

ORIGINAL PAPER

Revealing the seed proteome of the health benefitting grain amaranth (*Amaranthus cruentus* L.)

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Amaranth, a staple food source in ancient Aztec, Maya and Inca cultures, has been recognised as a 21st century crop. This superfood, known as Inca wheat, attracts the worldwide attention of researchers and farmers for its superior agronomical and technological properties but especially because of its exceptional nutritive value. A combination of two-dimensional electrophoresis (2-DE) with MS facilitating the effective differentiation of 13 classes of *Amaranthus cruentus* L. “Ficha” mature seed proteins was used in the current study. The best resolution results in the 2-DE were obtained using immobilised pH gradients strips with a pH range of 5–8. Out of the 461 spots detected, 249 were successfully identified by LC-MS/MS analysis, making this the most inclusive protein profile of mature amaranth seed. Unknown proteins represented the most abundant class of proteins (59), the second most abundant category was related to energy (46) and then to seed storage proteins (43). These results expand the knowledge of the amaranth seed proteome and it is expected that the data presented here will contribute to further in-depth characterisation of *Amaranthus* seeds.

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Keywords: *Amaranthus cruentus* L., pseudocereal, nutrition, seed, 2-DE, protein map

Introduction

The demand for natural products with highly nutritional and medical properties has risen over the past two decades. Plant species that have attracted the interest of farmers and breeders also include “minor” or “neglected” species like *Amaranthus*.

A number of reviews have summarised research focusing on the amaranth nutrient profile, its botany, biology, agronomy and technology (Teutonico & Knorr, 1985; Stallknecht & Schulz-Schaeffer, 1993; Paredes-López, 1994). Caselato-Sousa and Amaya-Farfán (2012) reported recent research specifically focused on

the highly nutritive amaranth grain and its use in clinical trials aimed at testing the possible benefits of the amaranth grain for human health and in the prevention of chronic disease. New findings on amaranth composition, application and processing were comprehensively reviewed by Venskutonis and Kraujalis (2013).

The *Amaranthus* seed, as a promising supplement to common grains, has received attention because of its unique nutritional properties including an appreciable amount of highly nutritive proteins, and its favourable amino acid composition, high unsaturated oil content, fat-soluble vitamins and its fibre content

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which is three times that of wheat. The valuable nutrient profile of the amaranth seed predetermines its uses as an alternative to conventional cereals. Because the content of prolamins was found to be extremely low in the amaranth seed (Barba de la Rosa et al., 1992; Gorinstein et al., 2002; Džunková et al., 2011), this new millennium crop may be primarily used by coeliac sufferers in their diet.

Amaranthus seed has been proposed as an excellent substitute for marine animals as a natural source of the steroidal hormone precursor squalene with anticancer and cholesterol-content reducing properties (Miettinen & Vanhanen, 1994; Rao, 1998; Smith, 2000; He, 2002).

Other bioactive components of this cereal-like seeds comprise a cancer-preventative peptide lunasin (Silva-Sánchez et al., 2008; Maldonado-Cervantes et al., 2010) and compounds with antioxidant properties such as flavonoids, phenolic acids, phytosterols, vitamin E, saponins, among others (Güçlü-Ustündağ & Mazza, 2007; Tikekar et al., 2008; Paško et al., 2009).

Because of the increasing importance of amaranth seed as a food ingredient, contributing significantly to human nutrient requirements, much research has been directed towards the identification and validation of its protein composition.

Advanced proteomics-based techniques have also been used to identify a broad spectrum of proteins in amaranth. Quiroga et al. (2007) characterised amaranth (*Amaranthus hypochondriacus* L.) globulin proteins, using two-dimensional electrophoresis, ion-exchange chromatography and RP-HPLC, sustaining the existence of genetic polymorphism in amaranth globulins. To enhance understanding of the amaranth stress-responsive genes/proteins, comparative proteomics was applied to study the differences in abundance of amaranth (*Amaranthus hypochondriacus* L.) leaf proteins exposed to drought stress (Huerta-Ocampo et al., 2009).

Recently, the *Amaranthus cruentus* L. cv Amaranteca seed proteome was characterised (Maldonado-Cervantes et al., 2014), identifying 43 proteins out of 400 2-DE gel spots. One most abundant spot was identified as a Late Embryogenesis Abundant (LEA) protein. This study was the first to reveal the presence of new LEA in amaranth seed.

In the current work, a proteome reference map containing 461 2-DE quantified gel spots representing 249 different identified proteins was constructed for the *Amaranthus cruentus* L. “Ficha” mature seed, making this the most inclusive amaranth protein profile. The data discussed in this report are primarily focused on the nutrition-associated seed proteins revealed in previous works.

Experimental

Mature seeds of the grain amaranth “Ficha” (*Ama-*

ranthus cruentus L.) used in this study were harvested from a location in Koliňany (Nitra region, Slovakia). The genotype “Ficha” is a medium early, light-coloured cultivar with large seeds (WTS; 0.85 g).

This location near Nitra has an average annual rainfall of 550 mm and average annual temperature of 11.1 °C. The soil type at the experimental site is a brown soil with pH of 7.4. During the growing season, no fertilisers or sprays were applied.

Total proteins content was extracted from 500 mg of mature seeds using the phenol-based extraction solution described in (Hajduch et al., 2005).

These were re-solubilised in 0.3 mL of immobilised pH gradient (IPG) buffer 8 M urea (Promega, Madison, WI, USA), 2 M thiourea (Sigma-Aldrich, Saint Louis, MI, USA), 2 mass % CHAPS (Merck, Darmstadt, Germany), 2 vol. % Triton™ X-100 (Sigma-Aldrich), and 50 mM DTT (Thermo Scientific, Waltham, MA, USA), quantified by Bradford assay (Sigma-Aldrich) and separated by two-dimensional electrophoresis, as described in Danchenko et al. (2009). IPG strips with pH of 3–10 (17 cm; Bio-Rad, Hercules, CA, USA) and pH 5–8 strips (17 cm; Bio-Rad, Hercules, CA, USA) were used for separation of the proteins (Fig. S1 in Supplementary data) in an isoelectric focusing (IEF) unit (Protean IEF Cell, Bio-Rad, Hercules, CA, USA). The second step of the analysis was performed using IPG strips of pH 5–8 (17 cm; Bio-Rad, Hercules, CA, USA). The second dimension SDS-PAGE was carried out using a Protean II xi Cell (Bio-Rad, Hercules, CA, USA), at 10 mA current for approximately 14 h until the dye migrated from the 12 mass % gel for pH 3–10 and pH 5–8, respectively.

The 2-DE gels were stained with colloidal Coomassie GS-800 (Serva, Heidelberg, Germany), digitalised using a GS-800 Calibrated Densitometer (Bio-Rad, Hercules, CA, USA) at 300 dpi and a 16-bit greyscale and analysed using ImageMaster software 4.9 (GE Healthcare, Uppsala, Sweden) in three independent biological replicates. The volumes of all spots detected in at least two out of three replicates were normalised as relative volumes to compensate for small differences in the sample loading or gels- staining. The standard deviation was calculated.

Protein spots were excised, digested with trypsin overnight at 37 °C and subjected to tandem mass spectrometry analysis as described by Klubicová et al. (2010).

The peptide mixtures were concentrated with a Concentrator plus (Eppendorf, Hamburg, Germany) and analysed by automated nanoflow reverse-phase chromatography using the nanoAcquity UPLC system (Waters, Manchester, UK) coupled to a Q-TOF Premier (Waters, Manchester, UK) tandem mass spectrometer. Data were acquired by MSE method, which performed alternate scans at low and high collision energies. Quadrupole analyzer operated in the radio-

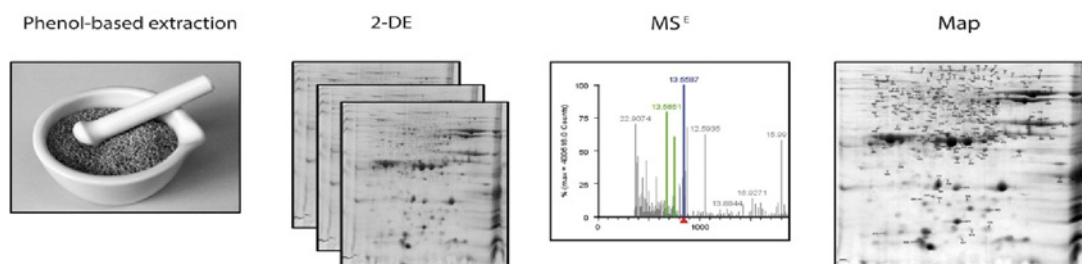


Fig. 1. Experimental workflow. Proteins were isolated from the amaranth grains, separated by 2D electrophoresis, and the 2-DE gels analysed using ImageMaster 4.9 software. Thereby, 461 proteins were quantified and 249 were identified using tandem mass spectrometry.

frequency mode, allowing all ions to reach TOF analyzer. Therefore, in a single full scan run, both precursor ions and accurate fragment masses were detected. In MS channel, data were collected at constant collision energy 4 eV and in MS/MS channel fragments were recorded while collision energy ramped from 20 eV to 35 eV. All the samples were analysed in duplicates and lock spray-calibrated against GFP. The data collected at low and elevated energy modes were processed using the ProteinLynx Global Server v. 2.4 (Waters, Manchester, UK).

Time alignment was used to initially correlate the precursor and fragment ions. The database search was then performed using the ion accounting algorithm which has been shown to be highly effective in the identification of proteins across a wide dynamic range (Klubicová et al., 2010). The algorithm incorporates a reverse-sequence/random decoy database to determine the false-positive identification rate. The peptides tentatively identified were score on the basis of their correlation to 14 different models of specific physico-chemical attributes (Li et al., 2001). This process was followed by a peptide-ranking process and by collapsing the identified peptides into proteins. The process ended when the rate of decoy protein identification exceeded the false-positive rate threshold which was set at 4 %. The resulting data were then queried against the UniProt database of *Amaranthaceae* sequences (Nov 2012; 2743 sequences) and UniProt *viridiplantae* database (Nov 2012; 1250542 sequences) containing sequences from green plants. During the database searches, one missed cleavage site was permitted. The precursor peptide mass tolerance was set at ± 0.0001 %, and the fragment mass tolerance at ± 0.1 m/z . The search was performed using the following criteria: fix modification was set at carbamidomethyl-C, variable modifications oxidation M, deamidation Q, deamidation N, acetylation N –terminus. Three or more consecutive fragment ions from the same series for each peptide and a minimum of two matched peptides were required for identification of a particular protein. Finally, the protein identifications were accepted after a manual inspection of the probabilistic-based PLGS assignment at a 95 % confidence level. Only those proteins which were found at least three

times out of six (three biological, two technical) replicates are listed in the tables.

In order to characterise those proteins that were denoted in the database as “unknown”, their sequences were searched against the complete NCBI database for possible homology using BLASTP search (NCBI, Bethesda MD, USA). When homologue proteins with a query coverage of at least 90 % and with the minimum Expected (E) 0.0003 were identified, the protein with the highest coverage was selected and the ‘unknown’ denotation was replaced with the prefix ‘similar to.’

To determine the sub-cellular location of the identified proteins, the prediction tools TargetP (<http://www.cbs.dtu.dk/services/TargetP/>), iPSORT (<http://ipsort.hgc.jp/>), and Predotar (<http://urgi.versailles.inra.fr/predotar/predotar.html>) were used. A schematic representation of the proteomics experimental set-up is given in Fig. 1.

Results and discussion

In the present study, an *Amaranthus cruentus* L. “Ficha” mature seed proteome map was established. In order to obtain a 2-DE reference protein map (Fig. 2), the proteins were separated by 2D-PAGE and visualised by Coomassie blue-staining. As a first attempt in establishing the amaranth mature seed proteome profile, a 2-DE gel electrophoresis was performed using pH 3–10 immobilised gradient strips (Fig. S1). Because most of the proteins were concentrated in the 5 to 8 pH range, a further gel electrophoresis using IPG pH 5–8 strips was performed. Only those spots present in at least two gels were considered for the database searches.

In total, 461 protein spots were quantified on pH 5–8 gels using ImageMaster 4.9 software (Table S1 in Supplementary data), excised from the gels and digested with trypsin. Subsequent LC-MS/MS (MS^E) analysis of the digested proteins resulted in 249 successful protein identifications (Table 1, Table S2). A database containing the sequences of the entire *Amaranthaceae* family was used in the first step of the identification process, since a limited number of *Amaranthus* sp. protein sequences are available. Unidentified

