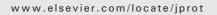
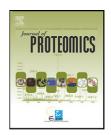


available at www.sciencedirect.com







The analysis of *Lupinus albus* root proteome revealed cytoskeleton altered features due to long-term boron deficiency

M. Alves^a, S. Moes^b, P. Jenö^b, C. Pinheiro^a, J. Passarinho^c, C.P. Ricardo^{a,*}

- ^a Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
- ^b Biozentrum of the University of Basel, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland
- ^c Instituto Nacional de Recursos Biológicos/L-INIA, Av. da República, 2784-505 Oeiras, Portugal

ARTICLEINFO

Article history: Received 13 December 2010 Accepted 1 March 2011 Available online 13 March 2011

Keywords:
Boron deficiency
Cytoskeleton
Lupinus albus
Proteomics
Root

ABSTRACT

Boron (B) deficiency greatly limits plants' growth and development. Since the root is the organ that first senses the deficiency, we have analyzed the adaptive responses of *Lupinus albus* roots to long-term B deficiency. Large morphological differences were observed between plants grown with or without B, and 265 polypeptides were found to be responsive to B deficiency out of a total of 406 polypeptides detected by two-dimensional electrophoresis in the *L. albus* root proteome. By using mass spectrometry techniques we were able to securely identify 128 of the responsive polypeptides that are related to cell wall metabolism, cell structure, defense, energy pathways and protein metabolism. The detection of multiple peptide isoforms is striking, suggesting that protein modification may have an important contribution during the plant response to long-term B deficiency. Furthermore, detected changes in cytoskeletal associated proteins indicate altered cytoskeletal biosynthesis and suggest that B may have an important contribution in this process.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Boron (B) has for long been known as an essential micronutrient for higher plants [1]. Since the B sufficiency range for plant growth and development is narrow, problems of both deficiency and toxicity can easily arise, as it has been observed in farmlands. B toxicity is characteristic of alkaline and saline soils, often associated with low rainfall and very scarce leaching, or it can be a consequence of over-fertilization and/or irrigation with water of high B levels [2]. However, B-deficient soils are more prevalent and widespread worldwide [3] than B-rich soils and so the deficiency is an important agriculture problem. It causes large yield losses, annually, which were reported for at least 132 crops in more than 80 countries [3]. In Portugal it was detected in woody crops (grapevine, olive, apple and pear trees) [3], as well as in herbaceous species, like the legume pastures [4].

The functioning of B in plant development has been associated with diverse physiological processes of both vegetative and reproductive growth [5,6], that involve, for instance, the metabolism of carbohydrates and RNA, respiration, lignification, cell wall synthesis and structure, and membrane transport. However, to date, the precise B roles in central plant biological processes remain elusive. So far, B is known to have a structural participation in the cell wall, through the borate cross-linking of rhamnogalacturonan-II chains [7–9], but the plethora of biochemical, physiological and anatomical effects due to B suppression [6] cannot be well explained by this role alone. This conclusion is strengthened by the recent indications that B might be also essential for development of animal cells [10,11], devoid of cell wall.

Due to the economic implications of B imbalance in plants and the elusive nature of the B role, studies involving this

^{*} Corresponding author. E-mail address: ricardo@itqb.unl.pt (C.P. Ricardo).

element in plant metabolism continue to be an important subject. More recently, molecular techniques, namely of transcriptomic [12–15] and proteomic nature [16–18], are being applied to such studies.

We have been studying B deficiency in the white lupin (Lupinus albus) [19,20], a winter grain legume crop valuable for its edible seed [21], that is nitrogen-fixing with reduced need for fertilizers, grows in infertile soils and can withstand adverse conditions. It is a species sensitive to B deficiency, a condition that is exacerbated by the environments under which L. albus grows, such as high light intensity, low temperature, drought and freely-draining sandy or gravelly soils [3].

In order to mimic low B field conditions, we have evaluated long-term B deficiency responses. Since nutrient imbalance is primarily sensed by the root system and because this organ is responsible for sending signals to the shoots for growth and developmental modulation [22], we have analyzed the *L. albus* root adaptive responses to long-term B deficiency. For this purpose, the root proteome of plants grown with and without B were analyzed by two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) techniques.

2. Material and methods

2.1. Plant material

Lupin seeds (Lupinus albus cv. Rio Maior) were pre-germinated in distilled water for 48 h, sown in white sand and grown under controlled conditions of temperature (19/25 °C, night/day), photoperiod (12 h) and light intensity (250 $\mu mol~m^{-2}~s^{-1}$, PAR). The plants were watered every other day with a nutritive solution [23] containing either 0 or 23.1 μM B [19]. For biomass analysis, roots and shoots were harvested 2, 3 and 4 weeks from sowing.

2.2. Protein extraction and solubilisation

Roots from four week-old plants were ground to a fine powder in liquid nitrogen, resuspended in a cold acetone solution containing 0.06 M DTT and 10% (w/v) TCA (12.5 mL/g) and kept at $-20\,^{\circ}\text{C}$ for 1 h. After a 15 min centrifugation at 27,200 g and 4 °C, the pellet was resuspended in cold acetone with 0.06 M DTT (25 mL/g) and kept at $-20\,^{\circ}\text{C}$ for 1 h. After centrifugation, the pellet was dried under vacuum and resuspended (0.05 g/mL) in a solubilization buffer containing 2 M thiourea, 7 M urea, 4% (w/v) CHAPS, 0.4% (v/v) Triton X-100, 0.06 M DTT and 1% (v/v) IPG buffer 3–10 NL (GE, Uppsala, Sweden). After 2 h dissolution at room temperature, the protein extracts were centrifuged at 15,000 g for 10 min and the supernatant collected and stored at $-80\,^{\circ}\text{C}$ until further use. The protein concentration was determined according to the Bradford method as modified by Ramagli [24].

2.3. Two-dimensional gel electrophoresis

For isoelectric focusing (IEF) electrophoresis, the IPGphor system was used (Amersham Biosciences, Uppsala, Sweden) with a non-linear pH gradient gel of 3–10 (IPGstrips, GE) loaded with $200\,\mu g$ of protein resolubilized in $8\,M$ urea, 4% (w/v) CHAPS,

0.06 M DTT and 0.5% (v/v) IPG buffer 3–10 NL (GE, Uppsala, Sweden). The IEF was carried out at 30 V for 12 h, followed by 200 V for 1 h, 500 V for 1.5 h, 1000 V for 1.5 h, and 8000 V for 6.5 h, at 20 °C. Prior to SDS-PAGE the IPGstrips were equilibrated for 2×15 min in a buffer solution containing 0.05 M Tris–HCl pH 8.8, 6 M urea, 30% (v/v) glycerol and 2% (w/v) SDS. 0.06 M DTT was added to the first equilibration step and 0.135 M iodoacetamide to the second one. The SDS-PAGE was performed on slab gels [25] and run at constant temperature of 15 °C. The 2-DE gels were stained with colloidal Coomassie Blue [26] and scanned using the ImageScanner (Amersham Biosciences, Uppsala, Sweden).

2.4. Two-dimensional gel analysis

Gels from independent biological triplicates were analyzed in ImageMaster 2D Platinum software v5.0 (GE, Uppsala, Sweden) for spot detection, measurement and matching. In order to correct for variation in gel staining that could affect protein spot intensities, the total spot volume of each gel was normalized to 100, and a volume % per spot calculated. A protein spot was kept for further analysis and declared reliable if at least n-1 volume values were available in each condition.

The protein spots found to be commonly expressed in the control and B deficiency 2-DE gels were statistically evaluated by two distinct methods. For the Kolmogorov–Smirnov test (p<0.05) the raw data were used whereas for the Student's t-test (p<0.05) a complete data matrix of volume % was generated by replacing the missing values in a condition by the mean of the existing values for that protein spot [27]. To eliminate the small between-condition bias observed in the two data sets the data were normalized and transformed according to Meunier et al. 2005 [28]. Only the spots having statistically significance in both statistical tests were considered as differentially expressed due to the deficiency.

2.5. In-gel digestion

The protein spots were rinsed with a washing solution of 50% (v/v) acetonitrile and 0.1 M ammonium bicarbonate for 4 h. Prior to digestion the dried spots were reduced with 0.01 M DTT for 2 h at 37 °C, then alkylated with 0.05 M iodoacetamide for 15 min at room temperature, in the dark. The gel spots were again rinsed with the washing solution for 2 h. The digestion was made overnight, with 125 ng of trypsin (Promega, Madison, WI) in 0.05 M ammonium bicarbonate at 37 °C. The peptides in the supernatant were collected and the gel pieces were extracted with a solution of 0.1% (v/v) acetic acid and 50% (v/v) acetonitrile. The extract was pooled with the tryptic peptides and dried in a speed vac. The pellet was redissolved in 0.1% (v/v) acetic acid and 2% (v/v) acetonitrile solution was used for mass spectrometric analysis.

2.6. MS/MS analysis

The trypsin digested proteins were analyzed by capillary liquid chromatography tandem MS (LC/MS/MS) using a set up of a trapping 300SB C-18 column (0.3 \times 50 mm) (Agilent Technologies, Basel, Switzerland) and a separating column (0.1 mm \times 10 cm) that had been packed with Magic 300 Å C18 reverse-phase material (5 μ m particle size, Michrom Bioresources, Auburn, CA, USA). The columns were connected on line to an Orbitrap FT

hybrid instrument (Thermo Finnigan, San Jose, CA, USA). A linear gradient from 2 to 80% of solvent B [0.1% (v/v) acetic acid and 80% (v/v) acetonitrile] in solvent A [0.1% (v/v) acetic acid and 2% (v/v) acetonitrile] was delivered with a Rheos 2200 pump (Flux Instruments, Basel, Switzerland) for 85 min at a flow rate of $100 \,\mu\text{L/min}$. A pre-column split was used to reduce the flow to approximately 100 nL/min. The injection of 10 µL of peptide digest was made by an auto-sampler thermostated to 4 °C and the eluting peptides ionized at 1.7 kV. Precursor ions were scanned between m/z range of 400-1600 in profile mode at a resolution of 60,000. MS/MS scans were triggered at 1000 ion counts and the fragmentation energy was set to 35% normalized collision energy. The mass spectrometer was operated in a datadependent fashion. The precursor scan was done in the Orbitrap, while the fragment ions were mass analyzed in the LTQ instrument. The five most intense signals of each precursor scan were selected for fragmentation. The MS/MS spectra were then searched against the NCBI non-redundant database, version August 15th 2008, using TurboSequest software [29]. The databank was searched with Bioworks version 3.3.1. SP1 by setting the precursor ion tolerance to 10 ppm, while the fragment ion tolerance was set to 0.5 Da. Cleavage rules were set to Fully enzymatic-cleaves at both ends, allowing 2 missed cleavages. In the databank search, carbamidomethylation of cysteine was set to fixed, while oxidation of methionine was set to variable modification. The SEQUEST search results were filtered to only show variable peptides; the Δ CN was set to 0.1; peptide and protein probability was set to 0.5 and 0.01, respectively. The Xcorr for singly, doubly, triply, and quadruply charged peptides was set to 1.50, 2.00, 2.50, and 3.00, respectively.

Protein coverage and unique peptide number were calculated by using Protein Coverage Summarizer software from the Pacific Northwest National Laboratory (Richland, WA, USA).

2.7. Statistical analysis

For the statistical analysis, the following software programs were used: in house software for the Kolmogorov–Smirnov test, the SigmaStat v3.10 software (Systat Software Inc, Erkrath, Germany) for the Student's t-test and the R program with the ade4 package [30] for a multivariate analysis (principal component analysis, PCA).

3. Results and discussion

3.1. Morphological effects of B deficiency

As a dicot plant, Lupinus albus is quite sensitive to B deficiency. Morphological alterations were already evident in two weeks deficient plants (Fig. 1). Despite no detectable effect in biomass at this time the roots were shorter, darker and lacked ramifications and proteoid formations. The morphological differences observed in the roots indicate that, at this early stage of development, the B present in the lupin seed was already insufficient to ensure adequate plant development. Three weeks from sowing, morphological alterations in the roots become more evident, and significant differences in biomass were found. In shoots, morphological differences due to B deficiency (darker and deformed leaves) become also



Fig. 1 – Morphological differences of Lupinus albus plants grown under B deficiency. The development of the plants grown with (C) or without B (BD) in the nutrient solution was monitored 2, 3 and 4 weeks after germination.

visible, but no significant biomass differences were detected. After 4 weeks the morphological differences were much more marked, with significant reduced biomass in both shoots and roots (Figs. 1 and 2).

3.2. Quantitative variations of the root proteome

As we considered that the lupin root system is very important for the plant ability to cope with long-term B deficiency, we analyzed by 2-DE the root proteomes of plants grown for 4 weeks with or without B (Fig. 3; Supplementary Fig. 1). 2-DE coupled to MS techniques and adequate statistical analysis, is a powerful tool to investigate the proteins whose expression is affected by the long-term B deficiency.

From a total of 406 reproducibly detected spots in the root proteome, 265 spots (65%) were responsive to B deficiency (Table 1). These include the specifically responsive spots, either suppressed (51%) or expressed *de novo* (10%) and those differentially expressed (4%). A PCA analysis was performed with all the reproducible protein spots (406) detected by 2-DE. This analysis shows that it is possible to distinguish the plants grown with or without B considering the first principal component (Fig. 4a), which explains 49% of the total variance (Table 2). In order to pinpoint the protein spots associated to this discrimination we performed two additional analyses. One involving the sub-set of spots common to both control and B deficiency treatments, i.e. non-responsive and differentially expressed spots (Fig. 4b), and the other analysis, the sub-set of

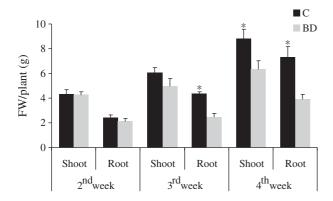


Fig. 2 – Shoots and roots fresh weight of *Lupinus albus* grown with (C) or without B (BD) for 2, 3 and 4 weeks after germination. Bars indicate standard errors. Significant changes were evaluated by the Student's t-test (*p<0.05).

spots specifically responsive to B deficiency (Fig. 4c). These analyses revealed that the common protein spots do not discriminate the treatments, whereas the spots specifically responsive to B deficiency gave a marked separation of the treatments (Table 2). The 265 spots reproducibly detected as responsive to B deficiency were analyzed by MS/MS techniques, and 213 were identified. In general, the analysis by LC–MS/MS techniques of the 2-DE gel spots reveals some spots containing multiple proteins [31], what we have found for 85 spots (40%) (Supplementary Table 1). Thus, only the remainder 128 spots were considered for secure quantification (Table 3; Supplementary Table 2).

3.3. Metabolic changes associated with B deficiency

In an attempt to identify the metabolic events associated with the plant root response to a long-term B deficiency, the identified proteins were grouped according to the biological functions annotated in the UniProt database (http://www.uniprot.org; Fig. 5)

The majority of the suppressed proteins are from classes related with plant growth and developmental processes. This observation is an indication of an adjusted biosynthetic flux that may be directly related with the reduced growth rates observed due to B deficiency. The fact that the up regulated or expressed de novo proteins belong to some of the same classes of the suppressed or down regulated proteins (energy pathways, protein metabolism, defense and cytoskeleton biosynthesis) can be an indication that the plant metabolism was not just impaired as a whole, but instead, was modified in a controlled manner.

The identified proteins showed in Table 3, were grouped according to the biological processes considered to be more relevant in relation to B deficiency.

3.3.1. Energy metabolism

Changes in several proteins that are related to cellular energy metabolism were observed in B deficient roots. Key enzymes of respiration, such as glucose-6-phospate-1-dehydrogenase (spot 700) from the pentose phosphate pathway, and the glycerladeyde-3-phosphate dehydrogenase (spot 146) from

the glycolysis pathways were suppressed. Also suppressed were several kinases, namely frutokinase (spot 421) and phosphoglycerate kinase (spots 493, 499 and 511), while the UDP-glucose pyrophosphorylase (spot 216) was expressed de novo. This enzyme is part of an alternative biochemical pathway for sucrose degradation that requires inorganic pyrophosphate, whereas the breakdown involving kinases requires two molecules of ATP [32]. The activation of bypass pathways allows the carbon flow to continue under stressful conditions by using alternative energy sources and thereby reducing ATP demand. Indeed, ATP availability could be compromised, since several ATPases were affected by B deficiency. A subunit B2 isoform from the V-type proton ATPase (spot 626) was suppressed as well as some other ATPase synthases subunit isoforms (spots 636, 640 and 646). A possible membrane damage caused by B deficiency, previously hypothesized to cause the reduced activity of proton-pumping ATPase observed in sunflower cells [33], could also be responsible for the release of membrane-bound proteins, as observed by the de novo expression of a V-type proton ATPase subunit A (spot 739) and B2 (spot 264). The de novo expression of pyruvate dehydrogenase E1 beta subunit component (spot 251) is also relevant since E1 protein levels correlate with the mitochondrial pyruvate dehydrogenase complex activity [34], and the higher expression of this enzyme correlates with metabolic and structural changes that accompany membrane remodeling [35].

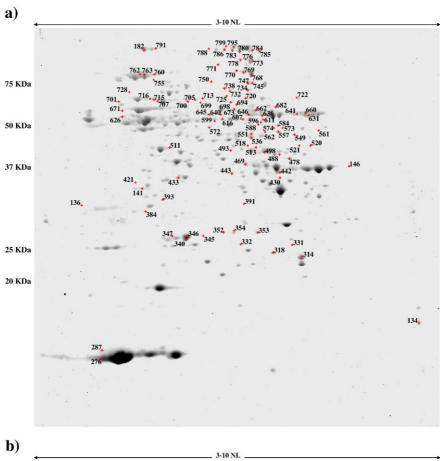
Therefore, these results indicate an effect of B deficiency on membranes, and it should be referred that a physiological role for B in membranes has been proposed by a wealth of information in which B deficiency has been shown to disrupt membrane-associated processes, including membrane potential and electron transport [6].

