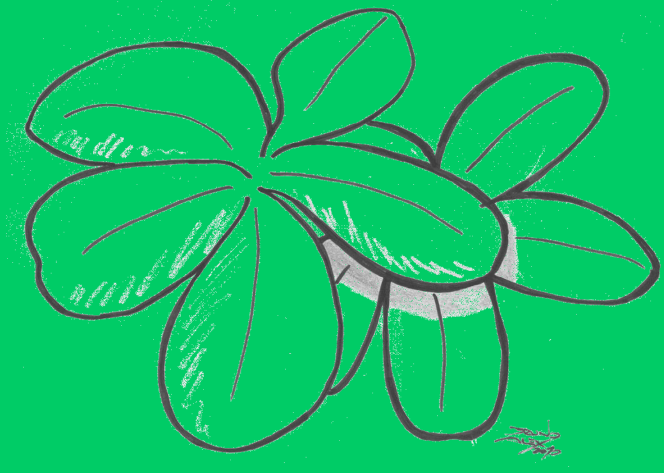
RESPONSES OF HIGHER PLANTS TO BORON DEFICIENCY



Marta Alexandra Marques Alves

June 2010
Oeiras, Portugal

Dissertation presented to obtain the degree of Doctorate in Biochemistry by Instituto de Tecnologia Química e Biológica of Universidade Nova de Lisboa

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Supervisor: Dr. Cândido Pinto Ricardo
Co-supervisor: Dr. Phillip Jackson

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To my best friend, husband and father of my child,
Paulo Oliveira

“Although nature commences with reason and ends in experience it is necessary for us to do the opposite that is to commence with experience and from this to proceed to investigate the reason.”

Leonardo da Vinci

(15th April 1452 – 2nd May 1519)

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SUMMARY

Despite being known for more than 80 years that B is essential for plant growth, the role of this element in plant metabolism remains elusive. Until now, B involvement in the plant cell wall structure is the only well-documented participation of B. However, this participation can not explain all the symptoms observed in B-deficient plants nor the requirement of B for early animal developmental processes.

The aim of this study was to acquire additional data on the role(s) of B in the growth and development of higher plants. For that purpose, *Lupinus albus*, an important crop plant, was used to study long-term B deficiency responses, characterizing the proteomes of the leaf apoplast (Chapter 2) and of the root system (Chapter 3). A metabolite analysis was also performed in the different organs (leaf-blades, petioles, apexes, hypocotyls and roots) of *L. albus* plants (Chapter 4). Some consistent responses were observed with these different approaches. The analysis of the metabolite content of *Lupinus albus* plants, showed minor changes in the content of sugars and absence of variation in malate suggests that the central carbohydrate metabolism is being little affected by B deficiency (Chapter 4). The proteome study of the root, revealed the differentially expression of UDP-glucose pyrophosphorylase and of several ATPases (Chapter 3), what appears to be related with the

activation of alternative energy sources. Thus, the carbon flow seems to be able to continue even under stressful conditions. Proteins related with the cell wall biosynthesis, namely of polysaccharide components, were suppressed due to B deficiency (Chapter 3), probably as a consequence of the structural cell wall damage. Some proteins related to protein folding and proteolytic processes were suppressed while others were *de novo* expressed (Chapter 3), pointing for a shift in the protein metabolism, probably to allow the plant to partially cope with B deficiency. Several proteins related with cytoskeleton biosynthesis were also affected by B deficiency, as for instance, tubulins and actins, that were *de novo* expressed (Chapter 3). The additional increased content of the branched-chain amino acids (Chapter 4), that could be related with cytoskeleton biosynthesis, points for some active involvement of B with cytoskeleton biosynthetic processes.

The long-term effects observed could result from both direct and indirect stress responses, but the indirect effects could have some degree of specificity to the stress, as suggested by the leaf apoplast study (Chapter 2), since the majority of the proteins that were commonly responsive to both B and water-deficit showed different patterns of expression.

Short-term B deficiency responses were also investigated, performing a transcriptional analysis with the model plant *Arabidopsis thaliana*

(Chapter 5). Altered expression of genes related with the cell wall was observed, as well as genes related to branched-chain amino acids. In analogy to what was discussed in the long-term responses, the increased expression of these genes could be related with impaired cytoskeleton biosynthesis. Genes related with sulphur metabolism were also found to be differentially expressed due to B deficiency. In particular, the expression of the three genes that encode for 5'-adenylylsulfate reductase, a key enzyme of the sulphate assimilation pathway, was decreased, what is a response with similarity to sulphur excess in the plant.

Thus, altered cytoskeleton biosynthesis seems to be a common response in short and long-term B deficiency studies, suggesting that a direct interaction of B with cytoskeleton may exist. Such B participation in plant metabolism is consistent with higher B requirement in the earlier animal development and reproductive stage in plants, where cytoskeleton is actively required for cell division processes.

SUMÁRIO

Apesar de ser conhecido há mais de 80 anos que o B é essencial para o crescimento das plantas, o seu papel no metabolismo das plantas continua por elucidar. Até agora, o envolvimento na estrutura da parede celular das plantas é a única e bem documentada participação do B que, no entanto, não parece ser suficiente para explicar todos os sintomas observados nas plantas devido à deficiência de B, nem o requisito de B nos processos iniciais do desenvolvimento animal.

O objectivo deste estudo foi o de reunir informação adicional sobre o papel do B no crescimento e desenvolvimento das plantas superiores. Para isso utilizámos uma importante cultura agrícola, o *Lupinus albus*, para os estudos das respostas à deficiência prolongada de B, caracterizando o proteoma do apoplasto da folha (Capítulo 2) e do sistema radicular (Capítulo 3). Foi também efectuada uma análise metabólica nos diferentes órgãos desta planta (folhas, pecíolos, ápices, hipocótilos e raízes). Estas diferentes abordagens permitiram obter algumas respostas consistentes. Observou-se uma pequena variação nos teores de açúcares e a ausência de variação nos teores de malato, o que sugere que o metabolismo central dos hidratos de carbono está a ser pouco afectado pela deficiência (Capítulo 4). O estudo do proteoma da raíz revelou uma expressão diferenciada da UDP-glucose fosforilase

e de várias ATPases (Capítulo 3), proteínas essas envolvidas na activação de vias metabólicas alternativas para a produção de energia. A planta consegue, desta forma, manter o fluxo de carbono apesar das condições de stress.

A biossíntese da parede celular parece ter sido afectada, dada a supressão de proteínas relacionadas com síntese de polissacáridos (Capítulo 3), provavelmente como consequência de danos causados na estrutura da parede celular. O rearranjo tridimensional das proteínas e certos processos proteolíticos foram modificados (capítulo 3), indicando uma provavel alteração do metabolismo das proteínas na deficiência de B.

Várias proteínas relacionadas com a biossíntese do citosqueleto foram também afectadas pela deficiência de B, como por exemplo tubulinas e actinas (Capítulo 3).

O aumento dos níveis de aminoácidos de cadeia ramificada que foi observado (Capítulo 4) poderá ser relacionado com o citosqueleto, o que poderá reforçar a hipótese da participação do B na biossíntese do citosqueleto.

Os efeitos da deficiência de B a longo prazo podem resultar de respostas directas ou indirectas ao stress, mas, todavia, mesmo sendo indirectos podem ter um certo grau de especificidade, como sugere o estudo do proteoma do apoplasto da folha (Capítulo 2). Com efeito, a maioria das proteínas que responderam tanto à deficiência de B como à seca mostraram diferentes padrões de expressão.

A análise dos transcriptos da planta modelo *Arabidopsis thaliana* (Capítulo 5) foi usada para investigar as respostas à deficiência de B a curto prazo. Verificou-se a alteração da expressão de genes relacionados com a parede celular, bem como de genes envolvidos com os aminoácidos de cadeia ramificada. Analogamente com o que foi discutido na resposta a longo prazo, o aumento da expressão destes genes poderá estar relacionada com danos na biossíntese do citosqueleto. Genes relacionados com o metabolismo do enxofre foram também diferencialmente expressos devido à deficiência de B. Em particular a expressão dos três genes que codificam para 5'-adenililsulfato redutase, a enzima chave da via da assimilação do enxofre, diminuiu, uma resposta que se assemelha a excesso de enxofre nas plantas.

Assim, os resultados obtidos tanto a longo como a curto prazo apontam para uma possível relação entre a deficiência de B e o citosqueleto. Esta sugestão é consistente com o elevado requisito de B tanto na fase reprodutiva das plantas como na fase inicial do desenvolvimento animal, nas quais o citosqueleto é activamente solicitado para processos de divisão celular.

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ABBREVIATIONS

ABBREVIATION	FULL FORM
ψpd	Water potential at pre-dawn
2-DE	Two-dimensional gel electrophoresis
A	Apexes
A4SA	Aspartate 4-semialdehyde
ABA	Abscisic acid
ACT	Actin
AI	Auto-inducer
APR	5'-Adenylyl sulphate reductase
APS	Ammonium persulphate
ATGH9CE	Glycosyl hydrolase 9C2
ATMGL	Methionine gamma-lyase
ATP	Adenosine triphosphate
B	Boron
BCAA	Branched-chain amino acid
BCAT	Branched-chain amino acid aminotransferase
BD	Boron deficiency
Ca	Calcium
CCR	Cinnamoyl-CoA reductase
cDNA	Complementary DNA
CHAPS	3-[3-Cholamidoproplyl(dimethylammonio)]-1-propanesulphonate
CND	Chloroplast nucleoid DNA
CoA	Coenzyme A
cRNA	Complementary RNA

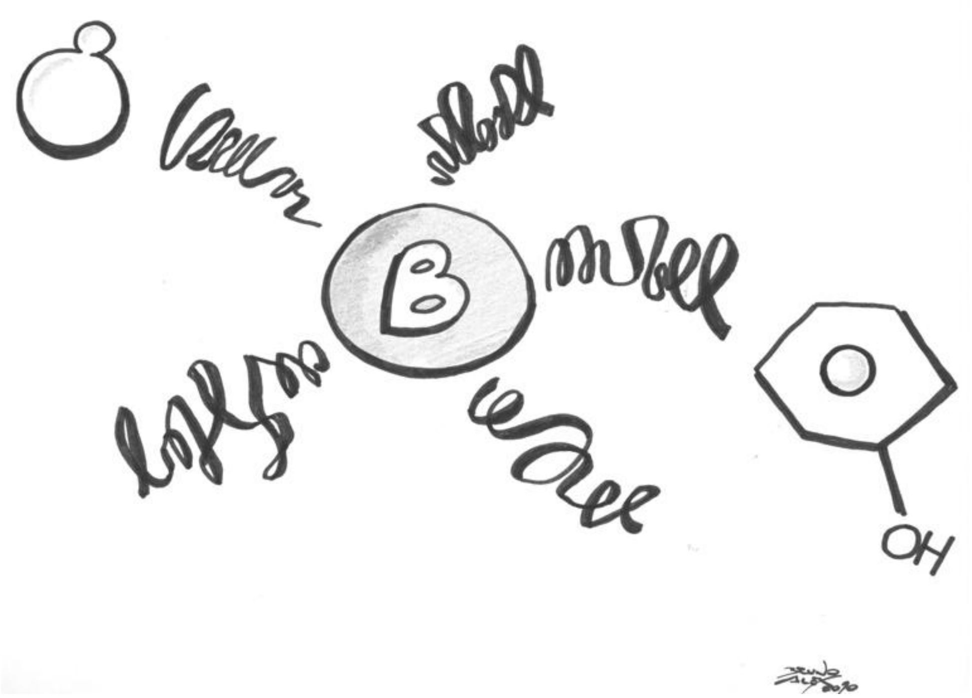
ABBREVIATION	**FULL FORM**
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DTT	Ditiothreitol
EXP5	Expasin5
FDR	False discovery rate
FMO GS-OX	Flavin-containing monooxygenases
FW	Fresh weight
GABA	γ-Aminobutyric acid
GO	Gene ontology
GTP	Guanosine triphosphate,
H	Hypocotyls
HPLC	High liquid performance chromatography
Hsp	Heat shock protein
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
IEF	Isoelectric-focusing
IPG	Immobilized pH gradient
IVT	*In vitro* transcription
L	Leaf-blades
LSU	Low sulphur upregulated
MALDI-TOF-TOF	Matrix assisted laser desorption/ionization-time of flight-time of flight
MCCA	3-Methylcrotonyl-CoA carboxylase
MDH	Malate dehydrogenase
MIOX	*myo*-Inositol oxygenase
MIP	Major intrinsic protein
mRNA	Messenger RNA

ABBREVIATION	**FULL FORM**
MS	Mass spectrometry
MS5	*Male sterility5*
MS/MS	Tandem mass spectrometry
MW	Molecular weight
NAC	Nascent polypeptide-associated complex
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced form of NADP
NL	Non linear
NMR	Nuclear magnetic resonance
NS1	Nicotianamine synthase
ODP	Ovule development protein
P	Petioles
PAGE	Polyacrylamide gel electrophoresis
PAR	Photosynthetic active radiation
pI	Isoelectric point
PME	Pectin methylesterase
PMEI	Pectin methylesterase inhibitor
PP2A	Protein phosphatase 2A subunit A3
PR	Pathogenesis Related
PVP	Polyvinylpyrrolidone
R	Roots
RG-II	Rhamnogalacturonan-II
RhaT	Rhamnosyltransferase
RNA	Ribonucleic acid

ABBREVIATION	**FULL FORM**
RT-qPCR	Reverse transcriptase quantitative real-time polymerase chain reaction
RuBisCO	Ribulose-1,5-bisphosphate carboxylase-oxygenase
SDR	Short-chain dehydrogenase/reductase
SDS	Sodium dodecyl sulphate
ST	Sulfotransferase
TAIR	The Arabidopsis information resource
TCA	Trichloroacetic acid
TF	Transcription factor
TRE	Trehalase
Tris	Tris (hydroxymethyl) aminomethane
TUB	Tubulin
UBC10	Ubiquitin-conjugating enzyme E2
UDP	Uridine diphosphate
USP	Universal stress protein
WD	Water-deficit

CHAPTER 1

GENERAL INTRODUCTION



INTRODUCTION

The importance of boron (B) for living organisms is quite puzzling. The dramatic effect of its deficiency in plants was first noticed almost one century ago, and current evidence suggests that B might be necessary for most organisms. Despite this, its precise functions in the metabolism of plants and other organisms remain to be elucidated. The study of B roles in metabolism is therefore, a subject of central interest in biology. In particular, in agriculture, B deficiency is a widespread problem and large yield losses are reported to occur annually. In Portugal, Olive (*Olea europaea*), Apple (*Malus domestica*) and Pear (*Pyrus communis*) are the fruit trees most affected by B deficiency. In the Douro region, grapevine "maromba" anomaly was found to be due to B deficiency, while in the Alentejo pastures this deficiency is known to causes a significant decrease in growth of legume plants.

THE CHEMISTRY OF BORON

The chemistry of B is unique and, after carbon, B might be the most intriguing and complex of elements (Greenwood and Earnshaw 1984). Along with other light elements, like lithium and berilium, B originates from the Big Bang nucleosynthesis or galactic cosmic-ray spallation (Revees 2001, Prochaska et al. 2003), and its abundance is extremely low: only about 10^{-9} times that of hydrogen and about 10^{-6} that of carbon. However, in spite of its low abundance in the Universe, B is widely distributed in the Earth's crust (from 5mg kg^{-1} in basalts to 100mg kg^{-1} in shales), in the sea water (1-10mg L^{-1}) and in the rivers (3-30μg L^{-1}) (Power and Woods 1997). Boron is a small atom with only three valence electrons that belongs to the semiconductor group of elements with properties intermediate between metals and non-metals. In the industry, B is widely used and the main commercial productions and materials are colemanite, ulexite, borax, borax-pentahydrate, sodium perborates as well as boric acid. The major usage of B includes insulators, fire prevention materials, enamel coating, laundry bleach (sodium perborate), glass fibers, borosilicate glass, frit, ceramic glazes, chemical fertilizers (as a trace element for agriculture) and herbicides (WHO 1998). Tetrahedral borate or boronate complexes have been shown to be involved in enzyme inhibition. Serine proteases were proposed to be inhibited by boronic acid compounds (Yang et al. 2003) and simple borates have been patented as protease stabilizers in liquid detergent formulations (Hora and Kivits 1981, Severson 1985).

At a near-neutral pH found in most biological fluids, B exists primarily as boric acid, $B(OH)_3$ and a small amount as borate

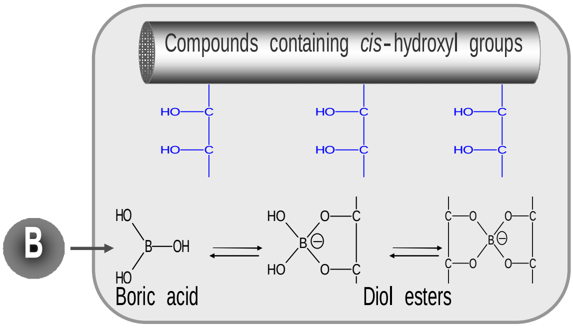


Figure 1.1. Esterification reactions of boric acid, $B(OH)_3$ with compounds containing *cis*-hydroxyl groups to form diol esters.

anion, $B(OH)^{4-}$ (Woods 1996). Both boric acid and borate readily form complexes with a wide variety of sugars and other compounds containing *cis*-hydroxyl groups (Loomis and Durst 1992) such as phenols, organic acids and some polymers (Figure 1.1; Boesekem 1949, Raven 1980). Except for complexes with polyols little is known about B binding inside the cell.

BORON IN PLANTS

Boron was established as an essential micronutrient for all vascular plants in the early 1920s (Warington 1923), but only recent research findings have improved our understanding of B interaction in plants for B uptake and transport processes, cell-wall formation and cellular membrane functions (Goldbach and Wimmer 2007, Bonilla et al. 2009). Boron participation in other processes was also proposed, including anti-oxidative systems (Cakmak and Römheld 1997, Koshiba et al. 2009) and signalling (Redondo-Nieto et al. 2001, González-Fontes et al. 2008).

Uptake and Transport

In soils, B occurs as borosilicates, which are resistant to weathering and not readily available to plants. Mobilisation of immobile forms of rock B occurs by acid-base, oxidation-reduction and dissolution-precipitation reactions. The dominant species in the soil, when B from primary silicates goes into solution is boric acid, $B(OH)_3$. This form of B is mobile and easily lost by leaching, but in this form it can be taken up by plants, held by organic matter or temporarily adsorbed on fine fractions (Nable et al. 1997). Depending on B availability, boric acid uptake by roots can de carried out by several molecular mechanisms such as passive diffusion across the lipid bilayers, facilitated transport by major intrinsic protein (MIP) channels, and also by energy-dependent high affinity transport systems. Whereas the first two represent passive uptake systems, which are constitutively present, the latter is induced by low B supply (Figure 1.2; Tanaka et al. 2008, Takano et al. 2008). Different proportions of sterols and longer chain fatty acids in the plasma membrane of root cells significantly altered B uptake in *Arabidopsis thaliana* mutants (Dordas and Brown 2000). Increased membrane rigidity, as a response to chilling temperature, results in a reduction of the membrane fluidity and permeability of root cells, possibly contributing to the inhibition of B uptake in chilling-sensitive species (Ye et al. 2003). Two members of the MIP family were identified as being up-regulated in B-deficient Arabidopsis plants. The *NIP5;1* gene was identified in roots (Takano et al. 2006) and the *NIP6;1* gene in shoots. The boric acid/borate exporter BOR1 is responsible for loading B into the xylem under both normal and B-deficient conditions (Takano et al. 2002) and its accumulation is controlled by post-transcriptional mechanisms according to B availability (Takano et al. 2005).

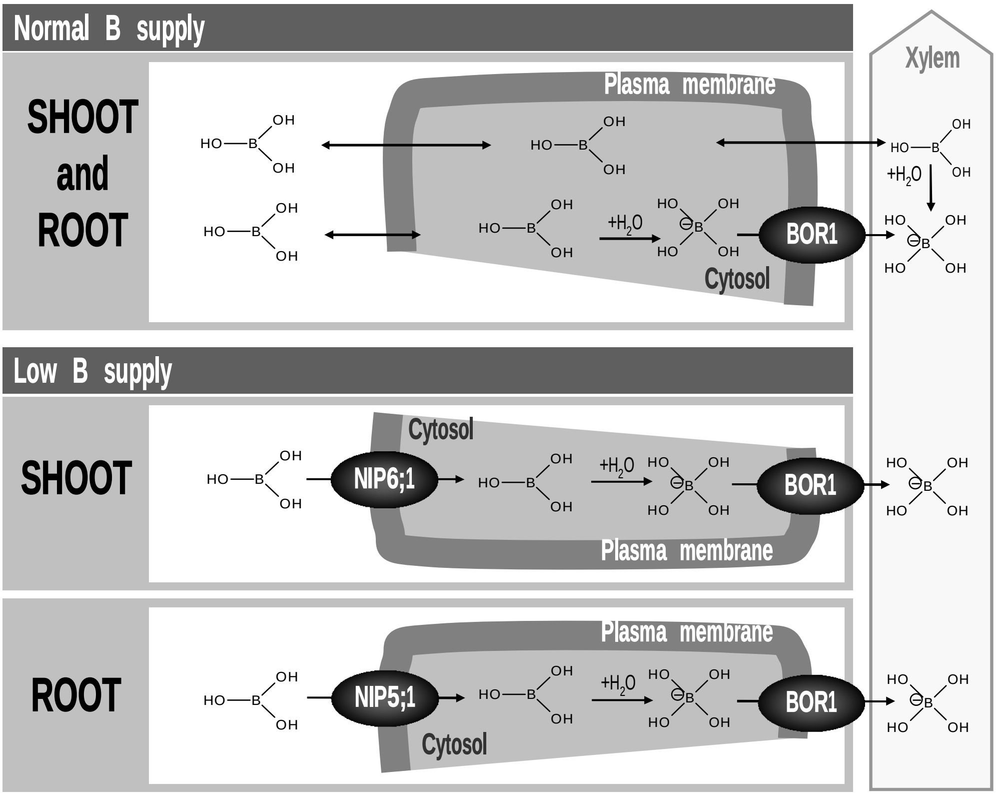


Figure 1.2. Model of B transport mechanisms in plants. Under normal B supply, B is passively transported as boric acid [$B(OH)_3$] across the plasma membrane. Under low B conditions, B is transported by boric channels, NIP5;1 in roots and NIP6;1 in young developing tissues. Under both normal and low B conditions the boric acid/borate exporter BOR1 is responsible for B loading into the xylem (Tanaka et al. 2008, Takano et al. 2008).