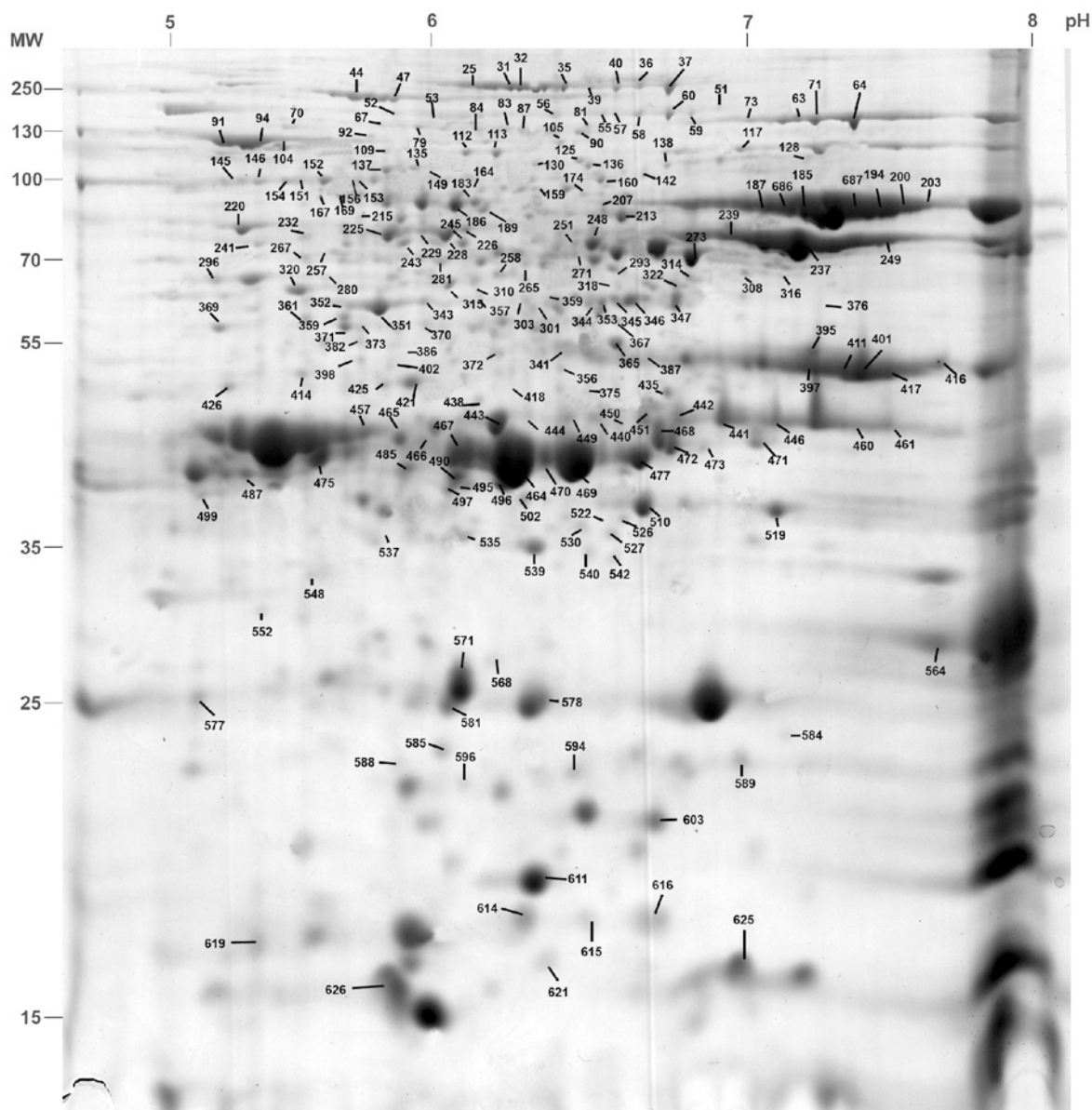


Fig. 2. Selected identified proteins were determined on 2-DE gel, and a protein map of *Amaranthus cruentus* “Ficha” grain was created. Numbers of spots correlating with spot ID are as follows: 25, 73, 83 – aminopeptidase; 31 – 50S ribosomal protein L15; 35 – cyclin d; 36, 37 – elongation factor 2; 39, 40 – aconitase; 44 – ClpC protease; 47 – acetolactate synthase; 51 – cyclin-D2-1-like; 53 – sucrose synthase; 55, 57, 58, 60 – pollen allergen MetE; 59 – Sal 3 pollen allergen; 64, 425, 487 – ribulose biphosphate carboxylase large subunit; 79 – NADH ubiquinone oxidoreductase; 84, 87, 314 – transketolase; 90 – glycyl-tRNA synthetase 1; 91, 94, 104 – heat shock 70 protein; 105 – delta aminolevulinic acid dehydratase; 109 – high molecular mass glutenin subunit, 112, 113 – stress-induced protein st1 like protein; 128, 174 – similar to magnesium transporter NIPA2-like; 130, 372, 615, 619, 621 – ribosomal protein S12; 138 – malic enzyme; 142 – pyruvate decarboxylase; 145, 160 – TCP 1 cpn60 chaperonin family protein; 149, 153, 200, 239, 273, 293 – choline monooxygenase; 151 – chaperonin 60kD; 154 – T complex protein 1 subunit epsilon; 159 – arginyl tRNA synthetase; 164 – phosphoglycerate dehydrogenase; 167 – elongation factor 1 alpha; 169 – betaine aldehyde dehydrogenase; 183, 186 – ketol acid reductoisomerase; 185 – ribulose biphosphate carboxylase small chain 2; 189 – glutathione reductase; 194 – 50S ribosomal protein L23-like; 207, 213 – ATP synthase subunit alpha; 215, 228, 232 – enolase; 220 – ATP synthase subunit beta; 225 – CP12; 226 – adenosylhomocysteinase; 241 – mitochondrial-processing peptidase subunit alpha; 243 – guanosine nucleotide diphosphate dissociation inhibitor 1; 245 – similar to xylose isomerase-like; 249 – aquaporin; 257 – helicase; 258 – gamma class glutathione transferase EF1Bgamma2; 265 similar to agamous-like MADS-box protein AGL18-like; 271 – 6 phosphogluconate dehydrogenase decarboxylating; 280 – glucose 1 phosphate adenyltransferase; 281 – flowering locus T-like protein FT1; 296 – similar to proteasome component (PCI) domain, 301 – isocitrate dehydrogenase NADP; 303 – luminal binding protein; 310, 387, 435 – malate dehydrogenase; 318 – similar to GDP-mannose 3,5-epimerase 1; 320 – actin; 341 – L-idonate 5-dehydrogenase; 343, 352 – phosphoglycerate kinase; 344, 353 – elongation factor Tu; 322, 346, 347 – alcohol dehydrogenase 1; 359 – uncharacterised; 361, 552 – similar to transcription factor MYB3; 365 – glycerol 3 phosphate acyltransferase; 369 – RelA homologue; 370 – F box protein; 371 – glutamine synthetase;

375 – maturase K; 376 – aspartate aminotransferase; 382 – MPF1-like-A; 386 – ribosomal protein L22p/L17e family protein; 395, 397, 442, 444, 446, 449, 451, 460, 461 – agglutinin; 401, 411, 416, 417 – glyceraldehyde 3 phosphate dehydrogenase; 414 – 60S acidic ribosomal protein P0; 418, 626 – NAD P H quinone oxidoreductase; 421 – lactoylglutathione lyase; 426 – enoyl ACP reductase; 438 – lactoylglutathione lyase; 440, 441, 450 – seed protein AmA1; 443 – hydroxypyruvate reductase; 464, 466, 467, 469, 470, 473, 477, 485, 490, 495, 496, 564 – 11S globulin; 468, 537 – ferredoxin-dependent glutamate synthase; 472 – glutamate 1 semialdehyde aminotransferase; 497 – proteasome subunit alpha type 3; 499 – 50S ribosomal protein L2; 502 – salt-tolerance protein 2; 519 – photosystem II 22 kDa protein; 526 – voltage-dependent anion channel protein; 527 – probable ATP synthase 24 kDa subunit; 530 – gibberellin 20 oxidase 1; 539, 540 – cystatin; 548 – 20 kDa chaperonin; 571 – similar to kinesin light chain-like; 578 – Inducer of CBF expression; 581 – delta12 oleic acid desaturase; 585 – thioredoxin-dependent peroxidase; 588 – allergen Pru p 1 06C; 594 – eukaryotic translation initiation factor 5A; 596 – ribosomal protein L16; 603 – 17 67 kDa heat shock protein; 611 – late embryogenesis abundant protein; 616 – nucleoside diphosphate kinase 1.

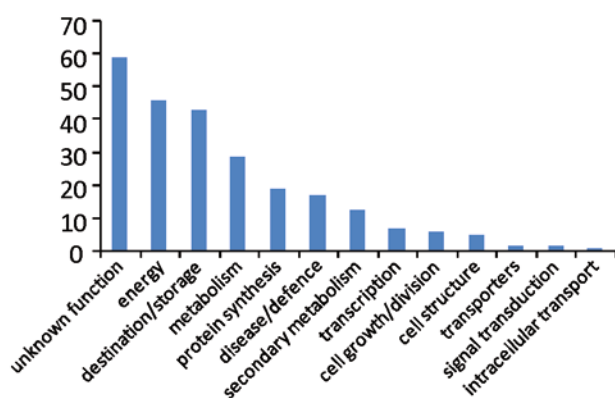


Fig. 3. The 249 identified amaranth grain proteins were classified into 13 categories in accordance with Bevan et al. (1998) as modified by Miernyk et al. (2011). The *x* axis represents the number of protein spots identified in each category, the *y* axis represents functional classes of proteins.

protein spots were searched against the *Viridiplantae* (green plants) database. The proteins identified were classified into 13 functional categories in accordance with Bevan et al. (1998) with minor modifications in accordance with (Fig. 3) Miernyk et al. (2011).

Proteins involved in energy (46) and seed storage proteins (43) dominated the 2-DE profile of mature amaranth seeds, followed by proteins of primary metabolism (29), protein synthesis (17), secondary metabolism (13). The identified proteins also include proteins involved in transcription (7), in cell growth (6), in cell structure (5), in signal transduction (2), transporters (2) and in intracellular transport (1). However, proteins with unknown functions (PUF) remain the most abundant class (59).

The proteins identified were localised using the prediction tools: TargetP, iPSORT and Predotar tools. Most of them are part of cytoplasm (153), followed by plastid (37), endoplasmic reticulum (ER, 26), mitochondrion (23) and the localisation of the remaining proteins (9) was unclear.

Seed storage proteins

In general, the nutritional quality of seeds depends

on the protein, starch and lipid content and composition, the level of vitamins, minerals, dietary fibre and other nutraceutical constituents and micro constituents. With regard to proteins, storage proteins are of particular importance because they determine not only the total protein content of the seed but also its quality for various uses.

Most of the results support the fact that easily digestible globulins and albumins are the major fractions of seed storage proteins (more than 50 % of the total fractional composition) in the amaranth grains, followed by alkali-soluble glutelins (Paredes-López, 1994; Gorinstein et al., 2002). Albumins and globulins have a well-balanced amino acid composition with a high content of essential amino acids, especially lysine and sulphur-containing amino acids (Dodok et al., 1997; Gorinstein et al., 2002; Písaříková et al., 2005; Nimbalkar et al., 2012). Alcohol-soluble prolamins lacking in essential amino acids represent a minor protein fraction of amaranth seeds (1.0–3.2 %). However, this fraction is known to be a major protein fraction in common cereals, with the exception of oats (Sontag-Strohm, 2008).

The category of protein destination and storage proteins was one of the dominant functional classes of the amaranth 2-DE profile obtained. 29 identified proteins can be attributed to a large number of seed storage proteins with a composite relative volume of 19.89 %, in which globulins are mainly represented.

Specifically, 14 proteins (spots 464, 466, 467, 469, 470, 473, 477, 485, 490, 495, 496, 562, 564, 568) out of 29 were identified as 11S globulin. A number of reports have revealed that amaranth and oat globulins and rice glutelin are highly homologous and are related to the legume 11S storage proteins (Okita et al., 1988; Bressani & Garcia-Vela, 1990; Segura-Nieto et al., 1994). An amaranth 11S globulin is one of the most abundant and important storage proteins of amaranth grains with a high content of essential amino acids, including lysine, tryptophan and tyrosine. Rascón-Cruz et al. (2004) reported that amaranth 11S globulin sequence could be used as a promising donor sequence to boost the functional and nutritional quality of food crops. The analysis performed by Maldonado-Cervantes et al. (2014) detected only 3 spots as 11S globulins in 2-DE *Amaranthus cruentus* L. cv Amaranteca in comparison with the present

Table 1. Selected identified proteins from amaranth grain related to nutrition attributes

Description	Species	Accession	Localization	Spot ID	PLGS Score	Cov/Pep	Theor MW/pI	Exp MW/pI
01 Metabolism								
01.01 Amino Acids								
Ketol acid reductoisomerase chloroplastic	<i>Spinacia oleracea</i>	Q01292	plastid	183	1926.64	28.9/23	63.7/6.6	70/6.3
Ketol acid reductoisomerase chloroplastic	<i>Spinacia oleracea</i>	Q01292	plastid	186	75.57	4.5/2	63.7/6.6	69/6.3
Acetolactate synthase	<i>Amaranthus sp Iowa</i>	Q38795	plastid	47	45.40	8.7/3	72.4/6.8	101/6.1
Glutamine synthetase	<i>Beta vulgaris</i>	Q9AXD1	cytoplasm	371	2056.95	21.3/8	39.1/5.1	46/6.0
Pollen allergen MetE	<i>Amaranthus retroflexus</i>	E3VW74	cytoplasm	55	289.36	16.0/9	83.7/6.1	95/6.7
Pollen allergen MetE	<i>Amaranthus retroflexus</i>	E3VW74	cytoplasm	57	122.61	7.4/4	83.7/6.1	95/6.7
Pollen allergen MetE	<i>Amaranthus retroflexus</i>	E3VW74	cytoplasm	58	809.11	27.7/22	83.7/6.1	94/6.7
Pollen allergen MetE	<i>Amaranthus retroflexus</i>	E3VW74	cytoplasm	60	880.33	16.1/9	83.7/6.1	94/6.8
01.05 Sugars								
Glucose 1 phosphate adenylyltransferase	<i>Spinacia oleracea</i>	Q43152	plastid	280	43.28	6.8/2	49.0/5.0	58/6.0
similar to xylose isomerase-like	<i>Vitis vinifera</i>	A5B1E9	cytoplasm	245	489.85	16.0/12	51.6/5.4	61/6.3
L-iditol 2-dehydrogenase	<i>Populus trichocarpa</i>	B9I4E5	cytoplasm	358	369.48	31.0/11	39.2/5.9	48/6.6
Sucrose synthase	<i>Prunus persica</i>	Q946X3	?	53	105.94	27.3/3	23.5/7.3	95/6.2
01.06 Lipids								
Enoyl ACP reductase	<i>Dimocarpus longan</i>	E9NVZ3	mitochondrial	426	599.44	24.6/11	35.9/10.0	38/5.7
Glycerol 3 phosphate acyltransferase chloroplastic	<i>Spinacia oleracea</i>	Q43869	plastid	365	48.36	8.3/3	52.1/5.6	46/6.6
Delta12 oleic acid desaturase	<i>Euphorbia lagascae</i>	Q6RXX0	cytoplasm	581	107.24	16.0/2	44.2/8.9	18/6.2
01.07. Cofactors								
L-idonate 5-dehydrogenase	<i>Vitis vinifera</i>	A5AIP9	cytoplasm	341	134.29	13.0/5	37.4/6.3	50/6.5
similar to GDP-mannose 3,5-epimerase 1	<i>Zea mays</i>	I1I416	cytoplasm	318	300.08	19.3/5	42.8/5.9	53/6.6
06. Protein storage and destination								
06.20 Storage proteins								
11S globulin	<i>Amaranthus hypochondriacus</i>	A2I9A6	ER	466	248.63	12.9/11	55.0/6.5	31/6.2
11S globulin	<i>Amaranthus hypochondriacus</i>	A2I9A6	ER	467	2191.87	12.3/5	55.0/6.5	30/6.3
11S globulin	<i>Amaranthus hypochondriacus</i>	A2I9A6	ER	490	636.82	12.5/18	55.0/6.5	29/6.3
High molecular weight glutenin subunit y10	<i>Triticum aestivum</i>	A9ZMG8	ER	109	89.55	10.5/3	69.7/7.3	84/6.1
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	496	1227.99	16.6/25	56.6/6.7	28/6.4
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	562	1736.64	19.0/22	56.6/6.7	20/7.6
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	564	668.96	8.2/6	56.6/6.7	20/7.5
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	568	1219.89	30.3/27	56.6/6.7	20/6.4

Table 1. (continued)