Several aconitate hydratase isoforms were suppressed (spots 780, 783, 784, 785, 786 and 788) or down regulated (spot 790) due to B deficiency and another form (spot 232) was de novo expressed. Since this protein spot has a considerable lower molecular mass than that annotated in the database, it may have been targeted for degradation during B deficiency. Considering that the aconitase cluster is lost under oxidative stress [36] and that oxidative damage is the major cause of cell death induced by B-deprivation in tobacco cells [14], the aconitase suppression or degradation could result from the oxidative damage caused by B deficiency.

3.3.2. Protein metabolism

The Rubisco large subunit-binding protein subunit β (spot 710) belongs to the heat shock protein 60 (Hsp60) family. Both this Hsp60 and a Hsp70 (spot 749) were *de novo* expressed under B deficiency and are molecular chaperones responsible for preventing irreversible aggregation of non-native proteins under both normal and stressful conditions [37]. Another protein involved in the protein folding processes, that was also *de novo* expressed under B deficiency, is a peptidylprolyl cis–trans isomerase (spot 291), that can additionally play important roles in protein degradation, signal transduction and mRNA processing [38].

The nascent polypeptide-associated complex (NAC) is a heterodimeric complex of α - and β -chains that is postulated to be involved in protein transport for an appropriate targeting of ribosome–nascent polypeptide complexes [39]. The *de novo*



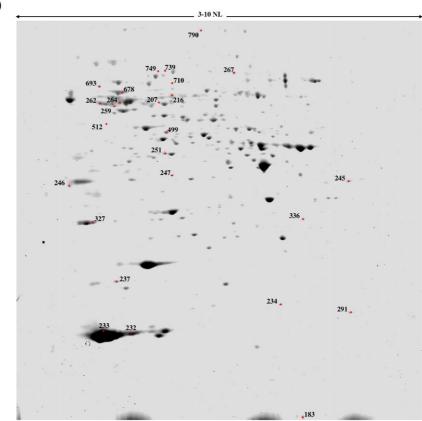


Fig. 3 – Representative 2-DE gels of *Lupinus albus* roots grown with (a) or without B (b) for 4 weeks. The gels were Coomassie Blue stained. Numbered proteins were identified by MS/MS (see Table 3). Labeled proteins in gel a) are those suppressed due to B deficiency, while those labeled in gel b) are differentially expressed or *de novo* expressed due to B deficiency.

Table 1 – Number of protein spots that have quantitative or qualitative variation due to B deficiency.

Protein spot expression	No. of protein spots
de novo expressed	42
Suppressed	209
Differentially expressed	
According to K–S test	33
According to Student's t-test	16
With both statistical tests	14
Total B responsive protein spots	265

expression of a NAC subunit α -like protein 2 (spot 247), might suggest altered protein translation and targeting, important for adaptive stress responses.

The CND41 (spot 259), that was *de novo* expressed, and the predicted protein A9PEP6 (spot 512), that was down regulated under B deficiency, have aspartic-type endopeptidase activity. This activity is apparently related with proteolitic processes implicated in post-mortem proteolysis of the 7S globular storage protein and in the degradation of extracellular pathogenesis-related (PR) proteins [40]. Indeed, a 7S seed storage protein, the globulin-1S allele (spot 682) and two pathogenesis-related (PR)-10 proteins (spots 276 and 287) were suppressed due to B deficiency.

Protein degradation is an ATP-demanding process, so its repression will decrease protein synthesis and turnover, thereby reducing ATP demand [41]. The fact that suppressed and *de novo* expressed proteins belong to the same metabolic class, points out for a shift in protein folding and proteolysis processes, that may lead to a redirected protein metabolism towards plant survival under stressful conditions.

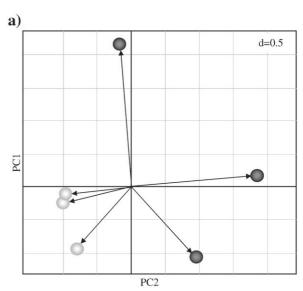
3.3.3. Amino acid metabolism

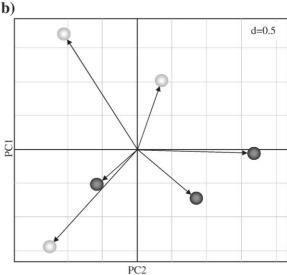
Some proteins related with amino acid metabolism were suppressed by B deficiency. The majority of these proteins (Adenosylhomocysteinase, spot 616; Cysteine synthase, spot 393 and 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, spots 773, 776 and 778) are related with the metabolism of sulfur containing amino acids. Such effect was not previously reported, although a relation of B deficiency with sulfur metabolism was reported through the reduction of the SH-containing compound, glutathione, higher glutathione reductase activity and glutathione S-transferase expression [5,18,42]. In our study, however, glutathione-S-transferase (spot 318) was suppressed due to the long-term B deficiency, whereas an increase in glutathione S-transferase proteins and genes has been reported in short-term B deficiency studies [18,43].

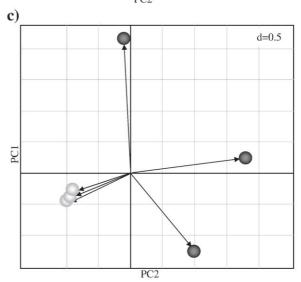
Fig. 4 – Principal component analysis of Lupinus albus root 2-DE protein profiles from plants grown with or without B. Each point summarizes either the complete protein profile (a), or the sub-set profile of commonly expressed proteins (b), or the sub-set profile of specifically B-responsive proteins (c). Black circles represent the control samples and the gray circles represent the B-deficient samples.

3.3.4. Cell wall metabolism

Altered cell wall metabolism can be envisaged considering the described participation of B in the cell wall structure. Two enzymes, UDP-glucose 6-dehydrogenase (spot 673) and GDP-







Component			Eigenv	alues		
	Whole da	lataset Common spots		Specifically res	ponsive spots	
	Total spots no.	% Variance	Total spots no.	% Variance	Total spots no.	% Variance
PC1	199	49	53	34	152	61
PC2	69	17	31	20	43	17
PC3	57	14	28	18	33	13
PC4	43	10	22	14	14	6
PC5	38	9	21	14	8	3
SUM	406	99	155	100	251	100

Table 3 – Protein identification of the 2-DE gel spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column FC are indicated the fold change of the differentially expressed proteins, the *de* novo expressed proteins (+) and the suppressed proteins (–).

ID a		Protein identification ^b	Species	FC	pI/MW (kDa)		
					Predicted ^c	Exp	
Energ	gy pathways						
146	P08477	Glyceraldehyde-3-phosphate dehydrogenase	Hordeum vulgare	-	-	8.2/38	
216	Q8W557	UDP-glucose pyrophosphorylase	Amorpha fruticosa	+	6.07/51.6	5.6/54	
232	P49608	Aconitate hydratase	Cucurbita maxima	+	5.74/98.0	5.1/15	
245	Q41712	Ascorbate peroxidase	Vigna unguiculata	+	5.64/27.0	8.6/31	
251	Q38799	Pyruvate dehydrogenase E1 component subunit	Arabidopsis thaliana	+	5.11/35.9	5.5/36	
264	Q40079	V-type proton ATPase subunit B2	Hordeum vulgare	+	5.12/53.7	5.0/53	
340	Q41712	Ascorbate peroxidase	Vigna unguiculata	_	5.64/27.0	6.3/26	
346	Q43758	Ascorbate peroxidase	Glycine max	_	5.51/27.1	6.4/27	
347	Q8H1K7	Ascorbate peroxidase	Retama raetam	_	5.88/23.6	5.7/27	
421	Q0JGZ6	Fructokinase-1	Oryza sativa	_	5.07/34.7	5.1/35	
442	Q8GZN3	Malate dehydrogenase	Lupinus albus	_	6.10/35.6	6.4/37	
443	Q8GZN3	Malate dehydrogenase	Lupinus albus	_	6.10/35.6	5.9/36	
469	Q40676	Fructose-bisphosphate aldolase	Oryza sativa	_	6.55/38.7	6.0/38	
478	Q9SXP2	Formate dehydrogenase 1, mitochondrial	Oryza sativa	_	6.20/39.3	6.6/40	
488	P52901	Pyruvate dehydrogenase E1 component subunit α-1	Arabidopsis thaliana	_	6.42/39.6	6.4/41	
493	P50318	Phosphoglycerate kinase	Arabidopsis thaliana	_	5.04/42.6	5.9/41	
198	P52902	Pyruvate dehydrogenase E1 component subunit	Pisum sativum	_	-	6.2/42	
499	Q9SAJ4	Phosphoglycerate kinase	Arabidopsis thaliana	-1.4	5.49/42.1	6.1/41	
511	P50318	Phosphoglycerate kinase	Arabidopsis thaliana	_	5.04/42.6	5.5/43	
536	Q7Y0W9	NADP-specific isocitrate dehydrogenase	Lupinus albus	_	6.13/46.1	6.0/46	
551	-	NADP-specific isocitrate dehydrogenase	Lupinus albus	_	5.99/46.0	6.0/47	
557	B0FGG5	Monodehydroascorbate reductase	Vaccinium corymbosum	_	5.78/47.4	6.3/48	
561	P93033	Fumarate hydratase 1	Arabidopsis thaliana	_	6.65/49.9	7.3/48	
562	B0FGG5	Monodehydroascorbate reductase	Vaccinium corymbosum		5.78/47.4	6.1/49	
		Enolase	•	-			
572	A9SGH3		Physcomitrella patens	-	5.22/46.1	5.7/50	
588	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating	Arabidopsis thaliana	-	5.62/53.3	6.1/50	
599	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating	Arabidopsis thaliana	-	5.62/53.3	5.7/52	
607	Q9LI00	6-phosphogluconate dehydrogenase, decarboxylating	Oryza sativa	-	5.85/52.7	6.0/53	
611	Q9LEJ0	Enolase 1	Hevea brasiliensis	-	5.57/47.8	6.1/73	
626	Q40079	V-type proton ATPase subunit B2	Hordeum vulgare	-	5.12/53.7	4.9/54	
631	Q9FNN5	Subunit of complex I	Arabidopsis thaliana	-	8.46/53.4	7.0/55	
636	P12862	ATP synthase subunit α	Triticum aestivum	-	5.70/55.3	6.1/55	
640	P12862	ATP synthase subunit α	Triticum aestivum	-	5.70/55.3	5.8/55	
641	P49357	Serine hydroxymethyltransferase 1	Flaveria pringlei	-	8.15/53.6	6.8/55	
646	P12862	ATP synthase subunit α	Triticum aestivum	-	5.70/55.3	5.0/43	
660	023254	Serine hydroxymethyltransferase	Arabidopsis thaliana	-	6.80/51.7	7.0/56	
662	B9SH74	Aldehyde dehydrogenase, putative	Ricinus communis	-	5.87/42.0	6.1/57	
700	Q42919	Glucose-6-phosphate 1-dehydrogenase	Medicago sativa	-	5.85/58.9	6.5/63	
738	O82663	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1	Arabidopsis thaliana	-	5.58/66.0	5.8/71	
739	P31405	V-type proton ATPase catalytic subunit A	Gossypium hirsutum	+	5.36/68.5	5.5/77	
771	Q9FGI6	NADH-ubiquinone oxidoreductase 75 kDa subunit	Arabidopsis thaliana	_	5.72/77.9	8.1/29	
780	P49608	Aconitate hydratase	Cucurbita maxima	_	5.74/98.0	5.9/13	

(continued on next page)

ID ^a		Protein identification ^b	Species	FC	pI/MW (kDa)		
ייי		110tem lacitimeaton	opecies .	10	Predicted c	• •	
Energ	y pathway:	S				1	
783	P49608	Aconitate hydratase	Cucurbita maxima	-	5.74/98.0	5.9/138	
784	P49608	Aconitate hydratase	Cucurbita maxima	-	5.74/98.0	6.0/138	
785	P49608	Aconitate hydratase	Cucurbita maxima	-	5.74/98.0	6.1/13	
786	P49608	Aconitate hydratase	Cucurbita maxima	-	5.74/98.0	6.0/13	
788	P49608	Aconitate hydratase	Cucurbita maxima	-	5.74/98.0	5.7/14	
790	P49608	Aconitate hydratase	Cucurbita maxima	-4.9	5.74/98.0	5.8/14	
	in metabolis						
134	O49886	Peptidyl-prolyl cis–trans isomerase	Lupinus luteus	-	8.71/18.3	9.6/17	
136	Q40682	Elongation factor 1-δ 2	Oryza sativa	-	4.40/24.5	4.2/32	
247	Q94JX9	Nascent polypeptide-associated complex subunit α -like protein 2	Arabidopsis thaliana	+	4.37/23.7	5.6/32	
259	Q9LS40	CND41, chloroplast nucleoid DNA binding protein-like	Arabidopsis thaliana	+	5.27/53.2	5.0/48	
291	O49886	Peptidyl-prolyl cis–trans isomerase	Lupinus luteus	+	8.71/18.3	8.7/17	
332	Q3HVM0	Proteasome subunit α type	Solanum tuberosum	-	5.40/28.1	5.9/26	
	A9TVH1	Proteasome subunit α type	Physcomitrella patens	-	5.92/27.2	5.7/27	
352	A5AXI5	Proteasome subunit α type	Vitis vinifera	-	6.11/27.2	5.8/27	
	A9TVH1	Proteasome subunit α type	Physcomitrella patens	-	5.91/27.3	5.9/27	
512	A9PEP6	Predicted protein	Populus trichocarpa	2.0	4.94/45.3	5.0/43	
513	Q9ZRU6	Elongation factor Tu	Catharanthus roseus	-	_	6.0/43	
573	A0FH76	EBP1	Solanum tuberosum	-	6.26/42.8	6.4/50	
584	Q0DDX2	26S protease regulatory subunit 7	Oryza sativa	-	6.03/47.7	6.3/50	
645	Q6K669	Leucine aminopeptidase 2	Oryza sativa	-	5.50/55.0	5.7/55	
593	P21239	RuBisCO large subunit-binding protein subunit α	Brassica napus	-2.2	4.78/57.0	4.9/61	
594	P21239	RuBisCO large subunit-binding protein subunit α	Brassica napus	-	4.78/57.0	5.9/61	
599	Q940P8	Putative uncharacterized protein	Arabidopsis thaliana	-	5.59/57.3	5.6/63	
701	P21239	RuBisCO large subunit-binding protein subunit α	Brassica napus	-	4.78/57.0	4.9/63	
705	Q93ZM7	Chaperonin CPN60-like 2	Arabidopsis thaliana	-	5.32/57.1	6.0/64	
707	Q05045	Chaperonin CPN60-1	Cucurbita maxima	-	5.09/57.4	5.3/63	
710	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana	+	5.26/58.1	5.6/64	
713	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana	-	5.26/58.1	5.6/65	
715	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana	-	5.26/58.1	5.3/65	
716	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana	-	5.26/58.1	5.3/65	
720	A5BFM5	Putative uncharacterized protein	Vitis vinifera	-	6.03/61.2	6.0/65	
722	A5BFM5	Putative uncharacterized protein	Vitis vinifera	-	6.03/61.2	6.7/66	
725	A5BFM5	Putative uncharacterized protein	Vitis vinifera	-	6.03/61.2	5.8/67	
728	A5BFM5	Putative uncharacterized protein	Vitis vinifera	-	6.03/61.2	5.0/69	
732	Q9M888	Putative uncharacterized protein	Arabidopsis thaliana	-	5.83/58.9	5.9/70	
749	P37900	Heat shock 70 kDa protein	Pisum sativum	+	5.18/66.7	5.8/66	
750	Q43468	Heat shock protein STI	Glycine max	_	5.81/63.6	5.7/77	
755	P37900	Heat shock 70 kDa protein	Pisum sativum	_	5.18/66.7	5.3/80	
760	P11143	Heat shock 70 kDa protein	Zea mays	_	5.22/70.6	5.3/89	
762	Q39043	Luminal-binding protein 2	Arabidopsis thaliana	_	5.08/71.1	5.2/87	
763	Q39043	Luminal-binding protein 2	Arabidopsis thaliana	_	5.08/71.1	5.2/88	
791	Q9LZF6	Cell division control protein 48 homolog E	Arabidopsis thaliana	_	5.08/90.0	5.3/14	
795	023755	Elongation factor 2	Beta vulgaris	_	5.93/93.8	5.9/14	
799	O23755	Elongation factor 2	Beta vulgaris	-	5.93/93.8	5.8/15	
Defer	ise response	2					
233	P52779	Protein LlR18B	Lupinus luteus	+	5.35/16.6	4.7/15	
234	Q93XI0	Pathogenesis-related 10	Lupinus albus	+	4.87/16.9	6.8/18	
276	Q93XI0	Pathogenesis-related 10	Lupinus albus	_	4.87/16.9	4.6/15	
287	Q93XI0	Pathogenesis-related 10	Lupinus albus	_	4.87/16.9	4.6/15	
318	Q0PN10	Glutathione S-transferase	Caragana korshinskii	_	6.86/25.8	6.3/25	
327	Q9SXM5	Acidic chitinase	Glycine max	+	5.01/31.9	4.7/26	
430	P23535	Glucan endo-1,3-β-glucosidase, basic isoform	Phaseolus vulgaris	-	8.75/35.2	6.4/36	
Γrans	scription						
314	-	Quinone reductase 2	Triticum monococcum	-	5.95/21.7	6.7/24	
Amin	ıo acids met	abolism					
393	A3RM06	Cysteine synthase	Glycine max	-	5.29/34.7	5.4/32	
520	P54260	Aminomethyltransferase	Solanum tuberosum	_	7.28/40.9	7.1/44	