Two potential BOR1 homologues were already found in a monocot plant, *Oryza sativa* (Nakagawa et al. 2007) and in eucalypt, a tree (Domingues et al. 2005).

Boron moves from the roots with the transpiration stream and accumulates in growing points of leaves and stems (Blevins and Lukaszewski 1998). Nevertheless, B transport in the phloem depends on the sugar or polyol molecules used by a particular plant. Several B transport molecules were isolated and characterized, such as sorbitol-B-sorbitol complexes from the floral nectar of peach and mannitol-B-mannitol complexes from phloem sap of celery. There is also evidence that dulcitol is a B transport molecule in some species (Blevins and Lukaszewski 1998). Although it seems that the B retranslocation is limited to sugar alcohol-producing plants, recently it was demonstrated that B is retranslocated from old tissues to younger tissues in response to short-term B deficiency in lupin, which does not produce sugar alcohols (Huang et al. 2008).

Cell wall

In the plant cell up to 90% of the cellular B is localized in the cell wall (Martini and Thellier 1993), an important structure for determining cell size and shape during plant development. The cross-linking of the pectic polysaccharide components by B was proposed to have an important contribution to the cell wall architecture, hence influencing the mechanical properties of growing cell walls (Kobayashi et al. 1996, O'Neill et al. 1996, 2001). Boron cross-links through the diol bridges of the rhamnogalacturonan-II (RG-II) molecules creating binding sites for calcium ions (Figure 1.3), which will further increase the strength of the B-RG-II complexes (Kobayashi et al. 1999).

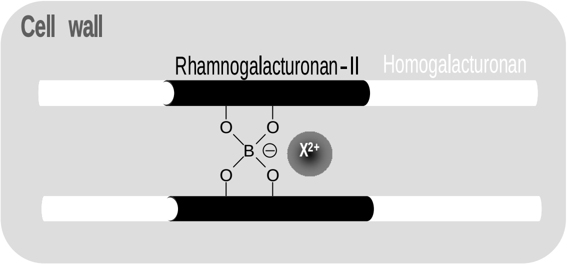


Figure 1.3. Model showing pectin rhamnogalacturonan-II molecules, of the plant cell wall, cross-linked by B. Through this cross-linking B contributes to the stabilization of the cell wall structure, additionally allowing a metal ion (X^{2+}) such as Ca, Mg, Pb, Ba or Sr to be bound to the B-RG-II complex (Ishii and Matsunaga 2001).

Genetic studies employing the *Arabidopsis thaliana mur1* mutant and the haploid *Nicotiana plumbaginifolia* callus mutant nolac-H18 indicated that B-RG-II cross-linking is essential for normal plant growth (O'Neill et al. 2001, Iwai et al. 2002). The correct establishing of an effective symbiosis between legume–*Rhizobium* is also dependent on B availability that seems to be an important component for the maintenance of the nodule cell wall structure (Bolaños et al. 1994, Bonilla et al. 1997).

Cellular membrane

A structural role for B in membranes has also been proposed due to the ability of B for linking or binding to hydroxyl-containing constituents, such as phosphoinositides, glycoproteins, glycolipids (Bonilla et al. 1997, Brown et al. 2002). Thus, B may play a role in the maintenance of membrane rheology, by modulating the hydration and fluidity of lipid bilayers (Verstraeten et al. 2005), and might explain the altered membrane composition observed in B-deficient plants. So, the lipid composition of membranes determines the magnitude and direction of B deficiency effects (Brown et al. 2002). Other observations that point to a structural role of B in membrane stabilization are modified permeability for potassium and sugars, altered membrane-bound calcium levels and damage of the peribacteroid membrane in nodules detected as a consequence of B deficiency (Goldbach and Wimmer 2007). Indeed, B is described to be an indispensable element for infection thread development and nodule invasion (Bolaños et al. 1996). In *Rhizobium*-plant interactions B seems to act as a modulator between the glycoprotein matrix of the plant and the bacterial cell surface. In the absence of B the glycoproteins can attach to the rhizobium cell surface, preventing the interaction with the plant cell membrane, thereby inhibiting cell invasion (Bolaños et al. 2004).

Several studies have indicated that B interferes with the functioning of enzymes and other proteins of the plasma membrane. For example, B deficiency alters the membrane potential, reduces the activity of Fe-reductase and proton-pumping ATPase, and thus the proton gradient across the plasma membrane. The plasma membrane–bound oxidoreductase activity was also inhibited within minutes of B deprivation (Goldbach and Wimmer 2007).

Despite the several observations for membrane damage upon B deficiency, there are not yet concluding remarks for a structural role for B in cellular membranes.

Oxidative damage

The accumulation of oxidative free radicals in cells, which is one of the indirect consequences of B deficiency in root and leaf cells, could be responsible for the impairment of membrane functions (Cakmak and Römheld 1997). In tobacco BY-2 cells grown under B deprivation, the accumulation of reactive oxygen species, higher lipid peroxidation and decreased cellular ascorbate levels were observed. The addition of lipophilic antioxidants suppressed the death of B-deprived cells, suggesting that oxidative damage is the immediate and major cause of cell death under B deficiency (Koshiba et al. 2009).

Photosynthesis

Photosynthesis has been shown to be affected by B deficiency. The existing evidence has been mostly obtained from *in vivo* experiments with plants grown under extended periods of B deficiency (Kastori et al. 1995, El-Shintinawy 1999), which raises the question whether such effects reflect a requirement for B in photosynthesis, or are related with the effects of B deficiency on other areas of plant metabolism. In fact, preliminary experiments with isolated spinach chloroplasts showed only weak effects, if any, under B-deficient conditions. It is therefore quite feasible that the effects observed in chloroplasts are secondary and caused by growth-inhibition, which could indirectly cause a reduced sink activity and an oversaturation of the electron acceptors of photosystems. These possible effects may increase the rate of photo-oxidative damage in response to further stresses (Goldbach and Wimmer 2007).

Signalling

The capacity of root exudates to induce the activity of nodulation genes is modulated by B nutrition in the host plant. Exudates derived from B-deficient pea plants led to a low level of *nod*-gene expression. Infection thread development was arrested at very early stages, and cell invasion by endocytosis was precluded, leading to

B-deficient nodules almost empty of bacteria (Redondo-Nieto et al. 2001).

González-Fontes et al. (2008) had proposed that B can have a role as a cellular signal through its direct or indirect interaction with transcription factors (TFs). The TF interaction with the target gene might be modulated by intercellular B levels due to an eventual direct linkage of B to the TF or to molecules that will then link to a TF. Recently, the WRKY6 was described to be a TF essential for the normal develpment of the roots of *A. thaliana* plants grown under low-B conditions (Kasajima et al. 2010).

BORON IN OTHER LIVING ORGANISMS

Only some 20 years ago it was considered that B was essential for animal development, including humans (Nielson 1997). Nowadays B is considered of nutritional importance for a broad spectrum of organisms, not only in plants and animals but also in bacteria and yeast.

Animals

The identification of a mammalian homolog of the *A. thaliana* BOR1 transporter, the NaBC1, suggests the need for minimum B levels in animal cells (Park et al. 2005). The finding that low concentrations of borate activate the MAPK pathway and that the knockdown of NaBC1 halted cell growth and proliferation provide further evidence for an essential functional role of B in animal metabolism. Several other reports showed that B was essential for embryonic development, at least for vertebrates, where B deprivation resulted in a high percentage of necrotic eggs and abnormal development of the gut in *Xenopus laevis* (Fort et al. 1999). The early stage of development is especially sensitive to B deficiency as described for zebrafish (Rowe and Eckhert 1999). It is interesting that Behrendt and Zoglauer (1996) also demonstrated that B was required during the early phase of somatic embryogenesis in the plant species, *Larix decidua*, where suspensor development formation was blocked without a B supply.

It has been found that physiological concentrations of B reduced the amount of insulin required to maintain plasma glucose (Bakken and Hunt 2003), but in a number of nutritional studies, the beneficial effects of B in animals seems to be related with bone metabolism. For example, B supplementation of a low B diet reduced gross-bone abnormalities in the vitamin D-deficient chick (Bai and Hunt 1996) and enhanced the apparent absorption and retention of calcium and phosphorus, and increased femur magnesium concentrations in vitamin D-deficient rats (Hegsted et al. 1991). In male pigs, bone lipid was lower and the bending moment higher when B was supplemented to a low-B diet (Armstrong et al. 2000). Thus, the effect of B on bone metabolism could be one of the essential functions of B in animals and humans.

Unicellular Organisms

Boron essentiality has been established for the growth of specific types of bacteria, such as heterocystous cyanobacteria (Bonilla et al. 1990) and actinomycetes of the genus *Frankia* (Bolaños et al. 1993). Both types of microorganisms require B for the stability of the envelopes that prevent access of nitrogenase-poisoning oxygen when grown under N_2-fixing conditions. Considering their different chemical composition, the stabilization of these envelopes by B is quite intriguing. In the heterocysts, B is present in an inner laminated layer formed of specific glycolipids. In contrast, in *Frankia*, B is found in a multilaminate vesicle wall composed of glycolipids and neutral lipids with a very high proportion of long-chain polyhydroxy, fatty acids or alcohols (Berry et al. 1991).

The discovery of a B-containing bacterial signal molecule, the auto-inducer AI-2, revealed a role for B in bacterial quorum sensing (Chen et al. 2002). Quorum sensing allows bacterial populations to monitor cell density, leading to coordinated alteration of gene expression, as the population increases (Coulthurst et al. 2002). The AI-2, identified as a furanosyl borate diester, is a signalling molecule for both, structure and function. The gene encoding the AI-2 synthase (luxS) is widely conserved, raising the possibility that AI-2 might serve as a universal bacterial signal for communication among species (Chen et al. 2002, Winans 2002). This molecule could also serve as a B transporter, in a way that B can be moved in or out of the cell, depending on growth or environmental conditions (Coulthurst et al. 2002).

Several B containing compounds with antibiotic activity are produced by several bacteria, such as *Sorangium cellulosum* and *Streptomyces* sp. For instance, the *Streptomyces antibioticus* is responsible for the production of boromycin, an antibiotic that inhibits the growth of Gram-positive bacteria (Rezanka and Sigler 2008) and the replication of HIV-1 (Kohno et al. 1996). Boron deficiency significantly decreases *Saccharomyces cerevisae* growth rate as well as population size. By analyzing log-phase mRNA of yeast grown without B, several genes were seen to be differentially expressed, namely those with functions in cell growth and maintenance (Becker et al. 2000, Bennett et al. 1999).

SCOPE OF THIS THESIS

The aim of this thesis is to increase the knowledge on the functions of B in higher plants. Two different plants, an important legume crop highly susceptible to B deficiency, *Lupinus albus*, and the model plant, *Arabidopsis thaliana*, were studied and several approaches were tested.

Since it is known that B participates in the cell wall structure, a first study consisted in the analysis of the protein profile of the leaf apoplast of B-deficient *L. albus* (Chapter 2).

Nutrient imbalance in soils is primary sensed by the root system, the organ responsible for sending signals to the shoot for growth modulation. Alterations in the protein pattern of *L. albus* roots due to B suppression were then analysed (Chapter 3).

Mineral stress can cause specific metabolic responses in the different plant organs. The analysis of metabolites of several organs of *L. albus* grown under B deficiency is described in Chapter 4.

To provide the insight into the early plant responses to B deficiency, a transcriptional analysis of *A. thaliana* was performed, as shown in Chapter 5.

The main conclusions of this work and proposed future perspectives are discussed in the last Chapter of this thesis (Chapter 6).

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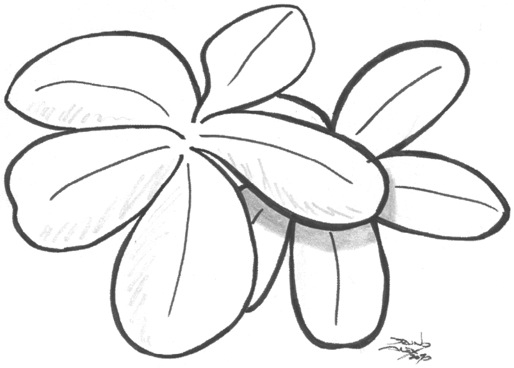
CHAPTER 2

ANALYSIS OF *Lupinus albus* LEAF APOPLASTIC PROTEINS IN RESPONSE TO BORON DEFICIENCY



Analysis of *Lupinus albus* leaf apoplastic proteins in response to boron deficiency

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CONTRIBUTION: The three first authors contributed equally for the plant growth, harvesting, electrophoresis technique and writing.

KEYWORDS: Apoplast, B deficiency, *Lupinus albus*, proteomics, PR-proteins.

SUMMARY

Boron (B) is essential for plant development and although its precise functions are not fully understood there is evidence for its importance in the extracellular matrix. So we have analysed by two-dimensional gel electrophoresis the effect of B deficiency in the soluble apoplast proteins of *Lupinus albus* leaf. Twenty-three polypeptide spots varied significantly between the control and the B deficiency patterns. Of these polypeptides only 9 could be identified by mass spectrometry techniques: PR-1 like protein, β-1,3-glucanases, class III chitinases, thaumatin like proteins and an expansin-like protein, all of them being involved in plant defence mechanisms. Only PR-1 like protein was *de novo* expressed under B deficiency, while the remaining proteins also responded to water stress. Although general response mechanisms seem to be triggered by both B and water stress, the pattern of protein expression was distinct, suggesting that under B deficiency specific regulatory mechanisms may be induced.

INTRODUCTION

Boron is an essential micronutrient for vascular plants and some green algae, but apparently is not required by fungi and bacteria (Marschner 1995). The role of this element in plant nutrition is still the least understood of all the mineral nutrients, which is surprising since on a molar basis its requirement is, at least for dicotyledons, higher than that of any other micronutrient (Marschner 1995). Boron is mainly localized in the plant cell wall, in fact, more than 90% of the total B in the cell is in that compartment (Blevins and Lukaszewski 1998), where it acts as a cross-linker (Matoh 1997, O'Neill et al. 1996, 2001). Matoh et al. (1993) isolated B-polysaccharide complexes from radish cell walls revealing that B was linked to rhamnogalacturonan-II molecules. This specific role of B is considered to be required for normal leaf expansion, but does not seem to explain all the observed effects of B deficiency in plant physiology (Dell and Huang 1997, Brown et al. 2002). Indeed, there is growing evidence showing that B plays essential roles in a number of membrane features and in the metabolism of carbohydrates, nucleic acids, indolacetic acid and phenols (Cakmak and Römheld 1997, Blevins and Lukaszewski 1998). It has been suggested that under B deficiency, phenolics concentrate through the stimulation of the enzymes phenylalanine-ammonium lyase and polyphenoloxidase (Cakmak and Römheld, 1997). Boron is also involved in lignification and nodulation processes (Bolaños et al. 1994).

The apoplast, that includes the cell wall, the intercellular spaces and other components external to the plasma membrane, is a dynamic compartment continuously changing during growth and development (Dietz 1997, Pignocchi and Foyer 2003). Due to the participation of B in the cell wall structure, the plant response to B deficiency is likely to induce changes in the protein profile of the apoplast. Such apoplastic response was observed in a number of stress conditions, such as osmotic stress (Marshall et al. 1999), heavy metal toxicity (Fecht-Christoffers et al. 2003, Kataoka et al. 2003), or cold stress (Marents et al. 1993). To get an insight into the apoplastic soluble proteins that react to B deficiency in *L. albus* leaves, we have analysed them by two-dimensional electrophoresis (2-DE) and mass spectrometry techniques. The identification of these proteins may give valuable information for understanding the metabolic processes that may be affected by B deficiency.

MATERIALS AND METHODS

Plant material

Lupin seeds (*Lupinus albus* cv. Rio Maior) pre-germinated in distilled water for 48h were sown in white sand and grown for 28 days under controlled temperature (19/25ºC, night/day), photoperiod (12h) and light intensity (250μmol m^{-2} s^{-1}, PAR). The plants were watered every other day with a nutrient solution (Arnon 1938) containing 0, 0.05, 0.46, 11.5, 23.1 or 46.1μM B.

The water-deficit was induced in 25 day old plants by suppressing watering for 3 days. The control plants were grown for 28 days with a suitable B concentration (23.1µM B). The leaf water potential was measured with a Sholander pressure chamber (PMS instrument Co, Corvallis, Oregon, USA) at pre-dawn (ψpd). At least 10 plants per experiment were analysed, in two independent experiments. Dry weight of lupin seeds was determined after oven drying for 48h at 80ºC.

Boron determination

Boron content in lupin seeds and in several substrates for plant growth (white sand, peat, vermiculite and perlite) was analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) at RAIZ (Instituto de Investigação da Floresta e Papel, Eixo, Portugal). The seeds were ashed overnight in an oven at 500 ºC and the ashes dissolved in 3N HCl. The substrates were extracted with 3N HCl (1:1) by shaking for 48h.

Extraction of soluble apoplastic proteins

Expanded leaves from 28 day old plants were used for the extraction of the soluble apoplastic proteins. This extraction was made using a vacuum infiltration/centrifugation technique based on the method of Parent and Asselin (1984) and Regalado and

Ricardo (1996). The leaf pieces were washed with distilled water, infiltrated in vacuum with 0.025M Tris–HCl pH 8.0 buffer, for 5 periods of 30s each and carefully blotted and centrifuged at 1480*xg* for 15min at 4ºC to collect the infiltrate. The protein samples were concentrated on a speed-vac and stored at -20ºC before use. Malate dehydrogenase (MDH, EC 1.1.1.37) was used as a cytosolic contamination marker of the apoplastic fluid, according to López-Millán et al. (2000).

Two-dimensional gel electrophoresis (2-DE)

Protein concentration in the extracts was determined according to Bradford (1976) and modified by Ramagli (1999). For isoelectric focusing (IEF) electrophoresis, the IPGphor system was used (Amersham Biosciences) with non linear (NL) pH gradient gels of 3–10 and 3–5.6 (IPGstrips, Amersham Biosciences) loaded with 25 µg or 60 µg of protein, respectively. Proteins were solubilized in 8M urea, 2% (w/v) CHAPS, 0.04M DTT and 0.5% (v/v) IPG buffer 3–10NL or/and 3.5–5NL (Amersham Biosciences). IEF was carried out at 30V for 12h, followed by 200V for 1h, 500V for 1.5h, 1000V for 1.5h, and 8000V for 6.5h, at 20ºC. Prior to SDS-PAGE the IPGstrips were equilibrated for 2x15min in a buffer solution containing 0.05M Tris–HCl pH 8.8, 6M urea, 30% (v/v) glycerol, 2% (w/v) SDS and a trace of Bromophenol Blue. To the first equilibration step DTT at 1% (w/v) was added and to the second one iodoacetamide at 4% (w/v). The SDS-PAGE was performed on slab gels (Laemmli 1970). The 2-DE gels were stained with colloidal Coomassie Blue (Neuhoff et al. 1985) or silver stained (Blum et al. 1987) and scanned using the ImageQuant v3.3 densitometer (Molecular Dynamics). After imaging, the gels were analysed by ImageMaster 2D Platinum software v5.0 (Amersham Biosciences). This analysis allowed to do spot detection, spot measurement, background subtraction and spot matching. The amount of a protein spot was expressed as the volume of that spot defined as the sum of the intensities of all the pixels of a spot. In order to correct the variability due to gel staining and to reflect the quantitative variations of protein spots intensity, the spot volumes were normalized as a percentage of the total volume in all of the spots present in the gel. To compare differences in protein abundance among the different samples, a one-way ANOVA ($p<0.05$) was performed for each spot, using the Ludesi software 2-DE interpreter (http://www.ludesi.com).

Protein identification by MS/MS

Spots were excised from colloidal Coomassie Blue stained gels and analysed by MALDI-TOF-TOF (matrix assisted laser desorption/ionization time of flight-time of flight) at the Technology

Facility, Department of Biology, University of York (UK). Database searching Similarity searching with the obtained partial amino acid sequences was performed at the EMBL-EBI server (http://www.dove.embl-heidelberg.de/Blast2/).