Description	Species	Accession	Localization	Spot ID	PLGS Score	Cov/Pep	Theor MW/pI	Exp MW/pI
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	464	1434.79	16.2/26	56.6/6.7	29/6.4
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	469	300.33	4.4/6	56.6/6.7	29/6.6
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	470	856.27	17.6/21	56.6/6.7	30/6.5
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	473	114.37	6.8/6	56.6/6.7	30/6.9
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	477	137.85	9.2/9	56.6/6.7	30/6.7
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	485	453.90	6.8/16	56.6/6.7	29/6.1
11S globulin seed storage protein	<i>Amaranthus hypochondriacus</i>	Q38712	ER	495	96.53	6.8/4	56.6/6.7	28/6.3
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	395	6593.93	61.5/40	34.9/6.7	41/7.2
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	397	1121.79	55.6/13	34.9/6.7	39/7.2
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	404	174.89	18.8/6	34.9/6.7	40/6.9
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	444	441.90	15.8/4	34.9/6.7	34/6.5
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	449	1457.26	42.1/14	34.9/6.7	33/6.6
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	451	7806.66	61.5/47	34.9/6.7	33/6.7
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	460	6729.44	53.6/44	34.9/6.7	32/7.3
Agglutinin	<i>Amaranthus hypochondriacus</i>	Q38719	cytoplasm	461	6316.09	61.5/27	34.9/6.7	32/7.4
Agglutinin	<i>Amaranthus caudatus</i>	Q6YNX3	cytoplasm	446	9215.30	54.3/43	34.9/6.7	33/7.1
Agglutinin	<i>Amaranthus caudatus</i>	Q6YNX3	cytoplasm	442	7614.45	54.6/38	34.9/6.7	33/6.8
Seed protein AmA1	<i>Amaranthus hypochondriacus</i>	Q8S390	cytoplasm	427	590.33	34.2/10	34.9/6.2	38/6.9
Seed protein AmA1	<i>Amaranthus hypochondriacus</i>	Q8S390	cytoplasm	440	2807.27	50.0/22	34.9/6.2	34/6.6
Seed protein AmA1	<i>Amaranthus hypochondriacus</i>	Q8S390	cytoplasm	441	6548.20	27.0/26	34.9/6.2	33/7.0
Seed protein AmA1	<i>Amaranthus hypochondriacus</i>	Q8S390	cytoplasm	450	2113.28	50.0/24	34.9/6.2	33/6.7
20 Secondary metabolism								
Betaine aldehyde dehydrogenase	<i>Halostachys caspica</i>	A4LAP2	cytoplasm	169	405.85	18.8/9	54.5/5.1	73/5.9
Betaine aldehyde dehydrogenase	<i>Amaranthus hypochondriacus</i>	O22477	cytoplasm	170	630.49	21.6/8	54.4/5.2	72/6.0
Betaine aldehyde dehydrogenase	<i>Amaranthus hypochondriacus</i>	O22477	cytoplasm	173	95.55	13.0/4	54.4/5.2	71/6.1
Choline monooxygenase	<i>Atriplex nummularia</i>	Q7XZR0	plastid	153	76.13	9.6/2	48.7/6.1	76/6.1
Choline monooxygenase	<i>Atriplex nummularia</i>	Q7XZR0	plastid	273	44.65	14.4/4	48.7/6.1	57/6.9
Choline monooxygenase chloroplastic	<i>Atriplex hortensis</i>	Q9LKN0	plastid	149	41.09	9.6/2	48.7/6.1	77/6.2
Choline monooxygenase chloroplastic	<i>Atriplex hortensis</i>	Q9LKN0	plastid	239	54.79	9.6/3	48.7/6.1	62/7.0
Choline monooxygenase chloroplastic	<i>Atriplex hortensis</i>	Q9LKN0	plastid	200	43.69	9.6/3	48.7/6.1	67/7.4
Choline monooxygenase chloroplastic	<i>Atriplex hortensis</i>	Q9LKN0	plastid	398	42.57	9.6/3	48.7/6.1	41/6.0
Choline monooxygenase	<i>Atriplex nummularia</i>	Q7XZR0	plastid	293	56	9.6/2	48.7/6.1	55/6.7

Table indicates protein name, species, accession (Uniprot accession number), localization, PLGS Score (ProteinLynxGlobal Server score), Cov/Pep (protein coverage and number of identified peptides matched to protein sequence), Theor MW/pI (theoretical molecular weight and pI), Exp MW/pI (experimental molecular weight and pI from gels).

study. The limited number of proteins identified in the most recent proteomic study of amaranth mature seeds could be due to the different extraction method and/or pH gradient.

A 2-DE survey of the amaranth seed proteome determined 4 protein spots as amaranth seed albumins AmA1 (spots 427, 440, 441, 450), non-allergenic storage protein, rich in all essential and sulphur-containing amino acids (Raina & Datta, 1992; Kim et al., 2001). Because of its well-balanced amino acid composition that correlates with WHO standards for optimal human nutrition, the sequence of AmA1 protein has already been used to improve the nutritional quality of wheat (Tamás et al., 2009) and potato (Chakraborty et al., 2000, 2010).

Moreover, proteins involved in the biosynthesis of essential amino acids, such as acetolactate synthase, also known as acetohydroxyacid synthase, (spots 47) and ketol-acid reductoisomerase (spots 183, 186) were determined. Both enzymes are associated with the biosynthesis of branched-chain amino acids, such as valine, leucine and isoleucine. Ketol-acid reductoisomerase was purified from barley (Durner et al., 1993), wheat (Donadini & Copeland, 2000) and rice (Lee et al., 2005). These essential branched-chain amino acids cannot be synthesised in animals and must be supplemented from the diet. Another protein of interest that has been determined was pollen allergen Met E (spots 55, 57, 58 and 60); this is known to contribute to methionine biosynthesis, which plays an important role in seed germination (Gallardo et al., 2002).

Around 20 % of amaranth seed storage proteins consist of the glutelin fraction (Zheleznov et al., 1997). As described by Džunková et al. (2011), the glutelin fraction is polymorphic in MW 50–65 kDa. However, in the present work, only one spot was identified as a high molecular mass glutenin subunit (HMW-GS; spot 109) with MW 84 kDa.

Many plant species contain carbohydrate-binding proteins, also known as lectins or agglutinins. In the present study several spots were also identified as agglutinins (spots 395, 397, 404, 444, 449, 451, 460, 461, 446 and 442). Hamid et al. (2013) reviewed the functions and applications of lectins in agriculture and medicine. Lectins are active in the plant's defence mechanism against pathogens (Fitches et al., 2008), play a role in embryogenesis, vegetative and reproductive development (Brill et al., 2001), exhibit an antimicrobial effect, antiviral (Hansen et al., 1989) and also anticancer activities (Bhutia et al., 2008; Platner et al., 2008; Lam & Ng, 2010a, 2010b). Lectins are abundant in legumes and grains and their large concentration in plant seeds such as *Caesalpinia bonduc* (Ajomale et al., 1997), *Dioclea reflexa* (Adeyemi et al., 1994), *Tetracarpidium conophorum* (Togun et al., 1994a, 1994b) and *Telfairia occidentalis* (Togun et al., 2004) decreases with growth, suggesting a role in plant germination.

Lipid and starch biosynthesis

During seed development, the accumulation of very important nutritive constituents and principal storage reserves such as lipids and starch occurs. Many of the enzymes identified are involved in carbon metabolism, such as starch and polysaccharides metabolism (4), glycolysis (15), gluconeogenesis (5), pentose phosphate (6), tricarboxylic acid (TCA) cycle (4), respiration (6), fermentation (4), photosynthesis (5) and lipid and sterol metabolism (3).

The lipid content in amaranth seeds is 2–3-times higher than in traditional cereals, such as wheat and barley (Alais & Linden, 1991; Haard & Chism, 1996; Erkan et al., 2006; Kumar et al., 2011; Kamal et al., 2012) or buckwheat (Steadman et al., 2001; Vojtíšková et al., 2012). It can vary from 5.83 % to 8.20 % (Saunders & Becker, 1984; León-Camacho et al., 2001; Berganza et al., 2003; Hlinková et al., 2013), but may be as high as 16.7 % and 19.3 %, as reported for some ornamental garden and weedy amaranths by Kraujalis and Venskutonis (2013) and Singhal and Kulkarini (1988). Amaranth lipids are of interest for their composition because of their high level of unsaturated fatty acids, particularly linoleic acid (33.3 % to 43.9 % in *A. cruentus*), which is important from a nutritional perspective (León-Camacho et al., 2001, Berganza et al., 2003; Hlinková et al., 2013). A few enzymes active in lipid metabolism such as enoyl ACP reductase (spot 426), glycerol 3 phosphate acyltransferase (spot 365) and delta12 oleic acid desaturase (spot 581) were identified. Enoyl-ACP reductase (ENR), which is active in the reduction of a trans carbon-carbon double bond to form saturated acyl-ACP, has been isolated from developing *Brassica napus* seeds (Slabas et al., 1986). The second enzyme of lipid metabolism identified in this study – glycerol 3 phosphate acyltransferase – is a first enzyme in phospholipids biosynthesis and triacylglycerol synthesis and has been isolated from pea leaves (Douady & Dubacq, 1987) and cocoa seeds (Fritz et al., 1986). Finally, delta12 oleic acid desaturase, active in the biosynthesis of polyunsaturated fatty acids through the conversion of oleic to linoleic acid, was reported as also playing a role in adaptation mechanisms to low temperature (Kargiotidou et al., 2008).

Several proteins participating in sucrose and polysaccharides metabolism were identified, one of which is sucrose synthase (SUS; spot 53), which catalyses the reversible conversion of sucrose and UDP to UDP-glucose and fructose. Baud et al. (2004) analysed the expression profiles of the *Arabidopsis thaliana* SUS multigene family under different conditions such as oxygen deprivation, dehydration, cold treatment and different sugar feedings. The authors revealed that the expression profiles of each member of the AtSUS family were distinct following these treatments. AtSUS also plays an important role in the development of

silique and seed (Fallahi et al., 2008).

In addition, glucose-1-phosphate adenylyltransferase (spot 280), also known as ADP-glucose pyrophosphorylase (AGP), was determined in the mature seed proteome investigation. The expression of this starch biosynthesis pivotal enzyme is controlled by sucrose (Lee et al., 2007). Several studies have demonstrated a significant increase in crop yield by manipulating starch biosynthesis through the introduction and over-expression of AGPases (Tuncel & Okita, 2013). These interesting findings have been reported in cereals such as wheat (Smidansky et al., 2007), rice (Lee et al., 2007) and maize (Hannah et al., 2012) as well as in potato (Stark et al., 1992).

Both proteins, SuS together with AGP, are involved in the production of ADP glucose (ADPG) linked to starch biosynthesis in maize endosperm (Li et al., 2013), pea seed (Déjardin et al., 1997) and potato tubers (Lloyd et al., 1999).

L-Iditol (sorbitol) 2-dehydrogenase (SDH; spot 358) catalyses the NAD⁺-dependent conversion of L-iditol to L-sorbose in fructose and mannose metabolism (Loescher, 1987; Yamaguchi et al., 1994). SDH is well characterised in the *Rosaceae* family of fruit trees (Loescher et al., 1982; Nosarszewski et al., 2004; Wang et al., 1999; Wu & Li, 2010) where it plays an important role in regulating sink strength and determining the quality of apple fruit. Although the expressed sequence tags of SDH-like sequences have also been generated from various non-*Rosaceae* species such as maize (Doehlert, 1987), soybean (Kuo et al., 1990), parasitic plant *Viscum album* (Wanek & Richter, 1993) or tomato (Ohta et al., 2005), their physiological role is unknown.

Other bioactive components

Amaranth, as an excellent source of micronutrients such as vitamins and minerals, also exhibits a relatively high antioxidant potential (Chlopicka et al., 2012; Paško et al., 2009, 2011; Alvarez-Jubete et al., 2010). The major antioxidant in plant cells is L-ascorbic acid (vitamin C), an essential small molecule covering a broad range of functions in plants. L-Ascorbate operates as an enzyme co-factor (Smirnoff & Wheeler, 2000), serves in signal transduction (Pignocchi & Foyer, 2003; Noctor et al., 2000), redox-signalling (Pignocchi & Foyer, 2003), cell division and growth (Kerk & Feldman, 1995), determination of flowering time (Barth et al., 2006) and is also active in stress and pathogen response (Noctor, 2006; Conklin & Barth, 2004). In addition, L-ascorbate acts as the biosynthetic precursor of oxalic and L-tartaric acids (DeBolt et al., 2007).

The proteins involved in the biosynthesis and catabolism of L-ascorbic acid were represented in the present work by two enzymes: first, GDP-mannose 3,5-epimerase (spot 318), an enzyme of critical importance

in L-ascorbate biosynthesis. This key metabolic intermediate forms GDP-L-glucose, a putative intermediate for the de novo biosynthesis of vitamin C in plants (Wolucka & Montagu, 2003; Gilbert et al., 2009). The second enzyme identified, L-idonate 5-dehydrogenase (spot 341), has been reported as active in the synthetic pathway of tartrate from ascorbate (DeBolt et al., 2007).

An important nutrient of whole grain with a notable effect on human health is glycine betaine (GB). Several studies suggest GB to have a pharmaceutical effect in the prevention of chronic disease and in helping to maintain heart, liver and kidney health, although epidemiological studies have yet to verify the hypothesised functions (Craig, 2004; Ueland, 2011). As an organic osmolyte, GB protects cells from environmental stress (Chen & Murata, 2008) and as a methyl-group donor, like folate (vitamin B group), can donate a methyl group to homocysteine to form the essential amino acid methionine (Cho et al., 2007).

Several spots were identified as two enzymes involved in GB biosynthesis such as betaine aldehyde dehydrogenase (BADH; spots 169, 170, 173) and choline monooxygenase (CMO; spots 153, 273, 293, 200, 149, 239, 398). Convincing evidence for the accumulation of BADH, which catalyses the final step of GB synthesis from choline under abiotic stress conditions such as salinity and water deficit, has been identified in many higher plants including *Triticum aestivum* L., *Hordeum vulgare* L., *Zea mays*, *Sorghum bicolor* L. and *Spinacia oleracea* (Chen & Murata, 2011). The accumulation of this osmoprotectant upon exposure to salt and water deficit has also been recognised in *Amaranthaceae* species – *A. hypochondriacus* L. (Gamboa et al., 1991; Valenzuela-Soto et al., 1994; Legaria et al., 1998) and *A. caudatus* L. (Weretilnyk et al., 1989; Russel et al., 1998), which was first known to accumulate GB and express CMO protein in response to salt stress (Wang et al., 1999). In addition, stress-induced accumulation of GB and CMO was later shown in *Amaranthus tricolor* (Meng et al., 2001; Bhuiyan et al., 2007), suggesting a key role and direct involvement of CMO in GB synthesis in *A. tricolor*.

Conclusions

In conclusion, an effective protein extraction methodology coupled with gel-LC-MS/MS proteomics has made possible the identification of 249 out of 461 quantified amaranth seed proteins. The current study catalogues these into 13 classes including the most abundant categories of unknown proteins, proteins related to energy and seed storage proteins, followed by proteins of primary metabolism, protein synthesis and secondary metabolism. The informative data about the predicted subcellular localisation showed more than half of the identified proteins to be localised in cytoplasm, which is consistent with their

function. 14 spots were identified as 11S globulin and 4 spots of Ama1 protein, representing protein fractions that give a high nutritional value to amaranth seed. The non-allergenic Ama1 protein, with well-balanced amino acid composition and high levels of essential amino acids, possesses the potential of further improving food and animal feed additives. Only 1 protein spot with a very low abundance was identified as glutenin, the major storage protein in conventional cereals as wheat or maize, very often associated with nutritive intolerances such as coeliac disease or gluten-sensitive enteropathy. An additional interesting identified protein is delta12 oleic acid desaturase which plays a role in the biosynthesis of polyunsaturated fatty acids with beneficial effects on health.

The present work represents the most extensive proteomic description of *Amaranthus cruentus* L. “Ficha” grain to date and provides a reference map as a useful tool that can accelerate *Amaranthus* proteomic studies.

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Supplementary data

Supplementary data associated with this article can be found in the online version of this paper (DOI: 10.1515/chempap-2016-0065).

