115474739	Putative flavoprotein wrbA	21.6/6.08	23.4/6.25	8	46	Unclear classification
117	128	gi|115461741	Putative dehydroascorbate reductase	23.7/5.81	24.2/6.25	9	63	Metabolism
119	66	gi|115440119	Similar to glutathione S-transferase I	24.3/5.77	27.2/6.28	5	45	Disease and defense
120	68	gi|125527970	Hypothetical protein OsI_04011	21.7/5.86	24.9/6.18	10	48	Unknown function
121	66	gi|158513205	Salt stress-induced protein	15.1/5.19	25.5/4.92	4	55	Disease and defense
122	76	gi|115449031	UMP-CMP kinase	23.7/5.16	26.6/5.15	8	38	Metabolism
123	93	gi|125537221	Hypothetical protein OsI_38931	20.0/5.61	25.6/5.44	11	69	Unknown function
124	119	gi|115447473	20S proteasome subunit alpha 2	25.8/5.39	26.2/5.59	12	54	Protein fate
126	80	gi|158512874	L-ascorbate peroxidase 1	27.2/5.31	26.5/5.77	8	48	Disease and defense
128	99	gi|115483096	Glutathione S-transferase	25.7/5.82	25.7/6.02	9	57	Disease and defense
129	101	gi|164375543	23 kDa polypeptide of photosystem II	20.1/5.56	23.8/6.10	8	41	Energy metabolism

Table 1. Contd.

130	94	gi|125555642	Hypothetical protein OsI_23273	17.6/5.54	20.3/5.70	7	62	Unknown function
131	86	gi|115489474	Ubiquitin-conjugating enzyme E2 variant	16.8/6.42	19.3/6.40	11	69	Protein fate
132	69	gi|223635304	Nucleoside diphosphate kinase 1	16.8/6/30	17.5/6.42	7	41	Metabolism
133	57	gi|297728629	Hypothetical protein	16.7/5.82	16.3/6.22	6	38	Unknown function
135	58	gi|671740	Ribulose-bisphosphate carboxylase	15.1/6.59	16.8/6.59	5	40	Energy metabolism
136	76	gi|149390789	60 S ribosomal protein l11	17.5/9.97	20.1/6.56	10	60	Protein synthesis
137	78	gi|115446541	Alkyl hydroperoxide reductase subunit C	28.3/5.67	24.0/4.66	6	36	Metabolism
140	58	gi|115434798	Alba, DNA/RNA-binding protein family protein	16.3/5.10	20.3/5.43	6	57	Protein synthesis
141	61	gi|42408425	Putative superoxide dismutase (Cu-Zn),	20.6/5.79	18.2/5.81	6	43	Disease and defense
142	65	gi|115456241	Similar to Actin-depolymerizing factor 3	16.1/5.72	17.6/5.75	5	50	Cell structure
143	66	gi|115455471	Translation initiation factor eIF-5A	17.8/5.71	19.3/5.59	6	42	Protein synthesis
144	86	gi|11466793	ATP synthase CF1 epsilon subunit	15.3/5.03	18.0/5.37	7	77	Energy metabolism
145	72	gi|115448731	Putativewound/stress protein	20.3/6.18	17.5/5.33	6	40	Disease and defense
146	72	gi|115462209	UspA domain containing protein	18.2/5.22	19.1/5.25	8	51	Unclear classification
147	62	gi|115452651	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 5	19.7/4.83	20.1/4.81	5	40	Energy metabolism
148	124	gi|115467820	Thioredoxin fold domain containing protein	15.3/4.88	19.0/4.78	10	74	Unclear classification
150	110	gi|115486523	Similar to Translationally controlled tumor protein	19.0/4.51	23.0/4.63	8	55	Disease and defense

Table 1. Contd.