RESULTS

Determination of suitable B concentration for L. albus *growth*

Since it was intended to induce B deficiency in the lupin plant, it was necessary to choose an adequate substrate with low B content to grow the plants. Vermiculite, peat, perlite and white sand were analysed and their B content (nmol B/ g dry weight), were found to be respectively, 530±30, 30±4, 17±2 and 1.8±0.1. So, white sand was considered the best substrate. The Arnon (1938) nutrient solution was used in the experiments after checking that its B concentration (46.1µM) was adequate for *L. albus* growth. Considering that lupin seed contained 1.31±0.06µmol B/g dry weight, an unavoidable source of B, for seeds with a mean dry weight of 0.5g, even when grown at 0µM B, the lupine plant would have at its disposal *ca.* 0.65µmol B. Several experiments were performed in which B concentration in the nutrient solution was varied: 0, 0.05, 0.46, 11.5, 23.1 and 46.1µM. The total biomass and the root/shoot ratio were determined under those conditions

(Figure 2.1). The B concentration in a range of 11.5–23.1μM was not limiting *L. albus* growth and 46.1μM B was marginally excessive. So we chose half the B concentration of Arnon’s solution (23.1μM B) as a suitable concentration for *L. albus* growth.

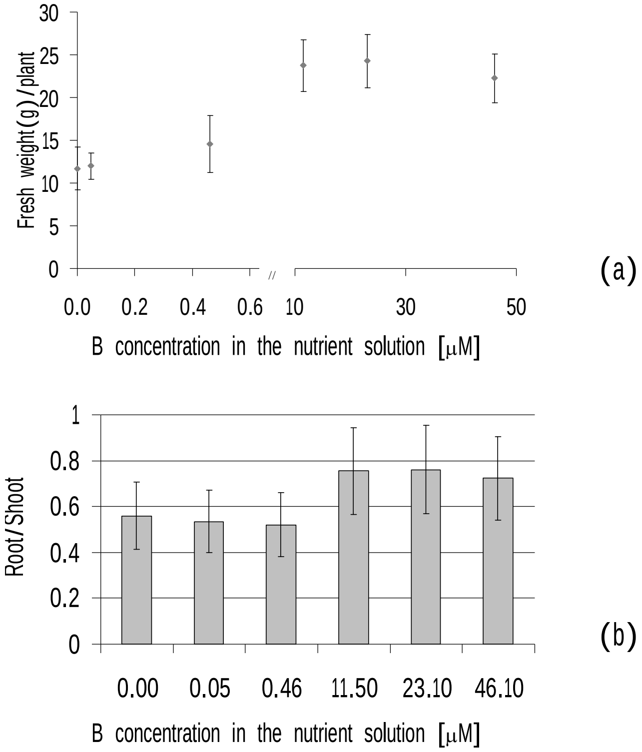


Figure 2.1. Effects of B concentrations in the nutrient solution on *L. albus* biomass production; (a) total biomass, (b) root/shoot ratio. At least ten plants per experiment were analysed, in two independent experiments and bars indicate standard errors.

Morphological and physiological effects of B deficiency

Morphological differences between the plants grown with 0 and 23.1μM B started to be evident from the 3rd to the 4th week from sowing (Figure 2.2). The most evident deficiency symptoms were necrosis of the terminal buds, darkened and structurally deformed leaves, cracking and breaking stems and petioles, and the absence of secondary and proteoid roots. It was also verified that the cotyledons became thicker and dark green and that their life span was extended. Despite the dramatic morphological effects, only a small decrease in ψpd was observed in B deficient plants when compared to that of water-deficit plants (Figure 2.3).

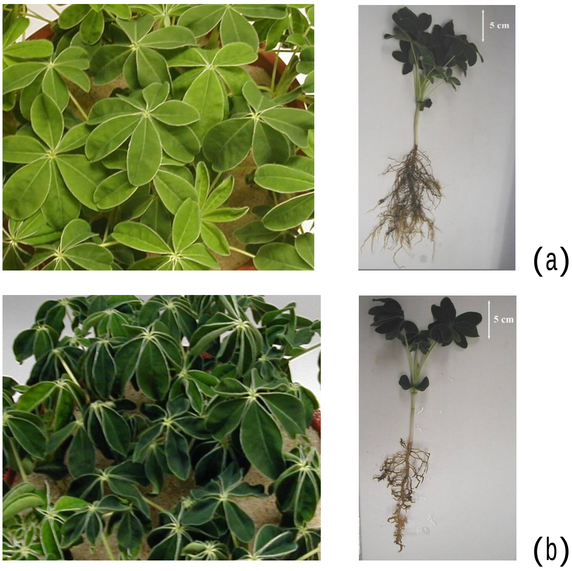


Figure 2.2. Morphological differences of L. albus plants grown in white sand culture for 28 days, with (a) 23.1μM B or (b) 0μM B in the nutrient solution.

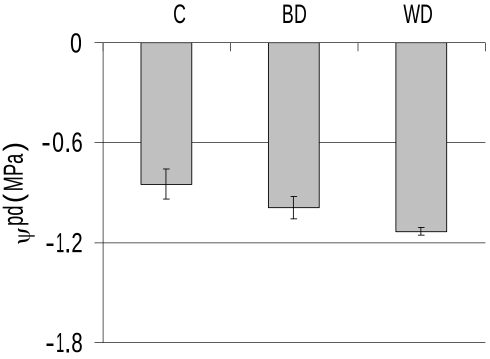


Figure 2.3. Leaf water potential (Ψpd) of 28 days old *L. albus*: control plants (Control), B deficient (BD) and water-deficit (WD) plants (bars indicating standard errors). The water potential was determined by Sholander pressure chamber measurements on the 5th leaf before the beginning of the illumination period.

Protein analysis by 2-DE

The activity of MDH was used as a marker of the contamination of the apoplastic samples with cytoplasm proteins. It was found that in

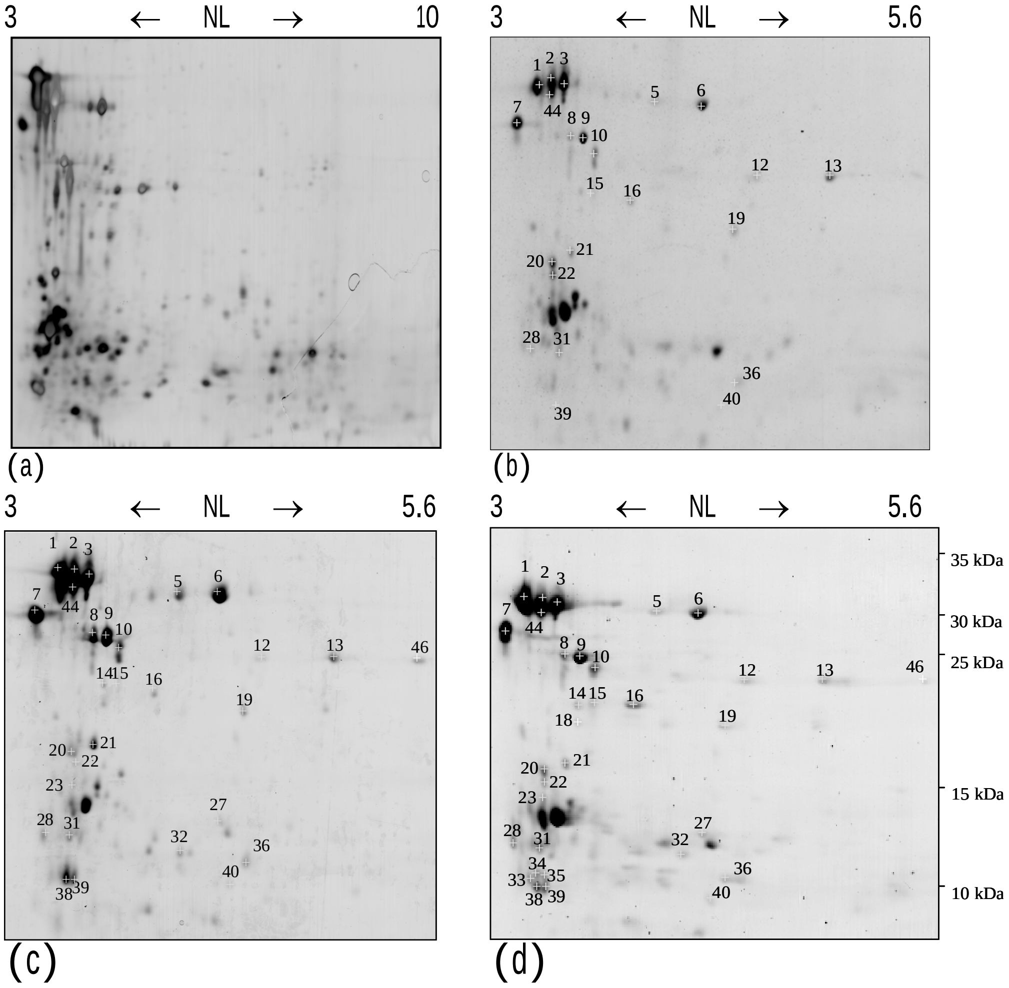


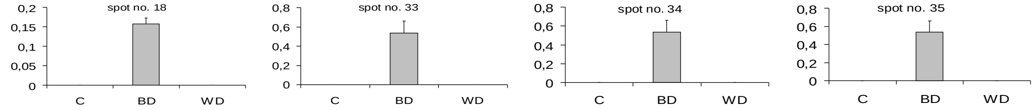
Figure 2.4. 2-DE gels from *L. albus* apoplast leaf proteins obtained from control (a, b), water deficit (c) and boron deficiency (d) plants. Gel (a) was loaded with 25 μg of proteins which were resolved using a NL pH gradient 3-10 and silver stained; gels (b, c, d) were loaded with 60μg proteins which were resolved using a NL pH gradient 3.0-5.6 and colloidal Coomassie Blue stained. Labelled proteins shown in the gels were selected for MS/MS identification.

the apoplastic samples this enzymatic activity was less than 3% (mean value of 4 replicates) of the activity of leaf homogenates. This indicates a negligible apoplastic contamination with cytoplasmatic components. 2-DE was carried out using IPGstrips with a pH gradient of 3-10NL and it was observed that the major soluble apoplastic proteins have an acidic pI (Figure 2.4a). Consequently a narrow pH gradient of 3-5.6NL was used to perform all the subsequent studies. The apoplastic soluble proteins collected from plants under water-deficit were used for a comparative purpose in order to identify the proteins that responded to general stress conditions (Pinheiro et al. 2005). The obtained protein profiles were analysed with ImageMaster 2D Platinum Software v5.0 for detection and matching of the protein spots and it was observed that 51 spots were present in the control and water-deficit gels and 55 spots in the B deficiency gels (Figure 4b-d). The statistical analysis made with Ludesi software 2-DE interpreter identified the polypeptides which were significantly altered by the imposition of the stress conditions ($p<0.05$) (Table 2.1).

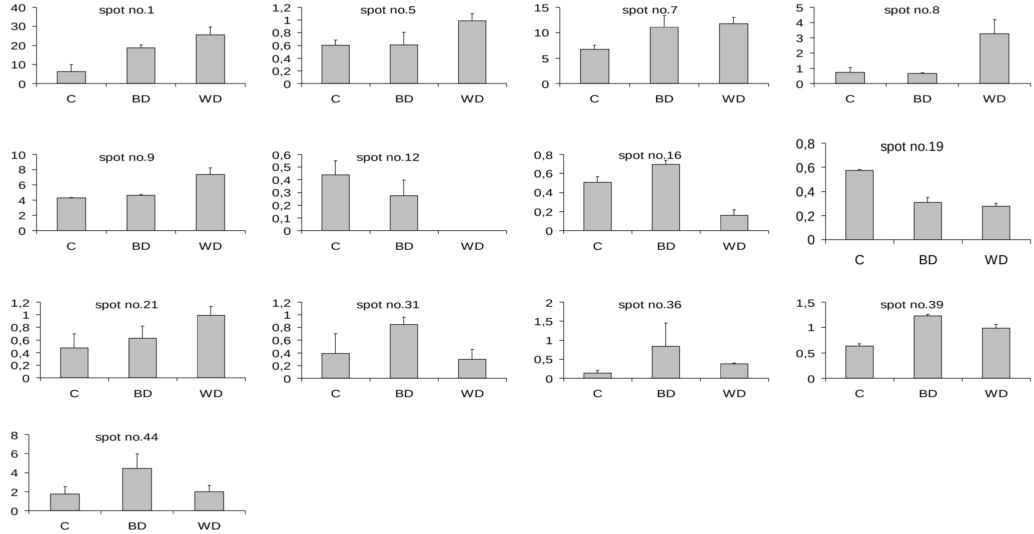
Three groups of polypeptides with particular expression patterns relative to the control could be defined: i) *de novo* expressed under B deficiency (4 polypeptides), ii) *de novo* expressed under B deficiency and/or water-deficit (6 polypeptides) and iii) already

Table 2.1. Relative abundances (% of normalized volume) of the individual apoplast spots whose levels were significantly altered in the stress conditions (bars indicating standard errors). C, control; BD, B deficiency; WD, Water-deficit.

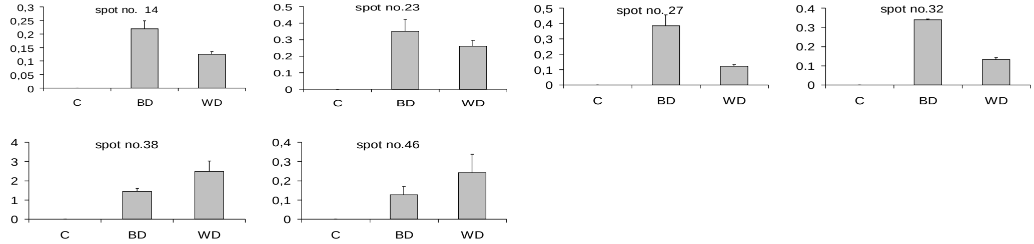
De novo expressed under BD



Responsive to BD and/or WD



De novo expressed under BD and WD



present in the control but responsive to B deficiency and water-deficit (13 polypeptides). MS/MS methods were used to try to identify these spots and others unaffected by the imposed stresses (spots 2, 3, 6, 10, 13, 15, 22 and 40).

Protein identification by MS/MS

Thirty one spots were selected for protein identification by MS/MS. We were able to identify proteins of 12 spots: two glucanase isoforms (Q01412), three acidic chitinase isoforms (O49876), four thaumatin-like proteins (P83332, P25096, Q8LKA8, O80327), one PR-1 like protein (Q9S9G8), one expansin-like protein (Q6K4C6) and one putative secretory protein (Q9FWU3) (Table 2.2). All these identified proteins are extracellular, since the presence of the signal peptide was predicted when using SignalP software (http://www.cbs.dtu.dk/services/SignalP). The spot 13 which is an exception, since it had no predicted signal peptide, was nonetheless identified as an extracellular protein. The polypeptides of the remaining 19 spots, although sequenced were not identified, which may be explained by the scarcity of information existing in lupin sequence databases.

Table 2.2. MS/MS identifications of *L. albus* leaf apoplast proteins resolved by 2-DE. C, control; BD, B deficiency; WD, water-deficit.

Spot	**Sequence** [b] **(TrEMBL Ac No.)** [c]		**pI** [d]	**MW** [d]
	***De novo* expressed under BD**			
33	PR-1 like protein (Q9S9G8)		9.45	10.8
Ions [a]	1126.56	LLTHGPGFNGN		
	1244.51	EPSKADYLGKH		
	1656.73	DSKADYLNAHNAAR		
	1731.81	KGMSDGKDKGVDPFGHG		
	Responsive to BD and/or WD			
1	Endo-β-1,3-glucanase (Q01412)		6.67	35.2
Ions [a]	1433.65	LYSPDEGTLQALK		
	1543.70	YLAVGNEVEPNDPK		
	2168.98	(LFA)RFETYLFAMFDENR		
7	Class III chitinase (O49876)		4.48	28.2
Ions [a]	1110.50	FNDLQTGYSDALK		
	1470.65	YGGVMLWNR		
8	Class III chitinase (O49876)		4.48	28.2
Ions [a]	1126.55	LITHPGGFNGN		
	1471.70	FNDLKTGYSDALK		
	2891.35	SSKGGADLVLCAGGAELDFNNGPDDAGKGA		
9	Class III chitinase (Q945U2)		9.18	30.9
Ions [a]	952.467	ALDGFSSKK		
	1755.87	AAGTFGDTKGEPLNLAHG		
	2891.35	RYPNFHGLDFDLEAGGMRVLDENPE		
	3336.95	SRTLLVVCVAAAAPSGGFMWAHLLSKVLLTPK		
16	Thaumatin-like protein (P25096)		4.84	21.5
Ions [a]	1562.71	GCTADINGKGCPSVLK		
	1819.74	DDKTFGGMFGPGGTNYR		
	2198.81	DTPLLHVPSLPYKSEPYSR		
31	Osmotin-like protein (Q8LKA8)		4.58	39.6
Ions [a]	1100.46	CGPDAYSYPK		
	1562.72	GCTADLNGKGCSPVLK		
	1801.74	VLKTSTFFGGPPECYR		
	1819.75	VMKTSTFTCVDATPYR		
39	Putative blight-associated protein/ Expansin like protein (Q6K4C6)		8.25	13.4
Ions [a]	1731.81	LTCTGATNKGVPKPCK		
	1747.81	DDCTKTNKGVPKPCK		
	959.503	TGLADLNAGK		
	2807.32	DCLLWLASMAEKSLDLTGVSVSSDPP		

Table 2.2 (cont) MS/MS identifications of *L. albus* leaf apoplast proteins resolved by 2-DE.
C, control; BD, B deficiency; WD, water-deficit.

Spot	**Sequence[b] (TrEMBL Ac No.)[c]**		**pI[d]**	**MW[d]**
44	Thaumatin-like protein (O80327)		5.01	22.9
Ions[a]	882.381	STEMEGGR		
	979.402	TGCSNNNGR		
	2119.01	STSCPANLNTVCLDVLKGGL		
	2327.99	CGNSGEKKCAKPGYMDCHK		
	Non responsive to BD or WD			
2	Thaumatin-like protein (P83332)		8.14	23.3
Ions[a]	1230.61	LSDLPAPAFSGR		
	1458.60	GTGCLAFNKPKYCG		
	2119.01	ARAHPANLNTVGCNGGVKLGIG		
	2133.02	VCSAGEARGKTVELMNKVVAA		
6	Endo-β-1,3-glucanase (Q01412)		6.67	35.2
Ions[a]	1384.70	ASPTKDTCAGRFM		
	1544.76	YLAVGNEVEPGGDPK		
	2724.54	AKYVLPVLAGNLKNALSSANIGAVGRK		
	2746.48	AKYVLPVNSSLANKLNAGLKLLGAYE		
13	Putative secretory protein (Q9FWU3)		7.89	28.7
Ions[a]	1061.18	AGMDAKLNNAG		
	1172.35	KGYAPSHSVVK		
	1295.46	AEDKLWKDYK		
	1367.54	RMDELGEKYAR		
	1735.99	LTYCGTPPAMPTDGGVR		
	1767.96	APYANPKVWHSPSGTR		
	2567.97	CDDPNNKLLGHRTMKKNNLVTR		

[a] Ion precursor (mass/charge unit)

[b] Proteins were sequenced by TOF/TOF at University of York

[c] Proteins were identified by http://www.dove.embl-heidelberg.de/Blast2/

[d] pI and MW were calculated by http://www.exapsy.org/

DISCUSSION

The apoplast, as the prime barrier between the plant and its environment is a critical compartment for studying plant responses to stressful conditions. In fact our analysis of that compartment in *L. albus* leaves revealed marked alterations in response to B deficiency and water-deficit. Apoplastic protein alterations were also reported in tobacco under salt stress (Dani et al. 2005) and in cowpea subjected to manganese toxicity (Fecht-Christoffers et al. 2003). Although the majority of *L. albus* proteins analysed gave good MS/MS spectra, only a few could be identified. This result may reflect the reduced number of studies concerning the apoplast and the scarcity of information available for *L. albus* proteins in databases.

The identified proteins can all be related to stress and plant defence responses, namely of the pathogenesis related type. Both PR and PR-like proteins (Van Loon and Van Strien 1999) are known to be induced in plant tissues by several biotic and abiotic stresses, such as fungal infection (Niderman et al. 1995), heavy metal toxicity (Rakwal et al. 1999), salinity (Esaka et al. 1994), high temperature (Margis-Pinheiro et al. 1994) and wounding (Ruperti et al. 2002). In previous studies it was observed that in the *L. albus* leaf apoplast PR-like proteins are constitutively expressed (Pinto and Ricardo 1995, Regalado and Ricardo 1996). We now found that endo-β-1,3-glucanases, thaumatin-like proteins and class III chitinases, were significantly increased under B deficiency as well as under water-deficit. The increased incorporation of glucose into the cell wall β-1,3-glucans, as a result of B deficiency may suggest a higher involvement of endo-β-1,3-glucanase in the cell wall metabolism under this stress (Dugger and Palmer 1985). Kobayashi *et al*. (2004) also observed an enhancement of endo-β-1,3-glucanase gene expression in tobacco cells under low B supply. In our study, of the 4 proteins *de novo* expressed under B deficiency only one could be identified, a PR-1-like protein belonging to a PR-1 gene family to which no putative function has been assigned (Green and Fluhr 1995).

