SHORT COMMUNICATION

The accumulation of abundant soluble proteins changes early in the development of the primary roots of maize (*Zea mays* L.)

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A reference database of the major soluble proteins of the primary root of the maize inbred line B73 was generated 5 days after germination (DAG) using a combination of 2-DE and MALDI-TOF MS. A total of 302 protein spots were detected with CBB in a pH 4–7 range and 81 proteins representing 74 distinct Genbank accessions were identified. Only 28% of the major proteins identified in 5 DAG primary roots were identified in similarly analyzed 9 DAG primary roots documenting remarkable changes in the accumulation of abundant soluble proteins early in primary root development.

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The morphology of root systems and the anatomical structures of individual roots reflect their functional roles of anchoring plants in soil and supplying water and nutrients [1]. The organization of the maize root system shows dramatic alterations during development. During the first days after germination (DAG) the embryonically formed primary root is the sole root that supports the growing maize seedling [2, 3]. Shortly after this a variable number of seminal roots is formed at the scutellar node. Subsequently, these early roots are replaced by a huge shoot-borne root system composed of crown and brace roots, which are initiated from below- and above-ground stem nodes, respectively [2, 3]. A common characteristic of all root types is the formation of branched lateral roots which considerably increases the absorbing surface of the root system. Longitudinally, maize roots can be grouped into several developmental zones [4] starting with the columella and the meristematic region at the root tip followed by a zone of elongation and differentiation, the latter characterized by the formation of root hairs, and finally lateral roots. These distinct zones develop in each root type early after initiation. The first differentiated cells marked by root hairs become visible around 3–5 days after a root has initiated [2, 3]. Shortly afterward, the first primordial structures of lateral roots can be detected [2, 3].

Although all plant organs contain the same complement of the genome, expression of genes varies widely among different organs and during the development and differentiation of a given plant organ such as a root [5]. Proteomic technology that combines the resolution of 2-DE with the sensitivity of MS allows for the detection of hundreds of proteins [6] that can provide important clues regarding the differential patterns of gene expression that occur during development.

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Abbreviations: AC, accession; **DAG**, days after germination; **MAGI**, maize assembled genomic islands; **MIPS**, Munich Information Center for Protein Sequences; **MOWSE**, molecular weight search; **MSU**, MS utilities; **NCBI**, National Center for Biotechnology Information; **TCEP**, tris[2-carboxyethyl] phosphine

In maize, thus far only a few proteome analyses have been published, including a survey of maize leaves [7], 6 DAG primary root tips [8], 9 DAG primary roots [9], the subcellular proteomes of mitochondria [10], and chloroplasts [11], and an analysis of the response of germinating maize embryos to fungal infection [12].

The objective of this study was to identify the most abundant soluble proteins in 5 DAG primary roots of maize *via* a combination of 2-D protein gel electrophoresis and MALDI-TOF MS and to compare this dataset of primary roots before lateral root emergence with a previously published dataset from our laboratory which identified the most abundant soluble proteins in 9 DAG maize primary roots [9], *i.e.*, after the formation of lateral roots.

The formation of the maize root system is characterized by dramatic alterations during early seedling development and leads to a complex root system starting with the emergence of a single primary root [2, 3]. In our experiments we analyzed the major soluble proteins of primary roots of the inbred line B73 (Schnable laboratory pedigree number: 98–5006) 5 DAG grown in paper rolls (Anchor Paper, St. Paul, MN) at 28°C in dark as described previously [13]. At this early developmental stage the primary root has already formed root hairs but no lateral roots are visible (Fig. 1A). Feulgen staining with Schiff's reagent is a means to detect lateral root primordia inside the primary root [14]. Staining intensity of the purple precipitate of Schiff's reagent in this procedure is directly proportional to the DNA content of a cell [14]. This staining technique demonstrated that lateral root initials, which are visible as violet dots, had just begun to form inside the primary root 5 DAG (Fig. 1B).