151	78	gi|115464167	Large subunit ribosomal protein LP2	11.6/4.22	17.6/4.22	5	30	Protein synthesis
152	92	gi|115474531	Calcium-binding protein CML	16.7/4.89	14.9/4.91	7	50	Signal transduction
153	175	gi|115476520	Similar to 14-3-3-like protein S94	29.0/4.78	29.6/4.84	24	71	Signal transduction
154	133	gi|115476928	Similar to TaWIN2	29.1/4.85	29.5/4.92	12	51	Signal transduction
155	107	gi|115454901	Putative tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	29.3/4.81	29.7/4.90	13	50	Signal transduction
157	91	gi|115440123	Chorismate mutase	36.7/5.52	31.3/5.14	10	35	Secondary metabolism
158	153	gi|115489664	Cysteine synthase A	33.9/5.39	33.0/5.51	13	63	Metabolism
159	66	gi|115461611	Translation initiation factor eIF-3 subunit 5	31.3/5.25	30.7/5.29	7	35	Protein synthesis
160	202	gi|115477092	Similar to Caffeoyl-CoA O-methyltransferase 2	27.9/5.11	28.5/5.28	15	68	Metabolism
162	144	gi|115436780	Photosystem II oxygen-evolving enhancer protein	35.1/6.10	31.2/5.35	11	49	Energy metabolism
163	141	gi|115480019	20S proteasome subunit beta 6	24.6/6.43	25.9/6.33	12	66	Protein fate
164	58	gi|218190960	Hypothetical protein OsI_07631	33.7/5.49	64.1/6.10	6	28	Unknown function
165	114	gi|125570679	Hypothetical protein OsJ_02079	26.3/5.33	26.8/5.41	13	62	Unknown function
166	93	gi|115456131	Xanthoxin dehydrogenase	29.8/5.91	34.4/6.20	9	47	Metabolism
167	87	gi|115471369	Calreticulin	48.5/4.47	57.8/4.55	12	35	Signal transduction
168	124	gi|218199368	Hypothetical protein OsI_25508	48.5/4.47	57.8/4.61	16	41	Unknown function
169	106	gi|115474481	Fructokinase	35.9/5.02	61.5/6.32	13	43	Metabolism
171	99	gi|89280711	ATP synthase F0 subunit 1	59.6/5.85	59.2/6.18	24	29	Energy metabolism

Table 1. Contd.

172	148	gi|11466794	ATP synthase CF1 beta subunit	54.0/5.47	58.0/5.60	14	35	Energy metabolism
173	95	gi|115437452	Ubiquitin carboxyl-terminal hydrolase 14	53.5/5.79	60.3/6.15	10	28	Protein fate
175	79	gi|54290767	Putative dehydroquinate dehydratase	55.0/5.86	58.2/6.30	13	36	Metabolism
176	63	gi|50428682	Putative TCP-1/cpn60 chaperonin family protein	57.6/5.56	63.1/6.02	9	30	Transporter
177	218	gi|115480571	UDP-glucose pyrophosphorylase	51.8/.543	55.3/5.96	21	62	Metabolism
178	77	gi|115444475	Hydroxymethylbilane synthase	38.9/7.06	38.7/6.17	7	31	Secondary metabolism
179	77	gi|115438875	Malate dehydrogenase	35.7/8.74	38.5/6.30	9	39	Energy metabolism
180	128	gi|11466784	ATP synthase CF1 alpha subunit	55.7/5.95	60.1/6.34	14	34	Energy metabolism
181	185	gi|115472953	Tubulin alpha	50.3/4.92	53.9/5.20	25	51	Cell structure
182	114	gi|115486523	Translationally controlled tumor protein-like	19.0/4.51	24.4/4.52	11	78	Disease and defense
183	82	gi|115478330	Similar to Plastid-specific 30S ribosomal protein 2	26.8/8.53	26.2/5.00	7	42	Protein synthesis
184	91	gi|218196757	Hypothetical protein OsI_19879	26.0/5.39	26.1/5.51	6	35	Unknown function
185	97	gi|27804768	Sedoheptulose-1,7-bisphosphatase precursor	42.7/5.64	43.0/4.97	11	32	Energy metabolism
187	84	gi|297724731	Hypothetical protein	49.3/5.35	54.0/5.80	10	32	Unknown function
189	116	gi|115452897	Uroporphyrinogen decarboxylase	43.0/6.15	40.2/5.87	12	42	Secondary metabolism
190	121	gi|115458044	Pyruvate/Phosphoenolpyruvate kinase	41.6/5.66	42.9/5.83	15	51	Metabolism
192	63	gi|115458588	26S proteasome regulatory subunit N6	47.8/5.87	40.2/4.91	8	28	Protein fate
193	83	gi|115482032	GDP-D-mannose 3', 5'-epimerase	43.1/5.75	49.3/6.15	11	35	Metabolism

Table 1. Contd.