Kobayashi et al. (2004) observed that B deficiency induces genes, namely of PR-like families, that are involved in the general response of plants to stressful conditions. The precise role of these several PR proteins in stress responses, and in particularly in B deficiency, is not fully understood. However it has been shown that some of them participate in specific processes such as antimicrobial activity (Broekaert et al. 1995, Epple et al. 1997) and antifreeze activity (Hon et al. 1995).

A putative blight associated protein, p12, described as an expansin-like protein (EXPL) soluble in the extracellular space (Kayim et al. 2004), was shown to increase under B deficiency. Expression of expansin genes correlates with growth of cells, and its increase also occurs during fruit ripening (Cosgrove 1997). However, the functional and potential wall-loosening activity of the EXPL proteins has not been established (Cosgrove 2003).

The presence in *L. albus* apoplast of three chitinase isoforms and two glucanase isoforms, that could result from post-translational modifications of the proteins (Dani *et al*. 2005), appears to be of some relevance. Salzer *et al*. (2000) when studying fungal infection and symbiosis in *Medicago truncatula* concluded that protein isoforms may have a crucial role in plant response to stress. It is also well documented that in addition to stress-specific adaptive responses plants have some other types of responses that protect them from more than one environmental stress (Chinnusamy et al. 2003). In this connection, it is relevant to note that the proteins that responded to both B deficiency and water-deficit in *L. albus* apoplast had a different pattern of expression. This seems to suggest that specific regulatory mechanisms are involved in those distinct stress conditions.

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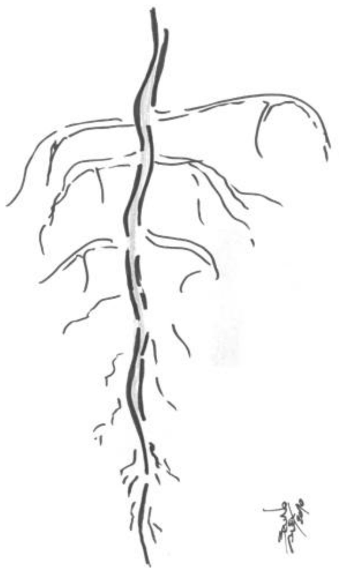
CHAPTER 3

THE ANALYSIS OF *Lupinus albus* ROOT PROTEOME REVEALED CYTOSKELETON ALTERED FEATURES DUE TO LONG-TERM BORON DEFICIENCY



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

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CONTRIBUTION: The first author performed all the experimental work and writing, with the exception of the technical MS analysis.

KEYWORDS: Boron deficiency, cytoskeleton, *Lupinus albus*, proteomics, root.

SUMMARY

Boron (B) deficiency in plants greatly limits their growth and development. Since the role of B in plant biology is not completely understood, we have analysed the adaptive responses of *Lupinus albus* roots to a long-term B deficiency. For this purpose, two-dimensional electrophoresis and mass spectrometry techniques were used to compare the root proteome of plants treated with or without B. Despite the large morphological differences observed, from the more than 790 protein spots detected, only 262 were responsive to B deficiency. The suppression of the majority of these polypeptides reflects the reduction of the biosynthetic fluxes. The cytoskeletal biosynthesis was also affected by B deficiency, probably indicating an important contribution of B in this process.

INTRODUCTION

Boron (B) has long been known to be an essential micronutrient for higher plants (Warington 1923), but to date, its precise roles in essential plant biological processes remain elusive. So far, B is known to have a structural role in the cell wall, through the borate cross-linking of rhamnogalacturonan-II (Kobayashi et al. 1996, O'Neill et al. 1996, 2001), but a plethora of biochemical, physiological and anatomical effects due to B suppression are not well explained by this role alone (Brown et al. 2002). Several authors proposed that B may be involvement in membrane features and cytoskeleton structural processes (Bassil et al. 2004, Bonilla et al. 2009). Since nutrient imbalance is primary sensed by the root system and because this is the organ responsible for sending signals to the shoots to modify growth and developmental processes (Fitter 2002), we have analysed the *Lupinus albus* L. plant root adaptive responses to long-term B deficiency. For this purpose, the root proteome of plants grown with and without B were analysed by two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) techniques.

MATERIALS AND METHODS

Plant Material

Lupin seeds (*Lupinus albus* cv. Rio Maior) were pre-germinated in distilled water for 48h, sown in white sand and grown under controlled conditions of temperature (19/25ºC, night/day), photoperiod (12h) and light intensity (250μmol m^{-2} s^{-1}, PAR). The plants were watered every other day with a nutritive solution (Arnon 1938) containing either 0 or 23.1μM B (Alves et al. 2006). The roots of *Lupinus albus* were harvested 28 days after sowing. Fresh weight statistical analysis was made with a Student's *t*-test ($p<0.05$) by using the SigmaStat v3.10 software (Systat Software, Inc.).

Protein extraction and solubilisation

Roots were ground to a fine powder in liquid nitrogen, resuspended in a cold acetone solution containing 60 mM DTT and 10% (w/v) TCA (12.5mL/g) and kept at -20ºC for 1h. After a 15min centrifugation at 27,200*xg* and 4ºC, the pellet was resuspended in cold acetone with 60 mM DTT (25mL/g) and kept at -20ºC for 1h. After centrifugation, the pellet was dried under vacuum and ressuspended (0.05g/mL) in a solubilization buffer containing 2M thiourea, 7M urea, 4% (w/v) CHAPS, 0.4% (v/v) Triton X-100, 60 mM DTT and 1% (v/v) IPG Buffer 3-10 NL (GE). After 2h dissolution at room temperature, the protein extracts were centrifuged at 15,000*xg* for 10min and the supernatant collected and stored at -80ºC until further use. The Protein concentration was determined according to the Bradford method as modified by Ramagli (Ramagli 1999).

Two-dimensional gel electrophoresis

For isoelectric focusing (IEF) electrophoresis, the IPGphor system was used (Amersham Biosciences) with a non-linear pH gradient gel of 3–10 (IPGstrips, GE) loaded with 200μg of protein resolubilized in 8M urea, 4% (w/v) CHAPS, 60mM DTT and 0.5% (v/v) IPG buffer 3–10NL (GE). The IEF was carried out at 30V for 12h, followed by 200V for 1h, 500V for 1.5h, 1000V for 1.5h, and 8000V for 6.5h, at 20ºC. Prior to SDS-PAGE the IPGstrips were equilibrated for 2x15min in a buffer solution containing 0.05M Tris–HCl pH 8.8, 6M urea, 30% (v/v) glycerol and 2% (w/v) SDS. The DTT at 1% (w/v) was added to the first equilibration step and 4% (w/v) iodoacetamide to the second one. The SDS-PAGE was performed on slab gels (Laemmli 1970) and run at constant temperature of 15ºC. The 2-DE gels were stained with colloidal Coomassie Blue (Neuhoff et al. 1985) and scanned using the ImageScanner (Amersham Biosciences).

Two-dimensional gel analysis

The gels were analysed in the ImageMaster 2D Platinum software v5.0 (GE) for spot detection, measurement and matching. In order to correct for variation in gel staining and for quantitative variations of protein spot intensities, the total spot volumes of each gel were normalized to 100, and % spot volumes calculated. The protein abundance analysis was performed according to Meunier et al. (2005), with minor changes. For each differentially expressed spot a Student's *t*-test ($p<0.05$) analysis was performed with SigmaStat v3.10 (Systat Software, Inc.).

In-gel digestion

The protein spots were rinsed with a washing solution of 50% (v/v) acetonitrile and 0.1M ammonium bicarbonate for 4h. Prior to digestion the dried spots were reduced with 0.01M DTT for 2h at 37ºC, then alkylated with 0.05M iodoacetamide for 15min at room temperature, in the dark. The gel spots were again rinsed with the washing solution for 2h. The digestion was made overnight, with 125ng of trypsin (Sequencing Grade, Promega) in 0.05M ammonium bicarbonate at 37ºC. 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 redissolved in 0.1% (v/v) acetic acid and 2% (v/v) acetonitrile solution was used for mass spectrometric analysis.

MS/MS analysis

The trypsin digested proteins were analysed by capillary liquid chromatography tandem MS (LC/MS/MS) using a set up of a trapping 300SB C-18 column (0.3 x 50mm) (Agilent Technologies, Basel, Switzerland) and a separating column (0.1mm x 10cm) that had been packed with Magic 300Å C18 reverse-phase material (5μm particle size, Michrom Bioresources, Inc., 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 85min at a flow rate of 100μL/min. A pre-column split was used to reduce the flow to approximately 100nL/min. The injection of 10μL of peptide digest was made by an auto-sampler thermostatted to 4ºC and the eluting peptides ionized at 1.7kV. The mass spectrometer was operated in a data-dependent fashion. The precursor scan was done in the Orbitrap set to 60,000 resolution, while the fragment ions were mass analysed in the LTQ instrument. A top five method was run so that the five most intense precursors were selected for fragmentation. The MS/MS spectra were then searched against the NCBI non-redundant database, version August 15th 2008, using TurboSequest software (Gatlin et al. 2000). The databank was searched with Bioworks version 3.3.1. SP1 by setting the precursor ion tolerance to 10ppm, while the fragment ion tolerance was set to 0.5Da. Cleavage rules were set to Fully enzymatic – cleaves at both ends, allowing 2 missed cleavages. Post filtering was set to the following parameters: ΔCN, 0.1; Xcorr versus charge state was 1.50 (1+), 2.00 (2+), 2.50 (3+); peptide probability, 0.01; protein probability 0.01.

RESULTS AND DISCUSSION

Morphological effects of B deficiency

As dicots, *Lupinus albus* plants are quite sensitive to B suppression. Plants grown under B deficiency for two weeks already showed morphological differences, with shorter and darker roots lacking secondary and proteoid roots, despite that no significant difference in the biomass were observed. The morphological differences observed in the roots indicate that the initial B present in the lupin seed is not enough for this early stage of development. Three weeks from sowing, the morphological differences in roots become more evident, accompanied by a significant difference in the biomass. In shoots, the

Figure 3.1. Morphological differences of *Lupinus albus* plants grown under B deficiency. The development of *L. albus* plants grown with (C) and without B (BD) in the nutrient solution was monitored two, three and four weeks after germination.

morphological differences due to B deficiency become visible, with darker and deformed leaves, but without significant biomass differences. After four weeks the morphological differences were much more marked, with significant reduced biomass in both shoots and roots (Figure 3.1, Figure 3.2).

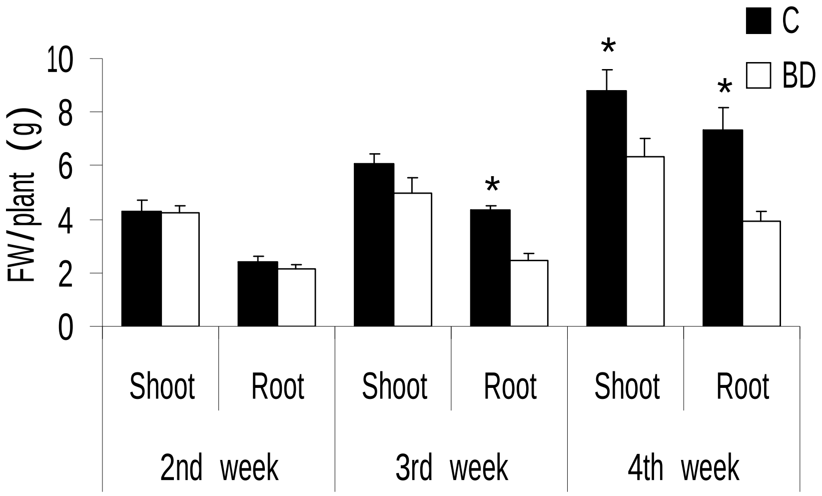


Figure 3.2. Fresh weight of *Lupinus albus* shoots and roots grown with (C) and without B (BD) for two, three and four weeks after seedling. Bars indicate standard errors. Significant changes were evaluated by the Student's *t*-test (*, $p<0.05$).

Quantitative variations of the root proteome

Considering the importance of the root system and the ability of the lupin plant to maintain a reduced growth under B deficiency, the root proteome of plants treated with or without B were analysed by 2-DE (Figure 3.3). This fine resolution system, coupled to MS

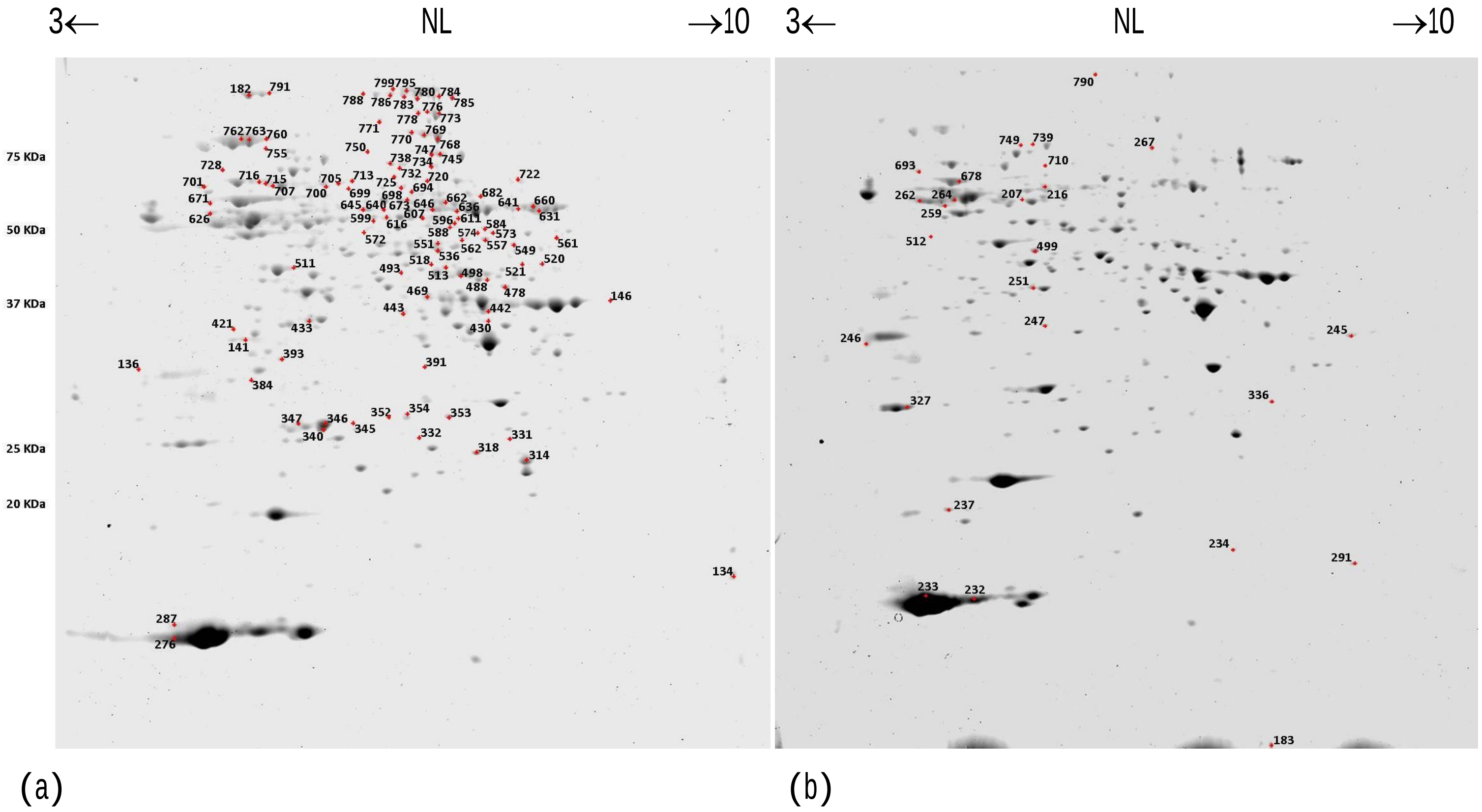


Figure 3.3. Representative 2-DE gels of *Lupinus albus* roots grown with (a) and without B (b). The gels were Coomassie Blue stained. Numbered proteins were identified by MS/MS (Table 3.1). Labelled proteins in gel a) are those suppressed due to B deficiency, while those labelled in gel b) are differentially expressed or *de novo* expressed due to B deficiency.

techniques and adequate statistical analysis, is a powerful tool to investigate the proteins that were differential expressed due to a long-term B deficiency.

Despite the large morphological changes observed due to B deficiency, the analysis of the root proteome revealed that 532 spots (67%) were non-responsive to B deficiency and that only 262 spots (33%) were responsive to B deficiency, either being suppressed (26%), expressed *de novo* (5%) or differentially expressed (2%) (Figure 3.4). The 262 spots reproducibly detected as responsive to B deficiency were analysed by MS/MS techniques, and, 213 were identified. However, 83 spots gave ambiguous identification (i.e. spots that had homology with two or more non-homologous proteins) and, thus only the remainder 130 spots were considered for secure quantification.

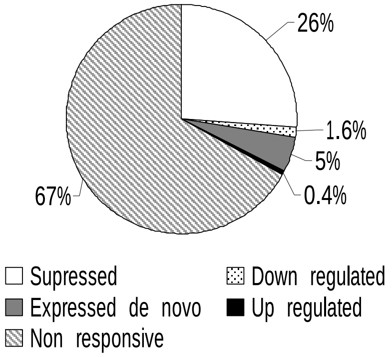


Figure 3.4. Percentage of polypeptides that was responsive to B deficiency in the *Lupinus albus* plant roots.

Metabolic changes associated with B deficiency

In an attempt to identify the metabolic events associated with the plant root ability to cope with long-term B deficiency the identified proteins were grouped according to their biological functions (Figure 3.5).

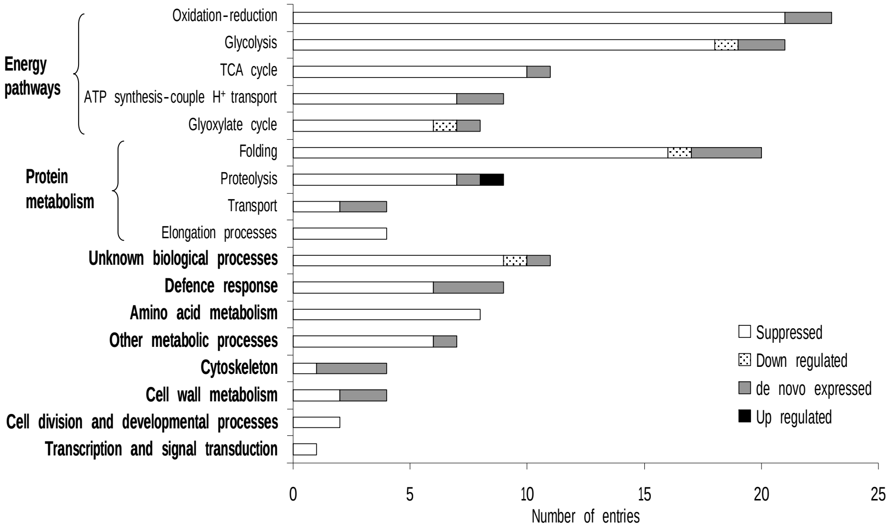


Figure 3.5. Functional classification of the proteins that had responded to B deficiency. The proteins of *Lupinus albus* plant roots identified were group according to the biological functions described in databases.

The majority of the suppressed proteins are from classes related with normal plant growth and developmental processes. This higher number of suppressed proteins is an indication of reduced 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, defence 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 most relevant biological processes where the identified proteins (Table 3.1) are involved will be discussed and related with B role in plant metabolism.