ID ^a		Protein identification ^b	Species	FC	pI/MW	(kDa)
					Predicted ^c	Exp
521	Q40108	Aspartate aminotransferase	Lupinus angustifolius	-	8.36/45.8	6.8/44
616	Q9SP37	Adenosylhomocysteinase	Lupinus luteus	-	5.64/53.3	5.8/53
773	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	Mesembryanthemum crystallinum	_	5.90/84.8	6.0/11
776	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	Mesembryanthemum crystallinum	-	5.90/84.8	6.0/11
778	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	Mesembryanthemum crystallinum	-	5.90/84.8	5.9/11
Cell ı	wall metabolisr	n				
267	P34105	NADP-dependent malic enzyme	Populus trichocarpa	+	6.50/65.2	6.4/61
518	Q93VR3	GDP-mannose 3,5-epimerase	Arabidopsis thaliana	-	5.85/42.8	6.0/44
673	Q96558	UDP-glucose 6-dehydrogenase	Glycine max	-	5.74/52.9	5.9/58
734	A9PGL9	Malic enzyme	Populus trichocarpa	-	7.61/54.6	6.0/70
745	P34105	NADP-dependent malic enzyme	Populus trichocarpa	-	6.50/65.2	6.0/75
747	P34105	NADP-dependent malic enzyme	Populus trichocarpa	_	6.50/65.2	6.0/75
Cytos	skeleton biosyn					
182	Q1G0Z1	Putative spindle disassembly related protein CDC48	Nicotiana tabacum	-	5.13/89.9	5.2/14
183	Q1G0Z1	Putative spindle disassembly related protein CDC48	Nicotiana tabacum	+	5.13/89.9	7.4/10
207	P20363	α -3/ α -5 tubulin chain	Arabidopsis thaliana	+	4.95/49.7	5.4/49
262	Q9STD0	β-tubulin	Zinnia elegans	+	4.75/50.1	4.9/49
331	P41916	GTP-binding nuclear protein Ran-1	Arabidopsis thaliana	-	6.39/25.3	6.7/26
336	P41916	GTP-binding nuclear protein Ran-1	Arabidopsis thaliana	+	6.39/25.3	7.4/26
Othei	r metabolic pro	cesses				
391	Q8LQJ6	Ethylene-responsive protein 2-like	Oryza sativa	-	10.86/12.6	6.0/31
574	Q7M1Z8	Globulin-2	Zea mays	-	6.16/49.9	6.3/50
596	P19595	UTP-glucose-1-phosphate uridylyltransferase	Solanum tuberosum	-	5.71/51.7	6.1/52
682	P15590	Globulin-1S allele	Zea mays	-	6.75/55.1	6.3/60
768	Q7SIC9	Transketolase	Zea mays	-	5.47/73.0	6.0/88
769	Q7SIC9	Transketolase	Zea mays	-	5.47/73.0	6.0/32
770	Q7SIC9	Transketolase	Zea mays	-	5.47/73.0	5.9/95
	nown biological	•				
141	Q8LPE5	Fructokinase-like protein	Cicer arietinum	-		5.1/35
237	Q9M328	Putative uncharacterized protein T18D12.90	Arabidopsis thaliana	+	5.66/17.8	5.1/20
246	Q8GYY8	Putative germin	Arabidopsis thaliana	+	8.39/23.5	4.4/30
353	Q2V987	Transcription factor APFI-like	Solanum tuberosum	-	7.05/29.1	5.8/27
384	Q9SMK5	Plasma membrane intrinsic polypeptide	Cicer arietinum	-	4.95/23.3	5.2/30
549	A5CB20	Putative uncharacterized protein	Vitis vinifera	-	8.42/54.2	6.7/47
671	Q7XCL2	Ubiquitin domain containing protein	Oryza sativa	-	4.71/59.3	4.9/58
678	Q94IC1	Betaine aldehyde dehydrogenase	Hordeum vulgare	-2.2	5.47/54.5	5.2/5

^a Spot numbers are corresponding to the numbers in Fig. 4.

mannose 3,5-epimerase (spot 518), involved in the carbohydrate metabolism directed for the cell wall biosynthesis were suppressed due to B deficiency. Several malic enzyme isoforms (spots 734, 745 and 747) were suppressed under B deficiency, and this enzyme is described to provide the NADPH used for the production of H_2O_2 in lignin biosynthesis [44]. Lignin production that results from the hydroxycinnamyl alcohols polymerization by peroxidases [45] could be compromised by B deficiency. The suppression of several ascorbate peroxidases isoforms (spots 340, 346 and 347), and the previously observation of ascorbate peroxidase inhibition in B-deficient squash roots [5] could be related with the increased content of

phenolic compounds described for B-deficient plants [46] and with the lower degree of lignification observed in some trees growing in low B content soils [47].

3.3.5. Defense responses

Several proteins related with defense responses were *de novo* expressed due to B deficiency, namely PR-10 (spot 234) and LIR18B protein (spot 233), both belonging to the PR-10 family, and an acidic chitinase (spot 327) that belongs to the PR-8 family [48]. The PR family proteins are known to be induced by several biotic and abiotic stresses [49]. An uncharacterized T18D12.90 protein (spot 237) and a putative germin-like protein (spot 246), that were *de*

^b Protein identification according to the UniProt database (http://www.uniprot.org).

^c Predicted pI and MW (kDa) were calculated by using an ExPASy tool (http://www.expasy.org).

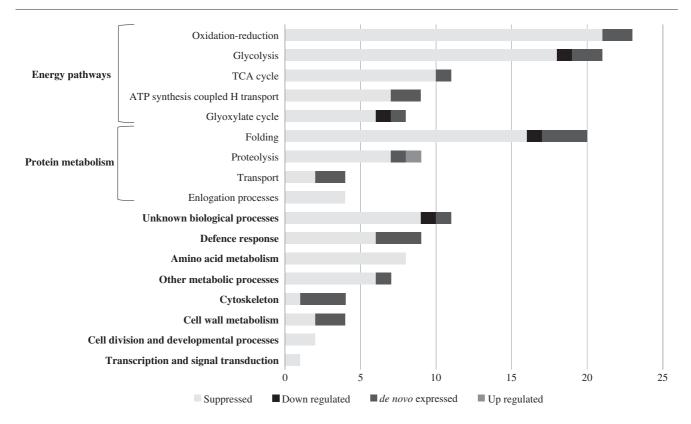


Fig. 5 – Functional classification of the Lupinus albus root proteins responsive to B deficiency. The proteins were grouped according to the biological functions described in databases.

novo expressed under B deficiency, have unknown biological functions, however, they have been also associated with various stress responses. For example, in *Arabidopsis thaliana* the protein T18D12.90 is described to be part of the universal stress protein (USP) family [50] and germin as well as germin-like proteins,

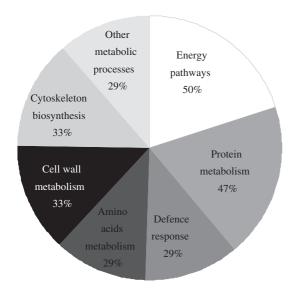


Fig. 6 – Percentage of protein isoforms identified in each metabolic class, of proteins responsive to B deficiency in Lupinus albus roots (see Table 3).

besides their involvement in stress responses, have been described to participate in a wide range of activities related to developmental processes and cell wall biosynthesis [51]. Several other studies had already reported increased defense proteins in association with B deficiency [19,42,52]. Updated evidence shows that PR proteins and other stress responsive proteins, may display additional functions in growth and developmental processes, by modulating signal molecules [53,54], however the association of these defense proteins with B is not yet understood.

3.3.6. Cytoskeleton biosynthesis

Several proteins related with cytoskeleton biosynthesis were affected by B deficiency. Under B deficiency we detected de novo expression of tubulins (spots 207 and 262), which are major components of microtubules. Another de novo expressed protein, which could be involved in the regulation of cytoskeletal assembly and organization, is a GTP-binding nuclear protein Ran-1 (spot 336) [55,56]. The de novo expression of a putative spindle disassembly-related protein CDC48 (spot 183) is probably the result of protein degradation processes since this protein spot has a considerable lower molecular mass than that one annotated in the database. Additionally, several other proteins related with cytoskeleton biosynthetic process, were found to be suppressed due to B deficiency. The putative proteins, still uncharacterized, from A. thaliana (spots 699 and 732) and from Vitis vinifera (spots 725, 720, 722 and 728) are described in the UniProt database to have sequence similarities to the TCP-1 chaperonin family that, in association with the Hsp70 molecular chaperones, can interact with

cytoskeleton components [57]. Elongation factors (spots 136 and 513), besides their participation in the translational apparatus, appear to have a second role as a regulator of cytoskeleton rearrangements [58]. Previous studies on altered cytoskeleton features were reported as increased levels of tubulins, actins and altered polymerization patterns of these cytoskeleton proteins in higher plants subjected to B deficiency [59,60]. Cytoskeleton is involved in diverse important cellular aspects, such as mitotic spindle formation, intracellular transport and control of cell shape [61]. So, modified cytoskeleton biosynthesis could still be a missing explanation for the B role in higher plants. Indeed, a possible role for B in cytoskeleton is supported by previous findings that B deficiency primarily disrupts processes where active cytoskeleton remodeling is required, such as the initial phases of differentiation, including pollen tube growth, anther development [62,63], somatic embryo formation [64] and early nodulation processes [65].

3.3.7. Protein isoforms in stress responses

One striking feature of this proteomic study is the detection of several protein isoforms associated with B deficiency. Indeed, a numerous group of proteins showed slight changes in pI and/or MW in response to the deficiency. The class with the major percentage of protein isoforms (Fig. 6) was that of energy pathways (50%) followed by the class of protein metabolism (47%). As previously discussed, the plant metabolism seems to be modified in a controlled manner in response to the long-term B deficiency, rather than being just impaired as a whole. Different protein isoforms were also found to have a crucial role in fungal infection and symbiosis studies in Medicago truncatula, and in the hydrogen peroxide responses of the rice seedling leaves [66,67]. The evidence for the participation of protein isoforms in several stress responses, points out for active functioning in regulatory processes that could be determinant for plants to cope with adverse conditions. This is an important and a very complex matter that is now emerging, and that in the near future may bring new insight on plant stress responses [68]. So, this could be a future in-depth study to perform in relation to B deficiency.

4. Conclusions

There appears to be a metabolic adjustment of the biosynthetic fluxes of the lupin root in response to B deficiency. The adaptive responses to the deficiency resulted in a reduction of important metabolic processes, namely, energy and protein metabolic processes, in which a higher number of protein isoforms was observed. Other common adaptive stress responses are related with defense proteins. Several other metabolic processes affected by the deficiency, such as cell wall metabolism, are in accordance with the known B participation in plant cell wall structure, and cytoskeleton biosynthesis. The high requirement of B in active cytoskeleton remodeling, such as in initial phases of differentiation [64], in reproductive processes [62,63] and in nodulation processes [69], is consistent with a possible role for B in cytoskeleton biosynthetic processes.

Supplementary materials related to this article can be found online at doi:10.1016/j.jprot.2011.03.002.

Acknowledgments

We are grateful to Dr. Phil Jackson (Instituto de Tecnologia Química e Biológica) for revising the manuscript and we thank the financial support from "Fundação para a Ciência e a Tecnologia" (FCT grant no. SFRH/BD/18273/2004). The Pacific Northwest National Laboratory (Richland, WA) is acknowledged concerning the utilization of Protein Coverage Summarizer software.

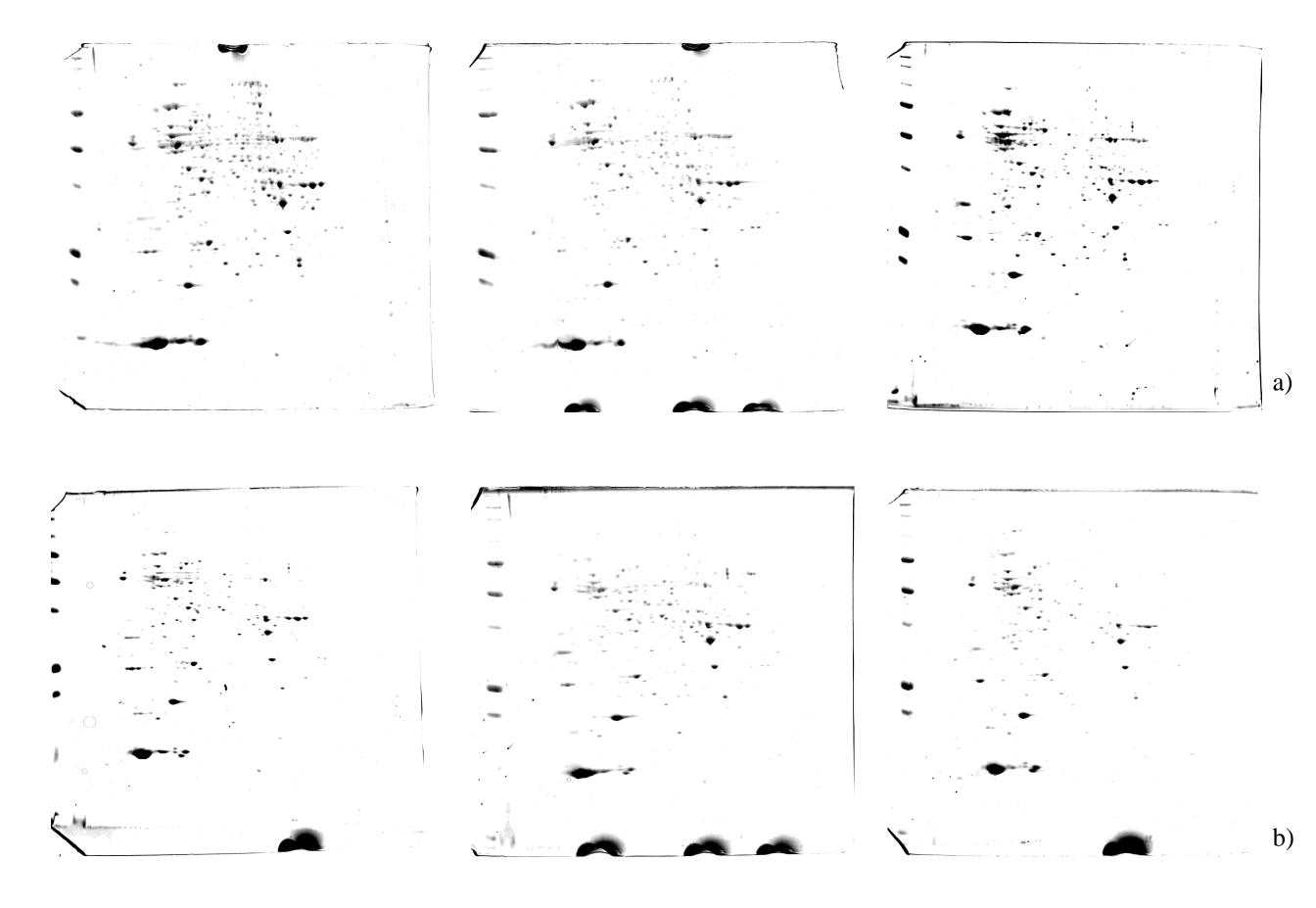
REFERENCES

- [1] Warington K. The effect of boric acid and borax on the broad bean and certain other plants. Ann Bot 1923;37:629–72.
- [2] Nable R, Bañuelos G, Paul J. Boron toxicity. Plant Soil 1997;193: 181–98.
- [3] Shorrocks VM. The occurrence and correction of boron deficiency. Plant Soil 1997;193:121–48.
- [4] Vale RR. Efeito da deficiência de boron na produção de matéria seca e semente de trevo subterrâneo. Pastagens Forragens 1995;16:9–19.
- [5] Cakmak I, Römheld V. Boron deficiency-induced impairments of cellular functions in plants. Plant Soil 1997;193:71–83.
- [6] Brown P, Bellaloui N, Wimmer M, Bassil E, Ruiz J, Hu H, et al. Boron in plant biology. Plant Biol 2002;4:203–23.
- [7] Kobayashi M, Matoh T, Azuma J. Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol 1996;110:1017–20.
- [8] O'Neill M, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill A, et al. Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross linked by a borate ester. J Biol Chem 1996;271:22923–30.
- [9] O'Neill M, Eberhard S, Albersheim P, Darvill A. Requirement of borate cross-linking of cell wall rhamnogalacturonan II for Arabidopsis growth. Science 2001;294:846–9.
- [10] Nielsen F. Boron in human and animal nutrition. Plant Soil 1997;193:199–208.
- [11] Park M, Li Q, Shecheynikov N, Muallem S, Zeng W. Borate transport and cell growth and proliferation: not only in plants. Cell Cycle 2005;4:24–6.
- [12] Unver T, Bozkurt O, Akkaya MS. Identification of differentially expressed transcripts from leaves of the boron tolerant plant Gypsophila perfoliata L. Plant Cell Rep 2008;27:1411–22.
- [13] Zeng C, Han Y, Shi L, Peng L, Wang Y, Xu F, et al. Genetic analysis of the physiological responses to low boron stress in Arabidopsis thaliana. Plant Cell Environ 2008;31:112–22.
- [14] Koshiba T, Kobayashi M, Matoh T. Boron nutrition of tobacco BY-2 cells. V. Oxidative damage is the major cause of cell death induced by boron deprivation. Plant Cell Physiol 2009;50:26–36.
- [15] Kasajima I, Ide Y, Hirai MY, Fujiwara T. WRKY6 is involved in the response to boron deficiency in Arabidopsis thaliana. Physiol Plant 2010;139:80–92.
- [16] Patterson J, Ford K, Cassin A, Natera S, Bacic A. Increased abundance of proteins involved in phytosiderophore production in boron-tolerant barley. Plant Physiol 2007;144: 1612–31.
- [17] Ahsan N, Renaut J, Komatsu S. Recent developments in the application of proteomics to the analysis of plant responses to heavy metals. Proteomics 2009;9:2602–21.
- [18] Wang Z, Wang Z, Shi L, Wang L, Xu F. Proteomic alterations of Brassica napus root in response to boron deficiency. Plant Mol Biol 2010;74:265–78.