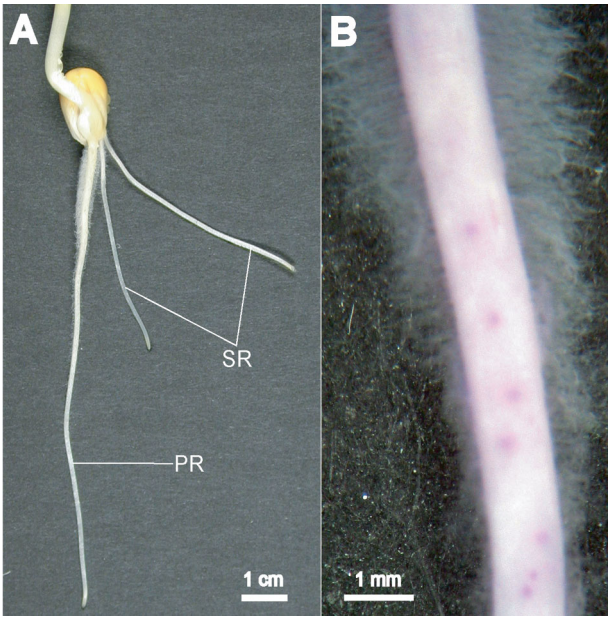

Figure 1. (A) Root system of a 5-day-old maize seedling consisting of a primary root (PR) and seminal roots (SR). Root hairs are present at the proximal end of the primary root but no lateral roots are visible at this developmental stage. (B) Close up of the proximal end of a 5-day-old B73 primary root stained with the Feulgen technique. Internal lateral root meristems stained purple.

Total proteins of 5 DAG primary roots were isolated *via* acetone precipitation [15] as described previously [11]. The protein pellet was dried in a speed vac and resuspended in a solution containing 7 M urea, 2 M thiourea, 4% CHAPS (Sigma, St. Louis, MO), 2 mM tris[2-carboxyethyl] phosphine (TCEP) (Pierce, Rockford, IL), 0.5% BioLytes 3/10 (BioRad, Hercules, CA), 40 mM Tris, and 0.001% orange G dye. Each protein extract was obtained from a pool of approximately 20 primary roots. IEF of soluble proteins of 5 DAG primary roots was performed with 1 mg of protein extract with the IPG Phor IEF unit (Amersham Pharmacia Biotech, Uppsala, Sweden) using 18 cm immobilized, linear pH 4–7 gradients. The voltage of the IEF was set to 90 000 Vh. Proteins were then separated according to their MWs on 12–18% SDS polyacrylamide gradient gels, stained with a modified colloidal CBB stain [16] and scanned with a BioRad GS 710 scanner (BioRad) as described previously [10]. A total of 302 proteins could be detected on these 2-D protein maps (Fig. 2). All spots displayed in Fig. 2 were consistently detected in three independent primary root preparations (data not shown).

A total of 156 proteins representing the proteins with the highest spot volume of this dataset were eluted from a representative gel and digested in-gel with trpysin as previously described [10]. The resulting peptide mixtures were analyzed *via* MALDI-TOF MS using a Voyager-DE PRO mass spectrometer (PerSeptive Biosystems, Framingham, MA) as previously described [10]. Proteins were identified *via* peptide mass fingerprints using the MS-Fit program of the protein prospector package (http://prospector.ucsf.edu/) and databases NCBI.nr (NCBI–National Center for biotechnology information) the maize EST contig and singlet database (www.maizegdb.org) (all databases as of 03.26.2004), and assembled genomic sequences from Version 3.1 of the Iowa State University Maize Assembled Genomic Islands (MAGI) project (www.plantgenomics.iastate.edu/maize). MSU (MS Utilities) softwarewas used to automate the MS-Fit identification tools [7]. A protein identified *via* the MS-Fit database search was accepted as true positive only if the following criteria similar to those used by [7] were met. First, the deviations between experimentally determined and predicted MWs of peptides were required to be less than 50 ppm and the difference between the smallest and largest deviation was not allowed to exceed 30 ppm. Second, at least four peptides were required to match the expected MWs and only one missed cleavage was allowed. Third, the matching tryptic fragments (peptides) were required to represent >10% of the protein. Fourth, the molecular weight search (MOWSE) score [17] which indicates the probability of a true positive identification was required to be higher than 1000. Finally, the experimentally determined MW of a protein was required to be within 20% of the predicted MW of its protein match.