194	81	gi|115438681	Sgt1	41.0/4.97	68.5/6.30	7	30	Signal transduction
195	146	gi|115453437	Sucrose synthase	93.4/5.94	48.0/5.95	20	28	Metabolism
196	140	gi|20146763	ATPase alpha subunit	55.2/5.95	60.4/6.28	17	37	Energy metabolism
198	76	gi|42408797	Putative aminoacylase	49.9/5.88	37.5/6.21	9	28	Metabolism
200	153	gi|222618527	Hypothetical protein OsJ_01941	56.2/5.94	55.4/6.04	17	43	Unknown function

a) SC stands for sequence coverage.

metabolism (9.88%), disease and defense (6.17%), signal transduction (6.17%), protein synthesis (4.94%), unclear classification (4.94%), transporter (4.32%), protein fate (4.32%), cell structure (3.08%), transcription (1.85%) (Figure 3). Proteins grouped under metabolism include those involved in the metabolism of amino acid, cofactors and vitamins, carbohydrate, nucleotide, sulfur, porphyrin and chlorophyll, nitrogen.The presence of 25 proteins (15.43%) with unknown function suggested that a large number of proteins had not been annotated in rice. Proteins associated with energy metabolism mainly function in glyoxylate and dicarboxylate metabolism, citrate cycle, oxidative phosphorylation metabolic pathways and carbon fixation in photosynthesis. Proteins related to cell structure include eukaryotic cytoskeleton proteins actin, tubulin beta 4, tubulin alpha-1 chain and tubulin alpha. Proteins devoted to protein fate were represented by disulfide-isomerase, 20S proteasome subunit alpha, 26S proteasome regulatory subunit N6. Within the protein synthesis group, the proteins were identified as 60S ribosomal protein 11, elongation factor Tu, translation initiation factor eIF-5A and eIF-3 subunit 5. The proteins grouped into transporter mainly contained heat shock of70 kDa proteins, V-type H+-transporting ATPase subunit A and B. The disease and defense group was composed of L-ascorbate peroxidase, Cu/Zn superoxide dismutase and glutathione S-transferase (GST). The remaining groups contained proteins related to signal transduction, secondary metabolism, transcription and unclear classification.

DISCUSSION

The accumulation of proteins associated with metabolism and energy metabolism could reflect the physiological activity of a plant tissue. In etiolated rice seedlings, the proteins devoted to metabolism and energy metabolism account for 38.89%. Koller et al. (2002) found that 25.8% proteins in rice roots, leaves, and seeds were relative to metabolism and energy metabolism. Our study suggested that the physiological activity of etiolated rice seedlings were much higher than the rice tissues reported. Cytoskeleton is the internal framework of a cell and is mainly involved in cell shape keeping, cell moving, chromosome separation in mitosis and meiosis (Wang et al., 2010). The basic components of cytoskeleton are microtubules and actin filaments. Microtubules are polymers of α- and β-tubulin dimers and are involved in many cellular events such as cell division, internal cell movements and formation of cytoskeleton. Actin filaments are dynamic polymers in cell cytoplasm and function in cell shape changes and chemotactic migration. In the proteomic map, we detected a large accumulation of beta-tubulin proteins (no.76). The high expression level of beta-tubulin proteins was consistent with the previous studies (Kang et al., 1994; Yang et al., 2006). Besides, eukaryotic cytoskeleton proteins actin (no.58) and actin depolymerizing factors 3 (no.142) also appeared in large accumulations.