- [19] Alves M, Francisco R, Martins I, Ricardo C. Proteomic analysis of the extracellular domain of Lupinus albus leaves in response to boron deficiency. Plant Soil 2006;279:1–11.
- [20] Alves M, Chicau P, Matias H, Passarinho J, Pinheiro C, Ricardo CP (in press) Metabolic analysis revealed altered amino acid profiles in *Lupinus albus* organs due to boron deficiency. Physiol Plant.
- [21] Gladstones JS, Atkins CA, Hamblin J, editors. Lupins as crop plants: biology, production and utilization. Wallingford, UK: CAB International; 1998.
- [22] Fitter A. Characteristics and functions of root systems. In: Waisel Y, Eshel A, Kafkafi U, editors. Plant roots: the hidden half. New York: CRC Press; 2002. p. 3–25.
- [23] Arnon D. Microelements in culture–solution experiment with higher plants. Am J Bot 1938;25:322–5.
- [24] Ramagli L. Quantifying protein in 2D PAGE solubilization buffers. In: Link Andrew J, editor. Methods in molecular biology — 2D proteome analysis protocols. Totowa, NJ: Humana Press; 1999. p. 95–105.
- [25] Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
- [26] Neuhoff V, Stamm R, Eibl H. Clear background and highly sensitive protein staining with Coomassie Blue dyes in polyacrylamide gels: a systematic analysis. Electrophoresis 1985;6:427–48.
- [27] Quackenbush J. Microarray data normalization and transformation. Nat Genet 2002;32:496–501.
- [28] Meunier B, Bouley J, Piec I, Bernard C, Picard B, Hocquette J-F. Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis. Anal Biochem 2005;340:226–30.
- [29] Gatlin C, Eng J, Cross S, Detter J, Yates 3rd J. Automated identification of amino acid sequence variations in proteins by HPLC/microspray tandem mass spectrometry. Anal Chem 2000;72:757–63.
- [30] Thioulouse J, Dray S. Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. J Stat Softw 2007;22:1–14.
- [31] Lim H, Eng J, Yates JR, Tollaksen SL, Giometti CS, Holden JF, et al. Identification of 2D-gel proteins: a comparison of MALDI/TOF peptide mass mapping to μ LC–ESI tandem mass spectrometry. J Am Soc Mass Spectrom 2003;14:957–70.
- [32] Stitt M. Pyrophosphate as an alternative energy donor in the cytosol of plant cells: an enigmatic alternative to ATP. Bot Acta 1998;111:167–75.
- [33] Ferrol N, Donaire J. Effect of boron on plasma membrane proton extrusion and redox activity in sunflower cells. Plant Sci 1992:86:41–7.
- [34] Luethy M, Gemel J, Johnston M, Mooney B, Miernyk J, Randall D. Developmental expression of the mitochondrial pyruvate dehydrogenase complex in pea (Pisum sativum) seedlings. Physiol Plant 2001;112:559–66.
- [35] Tovar-Méndez A, Miernyk J, Randall D. Regulation of pyruvate dehydrogenase complex activity in plant cells. FEBS J 2003:270:1043–9
- [36] Moeder W, Pozo O, Navarre D, Martin G, Klessig D. Aconitase plays a role in regulating resistance to oxidative stress and cell death in Arabidopsis and Nicotiana benthamiana. Plant Mol Biol 2007;63:273–87.
- [37] Timperio A, Giulia M, Zolla L. Proteomics applied on plant abiotic stress: role of heat shock proteins (HSP). J Proteomics 2008;71:391–411.
- [38] Nuc K, Nuc P, Slomski R. Yellow lupine cyclophilin transcripts are highly accumulated in the nodule meristem zone. Mol Plant-Microbe Interact 2001;14:1384–94.
- [39] Rospert S, Dubaquie Y, Gautschi M. Nascent-polypeptide associated complex. Cell Mol Life Sci 2002;59:1632–9.
- [40] Voigt G, Biehl B, Heinrichs H, Voigt J. Aspartic proteinase levels in seeds of different angiosperms. Phytochemistry 1997;44:389–92.

- [41] Hochachka P, Lutz P. Mechanism, origin and evolution of anoxia tolerance in animals. Comp Biochem Physiol 2001;130: 435–59
- [42] Han S, Chen L, Jiang H, Smith B, Yang L, Xie C. Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. J Plant Physiol 2008;165:1331–41.
- [43] Kobayashi M, Mutoh T, Matoh T. Boron nutrition of cultured tobacco BY-2 cells. IV. Genes induced under low boron supply. J Exp Bot 2004;55:1441–3.
- [44] Martinoia E, Rentsch D. Malate compartmentation-responses to a complex metabolism. Annu Rev Plant Physiol Plant Mol Biol 1994;45:447–67.
- [45] Higuchi T. Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 1990;24:23–63.
- [46] Blevins D, Lukaszewski K. Boron in plant structure and function. Annu Rev Plant Physiol Plant Mol Biol 1998;49: 481–500.
- [47] Dell B, Malajczuk N. Boron deficiency in eucalypt plantations in China. Can J Res 1994;24:2409–16.
- [48] Watanabe A, Nong V, Zhang D, Arahira M, Yeboah N, Udaka K, et al. Molecular cloning and ethylene-inducible expression of Chib1 chitinase from soybean. Biosci Biotechnol Biochem 1999;63:251–6.
- [49] Van Loon L, Van Strien E. The families of pathogenesisrelated proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol 1999;55:85–97.
- [50] Kvint K, Nachin L, Diez A, Nyström T. The bacterial universal stress protein: function and regulation. Curr Opin Microbiol 2003;6:140–5.
- [51] Bernier F, Berna A. Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? Plant Physiol Biochem 2001:39:545–54.
- [52] Reguera M, Bonilla I, Bolaños L. Boron deficiency results in induction of pathogenesis-related proteins from the PR-10 family during the legume-rhizobia interaction. J Plant Physiol 2010;167:625–32.
- [53] Kasprzewska A. Plant chitinases regulation and function. Cell Mol Biol Lett 2003;8:809–24.
- [54] Liu J-J, Ekramoddoullah A. The family 10 of plant pathogenesis-related proteins: their structure, regulation, and function in response to biotic and abiotic stresses. Physiol Mol Plant Pathol 2006;68:3–13.
- [55] Haizel T, Merkle T, Pay A, Fejes E, Nagy F. Characterization of proteins that interact with the GTP-bound form of the regulatory GTPase Ran in Arabidopsis. Plant J 1997;11:93–103.
- [56] Vernoud V, Horton A, Yang Z, Nielsen E. Analysis of the small GTPase gene superfamily of Arabidopsis. Plant Physiol 2003;131:1191–208.
- [57] Gatenby A, Viitanen P. Structural and functional aspects of chaperonin mediated protein folding. Annu Rev Plant Physiol Plant Mol Biol 1994;45:469–91.
- [58] Gromadski K, Wieden H, Rodnina M. Kinetic mechanism of elongation factor Ts-catalyzed nucleotide exchange in elongation factor Tu. Biochemistry 2002;41:162–9.
- [59] Yu Q, Wingender R, Schulz M, Baluska F, Goldbach H. Short-term boron deprivation induces increased levels of cytoskeletal proteins in Arabidopsis roots. Plant Biol 2001;3: 335–40.
- [60] Yu Q, Baluška F, Jasper F, Menzel D, Goldbach H. Short-term boron deprivation enhances levels of cytoskeletal proteins in maize, but not zucchini, root apices. Physiol Plant 2003;117:270–8.
- [61] Gunning B, Hardham A. Microtubules. Annu Rev Plant Physiol 1982:33:651–98.
- [62] Loomis W, Durst R. Chemistry and biology of boron. Biofactors 1992;3:229–39.
- [63] Rawson H. The developmental stage during which boron limitation causes sterility in wheat genotypes and the recovery of fertility. Aust J Plant Physiol 1996;23:709–17.

- [64] Behrendt U, Zoglauer K. Boron controls suspensor development in embryogenic cultures of Larix decidua. Physiol Plant 1996;97:321–6.
- [65] Bollaños L, de Esteban E, Lorenzo C, Fernandéz-Pascual M, de Felipe M, Gárate A, et al. Essentiality of boron for symbiotic dinitrogen fixation in pea (Pisum sativum)-Rhizobium nodules. Plant Physiol 1994;104:85–90.
- [66] Salzer P, Bonanami A, Beyer K, Vögeli-Lange R, Aeschbacher R, Lange J, et al. Differential expression of eight chitinase genes in Medicago truncatula roots during mycorrhiza
- formation, nodulation and pathogen infection. Mol Plant-Microbe Interact 2000;13:763–77.
- [67] Wan XY, Yu JY. Comparative proteomics analysis reveals an intimate protein network provoked by hydrogen peroxide stress in rice seedling leaves. Mol Cell Proteomics 2008;7:1469–88.
- [68] Ytterberg A, Jensen O. Modification-specific proteomics in plant biology. J Proteomics 2010;73:2249–66.
- [69] Bonilla I, Blevins D, Bolaños L. Boron functions in plants: looking beyond the cell wall. In: Taiz L, Zeiger E, editors. Plant physiology. Sunderland, MA: Sinauer Associates, Inc; 2009.



Supplementary Table 1.

		a .		pI/MW (kDa)		_	
ID		Protein identification ^a	Species	FC	Predicted ^b	Exp	Xcorr
225	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana	+	6.80/51.7	6.4/84	36.14
	O23264	Selenium-binding protein 1	Arabidopsis thaliana		5.37/54.1		20.16
252	Q10DV7	Actin-1	Oryza sativa	+	5.30/41.8	6.4/36	10.21
	Q96533	Alcohol dehydrogenase class-3	Arabidopsis thaliana		6.51/41.0		10.17
254	Q8GZN3	Malate dehydrogenase	Lupinus albus	+	6.10/35.6	6.3/38	60.19
	Q40676	Aldolase	Oryza sativa		6.55/38.7		16.20
255	Q9ZR33	Reversibly glycosylated polypeptide	Triticum aestivum	+	5.82/41.5	4.5/15	10.17
	Q8S3Q3	Putative uncharacterized protein	Oryza sativa		5.10/37.9		10.13
	A2Q396	Galactose mutarotase-like	Medicago truncatula		5.82/35.8		10.13
	D7SQC7	Unnamed protein product	Vitis vinifera				10.13
	P93819	Malate dehydrogenase	Arabidopsis thaliana		6.11/35.6		10.12
	Q40676	Fructose-bisphosphate aldolase	Oryza sativa		6.55/38.7		8.14
263	P12459	Tubulin β-1 chain	Glycine max	+	4.88/50.9	6.4/53	10.18
	Q9LEJ0	Enolase 1	Hevea brasiliensis		5.57/47.8		10.17
341	A9PHK1	Predicted protein	Populus trichocarpa	-	8.65/27.5	7.2/26	30.18
	Q3HVM0	Proteasome subunit α type	Solanum tuberosum		5.40/28.1		20.17
	Q9FMV1	Uncharacterized protein	Arabidopsis thaliana		9.01/27.6		20.14
	Q8H1K7	Ascorbate peroxidase	Retama raetam		5.88/23.6		16.19
357	Q7M1Z8	Globulin-2	Zea mays	-	6.16/49.9	6.6/26	30.14
	A9PHK1	Predicted protein	Populus trichocarpa		8.65/27.5		28.19
	Q9FMV1	Uncharacterized protein	Arabidopsis thaliana		9.01/27.6		20.16
	Q8S3Q3	Putative uncharacterized protein	Oryza sativa		5.10/37.9		18.19
358	A9U1X9	Predicted protein	Physcomitrella patens	-	6.75/31.1	6.2/28	10.18
	A9NZX3	Putative uncharacterized protein	Picea sitchensis		6.72/29.9		10.17

	P08477	Glyceraldehyde-3-phosphate dehydrogenase	Hordeum vulgare				10.15
363	P40941	ADP/ATP translocase 2	Arabidopsis thaliana	-	9.80/33.8	6.3/28	10.19
	Q9M7G2	Class I chitinase	Arabis lignifera				10.12
367	Q6W2J3	VDAC1.3	Lotus japonicus	-	6.71/29.6	7.8/29	40.21
	Q9SXU1	Proteasome subunit α type-7	Cicer arietinum		6.86/27.1		30.17
368	O24616	Proteasome subunit α type-7-B	Arabidopsis thaliana	-	8.73/27.3	6.0/29	10.15
	Q949H3	Putative class I chitinase	Hevea brasiliensis				10.15
	A9PI22	Putative uncharacterized protein	Populus trichocarpa		7.80/26.4		10.14
377	Q9SWE7	V-type proton ATPase subunit E	Citrus limon	-	7.13/26.3	5.3/30	20.16
	Q8W593	Probable lactoylglutathione lyase	Arabidopsis thaliana		5.27/32.2		20.15
	Q8S3Q3	Putative uncharacterized protein	Oryza sativa		5.10/37.9		18.13
410	Q8LPE5	Fructokinase-like protein	Cicer arietinum	2.2		5.1/34	30.15
	A2VC28	Spermidine synthase	Lotus japonicus				20.20
	P50346	60S acidic ribosomal protein P0	Glycine max		5.15/34.2		20.17
	P15590	Globulin-1 S allele	Zea mays		6.75/55.1		20.13
416	Q6VWJ5	Fructokinase 3	Solanum lycopersicum	-	5.57/41.5	7.5/34	30.17
	O80944	Aldo-keto reductase family 4 member C8	Arabidopsis thaliana		6.52/34.7		30.15
	O04397	Ferredoxin-NADP reductase	Nicotiana tabacum		8.10/35.4		20.16
418	A7LH72	GEM-like 1	Vitis vinifera	-	5.85/31.0	5.8/35	10.12
	Q39243	Thioredoxin reductase 1	Arabidopsis thaliana		6.96/39.6		8.16
420	Q9SPB8	Malate dehydrogenase	Glycine max	-	8.23/36.1	5.6/35	10.19
	Q0JGZ6	Fructokinase-1	Oryza sativa		5.07/34.7		10.14
422	Q94JJ0	Fructose-bisphosphate aldolase	Oryza sativa	-	8.81/42.0	7.0/35	30.18
	Q7Y256	Cysteine synthase	Betula pendula		6.38/38.2		18.12
428	A9P8P5	Putative uncharacterized protein	Populus trichocarpa	-1.6	4.94/35.3	3.4/35	20.18
	Q6Z1G7	Pyruvate dehydrogenase E1 component subunit β	Oryza sativa		5.25/39.9		20.11
431	Q6Z1G7	pyruvate dehydrogenase E1 component subunit β	Oryza sativa	-	5.25/39.9	5.3/36	20.16
	A9P8P5	Putative uncharacterized protein	Populus trichocarpa		4.94/35.3		20.11

110	0007013	Moloto dahridas comoso	Tamina all		6.01/25.5	6.0/27	70.20
446	Q8GZN2	Malate dehydrogenase	Lupinus albus	-	6.01/35.5	6.0/37	70.22
	P40691	Auxin-induced protein PCNT115	Nicotiana tabacum		7.10/33.9		18.16
452	Q8GZN2	Malate dehydrogenase	Lupinus albus	-	6.01/35.5	6.2/37	30.22
	Q40676	Fructose-bisphosphate aldolase	Oryza sativa		6.55/38.7		16.18
457	Q8GZN2	Malate dehydrogenase	Lupinus albus	-1.9	6.01/35.5	7.0/37	30.17
	Q94JJ0	Fructose-bisphosphate aldolase	Oryza sativa		8.81/42.0		20.14
	P08477	Glyceraldehyde-3-phosphate dehydrogenase	Hordeum vulgare				16.20
461	Q9M4M9	Fructose-bisphosphate aldolase	Persea americana	-	6.48/38.6	6.3/37	40.2
	Q8GZN2	Malate dehydrogenase	Lupinus albus		6.01/35.5		20.1
	Q9SMJ5	DTDP-glucose 4-6-dehydratase	Cicer arietinum		7.13/38.9		16.2
465	Q8GZN2	Malate dehydrogenase	Lupinus albus	-	6.01/35.5	6.0/38	40.1
	Q9M4M9	Fructose-bisphosphate aldolase	Persea americana		6.48/38.6		30.2
466	Q9ZR33	Reversibly glycosylated polypeptide	Triticum aestivum	-	5.82/41.5	5.7/38	20.1
	Q9M4M9	Fructose-bisphosphate aldolase	Persea americana		6.48/38.6		20.1
	A5B7D2	Putative uncharacterized protein	Vitis vinifera		5.26/40.6		20.1
471	O82705	RGP1 protein	Oryza sativa	+	8.21/39.5	5.6/39	10.1
	Q96533	Alcohol dehydrogenase class-3	Arabidopsis thaliana		6.51/40.7		10.1
	Q8S3Q3	Putative uncharacterized protein	Oryza sativa		5.10/37.9		10.1
	Q40676	Fructose-bisphosphate aldolase	Oryza sativa		6.55/38.7		8.19
476	O81796	Isocitrate dehydrogenase [NAD] regulatory subunit 3	Arabidopsis thaliana	-	6.13/37.1	6.5/52	20.2
	O82705	RGP1 protein	Oryza sativa		8.21/39.5		20.1
477	Q7XTJ3	Putative uncharacterized protein	Oryza sativa	-	6.16/46.1	5.9/40	30.2
	O81796	Isocitrate dehydrogenase [NAD] regulatory subunit 3	Arabidopsis thaliana		6.13/37.1		20.1
	O22263	Protein disulfide-isomerase like 2-1	Arabidopsis thaliana		5.65/37.2		20.1
487	Q96533	Alcohol dehydrogenase class-3	Arabidopsis thaliana		6.51/40.7	5.2/41	10.1
	B4FRH5	Succinyl-CoA ligase β-chain	Zea mays		6.08/45.2		10.1
	Q9XEF6	Hypothetical EIF-2-α	Arabidopsis thaliana		5.13/41.6		10.1
490	Q852R9	Pyruvate dehydrogenase E1 α subunit	Beta vulgaris	-	8.34/43.8	6.7/41	40.1