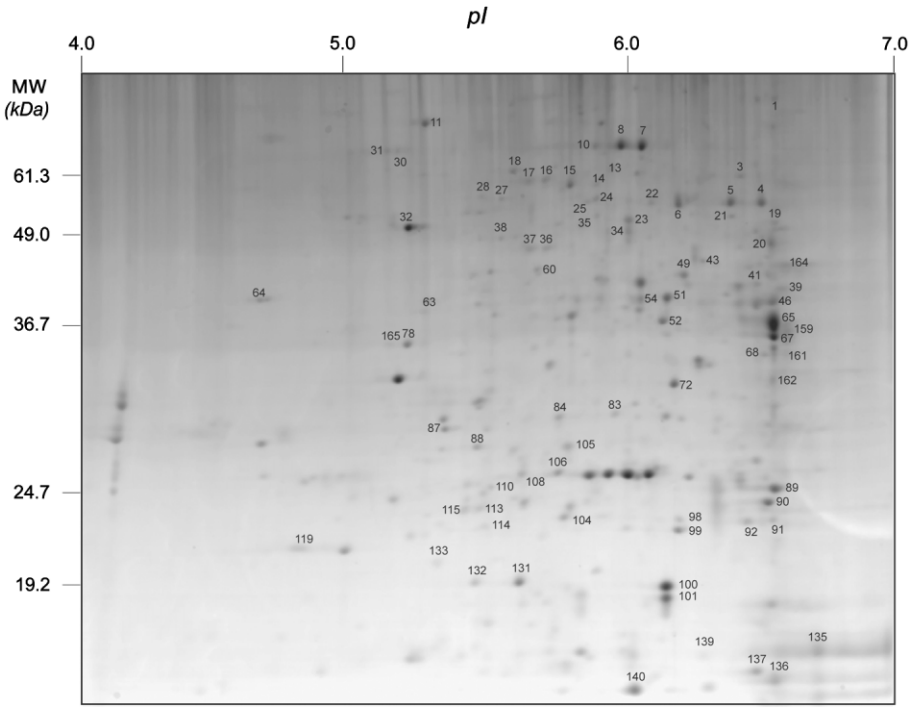

Figure 2. 2-D map of proteins isolated from 5-day-old primary roots of the inbred line B73. Proteins were separated in the first dimension according to their p*I*s on an IPG strip 4–7 and in the second dimension according to their masses (MW kDa) on a linear 12–18% SDS-polyacrylamide gradient gel. Proteins were stained with colloidal CBB. Protein spots that were identified *via* MALDI-TOF spectrometry are numbered on the map.

Table 1 summarizes the result of the MS-Fit database searches. This procedure led to the identification of 81 (52%) of the 156 proteins. This is similar to the recovery rates we obtained from different datasets generated in our laboratory where we analyzed the maize mitochondrial proteome [10] and were able to identify 51% of the proteins subjected to MS and a 9 DAG primary root dataset [9] where we were able to identify 70% of the proteins subjected to MS. The 81 proteins identified in this study represented 74 different Genbank accessions (ACs). Interestingly, in this dataset 94% of the identified proteins were identified *via* distinct Genbank ACs while in previous maize datasets from our laboratory only 58% [10] and 70% [9] of the identified proteins represented distinct Genbank ACs.

It was possible to assign functions to 43 (57%) of the 74 different genes. While predicted functions were immediately accessible for proteins identified *via* the NCBI protein database, hits to the EST and genomic MAGI databases were functionally annotated *via* blastx db searches [18]. Proteins with known or predicted function were annotated according to the Munich Information Center for Protein Sequences (MIPS) [19]. As expected, primary and secondary metabolism accounted for the highest proportion of the annotated proteins (26%). This also reflects the dominant position of this functional class in the fully sequenced rice and *Arabidopsis* genomes [20, 21]. Interestingly, proteins related to defense and to interaction with the environment were also very abundant among the dominant genes of the early maize primary root proteome (18%) while all other classes were represented by only a few proteins. In contrast, in a 2-wk-old leaf proteome of maize [7], a 9 DAG primary maize root dataset [9], and a 49 DAG rice root dataset [22], only 4, 12, and 7% of the identified ACs were related to defense or environmental interaction, respectively. These observations suggest that the relative fraction of the highly expressed proteins involved in defense and environmental interaction may decrease during root development. Consistent with this view, some of the proteins detected in the current study that are related to defense and environmental interaction are known to be developmentally regulated (*e.g.*, annexin spot 161 [23]; cytochrome P450 [24]; SOD4 [25]). This might explain their abundance during this very early stage of root development, while they were not identified at a later stage of root development (9 DAG) in another dataset from our laboratory which was obtained under the identical experimental conditions [9].