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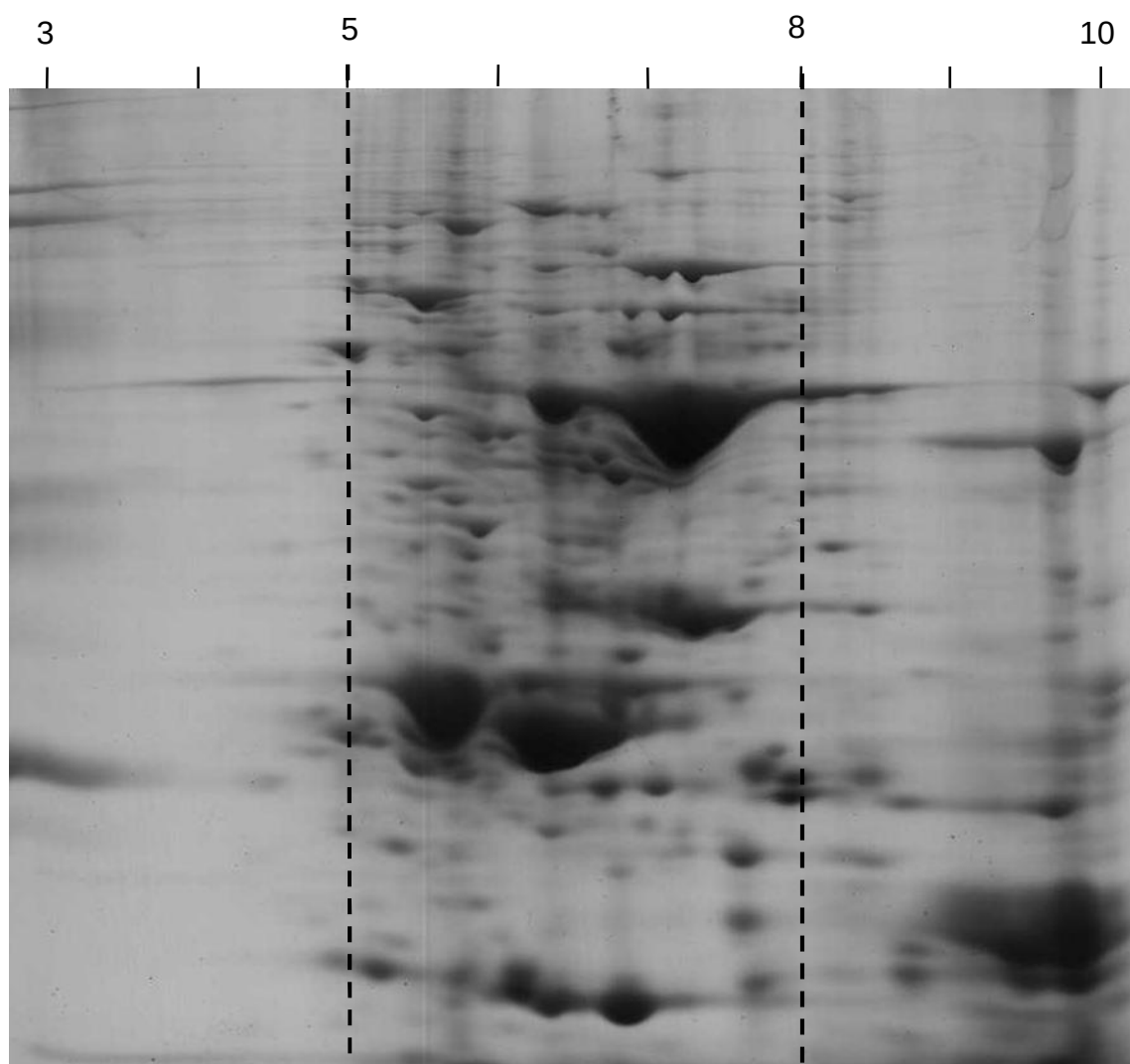
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Supplemental Figure 1. 2-DE gel image of amaranth grain obtained using IPG strips with pH 3-10.

Supplemental table 1.

Group ID	A1_crop	A2_crop	A3_crop	AVERAGE	STDEV
7	0,0273	0,0304	0,0219	0,0265	0,0043
12	0,0276	0,0672	0,0102	0,0350	0,0292
14	0,0652	0,0969	0,0198	0,0606	0,0388
19	0,0361	0,0509	0,0288	0,0386	0,0113
21	0,0786	0,0523	0,0459	0,0589	0,0173
23	0,0305	0,0473	0,0357	0,0378	0,0086
24	0,0323	0,0326	0,0300	0,0316	0,0014
25	0,0545	0,0730	0,0757	0,0677	0,0115
30	0,0737	0,0840	0,0799	0,0792	0,0052
31	0,1165	0,0827	0,1007	0,1000	0,0169
32	0,0948	0,0656	0,1003	0,0869	0,0186
33	0,1033	0,0908	0,1038	0,0993	0,0074
34	0,0641	0,0445	0,0594	0,0560	0,0103
35	0,0699	0,0928	0,0720	0,0782	0,0127
36	0,0817	0,0759	0,0770	0,0782	0,0031
37	0,1062	0,1159	0,1425	0,1215	0,0188
38	0,0416	0,0875	0,0321	0,0538	0,0296
39		0,0326	0,0251	0,0289	0,0054
40	0,0679	0,0708	0,0396	0,0594	0,0172
42	0,0874	0,1462	0,0704	0,1014	0,0398
43	0,0109		0,0115	0,0112	0,0004
44	0,1546	0,2208	0,1213	0,1656	0,0507
45	0,0931	0,0804	0,0776	0,0837	0,0082
46	0,0541		0,0788	0,0664	0,0175
47	0,0581	0,2373	0,0589	0,1181	0,1032
50		0,2228	0,1772	0,2000	0,0322
51	0,0274	0,0505	0,0122	0,0301	0,0193
52	0,0273	0,0846	0,0192	0,0437	0,0357
53	0,0166	0,0406	0,0146	0,0239	0,0145
55		0,0376	0,0389	0,0382	0,0009
56	0,0361	0,0541	0,0417	0,0440	0,0092
57	0,0328	0,0348	0,0241	0,0306	0,0057
58	0,0507	0,0684	0,0407	0,0533	0,0140
59	0,0305	0,0573	0,0162	0,0347	0,0209
60	0,0757	0,0978	0,0606	0,0780	0,0187
63	0,1767	0,1356	0,0685	0,1269	0,0546
64	0,7302	0,8241	0,2212	0,5918	0,3244
65	0,1987	0,2128	0,1794	0,1970	0,0167
67		0,0262	0,0195	0,0229	0,0048
68	0,0147	0,0252	0,0175	0,0191	0,0054
69	0,0648	0,0807	0,1042	0,0832	0,0198
70	0,0664	0,0429	0,0170	0,0421	0,0247
71	0,2874	0,3237	0,1304	0,2472	0,1028
73	0,1008	0,0498	0,0727	0,0744	0,0255
74	0,1489	0,1876	0,1153	0,1506	0,0362
75		0,0263	0,0906	0,0584	0,0455
76	0,0176	0,0432	0,0279	0,0296	0,0129
79	0,0195	0,0493	0,0409	0,0366	0,0154

81	0,0210	0,0163	0,0189	0,0187	0,0024
82	0,0140	0,0180	0,0145	0,0155	0,0022
83	0,0262	0,0270	0,0314	0,0282	0,0028
84	0,0136	0,0265	0,0240	0,0213	0,0068
85	0,0612	0,0559	0,0332	0,0501	0,0148
87	0,0319	0,0260	0,0439	0,0339	0,0091
90	0,0198	0,0489	0,0460	0,0382	0,0161
91	0,2338		0,3187	0,2763	0,0600
92	0,0112	0,0371	0,0159	0,0214	0,0138
94	0,0347		0,6539	0,3443	0,4378
95	0,0234	0,0294	0,0176	0,0235	0,0059
96	0,0231	0,0532	0,0092	0,0285	0,0225
97		0,0337	0,0161	0,0249	0,0124
99	0,0243	0,0084	0,0096	0,0141	0,0089
102	0,0153	0,0183	0,0216	0,0184	0,0031
103	0,0146		0,0079	0,0112	0,0047
104	0,0579	0,0341	0,0621	0,0514	0,0151
105	0,0343	0,0222	0,0229	0,0265	0,0068
107	0,0217		0,0168	0,0193	0,0034
109	0,0128	0,0331	0,0161	0,0206	0,0109
110	0,0165	0,0508	0,0225	0,0299	0,0183
111	0,0144	0,0186	0,0090	0,0140	0,0048
112	0,0275	0,0410	0,0395	0,0360	0,0074
113	0,0403	0,0455	0,0474	0,0444	0,0036
114	0,3486	0,3865	0,1481	0,2944	0,1281
115	0,1067	0,2853	0,1633	0,1851	0,0913
116	0,0810	0,0346	0,0450	0,0535	0,0244
117	0,1324	0,0671	0,0608	0,0868	0,0396
119	0,0271	0,0258	0,0242	0,0257	0,0015
120	0,0462	0,0491	0,0647	0,0533	0,0100
124	0,0229		0,0273	0,0251	0,0031
125	0,0201	0,0646	0,0333	0,0393	0,0229
126	0,0300	0,0655	0,0406	0,0454	0,0182
127	0,0326	0,0070	0,0313	0,0236	0,0144
128		0,0779	0,0370	0,0574	0,0289
129	0,0157	0,0111	0,0147	0,0139	0,0024
130	0,0143	0,0186	0,0239	0,0189	0,0048
131	0,0882	0,1275	0,0829	0,0995	0,0244
132		0,0292	0,0376	0,0334	0,0059
135	0,0228	0,0446	0,0285	0,0320	0,0113
136	0,0181	0,0382	0,0372	0,0312	0,0113
137	0,0340	0,0681	0,0496	0,0505	0,0171
138	0,0222	0,0177	0,0225	0,0208	0,0027
142	0,0209	0,0250	0,0342	0,0267	0,0069
143	0,0493	0,0793	0,0563	0,0616	0,0157
145	0,0625	0,1000	0,0744	0,0789	0,0191
146		0,1093	0,0503	0,0798	0,0417
147		0,0878	0,0237	0,0558	0,0453
148	0,0482	0,0533	0,0266	0,0427	0,0141
149	0,0241	0,0083	0,0201	0,0175	0,0082

150	0,0410	0,0330	0,0652	0,0464	0,0167
151	0,0713	0,1700	0,0826	0,1080	0,0540
152	0,0749	0,2277	0,0842	0,1289	0,0857
153	0,0300		0,0381	0,0341	0,0057
154	0,0645	0,1342	0,0593	0,0860	0,0419
155		0,1311	0,0830	0,1071	0,0340
156	0,0355		0,0363	0,0359	0,0005
159	0,0187	0,0180	0,0234	0,0201	0,0029
160	0,0302	0,0342	0,0505	0,0383	0,0107
163	0,0215	0,0245	0,0212	0,0224	0,0018
164	0,0181	0,0235	0,0184	0,0200	0,0030
165	0,0313	0,0256	0,0475	0,0348	0,0113
166	0,0257	0,0304	0,0374	0,0311	0,0059
167	0,0193	0,0491	0,0249	0,0311	0,0159
168	0,0213	0,0133	0,0243	0,0196	0,0056
169	0,0358		0,0347	0,0353	0,0008
170	0,1312	0,1524	0,0496	0,1110	0,0543
172	0,0168		0,0496	0,0332	0,0232
173	0,1988	0,2529	0,1961	0,2159	0,0320
174	0,0115	0,0223	0,0164	0,0167	0,0054
177	0,0454	0,0695	0,0547	0,0565	0,0122
178		0,0223	0,0127	0,0175	0,0068
179	0,0465		0,0358	0,0411	0,0075
180	2,8170	3,4543	2,0682	2,7798	0,6938
181	0,2648	0,2974	0,2851	0,2824	0,0165
183	0,0584	0,0451	0,0667	0,0568	0,0109
184	0,0351		0,0247	0,0299	0,0074
185	0,8773	1,4353	0,7673	1,0266	0,3582
186	0,2123	0,2241	0,2140	0,2168	0,0064
187	0,3129	0,5140	0,4886	0,4385	0,1095
189	0,0578	0,0853	0,0667	0,0699	0,0140
193	0,8064	1,2795	0,5019	0,8626	0,3918
194		0,3470	0,8762	0,6116	0,3742
196	0,0399		0,0437	0,0418	0,0026
197	0,1648		0,4694	0,3171	0,2154
198	0,0267		0,0311	0,0289	0,0031
200	0,8762		0,2752	0,5757	0,4250
203	0,6621		0,2370	0,4496	0,3005
204	0,0104		0,0056	0,0080	0,0034
206	0,0293		0,0406	0,0349	0,0080
207	0,0408	0,0288	0,0893	0,0530	0,0320
211	0,0371	0,0453	0,0198	0,0340	0,0130
212	0,0201		0,0288	0,0245	0,0062
213	0,1807	0,1275	0,1408	0,1497	0,0277
214		0,0571	0,0257	0,0414	0,0223
215	0,0626	0,0950	0,0808	0,0795	0,0163
216	0,0222	0,0141	0,0289	0,0217	0,0074
217	0,0630		0,0244	0,0437	0,0273
219	0,0203	0,0569	0,0157	0,0310	0,0225
220	0,1560	0,3627	0,2180	0,2456	0,1060

222	0,6521	0,3360	0,4310	0,4730	0,1622
223	0,0351		0,0373	0,0362	0,0016
224	0,0164		0,0275	0,0219	0,0078
225	0,2448	0,1721	0,2395	0,2188	0,0405
226	0,0688		0,0628	0,0658	0,0043
228	0,1858	0,2835	0,2849	0,2514	0,0568
229	0,0542		0,0860	0,0701	0,0225
231	0,0637		0,0602	0,0619	0,0025
232	0,0419	0,0603	0,0428	0,0483	0,0104
233	0,0482	0,0347	0,0306	0,0378	0,0092
234	0,0288	0,0543	0,0289	0,0373	0,0147
236	0,7811	0,9471	0,7225	0,8169	0,1165
237	2,9680	3,1335	1,4546	2,5187	0,9253
238		0,2865	0,4121	0,3493	0,0888
239	0,1540	0,3502	0,3661	0,2901	0,1181
240	0,0262	0,0289	0,0449	0,0334	0,0101
241	0,0691	0,0621	0,0512	0,0608	0,0090
243	0,0525	0,1201	0,0714	0,0813	0,0349
244	0,0372		0,0281	0,0326	0,0064
245	0,0424	0,0604	0,0629	0,0553	0,0112
246	0,2668	0,2837	0,5332	0,3612	0,1492
248	0,2928	0,3729	0,1716	0,2791	0,1013
249	0,4307	0,0956	0,4289	0,3184	0,1929
250	0,0456		0,0247	0,0352	0,0148
251	0,0154		0,0167	0,0160	0,0009
252	0,6257	0,4474	0,6436	0,5722	0,1085
253	0,0414	0,0694	0,0505	0,0538	0,0143
254	0,5579	0,4185	0,5808	0,5191	0,0879
255		0,0302	0,0193	0,0248	0,0077
257	0,0602		0,0344	0,0473	0,0183
258	0,0736	0,0787	0,0945	0,0822	0,0109
260		0,0326	0,0291	0,0309	0,0025
261		0,0383	0,0187	0,0285	0,0139
265	0,0737		0,0920	0,0829	0,0130
266	0,3611	0,1848	0,4215	0,3225	0,1230
267	0,1184	0,0914	0,0661	0,0920	0,0262
268	0,0396		0,0514	0,0455	0,0083
269	0,0617		0,0207	0,0412	0,0290
270	0,0330	0,0409	0,0550	0,0430	0,0112
271	0,0282	0,0227	0,0428	0,0313	0,0104
273	0,4695	0,5389	0,4309	0,4797	0,0547
275	0,0654	0,0835	0,0980	0,0823	0,0163
277		0,0377	0,0084	0,0231	0,0207
278		0,0995	0,0436	0,0716	0,0396
279	0,0435		0,0179	0,0307	0,0181
280	0,0259		0,0562	0,0410	0,0214
281	0,0432	0,0496	0,0577	0,0502	0,0073
282	0,0664	0,0646	0,0827	0,0712	0,0100
284	0,5855	0,6384	0,4002	0,5414	0,1251
285	0,1118	0,1098	0,1479	0,1232	0,0214