 496 Q852R9 Pyruvate dehydrogenase E1 α subunit P19168 Chalcone synthase 3 	Beta vulgaris	_	0.04/40.0		
P19168 Chalcone synthase 3			8.34/43.8	6.5/41	70.19
	Glycine max		5.89/42.4		20.15
Q9SXP2 Formate dehydrogenase 1, mitochondrial	Oryza sativa		6.20/39.3		18.17
497 P52903 Pyruvate dehydrogenase E1 component subr	nit α Solanum tuberosum	-	6.38/40.4	6.1/41	50.17
Q30D01 Putative 3-dehydroquinate synthase	Fagus sylvatica		8.38/50.1		40.17
A9NUF2 Putative uncharacterized protein	Picea sitchensis		8.65/49.5		20.17
500 Q96255 Phosphoserine aminotransferase	Arabidopsis thaliana	-		6.7/42	40.15
P50318 Phosphoglycerate kinase	Arabidopsis thaliana		5.04/42.6		20.25
501 Q30D01 Putative 3-dehydroquinate synthase	Fagus sylvatica	-	8.38/50.1	6.4/42	20.18
Q852R9 Pyruvate dehydrogenase E1 α subunit	Beta vulgaris		8.34/43.8		20.16
A5B2Z7 Putative uncharacterized protein	Vitis vinifera		7.17/43.9		20.16
502 P50318 Phosphoglycerate kinase	Arabidopsis thaliana	-	5.04/42.6	6.2/42	30.24
B5AGU9 12-oxophytodienoate reductase-like protein	Artemisia annua		7.72/43.2		20.21
A5B6G3 Putative uncharacterized protein	Vitis vinifera		8.12/49.4		20.14
503 P50318 Phosphoglycerate kinase	Arabidopsis thaliana	-	5.04/42.6	5.7/42	30.22
Q9XQ94 Glutamine synthetase	Medicago sativa		5.25/41.7		18.19
504 O82478 Alcohol dehydrogenase Adh-1	Glycine max	-		5.9/42	40.18
Q852R9 Pyruvate dehydrogenase E1 α subunit	Beta vulgaris		8.34/43.8		20.16
A5B2Z7 Putative uncharacterized protein	Vitis vinifera		7.17/43.9		20.16
507 O82478 Alcohol dehydrogenase Adh-1	Glycine max	-		6.2/42	30.19
P30074 Chalcone synthase 2	Medicago sativa		5.97/42.7		30.17
P52902 Pyruvate dehydrogenase E1 component subr	nnit α Pisum sativum				20.17
A5B2Z7 Putative uncharacterized protein	Vitis vinifera		7.17/43.9		20.16
509 Q9FUZ6 Elongation factor Tu	Zea mays	-	5.99/48.5	6.0/43	60.19
P50318 Phosphoglycerate kinase	Arabidopsis thaliana		5.04/42.6		20.25
510 P26563 Aspartate aminotransferase P2	Lupinus angustifolius	-	6.23/44.7	6.6/43	68.23
A9PGP7 Chorismate synthase	Populus trichocarpa		8.96/47.0		20.22

	Q96255	Phosphoserine aminotransferase	Arabidopsis thaliana				20.18
534	Q9SA73	T5I8.3 protein	Arabidopsis thaliana	-	6.35/44.5	6.7/46	40.16
	Q9LDQ7	S-adenosylmethionine synthase	Camellia sinensis		5.34/42.8		20.12
547	Q7Y0W8	Isocitrate dehydrogenase	Lupinus albus	-	5.99/46.0	6.1/47	28.15
	Q5KT13	Tryptophan synthase β subunit	Polygonum tinctorium		6.45/51.8		20.21
553	Q9XFS9	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Arabidopsis thaliana	-		5.8/47	20.17
	Q9LJL7	DNA/RNA binding protein-like	Arabidopsis thaliana		5.73/54.3		20.17
556	Q7Y0W8	Isocitrate dehydrogenase	Lupinus albus	-	5.99/46.0	5.9/47	46.23
	Q9XFS9	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Arabidopsis thaliana				30.20
566	A9PHT1	Putative uncharacterized protein	Populus trichocarpa	-	6.9/54.0	6.5/49	20.22
	A0FH76	EBP1	Solanum tuberosum		6.26/42.8		20.15
569	A9SGH3	Enolase	Physcomitrella patens	-	5.22/46.1	6.3/49	20.15
	P35683	Eukaryotic initiation factor 4A-1	Oryza sativa		5.37/47.1		18.24
578	A7PRX3	Putative uncharacterized protein	Vitis vinifera	4.0	8.44/55.4	7.3/50	20.18
	A9PHT1	Putative uncharacterized protein	Populus trichocarpa		6.90/54.0		20.17
585	A9SGH3	Enolase	Physcomitrella patens	-	5.22/46.1	5.7/50	20.16
	Q9FFR3	6-phosphogluconate dehydrogenase	Arabidopsis thaliana		5.62/53.3		18.14
592	O23960	Acetyl-CoA carboxylase	Glycine max	-	7.22/58.9	6.0/51	78.16
	A7PFD2	Putative uncharacterized protein	Vitis vinifera		6.56/53.3		40.16
593	Q9M434	Enolase	Lupinus luteus	-	5.14/47.8	5.2/50	50.20
	P17614	ATP synthase subunit β	Nicotiana plumbaginifolia		5.13/54.1		30.18
600	Q8W557	UDP-glucose pyrophosphorylase	Amorpha fruticosa	-	6.07/51.6	5.7/52	30.17
	O49169	Elongation factor 1-α	Manihot esculenta		9.20/49.4		20.16
604	A9PCX3	Serine hydroxymethyltransferase	Populus trichocarpa	+	7.18/51.9	5.5/51	38.20
	P19023	ATP synthase subunit β	Zea mays		5.19/54.1		28.17
	Q8W557	UDP-glucose pyrophosphorylase	Amorpha fruticosa		6.07/51.6		20.18
	P17614	ATP synthase subunit β	Nicotiana plumbaginifolia		5.13/54.1		20.17

	O49169	Elongation factor 1-α	Manihot esculenta		9.20/49.4		20.16
	P68172	Adenosylhomocysteinase	Nicotiana sylvestris		5.51/53.1		18.15
613	Q9ZTB2	Nodulin	Glycine max	-	5.74/52.6	5.9/53	40.16
	Q9SP37	Adenosylhomocysteinase	Lupinus luteus		5.64/53.3		20.18
619	P93508	Calreticulin	Ricinus communis	-1.4	4.36/45.5	4.3/52	38.18
	O04275	ATP synthase subunit β	Pisum sativum		6.63/60.2		20.17
621	Q9LEJ0	Enolase 1	Hevea brasiliensis	-	5.57/47.8	5.4/54	20.20
	O04275	ATP synthase subunit β	Pisum sativum		6.63/60.2		20.17
622	P19023	ATP synthase subunit β	Zea mays	-	5.19/54.1	5.3/53	28.19
	Q9LEJ0	Enolase 1	Hevea brasiliensis		5.57/47.8		20.16
624	O04275	ATP synthase subunit β	Pisum sativum	-1.6	6.63/60.2	5.2/52	30.19
	Q9LEJ0	Enolase 1	Hevea brasiliensis		5.57/47.8		20.23
625	Q40079	V-type proton ATPase subunit B 2	Hordeum vulgare	+	5.12/53.7	4.8/53	58.20
	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana		6.80/51.7		36.19
	Q9M5K3	Dihydrolipoyl dehydrogenase 1	Arabidopsis thaliana		6.24/49.9		30.17
628	Q8H2T7	Putative NADH dehydrogenase	Oryza sativa	-	8.31/55.3	6.9/55	78.24
	Q5F2M7	Pyruvate kinase	Glycine max		6.80/54.4		48.22
	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana		6.80/51.7		26.20
632	Q40079	V-type proton ATPase subunit B 2	Hordeum vulgare	+	5.12/53.7	5.6/53	58.18
	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana		6.80/51.7		48.22
	O23264	Selenium-binding protein 1	Arabidopsis thaliana		5.37/54.1		40.19
	A8IKE1	Alanine aminotransferase 1	Glycine max		5.32/53.3		30.21
	P31023	Dihydrolipoyl dehydrogenase	Pisum Sativum		6.06/49.7		30.18
	Q9LEJ0	Enolase 1	Hevea brasiliensis		5.57/47.8		20.22
634	P93508	Calreticulin	Ricinus communis	-	4.36/45.5	4.3/55	28.13
	Q70Z18	Nucleosome assembly protein 1-like protein 2	Nicotiana tabacum		4.34/43.2		20.23
637	Q9FNN5	NADH dehydrogenase	Arabidopsis thaliana	-	8.46/53.4	6.7/55	30.18
	P12862	ATP synthase subunit α	Triticum aestivum		5.70/55.3		30.14

638	P12862	ATP synthase subunit α	Triticum aestivum	-	5.70/55.3	6.2/55	40.18
	Q9M5K3	Dihydrolipoyl dehydrogenase 1	Arabidopsis thaliana		6.24/49.9		20.17
639	Q9ZTB2	Nodulin	Glycine max	-	5.74/52.6	5.9/55	40.17
	P12862	ATP synthase subunit α	Triticum aestivum		5.70/55.3		20.14
644	O23264	Selenium-binding protein 1	Arabidopsis thaliana	-2.2	5.37/54.1	5.7/55	30.19
	Q9SSV4	Inositol-3-phosphate synthase	Nicotiana paniculata		5.44/56.4		20.18
652	Q9FNN5	NADH dehydrogenase	Arabidopsis thaliana	-	8.46/53.4	7.1/55	60.19
	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana		6.80/51.7		54.21
	Q9ZWH7	Catalase	Oryza sativa		7.36/56.2		20.20
653	P31023	Dihydrolipoyl dehydrogenase	Pisum Sativum	-	6.06/49.7	6.4/55	40.18
	P12862	ATP synthase subunit α	Triticum aestivum		5.70/55.3		30.17
656	P31023	Dihydrolipoyl dehydrogenase	Pisum Sativum	-	6.06/49.7	6.9/56	50.17
	P12862	ATP synthase subunit α	Triticum aestivum		5.70/55.3		30.12
	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana		6.80/51.7		26.16
657	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana	-	6.80/51.7	6.6/56	26.21
	P31023	Dihydrolipoyl dehydrogenase	Pisum Sativum		6.06/49.7		20.16
661	P12862	ATP synthase subunit α	Triticum aestivum	-	5.70/55.3	6.2/57	30.16
	Q9C5J7	6-phosphofructokinase 7	Arabidopsis thaliana		6.87/53.5		18.12
669	Q96558	UDP-glucose 6-dehydrogenase	Glycine max	-	5.74/52.9	5.7/58	36.18
	P12862	ATP synthase subunit α	Triticum aestivum		5.70/55.3		30.16
670	P12862	ATP synthase subunit α	Triticum aestivum	-	5.70/55.3	6.0/58	30.14
	A7Q2D6	Putative uncharacterized protein	Vitis vinifera		6.04/58.3		14.18
672	Q9FZE1	T1K7.6 protein	Arabidopsis thaliana	-	5.84/53.0	6.0/58	20.17
	A7Q2D6	Putative uncharacterized protein	Vitis vinifera		6.04/58.3		14.17
677	B4F972	Glutamate decarboxylase	Zea mays	-	5.64/55.2	5.3/59	10.11
	Q9LEJ0	Enolase 1	Hevea brasiliensis		5.57/47.8		10.11
680	A7R2K3	Putative uncharacterized protein	Vitis vinifera	-	5.09/44.0	5.4/59	10.10
	A5ASG9	Putative uncharacterized protein	Vitis vinifera		8.82/57.9		10.10

709	Q05045	Precursor Chaperonin CPN60-1, mitochondrial	Cucurbita maxima	-1.7	5.09/57.4	5.4/62	184.23
	Q76E42	Glucose-6-phosphate isomerase	Oryza sativa		5.88/68.3		28.19
711	P21343	Pyrophosphate-fructose 6-phosphate 1-phosphotransferase	Solanum tuberosum	-	6.2/60.4	6.0/65	60.19
	P15590	subunit β Globulin-1 S allele	Zea mays		6.75/55.1		30.19
	Q653F6	Putative t-complex protein 1 theta chain	Oryza sativa		6.16/60.3		20.20
719	P21343	Pyrophosphate-fructose 6-phosphate 1-phosphotransferase subunit β	Solanum tuberosum	-	6.20/60.4	6.0/65	40.15
	Q653F6	Putative t-complex protein 1 theta chain	Oryza sativa		6.16/60.3		30.19
	P15590	Globulin-1 S allele	Zea mays		6.75/55.1		30.18
724	Q9FMP3	Dihydropyrimidinase	Arabidopsis thaliana	-	5.58/58.0	5.9/66	10.21
	Q9LIR4	Putative dihydroxyacid dehydratase	Arabidopsis thaliana		5.85/64.9		10.13
	A9P0C2	Putative uncharacterized protein	Picea sitchensis		5.84/34.7		10.12
740	P31405	V-type proton ATPase catalytic subunit A	Gossypium hirsutum	-3.5	5.36/68.5	5.7/73	98.22
	Q9ZSQ4	Phosphoglucomutase	Populus tremula		5.49/63.1		58.19
	Q38931	70 kDa peptidyl-prolyl isomerase	Arabidopsis thaliana		5.24/61.5		20.16
741	B9RHV0	ATP synthase α	Ricinus communis	-	5.31/63.2	5.5/74	20.19
	Q38931	70 kDa peptidyl-prolyl isomerase	Arabidopsis thaliana		5.24/61.5		20.16
744	B8PUQ5	Malic enzyme	Triticum aestivum	-	6.51/71.0	6.2/74	28.15
	P15590	Globulin-1 S allele	Zea mays		6.75/55.1		20.17
772	Q43644	NADH-ubiquinone oxidoreductase 75 kDa subunit	Solanum tuberosum	-	5.52/77.1	6.0/106	10.17
	Q6K8J4	Putative uncharacterized protein	Oryza sativa		5.64/82.2		10.14
792	O23755	Elongation factor 2	Beta vulgaris	-	5.93/93.8	6.0/142	60.16
	P49608	Aconitate hydratase	Cucurbita maxima		5.74/98.0		30.13

 ^a Protein identification according to the UniProt database (http://www.uniprot.org)
 ^b Predicted pI and MW (kDa) were calculated by using an ExPASy tool (http://www.expasy.org)
 ^c Cross-correlation score (Xcorr)

Supplementary Table 2.

		Protein identification ^b					a		
ID ^a		Polypetides	Species	MH+	Z	P ^c	Xcorr ^d	Cov (%)	UnPep
	Energy path	ways							
146	P08477	Glyceraldehyde-3-phosphate dehydrogenase	Hordeum vulgare			3.14X10 ⁻⁵	18.15	10.8	2
		RVPTVDVSVVDLTVRL RAASFNIIPSSTGAAKA		1498.84753 1434.75867	2 2	3.14X10 ⁻⁵ 1.85X10 ⁻²	3.04 2.69		
216	Q8W557	UDP-glucose pyrophosphorylase	Amorpha fruticosa			1.03X10 ⁻⁷	86.20	18.9	7
		KSAVAGLNQISENEKS RANPENPTVELGPEFKK RYLSGEAQHVEWSKI KLEIPDGAVIANKD KGGTLISYEGRV RLVVDDFLPLPSKG KKVSNFLSRF KVSNFLSRF KYGSNVPLLLMNSFNTHDDTQKI		1459.73877 1641.81189 1533.73328 1239.69434 1052.53711 1342.76172 950.54181 822.44684 2410.13435	2 2 2 2 2 2 2 2 2 4	1.03X10 ⁻⁷ 1.82X10 ⁻⁵ 2.59X10 ⁻⁵ 2.49X10 ⁻³ 4.15X10 ⁻² 2.11X10 ⁻² 3.41X10 ⁻² 3.64X10 ⁻² 1.14X10 ⁻¹	3.93 3.29 3.17 2.94 2.64 2.60 2.42 2.38 1.63		
232	P49608	Aconitate hydratase	Cucurbita maxima			3.28X10 ⁻⁶	30.26	5.9	3
		RSENAVQANMELEFQRN KSAGQDTIVLAGAEYGSGSSRD KTSLAPGSGVVTKY		1781.81229 1925.91992 1116.62585	3 3 2	3.28X10 ⁻⁶ 3.46X10 ⁻³ 1.99X10 ⁻⁴	5.14 2.91 2.18		
245	Q41712	Ascorbate peroxidase	Vigna unguiculata			3.49X10 ⁻⁵	46.23	28.4	5
		KAMGLSDQDIVALSGGHTIGAAHKERS KAMGLSDQDIVALSGGHTIGAAHKE RSGFEGPWTSNPLIFDNSYFKE KTGGPFGTIKH KSYPTVSADYQKA		2550.27291 2265.12921 2306.07642 877.47778 1258.59497	4 4 2 2 2 2	5.26X10 ⁻⁴ 3.49X10 ⁻⁵ 6.42X10 ⁻⁴ 1.39X10 ⁻¹ 9.61X10 ⁻⁴	4.16 3.69 2.71 2.30 2.19		
251	Q38799	Pyruvate dehydrogenase E1 component subunit	Arabidopsis thaliana			1.17X10 ⁻⁶	8.18	5.0	1
		RDALNSAIDEEMSADPKV		1721.75344	2	1.17X10 ⁻⁶	3.59		
264	Q40079	V-type proton ATPase subunit B2	Hordeum vulgare			6.38X10 ⁻⁵	28.15	8.1	3
		KTPVSLDMLGRI KAVVQVFEGTSGIDNKY KYQEIVNIRL		1104.57177 1563.80127 1034.56287	2 2 2	6.38X10 ⁻⁵ 2.15X10 ⁻⁴ 2.53X10 ⁻¹	2.98 2.73 2.21		
340	Q41712	Ascorbate peroxidase	Vigna unguiculata			1.64X10 ⁻⁴	28.23	19.6	3
		KSYPTVSADYQKA KAMGLSDQDIVALSGGHTIGAAHKE KTGGPFGTIKH		1258.59497 2265.12921 877.47778	3 2 2	1.08X10 ⁻³ 1.64X10 ⁻⁴ 1.54X10 ⁻³	3.37 2.65 2.33		
346	Q43758	Ascorbate peroxidase	Glycine max			2.11X10 ⁻⁴	30.17	14.8	3