The root system has evolved a considerable plasticity in reaction to exogenous stimuli due to its direct exposure to the biotic and abiotic environment of the soil [26]. Roots can, for example, exploit niches of enhanced nitrogen or phosphorus concentration in the soil by elongating their lateral roots into nutrient-rich regions of the rhizosphere [27, 28]. Consistent with this developmental plasticity proteins were detected that are reported to be inducible under specific environmental conditions including the application of exogenous abscisic acid (annexin, spot 161 [23]; SOD4, spot 140 [25]), abscisic acid and salt (r40c1, spot 65, 67, 68 [29]; r40g2, spot 159 [29]), cold shock (spot 101), and drought (spot 100).

The flexibility of the young root system is also supported by the accumulation of proteins that confer protection from pathogens. We identified, *e.g.*, a selenium binding protein

Table 1. Primary root proteins of the inbred line B73 (5 DAG) identified after 2-D separation and MALDI-TOF analysis of trypsin-digested proteins matched against the NCBI.nr protein db, the ZMtuc maize EST contig db, the EST singletons of the dbEST.others db, and the ISU maize genomic sequences assembly db (MAGI) entries

Spot number	MOWSE score[a]	Peptides matched (n)[b]	% Protein covered[c]	MW gel/ predicted, kDa[d]	p*I* gel/ predicted[e]	AC[f]	Function [*species*] Genbank AC[g]
Cellular organization[h]							
36	1.14e + 3	5	16	46.2/42.7	5.71/5.30	NCBI	Actin [*P. sativum*] T06788
Defense/interaction with environment							
13	9.74e + 5	8	16	60.6/53.1	5.97/5.66	ZMtuc03–08–11.10324	Selenium binding protein [*O. sativa*] NP_914832.1
38	7.30e + 3	4	38	47.8/57.8	5.54/9.07	CA404041	Cytochrome P450 [*O. sativa*] NP_915570.1
65	2.11e + 4	7	20	37.0/38.8	6.54/6.30	NCBI	r40c1 protein [*O. sativa*] T03911
67	2.20e + 3	6	17	35.9/38.8	6.54/6.30	NCBI	r40c1 protein [*O. sativa*] T03911
68	4.06e + 4	5	24	34.9/41.7	6.48/6.25	MAGI_79568	r40c1 protein [*O. sativa*] NP_912421.1
89	8.76e + 4	7	34	25.4/24.0	6.54/6.31	MAGI_6874	Peroxiredoxin [*H. vulgare*] P52572
99	6.10e + 3	5	31	22.7/25.2	6.18/6.71	NCBI	Mn-superoxide dismutase [*Z. mays*] P41980
100	1.17e + 4	4	39	19.4/15.9	6.14/5.78	ZMtuc03–08–11.1823	Drought inducible protein [*S. officinalis*] BAB68268.1
101	2.87e + 7	8	70	18.7/18.7	6.14/6.28	ZMtuc03–08–11.13822	Cold shock protein-1 [*O. sativa*] BAC66711.1
106	1.24e + 7	9	44	26.6/27.3	5.75/5.28	ZMtuc03–08–11.13269	L-ascorbate peroxidase [*Z. mays*] S49914
140	1.45e + 3	4	24	15.1/15.1	6.02/5.65	ZMtuc03–08–11.26531	SOD4 superoxide dismutase [*Z. mays*] P23345
159	2.31e + 4	5	17	36.3/38.5	6.59/6.80	MAGI_59918	r40g2 protein [*O. sativa*] BAC83804.1
161	9.50e + 5	10	28	34.5/35.3	6.58/6.81	ZMtuc03–08–11.5515	Annexin P35 [*Z. mays*] T02975
162	2.48e + 4	8	25	32.7/26.6	6.54/6.88	ZMtuc03–08–11.24194	Yd-2 linked protein [*O. sativa*] NP_917347.1
Energy							
16	2.34e + 5	8	22	59.1/59.1	5.70/6.13	NCBI	F-1-ATPase subunit 2 [*Z. mays*] P19023
17	8.84e + 5	6	34	58.9/59.1	5.64/6.01	ZMtuc03–08–11.9030	ATP synthase beta chain, mitochondrial [*Z. mays*] P19023
39	1.58e + 3	5	18	39.5/36.5	6.56/6.40	NCBI	Glyceraldehyde-3-phosphate dehydrogenase [*Z. mays*] Q09054
Metabolism							
3	1.40e + 3	4	14	59.5/59.4	6.41/6.69	ZMtuc03–08–11.26625	Rf2 nuclear restorer protein [*Z. mays*] T03983
8	8.86e + 7	15	25	64.2/64.2	6.00/6.23	NCBI	Beta-D-glucosidase [*Z. mays*] P49235
10	6.34e + 3	11	15	64.1/64.2	5.91/6.23	NCBI	Beta-D-glucosidase [*Z. mays*] P49235
14	2.35e + 3	6	18	55.4/48.1	5.90/5.20	NCBI	Enolase [*Z. mays*] P26301
15	2.63e + 4	9	24	55.2/48.1	5.84/5.20	NCBI	Enolase [*Z. mays*] P26301
21	2.90e + 3	4	16	51.8/56.1	6.38/9.59	MAGI_74164	Phospholipid/glycerol acyltransferase [*A. thaliana*] NP_181346.1
24	6.77e + 3	5	19	55.4/49.8	5.90/5.88	ZMtuc03–08–11.10829	Aminoacylase [*O. sativa*] BAD10058.1
34	4.32e + 5	8	32	49.1/43.2	6.01/5.93	NCBI	*S*-adenosylmethionine synthetase [*O. sativa*] 17529621