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

Table 3.1. Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

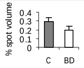
ID[a]		**Protein identification[b]**	**Species**	**pI/MW (kDa)**		**Expr. level**
				Predicted[c]	**Exp**	
	Energy pathways					
146	P08477	Glyceraldehyde-3-phosphate dehydrogenase	*Hordeum vulgare*	---	8.2/38	S
216	Q8W557	UDP-glucose pyrophosphorylase	*Amorpha fruticosa*	6.07/51.6	5.6/54	N
232	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	5.1/15	N
245	Q41712	Ascorbate peroxidase	*Vigna unguiculata*	5.64/27.0	8.6/31	N
251	Q38799	Pyruvate dehydrogenase E1 component subunit β	*Arabidopsis thaliana*	5.11/35.9	5.5/36	N
264	Q40079	V-type proton ATPase subunit B2	*Hordeum vulgare*	5.12/53.7	5.0/53	N
340	Q41712	Ascorbate peroxidase	*Vigna unguiculata*	5.64/27.0	6.3/26	S
346	Q43758	Ascorbate peroxidase	*Glycine max*	5.51/27.1	6.4/27	S
347	Q8H1K7	Ascorbate peroxidase	*Retama raetam*	5.88/23.6	5.7/27	S
421	Q0JGZ6	Fructokinase-1	*Oryza sativa*	5.07/34.7	5.1/35	S
442	Q8GZN3	Malate dehydrogenase	*Lupinus albus*	6.10/35.6	6.4/37	S
443	Q8GZN3	Malate dehydrogenase	*Lupinus albus*	6.10/35.6	5.9/36	S
469	Q40676	Fructose-bisphosphate aldolase	*Oryza sativa*	6.55/38.7	6.0/38	S
478	Q9SXP2	Formate dehydrogenase 1, mitochondrial	*Oryza sativa*	6.20/39.3	6.6/40	S
488	P52901	Pyruvate dehydrogenase E1 component subunit α-1	*Arabidopsis thaliana*	6.42/39.6	6.4/41	S
493	P50318	Phosphoglycerate kinase	*Arabidopsis thaliana*	5.04/42.6	5.9/41	S
498	P52902	Pyruvate dehydrogenase E1 component subunit α	*Pisum sativum*	---	6.2/42	S
499	Q9SAJ4	Phosphoglycerate kinase	*Arabidopsis thaliana*	5.49/42.1	6.1/41	
511	P50318	Phosphoglycerate kinase	*Arabidopsis thaliana*	5.04/42.6	5.5/43	S
536	Q7Y0W9	NADP-specific isocitrate dehydrogenase	*Lupinus albus*	6.13/46.1	6.0/46	S
551	Q7Y0W8	NADP-specific isocitrate dehydrogenase	*Lupinus albus*	5.99/46.0	6.0/47	S

Table 3.1 (cont) Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

ID[a]		Protein identification[b]	Species	pI/MW (kDa)		Expr. level
				Predicted[c]	Exp	
557	B0FGG5	Monodehydroascorbate reductase	*Vaccinium corymbosum*	5.78/47.4	6.3/48	S
561	P93033	Fumarate hydratase 1	*Arabidopsis thaliana*	6.65/49.9	7.3/48	S
562	B0FGG5	Monodehydroascorbate reductase	*Vaccinium corymbosum*	5.78/47.4	6.1/49	S
572	A9SGH3	Enolase	*Physcomitrella patens*	5.22/46.1	5.7/50	S
588	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating	*Arabidopsis thaliana*	5.62/53.3	6.1/50	S
599	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating	*Arabidopsis thaliana*	5.62/53.3	5.7/52	S
607	Q9LI00	6-phosphogluconate dehydrogenase, decarboxylating	*Oryza sativa*	5.85/52.7	6.0/53	S
611	Q9LEJ0	Enolase 1	*Hevea brasiliensis*	5.57/47.8	6.1/73	S
626	Q40079	V-type proton ATPase subunit B2	*Hordeum vulgare*	5.12/53.7	4.9/54	S
631	Q9FNN5	Subunit of complex I	*Arabidopsis thaliana*	8.46/53.4	7.0/55	S
636	P12862	ATP synthase subunit alpha	*Triticum aestivum*	5.70/55.3	6.1/55	S
640	P12862	ATP synthase subunit alpha	*Triticum aestivum*	5.70/55.3	5.8/55	S
641	P49357	Serine hydroxymethyltransferase 1	*Flaveria pringlei*	8.15/53.6	6.8/55	S
646	P12862	ATP synthase subunit alpha	*Triticum aestivum*	5.70/55.3	5.0/43	S
660	O23254	Serine hydroxymethyltransferase	*Arabidopsis thaliana*	6.80/51.7	7.0/56	S
662	B9SH74	Aldehyde dehydrogenase, putative	*Ricinus communis*	5.87/42.0	6.1/57	S
700	Q42919	Glucose-6-phosphate 1-dehydrogenase	*Medicago sativa*	5.85/58.9	6.5/63	S
738	O82663	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1	*Arabidopsis thaliana*	5.58/66.0	5.8/71	S
739	P31405	V-type proton ATPase catalytic subunit A	*Gossypium hirsutum*	5.36/68.5	5.5/77	N
771	Q9FGI6	NADH-ubiquinone oxidoreductase 75 kDa subunit	*Arabidopsis thaliana*	5.72/77.9	8.1/29	S
780	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	5.9/135	S
783	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	5.9/138	S
784	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	6.0/138	S

Table 3.1 (cont) Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

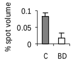
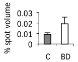
ID[a]		Protein identification[b]	Species	pI/MW (kDa)		Expr. Level
				Predicted[c]	Exp	
785	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	6.1/136	S
786	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	6.0/138	S
788	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	5.7/143	S
790	P49608	Aconitate hydratase	*Cucurbita maxima*	5.74/98.0	5.8/140	
	Protein metabolism					
134	O49886	Peptidyl-prolyl cis-trans isomerase	*Lupinus luteus*	8.71/18.3	9.6/17	S
136	Q40682	Elongation factor 1-delta 2	*Oryza sativa*	4.40/24.5	4.2/32	S
247	Q94JX9	Nascent polypeptide-associated complex subunit alpha-like protein 2	*Arabidopsis thaliana*	4.37/23.7	5.6/32	N
259	Q9LS40	CND41, chloroplast nucleoid DNA binding protein-like	*Arabidopsis thaliana*	5.27/53.2	5.0/48	N
291	O49886	Peptidyl-prolyl cis-trans isomerase	*Lupinus luteus*	8.71/18.3	8.7/17	N
332	Q3HVM0	Proteasome subunit alpha type	*Solanum tuberosum*	5.40/28.1	5.9/26	S
345	A9TVH1	Proteasome subunit alpha type	*Physcomitrella patens*	5.92/27.2	5.7/27	S
352	A5AXI5	Proteasome subunit alpha type	*Vitis vinifera*	6.11/27.2	5.8/27	S
354	A5ALB2	Proteasome subunit alpha type	*Vitis vinifera*	5.91/27.3	5.9/27	S
512	A9PEP6	Predicted protein	*Populus trichocarpa*	4.94/45.3	5.0/43	
513	Q9ZRU6	Elongation factor Tu	*Catharanthus roseus*	--	6.0/43	S
573	A0FH76	EBP1	*Solanum tuberosum*	6.26/42.8	6.4/50	S
584	Q0DDX2	26S protease regulatory subunit 7	*Oryza sativa*	6.03/47.7	6.3/50	S
645	Q6K669	Leucine aminopeptidase 2	*Oryza sativa*	5.50/55.0	5.7/55	S

Table 3.1 (cont) Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

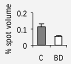
ID[a]		Protein identification[b]	Species	pI/MW (kDa)		Expr. Level
				Predicted[c]	Exp	
693	P21239	RuBisCO large subunit-binding protein subunit α	*Brassica napus*	4.78/57.0	4.9/61	
694	P21239	RuBisCO large subunit-binding protein subunit α	*Brassica napus*	4.78/57.0	5.9/61	S
699	Q940P8	Putative uncharacterized protein	*Arabidopsis thaliana*	5.59/57.3	5.6/63	S
701	P21239	RuBisCO large subunit-binding protein subunit α	*Brassica napus*	4.78/57.0	4.9/63	S
705	Q93ZM7	Chaperonin CPN60-like 2	*Arabidopsis thaliana*	5.32/57.1	6.0/64	S
707	Q05045	Chaperonin CPN60-1	*Cucurbita maxima*	5.09/57.4	5.3/63	S
710	P21240	RuBisCO large subunit-binding protein subunit β	*Arabidopsis thaliana*	5.26/58.1	5.6/64	N
713	P21240	RuBisCO large subunit-binding protein subunit β	*Arabidopsis thaliana*	5.26/58.1	5.6/65	S
715	P21240	RuBisCO large subunit-binding protein subunit β	*Arabidopsis thaliana*	5.26/58.1	5.3/65	S
716	P21240	RuBisCO large subunit-binding protein subunit β	*Arabidopsis thaliana*	5.26/58.1	5.3/65	S
720	A5BFM5	Putative uncharacterized protein	*Vitis vinifera*	6.03/61.2	6.0/65	S
722	A5BFM5	Putative uncharacterized protein	*Vitis vinifera*	6.03/61.2	6.7/66	S
725	A5BFM5	Putative uncharacterized protein	*Vitis vinifera*	6.03/61.2	5.8/67	S
728	A5BFM5	Putative uncharacterized protein	*Vitis vinifera*	6.03/61.2	5.0/69	S
732	Q9M888	Putative uncharacterized protein	*Arabidopsis thaliana*	5.83/58.9	5.9/70	S
749	P37900	Heat shock 70 kDa protein	*Pisum sativum*	5.18/66.7	5.8/66	N
750	Q43468	Heat shock protein STI	*Glycine max*	5.81/63.6	5.7/77	S
755	P37900	Heat shock 70 kDa protein	*Pisum sativum*	5.18/66.7	5.3/80	S
760	P11143	Heat shock 70 kDa protein	*Zea mays*	5.22/70.6	5.3/89	S
762	Q39043	Luminal-binding protein 2	*Arabidopsis thaliana*	5.08/71.1	5.2/87	S
763	Q39043	Luminal-binding protein 2	*Arabidopsis thaliana*	5.08/71.1	5.2/88	S
791	Q9LZF6	Cell division control protein 48 homolog E	*Arabidopsis thaliana*	5.08/90.0	5.3/142	S

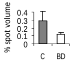
Table 3.1 (cont) Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

ID[a]		Protein identification[b]	Species	pI/MW (kDa)		Expr. Level
				Predicted[c]	Exp	
795	O23755	Elongation factor 2	*Beta vulgaris*	5.93/93.8	5.9/146	S
799	O23755	Elongation factor 2	*Beta vulgaris*	5.93/93.8	5.8/150	S
	Defence response					
233	P52779	Protein LIR18B	*Lupinus luteus*	5.35/16.6	4.7/15	N
234	Q93XI0	Pathogenesis-related 10	*Lupinus albus*	4.87/16.9	6.8/18	N
276	Q93XI0	Pathogenesis-related 10	*Lupinus albus*	4.87/16.9	4.6/15	S
287	Q93XI0	Pathogenesis-related 10	*Lupinus albus*	4.87/16.9	4.6/15	S
318	Q0PN10	Glutathione S-transferase	*Caragana korshinskii*	6.86/25.8	6.3/25	S
327	Q9SXM5	Acidic chitinase	*Glycine max*	5.01/31.9	4.7/26	N
430	P23535	Glucan endo-1,3-β-glucosidase, basic isoform	*Phaseolus vulgaris*	8.75/35.2	6.4/36	S
	Transcription					
314	Q5EI63	Quinone reductase 2	*Triticum monococcum*	5.95/21.7	6.7/24	S
	Amino acids metabolism					
393	A3RM06	Cysteine synthase	*Glycine max*	5.29/34.7	5.4/32	S
520	P54260	Aminomethyltransferase	*Solanum tuberosum*	7.28/40.9	7.1/44	S
521	Q40108	Aspartate aminotransferase	*Lupinus angustifolius*	8.36/45.8	6.8/44	S
616	Q9SP37	Adenosylhomocysteinase	*Lupinus luteus*	5.64/53.3	5.8/53	S
773	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	*Mesembryanthemum crystallinum*	5.90/84.8	6.0/114	S
776	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	*Mesembryanthemum crystallinum*	5.90/84.8	6.0/119	S
778	P93263	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	*Mesembryanthemum crystallinum*	5.90/84.8	5.9/116	S

Table 3.1 (cont) Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

ID[a]		Protein identification[b]	Species	pI/MW (kDa)		Expr. Level
				Predicted[c]	Exp	
	Cell wall metabolism					
267	P34105	NADP-dependent malic enzyme	*Populus trichocarpa*	6.50/65.2	6.4/61	N
518	Q93VR3	GDP-mannose 3,5-epimerase	*Arabidopsis thaliana*	5.85/42.8	6.0/44	S
673	Q96558	UDP-glucose 6-dehydrogenase	*Glycine max*	5.74/52.9	5.9/58	S
734	A9PGL9	Malic enzyme	*Populus trichocarpa*	7.61/54.6	6.0/70	S
745	P34105	NADP-dependent malic enzyme	*Populus trichocarpa*	6.50/65.2	6.0/75	S
747	P34105	NADP-dependent malic enzyme	*Populus trichocarpa*	6.50/65.2	6.0/75	S
	Cytoskeleton biosynthesis					
182	Q1G0Z1	Putative spindle disassembly related protein CDC48	*Nicotiniana tabacum*	5.13/89.9	5.2/149	S
183	Q1G0Z1	Putative spindle disassembly related protein CDC48	*Nicotiniana tabacum*	5.13/89.9	7.4/10	N
207	P20363	α-3/α-5 tubulin chain	*Arabidopsis thaliana*	4.95/49.7	5.4/49	N
262	Q9STD0	β-tubulin	*Zinnia elegans*	4.75/50.1	4.9/49	N
331	P41916	GTP-binding nuclear protein Ran-1	*Arabidopsis thaliana*	6.39/25.3	6.7/26	S
336	P41916	GTP-binding nuclear protein Ran-1	*Arabidopsis thaliana*	6.39/25.3	7.4/26	N
	Other metabolic processes					
391	Q8LQJ6	Ethylene-responsive protein 2-like	*Oryza sativa*	10.86/12.6	6.0/31	S
574	Q7M1Z8	Globulin-2	*Zea mays*	6.16/49.9	6.3/50	S
596	P19595	UTP-glucose-1-phosphate uridylyltransferase	*Solanum tuberosum*	5.71/51.7	6.1/52	S
682	P15590	Globulin-1 S allele	*Zea mays*	6.75/55.1	6.3/60	S
768	Q7SIC9	Transketolase	*Zea mays*	5.47/73.0	6.0/88	S
769	Q7SIC9	Transketolase	*Zea mays*	5.47/73.0	6.0/32	S
770	Q7SIC9	Transketolase	*Zea mays*	5.47/73.0	5.9/95	S

Table 3.1 (cont) Protein identification of the spots selected as responsive to B deficiency. Proteins were grouped according to the most relevant biological process where they are involved. In the column of expression level, the *de novo* expressed proteins are marked as N and the suppressed as S. The differentially expressed proteins have their percentage of volume represented.

ID[a]		**Protein identification**[b]	**Species**	**pI/MW (kDa)**		**Expr. Level**
				Predicted[c]	**Exp**	
	Unknown biological processes					
141	Q8LPE5	Fructokinase-like protein	*Cicer arietinum*	---	5.1/35	S
237	Q9M328	Putative uncharacterized protein T18D12.90	*Arabidopsis thaliana*	5.66/17.8	5.1/20	N
246	Q8GYY8	Putative germin	*Arabidopsis thaliana*	8.39/23.5	4.4/30	N
353	Q2V987	Transcription factor APFI-like	*Solanum tuberosum*	7.05/29.1	5.8/27	S
384	Q9SMK5	Plasma membrane intrinsic polypeptide	*Cicer arietinum*	4.95/23.3	5.2/30	S
549	A5CB20	Putative uncharacterized protein	*Vitis vinifera*	8.42/54.2	6.7/47	S
671	Q7XCL2	Ubiquitin domain containing protein	*Oryza sativa*	4.71/59.3	4.9/58	S
678	Q94IC1	Betaine aldehyde dehydrogenase	*Hordeum vulgare*	5.47/54.5	5.2/58	

[a] Spot numbers are corresponding to the numbers in Figure 3.3.

[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)

inorganic pyrophosphate, whereas the breakdown involving kinases requires two molecules of ATP (Stitt 1998). 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, several ATPases were affected by B deficiency as it will be discussed below.

For the V-type proton ATPase some subunits were expressed *de novo* (subunits A, spot 739 and B2, spot 264) and others suppressed (a subunit B2 isoform, spot 626) as well as some ATPase synthases subunit isoforms (spots 636, 640 and 646). The reduced activity of proton-pumping ATPase, as previously observed in sunflower cells, was related with possible membrane damage caused by B deficiency (Ferrol and Donaine 1992). The expression *de novo* of pyruvate dehydrogenase E1 component subunit beta (spot 251) is also relevant since E1 protein levels correlate with mitochondrial pyruvate dehydrogenase complex activity (Luethy et al. 2001), and higher expression of this enzyme correlates with metabolic and structural changes that accompany membrane expansion and remodelling (Tovar-Méndez et al. 2003).

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 (Brown et al. 2002).

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 expressed *de novo*. 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 (Moeder et al. 2007) and that oxidative damage is the major cause of cell death induced by B-deprivation in tobacco cells (Koshiba et al. 2009), the aconitase suppression or degradation probably results from the oxidative damage caused by B deficiency.

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 expressed *de novo* under B deficiency and are molecular chaperones responsible for preventing irreversible aggregation of non-native proteins under both normal and stressful conditions (Timperio et al. 2008). Another protein involved in the protein folding processes, that was also expressed *de novo* 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 (Nuc et al. 2001).

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 (Rospert et al. 2002). The expression *de novo* of a NAC subunit α-like protein 2 (spot 247), might suggest altered protein translation and targeting, important for adaptive stress responses.

Both the CND41 (spot 259) that was expressed *de novo*, and the predicted protein (A9PEP6; spot 512) that was up 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 the degradation of extracellular pathogenesis-related (PR) proteins (Voigt et al. 1997). 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.

The protein degradation is an ATP-demanding process, so its repression will decrease protein synthesis and turnover, thereby reducing ATP demand (Hochachka and Lutz 2001). 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.

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 sulphur containing amino acids. Studies on the transcriptional profile of *Arabidopsis thaliana* plants grown under B deficiency suggested altered sulphur metabolism (Chapter 5), but the relationship between B role in plants and sulphur metabolism remains unclear.

Cell wall metabolism

Some proteins associated with the cell wall metabolism were suppressed due to B deficiency. Both the UDP-glucose 6-dehydrogenase (spot 673) and the GDP-mannose 3,5-epimerase (spot 518) are involved in the carbohydrate metabolism directed for the cell wall biosynthesis. Several malic enzyme isoforms (spots 734, 745 and 747), that were suppressed under B deficiency, are described to provide the NADPH used for the production of H_2O_2 in lignin biosynthesis (Martinoia and Rentsch 1994). Lignin production that results from the hydroxycinnamyl alcohols polymerization by peroxidases (Higuchi 1990) could be compromised by B deficiency, as observed by the lower degree of lignification of some trees growing in a low B content soil (Dell and Malajczuk 1994). 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 (Cakmak and Römheld 1997) could be related with the phenol increased content described for B-deficient plants (Blevins and Lukaszewski 1998).

In a transcriptional analysis of B deficient *Arabidopsis thaliana* roots, it was found that several genes related with cell wall metabolism were down regulated (Camacho-Cristóbal et al. 2008). The known participation of B in cell wall structure may explain the impaired cell wall metabolism observed in *L. albus*, however this is not enough to explain all the symptoms observed due to B deficiency.

Defence responses

Several proteins related with defence responses were expressed *de novo* due to B deficiency, namely PR-10 (spot 234) and LIR18B protein (spot 233), both belongs to the PR-10 family, and an acidic chitinases (spot 327) that belongs to the PR-8 family

(Watanabe et al. 1999). The PR family proteins are known to be induced by several biotic and abiotic stresses (Van Loon and Van Strien 1999). An uncharacterized T18D12.90 protein (spot 237) and a putative germin-like protein (spot 246), that were expressed *de novo* under B deficiency, have unknown biological functions however, they have been also associated with various stress responses. For example, in *A. thaliana* the protein T18D12.90 is described to be part of the universal stress protein (USP) family (Kvint et al. 2003). Germin and germin-like proteins, besides their involvement in stress responses, have been described to participate in a wide range of activities related to developmental processes and cell wall biosyntheses (Bernie and Berna 2001). Several other studies had already reported increased defence proteins in association with B deficiency (Alves et al. 2006, Kobayashi et al. 2004, Reguera et al. 2010). Updated evidence shows that PR proteins and other stress responsive proteins may display additional functions in growth and developmental processes by modulating signal molecules (Kasprzewska 2003, Liu and Ekramoddoullah 2006), however the association of these defence proteins with B is not yet understood.

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, and a putative actin from *Dictyostelium discoideum* (Q553U6). Another expressed *de novo* protein, which could be involved in the regulation of cytoskeletal assembly and organization, was a GTP-binding nuclear protein Ran-1 (spot 336) (Haizel et al. 1997, Vernoud at al. 2003). 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 the annotated in the database. Beside this last protein, we have detected several others, which were suppressed due to B deficiency, related with cytoskeleton biosynthetic process. The putative uncharacterized proteins from *A. thaliana* (spots 699 and 732) and from *V. vinifera* (spots 725, 720, 722 and 728) belong to the TCP-1 chaperonin family that, in association with the Hsp70 molecular chaperones, can interact with cytoskeletal components (Gatenby and Viitanen 1994). 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 (Gromadski et al. 2002).

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 (Yu et al. 2001, 2003). Since cytoskeleton is involved in diverse important cellular aspects, such as mitotic spindle formation, intracellular transport and control of cell shape (Gunning and Hardham 1982), a modified cytoskeleton biosynthesis can be a possible explanation for the missing part of 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 remodelling is required, such as the initial phases of differentiation, including pollen tube growth, anther development (Loomis and Durst 1992, Rawson 1996), somatic embryo formation (Behrendt and Zoglauer 1996) and early nodulation processes (Bonilla et al. 1994).

Protein isoforms in stress responses

One striking feature of this proteomic study is the detection of several changes in protein isoforms as a consequence of B deficiency. A numerous group is that of the proteins that showed slight changes in pI and/or MW in response to the deficiency. Different protein isoforms were already found to have a crucial role in fungal infection and symbiosis studies in *Medicago truncatula*

(Salzer et al. 2000). This may indicate that isoforms could have an active participation in regulatory processes, determinant for plants to cope with stressful conditions.