		KSYPTVSADYQKA KSYPTVSADYQKA		1258.59497 1258.59497	3	2.52X10 ⁻² 1.65X10 ⁻²	3.43 3.40		
		KTGGPFGTIKH KGSDHLRDVFGKA		877.47778 1230.62256	2 2	1.21X10 ⁻² 2.11X10 ⁻⁴	3.18 2.57		
		KGSDHLRDVFGKA		1230.62256	2	2.72X10 ⁻²	2.37		
		KSYPTVSADYQKA		1258.59497	2	6.33X10 ⁻⁴	2.03		
347	Q8H1K7	Ascorbate peroxidase	Retama raetam			1.41X10 ⁻⁴	16.21	17.7	2
		KAMGLSDQDIVALSGGHTIGAAHKE RLAWHSAGTFDVKT		2265.12921 1331.67432	3 4	1.41X10 ⁻⁴ 4.47X10 ⁻²	4.12 2.63		
421	Q0JGZ6	Fructokinase-1	Oryza sativa			1.35X10 ⁻⁷	10.17	5.3	1
		KAPGGAPANVAIAVARL		1334.75391	2	1.35X10 ⁻⁷	3.43		
442	Q8GZN3	Malate dehydrogenase	Lupinus albus			5.65X10 ⁻⁴	30.15	11.7	3
		RLNVQVSDVKN		1001.56256	2	3.81X10 ⁻³	3.07		
		KLDLTAEELSEEKA KINOCL CIDEESBY		1376.67908	2 2	6.08X10 ⁻⁴ 5.65X10 ⁻⁴	2.91		
443	09C7N2	KIVQGLGIDEFSRK Molete dehydrogenese	Luninus albus	1333.71106	2	4.74X10 ⁻⁶	2.91 40.19	11.7	4
443	Q8GZN3	Malate dehydrogenase KLDLTAEELSEEKA	Lupinus albus	1376.67908	2	4.74X10 4.74X10 ⁻⁶	3.68	11./	4
		KIVQGLGIDEFSRK		1376.67908	2	4.74X10 1.72X10 ⁻⁵	3.08		
		RLNVQVSDVKN		1001.56256	2	2.67X10 ⁻³	2.69		
		KKLDLTAEELSEEKA		1504.77405	2	1.23X10 ⁻²	2.05		
469	Q40676	Fructose-bisphosphate aldolase	Oryza sativa			7.43X10 ⁻⁵	8.17	4.5	1
		KGILAADESTGTIGKR		1332.70056	2	7.43X10 ⁻⁵	3.50		
478	Q9SXP2	Formate dehydrogenase 1, mitochondrial	Oryza sativa			1.81X10 ⁻⁵	20.22	3.5	1
		KYEEDLDAMLPKC		1084.64734	2	5.07X10 ⁻⁵	3.71		
		KYEEDLDAMLPKC		956.55237	2	1.79X10 ⁻¹	2.98		
488	P52901	Pyruvate dehydrogenase E1 component subunit α-1	Arabidopsis thaliana			1.81X10 ⁻⁴	40.17	9.5	4
		RRMEIAADSLYKA		1312.65656	3	1.02X10 ⁻²	3.33		
		KRGDYVPGLKV RMEIAADSLYKA		1004.55237 1140.56055	2 2	1.64X10 ⁻² 1.81X10 ⁻⁴	2.68 2.61		
		KWEIAADSETKA KGPIILEMDTYRY		1323.66131	2	1.23X10 ⁻¹	2.06		
493	P50318	Phosphoglycerate kinase	Arabidopsis thaliana			9.24X10 ⁻⁵	28.17	9.4	3
		KGVTTIIGGGDSVAAVEKV		1573.84314	2	4.57X10 ⁻⁴	2.90		
		KELDYLVGAVSNPKR		1404.73694	2	9.24X10 ⁻⁵	2.56		
		KRPFAAIVGGSKV		1102.63672	2	3.47X10 ⁻²	2.53		
498	P52902	Pyruvate dehydrogenase E1 component subunit	Pisum sativum			3.40X10 ⁻⁵	40.18	9.6	4
		KGYGVEAFGVDRK		1169.55859	2	2.98X10 ⁻²	3.64		
		KNGPIILEMDTYRY KDCIITAYRD		1437.70424 1011.49279	2 2	3.40X10 ⁻⁵ 3.19X10 ⁻¹	2.91 2.51		
		KGYGVEAFGVDRKE		1297.65356	2	$2.05X10^{-3}$	2.46		
499	Q9SAJ4	Phosphoglycerate kinase	Arabidopsis thaliana			4.10X10 ⁻¹²	66.25	20.0	6
	-	KFLKPSVAGFLMQKE		1465.82361	3	1.54X10 ⁻³	4.91		
		KFLKPSVAGFLMQKE		1481.81848	3	1.43X10 ⁻²	4.85		

511	DE0219	KLAALADVYVNDAFGTAHRA KKLAALADVYVNDAFGTAHRA RVDLNVPLDDNSNITDDTRI KELDYLVGAVANPKK KYSLKPLVPRL	Aa.l.: J	1903.96606 2032.06104 2015.95166 1388.74207 1072.65137	3 4 2 2 2	3.78X10 ⁻⁵ 6.40X10 ⁻³ 4.10E ⁻¹ 2 3.19X10 ⁻⁵ 6.58X10 ⁻⁵ 2.76X10 ⁻⁴	3.75 3.58 3.56 3.53 2.23	7.1	2
511	P50318	Phosphoglycerate kinase KELDYLVGAVSNPKR	Arabidopsis thaliana	1404.73694	2	6.18X10 ⁻⁴	20.16 3.26	7.1	2
		KGVTTIIGGGDSVAAVEKV		1573.84314	2	2.76X10 ⁻⁴	2.92		
536	Q7Y0W9	NADP-specific isocitrate dehydrogenase	Lupinus albus			9.69X10 ⁻⁶	106.21	26.0	9
		KLEAACIGAVESGKM RAFAEASMTTAYEKK RLIDDMVAYAVKS RLIDDMVAYAVKS KTIESEAAHGTVTRH KINVANPIVEMDGDEMTRV RAFAEASMTTAYEKK KFEAAGIWYEHRL KYYDLGLPYRD KLLDFTQKL RAFAEASMTTAYEKKW		1304.65148 1435.64097 1253.64460 1237.64966 1371.68628 1935.87865 1419.64612 1378.65381 1159.57825 864.48254 1563.73594	2 2 2 2 2 3 2 3 2 2 2 2	8.63X10 ⁻⁴ 4.35X10 ⁻⁵ 2.43X10 ⁻⁴ 1.56X10 ⁻⁵ 9.69X10 ⁻⁶ 8.26X10 ⁻⁵ 1.82X10 ⁻³ 7.05X10 ⁻² 2.49X10 ⁻⁴ 1.19X10 ⁻¹ 4.13X10 ⁻¹	3.82 3.71 3.68 3.59 3.50 2.88 2.68 2.66 2.55 2.54 2.14		
551	Q7Y0W8	NADP-specific isocitrate dehydrogenase	Lupinus albus			1.67X10 ⁻⁴	30.24	10.7	3
		KKLEEACIGAVESGKM RLIDDMVAYAVKS RAFADASMTTAYEKK	,	1490.75192 1253.64460 1421.62532	3 2 2	3.65X10 ⁻⁴ 2.84X10 ⁻⁴ 1.67X10 ⁻⁴	4.88 3.03 2.82		
557	B0FGG5	Monodehydroascorbate reductase	Vaccinium corymbosum			6.54X10 ⁻⁶	20.21	2.8	1
		KLTDFGVQGADAKN KAYLFPESPARL		1221.61096 1150.58911	2 2	6.54X10 ⁻⁶ 2.62X10 ⁻²	2.65 2.02		
561	P93033	Fumarate hydratase 1	Arabidopsis thaliana			1.27X10 ⁻⁵	20.17	5.9	2
		RDTFGPIQVPSDKL KVNMEYGLDPTIGKA		1303.65283 1452.70391	2 2	1.27X10 ⁻⁵ 2.12X10 ⁻²	3.35 2.23		
562	B0FGG5	Monodehydroascorbate reductase	Vaccinium corymbosum			8.42X10 ⁻⁴	20.18	2.8	1
		KLTDFGVQGADAKN KAYLFPESPARL		1221.61096 1150.58911	2 2	8.42X10 ⁻⁴ 1.45X10 ⁻¹	3.67 2.22		
572	A9SGH3	Enolase	Physcomitrella patens			5.78X10 ⁻⁴	20.18	2.6	1
		RIEEELGNVRY RGNPTVEVDLVTDRV		1058.54761 1414.71729	2 2	5.99X10 ⁻³ 5.78X10 ⁻⁴	3.69 2.22		
588	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating	Arabidopsis thaliana			4.63X10 ⁻⁴	18.18	5.1	2
		KICSYAQGMNLLRA KGFPISVYNRT		1441.69263 1052.55237	2 2	1.68X10 ⁻² 4.63X10 ⁻⁴	2.88 2.26		
599	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating	Arabidopsis thaliana			4.64X10 ⁻⁶	24.23	9.0	3
		KGLLYLGMGVSGGEEGARN KICSYAQGMNLLRA KGFPISVYNRT		1681.82140 1441.69263 1052.55237	2 2 2	4.64X10 ⁻⁶ 7.84X10 ⁻² 3.95X10 ⁻¹	4.66 2.76 2.42		
607	Q9LI00	6-phosphogluconate dehydrogenase, decarboxylating	Oryza sativa			7.76X10 ⁻⁴	16.16	2.9	1

		RDRLPANLVQAQRD KGLLYLGMGVSGGEEGARN		1380.77063 1681.82140	3 2	4.06X10 ⁻¹ 7.76X10 ⁻⁴	3.28 2.48		
611	Q9LEJ0	Enolase 1	Hevea brasiliensis			2.14X10 ⁻⁴	10.12	3.8	1
		KVNQIGSVTESIEAVKM		1573.84314	2	2.14X10 ⁻⁴	2.49		
626	Q40079	V-type proton ATPase subunit B2	Hordeum vulgare			2.29X10 ⁻⁴	58.19	14.1	6
		KAVVQVFEGTSGIDNKY RKFVAQGAYDTRN KFVAQGAYDTRN KTPVSLDMLGRI KYQEIVNIRL RTVSGVAGPLVILDKV		1563.80127 1255.64294 1127.54797 1104.57177 1034.56287 1368.80969	2 3 2 2 2 2	2.29X10 ⁻⁴ 2.04X10 ⁻² 1.35X10 ⁻² 4.51X10 ⁻⁴ 1.47X10 ⁻¹ 1.04X10 ⁻²	3.88 3.30 3.17 3.12 2.72 2.15		
631	Q9FNN5	Subunit of complex I	Arabidopsis thaliana			5.16X10 ⁻⁵	40.18	13.8	4
		KLEEIDMLQEVTKQ KAVQSGLGTAAVIVMDKS RASAAYIYIRG KVSDGRPSYLVVNADESEPGTCKD		1463.72979 1575.84107 1027.55713 2380.10853	2 3 2 3	5.16X10 ⁻⁵ 1.49X10 ⁻² 3.97X10 ⁻⁴ 6.11X10 ⁻⁴	3.61 3.48 3.27 2.60		
636	P12862	ATP synthase subunit α	Triticum aestivum			3.51X10 ⁻⁴	30.16	7.5	3
		RTGSIVDVPAGKA RVVSVGDGIARV RAAELTTLLESRM		1043.57312 972.54724 1203.65796	2 2 2	1.31X10 ⁻³ 3.51X10 ⁻⁴ 3.53X10 ⁻³	3.20 2.85 2.68		
640	P12862	ATP synthase subunit α	Triticum aestivum			1.01X10 ⁻⁴	30.14	7.5	3
		RAAELTTLLESRM RTGSIVDVPAGKA RVVSVGDGIARV		1203.65796 1043.57312 972.54724	2 2 2	7.28X10 ⁻² 2.42X10 ⁻³ 1.01X10 ⁻⁴	2.80 2.59 2.38		
641	P49357	Serine hydroxymethyltransferase 1	Flaveria pringlei			1.60X10 ⁻⁵	18.17	6.8	2
		KNTVPGDVSAMVPGGIRM RLNESTGYIDYDQLEKS		1585.80027 1787.83337	3 2	2.69X10 ⁻² 1.60X10 ⁻⁵	2.92 2.25		
646	P12862	ATP synthase subunit α	Triticum aestivum			2.54X10 ⁻⁴	16.17	7.7	4
		RTGSIVDVPAGKA RAAELTTLLESRM RVVSVGDGIARV KRTGSIVDVPAGKA		1043.57312 1203.65796 972.54724 1199.67432	2 2 2 2	5.34X10 ⁻² 4.89X10 ⁻⁴ 3.60X10 ⁻⁴ 1.45X10 ⁻²	3.25 3.17 2.71 2.58		
660	O23254	Serine hydroxymethyltransferase	Arabidopsis thaliana			1.52X10 ⁻⁶	26.21	9.3	3
		KANAVALGNYLMSKG KLLICGGSAYPRD KISATSIYFESLPYKV		1367.69876 1206.62995 1618.83630	2 2 2	6.89X10 ⁻⁶ 6.35X10 ⁻⁵ 1.52X10 ⁻⁶	3.97 3.33 3.10		
662	B9SH74	Aldehyde dehydrogenase, putative	Ricinus communis			4.35X10 ⁻⁶	10.22	4.4	1
		RSGVESGATLETGGDRF		1435.66589	2	4.35X10 ⁻⁶	4.31		
700	Q42919	Glucose-6-phosphate 1-dehydrogenase	Medicago sativa			3.03X10 ⁻⁴	10.13	2.5	1
		RGPAEADELLEKA		1171.58411	2	3.03X10 ⁻⁴	2.53		
738	O82663	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1	Arabidopsis thaliana			6.48X10 ⁻⁴	20.13	5.4	2

		KGSDWLGDQDAIQYMCRE RTQETLEEGCQLIDKA		1930.80582 1663.78434	2 2	6.48X10 ⁻⁴ 1.53X10 ⁻³	2.59 2.37		
739	P31405	V-type proton ATPase catalytic subunit A	Gossypium hirsutum			1.05X10 ⁻⁵	60.17	10.9	6
		KLAADTPLLTGQRV KDTVLELEFQGVKK RSGDVYIPRG KITYIAPPGQYSLKD REDYLAQNAFTPYDKF KRSGDVYIPRG		1255.70044 1377.72607 906.46796 1450.79407 1674.76465 1062.56909	2 2 2 2 2 2 2	1.05X10 ⁻⁵ 8.44X10 ⁻³ 4.67X10 ⁻² 7.02X10 ⁻² 2.35X10 ⁻⁴ 1.32X10 ⁻¹	3.49 3.10 2.57 2.41 2.28 2.13		
771	Q9FGI6	NADH-ubiquinone oxidoreductase 75 kDa subunit	Arabidopsis thaliana			1.51X10 ⁻⁵	26.15	6.0	3
		RGSGEEIGTYVEKL RLNEDINEEWISDKT RFASEVAGVQDLGILGRG		1268.60046 1604.74390 1631.87512	2 2 2	4.94X10 ⁻² 1.87X10 ⁻³ 1.51X10 ⁻⁵	2.69 2.68 2.48		
780	P49608	Aconitate hydratase	Cucurbita maxima			6.35X10 ⁻⁹	40.22	7.0	4
		RSENAVQANMELEFQRN KSAGQDTIVLAGAEYGSGSSRD KTSLAPGSGVVTKY KGPMLLGVKA		1781.81229 1925.91992 1116.62585 830.48044	3 3 2 2	6.35X10 ⁻⁹ 1.43X10 ⁻⁴ 1.68X10 ⁻⁵ 4.07X10 ⁻¹	4.42 4.09 2.63 2.56		
783	P49608	Aconitate hydratase	Cucurbita maxima			1.25X10 ⁻⁷	40.18	7.0	4
		RSENAVQANMELEFQRN KSAGQDTIVLAGAEYGSGSSRD KTSLAPGSGVVTKY KGPMLLGVKA		1781.81229 1925.91992 1116.62585 830.48044	2 3 2 2	1.25X10 ⁻⁷ 1.79X10 ⁻³ 9.20X10 ⁻⁴ 2.17X10 ⁻¹	3.42 2.71 2.60 2.53		
784	P49608	Aconitate hydratase	Cucurbita maxima			3.92X10 ⁻⁵	40.17	7.0	4
		KSAGQDTIVLAGAEYGSGSSRD RSENAVQANMELEFQRN KTSLAPGSGVVTKY KGPMLLGVKA		1925.91992 1781.81229 1116.62585 830.48044	3 2 2 2	1.05X10 ⁻³ 3.92X10 ⁻⁵ 1.93X10 ⁻⁴ 2.58X10 ⁻¹	2.58 2.57 2.44 2.34		
785	P49608	Aconitate hydratase	Cucurbita maxima			1.33X10 ⁻⁷	30.20	4.6	3
		RSENAVQANMELEFQRN KTSLAPGSGVVTKY KGPMLLGVKA		1781.81229 1116.62585 830.48044	2 2 2	1.33X10 ⁻⁷ 2.94X10 ⁻⁴ 2.01X10 ⁻²	4.06 2.39 2.31		
786	P49608	Aconitate hydratase	Cucurbita maxima			1.49X10 ⁻⁴	20.14	4.3	2
		RSENAVQANMELEFQRN KSAGQDTIVLAGAEYGSGSSRD		1781.81229 1925.91992	2 2	1.49X10 ⁻⁴ 6.51X10 ⁻⁴	2.77 2.66		
788	P49608	Aconitate hydratase	Cucurbita maxima			1.35X10 ⁻⁴	30.15	5.5	3
		RSENAVQANMELEFQRN KGPMLLGVKA KSAGQDTIVLAGAEYGSGSSRD		1781.81229 830.48044 1925.91992	2 2 2	1.35X10 ⁻⁴ 3.21X10 ⁻¹ 3.15X10 ⁻³	3.05 2.23 2.08		
790	P49608	Aconitate hydratase	Cucurbita maxima			2.30X10 ⁻⁷	40.21	7.0	4
		RSENAVQANMELEFQRN KTSLAPGSGVVTKY KSAGQDTIVLAGAEYGSGSSRD KGPMLLGVKA		1781.81229 1116.62585 1925.91992 830.48044	2 2 2 2	2.30X10 ⁻⁷ 2.79X10 ⁻⁵ 1.47X10 ⁻² 4.72X10 ⁻¹	3.64 2.49 2.36 2.18		