Actin depolymerizing factors 3 is a member of microfilament proteins and plays roles in regulating actin filaments assembly (Didry et al., 1998). Moustafa (2009) found that cytoskeleton proteins play a crucial role in the differentiation and development of Alaska pea seedlings. In consideration

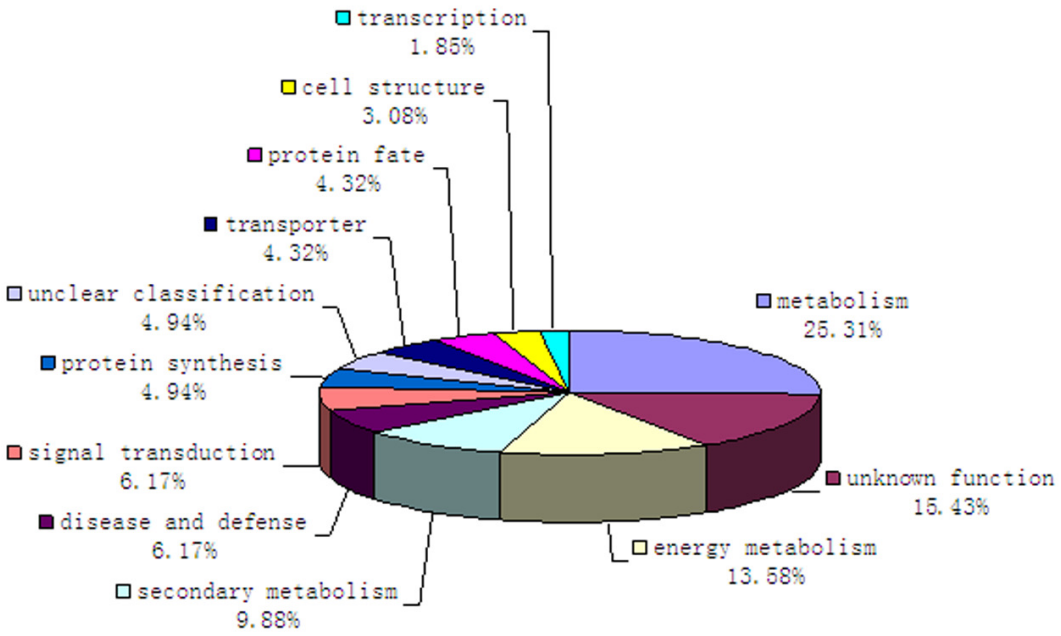

Figure 3. Functional categories and the corresponding percentage of the proteins that were chosen for the reference map.

of the functions of cytoskeleton, the abundance of the cytoskeleton-associated proteins may suggest that cytoskeletons are also important for rice seedlings differentiation and development. Sucrose synthase (no.195), the enzyme related with sugar import, cell wall synthesis and skin strength (Sun et al., 1992; Sung et al., 1989) is important for plants growth and development. It demonstrated that high sucrose synthase activity is needed for the early phase of organ development (Chan et al., 1990). Moreover, UDP-glucose pyrophosphorylase (no.177), the enzyme which takes part in cellulose synthesis is also present in this study. Since cellulose is the basic component of cell wall, the relative high abundance of spots 195 and 177 might provide substance basis for the rapid elongation process of the etiolated rice seedling stems. What is noticeable is that Sgt1 (no.194) also expressed at a high level. Sgt1 is a binding partner of heat shock proteins and is up-regulated by heat shock. This protein is homologous to the yeast SGT1 which takes part in the kinetochore complex assembling and is involved in the transitions of G1/S and G2/M in cell cycle (Komatsu, 2005).