287	0,0314	0,0513	0,0300	0,0376	0,0119
288	0,0267		0,0323	0,0295	0,0040
289	0,0346		0,0589	0,0468	0,0172
290	0,0300		0,0286	0,0293	0,0010
292	0,0261		0,0207	0,0234	0,0038
293	0,0442	0,0472	0,0498	0,0471	0,0028
294	0,0415		0,0290	0,0353	0,0088
295	0,0391	0,0646	0,0318	0,0452	0,0172
296		0,1023	0,0809	0,0916	0,0152
297	0,1976	0,3212	0,2270	0,2486	0,0646
298		0,0326	0,0302	0,0314	0,0017
300	0,0579		0,0330	0,0455	0,0176
301	0,0525	0,0331	0,0475	0,0444	0,0101
303	0,0263	0,0325	0,0620	0,0403	0,0191
305	0,0269		0,0535	0,0402	0,0188
307	0,0171		0,0153	0,0162	0,0013
308	0,0486		0,0418	0,0452	0,0048
309	0,0310	0,0207	0,0511	0,0343	0,0155
310	0,0721	0,0389	0,0847	0,0652	0,0236
311	0,0136	0,0202	0,0245	0,0194	0,0055
312	0,0379		0,0272	0,0326	0,0076
313	0,0125		0,0260	0,0193	0,0096
314	0,0246	0,1147	0,0692	0,0695	0,0451
315	0,0546	0,0755	0,0791	0,0697	0,0133
316		0,1560	0,0171	0,0865	0,0983
317	0,0201	0,0409	0,0264	0,0291	0,0106
318	0,0103		0,0173	0,0138	0,0050
320	0,1609	0,2823	0,1674	0,2035	0,0683
321		0,0465	0,0219	0,0342	0,0174
322	0,0652	0,0244	0,0511	0,0469	0,0207
323	0,0173		0,0242	0,0208	0,0049
325	0,0229	0,0238	0,0399	0,0288	0,0096
327	0,0150	0,0524	0,0162	0,0279	0,0213
332	0,0192	0,0199	0,0209	0,0200	0,0008
333		0,0426	0,0309	0,0367	0,0083
334		0,0310	0,0477	0,0394	0,0118
335		0,0341	0,0485	0,0413	0,0102
336	0,0420		0,0760	0,0590	0,0240
337	0,0413	0,0265	0,0289	0,0322	0,0080
338	0,0385		0,0384	0,0385	0,0001
339	0,0359	0,0361	0,0463	0,0394	0,0060
341	0,0333		0,0561	0,0447	0,0161
343	0,0548	0,0484	0,0636	0,0556	0,0076
344	0,0434	0,0403	0,0859	0,0565	0,0255
345	0,0991	0,0694	0,1013	0,0899	0,0178
346	0,2185	0,1997	0,2377	0,2186	0,0190
347	0,2687	0,1025	0,2493	0,2068	0,0909
348	0,0507	0,0552	0,0533	0,0531	0,0023
350	0,0494	0,0551	0,0859	0,0635	0,0197
351	0,3612	0,4457	0,3281	0,3783	0,0607

352		0,0817	0,0828	0,0822	0,0008
353	0,0434	0,0811	0,0800	0,0682	0,0215
354	0,0653	0,0595	0,0400	0,0549	0,0133
356	0,0276		0,0271	0,0273	0,0003
357	0,0720	0,0727	0,1234	0,0894	0,0295
358	0,0297	0,0399	0,0385	0,0360	0,0055
359	0,0541		0,0707	0,0624	0,0117
361	0,1043	0,0754	0,0641	0,0813	0,0207
363	0,0556		0,0836	0,0696	0,0198
364	0,0255	0,0207	0,0206	0,0222	0,0028
365	0,0569	0,0813	0,0593	0,0659	0,0134
366		0,0846	0,0653	0,0750	0,0136
367	0,0454	0,0177	0,0380	0,0337	0,0143
369	0,0445	0,1038	0,0863	0,0782	0,0305
370	0,0432	0,1406	0,0524	0,0787	0,0538
371	0,1144	0,1560	0,1040	0,1248	0,0275
372		0,0854	0,0482	0,0668	0,0263
373		0,0684	0,0569	0,0626	0,0081
374	0,0861	0,0707	0,0696	0,0755	0,0092
375	0,0866	0,0854	0,0642	0,0787	0,0126
376	0,0505	0,0520	0,0243	0,0423	0,0155
377		0,0237	0,0217	0,0227	0,0014
378	0,0492	0,1172	0,0553	0,0739	0,0376
379	0,0132		0,0209	0,0170	0,0054
380	0,0234	0,0196	0,0129	0,0186	0,0053
381	0,0532	0,1024	0,0437	0,0664	0,0315
382	0,0603	0,0615	0,0468	0,0562	0,0082
383		0,0381	0,0246	0,0313	0,0096
385		0,0383	0,0259	0,0321	0,0088
386	0,0229		0,0314	0,0271	0,0060
387	0,2207	0,2233	0,2000	0,2146	0,0128
388	0,0356		0,0309	0,0333	0,0034
389	0,1648	0,2480	0,2554	0,2227	0,0503
390		0,2077	0,2022	0,2049	0,0039
391	0,0239		0,0430	0,0334	0,0135
392		0,0109	0,0309	0,0209	0,0141
393	0,0597	0,0544	0,0574	0,0572	0,0027
394	0,0244		0,0177	0,0211	0,0047
395	0,4541	0,6367	0,3502	0,4803	0,1450
397	0,5376	1,2025	1,4764	1,0722	0,4828
398	0,0470		0,0403	0,0437	0,0047
400	0,2301	0,2433	0,3744	0,2826	0,0798
401	1,7523	3,2827	1,4484	2,1611	0,9831
402		0,0315	0,0470	0,0393	0,0109
404	0,0826		0,2624	0,1725	0,1271
405	0,0235	0,0395	0,0196	0,0275	0,0105
406	0,2410	0,1690	0,1056	0,1719	0,0677
407	0,2383	0,2546	0,1825	0,2251	0,0378
408	0,2607	0,0660	0,3649	0,2305	0,1517
409	0,3628	0,4987	0,4001	0,4206	0,0702

411	0,5029	0,8922	0,4824	0,6258	0,2309
413	0,0288		0,0268	0,0278	0,0014
414	0,0683	0,0939	0,0796	0,0806	0,0128
415	0,0289		0,0284	0,0286	0,0004
416	0,8137	0,5479	0,3057	0,5558	0,2541
417	1,3808		0,8345	1,1076	0,3863
418	0,0313		0,0476	0,0395	0,0116
420	0,0302		0,0509	0,0406	0,0147
421	0,1654	0,2078	0,1971	0,1901	0,0220
425	0,1049	0,0857	0,0547	0,0818	0,0254
426		0,0931	0,0708	0,0819	0,0158
427	0,0426	0,1293	0,0430	0,0716	0,0500
428	0,0315	0,0690	0,0424	0,0476	0,0193
431	0,0284		0,0328	0,0306	0,0031
433	0,0251	0,0325	0,0341	0,0306	0,0048
434	0,0365		0,0240	0,0302	0,0088
435	0,0834	0,0795	0,0902	0,0844	0,0054
437	0,0099	0,0333	0,0284	0,0239	0,0124
438	0,0405	0,0293	0,0366	0,0355	0,0057
439	0,1622		0,1345	0,1483	0,0196
440	0,1258	0,1290	0,2699	0,1749	0,0823
441	0,4639	0,5729	0,8270	0,6213	0,1863
442		0,2904	0,5063	0,3984	0,1527
443	0,8578	0,6339	0,7381	0,7433	0,1121
444		0,1869	0,0980	0,1424	0,0629
445	1,3329	1,2091	1,1162	1,2194	0,1087
446	0,7334		0,6625	0,6979	0,0501
449	0,1077	0,2950	0,1423	0,1817	0,0997
450	0,0326	0,0640	0,0383	0,0450	0,0167
451	0,1102	0,2055	0,1927	0,1695	0,0517
452	0,4146	0,4700	0,3417	0,4087	0,0644
453		2,2191	1,6976	1,9583	0,3688
454	0,8546	0,8774	0,4208	0,7176	0,2573
456	0,4442		0,5102	0,4772	0,0467
457	0,3597	0,9637	0,5388	0,6208	0,3102
458	1,2198	1,8555	1,0953	1,3902	0,4077
460	0,3687	0,7648	0,5162	0,5499	0,2002
461	0,3294	0,3113	0,3757	0,3388	0,0332
464	4,6659	3,9214	4,7524	4,4466	0,4568
465	0,0043	0,6188	0,2020	0,2750	0,3137
466	0,2152	0,0423	0,0921	0,1165	0,0890
467	1,3617	1,8000	1,2225	1,4614	0,3014
468	0,4320	0,5642	0,3184	0,4382	0,1230
469	3,6526	3,8274	2,8814	3,4538	0,5034
470	0,5249	0,3148	0,4400	0,4266	0,1057
471	0,3890		0,2805	0,3347	0,0767
472	1,1378		0,3705	0,7542	0,5426
473	0,0772	0,4640	0,1371	0,2261	0,2082
474	0,1770		0,2594	0,2182	0,0583
475	0,5799		0,6242	0,6020	0,0313

476		0,0414	0,0318	0,0366	0,0068
477	1,1186	1,6645	1,1731	1,3187	0,3007
478	0,0583	0,2374	0,0838	0,1265	0,0969
482		0,9024	0,7887	0,8456	0,0804
483	0,4114		0,1025	0,2570	0,2184
485	0,2012	0,2431	0,2530	0,2325	0,0275
486		0,2313	0,1878	0,2096	0,0308
487		0,2867	0,3179	0,3023	0,0221
489	0,0969		0,0946	0,0957	0,0016
490	0,2987	0,2954	0,2671	0,2871	0,0173
493	0,0316	0,0761	0,0260	0,0446	0,0275
494	0,1097	0,2351	0,1261	0,1570	0,0682
495	0,1363		0,1284	0,1323	0,0056
496	0,1711	0,2506	0,1789	0,2002	0,0438
497		0,1898	0,1594	0,1746	0,0215
499		0,0918	0,0752	0,0835	0,0118
501	0,2425	0,3040	0,2289	0,2585	0,0400
502	0,1214	0,0791	0,1026	0,1011	0,0212
503	0,0467	0,0690	0,0039	0,0398	0,0331
504	0,0817	0,0969	0,1103	0,0963	0,0143
507	0,0269		0,0387	0,0328	0,0084
510	0,5410	0,6151	0,5042	0,5535	0,0565
511	0,2638	0,0787	0,2528	0,1984	0,1039
512	0,1760	0,2911	0,2009	0,2226	0,0606
516		0,0325	0,0309	0,0317	0,0011
517	0,1459	0,3073	0,1884	0,2139	0,0837
519	0,5659	0,6752	0,4169	0,5526	0,1296
520	0,1143		0,0137	0,0640	0,0712
522		0,0515	0,0400	0,0458	0,0082
526	0,0351	0,1357	0,0552	0,0753	0,0532
527	0,0610	0,0550	0,0690	0,0617	0,0070
528	0,0416		0,0479	0,0448	0,0045
530	0,0235		0,0526	0,0380	0,0206
531	0,0292	0,1238	0,2248	0,1259	0,0978
532	0,0540		0,0619	0,0580	0,0056
534	0,0540	0,0625	0,0964	0,0709	0,0224
535	0,2746	0,1393	0,0846	0,1662	0,0978
537	0,2018	0,5112	0,1722	0,2951	0,1878
538	0,0860		0,0893	0,0876	0,0023
539	0,3014	0,2740	0,3076	0,2943	0,0179
540	0,0445		0,0508	0,0477	0,0044
541	0,1051	0,0594	0,0399	0,0681	0,0334
542	0,0395		0,0396	0,0396	0,0001
544	0,0342		0,0280	0,0311	0,0044
547	0,1550		0,1453	0,1501	0,0069
548	0,2516	0,1529	0,0950	0,1665	0,0792
549	0,7478	0,5727	0,5806	0,6337	0,0989
550	0,1104	0,5688	0,1039	0,2610	0,2665
551		0,1416	0,2630	0,2023	0,0858
552	0,0174	0,1019	0,0725	0,0639	0,0429

553	0,0110	0,1059	0,0855	0,0675	0,0500
554	0,0491		0,0923	0,0707	0,0306
560	0,1093	0,1312	0,0965	0,1123	0,0175
561	0,1230	0,1356	0,1704	0,1430	0,0246
562	0,7918		1,0993	0,9456	0,2175
563	0,0790		0,0658	0,0724	0,0093
564	1,8297	1,2390	1,2122	1,4270	0,3490
566	0,1644	0,0599	0,0760	0,1001	0,0562
568		0,0749	0,0883	0,0816	0,0094
571	0,4668	0,4586	0,5225	0,4826	0,0348
572	0,3780	0,5099	0,3612	0,4163	0,0815
573	1,1409	0,8329	0,9414	0,9717	0,1563
574	0,1213	0,1748	0,1437	0,1466	0,0268
575	3,5438	5,2595	3,1779	3,9937	1,1114
577		0,2608	0,2082	0,2345	0,0372
578	1,1780	1,3873	1,1809	1,2487	0,1200
579	0,2947	0,2394	0,2663	0,2668	0,0277
580	0,7410	1,6042	0,4065	0,9172	0,6180
581	0,2790	0,3951	0,3831	0,3524	0,0639
583		0,0117	0,0053	0,0085	0,0045
584	0,0818		0,0508	0,0663	0,0219
585	0,0971	0,2017	0,1425	0,1471	0,0525
586	0,2070	0,2675	0,2419	0,2388	0,0304
588		0,1295	0,0957	0,1126	0,0239
589	0,1945		0,1967	0,1956	0,0016
590		0,1466	0,1345	0,1405	0,0085
593	0,1158	0,0707	0,1297	0,1054	0,0308
594	0,1961	0,1765	0,1663	0,1796	0,0151
596	0,1022	0,1251	0,0942	0,1072	0,0161
597	0,2917	0,3147	0,4106	0,3390	0,0630
598	0,2427	0,2154	0,2406	0,2329	0,0152
601	0,5563	0,6806	0,6242	0,6204	0,0623
602	0,1225	0,2602	0,2670	0,2165	0,0816
603	0,4119	0,3951	0,4936	0,4335	0,0527
605	0,2467	0,2614	0,1962	0,2348	0,0342
606	0,1381	0,0048	0,0048	0,0492	0,0770
607	0,2014	0,2467	0,2815	0,2432	0,0402
608	0,0604		0,0896	0,0750	0,0207
610	0,0482		0,1451	0,0967	0,0685
611	1,1400	0,8657	1,1866	1,0641	0,1734
614	0,4699	0,4119	0,4949	0,4589	0,0426
615	0,1713		0,2212	0,1963	0,0353
616	0,3805		0,6313	0,5059	0,1773
617	1,3669		1,5563	1,4616	0,1340
618	0,6571		0,5248	0,5910	0,0935
619	0,4657	0,2529	0,4172	0,3786	0,1115
620		0,3265	0,1241	0,2253	0,1432
621	0,1246		0,1570	0,1408	0,0229
624	0,2742		0,3846	0,3294	0,0781
625	1,2710		1,2681	1,2696	0,0021

626	1,2093		2,0642	1,6368	0,6044
628	1,1401	0,4522	0,7776	0,7900	0,3441
629		0,1623	0,7775	0,4699	0,4351
632	0,1150		0,0963	0,1056	0,0133
633	1,6004		2,0341	1,8173	0,3066
635	0,4291		0,2900	0,3595	0,0984
646	1,8220		1,1318	1,4769	0,4881
665	0,0065		0,0666	0,0365	0,0425
666	0,0159		0,1386	0,0773	0,0868
673	0,0058		0,0348	0,0203	0,0205
685	5,6990	5,6964	2,8009	4,7321	1,6725
686	0,4807	0,5751	0,4028	0,4862	0,0863
687	0,9588	1,0631	0,5956	0,8725	0,2454

Supplemental table 2.