	Protein metal	bolism							
134	O49886	Peptidyl-prolyl cis-trans isomerase	Lupinus luteus			4.08X10 ⁻⁵	30.15	16.8	2
		RIVMELYADTTPRT		1424.70899	2	4.08X10 ⁻⁵	3.00		
		RIVMELYADTTPRT KTSRPVTIADCGQLS		1408.71411 1504.74242	2 2	1.42X10 ⁻² 1.02X10 ⁻³	2.63 2.16		
136	Q40682	Elongation factor 1-δ2	Oryza sativa			8.31X10 ⁻⁴	20.16	8.9	2
		KLDEYLLTRS RSYISGYQASKD		1022.55170 1103.53674	2 2	6.43X10 ⁻² 8.31X10 ⁻⁴	3.11 2.41		
247	Q94JX9	Nascent polypeptide-associated complex subunit α-	Arabidopsis thaliana	1103.33074		4.82X10 ⁻⁴	20.18	13.4	2
,	Q) 1012)	like protein 2 KSPHSETYVIFGEAKI	Thuo tao pala manana	1564.76416	3	3.79X10 ⁻³	3.57	10	_
		KNVLFFISKPDVFKS		1553.87268	2	4.82X10 ⁻⁴	2.11		
259	Q9LS40	CND41, chloroplast nucleoid DNA binding protein- like	Arabidopsis thaliana			6.19X10 ⁻⁶	10.13	3.4	1
		KATSFSYCLVDRDSGKS		1705.78501	2	6.19X10 ⁻⁶	2.51		
91	O49886	Peptidyl-prolyl cis-trans isomerase	Lupinus luteus			6.42X10 ⁻⁵	40.16	22.7	3
		KTSRPVTIADCGQLS		1504.74242	2	6.42X10 ⁻⁵	3.21		
		RIVMELYADTTPRT RIVMELYADTTPRT		1424.70899 1408.71411	2 2	2.34X10 ⁻³ 8.94X10 ⁻⁴	3.11 2.85		
		KFADENFIKR		983.48328	2	1.92X10 ⁻¹	2.65		
332	Q3HVM0	Proteasome subunit α type	Solanum tuberosum			9.01X10 ⁻⁴	20.16	11.3	2
		KAAAIGANNQAAQSILKQ		1540.84424	3 2	1.16X10 ⁻²	3.16		
345	A9TVH1	KDGVVLVGEKK Proteasome subunit α type	Physcomitrella patens	915.51459	2	9.01X10 ⁻⁴ 1.85X10 ⁻⁶	3.06 20.17	11.4	2
, 15	71,71,111	KYIGLLATGMTADAKS	1 hyscommena patens	1440.74029	2	1.85X10 ⁻⁶	3.31	11.1	_
		KAAGITSIGVRG		944.55237	2	1.30X10 ⁻¹	2.16		
352	A5AXI5	Proteasome subunit α type	Vitis vinifera			9.71X10 ⁻⁸	50.23	27.7	5
		RDGPQLYMVEPSGVSYRY		1813.84253	2 2	9.71X10 ⁻⁸	4.31		
		RHSGMAVAGLAADGRQ KAVDNSGTVIGIKC		1328.63756 1173.64734	2	4.00X10 ⁻⁴ 3.71X10 ⁻³	3.17 3.15		
		RVFQIEYAAKA		1068.57239	2	1.29X10 ⁻³	2.68		
		KIIYGVHDEAKD		1144.59973	2	1.18X10 ⁻³	2.57		
354	A9TVH1	Proteasome subunit α type	Physcomitrella patens			4.10X10 ⁻⁸	10.21	6.5	1
		KYIGLLATGMTADAKS		1440.74029	2	2.46X10 ⁻⁵	4.10		
512	A9PEP6	Predicted protein	Populus trichocarpa			1.86X10 ⁻⁶	50.21	14.6	4
		KAFVDSGAQSTIISKS		1423.74268	2	1.86X10 ⁻⁶	4.30		
		RYKGIAHGVGQSEILGRI RVGGGEVSVPFLOEKD		1684.91296 1445.76343	3 2	2.77X10 ⁻³ 1.89X10 ⁻²	4.25 4.14		
		RGIAHGVGOSEILGRI		1393.75464	2	2.96X10 ⁻⁴	3.03		
		KLVELGFGRE		890.50940	2	4.45X10 ⁻²	2.24		
513	Q9ZRU6	Elongation factor Tu	Catharanthus roseus			7.37X10 ⁻⁶	50.22	21.7	4
		RGSALSALQGTNEEIGRK		1602.80823	2	1.54X10 ⁻³	4.48		
					_				

		KFPGDEIPIIRG RTADITGKVELPENVKM KLMDAVDEYIPDPVRV RGSALSALQGTNEEIGRKA		1156.63611 1613.87451 1648.78870 1730.90320	2 2 2 2	1.40X10 ⁻⁴ 7.37X10 ⁻⁶ 4.97X10 ⁻⁴ 2.67X10 ⁻⁴	3.09 2.54 2.23 2.20		
573	A0FH76	EBP1	Solanum tuberosum			9.96X10 ⁻⁵	20.15	6.7	1
		KVVLSVSNPDTRV KIVEGVLSHQMKQ		1186.64258 1256.66673	2 2	9.96X10 ⁻⁵ 3.97X10 ⁻²	2.95 2.90		
584	Q0DDX2	26S protease regulatory subunit 7	Oryza sativa			7.39X10 ⁻⁵	40.13	13.4	4
		RSVCTEAGMYAIRA KGVLCYGPPGTGKT KTYGLGPYSTSIKK RFDDGVGGDNEVQRT		1373.61880 1205.59832 1286.66272 1407.61353	2 2 2 2	7.39X10 ⁻⁵ 1.10X10 ⁻² 9.88X10 ⁻² 4.89X10 ⁻²	2.46 2.40 2.05 2.04		
645	Q6K669	Leucine aminopeptidase 2	Oryza sativa			1.03X10 ⁻⁴	10.15	2.7	1
		KFDMGGSAAVFGAAKA		1344.62526	2	1.03X10 ⁻⁴	2.03		
693	P21239	RuBisCO large subunit-binding protein subunit α	Brassica napus			1.09X10 ⁻⁷	64.20	15.4	7
		KVGAATETELEDRK KTNDSAGDGTTTASVLARE RGYISPQFVTNPEKL KELSETDSVYDSEKL KDSTTLIADAASKDELQARI KVGAATETELEDRKL KDSTTLIADAASKD		1290.61719 1636.77734 1479.74780 1501.65405 1904.95593 1418.71216 1192.60559	2 2 2 2 3 2 2	4.83X10 ⁻⁶ 1.09X10 ⁻⁷ 1.18X10 ⁻⁶ 4.54X10 ⁻⁴ 3.04X10 ⁻⁷ 3.24X10 ⁻³ 2.99X10 ⁻²	4.02 3.98 3.55 3.01 2.87 2.83 2.58		
694	P21239	RuBisCO large subunit-binding protein subunit α	Brassica napus			4.71X10 ⁻⁴	26.14	9.0	3
		KDSTTLIADAASKDELQARI RGYISPQFVTNPEKL KVGAATETELEDRK		1904.95593 1479.74780 1290.61719	2 2 2	4.71X10 ⁻⁴ 3.06X10 ⁻³ 8.42X10 ⁻³	2.80 2.56 2.34		
699	Q940P8	Putative uncharacterized protein	Arabidopsis thaliana			2.47X10 ⁻⁴	30.20	8.2	3
		RVDEIITCAPRR RGASHHVLDEAERS RMASFVGAMAISDLVKS		1173.59323 1320.62915 1555.78586	2 3 2	2.80X10 ⁻⁴ 2.47X10 ⁻⁴ 1.06X10 ⁻³	3.58 3.36 2.53		
701	P21239	RuBisCO large subunit-binding protein subunit $\boldsymbol{\alpha}$	Brassica napus			8.30X10 ⁻⁸	74.20	15.6	8
		KTNDSAGDGTTTASVLARE RGYISPQFVTNPEKL KDSTTLIADAASKDELQARI KVGAATETELEDRK KELSETDSVYDSEKL KKELSETDSVYDSEKL KVGAATETELEDRKL KOSTTLIADAASKD		1636.77734 1479.74780 1904.95593 1290.61719 1501.65405 1629.74902 1418.71216 1192.60559	2 2 3 2 2 3 2 2 2	8.30X10 ⁻⁸ 2.78X10 ⁻⁶ 1.54X10 ⁻⁷ 1.76X10 ⁻⁶ 4.99X10 ⁻⁵ 3.00X10 ⁻² 9.83X10 ⁻⁵ 4.92X10 ⁻²	4.03 3.98 3.82 3.80 3.02 2.98 2.71 2.19		
705	Q93ZM7	Chaperonin CPN60-like 2	Arabidopsis thaliana			6.91X10 ⁻⁵	30.19	6.3	3
		KSVAAGVNVMDLRV RVTDALNATRA KLSGGVAVFKV		1247.64125 960.51086 877.51416	2 2 2	6.91X10 ⁻⁵ 1.40X10 ⁻³ 2.06X10 ⁻³	3.70 2.57 2.26		
707	Q05045	Chaperonin CPN60-1	Cucurbita maxima			3.95X10 ⁻⁵	124.23	22.4	11

		KIGGASEAEVGEKK KGVEDLADAVKV KAAVEEGIVPGGGVALLYASKE KIGVQIIQNALKT KLLEQDDPDLGYDAAKG KEGVITISDGKT RVTDALNATKA KDDTVILDGAGDKKA KIGGASEAEVGEKKD KSVASGMNAMDLRR KDRVTDALNATKA KSVASGMNAMDLRR KSVASGMNAMDLRR		1146.56372 1016.52588 1901.03784 1196.73608 1662.78577 1018.54150 932.50476 1346.67981 1274.65869 1267.57693 1203.63281 1283.57185 1251.58203	2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	3.95X10 ⁻⁵ 1.28X10 ⁻² 6.29X10 ⁻² 8.87X10 ⁻³ 1.00X10 ⁻⁴ 1.32X10 ⁻² 3.17X10 ⁻³ 1.30X10 ⁻¹ 1.03X10 ⁻³ 7.30X10 ⁻² 1.07X10 ⁻¹ 1.37X10 ⁻¹ 1.27X10 ⁻²	4.51 3.16 2.97 2.78 2.52 2.48 2.45 2.27 2.16 2.15 2.15 2.10 2.01		
710	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana			5.73X10 ⁻⁷	40.19	10.2	4
		KYEDLMAAGIIDPTKV KLADLVGVTLGPKG REVELEDPVENIGAKL KVVAAGANPVLITRG		1552.75634 1182.70923 1541.76929 1280.76855	2 2 2 2	5.73X10 ⁻⁷ 1.00X10 ⁻⁴ 3.81X10 ⁻³ 1.43X10 ⁻³	3.80 3.40 3.19 2.20		
713	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana			8.09X10 ⁻⁵	30.17	7.5	3
		REVELEDPVENIGAKL KLADLVGVTLGPKG KVVAAGANPVLITRG		1541.76929 1182.70923 1280.76855	2 2 2	6.86X10 ⁻³ 8.09X10 ⁻⁵ 3.35X10 ⁻⁴	3.39 3.15 2.99		
715	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana			3.69X10 ⁻⁴	40.17	10.2	4
		REVELEDPVENIGAKL KLADLVGVTLGPKG KVVAAGANPVLITRG KYEDLMAAGIIDPTKV		1541.76929 1182.70923 1280.76855 1552.75634	2 2 2 2	1.23X10 ⁻² 1.73X10 ⁻³ 1.82X10 ⁻³ 3.69X10 ⁻⁴	2.91 2.77 2.48 2.35		
716	P21240	RuBisCO large subunit-binding protein subunit β	Arabidopsis thaliana			2.54X10 ⁻⁵	40.15	9.7	4
		KLADLVGVTLGPKG KYEDLMAAGIIDPTKV REVELEDPVENIGAKL RKGVVTLEEGKS		1182.70923 1552.75634 1541.76929 1059.60449	2 2 2 2	2.54X10 ⁻⁵ 6.99X10 ⁻⁴ 7.84X10 ⁻² 2.26X10 ⁻¹	2.94 2.79 2.29 2.03		
720	A5BFM5	Putative uncharacterized protein	Vitis vinifera			1.34X10 ⁻⁵	10.18	3.4	1
		KNSTVVAGGGAIDMEISRY		1692.82212	2	1.34X10 ⁻⁵	3.52		
722	A5BFM5	Putative uncharacterized protein	Vitis vinifera			1.03X10 ⁻⁴	10.16	3.4	1
		KNSTVVAGGGAIDMEISRY		1692.82212	2	1.03X10 ⁻⁴	3.11		
725	A5BFM5	Putative uncharacterized protein	Vitis vinifera			6.36X10 ⁻⁵	10.16	3.4	1
		KNSTVVAGGGAIDMEISRY		1692.82212	2	6.36X10 ⁻⁵	3.18		
728	A5BFM5	Putative uncharacterized protein	Vitis vinifera			2.21X10 ⁻⁵	10.15	3.4	1
		KNSTVVAGGGAIDMEISRY		1692.82212	2	2.21X10 ⁻⁵	3.03		
732	Q9M888	Putative uncharacterized protein	Arabidopsis thaliana			2.93X10 ⁻⁶	40.20	11.2	4

		KEMQIQNPTAIMIART RLVEGLVLDHGSRH KTPVVMGDEPDKEILKM RVLNPNAEVLNKS		1647.81929 1294.71143 1686.86186 1210.67896	2 2 3 2	8.62X10 ⁻⁵ 2.93X10 ⁻⁶ 7.93X10 ⁻³ 2.01X10 ⁻¹	4.04 3.82 2.55 2.01		
749	P37900	Heat shock 70 kDa protein	Pisum sativum			7.25X10 ⁻⁴	36.14	7.4	4
		RIAGLDVQRI KEIEDAVSDLRT KDVDEVLLVGGMTRV RTTPSVVAFNQKS		871.49957 1146.56372 1419.71481 1191.63684	2 2 2 2	9.95X10 ⁻² 7.25X10 ⁻⁴ 1.76X10 ⁻² 4.13X10 ⁻²	2.83 2.82 2.54 2.03		
750	Q43468	Heat shock protein STI	Glycine max			2.62X10 ⁻⁵	38.17	9.5	4
		KALELDDEDISYLTNRA KLGAMPEGLKDAEKC KELEQQEYFDPKL RAAVYLEMGKF		1766.84436 1374.69334 1425.65320 997.50229	2 3 2 2	2.62X10 ⁻⁵ 9.81X10 ⁻³ 1.34X10 ⁻³ 8.22X10 ⁻³	3.32 3.30 2.22 2.10		
755	P37900	Heat shock 70 kDa protein	Pisum sativum			2.93X10 ⁻⁶	40.19	8.7	4
		RIINEPTAAALSYGMNNKE KDVDEVLLVGGMTRV RTTPSVVAFNQKS KEIEDAVSDLRT		1822.90038 1419.71481 1191.63684 1146.56372	2 2 2 2	2.93X10 ⁻⁶ 1.06X10 ⁻³ 1.64X10 ⁻³ 5.59X10 ⁻³	3.60 2.87 2.80 2.72		
760	P11143	Heat shock 70 kDa protein	Zea mays			8.73X10 ⁻⁶	10.16	2.0	1
		KNALENYAYNMRN		1374.61068	2	8.73X10 ⁻⁶	3.24		
762	Q39043	Luminal-binding protein 2	Arabidopsis thaliana			2.78X10 ⁻⁷	50.18	9.0	5
		KDAGVIAGLNVARI RARFEELNNDLFRK KVFSPEEISAMILTKM RFEELNNDLFRK KNGHVEIIANDQGNRI		1155.64807 1523.76013 1580.82402 1296.62195 1536.75134	2 2 2 2 2	1.59X10 ⁻⁴ 2.78X10 ⁻⁷ 3.59X10 ⁻³ 3.51X10 ⁻² 2.59X10 ⁻⁴	3.30 2.76 2.56 2.31 2.25		
763	Q39043	Luminal-binding protein 2	Arabidopsis thaliana			1.71X10 ⁻⁴	58.21	12.9	7
		RARFEELNNDLFRK KDAGVIAGLNVARI KNGHVEIIANDQGNRI RFEELNNDLFRK KSQIDEIVLVGGSTRI KEAEEFAEEDKKV KFDLTGVPPAPRG		1523.76013 1155.64807 1536.75134 1296.62195 1473.79077 1324.59033 1169.63135	3 2 3 2 2 2 2	1.03X10 ⁻² 1.71X10 ⁻⁴ 5.45X10 ⁻⁴ 1.54X10 ⁻³ 8.76X10 ⁻³ 3.20X10 ⁻³ 7.02X10 ⁻²	4.20 3.94 3.91 3.33 2.81 2.15 2.10		
791	Q9LZF6	Cell division control protein 48 homolog E	Arabidopsis thaliana			1.28X10 ⁻⁶	50.18	6.8	5
		KYQAFAQTLQQSRG KYTQGFSGADITEICQRA RKYQAFAQTLQQSRG KLAEDVDLERI KDFSTAILERK		1440.72302 1845.84359 1568.81799 1059.53174 1051.54187	2 2 2 2 2	2.55X10 ⁻² 1.28X10 ⁻⁶ 3.94X10 ⁻⁴ 1.14X10 ⁻² 1.25X10 ⁻¹	3.54 2.87 2.84 2.68 2.33		
795	O23755	Elongation factor 2	Beta vulgaris			1.46X10 ⁻⁴	50.15	8.1	5
		KDLQDDFMGGAEIIKS RGFVQFCYEPIKQ		1567.73085 1387.67148	2 2	2.69X10 ⁻³ 5.67X10 ⁻²	2.97 2.51		