Since this protein participates in cell division, the abundance of Sgt1 was essential for the rapid cell proliferation and played a key role in the rapid elongation process of etiolated seedling stems. Reactive oxygen species (ROS) are universal in all plant cells and their contents will be increased in adverse conditions (Lamb and Dixon, 1997). The reactive and toxic ROS are harmful to all living cells. The main origination of ROS is from oxidizing metabolic process or electron transport chains in chloroplasts, mitochondria and microbodies (Dat et al., 2000). From the reference map, we found L-ascorbate peroxidase (no.94), Cu/Zn superoxide dismutase (no.141) and GST (no.128) with strong appearance. These proteins are common in plant cells that are involved in eliminating the ROS. Because the formation of the etiolate morphology needs a large amount of energy (McNellis and Deng, 1995), the oxidizing metabolic process must proceed rapidly to produce enough energy to meet the requirement, thus, the contents of ROS would increase in etiolated rice seedlings and cause the large accumulations of the aforementioned three antioxidants eventually (Komatsu et al., 1999). Studies showed that the contents of these antioxidants in etiolated rice seedlings will be down-regulated when exposed to light (Yang et al., 2007). The reason may be that once the etiolated seedlings are exposed to light, the elongation rate of the seedling stems will reduce significantly. Corresponding, the energy required for elongation will also reduce significantly. As a result, the oxidizing metabolic process will slow down and the production of ROS will also decrease, thus the contents of the antioxidants will be down-regulated in de-etiolated seedlings.

Heat shock proteins are one of the most important chaperones and are universal in all living cells. These proteins mainly function in stabilizing protein complexes against aggregation, folding the newly translated polypeptides, preventing proteins from denaturing and eliminating the damaged proteins (Schlesinger, 1990). In this study we detected heat shock proteins 70 (no. 69) accumulated at a relative high level. Heat shock proteins 70 might function in three ways in etiolated rice

seedlings: protecting the damaged proteins caused by the ROS that was produced during the rapid oxidizing metabolic process, maturing and stabilizing the abundant protein complexes required for the high rate of elongation of the stems, eliminating the abnormal proteins produced during the abnormal metabolic pathway caused by darkness. Chloroplast ATP synthase (CF_0 CF_1-ATPase) is a light regulated enzyme that utilizes the energy generated by the electrochemical gradient transformed from the sunshine to synthetize ATP from ADP and inorganic phosphate (Mills and Richter, 1991). The enzyme is an integral membrane protein with the CF_1 portion sticks into stroma and the CF_0 portion integrated into the thylakoid membrane. In this study, we found three subunits of ATP synthase CF_1 (alpha, beta, epsilon), but did not find any subunits of ATP synthase CF_0.

The reason may be that CF_0 is a transmembrane protein and is difficult to isolate during the protein extraction process or unable to be resolved by IEF and 2-DE procedures. Although, the etiolated seedlings grew in darkness and were unable to perform photosynthesis, the most abundant protein in the map was ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large chain (no.18). Rubisco is the most abundant protein in nature and catalyses the key carboxylation reaction of photosynthetic carbon fixation in the Calvin cycle (Lundqvist and Schneider, 1991). The strongest appearance of Rubisco large chain implied that light was not necessary for the synthesis of this protein. Intriguingly, although root is not a photosynthetic organ, several subunits of Rubisco have been found in *Arabidopsis thaliana* and rice roots (Song et al., 2007). In addition, we also found the precursor of sedoheptulose-1,7-bisphosphatase which serves as another crucial enzyme in photosynthetic carbon assimilation cycle. Protochlorophyllide reductase is a key enzyme that catalyzes the light-dependent reduction of protochlorophyllide to chlorophyllide during the biosynthesis of chlorophylls and bacteriochlorophylls (Wilks and Timko, 1995). Chlorophyll is a kind of pigment in chloroplasts that endows plants with green color. As the photoreceptors of light in photosynthesis, chlorophyll turns CO_2 and water into carbohydrates utilizing the energy switches from the sunlight.

The abundance of protochlorophyllide reductase (no. 17) presented in this study indicated that, once the etiolated seedlings are exposed to light, this enzyme will be applied to synthesize chlorophylls rapidly and the chloroplasts will be competent for Rubisco and sedoheptulose-1,7-bisphosphatase to function in the Calvin cycle. S-adenosylmethionine synthetase (no. 27, 30) also appeared in a remarkable abundance in this study. This enzyme is involved in the biosynthesis of S-adenosylmethionine, which is the precursor of phytohormone ethylene. Phytohormones ethylene takes part in many important cellular processes in plants such as cell differentiation, cell expansion. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness and this might explain the strong presence of S-adenosylmethionine synthetase in etiolated rice seedlings. Calreticulin (no.167) is an important calcium-binding protein that participates in integrin-dependent Ca^{2+} signaling, cell adhesion and chaperoning. As a multifunctional protein, calreticulin involved in the regulation of a wide range of developmental and physiological processes in eukaryotic organism (Ferreira et al., 2004). In rice proteome, calreticulin had been identified as a functional phosphoprotein, and the overexpression of calreticulin will inhibit callus and seedling growth (Shen et al., 2003). Komatsu (2005) described some analyses and proposed that calreticulin acted like a negative repressor in the differentiation system and in development.