CONCLUSIONS

There was a clear 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 in energy and protein metabolic processes. Other common adaptive stress responses are related with defence proteins. Several other metabolic processes were affected by the deficiency, such as cell wall metabolism, as expected since B is known to participate in plant cell wall structure, and cytoskeleton biosynthesis. The high requirement of B in active cytoskeleton remodelling, such as in initial phases of differentiation (Behrendt and Zoglauer 1996), in reproductive processes (Loomis and Durst 1992, Rawson 1996) and in nodulation processes, is consistent with a possible role for B in cytoskeleton biosynthetic processes.

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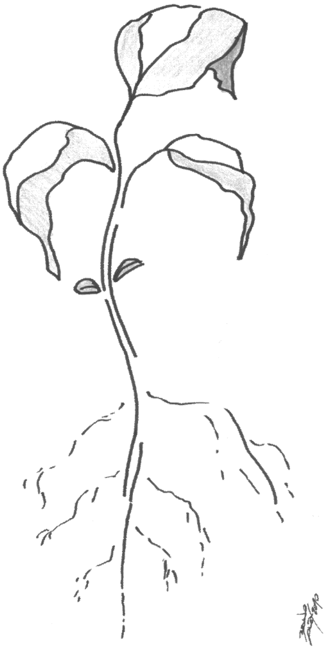
CHAPTER 4

METABOLIC ANALYSIS REVEALED ALTERED AMINO ACID PROFILES IN *Lupinus albus* ORGANS DUE TO BORON DEFICIENCY



Metabolic analysis revealed altered amino acid profiles in *Lupinus albus* organs due to boron deficiency

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CONTRIBUTION: The first author had the major contribution for all the work described, with the exception of free amino acid HPLC analysis and in handling of the NMR spectrometer.

KEYWORDS: *Lupinus albus*, B deficiency, metabolite, branched-chain amino acids, glycine.

SUMMARY

In B-deficient *Lupinus albus*, marked changes in the free amino acid content of leaf-blades, petioles, apexes, hypocotyls and roots were observed while the absence of variation in malate, and the minor changes in the content of sugars, can be an indication that the central carbohydrate metabolism is little affected by B deficiency. The changes observed in the different organs were qualitatively different, with increases in some amino acids while others decreased, suggesting that the observed alteration in the free amino acid content is not the result of an indiscriminate effect on protein metabolism. Increased branched-chain amino acids content was observed in all the studied organs, which supports the hypothesis of the involvement of B with the cytoskeleton. Glycine decreased in leaf-blades and active growing organs (apexes and roots), but was not detected in petioles and hypocotyls. Considering the proposed role of glycine in plant signalling processes, there could also be an association of this amino acid with the decreased growth rates observed in the active growing organs of *L. albus* plants due to B deficiency.

INTRODUCTION

Boron is an essential microelement for higher plants (Warington 1923) that is considered to be important for plant cell wall structure through its formation of diester bridges between rhamnogalacturonan-II molecules (O'Neill et al. 1996, 2001, Kobayashi et al. 1999). However, this participation does not seem to explain all the plant symptoms due to B deprivation, or the recent evidence of B requirement in animal metabolism (Nielson 1997). Several recent reviews propose the additional participation of B in metabolic supporting activities (Brown et al. 2002, Goldbach and Wimmer 2007, Bonilla et al. 2009).

Free amino acids, besides their obvious role of nitrogen storage molecules and participation in protein synthesis, can also act as signalling molecules. Recently, amino acids were proposed to regulate multiple processes related to gene expression, not only on a global scale, but also by inducing preferential changes in the translation of mRNA encoding particular proteins or family of proteins (Kimball and Jefferson 2006).

Despite the increasing recognition of the importance of free amino acids in plant metabolism this matter has been little studied and there is also a lack of information on the effect of stress on the free amino acids of distinct plant organs. The fact that B deficiency virtually affects every aspect of plant metabolism, prompted us to investigate the effects of B deficiency on the free amino acid content of several organs (leaf-blades, petioles, apexes, hypocotyls and roots) of *Lupinus albus* plants.

MATERIALS AND METHODS

Plant material

Lupin seeds (*Lupinus albus* cv. Rio Maior) were pre-germinated in distilled water for 48h and then sown in white sand and grown under controlled conditions of temperature (19/25ºC, night/day) and light (intensity of 250μmol m^{-2} s^{-1} PAR and 12h photoperiod). The plants were watered every other day with a nutritive solution (Arnon 1938) containing either 0 or 23.1μM B (Alves et al. 2006). The different organs of the control and B-deficient *L. albus*: leaf-blade, petiole, apex, hypocotyl and root were harvested 28 days after germination, frozen in liquid nitrogen and stored at -80ºC.

Metabolite analysis

For the analysis of metabolites, the frozen samples (3-10g, in triplicate) were ground to a fine powder and then boiled in ddH_2O (4mL/g fresh weight) for 5min before centrifugation (15min; 14,000*xg*) and recovery of the supernatant. The supernatant was lyophilized to dryness and resuspended in 2mL of an aqueous solution containing D_2O (5.8M), Na_2EDTA (2.5mM) and NaN_3 (2.5mM).

The major soluble metabolites were analysed with ^{13}C-nuclear magnetic resonance (^{13}C-NMR) in a Bruker Avance II NMR spectrometer (400MHz) at 100MHz using a broadband probe head (5mm diameter), with the following parameters: number of scans 1500; repetion delay 1.86s; sweep width 24kHz and a 30° pulse flip angle. Proton decoupling was applied during the acquisition only. The temperature of the probe head was kept at 25ºC. Data was processed with 4Hz exponential line broadening. Unequivocally identified resonances from each metabolite were integrated using the Mnova software v5.1.1 (Mestrelab Research S.L.). Fully relaxed spectra, with a 31.36s repetition delay, were acquired and used to calculate area saturation correction factors of the relevant resonances. Chemical shifts are expressed in ppm relative to dioxan (66.66ppm) that was also used as an internal concentration standard (0.194M) for the metabolite quantification. Resonances due to asparagine, malate, *myo*-inositol, sucrose, glucose and fructose were identified by adding a 100mM solution of the pure compound to a tissue extract.

Free amino acids were analysed by high performance liquid chromatography (HPLC). The samples were previously cleaned-up with solid phase extraction (Sep-Pak® C-18 Cartridges) to remove low molecular weight contaminants. After derivatization with phenylisothiocyanate, the free amino acids were analyzed by HPLC (Alliance 2695, Waters) using a RP C18 (Nova-Pak, 60Å, 4µm, 3.9 x 300mm) at an absorbance of 254nm (PDA 2996, Waters). The eluents used for the chromatographic separation were (A) 0.07M sodium acetate, pH 6.55 with 2.5% acetonitrile, and (B) 45% acetonitrile and 15% methanol (Cohen et al. 1986). The free amino acids analysed were alanine, β-alanine, aminoadipic acid, α-aminobutyric acid, γ-aminobutyric acid, β- aminoisobutyric acid, arginine, aspartic acid, carnosine, citrulline, cysteic acid, cystine, glycine, glutamic acid, glutamine, histidine, hydroxylysine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, taurine, threonine, tryptophan, tyrosine and valine.

Statistical analysis

Significant changes in metabolite concentrations between stress and control were evaluated by the Student's *t*-test ($p<0.05$) using the software SigmaStat v3.10 (Systat Software, Inc.) and correlation analysis performed with Microsoft Excel analysis ToolPak.

RESULTS AND DISCUSSION

Biomass analysis of L. albus *plants grown under B deficiency*

The morphological symptoms of B deficiency in *Lupinus albus* plants are quite evident four weeks after germination, and are expressed as necrosis of the terminal bud, darkened and deformed leaf-blades, cracked petioles and hypocotyls and marked reduction in root development (Alves et al. 2006). Roots showed the greatest decrease in biomass (47%), followed by petioles and leaf-blades (43% and 37%, respectively; Figure 4.1). No statistically significant differences were found for apexes (16% decrease) or hypocotyls (15% increase).

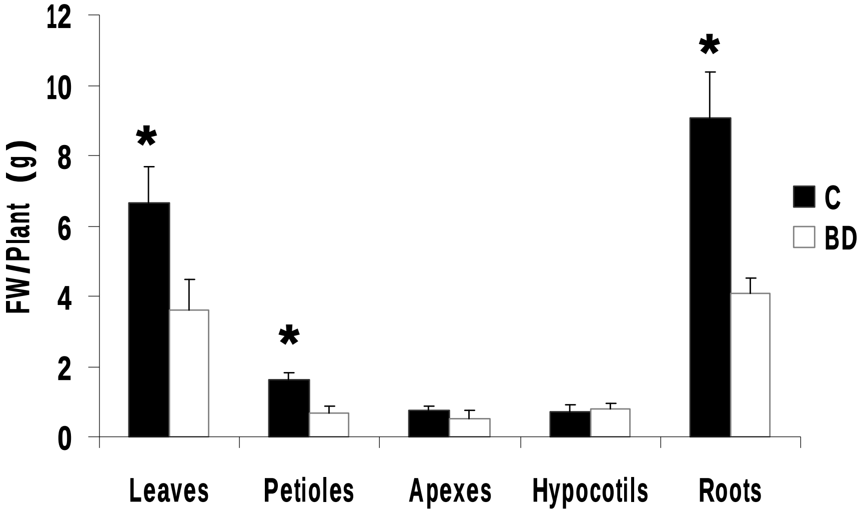

Figure 4.1. Biomass analysis of *Lupinus albus* plants grown under B deficiency. Fresh weight (FW) of the leaf-blades, petioles, apexes, hypocotyls and roots of the *L. albus* grown with (C) or without B (BD). Significant changes were evaluated by the Student's *t*-test (*, $p<0.05$).

Metabolite analysis

The metabolite changes observed in the different organs of *Lupinus albus* plants grown for four weeks under B deficiency are shown in Table 4.1. Concerning carbohydrates, sucrose content of B-deficient plants was increased in all organs, while fructose and glucose increased in leaf-blades, but decreased in the other organs. This observation seems to indicate a reduced sucrose consumption that could be due to stress resulting from B deficiency. In the leaf-blades the excess of sucrose was probably hydrolysed, leading to the increased content of the two reducing sugars, while in the remaining organs sucrose could accumulate and not be hydrolysed. Therefore, the reduced plant growth observed due to B deficiency does not seem to result from sugar limitation.

The *myo*-inositol content showed no change in leaves of B-deficient plants. A high correlation was observed between *myo*-inositol and sucrose (r=0.99) and with glucose (r=0.90), indicating that these compounds have similar metabolic behaviour under B deficiency.

Malate is a key product of plant metabolism that is thought to be the ultimate product of glycolysis, and is seen as an important marker of this pathway (Schulze et al. 2002). In *L. albus*, malate is known to accumulate in large amounts in shoots (Peñaloza et

Table 4.1. The concentration (nmol/g fresh weight) of the major soluble compounds identified by ^{13}C-NMR and of the free amino acids identified by HPLC in the different organs of *L. albus* grown with or without B. Data are the means ± sd. Statistical analysis was made with Student's *t*-test ($p<0.05$) and statistical significant changes resulting from B deficiency are marked for decreases (🟩) or increases (🟥) in the metabolite.

Metabolism	Compound	Treatment	Leaf-blades	Petioles	Apex	Hypocotyls	Roots
Carbohydrates	Sucrose	BD	🟥 (1.0±0.3)x10^{6}	🟥 (1.4±0.1)x10^{6}	(6.5±0.5)x10^{5}	(1.4±0.2)x10^{6}	(5±2)x10^{5}
		C	<1x10^{5}	(6.4±0.9)x10^{5}	(6.1±0.3)x10^{5}	(8±2)x10^{5}	(2.2±0.9)x10^{5}
	Fructose	BD	(7±3)x10^{5}	🟩 <1x10^{5}	<1x10^{5}	<1x10^{5}	<1x10^{5}
		C	(3±2)x10^{5}	(3.2±0.5)x10^{5}	<1x10^{5}	<1x10^{5}	(3±1)x10^{5}
	Glucose	BD	(9±3)x10^{5}	(1.4±0.4)x10^{6}	🟩 (3±1)x10^{5}	<1x10^{5}	0.1±0.1
		C	(3±2)x10^{5}	(2.4±0.8)x10^{6}	(7.4±0.3)x10^{5}	<1x10^{5}	(1.9±0.6)x10^{5}
	myo-Inositol	BD	(3±2)x10^{5}	<1x10^{5}	<1x10^{5}	<1x10^{5}	<1x10^{5}
		C	(1.9±0.6)x10^{5}	<1x10^{5}	<1x10^{5}	<1x10^{5}	<1x10^{5}
TCA cycle	Malate	BD	(4±1)x10^{6}	(5.6±0.6)x10^{6}	(2.6±0.7)x10^{6}	(2.4±0.6)x10^{6}	(1.7±0.4)x10^{6}
		C	(6±1)x10^{6}	(5.6±0.5)x10^{6}	(4.1±0.6)x10^{6}	(3.4±0.9)x10^{6}	(1.6±0.4)x10^{6}
Aromatic amino acids	Tyrosine	BD	🟥 0.14±0.02	<4x10^{-5}	<4x10^{-5}	🟥 0.12±0.06	<4x10^{-5}
		C	<4x10^{-5}	<4x10^{-5}	<4x10^{-5}	<4x10^{-5}	<4x10^{-5}
Branched-chain amino acids (BCCAs)	Isoleucine	BD	0.4±0.1	🟥 0.16±0.04	🟥 0.29±0.04	🟥 0.24±0.07	🟥 0.11±0.03
		C	0.17±0.07	0.07±0.01	0.17±0.04	<4x10^{-5}	<4x10^{-5}
	Leucine	BD	🟥 0.17±0.02	🟥 0.11±0.05	🟥 0.14±0.04	🟥 0.16±0.03	🟥 0.07±0.03
		C	0.08±0.03	<4x10^{-5}	<4x10^{-5}	<4x10^{-5}	<4x10^{-5}
	Valine	BD	🟥 0.5±0.1	🟥 0.5±0.2	🟥 1.3±0.2	🟥 0.6±0.1	0.22±0.08
		C	0.24±0.04	0.19±0.05	0.4±0.1	0.23±0.08	0.11±0.02
GABA shunt	Alanine	BD	1.0±0.3	🟥 0.48±0.05	🟥 1.2±0.1	🟥 0.8±0.3	0.4±0.2
		C	1.0±0.2	0.23±0.05	0.6±0.2	0.30±0.06	0.25±0.04
	GABA	BD	1.2±0.4	🟥 0.73±0.07	1.6±0.6	🟥 0.9±0.2	🟥 0.3±0.1
		C	2.0±0.4	0.33±0.03	0.7±0.4	0.27±0.04	0.13±0.01

Table 4.1 (cont) The concentration (nmol/g fresh weight) of the major soluble compounds identified by ^{13}C-NMR and of the free amino acids identified by HPLC in the different organs of *L. albus* grown with or without B. Data are the means ± sd. Statistical analysis was made with Student's *t*-test (p<0.05) and statistical significant changes resulting from B deficiency are marked for decreases (🟩) or increases (🟥) in the metabolite.

Metabolism	Compound	Treatment	Leaf-blades	Petioles	Apex	Hypocotyls	Roots
Glutamate family amino acids	Glutamate	BD	0.44±0.03	0.64±0.05	1.2±0.2	0.6±0.2	0.57±0.04
		C	0.42±0.07	0.5±0.1	1.1±0.4	0.55±0.05	0.48±0.06
	Arginine	BD	0.24±0.03	0.27±0.05	0.5±0.2	0.1±0.2	0.20±0.02
		C	0.25±0.04	0.20±0.02	0.31±0.07	0.22±0.05	0.196±0.003
	Histidine	BD	🟥 0.17±0.03	🟥 0.3±0.1	🟥 0.7±0.3	🟥 0.62±0.04	0.13±0.06
		C	<4x10^{-5}	0.05±0.04	0.13±0.04	0.13±0.06	0.074±0.005
	Proline	BD	0.3±0.1	🟥 0.8±0.3	🟥 0.8±0.4	🟥 0.5±0.1	0.2±0.1
		C	0.15±0.04	0.07±0.01	0.12±0.05	<4x10^{-5}	0.15±0.08
Asparate family amino acids	Aspartic acid	BD	🟩 0.9±0.2	🟥 1.26±0.05	1.8±0.2	1.0±0.2	0.6±0.2
		C	1.45±0.07	0.88±0.09	1.4±0.5	0.6±0.2	0.37±0.02
	Lysine	BD	0.14±0.05	🟥 0.13±0.02	0.17±0.04	🟥 0.14±0.01	0.08±0.02
		C	0.20±0.03	0.07±0.02	0.13±0.02	0.08±0.01	0.07±0.02
	Threonine	BD	0.23±0.07	🟥 0.28±0.08	🟥 0.44±0.03	🟥 0.28±0.08	0.10±0.04
		C	0.16±0.04	0.09±0.01	0.18±0.07	0.09±0.04	0.07±0.01
	Asparagine	BD	(7±3)x10^{5}	🟥 (3.0±0.5)x10^{6}	🟥 (2.4±0.7)x10^{6}	🟥 (2.3±0.4)x10^{6}	<1x10^{5}
		C	(2±2)x10^{5}	(6±3)x10^{5}	(3.5±0.9)x10^{5}	<1x10^{5}	<1x10^{5}
Others	Glycine	BD	🟩 <4x10^{-5}	<4x10^{-5}	🟩 <4x10^{-5}	<4x10^{-5}	🟩 <4x10^{-5}
		C	0.21±0.08	<4x10^{-5}	0.13±0.03	<4x10^{-5}	0.06±0.01

al. 2002), but under stressful conditions, such as water-deficit, the malate content was decreased in all organs, but most markedly in the leaves and roots (Pinheiro et al. 2004). Under B deficiency, the absence of variation in malate, and the minor changes in the content of sugars, can be an indication that the central carbohydrate metabolism is little affected by B deficiency (Figure 4.2).

Regarding the amino acids, we observed marked changes in their content due to B deficiency, but distinct patterns of variation were observed between them and also between the several organs (Table 4.1, Figure 4.2). The fact that in every organ some amino acids increased while others decreased, suggests that the observed alterations are not the result of indiscriminate protein degradation. For instance, in roots, despite the small number of amino acids changed due to B deficiency the content of leucine, valine, isoleucine and GABA increased but the content of glycine decreased. In the leaf-blades, the additional changes were observed: tyrosine, leucine, valine, isoleucine and histidine increased, while aspartic acid and glycine decreased. Such an increase in tyrosine could be related to the known increase in phenol content of leaves as a consequence of B deficiency (Blevins and Lukaszewski, 1998). Changes in tyrosine could also be important due to the participation of this amino acid in the synthesis of signalling molecules responsible for growth modulation processes (Lea and Forde 1994).

The increase of the branched-chain amino acids (BCAAs), leucine, isoleucine and valine, in leaf-blades appears to be an interesting observation since, in animal tissues BCAAs are known to participate in the regulation of skeletal muscle protein synthesis, leucine being

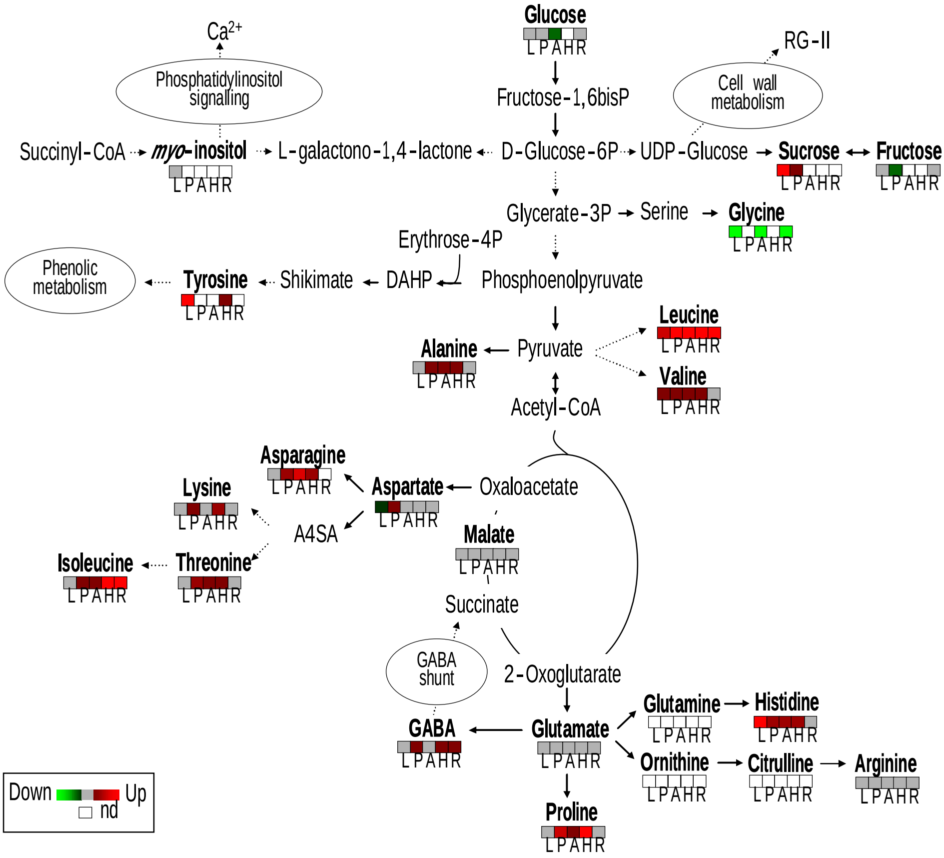

Figure 4.2. Metabolic sequences affected by B deficiency in *L. albus* plants, as predicted by the analysed metabolites of leaf-blades (L), petioles (P), apexes (A), hypocotyls (H) and roots (R). Decreased metabolite contents are in green and increased changes in red. Non altered content is in grey, whereas white represents non detected metabolites. Abbreviations: A4SA, aspartate 4-semialdehyde; DAHP, 3-Deoxy-D-arabino-heptulosonate-7-phosphate; RG-II, rhamnogalacturonan-II.

the most effective with regard to the regulation of these proteins (Kimball and Jefferson 2006, Rhoads and Wu 2009). The importance of actin in the cytoskeleton and the observation that B deficiency affects the morphology of leaf-blades and roots, may suggest some association between the increase in BCAAs and some developmental processes affected by B deficiency. Enhanced levels of cytoskeletal proteins and abnormal polymerization patterns, in Arabidopsis and maize roots, were observed in short-term B deficiencies (Yu et al. 2001, 2003), which could be related to the postulated hypothesis of BCAAs influencing cytoskeleton structure. The BCAAs also markedly increased in *Arabidopsis* when subjected to drought stress (Urano et al. 2009).