799	O23755	RIMGPNYVPGEKKD RVFYASQLTAKPRL RIRPVLTVNKM Elongation factor 2	Beta vulgaris	1348.69295 1380.76343 1039.66223	2 2 2	6.45X10 ⁻² 1.46X10 ⁻⁴ 2.13X10 ⁻² 8.19X10 ⁻⁴	2.38 2.18 2.09 30.14	3.3	3
		RRVFYASQLTAKPRL RVFYASQLTAKPRL RGFVQFCYEPIKQ	Ü	1536.86450 1380.76343 1387.67148	3 2 2	8.19X10 ⁻⁴ 5.07X10 ⁻³ 1.33X10 ⁻²	2.87 2.49 2.23		
	Defence respon	se							
233	P52779	Protein LlR18B	Lupinus luteus			1.93X10 ⁻⁵	50.16	29.5	5
		KFHTKGDVLSDAVRE KGDVLSDAVREEAKA KLLSGPDGGSIGKI KGDVLSDAVRE KAVEGYVLANPNY		1444.75427 1388.70154 1100.59460 931.48431 1309.64233	3 2 2 2 2	5.84X10 ⁻² 2.71X10 ⁻² 1.93X10 ⁻⁵ 4.57X10 ⁻² 2.89X10 ⁻⁵	3.22 2.74 2.73 2.67 2.52		
234	Q93XI0	Pathogenesis-related 10	Lupinus albus			5.74X10 ⁻⁶	86.28	51.9	9
		KAIENYLSAHPEYN KLVEGVNGGSIGKV KKLTLIEGGETKY KALVKDADTIIPKA KAVEAIQSVETVEGNGGPGTIKK KAVEAIQSVETVEGNGGPGTIKK KLTLIEGGETKY KVRGDAFFKA KDADTIIPKA		1520.70154 1129.62122 1188.68347 1283.75696 2056.05566 2184.15063 1060.58850 939.50470 872.47235	2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2	2.13X10 ⁻⁷ 6.04X10 ⁻⁶ 4.39X10 ⁻⁴ 5.74X10 ⁻⁶ 8.19X10 ⁻⁶ 6.14X10 ⁻³ 2.46X10 ⁻⁴ 6.36X10 ⁻² 1.01X10 ⁻¹	3.89 3.59 3.49 3.45 3.39 3.19 2.87 2.32 2.23		
276	Q93XI0	Pathogenesis-related 10	Lupinus albus			1.45X10 ⁻⁶	56.25	44.3	6
		KAVEAIQSVETVEGNGGPGTIKK KAIENYLSAHPEYN KLVEGVNGGSIGKV KLTLIEGGETKY KKLTLIEGGETKY KDADTIIPKA		2056.05566 1520.70154 1129.62122 1060.58850 1188.68347 872.47235	3 2 2 2 2 2 2	1.45X10 ⁻⁶ 4.02X10 ⁻⁶ 1.99X10 ⁻⁵ 1.15X10 ⁻³ 8.54X10 ⁻³ 1.78X10 ⁻¹	5.03 3.44 3.44 2.98 2.61 2.34		
287	Q93XI0	Pathogenesis-related 10	Lupinus albus			4.84X10 ⁻⁵	50.16	34.8	5
		KAIENYLSAHPEYN KLVEGVNGGSIGKV KLTLIEGGETKY KKLTLIEGGETKY KALVKDADTIIPKA		1520.70154 1129.62122 1060.58850 1188.68347 1283.75696	2 2 2 2 2 2	4.84X10 ⁻⁵ 4.16X10 ⁻² 1.06X10 ⁻¹ 3.58X10 ⁻² 2.44X10 ⁻¹	2.75 2.67 2.52 2.43 2.31		
318	Q0PN10	Glutathione S-transferase	Caragana korshinskii			7.49X10 ⁻⁵	18.16	6.4	1
		KSPLLPSDPYQRA KGIKYEYKEEDLRN		1272.65833 1542.77991	3 2	1.68X10 ⁻¹ 7.49X10 ⁻⁵	2.78 2.78		
327	Q9SXM5	Acidic chitinase	Glycine max			6.51X10 ⁻⁴	20.17	3.7	1
		KYGGVMLWNRF KYGGVMLWNRF		1095.54041 1111.53533	2 2	6.51X10 ⁻⁴ 8.37X10 ⁻³	3.31 3.24		
430	P23535	Glucan endo-1,3-β-glucosidase, basic isoform	Phaseolus vulgaris			5.46X10 ⁻⁵	20.12	8.9	2

		RNVLNFWPSVKI RDISLPYALFTSPNVVVRD		1203.65210 1891.03235	2 2	3.73X10 ⁻² 5.46X10 ⁻⁵	2.49 2.47		
	Transcription								
314	Q5EI63	Quinone reductase 2	Triticum monococcum			8.81X10 ⁻⁴	10.14	6.4	1
		KAFFDATGGLWRE		1240.61096	2	8.81X10 ⁻⁴	2.13		
	Amino acids r	netabolism							
393	A3RM06	Cysteine synthase	Glycine max			2.34X10 ⁻⁴	10.15	4.6	1
		RAFGAEVYLTDPAKG		1381.69983	2	2.34X10 ⁻⁴	2.74		
520	P54260	Aminomethyltransferase	Solanum tuberosum			1.15X10 ⁻⁵	20.16	6.9	2
		RVGFFSSGPPPRS		1147.58948	2 2	1.15X10 ⁻⁵ 5.69X10 ⁻²	3.15		
521	O40108	RAEGGFLGAEVILKQ Aspartate aminotransferase	Lupinus angustifolius	1303.72559		1.60X10 ⁻⁶	2.62 88.22	22.4	8
027	Q 10100	KLIFGADSPAIQENRV RNKEYLPIVGVADFNKL RRVEQQLVNEASRN RVGALSIVSKS KEYHIYLTSDGRI RVEQQLVNEASRN RTEEGKPLVLNVVRR RVTTVQCLSGTGSLRV RQQLFDALQSRG		1530.79114 1706.91125 1428.75537 873.54041 1353.64331 1272.65430 1453.83728 1478.76315 1205.62732	2 3 2 2 2 2 2 3 2 2 2	9.27X10 ⁻⁶ 3.77X10 ⁻³ 1.60X10 ⁻⁶ 1.08X10 ⁻³ 1.20X10 ⁻³ 4.28X10 ⁻³ 6.46X10 ⁻⁴ 2.44X10 ⁻² 5.28X10 ⁻³	4.46 4.03 3.77 3.35 3.17 2.83 2.62 2.44 2.13	22	· ·
616	Q9SP37	Adenosylhomocysteinase	Lupinus luteus			1.42X10 ⁻⁶	20.16	5.6	2
		RVVGVSEETTTGVKR RSEFGPSQPFKG		1305.68970 1123.54187	2 2	1.42X10 ⁻⁶ 6.37X10 ⁻⁴	3.25 2.27		
773	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	Mesembryanthemum crystallinum			5.10X10 ⁻⁵	48.17	8.9	5
		KYLFAGVVDGRN KAGINVIQIDEAALRE RIPPTEELADRI KFALESFWDGKS KYGAGIGPGVYDIHSPRI		1096.57861 1482.82751 1140.58948 1199.57312 1658.82849	2 2 2 2 2	5.10X10 ⁻⁵ 5.23X10 ⁻⁵ 6.20X10 ⁻⁴ 4.81X10 ⁻² 3.35X10 ⁻³	3.32 3.09 2.62 2.17 2.05		
776	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	Mesembryanthemum crystallinum			1.90X10 ⁻⁵	20.20	3.9	2
		KYGAGIGPGVYDIHSPRI KYLFAGVVDGRN		1658.82849 1096.57861	2 2	1.90X10 ⁻⁵ 7.67X10 ⁻⁴	2.96 2.79		
778	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	Mesembryanthemum crystallinum			1.81X10 ⁻⁴	48.17	8.9	5
		KYLFAGVVDGRN KYGAGIGPGVYDIHSPRI KAGINVIQIDEAALRE KFALESFWDGKS KISEEEYVKA		1096.57861 1658.82849 1482.82751 1199.57312 996.48840	2 2 2 2 2	1.81X10 ⁻⁴ 5.52X10 ⁻² 1.83X10 ⁻² 4.92X10 ⁻³ 1.68X10 ⁻¹	3.34 2.93 2.61 2.17 2.01		

267	P34105	NADP-dependent malic enzyme	Populus trichocarpa			4.15X10 ⁻⁶	20.18	5.1	2
		KAIFASGSPFDPVEYEGKV		1813.86426	2	4.15X10 ⁻⁶	3.51		
710	00011700	KAYELGLATRL		993.53638	2	1.82X10 ⁻¹	2.30	0.0	2
518	Q93VR3	GDP-mannose 3,5-epimerase	Arabidopsis thaliana	1429 65221	2	7.72X10 ⁻⁴	30.13	9.0	3
		KHYNKDFGIECRI RFEMWGDGLQTRS		1438.65321 1355.60486	3 2	2.47X10 ⁻³ 7.72X10 ⁻⁴	2.50 2.25		
		KLATEELCKH		963.48156	2	9.30X10 ⁻²	2.03		
673	Q96558	UDP-glucose 6-dehydrogenase	Glycine max			2.00X10 ⁻⁵	18.18	6.0	2
		KAADLTYWESAARM		1353.64331	2	2.00X10 ⁻⁵	3.57		
		RILTTNLWSAELSKL		1475.81042	2	3.07X10 ⁻⁴	2.78		
734	A9PGL9	Malic enzyme	Populus trichocarpa			$1.36X10^{-6}$	10.18	3.4	1
		KAIKPTVLIGTSGVGKT		1440.87842	2	1.36X10 ⁻⁶	3.58		
745	P34105	NADP-dependent malic enzyme	Populus trichocarpa			6.88X10 ⁻⁶	20.16	4.2	2
		KAYELGLATRL		993.53638	2	4.03X10 ⁻³	3.23		
		KSIQVIVVTDGERI		1315.72156	2	6.88X10 ⁻⁶	2.75		
747	P34105	NADP-dependent malic enzyme	Populus trichocarpa			1.13X10 ⁻⁵	20.17	4.2	2
		KSIQVIVVTDGERI KAYELGLATRL		1315.72156 993.53638		1.13X10 ⁻⁵ 2.57X10 ⁻³	3.38 3.13		
	Cytoskeleton b			773.33036		2.57X10	3.13		
182	Q1G0Z1	Putative spindle disassembly related protein CDC48	Nicotiana tabacum			2.11X10 ⁻⁵	60.19	8.5	5
102	QIGOZI	REDENRLDEIGYDDVGGVRK	Niconana tabacum	2050.93115	3	1.20X10 ⁻⁴	3.58	6.5	3
		RGILLYGPPGSGKT		1158.65173	2	2.11X10 ⁻⁵	3.38		
		KYQAFAQTLQQSRG		1440.72302	2	8.68X10 ⁻⁴	3.24		
		RKYQAFAQTLQQSRG		1568.81799	3	4.52X10 ⁻²	2.67		
		KYTQGFSGADITEICQRA RLGDVVSVHQCPDVKY		1845.84359 1552.77880	2 2	2.62X10 ⁻² 4.86X10 ⁻²	2.27 2.11		
183	Q1G0Z1	Putative spindle disassembly related protein CDC48	Nicotiana tabacum	1332.77000		1.26X10 ⁻⁴	40.19	7.8	3
	4	REDENRLDEIGYDDVGGVRK		2050.93115	3	5.51X10 ⁻²	3.80		
		RLVVDEAINDDNSVVALHPDTMEKL		2540.21847	3	1.76X10 ⁻³	3.10		
		KYTQGFSGADITEICQRA		1845.84359	2	1.26X10 ⁻⁴	2.40		
		RGILLYGPPGSGKT		1158.65173	2	8.07X10 ⁻²	2.15		
207	P20363	α -3/ α -5 tubulin chain	Arabidopsis thaliana			5.35X10 ⁻⁴	24.16	11.6	3
		RAVCMISNNTAVAEVFSRI RSLDIERPTYTNLNRL		1884.89424 1691.87109	3	4.94X10 ⁻³ 5.35X10 ⁻⁴	2.90 2.74		
		RAVFVDLEPTVIDEVRT		1701.90576	2	1.38X10 ⁻³	2.74		
262	Q9STD0	β-tubulin	Zinnia elegans			2.65X10 ⁻⁴	8.14	3.8	1
		RAVLMDLEPGTMDSIRS		1679.79788	2	2.65X10 ⁻⁴	2.79		
		RAVLMDLEPGTMDSIRS		1679.79788	2	8.20X10 ⁻²	2.11		
331	P41916	GTP-binding nuclear protein Ran-1	Arabidopsis thaliana			5.02X10 ⁻⁶	18.14	11.3	2
		KNLQYYEISAKS		1228.62085	2	7.36X10 ⁻³	2.83		
		RFYCWDTAGQEKF		1404.58888	2	5.02X10 ⁻⁶	2.74		

336	P41916	GTP-binding nuclear protein Ran-1 KNLQYYEISAKS	Arabidopsis thaliana	1228.62085	2	9.79X10 ⁻⁵ 1.75X10 ⁻³	18.13 2.50	11.3	2
	Other metab	RFYCWDTAGQEKF olic processes		1404.58888	2	9.79X10 ⁻⁵	2.32		
391	Q8LQJ6	Ethylene-responsive protein 2-like	Oryza sativa			5.51X10 ⁻⁴	8.12	12.4	1
		RIAAPYDLVMQTKQ	•	1365.70826	2	5.51X10 ⁻⁴	2.09		
574	Q7M1Z8	Globulin-2	Zea mays			6.36X10 ⁻⁵	70.17	18.9	7
		KQSKGEITTASEEQIRE KVFLAGTNSALQKM RVVMLLSPVVSTSGRF KGEITTASEEQIRE RVAELEAAPRA KEGEGVIVLLRG RLLDMDVGLANIARG		1676.84497 1248.69470 1460.81413 1333.65942 955.52069 1084.63611 1416.75153	3 2 2 2 2 2 2 2	4.49X10 ⁻² 2.48X10 ⁻¹ 6.36X10 ⁻⁵ 2.81X10 ⁻³ 3.85X10 ⁻⁴ 3.19X10 ⁻¹ 9.92X10 ⁻³	3.34 3.16 2.90 2.88 2.83 2.27 2.19		
596	P19595	UTP-glucose-1-phosphate uridylyltransferase	Solanum tuberosum			5.83X10 ⁻⁶	20.19	6.3	2
		KSAVAGLNQISENEKS KLEIPDGAVIANKD		1459.73877 1239.69434	2 2	5.83X10 ⁻⁶ 2.80X10 ⁻⁴	3.79 2.43		
682	P15590	Globulin-1 S allele	Zea mays			1.88X10 ⁻⁵	20.17	4.7	2
		KAEEVDEVLGSRR KVFLAGADNVLQKL		1203.58521 1274.71033	2 2	1.88X10 ⁻⁵ 2.22X10 ⁻³	3.42 2.67		
768	Q7SIC9	Transketolase	Zea mays			5.82X10 ⁻⁶	20.18	4.1	2
		KVTTTIGFGSPNKA KANSYSVHGSALGAKE		1221.64734 1361.68079	2 2	9.35X10 ⁻⁵ 5.82X10 ⁻⁶	3.59 2.25		
769	Q7SIC9	Transketolase	Zea mays			8.39X10 ⁻⁵	20.18	3.4	2
		KVTTTIGFGSPNKA KNPYWFNRD		1221.64734 996.46863	2 2	8.39X10 ⁻⁵ 2.23X10 ⁻²	2.42 2.35		
770	Q7SIC9	Transketolase	Zea mays			8.77X10 ⁻⁵	10.16	2.1	1
		KVTTTIGFGSPNKA		1221.64734	2	8.77X10 ⁻⁵	3.25		
	Unknown biological processes								
141	Q8LPE5	Fructokinase-like protein	Cicer arietinum			1.82X10 ⁻⁵	40.15	22.3	4
		KFANACGAITTTKK KIVDDQSILEDEARL RTALAFVTLRA RLPLWPSPEEARN		1254.61470 1502.73328 991.59351 1294.67896	2 2 2 2	1.04X10 ⁻² 4.79X10 ⁻⁵ 2.82X10 ⁻³ 1.82X10 ⁻⁵	2.97 2.88 2.70 2.63		
237	Q9M328	Putative uncharacterized protein T18D1290	Arabidopsis thaliana			2.81X10 ⁻⁴	14.15	6.9	1
		KLDSIVMGSRG KLYWGDARQ		977.50842 880.43115	2 2	2.81X10 ⁻⁴ 5.56X10 ⁻²	3.00 2.01		
246	Q8GYY8	Putative germin	Arabidopsis thaliana			1.17X10 ⁻⁵	18.21	12.3	2

	RIDYAPGGLNPPHTHPRA RGLVHFQKN		1741.87683 828.47266	2 2	1.17X10 ⁻⁵ 8.36X10 ⁻²	3.26 2.31		
353 Q2V987	Transcription factor APFI-like	Solanum tuberosum			1.76X10 ⁻⁴	30.19	10.1	2
	KNAMVAAGALVRQ KDEEYDSMLGVVRE KNAMVAAGALVRQ		1088.58809 1428.63114 1072.59314	2 2 2	7.06X10 ⁻³ 1.76X10 ⁻⁴ 1.35X10 ⁻³	3.70 3.16 2.99		
384 Q9SMK5	Plasma membrane intrinsic polypeptide	Cicer arietinum			4.64X10 ⁻⁴	10.12	5.8	1
	KVSTFIVTEEKV		1152.61462	2	4.64X10 ⁻⁴	2.43		
549 A5CB20	Putative uncharacterized protein	Vitis vinifera			1.22X10 ⁻⁴	18.20	3.6	1
	KAGGVCIADEVQTGFGRT RHDIIGDVRG		1636.77478 924.48975	2 2	1.22X10 ⁻⁴ 1.21X10 ⁻²	4.03 2.97		
671 Q7XCL2	Ubiquitin domain containing protein	Oryza sativa			2.51X10 ⁻⁴	10.12	3.1	1
	RAMSNIESSPEGFNMLRR		1814.80476	2	2.51X10 ⁻⁴	2.38		
678 Q94IC1	Betaine aldehyde dehydrogenase	Hordeum vulgare			2.55X10 ⁻⁵	10.13	2.8	1
	RLGPVVSEGQYEKI		1305.66846	2	2.55X10 ⁻⁵	2.63		

^a Spot numbers are corresponding to the numbers in Figure 4.

^b Protein identification according to the UniProt database (http://www.uniprot.org)

^c Probability of a false identification (P)

^d Cross-correlation score (Xcorr)