Table 1. Continued

Spot number	MOWSE score[a)]	Peptides matched (n)[b)]	% Protein covered[c)]	MW gel/ predicted, kDa[d)]	p*I* gel/ predicted[e)]	AC[f)]	Function [*species*] Genbank AC[g)]
35	4.32e + 3	4	18	51.6/40.6	5.91/8.14	BG320677	NAD-dependent isocitrate dehydrogenase [*O. sativa*] BAD16830.1
49	9.59e + 3	6	47	40.7/40.7	6.20/6.43	ZMtuc03–08–11.6389	Protein disulfide isomerase [*O. sativa*] NP_908816.1
51	4.91e + 3	6	23	38.8/35.6	6.14/5.77	NCBI	Malate dehydrogenase [*Z. mays*] T02935
52	5.02e + 6	10	40	36.9/34.2	6.13/5.91	NCBI	Cysteine synthase [*Z. mays*] P80608
64	1.54e + 5	4	64	38.8/39.0	4.68/8.83	ZMtuc02–12–23.19591	Lipase [*O. sativa*] AAS91011.1
72	5.95e + 6	10	32	32.4/32.4	6.17/5.59	ZMtuc03–08–11.10346	Glyoxalase I [*Z. mays*] AAP76396.1
83	1.37e + 4	5	23	30.6/25.5	5.98/5.43	ZMtuc03–08–11.18626	Inorganic pyrophosphatase [*O. sativa*] AAT07613.1
84	4.72e + 3	4	20	30.4/25.5	5.75/5.43	ZMtuc03–08–11.18626	Inorganic pyrophosphatase [*O. sativa*] AAT07613.1
91	9.91e + 4	5	26	23.4/21.7	6.54/6.06	ZMtuc03–08–11.8209	1,4-benzoquinone reductase [*O. sativa*] NP_916411.1
92	2.55e + 4	4	20	23.2/21.7	6.44/6.06	ZMtuc03–08–11.8209	1,4-benzoquinone reductase [*O. sativa*] NP_916411.1
98	2.79e + 4	4	20	23.4/21.7	6.19/6.06	ZMtuc03–08–11.8209	1,4-benzoquinone reductase [*O. sativa*] NP_916411.1
104	3.37e + 4	6	25	22.3/23.2	5.70/5.43	ZMtuc03–08–11.14478	UMP/CMP kinase a [*O. sativa*] AAF23371.1
164	4.57e + 3	7	16	42.7/39.3	6.58/7.16	NCBI	UDP-glucuronic acid decarboxylase [*O. sativa*] 18447934
Secondary metabolism							
87	8.62e + 3	4	21	29.7/27.8	5.34/5.11	ZMtuc02–12–23.6818	Caffeoyl-CoA *O*-methyltransferase 1 [*O. sativa*] BAA81774.1
88	2.72e + 3	6	28	28.5/23.9	5.46/5.96	NCBI	Glutathione S-transferase III(b) [*Z. mays*] T52083
90	7.18e + 3	6	27	24.5/23.8	6.52/6.05	NCBI	Glutathione S-transferase III [*Z. mays*] P04907
Protein fate							
46	4.87e + 3	4	93	38.6/32.6	6.54/5.79	AI691647	Protein phosphatase 2A regulatory A subunit [*L. perenne*] AAM94368.1
135	2.86e + 3	7	42	16.5/18.3	6.69/8.91	NCBI	Cyclophilin [*Z. mays*] P21569
136	7.90e + 3	5	32	15.5/16.4	6.54/6.51	ZMtuc03–08–11.25670	Ubiquitin-conjugating protein [*A. thaliana*] NP_565834.1
Protein synthesis							
43	4.80e + 3	6	18	43.4/48.4	6.27/6.04	NCBI	Tanslational elongation factor EF-TuM [*Z. mays*] AAG32661
Transcription							
54	2.40e + 3	6	25	38.7/45.8	6.04/8.80	MAGI_102507	RING-H2 zinc finger protein [*O. sativa*] BAD17101.1I
105	2.03e + 3	4	19	28.6/24.9	5.79/6.45	NCBI	LIM-domain protein [*A. thaliana*] T02467