Conclusion

It is reported that the major proteins involved in rice growth and regulation in etiolated seedlings were the same as in green seedlings (Komatsu et al., 1999), so, to construct the proteomic reference map for etiolated seedlings would be beneficial to understand the development of green seedlings. Besides, due to low expression level of RuBisCO in etiolated seedlings, we can detect many proteins of low abundance which are usually very hard to observe in a proteomic map of rice growing under light. After all, our comprehensive reference map will also be beneficial for subsequent systematic analysis of the growth and development of rice seedlings.

ACKNOWLEDGEMENTS

This work was supported by the ‘key project of genetically modified organisms breeding’ (2009ZX08012-018B) and the grants from Jiangsu Sci-Tech Support Project-Agriculture (No. BE2008379).

REFERENCES

Anderson NL, Anderson NG (1998). Proteome and proteomics: new technologies, new concepts, and new words. Electrophoresis, 19: 1853-1861.

Bevan M, Bancroft I, Bent E (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of Arabidopsis thaliana. Nature, 391: 485-488.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Carpentier SC, Witters E, Laukens K, Deckers P, Swennen R, Panis B (2005). Preparation of protein extracts from recalcitrant plant tissues: an evaluation of different methods for two-dimensional gel electrophoresis analysis. Proteomics, 5: 2497-2507.

Chan HY, Ling TY, Juang RH, Ting IN, Sung HY, Su JC (1990). Sucrose synthase in rice plants: growth-associated changes in tissue specific distributions. Plant. Physiol., 94: 1456-1461.