Histidine, which increased in leaf-blades, was also found to increase in heavy metal stress and its participation as a chelator or transporter of metal ions was proposed. In addition, histidine is involved in several biosynthetic pathways, such as that of purines, pyrimidines, pyridine nucleotides, folates and tryptophan (Stepansky and Leustek 2006), all of which could have been affected by the B-deficiency.

The decrease in the leaf-blades of aspartic acid and glycine could be significant to the B deficiency response. Aspartic acid serves as the nitrogen donor in numerous aminotransferase reactions and could be related with decreased nitrate content due to B deficiency, an observation described in tobacco leaves (Camacho-Cristóbal and González-Fontes 1999).

In what concerns glycine, this amino acid was also decreased in the apexes and roots (active growing organs), but was not detected in petioles and hypocotyls.

Glycine has a well established role in the signalling processes of the mammalian central system and in plants there is also evidence for glycine participation in signalling processes. The postulated binding of glycine to glutamate receptors could explain why the increased levels of glycine induced higher cytosolic calcium concentrations in *Arabidopsis* (Dubos et al. 2003). It is noteworthy to refer the observed amelioration of some B deficiency symptoms after the addition of calcium (Bolaños et al. 2004).

Comparing amino acid changes in leaf-blades to those occurring in petioles, apexes and hypocotyls, it is evident that a higher number of amino acids were statistically affected by B deficiency in these three organs. In addition to the specific metabolic changes that might be occurring in each organ, the possibility of some amino acids being translocated from the leaf-blades (Fischer et al. 1998) should not be excluded. For instance, asparagine, which increased only slightly in the leaf-blades under B deficiency, is one form of N-transport from source to sink tissues (Lea et al. 2007) and it did markedly increase in apexes, petioles and hypocotyls. Such an accumulation in the petioles and hypocotyls may be an indication of temporary functioning of these organs as transient stores, in response to B deficiency, similarly to what was previously suggested for *L. albus* hypocotyls in response to drought stress (Pinheiro et al. 2004).

The asparagine increased content is highly correlated with the proline increase (r=0.98) under B-deficient conditions, and the two amino acids are known to accumulate under conditions of stress. Proline, in particular, is associated with a wide range of biotic and abiotic stresses (Verbruggen and Hermans 2008). The most common proposed hypothesis is that proline acts as an osmotic and a protective agent for cytosolic enzymes and membrane structures, but it may also be part of stress signalling processes responsible for adaptive stress responses (Verbruggen and Hermans 2008). The observed proline significant increase in the petioles, apexes and hypocotyls can be envisage as an important protective role in the apexes, but it is less clear why this amino acid should also highly accumulate in both petioles and hypocotyls.

GABA is another amino acid commonly associated with biotic and abiotic stresses (Fait et al. 2007). GABA and alanine, both increased in the petioles, apexes and hypocotyls, showing a high correlation value (r=0.90), and possibly their production resulted from a common metabolic process, known as the GABA shunt. The accumulation of alanine and GABA in low oxygen stressed plants has been reported to be partially associated with the GABA shunt (Miyashita and Good 2008). When GABA production was inhibited, the accumulation of reactive oxygen species was prevented (Fait et al. 2005). Thus, it could be considered that increased GABA levels could be related to the described accumulation of reactive oxygen species in B-deficient plants (Cakmak and Römheld 1997, Koshiba et al. 2009).

CONCLUSIONS

The plant responses to stress constitute a complex framework of metabolic events that also result from complex plant organs interactions. This complexity is well expressed in the free amino acids profile that was quite distinct in the different organs of *Lupinus albus* grown under B deficiency. The observed changes in the several amino acids indicate that B deficiency is affecting many aspects of the plant metabolism including certain signalling processes. The increase in BCAAs content in all the studied organs supports the hypothesis of damaged cytoskeleton structure due to B deficiency. Boron requirement in dynamic microtubule rearrangements, like in the early phases of tissue differentiation in zebra fish (Rowe and Eckert 1999) and in *Larix* embryogenesis (Behrendt and

Zoglauer 1996) is consistent with B involvement in the organization of the cytoskeleton structure.

Thus, amino acid biosynthesis seems to be, directly or indirectly, associated with various aspects of plant response to B deficiency.

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CHAPTER 5

TRANSCRIPTIONAL ANALYSIS OF SHORT-TERM BORON DEFICIENCY IN ARABIDOPSIS REVEALED ALTERED SULPHUR METABOLISM

Transcriptional analysis of short-term boron deficiency in Arabidopsis indicates altered sulphur metabolism

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CONTRIBUTION: The first author performed all the experimental work and writing with the exception of the GeneChip hybridization, GeneChip data analysis and boron quantification.

KEYWORDS: *Arabidopsis thaliana*, Boron deficiency, Calcium deficiency, cytoskeleton, sulphur metabolism.

SUMMARY

For more than 80 years, boron (B) has been recognized as an essential microelement for higher plants, but in spite of this its precise role remains elusive. In order to gain insight into the participation of B in the metabolism of higher plants, we have analysed *Arabidopsis thaliana* for transcriptional alterations caused by short-term B deficiency. Given that several studies suggest an interaction between B and calcium (Ca) in plants, the transcriptional profile of *A. thaliana* plants under Ca-deficiency was also analysed. The microarray analysis revealed that the expression of 102 genes was altered 2 days after withholding B and 208 by day 4. For the Ca deficiency, 2 days after Ca withholding morphological symptoms were already evident and more than 2,500 genes were altered, and at 4 days the plants showed necrotic symptoms and only 270 genes were differentially expressed. Of the 298 B-responsive genes, 37 were identified has having the same expression trend at 2 and 4 days of deficiency (15 up-regulated and 22 down-regulated) and, so, were selected for further analysis. Thirteen genes that had a fold-change higher than 2 were chosen for RT-qPCR analysis and since we were able to validate all of them we included all the 37 genes for the detailed discussion. In this set, we found genes related with cell wall biosynthesis (24%), sulphur metabolism (24%), transcriptional factors and hormones

(18%), while genes related with miscellaneous and unknown functions represent 13% and 16%, respectively. Altered cell wall biosynthesis is a process already described for B-deficient plants, whereas altered sulphur metabolism due to B deficiency is a rather novel observation.

INTRODUCTION

Boron (B) is known to be an essential micronutrient for higher plants since 1923 (Warington 1923), but only in the 90's it was reported to participate in the cell wall structure as a cross linker of the cell wall pectin, rhamnogalacturonan-II (RG-II) (Kobayashi et al. 1996, O'Neill et al. 1996, 2001). However, this participation does not seem enough to explain all the symptoms of B deficiency in plants.

There are symptoms of short-term B deficiency that remain unexplained, such as the quantitative and qualitative changes observed in the phenolic metabolism of tobacco (Camacho-Cristóbal et al. 2002) and the increased levels of cytoskeletal proteins in both Arabidopsis and maize roots (Yu et al. 2001, 2003). The enhancement of cytoskeleton protein levels and the altered polymerization patterns were hypothesized to be involved in the mechanical reinforcement of the cell periphery complex. Another observed symptom that results from short-term B deprivation is the inhibition of pectin endocytosis from the cell walls of meristematic cells in maize and wheat root apices, which could be related to endocytosis-mediated pectin signalling (Yu et al. 2002). Additionally, it has been suggested that the metabolic functions of calcium (Ca) and B in plants may be inter-linked. For instance, in nodulation processes some effects of B deficiency were ameliorated by Ca addition (Redondo-Nieto et al. 2003, Koshiba et al. 2010), and genetic studies showed that the expression of some genes affected by B deficiency could be reversed by Ca supplementation (Bolaños et al. 2004). Stability studies of different B fractions suggested that B cross-link of RG-II may result in conformational changes that can create binding sites for Ca ions, which increase pectin association and rigidity (Kobayashi et al. 1999). Since little is known about the roles of B in higher plants, and given the possible interactions between B and Ca, we have analysed the transcriptional profile of *Arabidopsis thaliana* plants subjected to 2 and 4 days of B or Ca deficiency. Our results provide insight into the early responses to B deprivation in Arabidopsis.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana cv. Columbia seeds were sown in 1L plastic containers filled with perlite, which has a low B content (Alves et al. 2006). After a period of 2 days at 4ºC, the containers were transferred to a growth chamber (Fitoclima 700 EDT4, Aralab) at a temperature of 16/22ºC (night/day), with a 16h photoperiod and a light intensity of 150μmol m^{-2} s^{-1}, PAR. The plants were watered every other day with a complete nutrient solution (Arnon 1938) in which the B concentration was 25μM and the Ca concentration 3mM. Boron or Ca deficiencies were imposed 17 days after sowing by daily watering the plants with a B-free nutrient solution or with a Ca-free nutrient solution corrected for the nitrate content with magnesium and potassium salts.

The Arabidopsis plants were harvested 2 and 4 days after B or Ca suppression and kept frozen at -80ºC until further use. The Arabidopsis plants grown for longer than 12 days under B or Ca deficiency were only used for morphological analysis. For dry weight determinations, the plants were oven-dried for 48h at 80ºC.

Boron quantification

Boron was quantified following the Azomethine-H method (Sungur and Okur 2009) adapted for micro-assays. The plants were ashed overnight in an oven at 550ºC and the ashes dissolved in 3N HCl. The solution was filtered through a 0.45µm filter and 20 µL were used for analysis. To each well were added 20µL of water, 80µL of azomethine-H solution [0.45% (w/v) azometinhe-H and 2% (w/v) ascorbic acid], and 80µL of buffer solution at pH 5.7 [48% (w/v) ammonium acetate, 3% (w/v) EDTA disodium salt and 24% (v/v) acetic acid]. The mixture was kept at room temperature for 40min. The absorbance was measured in a UV/Vis 96-well plate reader (Powerwave xs, Bioteck) at 420nm. A calibration curve was prepared using different concentrations of aqueous B solutions. All analyses were performed in triplicate.

RNA Isolation, Target Synthesis and Hybridization to Affymetrix GeneChips

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Concentration and purity were determined by spectrophotometry and integrity was confirmed using an Agilent 2100 Bioanalyzer with a RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, CA). The RNA was processed for use on Affymetrix (Santa Clara, CA, USA) Arabidopsis ATH1 Genome Arrays, according to the manufacturer's One-Cycle Target Labelling Assay. Briefly, 5µg of total RNA spiked with Poly-A RNA controls (GeneChip Expression GeneChip Eukaryotic Poly-A RNA Control Kit;

Affymetrix) was used in a reverse transcription reaction (One-Cycle DNA synthesis kit; Affymetrix) to generate first-strand cDNA. After second-strand synthesis, double-stranded cDNA was used in an *in vitro* transcription (IVT) reaction to generate biotinylated cRNA (GeneChip Expression 3'-Amplification Reagents for IVT-Labeling; Affymetrix). Size distribution of the cRNA and fragmented cRNA, respectively, was assessed using an Agilent 2100 Bioanalyzer with a RNA 6000 Nano Assay. From the fragmented cRNA 15µg were used in a 300µL hybridization containing added hybridization controls. From the mixture, 200µL were hybridized on arrays for 16h at 45ºC. Standard post hybridization wash and double-stain protocols (EukGE-WS2v4) were used on an Affymetrix GeneChip Fluidics Station 450. Arrays were scanned on an Affymetrix GeneChip scanner 3000 7G.

GeneChip Data Analysis

Scanned arrays were analyzed first with GCOS 1.4 software to obtain Absent/Present calls and for subsequent analysis with DNA-Chip Analyzer (dChip; release Jan 24, 2008) (http://www.dchip.org, Wong Lab, Harvard). The arrays were normalized to a baseline array with median CEL intensity of 160 by applying an Invariant Set Normalization Method (Li and Wong 2001a). Normalized CEL intensities of the 12 arrays (2 per treatment) were used to obtain model-based gene expression indices based on a Perfect Match-only model (Li and Hung Wong, 2001b). Replicate data for the same sample type were weighted gene-wise by using inverse squared standard error as weights. Only genes called Present in at least one of the 12 arrays were kept for downstream analysis (17,797 genes). Thus, genes called Absent in all arrays were excluded. Li and Hung Wong (2001b) have shown that using the lower confidence bound of the fold change as a conservative estimate of the fold change is a more reliable as a ranking statistic for changes in gene expression. Therefore, all genes compared were considered to be differentially expressed if the lower confidence bound of the fold-change was greater than 90% and if above 1.2, between experiment and baseline, in both data sets. The lower confidence bound criterion means that we can be 90% confident that the fold-change is a value between the lower confidence bound and a variable upper confidence bound.

The data set for the 2 and 4 days of deficiency were analysed separately or jointly. In the separated analysis, a cut-off by FDR of 7.8 or 1.9 for 2 days of B or Ca deficiency, and 33.7 or 55.2 for 4 days of B or Ca deficiency, respectively was used.

Annotations for the 22,392 *Arabidopsis thaliana* transcripts that are represented on the GeneChip Arabidopsis ATH1 Genome Array were obtained from the NetAffx database (http://www.affymetrix.com) as of January 2008 and imported into dChip using ChipInfo software (Zhong et al. 2003).

Reverse transcriptase quantitative real-time PCR (RT-qPCR) analysis

The mRNA from duplicate samples was isolated from Arabidopsis plant as previously described. A first-strand cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. The primers used for the RT-qPCR were designed using the Roche Applied Science software (ProbeFinder v. 2.44 at http://qpcr.probefinder.com) for the thirteen genes: At5g24660, At1g62560, At1g65860, At4g04610, At1g64390, At4g15920, At4g26260, At1g10070, At1g79700, At1g03090, At2g19800, At1g21400, At1g76410 and At1g64660. The specificity of each primer pair was checked against the *Arabidopsis* genome using WU-BLAST 2.0 (http://www.arabidopsis.org). The RT-qPCR was performed with an iQ5 detection system (Bio-Rad) by using the SYBR Green supermix reagent (Bio-Rad) according to the manufacturer's instructions. The following standard thermal profile was used for all PCRs: Stage 1, 1 cycle at 95ºC for 3min; Stage 2, 55 cycles at 95ºC for 10s, primer specific annealing temperature for 20s and 72ºC for 30s; Stage 3 (melting curve), from 65ºC to

95ºC with increments of 0.5ºC/min. For primer sequences and annealing temperatures, see Supplementary Table 5.2.

A pool of cDNA from the control, B deficiency, and Ca deficiency samples was used for primer efficiency assays and the Ct values generated for each primer pair set over a range of dilutions were used to calculate the primer efficiency. The relative quantification method (ΔΔCt) was used to asses the relative expression of each gene analysed (Livak and Schmittgen, 2001) with two housekeeping genes, At5g53300, an *ubiquitin-conjugating enzyme E2 10 (UBC10)* and At1g13320, *protein phosphatase 2A subunit A3 (PP2A)* (Czechowski et al. 2005).

Gene annotation

For gene annotation, we have used the updated TAIR9 (The Arabidopsis Information Resource at http://www.arabidopsis.org) annotation. Genes were classified using the Gene Ontology categories to group functionally-related genes.

RESULTS AND DISCUSSION

Morphological symptoms of B deficiency

Seventeen-day-old *Arabidopsis thaliana* plants were subjected to B or Ca deficiency. The morphological symptoms of the B-deficient plants were not visible 2 days after withholding B, but after 4 days, the plants showed slightly darker leaves. In contrast, the morphological symptoms of Ca-deficient plants were already visible 2 days after withholding Ca. Plants showed yellowish and curled leaves and reduced growth rates, while after 4 days of imposed deficiency, some symptoms of leaf necrosis were also visible. The plants grown under B deficiency for a few more weeks seemed to have arrested development, while the plants subjected to Ca deficiency died (data not shown).

Boron levels in the different treatments after 4 days of deficiency

Control plants were seen to contain 27±3μg B/g dry weight, whereas the B content of plants grown under B deficiency for 4 days was reduced to 18±3μg B/g dry weight (*t*-test $p<0.05$). In Ca-deficient plants, no effect on B content was observed 27±2μg B/g dry weight (*t*-test $p<0.05$).

Gene expression analysis and functional categorization

The Arabidopsis GeneChip ATH1 oligonucleotide array, representing 22,392 annotated genes of the Arabidopsis genome, was used to study the gene expression profile of plants subjected to either B or Ca deficiency for 2 and 4 days. Two days after B deficiency, 102 genes were found to be differentially expressed while 4 days after B deficiency twice as many (208) were differentially expressed. A different behaviour was observed for the Ca deficiency treatment. Two days after Ca deficiency 2,975 genes were found to be differentially expressed, while 4 days after Ca deficiency, only one tenth of the genes (270) were differentially expressed (Supplementary Table 5.1). The different sets of genes were categorized into biological processes (Figure 5.1), according to annotations present in *A. thaliana* database (TAIR9; http://www.arabidopsis.org).

The description of Ca as a versatile messenger mediating a variety of responses related with growth and stress conditions (Hepler 2005), might explain the higher number of the differentially expressed genes observed after only 2 days of Ca deficiency. It is likely that this results from a complexity of down-stream responses (secondary effects) which could also include adaptive responses towards the management of Ca deficiency. The reduced number of genes expressed 4 days after Ca deficiency (Figure 5.1a) could possibly be the expression of necrotic processes taking place. This dramatic effects observed in Ca-deficient plants precluded any direct comparison of the transcriptomics of Ca and B deficiency.

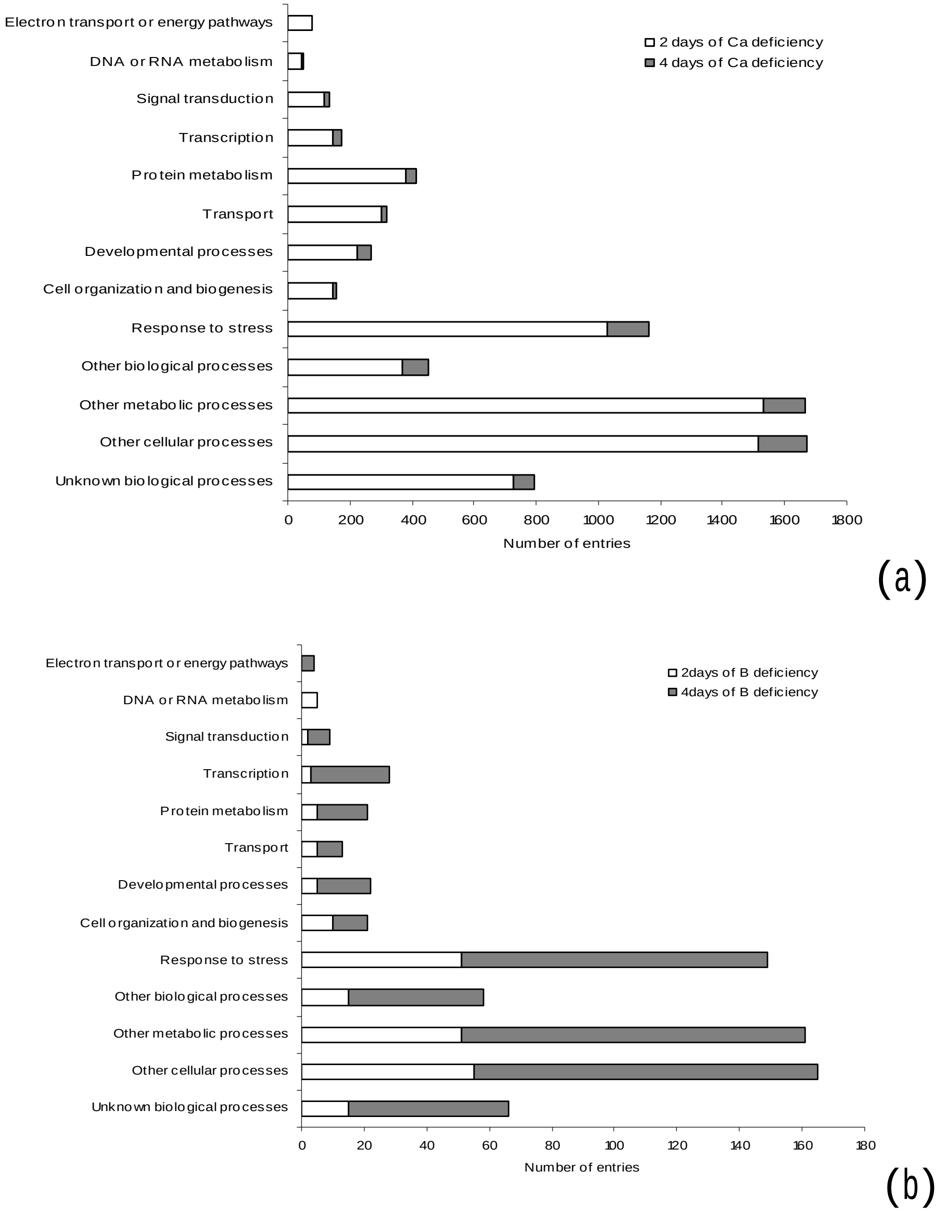

Figure 5.1. Biological functional categorization of the differentially expressed genes due to Ca or B deficiency (Supplementary Table 5.1). The genes that were differentially expressed 2 (☐) and 4 days (■) after Ca (a) or B deficiency (b) were grouped according to gene ontology (GO) classification annotated in TAIR9 database (http://www.arabidopsis.org).