Table 1. Continued

Spot number	MOWSE score[a)]	Peptides matched (n)[b)]	% Protein covered[c)]	MW gel/ predicted, kDa[d)]	p*I* gel/ predicted[e)]	AC[f)]	Function [*species*] Genbank AC[g)]
Transport							
18	3.68e + 5	8	13	60.2/54.1	5.59/5.07	ZMtuc03–08–11.6793	V-ATPase B subunit [*O. sativa*] AAK54617.1
Unclassified							
63	1.33e + 7	9	31	38.5/34.8	5.28/4.90	MAGI_2594	Late embryogenesis abundant protein [*O. sativa*] AAS07355.1
119	9.73e + 3	5	27	21.6/18.7	4.81/4.52	ZMtuc03–08–11.15619	Translationally controlled tumor protein-like protein [*Z. mays*] AAN40686.1
Unknown							
1	2.17e + 5	8	18	84.0/84.0	6.54/6.04	ZMtuc03–08–11.14232	Hypothetical protein [*O. sativa*] CAE03021.3
4	9.70e + 3	4	69	54.2/n.p.	6.49/n.p.	CB381244	None
5	1.09e + 3	4	30	54.3/n.p.	6.38/n.p.	ZMtuc03–08–11.18847	None
6	1.19e + 4	4	50	54.0/53.6	6.18/9.30	MAGI_84332	Hypothetical protein [*O. sativa*] NP_916857.1
7	1.24e + 3	6	12	64.2/63.3	6.04/8.51	NCBI	Hypothetical protein [*O. sativa*] BAA99525
11	1.88e + 3	5	14	72.6/65.1	5.27/5.83	ZMtuc03–08–11.20041	Hypothetical protein [*O. sativa*] CAD41779.2
19	1.03e + 3	4	26	50.7/53.2	6.54/9.89	ZMtuc03–08–11.11868	Hypothetical protein [*O. sativa*] NP_911228.1
22	1.00e + 4	4	82	54.5/n.p.	6.08/n.p.	ZMtuc03–08–11.8504	None
23	2.01e + 3	5	15	51.1/60.3	6.01/9.27	ZMtuc03–08–11.12133	Hypothetical protein [*O. sativa*] NP_914178.1
25	5.02e + 3	6	31	55.0/n.p.	5.87/n.p.	ZMtuc03–08–11.3672	None
27	2.52e + 3	4	18	55.0/n.p.	5.55/n.p.	MAGI_64759	None
28	4.39e + 3	4	46	55.5/n.p.	5.47/n.p.	ZMtuc03–08–11.20824	None
30	1.97e + 4	4	71	63.1/n.p.	5.17/n.p.	CD964379	None
31	1.43e + 4	4	48	63.3/n.p.	5.14/n.p.	MAGI_59363	None
32	1.58e + 4	6	18	50.0/41.7	5.21/5.08	MAGI_105751	Hypothetical protein [*O. sativa*] BAC78584.1
37	7.15e + 3	4	22	46.0/n.p.	5.64/n.p.	ZMtuc03–08–11.15119	None
41	1.19e + 5	4	94	39.9/n.p.	6.48/n.p.	MAGI_52135	None
60	1.46e + 4	4	37	41.8/46.3	5.67/8.93	CF055621	Hypothetical protein [*O. sativa*] NP_918594.1
78	1.07e + 3	4	21	35.3/n.p.	5.21/n.p.	CF041608	None
108	3.02e + 3	4	85	26.5/n.p.	5.62/n.p.	ZMtuc03–08–11.17144	None
110	7.95e + 3	4	55	25.6/n.p.	5.51/n.p.	MAGI_23938	None
113	4.34e + 3	4	18	24.5/n.p.	5.46/n.p.	ZMtuc03–08–11.24157	None
114	1.70e + 3	4	54	23.0/n.p.	5.49/n.p.	MAGI_107657	None
115	4.30e + 3	4	49	23.9/n.p.	5.41/n.p.	MAGI_80094	None
131	3.89e + 6	9	36	19.7/19.7	5.61/5.91	ZMtuc03–08–11.5252	Hypothetical protein [*O. sativa*] BAC78567.1