Description	Species	Accession	localization	spot ID	PLGS Score	Cov/Pep	Theoretical	
							MW/pi	Exp MW/pi
01 Metabolism								
01.01 Amino Acids								
Hydroxypyruvate reductase	Chlamydomonas reinhardtii	A8IP17	?	443	110.56	8.4/2	44.7/8.4	33/6.4
Aminopeptidase	Micromonas sp strain RCC299	C1EA64	plastid	25	119.46	2.6/4	63.0/7.7	105/6.3
Aminopeptidase	Micromonas sp strain RCC299	C1EA64	plastid	83	53.95	5.1/4	63.0/7.7	90/6.4
Aminopeptidase	Micromonas sp strain RCC299	C1EA64	plastid	73	166.29	2.6/4	63.0/7.7	92/7.0
Aspartate aminotransferase	Gossypium hirsutum	H2D5S4	cytoplasm	376	157.64	7.9/6	44.0/7.8	45/7.2
Ketol acid reductoisomerase chloroplastic	Spinacia oleracea	Q01292	plastid	183	1926.64	28.9/23	63.7/6.6	70/6.3
Ketol acid reductoisomerase chloroplastic	Spinacia oleracea	Q01292	plastid	186	75.57	4.5/2	63.7/6.6	69/6.3
Acetolactate synthase	Amaranthus sp Iowa	Q38795	plastid	47	45.40	8.7/3	72.4/6.8	101/6.1
Ferredoxin dependent glutamate synthase chloroplastic	Spinacia oleracea	Q43155	cytoplasm	537	48.87	2.0/3	165.3/5.8	26/6.1
Ferredoxin dependent glutamate synthase chloroplastic	Spinacia oleracea	Q43155	cytoplasm	468	41.94	1.0/3	165.3/5.8	31/6.8
phosphoglycerate dehydrogenase (92%)	Gossypium hirsutum	B9HS53	cytoplasm	164	143.52	10.9/5	57.2/5.7	73/6.3
Adenosylhomocysteinase	Beta vulgaris	Q4H1G1	cytoplasm	226	333.89	25.7/12	53.4/5.8	64/6.3
Glutamine synthetase	Beta vulgaris	Q9AXD1	cytoplasm	371	2056.95	21.3/8	39.1/5.1	46/6.0
Pollen allergen MetE Fragment	Amaranthus retroflexus	E3VW74	cytoplasm	55	289.36	16.0/9	83.7/6.1	95/6.7
Pollen allergen MetE Fragment	Amaranthus retroflexus	E3VW74	cytoplasm	57	122.61	7.4/4	83.7/6.1	95/6.7
Pollen allergen MetE Fragment	Amaranthus retroflexus	E3VW74	cytoplasm	58	809.11	27.7/22	83.7/6.1	94/6.7
Pollen allergen MetE Fragment	Amaranthus retroflexus	E3VW74	cytoplasm	60	880.33	16.1/9	83.7/6.1	94/6.8
Sal k 3 pollen allergen Fragment	Salsola kali	C1KEU0	cytoplasm	59	237.60	13.6/9	83.5/5.9	94/6.9
01.03 Nucleotides								
Nucleoside diphosphate kinase 1	Spinacia oleracea	Q02254	cytoplasm	616	1303.43	23.0/4	16.3/6.5	12/6.8
RelA homolog	Suaeda japonica	Q84KS9	plastid	369	83.67	6.4/3	79.1/6.4	46/5.7
01.05 Sugars								
Glucose 1 phosphate adenyltransferase Fragment	Spinacia oleracea	Q43152	plastid	280	43.28	6.8/2	49.0/5.0	58/6.0
similar to xylose isomerase-like (99%)	Vitis vinifera	A5B1E9	cytoplasm	245	489.85	16.0/12	51.6/5.4	61/6.3
L-iditol 2-dehydrogenase (100%)	Populus trichocarpa	B9I4E5	cytoplasm	358	369.48	31.0/11	39.2/5.9	48/6.6
Sucrose synthase Fragment	Prunus persica	Q946X3	?	53	105.94	27.3/3	23.5/7.3	95/6.2
01.06 Lipids								
Enoyl ACP reductase Fragment	Dimocarpus longan	E9NVZ3	mitochondrial	426	599.44	24.6/11	35.9/10.0	38/5.7
Glycerol 3 phosphate acyltransferase chloroplastic	Spinacia oleracea	Q43869	plastid	365	48.36	8.3/3	52.1/5.6	46/6.6
Delta12 oleic acid desaturase	Euphorbia lagascae	Q6RXX0	cytoplasm	581	107.24	16.0/2	44.2/8.9	18/6.2
01.07.Cofactors								
L-idonate 5-dehydrogenase (100%)	Vitis vinifera	A5AIP9	cytoplasm	341	134.29	13.0/5	37.4/6.3	50/6.5
similar to GDP-mannose 3,5-epimerase 1 (95%)	Zea mays	I1I416	cytoplasm	318	300.08	19.3/5	42.8/5.9	53/6.6
02 Energy								
02.01 Glycolysis								
Glyceraldehyde 3 phosphate dehydrogenase	Beta vulgaris	A3FMH0	cytoplasm	416	3569.20	28.5/21	36.6/6.9	39/7.5

Glyceraldehyde 3 phosphate dehydrogenase Fragment	Spinacia oleracea	D7SA11	cytoplasm	401	2014.29	33.2/6	21.2/8.2	38/7.3
Glyceraldehyde 3 phosphate dehydrogenase Fragment	Spinacia oleracea	D7SA11	cytoplasm	408	551.87	24.3/3	21.2/8.2	40/7.0
Glyceraldehyde 3 phosphate dehydrogenase Fragment	Spinacia oleracea	D7SA11	cytoplasm	409	130.40	14.4/2	21.2/8.2	40/7.1
Glyceraldehyde 3 phosphate dehydrogenase Fragment	Spinacia oleracea	D7SA11	cytoplasm	411	1844.96	27.7/4	21.2/8.2	38/7.3
Glyceraldehyde 3 phosphate dehydrogenase	Beta vulgaris	A3FMH0	cytoplasm	417	873.9	24.3/3	36.6/6.9	38/7.4
6 phosphofructokinase	Medicago truncatula	G7L7C5	cytoplasm	212	69.70	7.3/2	39.1/6.6	67/6.7
Fructose bisphosphate aldolase cytoplasmic isozyme	Spinacia oleracea	P29356	cytoplasm	400	1391.08	10.1/9	38.4/5.9	40/6.9
Phosphoglycerate kinase putative	Arabidopsis thaliana	Q8LFV7	cytoplasm	352	427.82	14.7/4	42.1/5.3	50/6.0
Phosphoglycerate kinase putative	Arabidopsis thaliana	Q8LFV7	cytoplasm	343	1125.09	18.0/6	42.1/5.3	50/6.2
Enolase	Spinacia oleracea	Q9LEE0	cytoplasm	163	1417.87	29.7/8	48.1/5.4	74/6.4
Enolase	Spinacia oleracea	Q9LEE0	cytoplasm	215	4092.60	36.9/17	48.1/5.4	67/6.1
Enolase	Spinacia oleracea	Q9LEE0	cytoplasm	228	8783.77	41.9/22	48.1/5.4	63/6.2
Enolase	Spinacia oleracea	Q9LEE0	cytoplasm	232	117.05	14.0/4	48.1/5.4	63/5.9
Pyruvate dehydrogenase E1 alpha subunit	Beta vulgaris	Q852R9	mitochondrial	166	47.8	7.6/2	43.7/8.2	73/6.6
<i>02.02 Gluconeogenesis</i>								
Malic enzyme	Ricinus communis	B9RKI6	cytoplasm	138	318.92	6.1/3	65.1/5.9	79/6.8
Malate dehydrogenase Fragment	Pinus sylvestris	E3V248	mitochondrial	435	4634.14	19.7/6	14.5/9.8	36/6.8
Malate dehydrogenase	Glycine max	I1LXZ7	ER	388	460.05	10.2/3	41.0/6.1	43/6.9
Malate dehydrogenase Fragment	Hyparrhenia rufa	Q2MG94	cytoplasm	310	265.64	9.1/2	38.2/5.3	54/6.3
Malate dehydrogenase cytoplasmic	Beta vulgaris	Q9SML8	cytoplasm	387	5307.80	38.9/20	35.4/5.8	43/6.7
<i>02.07 Pentose Phosphate</i>								
Transketolase chloroplastic	Spinacia oleracea	O20250	plastid	84	66.97	3.2/2	80.2/6.2	90/6.3
Transketolase chloroplastic	Spinacia oleracea	O20250	plastid	87	82.30	7.7/3	80.2/6.2	89/6.5
Transketolase chloroplastic	Spinacia oleracea	H6ULZ6	plastid	314	68.07	12.7/2	24.9/9.3	54/6.9
6 phosphogluconate dehydrogenase decarboxylating	Spinacia oleracea	Q94KU1	cytoplasm?	287	284.64	26.9/12	53.2/6.0	57/6.8
6 phosphogluconate dehydrogenase decarboxylating	Spinacia oleracea	Q94KU2	plastid	271	267.99	11.0/4	58.3/5.4	58/6.6
CP12 (100%)	Spinacia oleracea	O24360	plastid	225	57.39	12.1/3	13.6/4.6	63/6.1
<i>02.10 TCA pathway</i>								
Putative aconitase	Capsicum chinense	B1Q486	?	40	342.13	16.5/22	108.3/7.1	103/6.7
Cytosolic aconitase	Pyrus pyrifolia	G3BMW4	plastid	39	52.76	12.0/6	97.9/5.9	104/6.6
Isocitrate dehydrogenase NADP	Pyrus pyrifolia	G3BMV7	cytoplasm	309	208.66	12.1/7	46.2/6.6	54/6.6
Isocitrate dehydrogenase NADP	Arabidopsis thaliana	Q8RYD5	cytoplasm	301	192.85	15.9/6	45.7/6.1	55/6.4
<i>02.13 Respiration</i>								
ATP synthase subunit alpha Fragment	Celosia cristata	E5DK56	cytoplasm	207	103.52	13.5/4	37.3/7.2	68/6.7
ATP synthase subunit alpha	Beta vulgaris	E6ZE62	cytoplasm	213	8658.62	50.8/69	55.0/5.9	66/6.7
ATP synthase subunit alpha	Beta vulgaris	E8ZCA7	cytoplasm	206	987.37	34.0/16	55.0/5.9	68/6.5
ATP synthase subunit beta mitochondrial	Hevea brasiliensis	P29685	mitochondrial	220	1926.87	43.2/56	60.2/5.9	65/5.7
probable ATP synthase 24 kDa subunit, mitochondrial-like (100%)	Vitis vinifera	D7SW76	mitochondrial	527	97.45	6.3/3	27.7/9.7	26/6.6
ATP synthase subunit alpha	Beta vulgaris	E6ZE62	cytoplasm	24	116.7	9.3/3	55/5.9	106/6.6
<i>02.16 Fermentation</i>								
Pyruvate decarboxylase	Gossypium hirsutum	D2D327	cytoplasm	142	134.04	7.4/3	60.4/6.2	77/6.8