Chory J, Chatterjee M, Cook RK (1996). From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc. Natl. Acad. Sci. U. S. A., 93: 12066-12071.
Clouse SD (2001). Integration of light and brassinosteroid signals in etiolated seedling growth. Trends Plant Sci., 6: 443-445.
Dat J, Vandenabeele S, Vranova E, Van MM, Inze D, Van BF (2000). Dual action of the active oxygen species during plant stress responses. Cell Mol. Life Sci., 57: 779-795.
Didry D, Carlier MF, Pantaloni D (1998). Synergy between actin depolymerizing factor/cofilin and profilin in increasing actin filament turnover. J. Biol. Chem., 273: 25602-25611.
Ferreira V, Molina MC, Valck C, Rojas A, Aguilar L, Ram¨¢rez G, Schwaeble W, Ferreira A (2004). Role of calreticulin from parasites in its interaction with vertebrate hosts. Mol. Immunol., 40: 1279-1291.
Goff SA, Ricke D, Lan TH (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonic*a). Science, 296: 92-100.
Kang MS, Choi YJ, Kim MC, Lim CO, Hwang I, Cho MJ (1994). Isolation and characterization of two beta-tubulin cDNA clones from rice. Plant Mol. Biol., 26: 1975-1979.
Kerim T, Imin N, Weinman JJ, Rolfe BG (2003). Proteome analysis of male gametophyte development in rice anthers. Proteomics, 3: 738-751.
Koller A, Washburn MP, Lange BM, Andon NL, Deciu C, Haynes PA, Hays L, Schieltz D, Ulaszek R, Wei J, Wolters D, Yates JR (2002). Proteomic survey of metabolic pathways in rice. Proc. Natl. Acad. Sci. U. S. A., 99: 11969-11974.
Komatsu S (2005). Rice proteome database: a step toward functional analysis of the rice genome. Plant. Mol. Biol., 59: 179-190.
Komatsu S, Kojima K, Suzuki K, Ozaki K, Higo K (2004). Rice Proteome Database based on two-dimensional polyacrylamide gel electrophoresis: its status in 2003. Nucleic Acids Res., 32: D388-392.
Komatsu S, Muhammad A, Rakwal R (1999). Separation and characterization of proteins from green and etiolated shoots of rice (*Oryza sativa* L.): towards a rice proteome. Electrophoresis, 20: 630-636.
Lamb C, Dixon RA (1997). The oxidative burst in plant disease resistance. Annu. Rev. Plant. Physiol. Plant. Mol. Biol., 48: 251-275.
Liang Y, Chen H, Tang M, Shen S (2007). Proteome analysis of an ectomycorrhizal fungus *Boletus edulis* under salt shock. Mycol. Res., 111: 939-946.
Lundqvist T, Schneider G (1991). Crystal structure of activated ribulose-1,5-bisphosphate carboxylase complexed with its substrate, ribulose-1,5-bisphosphate. J. Biol. Chem., 266: 12604-12611.
McNellis TW, Deng XW (1995). Light control of seedling morphogenetic pattern. Plant Cell, 7: 1749-1761.
Mills DA, Richter ML (1991). Nucleotide binding to the isolated beta subunit of the chloroplast ATP synthase. J. Biol. Chem., 266: 7440-7444.
Moustafa MFM (2009). Status of cytoskeleton proteins during initial germination of dark-grown pea (*Pisum sativum*) seedlings. J. Environ. Stud., 2: 99-106.
Muccilli V, Licciardello C, Fontanini D, Russo MP, Cunsolo V, Saletti R, Recupero GR, Foti S (2009). Proteome analysis of *Citrus sinensis* L. (Osbeck) flesh at ripening time. J. Proteomics, 73: 134-152.
Schlesinger MJ (1990). Heat shock proteins. J. Biol. Chem., 265: 12111-12114.
Shen S, Sharma A, Komatsu S (2003). Characterization of proteins responsive to gibberellin in the leaf-sheath of rice (*Oryza sativa* L.) seedling using proteome analysis. Biol. Pharm. Bull., 26: 129-136.
Somerville C, Somerville S (1999). Plant functional genomics. Science, 285: 380-383.
Song X, Ni Z, Yao Y, Xie C, Li Z, Wu H, Zhang Y, Sun Q (2007). Wheat (Triticum aestivum L) root proteome and differentially expressed root proteins between hybrid and parents. Proteomics, 7: 3538-3557.
Sun J, Loboda T, Sung SJ, Black CC (1992). Sucrose synthase in wild tomato, Lycopersicon chmielewskii, and tomato fruit sink strength. Plant. Physiol., 98: 1163-1169.
Sung SJ, Xu DP, Black CC (1989). Identification of actively filling sucrose sinks. Plant. Physiol., 89: 1117-1121.
Tsugita A, Kawakami T, Uchiyama Y, Kamo M, Miyatake N, Nozu Y (1994). Separation and characterization of rice proteins. Electrophoresis, 15: 708-720.
Wang C, Zhang L, Yuan M, Ge Y, Liu Y, Fan J, Ruan Y, Cui Z, Tong S, Zhang S (2010). The microfilament cytoskeleton plays a vital role in salt and osmotic stress tolerance in Arabidopsis. Plant. Biol. (Stuttg), 12: 70-78.
Wilks HM, Timko MP (1995). A light-dependent complementation system for analysis of NADPH: protochlorophyllide oxidoreductase: identification and mutagenesis of two conserved residues that are essential for enzyme activity. Proc. Natl. Acad. Sci. U. S. A., 92: 724-728.
Xu C, Xu Y, Huang B (2008). Protein extraction for two-dimensional gel electrophoresis of proteomic proifling in turfgrass. Crop Sci. Soc. Am., 48: 1608-1614.
Yang P, Chen H, Liang Y, Shen S (2007). Proteomic analysis of de-etiolated rice seedlings upon exposure to light. Proteomics, 7: 2459-2468.
Yang P, Liang Y, Shen S, Kuang T (2006). Proteome analysis of rice uppermost internodes at the milky stage. Proteomics, 6: 3330-3338.
Yu J, Hu S, Wang J (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science, 296: 79-92.