Table 1. Continued

Spot number	MOWSE score[a)]	Peptides matched (n)[b)]	% Protein covered[c)]	MW gel/ predicted, kDa[d)]	p*I* gel/ predicted[e)]	AC[f)]	Function [*species*] Genbank AC[g)]
132	2.84e + 3	4	18	19.7/19.7	5.45/7.07	ZMtuc03–08–11.5252	Hypothetical protein [*O. sativa*] BAC78567.1
133	5.13e + 3	4	50	20.7/n.p.	5.32/n.p.	MAGI_62106	None
137	5.73e + 7	7	59	15.8/n.p.	6.47/n.p.	ZMtuc03–08–11.1885	None
139	1.84e + 3	4	71	16.4/n.p.	6.28/n.p.	MAGI_46977	None
165	1.94e + 4	6	46	35.0/n.p.	5.10/n.p.	ZMtuc03–08–11.5304	None

a) MOWSE score: Statistical probability of true positive identification of predicted proteins (cutoff value: 1e + 3) calculated *via* MS-Fit software (http://prospector.ucsf.edu/). Maximum allowed MW deviation of experimental and predicted peptide fragments: 50 ppm; allowed missed cleavage: 1.
b) Peptides matched (*n*): number of peptides matching predicted protein sequences (cutoff value: $n = 4$).
c) % EST/protein covered: percentage of predicted protein sequence covered by matched peptides (cutoff value: 10%).
d) MW gel/MW predicted: molecular mass of protein on gel/predicted protein (MW gel = MW predicted ± 20%); n.p.: no prediction (proteins without functional annotation).
e) p*I* gel/p*I* predicted: isoelectric point of protein on gel/predicted protein.
f) EST, EST contig (ZMtuc), or genomic sequence (MAGI) ACs of predicted protein; NCBI: Proteins identified *via* the annotated NCBI protein database.
g) Function: for ESTs obtained *via* blastx; for proteins predicted *via* NCBI according to functional NCBI annotation (cutoff value: 1e–10).
h) Functional category: classification of the proteins according to the Arabidopsis MATDB (http://mips.gsf.de/proj/thal/db/ [19]).

(spot 13) which is known to enhance resistance to blast fungi and bacterial blight in transgenic rice plants overexpressing this gene [30]. Moreover, a cytochrome P450 (spot 38) which belongs to a class of proteins known to play a role in the degradation of environmental toxins and mutagens [24] was identified. Also identified were peroxidases (spot 89, 106) and superoxide dismutases (spots 99, 140) which regulate the accumulation of toxic reactive oxygen species, including superoxide anion radical [31]. These reactive oxygen species serve both as substrates in metabolism and act as signals during development [32]. Finally, a protein similar to YLP (Spot 162), which confers resistance to barley yellow dwarf virus, was identified.

In summary, a considerable number of proteins involved in defense and reaction to environmental stimuli were identified in young maize primary roots. Damage of the primary root at this early stage of development would most likely result in the death of the seedling. Thus, expression of these proteins involved in defense and reaction to environmental stimuli might be a mechanism to ensure maximum protection of the root system from exogenous damage.

The sequences of proteins identified in the proteome of 5-day-old primary roots were compared to the previously published datasets derived from 6 DAG maize root tips [8], 9-day-old maize primary roots [9], and 14 DAG seedling leaves [7] of maize (Table 2). Maize protein sequences identified from the NCBI protein database were directly compared to the other maize seedling proteome datasets [7–9] *via* the blastp algorithm [18]. In cases where proteins were identified *via* EST sequences these sequences were compared to proteins in other datasets *via* the blastx algorithm or *versus* ESTs from other datasets *via* the blastn algorithm [18]. The maize protein dataset from the current study was set as the query file to search these maize data sets using the different blast algorithms with an *E*-value cutoff of 1e–10.