Alcohol dehydrogenase 1 Fragment	Spinacia oleracea	E1A4D0	cytoplasm	322	230.65	17.8/11	35.8/6.6	53/6.8
Alcohol dehydrogenase 1 Fragment	Spinacia oleracea	E1A4D0	cytoplasm	346	634.02	22.9/13	35.8/6.6	50/6.7
Alcohol dehydrogenase 1 Fragment	Spinacia oleracea	E1A4D0	cytoplasm	347	574.52	17.8/22	35.8/6.6	49/6.8
<i>02.20 E-transport</i>								
NADH ubiquinone oxidoreductase putative	Ricinus communis	B9T118	mitochondrial	79	74.25	5.6/3	80.7/6.6	91/6.2
<i>02.30 Photosynthesis</i>								
Photosystem II 22 kDa protein	Arabidopsis thaliana	F4IEG8	plastid	519	158.82	12.7/3	22.0/10.0	27/7.1
Ribulose biphosphate carboxylase large chain Fragment	Atriplex patula	G8D4D3	cytoplasm	487	129.71	27.2/4	22.4/5.4	29/5.7
Ribulose biphosphate carboxylase large chain Fragment	Forchhammeria watsonii	Q5VGZ2	cytoplasm	425	95.63	12.0/2	32.1/5.9	38/6.1
Ribulose biphosphate carboxylase large subunit Fragment	Manocharisma albicans	A2VA68	cytoplasm	64	43.61	17.9/3	27.6/6.2	90/7.3
Ribulose biphosphate carboxylase small chain 2 chloroplastic	Amaranthus hypochondriacus	Q9XGX5	plastid	185	113.44	23.9/3	20.4/8.8	68/7.2
03 Cell growth/division								
<i>03.22 Cell cycle</i>								
Cyclin d putative	Ricinus communis	B9SG00	cytoplasm	35	75.18	18.1/2	31.7/9.3	104/6.6
Cystatin	Amaranthus hypochondriacus	Q0GPA4	ER	539	10411.60	56.3/44	27.7/5.6	25/6.5
Cystatin	Amaranthus hypochondriacus	Q0GPA4	ER	540	524.04	17.0/3	27.7/5.6	25/6.6
cyclin-D2-1-like (100%)	Brachypodium distachyon	I1IPV4	cytoplasm	51	67.61	15.5/2	35.2/4.8	96/7.0
<i>03.30 seed maturation</i>								
Late embryogenesis abundant protein	Vigna radiata	A4VBF1	cytoplasm	611	277.62	17.9/3	12.2/9.1	13/6.4
<i>03.99 Others</i>								
Maturase K 2	Anacampseros vulcanensis	Q5J2V7	cytoplasm	375	78.04	3.0/2	60.9/10.0	46/6.5
04 Transcription								
Helicase	Cucumis melo	E5GB87	cytoplasm	257	835.83	34.4/12	46.9/5.2	60/6.0
similar to transcription factor MYB3 (93%)	Arabidopsis thaliana	D7KLT8	cytoplasm	552	56.29	7.8/4	29.3/8.2	22/5.8
similar to transcription factor MYB3 (93%)	Arabidopsis thaliana	D7KLT8	cytoplasm	428	250.36	14.8/4	29.3/8.2	38/6.9
similar to transcription factor MYB3 (93%)	Arabidopsis thaliana	D7KLT8	cytoplasm	361	163.52	7.8/4	29.3/8.2	47/5.9
similar to transcription factor MYB3 (93%)	Arabidopsis thaliana	D7KLT8	cytoplasm	364	149.34	7.8/3	29.3/8.2	47/7.0
similar to agamous-like MADS-box protein AGL18-like (96%)	Glycine max	I1JGA3	cytoplasm	265	134.33	5.7/3	29.9/5.7	59/6.4
MPF1-like-A (100%)	Withania sp. W009	C9X3L8	?	382	56.32	14.9/3	22.3/9.6	44/6.0
05 Protein synthesis								
<i>05.01 Ribosomal proteins</i>								
Ribosomal protein S12 Fragment	Celosia cristata	G0YEE8	mitochondrial	619	224.31	25.9/3	6.4/11.4	11/5.7
Ribosomal protein S12 Fragment	Celosia cristata	G0YEE8	mitochondrial	130	223.50	25.9/3	6.4/11.4	80/6.5
Ribosomal protein S12	Beta vulgaris	Q5U6C5	cytoplasm	621	206.88	16.8/4	14.2/12.0	11/6.5
Ribosomal protein S12	Beta vulgaris	Q5U6C5	cytoplasm	615	133.06	12.0/3	14.2/12.0	12/6.6
Ribosomal protein S12 Fragment	Celosia cristata	G0YEE8	plastid	372	121.19	25.9/3	6.4/11.4	46/6.2
50S ribosomal protein L2 chloroplastic	Spinacia oleracea	P06509	plastid	499	49.27	8.1/2	29.8/11.6	28/5.6
50S ribosomal protein L15 chloroplastic Fragments	Spinacia oleracea	P22798	plastid	31	35.11	29.7/2	9.8/10.3	104/6.4
Ribosomal protein L16 Fragment	Nymphaea sp Bergthorsson	Q5IA34	cytoplasm	596	63.37	10.3/4	18.2/11.0	16/6.3
Putative 60S acidic ribosomal protein P0 Fragment	Zinnia elegans	Q8LNX8	cytoplasm	414	673.30	11.8/2	21.9/10.0	39/5.9
50S ribosomal protein L23-like (100%)	Glycine max	I1K4J4	plastid	194	114.84	35.1/2	11.1/10.3	67/7.4

Ribosomal protein L22p/L17e family protein (100%)	Arabidopsis thaliana	Q8LDU0	mitochondrial	386	141.26	11.4/4	30.4/11.3	43/6.1
<i>05.04 Translation factors</i>								
Eukaryotic translation initiation factor 5A	Medicago truncatula	G7JWN5	cytoplasm	594	345.30	24.1/3	15.5/5.8	16/6.6
<i>05.10 tRNA synthase</i>								
Arginyl tRNA synthetase putative	Ricinus communis	B9RKG2	cytoplasm	159	88.97	12.5/5	66.8/5.8	76/6.5
glycyl-tRNA synthetase 1, mitochondrial-like (100%)	Vitis vinifera	D7SH83	cytoplasm	90	165.26	10.8/9	76.3/5.8	89/6.6
<i>05.99 Others</i>								
Elongation factor Tu	Glycine max	I1N9E6	mitochondrial	344	56.07	7.5/4	49.1/6.4	49/6.6
Elongation factor Tu	Glycine max	I1N9E6	mitochondrial	353	565.74	13.7/10	49.1/6.4	49/6.6
Elongation factor 2	Beta vulgaris	O23755	cytoplasm	36	116.62	8.1/7	93.7/5.9	104/6.8
Elongation factor 2	Beta vulgaris	O23755	cytoplasm	37	2150.85	27.5/42	93.7/5.9	103/6.8
Elongation factor 1 alpha	Salsola komarovii	Q8H9A9	cytoplasm	167	162.36	23.0/15	49.3/9.4	73/5.9
06. Protein storage and destination								
<i>06.01 Folding and stability</i>								
Heat shock 70 protein	Spinacia oleracea	O50047	mitochondrial	104	290.91	26.4/11	72.3/5.4	86/5.8
Chaperonin 60kD ch60 putative	Ricinus communis	B9RWQ2	mitochondrial	151	215.31	10.8/8	61.3/5.7	77/5.9
T complex protein 1 subunit epsilon	Arabidopsis thaliana	F4IAR7	cytoplasm	154	288.18	16.6/13	51.1/5.2	76/5.8
TCP 1 cpn60 chaperonin family protein	Arabidopsis thaliana	F4J7H2	cytoplasm	160	1575.05	21.8/16	59.5/6.2	76/6.6
putative TCP-1/cpn60 chaperonin family protein (100%)	Zea mays	C0P530	plastid	145	257.19	11.7/8	61.7/5.2	76/5.7
20 kDa chaperonin, chloroplastic-like (100%)	Vitis vinifera	D7SIZ7	plastid	548	208.45	13.1/3	26.3/9.8	24/5.9
<i>06.13 Proteolysis</i>								
Proteasome subunit alpha type 3	Spinacia oleracea	O24362	cytoplasm	497	483.56	22.9/11	27.3/6.1	28/6.2
similar to Proteasome component (PCI) domain protein	Theobroma cacao	A9PB47	cytoplasm	296	48.5	10.3/3	45.2/4.9	55/5.7
ClpC protease	Spinacia oleracea	O98447	plastid	44	44.42	10.2/6	99.4/9.0	102/6.0
26S protease regulatory subunit 7	Spinacia oleracea	Q41365	cytoplasm	250	275.49	18.1/9	47.7/6.1	61/6.7
F box protein (100%)	Medicago truncatula	G7JM44	cytoplasm	370	57.23	18.3/4	28.0/9.2	46/6.1
26S proteasome ATPase subunit Fragment	Pisum sativum	Q6GX77	cytoplasm	250	185.64	23.7/4	23.6/6.9	61/6.7
mitochondrial-processing peptidase subunit alpha (100%)	Vitis vinifera	D7TBD9	mitochondrial	241	238.06	18.6/7	54.4/5.6	62/5.8
<i>06.20 Storage proteins</i>								
11S globulin	Amaranthus hypochondriacus	A2I9A6	ER	466	248.63	12.9/11	55.0/6.5	31/6.2
11S globulin	Amaranthus hypochondriacus	A2I9A6	ER	467	2191.87	12.3/5	55.0/6.5	30/6.3
11S globulin	Amaranthus hypochondriacus	A2I9A6	ER	490	636.82	12.5/18	55.0/6.5	29/6.3
High molecular weight glutenin subunit γ10	Triticum aestivum	A9ZMG8	ER	109	89.55	10.5/3	69.7/7.3	84/6.1
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	496	1227.99	16.6/25	56.6/6.7	28/6.4
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	562	1736.64	19.0/22	56.6/6.7	20/7.6
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	564	668.96	8.2/6	56.6/6.7	20/7.5
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	568	1219.89	30.3/27	56.6/6.7	20/6.4
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	464	1434.79	16.2/26	56.6/6.7	29/6.4
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	469	300.33	4.4/6	56.6/6.7	29/6.6
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	470	856.27	17.6/21	56.6/6.7	30/6.5
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	473	114.37	6.8/6	56.6/6.7	30/6.9

11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	477	137.85	9.2/9	56.6/6.7	30/6.7
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	485	453.90	6.8/16	56.6/6.7	29/6.1
11S globulin seed storage protein Fragment	Amaranthus hypochondriacus	Q38712	ER	495	96.53	6.8/4	56.6/6.7	28/6.3
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	395	6593.93	61.5/40	34.9/6.7	41/7.2
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	397	1121.79	55.6/13	34.9/6.7	39/7.2
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	404	174.89	18.8/6	34.9/6.7	40/6.9
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	444	441.90	15.8/4	34.9/6.7	34/6.5
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	449	1457.26	42.1/14	34.9/6.7	33/6.6
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	451	7806.66	61.5/47	34.9/6.7	33/6.7
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	460	6729.44	53.6/44	34.9/6.7	32/7.3
Agglutinin	Amaranthus hypochondriacus	Q38719	cytoplasm	461	6316.09	61.5/27	34.9/6.7	32/7.4
Agglutinin	Amaranthus caudatus	Q6YNX3	cytoplasm	446	9215.30	54.3/43	34.9/6.7	33/7.1
Agglutinin	Amaranthus caudatus	Q6YNX3	cytoplasm	442	7614.45	54.6/38	34.9/6.7	33/6.8
Seed protein AmA1	Amaranthus hypochondriacus	Q8S390	cytoplasm	427	590.33	34.2/10	34.9/6.2	38/6.9
Seed protein AmA1	Amaranthus hypochondriacus	Q8S390	cytoplasm	440	2807.27	50.0/22	34.9/6.2	34/6.6
Seed protein AmA1	Amaranthus hypochondriacus	Q8S390	cytoplasm	441	6548.20	27.0/26	34.9/6.2	33/7.0
Seed protein AmA1	Amaranthus hypochondriacus	Q8S390	cytoplasm	450	2113.28	50.0/24	34.9/6.2	33/6.7
<i>06.21 Allergens</i>								
allergen Pru p 1 06C	Prunus dulcis x Prunus persica	B6CQT1	cytoplasm	588	110.77	23.8/2	17.3/4.8	16/6.1
07 Transporters								
similar to magnesium transporter NIPA2-like (98%)	Glycine max	C6TK16 plasm/membrane		128	80.20	29.2/2	14.4/10.1	80/7.2
similar to magnesium transporter NIPA2-like (98%)	Glycine max	C6TK16 plasm/membrane		174	108.80	29.2/2	14.4/10.1	72/6.6
08 Intracellular transport								
<i>08.07 Vesicular</i>								
guanosine nucleotide diphosphate dissociation inhibitor 1 (100%)	Arabidopsis thaliana	B9DFX1	cytoplasm	243	738.43	23.7/21	48.4/6.0	61/6.1
09 Cell structure								
<i>09.04 Cytoskeleton</i>								
Actin Fragment	Suaeda maritima	B9VNV6	cytoplasm	320	6003.79	56.8/26	21.8/5.1	52/5.9
similar to kinesin light chain-like (99%)	Brachypodium distachyon	I1H7X6	mitochondrial	571	134.94	5.1/2	60.0/8.2	19/6.3
<i>09.07 ER/Golgi</i>								
Luminal binding protein	Spinacia oleracea	Q42434	ER	303	1826.52	19.8/13	73.5/4.8	55/6.4
<i>09.16 Mitochondrion</i>								
Voltage dependent anion channel protein	Spinacia oleracea	Q41368	cytoplasm	526	103.52	6.2/3	29.6/6.7	26/6.7
<i>09.99 Others</i>								
Aquaporin	Atriplex canescens	H2DPZ8	cytoplasm	249	52.01	18.4/2	30.2/9.2	60/7.4
10 Signal Transduction								
<i>10.0404 Kinases</i>								
Non receptor serine threonine protein kinase	Medicago truncatula	G7IUD9	cytoplasm	379	88.87	18.1/4	43.4/8.1	45/6.9
Flowering locus T like protein FT1 Fragment	Beta vulgaris	E5KIA6	?	281	76.61	61.7/2	7.1/9.9	57/6.2
11 Disease/defence								
<i>11.05 Stress responses</i>								

Stress induced protein sti1 like protein	Atriplex canescens	H9B3R7	cytoplasm	112	93.85	8.1/4	51.3/5.4	83/6.3
Stress induced protein sti1 like protein	Atriplex canescens	H9B3R7	cytoplasm	113	179.66	16.8/7	51.3/5.4	83/6.4
Salt tolerance protein 2	Beta vulgaris	Q711N3	cytoplasm	502	59.99	5.3/2	38.4/5.2	27/6.4
Heat shock protein 83 Fragment	Beta vulgaris	Q1H8M1	cytoplasm	50	317.10	12.6/6	39.9/4.8	98/5.6
Heat shock protein 101	Triticum durum	Q334I0	cytoplasm	23	96.47	11.9/8	101.0/5.8	105/6.6
Cytosolic heat shock 70 protein	Spinacia oleracea	Q41374	cytoplasm	91	1678.54	33.8/23	70.8/5.0	88/5.7
Cytosolic heat shock 70 protein	Spinacia oleracea	Q41374	cytoplasm	94	3024.86	30.0/28	70.8/5.0	87/5.8
17 67 kDa heat shock protein	Helianthus annuus	Q8VXJ4	cytoplasm	603	337.96	23.9/7	17.7/6.2	14/6.8
Putative thioredoxin dependent peroxidase	Citrus hybrid cultivar	A1ECJ6	cytoplasm	585	158.88	10.5/3	17.3/5.0	17/6.2
Glutathione reductase chloroplastic Fragment	Spinacia oleracea	Q43154	plastid	198	170.59	14.5/5	52.6/5.4	69/6.5
Glutathione reductase chloroplastic Fragment	Spinacia oleracea	Q43154	plastid	189	70.5	12.7/3	52.6/5.4	68/6.3
Inducer of CBF expression	Medicago truncatula	G7IR54	cytoplasm	578	276,9012	17.3/2	17.2/7.2	18/6.4
11.06 Detoxification								
Putative lactoylglutathione lyase	Brassica campestris	A8CF50	cytoplasm	421	424.59	10.2/5	31.9/5.2	38/6.2
Putative lactoylglutathione lyase	Brassica campestris	A8CF50	cytoplasm	438	495.07	7.8/2	31.9/5.2	36/6.3
Gamma class glutathione transferase EF1Bgamma2	Populus trichocarpa	A9PE52	cytoplasm	258	580.81	12.1/9	47.8/5.7	59/6.4
NAD P H quinone oxidoreductase	Amaranthus hybridus	A7U3X0	cytoplasm	626	219.91	28.9/2	15.1/5.9	10/6.1
NAD P H quinone oxidoreductase	Amaranthus spinosus	A7U3X1	cytoplasm	418	113.60	28.9/2	15.0/5.9	39/6.4
13 PUF								
Brother of FT AND TFL1 like protein BFT1	Beta vulgaris	E3UKE9	cytoplasm	52	45.94	23.0/2	19.5/8.5	96/6.1
Fgenesh protein 60	Beta vulgaris	Q1ZY19	cytoplasm	351	52.94	5.3/2	56.2/6.4	49/6.1
Putative GTP binding protein	Oryza sativa	Q7EZT3	cytoplasm	229	126.02	1.7/2	50.3/6.2	65/6.2
Putative GTP binding protein	Oryza sativa	Q7EZT3	cytoplasm	127	149.42	6.1/3	50.3/6.2	80/7.1
Putative TAG factor protein	Lupinus angustifolius	Q8GSE7	cytoplasm	471	88.51	24.1/5	32.2/6.3	31/7.0
Uncharacterized protein	Beta vulgaris	A2I5E8	cytoplasm	625	45.67	11.7/6	24.5/4.3	11/7.0
Uncharacterized protein	Beta vulgaris	A2I5E8	cytoplasm	56	185.54	14.4/7	24.5/4.3	94/6.5
Uncharacterized protein	Beta vulgaris	A2I5E8	cytoplasm	67	50.01	14.4/3	24.5/4.3	92/6.1
Uncharacterized protein	Beta vulgaris	A2I5E8	cytoplasm	261	92.92	9.5/2	24.5/4.3	59/5.8
Uncharacterized protein	Vitis vinifera	A5C2S6	cytoplasm	125	416.11	14.8/12	59.8/5.7	81/6.6
Uncharacterized protein	Picea sitchensis	A9NQ96	mitochondrial	475	139.94	16.8/2	17.5/8.2	30/5.9
Uncharacterized protein	Picea sitchensis	A9NV22	plastid	146	154.58	15.4/7	65.5/5.5	77/5.7
Uncharacterized protein	Zea mays	B4FQH5	plastid	398	163.28	41.4/2	10.8/11.5	41/6.0
Uncharacterized protein	Ricinus communis	B9SEC8	mitochondrial	402	110.34	38.8/2	7.5/11.1	40/6.1
Uncharacterized protein	Sorghum bicolor	C5WZR7	cytoplasm	359	47.50	4.7/4	36.6/9.1	48/6.0
Uncharacterized protein	Sorghum bicolor	C5X4W3	plastid	560	65.56	3.9/3	81.0/4.7	21/6.1
Uncharacterized protein	Sorghum bicolor	C5Y1I3	cytoplasm	687	185.16	72.0/2	5.7/5.5	68/7.3
Uncharacterized protein	Sorghum bicolor	C5YX72	cytoplasm	251	54.87	18.4/2	18.3/8.7	61/6.6
Uncharacterized protein	Sorghum bicolor	C5Z454	cytoplasm	522	92.43	4.6/3	54.4/6.2	27/6.5
Uncharacterized protein	Sorghum bicolor	C5Z454	cytoplasm	373	57.74	4.6/3	54.4/6.2	46/6.0
Uncharacterized protein	Sorghum bicolor	C5Z589	?	348	68.08	10.2/2	53.0/5.2	50/7.0
Uncharacterized protein	Glycine max	C6SWF7	cytoplasm	614	105.43	21.9/3	12.7/5.7	12/6.4