Our study of 5 DAG maize primary roots yielded 74 distinct proteins. The 9 DAG primary root dataset generated in our laboratory [9] yielded 47 distinct proteins, while the 6 DAG root tip dataset [8] and the 14 DAG leaf dataset [7] yielded 31 and 73 distinct proteins, respectively. All of these datasets were generated with CBB stained 2-D gels that allow for the identification of only the major components of the soluble proteomes. Similarity searches of the 5 DAG primary root proteome dataset as a query file with the other maize seedling datasets revealed that 41% (30/74) of the proteins present in our dataset were detected in at least one of the other datasets. Hence, 44 proteins isolated from 5-day-old roots have not been found in previous proteome studies of maize seedlings. While 39% (12/31) of the distinct proteins in the 6 DAG root tip dataset [8] displayed similarity to proteins identified in 5 DAG roots, as expected [7] only 26% of the proteins in the leaf dataset exhibited similarity to proteins extracted from 5 DAG primary roots. When comparing the functional categories of the proteins that were present in more than one dataset with the classification of all proteins in this dataset (Table 1) it was interesting that metabolism (37%), defense (23%), and energy (10%) were overrepresented, which supports the notion that these fractions

Table 2. Similar proteins identified in various maize seedling datasets

5 DAG primary root (74)[a)]	Function	9 DAG primary root (47)[9]	6 DAG primary root tips (31)[8]	6 DAG leaves (73) [7]
3[b)]	Rf2 nuclear restorer		48b	
8	Beta-D-glucosidase		8	16
14	Enolase		12, 39	
16, 17, 18	ATPase	7, 8, 10, 86	11, 48a	1, 9, 23
34	*S*-adenosylmethionine synthetase		29	
36	Actin	15	4	37, 38, 40
39	Glyceraldehyde-3-phosphate DH	49, 51	5, 6	206, 207, 210
43	Translation elongation factor		45	
51	Malate DH		19	62
52	Cysteine synthase			36
63	Late embryogenesis abundant protein	21		
65, 68, 159	r40c1/r40g2	46		
72	Glyoxylase	96		
87	Caffeoyl-CoA-*O*-methyltransferase	28		
88, 90	Glutathione-transferase III	72		104
89	Peroxiredoxin			108
91	1,4 benzoquinone reductase	69		
99	Mn superoxide dismutase	70		129
100, 137, 165	Drought inducible/unknown protein	26, 81		143, 154
104	UMP/CMP kinase a			110
106	L-ascorbate peroxidase	41, 43, 76		103
119	Tumor like protein	29		
131	Hypothetical protein	65		

a) Numbers in parenthesis in the header indicate the number of different Genbank ACs represented in each dataset. Six DAG leaves dataset actually contained 76 different Genbank ACs, however only 73 are still accessible.

b) Each protein spot number in the table represents a different Genbank AC. Thus, Genbank ACs that were identified several times in a dataset are represented only by one number in this table.

play a general role in various organs and developmental stages. Interestingly, proteins of unknown function or as yet unclassified proteins which made up 43% of the identified protein ACs in our 5 DAG primary root dataset (Table 1) represented only 20% of the proteins found in more than one maize seedling dataset. This might indicate that these proteins have developmental stage specific functions which remain to be elucidated.

Of particular interest was the comparison of the 5 DAG primary root dataset with the 9 DAG primary root dataset [9] previously generated in our laboratory with the same maize genotype (B73) grown under the identical experimental conditions. Only 28% (21/74) of the proteins identified in the 5 DAG primary root dataset were detected in 9 DAG primary roots and only 40% (21/47) of the proteins identified in the 9 DAG primary root proteome were isolated from 5 DAG primary roots. These analyses, therefore, demonstrate that there is a remarkable difference in the accumulation of the most abundant proteins between these two early stages of root development separated by only 4 days of growth. This notion is supported by the comparison of the 2-D protein maps of the 5 DAG and 9 DAG primary root proteomes. A total of 302 and 150 proteins were detected in the 5 DAG and 9 DAG roots, respectively. Only about 30% of the 150 proteins that were detected on the 9 DAG primary root map had an equivalent on the 5 DAG primary root map. Although one has to be cautious about such comparisons due to the limited number of detected proteins and the different sizes of the two datasets, this comparison provides further support for the very significant changes that occur in the accumulation of proteins during early root development.

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