Uncharacterized protein	Glycine max	C6TLM7	plastid	187	86.94	9.1/2	24.9/6.6	68/7.1
Uncharacterized protein	Arabidopsis lyrata	D7L4A9	mitochondrial	152	264.98	11.1/11	61.3/5.4	76/5.9
Uncharacterized protein	Arabidopsis lyrata	D7LVQ4	ER	589	41.10	7.1/2	37.7/8.7	16/7.0
Uncharacterized protein	Arabidopsis lyrata	D7MBZ4	cytoplasm	179	66.84	7.3/2	36.8/9.1	71/6.1
Uncharacterized protein	Vitis vinifera	D7SS45	?	510	237.47	22.7/4	7.5/10.0	27/6.7
Uncharacterized protein	Volvox carteri	D8UJQ0	mitochondrial	541	54.83	15.3/2	15.0/10.4	25/7.2
Uncharacterized protein	Chlorella variabilis	E1ZLY5	mitochondrial	117	273.09	4.6/3	19.4/10.9	83/7.0
Uncharacterized protein	Chlorella variabilis	E1ZLY5	mitochondrial	345	236.22	12.1/4	19.4/10.9	50/6.7
Uncharacterized protein	Chlorella variabilis	E1ZU40	ER	70	680.11	10.0/4	33.7/9.1	92/5.8
Uncharacterized protein	Beta vulgaris	F4NCI9	cytoplasm	535	59.70	7.8/2	49.6/6.4	26/6.3
Uncharacterized protein	Beta macrocarpa	G2XLR9	cytoplasm	136	42.52	15.7/2	17.1/9.3	79/6.6
Uncharacterized protein	Aegilops longissima	G8CNC3	cytoplasm	315	2372.37	34.6/22	30.3/4.7	53/6.3
Uncharacterized protein	Ceratopteris richardii	H8WFA4	cytoplasm	325	50.88	13.7/2	20.6/7.3	53/6.8
Uncharacterized protein	Coccomyxa subellipsoidea	I0Z1T9	cytoplasm	407	63.82	6.6/4	42.7/6.4	40/6.7
Uncharacterized protein	Brachypodium distachyon	I1H9T4	cytoplasm	457	69.58	31.4/3	21.7/4.1	32/6.0
Uncharacterized protein	Brachypodium distachyon	I1I2G4	ER	81	91.75	23.3/3	13.5/12.2	91/6.6
Uncharacterized protein	Brachypodium distachyon	I1IF68	?	63	56.93	38.5/3	13.0/5.2	92/7.2
Uncharacterized protein	Glycine max	I1L6W0	cytoplasm	135	269.16	10.4/10	60.8/5.4	79/6.2
Uncharacterized protein	Glycine max	I1MIA6	?	156	262.74	2.9/3	61.4/7.3	76/6.0
Uncharacterized protein	Medicago truncatula	I3S6L5	cytoplasm	357	413.23	21.6/4	10.6/8.9	49/6.5
Uncharacterized protein	Oryza brachyantha	J3LIK1	cytoplasm	237	136.03	30.2/4	13.4/9.8	59/7.2
Uncharacterized protein	Oryza brachyantha	J3N8C8	cytoplasm	71	50.66	11.5/2	47.6/7.9	91/7.2
Uncharacterized protein	Beta vulgaris	Q0PEL5	cytoplasm	356	67.62	12.0/2	29.8/10.9	49/6.5
Predicted protein	Ostreococcus lucimarinus	A4RXT4	cytoplasm	203	291.79	59.4/2	11.1/7.4	68/7.5
Predicted protein	Chlamydomonas reinhardtii	A8HPZ7	mitochondrial	584	98.22	26.6/2	30.0/9.2	17/7.1
Predicted protein	Physcomitrella patens	A9RGW9	cytoplasm	267	190.69	5.1/3	18.8/9.9	59/5.9
Predicted protein	Physcomitrella patens	A9SJE6	mitochondrial	686	208.99	7.0/3	46.3/5.9	68/7.1
Predicted protein	Physcomitrella patens	A9T1X4	cytoplasm	92	209.15	10.7/3	12.4/10.0	89/6.1
Predicted protein	Populus trichocarpa	B9HNC0	cytoplasm	248	402.52	19.4/4	17.9/7.4	60/6.6
Predicted protein	Populus trichocarpa	B9IGY9	cytoplasm	137	566.57	16.6/14	61.1/5.3	79/6.1
Predicted protein	Populus trichocarpa	B9N2L3	ER	465	199.24	2.4/2	55.9/6.0	31/6.1
uncharacterized protein	Ostreococcus tauri	Q00Y82	cytoplasm	308	333.58	49.6/3	13.7/10.6	54/7.0
expressed protein (100%)	Chlorella variabilis	E1Z287	ER	542	152.57	3.0/5	55.0/9.1	24/6.7
expressed protein (100%)	Chlorella variabilis	E1Z287	ER	577	94.62	3.0/3	55.0/9.1	19/5.6
RING-H2 finger protein ATL8-like (100%)	Brachypodium distachyon	I1IE86	ER	288	67.51	30.1/2	16.0/5.5	57/6.5
DUF185-domain-containing protein (100%)	Coccomyxa subellipsoidea	I0YJQ5	cytoplasm	316	200.97	8.9/2	97.0/5.5	54/7.1
zinc finger CCCH domain-containing protein 37-like (100%)	Glycine max	I1KW27	cytoplasm	32	174.92	1.6/2	55.1/7.5	104/6.4
20 Secondary metabolism								
Gibberellin 20 oxidase 1	Gossypium hirsutum	Q2QCX5	cytoplasm	530	62.49	12.8/2	41.4/5.2	26/6.5
Delta aminolevulinic acid dehydratase chloroplastic	Spinacia oleracea	P24493	plastid	105	73.26	9.0/2	47.3/5.8	86/6.5
Betaine aldehyde dehydrogenase	Halostachys caspica	A4LAP2	cytoplasm	169	405.85	18.8/9	54.5/5.1	73/5.9

Betaine aldehyde dehydrogenase	Amaranthus hypochondriacus	O22477	cytoplasm	170	630.49	21.6/8	54.4/5.2	72/6.0
Betaine aldehyde dehydrogenase	Amaranthus hypochondriacus	O22477	cytoplasm	173	95.55	13.0/4	54.4/5.2	71/6.1
Choline monooxygenase	Atriplex nummularia	Q7XZR0	plastid	153	76.13	9.6/2	48.7/6.1	76/6.1
Choline monooxygenase	Atriplex nummularia	Q7XZR0	plastid	273	44.65	14.4/4	48.7/6.1	57/6.9
Choline monooxygenase chloroplastic	Atriplex hortensis	Q9LKN0	plastid	149	41.09	9.6/2	48.7/6.1	77/6.2
Choline monooxygenase chloroplastic	Atriplex hortensis	Q9LKN0	plastid	239	54.79	9.6/3	48.7/6.1	62/7.0
Choline monooxygenase chloroplastic	Atriplex hortensis	Q9LKN0	plastid	200	43.69	9.6/3	48.7/6.1	67/7.4
Choline monooxygenase chloroplastic	Atriplex hortensis	Q9LKN0	plastid	398	42.57	9.6/3	48.7/6.1	41/6.0
Choline monooxygenase	Atriplex nummularia	Q7XZR0	plastid	293	56	9.6/2	48.7/6.1	55/6.7
Glutamate 1 semialdehyde aminotransferase Fragment	Amaranthus tricolor	Q9AVF5	cytoplasm	472	57.44	15.8/2	32.9/4.9	30/6.8

SUPPLEMENTARY DATA

Revealing the seed proteome of the health benefitting grain amaranth (*Amaranthus cruentus* L.)

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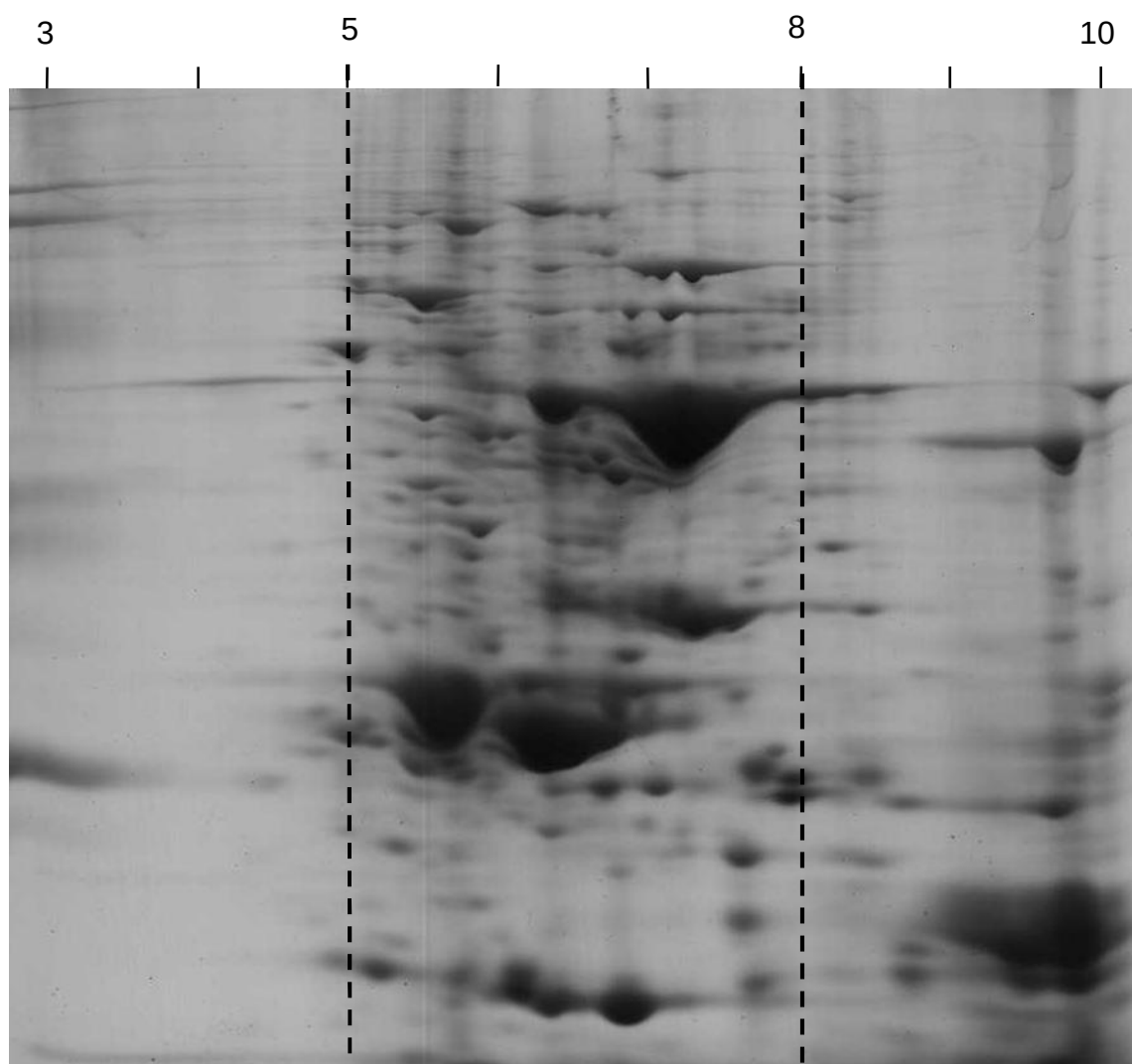
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SUPPLEMENTARY INFORMATION

Supplemental Figure 1. 2-DE gel image of amaranth grain obtained using IPG strips with pH 3-10.

Supplemental Table 1. The relative spot volumes (%V) of all 461 quantified proteins presented on 2-DE gels of pH 5-8. The table shows the triplicate analysis data of proteins of *Amaranthus cruentus* L. grains, average, standard deviation - STDEV.

Supplemental Table 2. The complete list of 249 proteins identified from mature amaranth grains that were classified into 13 functional categories. The table shows: Protein description, Protein name; Accession number, UniProt accession number; Localization, subcellular localization; Spot ID, spot number in gels obtained by ImageMaster analysis; PLGS Score, score provided by Protein Lynx software; Pep/Cov, number of identified peptides/coverage in %; Theor MW/pI, theoretical MW/pI value; Exp MW/pI, experimental MW/pI value.



Supplemental Figure 1. 2-DE gel image of amaranth grain obtained using IPG strips with pH 3-10.