Boron is reported to cross-link cell wall pectin (RG-II) molecules, and its deficiency results in damaged cell wall structure (Kobayashi et al. 1996, O'Neill et al. 1996, 2001). In our study, genes related with cell organization and biogenesis (Figure 5.1b), namely cell elongation, cell wall loosening, xyloglucan biosynthesis and lignin biosynthetic process were altered by B deficiency. A recent report on B-deficient *Arabidopsis thaliana* root revealed that the majority of the cell wall genes that were down-regulated are associated with the cell elongation process, cell wall loosening and xyloglucan biosynthesis (Camacho-Cristóbal et al. 2008).

A structural role for B in membranes has also been proposed and it can explain a large number of reported effects of B deficiency in processes that are related with cellular membrane damage (Brown et al. 2002). In our study we observed that B deficiency altered the expression of genes related with lipid processing, namely lipid transport-related processes (that is partially represented by altered transport 2 days after B deficiency; Figure 5.1b) and lipid metabolic processing (that is partially represented in other metabolic processes 4 days after B deficiency; Figure 5.1b). Altered expression of genes related with K^+ transport was also observed 4 days after the imposition of B deficiency. Altered lipid biosynthesis and increased effluxes of K^+ due to B deficiency have already been reported (Belver et al. 1983, Schon et al. 1990, Cakmak et al. 1995). An important plasma membrane boric channel, NIP5;1, previously described to be crucial for B import into cell roots under B limitation (Takano et al. 2006), was also observed to be up regulated 4 days after B deficiency (Supplementary Table 5.1).

Boron has also been associated with cytoskeleton biosynthesis, reproductive stage and organ morphogenesis, three events represented in the developmental processes (Figure 5.1b). In our study, we observed the up-regulation of *Actin3* (*ACT3*) and *Tubulin1* (*TUB1*) after 2 days of B deficiency. Yu et al. (2001) observed increased levels of cytoskeleton proteins and altered polymerization patterns in *A. thaliana* roots subjected to short-term B deficiency. An alteration in the expression of genes related with the reproductive stage and organ morphogenesis is in agreement with the referred higher requirement of B in plant reproductive structures (Loomis and Durst 1992, Rawson 1996).

We also detected changes into the expression of genes related with responses to stress (Figure 5.1b) in particular with oxidative stress, other stresses (e. g. cadmium stress) and defence responses. In a recent study, the major cause of cell death in B-deficient tobacco cells was associated to oxidative damage (Koshiba et al. 2009), and several defence-related responses have been reported in B-deficient plants (Kobayashi et al. 2004, Alves et al. 2006, Reguera et al. 2010). Additionally, the expression of genes related with proteolysis and protein folding processes were also altered as a consequence of B deficiency as represented by altered protein metabolism in Figure 5.1b.

In the other metabolic processes (Figure 5.1b) we also observed the altered expression of several genes related with amino acid (mainly the branched-chain amino acids, methionine and threonine) and with glucosinolate metabolism. Free amino acids were suggested to regulate processes related to gene expression, not only on a global scale, but also in relation to translation of a particular group of proteins (Kimball and Jefferson 2006). Glucosinolate biosynthetic processing is modulated by either normal or stressful conditions, for example, wounding (Grubb and Abel 2006).

We detected several genes encoding transcription factors, which are included in the transcription class (Figure 5.1b), that were altered by B deficiency. Previously, Kasajima et al. (2010) reported that the *A. thaliana* transcription factor WRKY6, regulates several genes under B deficiency.

The increased number of differentially expressed genes from the 2nd to the 4th day of B deficiency (Figure 5.1 b) could reflect the transition from early or immediate responses to B deficiency (primary), to down-stream responses (secondary effects) which could include adaptive responses towards the management of B deficiency, but also non-specific responses to general stress.

Therefore, we have preferentially selected genes which were similarly differentially expressed after 2 and 4 days of B deficiency (Table 5.1).

Among the 37 genes selected (Supplementary Table 5.1), only the differential expression of one (At3g44450) was common to both B and Ca deficiencies, being down- and up-regulated, respectively. The remaining 36 genes were specifically responsive to B deficiency. Considering that the differential regulation of a chosen set of genes (13 genes) was validated by RT-qPCR analysis (Supplementary Table 5.2), the 37 genes selected are discussed in more detail below.

Cell wall biosynthesis

Several genes related with cell wall biosynthesis were differentially expressed under B deficiency. It has been reported that B is an essential element for the cell wall structure, through the cross-linking of rhamnogalacturonan molecules (Kobayashi et al. 1996, O'Neill et al. 1996, 2001). Polygalacturonans are abundant constituents of pectin and several enzymes are responsible for pectin modifications, including pectin methylesterases (Giovane et al. 2004). We observed the increased gene expression of a *pectin methylesterase*

Table 5.1. Functional classification of genes differentially expressed due to B deficiency. The thirty seven genes selected were expressed 2 and 4 days after B deficiency and were classified according to their biological functions.

Probe Set[1]	AGI ID[2]	Curator Summary	Expr.	Biological Processes
Cell wall biosynthesis				
266693_at	At2g19800	Myo-inositol oxygenase 2 (MIOX2)	Up	L-ascorbic acid biosynthesis; inositol catabolism; ox/red
254001_at	At4g26260	Myo-inositol oxygenase 4 (MIOX4)	Up	L-ascorbic acid biosynthesis; inositol catabolism; ox/red
264898_at	At1g23205	Pectin methylesterase inhibitor	Up	Pectinesterase inhibitor activity
259736_at	At1g64390	Glycosyl hydrolase 9C2 (ATGH9C2)	Down	Cell wall organization; cellulose catabolism
258003_at	At3g29030	Expansin (EXP5)	Down	Cell wall modification during multidimensional cell growth
263477_at	At2g31790	UDP-glucoronosyl/UDP-glucosyl transferase	Down	Metabolic process
260955_at	At1g06000	Flavonol-7-O-rhamnosyltransferase (7RhaT)	Down	Flavonol biosynthetic process
264931_at	At1g60590	Pectinase, putative	Down	Carbohydrate metabolic process
253638_at	At4g30470	Cinnamoyl-CoA reductase-related (CCR)	Down	Lignin biosynthetic process
Sulphur metabolism				
261957_at	At1g64660	Methionine gamma-lyase (ATMGL)	Up	Methionine catabolic process via 2-oxobutanoate
265121_at	At1g65860	Flavin-containing monooxygenase 1 (FMO GS-OX1)	Down	Glucosinolate biosynthesis from homoMet; Ox/Red
261913_at	At1g62560	Flavin-containing monooxygenase 3 (FMO GS-OX3)	Down	Glucosinolate biosynthesis from homoMet; Ox/Red
254343_at	At4g04610	5'-adenylylsulfate reductase (APR1)	Down	Sulfate assimilation and reduction; Cys biosynthesis; Ox/Red
264745_at	At1g62180	5'-adenylylsulfate reductase 2 (APR2)	Down	Sulfate assimilation and reduction; Cys biosynthesis; Ox/Red
255284_at	At4g21990	5'-adenylylsulfate reductase 3 (APR3)	Down	Sulfate assimilation and reduction; Cys biosynthesis; Ox/Red
249752_at	At5g24660	Response to low sulfur 2 (LSU2)	Down	Unknown
260385_at	At5g07010	Sulfotransferase (AtST2a)	Down	Jasmonic acid metabolic process, response to jasmonic acid stimulus
250662_at	At1g74090	Desulphoglucosinolate sulfotransferase (AtST5b)	Down	Glucosinolate biosynthetic process
Branched-chain amino acid (BCAA) catabolism				
264524_at	At1g10070	Branched-chain amino acid transaminase 2 (BCAT2)	Up	BCAA biosynthesis/catabolism
263118_at	At1g03090	3-methylcrotonyl-CoA carboxylase 1 (MCCA1)	Up	Leucine catabolic process

Table 5.1 (cont) Functional classification of genes differentially expressed due to B deficiency. The thirty seven genes selected were expressed 2 and 4 days after B deficiency and were classified according to their biological functions.

Probe Set[1]	AGI ID[2]	Curator Summary	Expr.	Biological Processes
Transcriptional factors and hormones				
261395_at	At1g79700	Ovule development protein (ODP), putative	Up	Regulation of transcription, DNA-dependent; transcription
259982_at	At1g76410	ATL1R	Up	Protein binding, zinc ion binding
259230_at	At3g07780	OBE1	Up	Regulation of gene expression
266015_at	At2g24190	Short-chain dehydrogenase/reductase 1 (SDR1)	Up	Metabolic process
263739_at	At2g21320	DBB1a	Down	Regulation of transcription
254231_at	At4g23810	WRKY53	Down	Regulation of transcription
248353_at	At5g52320	Cytochrome P450 (CYP96A4)	Down	Unknown
Miscellaneous				
254197_at	At4g24040	Trehalase 1 (TRE1)	Up	Trehalose catabolic process
261177_at	At1g04770	Male sterility 5 (MS5)	Up	Unknown
249527_at	At5g38710	Proline oxidase, putative	Up	Glutamate biosynthetic process, proline catabolic process
250832_at	At5g04950	Nicotianamine synthase 1 (NAS1)	Down	Nicotianamine biosynthetic process
249063_at	At5g44110	ABC transporter AtNAP2	Down	Response to red light
Unknown				
264467_at	At1g10140	Expressed protein	Up	Unknown
247374_at	At5g63190	MA3 domain-containing protein	Up	Unknown
252478_at	At3g46540	Epsin N-terminal homology (ENTH) domain-containing protein	Down	Unknown
245524_at	At4g15920	Expressed protein	Down	Unknown
252661_at	At3g44450	Expressed protein	Down	Unknown

[1] Affymetrix microarray ATH1

[2] From the TAIR9 (The Arabidopsis Information Resource) database (http://www.arabidopsis.org)

inhibitor (PMEI) and the decreased gene expression of a putative *pectinase*. Previous work (Camacho-Cristóbal et al. 2008) reported the decreased gene expression of two pectin methylesterases (PME) in B-deficient Arabidopsis roots, which suggests impaired pectin demethylation due to B deficiency.

The cellulose framework is thought to be embedded in the pectic polysaccharide matrix. We also detected decreased expression of genes involved in cellulose biosynthesis, namely the *glycosyl hydrolase 9C2 (ATGH9CE)*, involved in cellulose degradation and in an *expansin5* (*EXP5*). This data indicates that cell elongation was impaired or at least reduced due to B deficiency. Indeed, endoglycosidases and expansins are thought to be needed to modify the cell wall architecture in order to allow the incorporation of new material and consequent cell elongation (Gibeaut and Carpita 1994, Cosgrove et al. 2002).

Boron deficiency also induced several changes that interfere with the formation of cell wall precursors. *myo*-Inositol oxygenase (MIOX) is a key enzyme for the oxygenative cleavage of inositol into glucuronic acid, and has been associated with the control of carbohydrates flux to the cell wall (Kanter et al. 2005). From this multigene family, two (MIOX2 and 4) were up-regulated due to B deficiency. This observation suggests an increase in the synthesis of glucuronic acid, the main precursor for important residues of plant cell wall polymers, and an important substrate for nucleotide sugar inter-conversions (Kanter et al. 2005). The converted nucleotide sugars can then be used as substrates for glycosyltransferases, which are involved in the transfer of glycosyl residues to a wide range of accepter molecules, and are responsible for regulating properties, such as bioactivity or solubility (Ross et al. 2001). Two genes, encoding a *glucosyltransferase* and a *rhamnosyltransferase (7RhaT)*, were down-regulated due to B deficiency, reinforcing the observation that indicates perturbations in the cell wall metabolism. Furthermore, the gene expression of *trehalase 1* (*TRE1*), an enzyme responsible for trehalose degradation, was up-regulated under B deficiency. Trehalase is a plasma membrane-bound enzyme with its catalytic domain oriented towards the cell wall (Frison et al. 2007). The possible decreased content of trehalose, a recognized important sugar, could be related with carbohydrate partitioning and allocation (Müller et al. 2001). It seems that regulation of endogenous trehalose levels requires the transport of this sugar out of the cell (Frison et al. 2007). Thus, B deficiency, by altering the cell wall structure could also be interfering with trehalose metabolism and signalling in the plant.

Lignin is an important constituent of the secondary cell wall, and is composed of a complex network of aromatic polymers derived from the phenylpropanoid pathway. The *cinnamoyl-CoA reductase (CCR)* gene that encodes a key enzyme for the formation of lignin monomers (Lacombe et al. 1997) was also down regulated due to B deficiency. A reduction in *CCR* gene expression was reported to decrease lignin biosynthesis (Piquemal et al. 1998), while the fraction of soluble phenols increases (van der Rest et al. 2006). Indeed, an increase in phenol content of B-deficient plants has been widely described (Brown et al. 2002).

Boron deficiency thus seems to affect cell wall biosynthesis at different levels (Figure 5.2). Probably, the damage of the pectin network compromises the stability of the embedded and adjacent matrixes. It seems that on one hand the plant is reducing cell wall related processes by decreasing the expression of the cell wall related genes, but on the other hand is trying to rescue the wall damage by increasing glucuronate biosynthesis.

Sulphur metabolism

Sulphur, in its reduced form, is involved in the biosynthesis of compounds of primary and secondary metabolism (Schmidt and Jäger 1992). The expression of an unknown gene, described as *Low sulphur up-regulated 2* gene (*LSU2*) (Lewandowska et al. 2010), was down-regulated due to B deficiency. Additionally, the three genes (*APR1*, *2* and *3*) that encode 5'-adenylylsulfate reductases (APRs) were also down-regulated by B deficiency. APR is encoded by a multigene family (Gutierrez-Marcos et al 1996) and plays a key role in the regulation of the sulphate assimilation pathway (Vauclare et al. 2002). Taken together, these results indicate that sulphur metabolism is being significantly affected by B

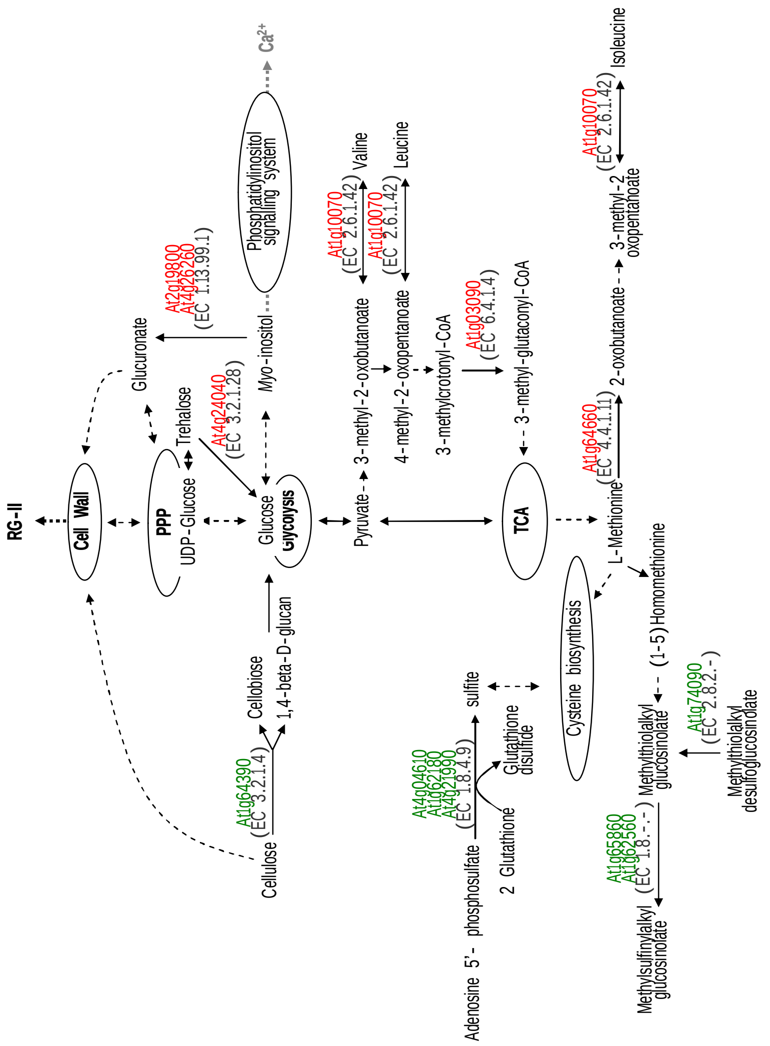

Figure 5.2. Metabolic overview of the pathways affected by B deficiency. Genes are marked either red (up regulated) or green (down regulated); see Table 5.1. For enzymes the respective EC number is indicated.

deficiency. Once inside the plant, sulphur can be fixed into cystein and thereafter interconverted in methionine (Saito 2004). The catabolism of methionine is often related with homeostatic management of the excess of sulphur-containing amino acids. Thus, our observation of increased expression of the gene encoding *methionine gamma-lyase (ATMGL)* responsible for the degradation of methionine, can be directly related with the decrease in the sulphate assimilation pathway (Rennenberg 1984). It appears that B deficiency induces an effect similar to that of sulphur excess in plants. The expression of other genes related with sulphur metabolism was also decreased by B deficiency (Table 5.1), namely two flavin-containing monooxygenases and two sulfotransferases. These enzymes have been described as participants in sulphur-related steps in glucosinolate and jasmonate biosyntheses. The two *flavin-containing monooxygenases (FMO GS-OX1 and 3)* and a *desulphoglucosinolate sulfotransferase (AtST5b)* are required for glucosinolate biosynthesis; the FMO in the sulphur redox reactions (Grubb and Abel 2006), and the ST in the sulfation of desolfoglucosinolates, preferentially those derived from methionine (Piotrowski et al. 2004, Klein and Papenbrock 2006). Glucosinolates are sulphur-rich compounds that are differentially regulated at different developmental stages and conditions of stress, e. g. altered mineral nutrition (Halkier and Gershenzon 2006, Grubb and Abel 2006). The *sulfotransferase AtST5b* is a close relative of the previous *AtST2a* that has been described as a hydroxyjasmonate sulfotransferase (Klein and Papenbrock 2006), proposed to regulate the levels of jasmonate and/or hydroxyjasmonate in plants (Gidda et al. 2003). Therefore, B may have an important impact on jasmonate biosynthesis.

Our work provides an indication that B deficiency affects several aspects of sulphur metabolism in plants (Figure 5.2). However, in our research of the literature, we only found a reference to decreased glutathione levels and increased gene expression of glutathione-S-transferase due to B deficiency (Lukaszewski and Blevins 1996, Kobayashi et al. 2004).

A link between sulphur and nitrogen metabolism has been known for many years, the deprivation of one leading to a disruption of the metabolism of the other (Koprivova et al. 2000). Our observation of the decreased expression of a nitrogen-related gene, *nicotianamine synthase (NS1)*, an enzyme reported to be induced by nitrate, (Wang et al. 2003) suggests a decrease in nitrogen metabolism. Previous studies on B deficiency also indicate a decrease in nitrogen metabolism (Camacho-Cristóbal and González-Fontes 1999, Beato et al. 2010).

Branched-chain amino acid catabolism

The branched-chain amino acid aminotransferase (BCAT) is responsible for the translocation of amino groups for both the biosynthesis and catabolism of the branched-chain amino acids (BCAAs), isoleucine, leucine and valine (Schuster and Binder 2005). The increased expression of *BCAT2*, a member of this gene family, and the repression of the *3-methylcrotonyl-CoA carboxylase 1* (*MCCA1*), which is described to participate in leucine catabolism (Anderson et al. 1998), may indicate that *BCAT2* is operating towards BCAAs degradation, in particular of leucine (Figure 5.2). In a recent study with *A. thaliana*, another member of the branched-chain amino acid aminotransferase, *BCAT4*, was also found to be up-regulated by B deficiency (Kasajima et al. 2010). In animals, the BCAA were described to stimulate muscle protein synthesis, leucine being the most effective with regard to the regulation of these proteins (Rhoads and Wu 2009). Since actin is an important muscle and also a cytoskeleton component (Hussey et al. 2002), changes in BCAAs content, in particular leucine, could interfere with cytoskeleton rearrangement. Under short-term B deficiency, increased levels of cytoskeletal proteins and altered polymerization patterns were already reported (Yu et al. 2001, 2003). Cytoskeleton altered biosynthesis is a hypothesis consistent with the knowledge that B plays a critical role in reproduction where an actively changing cytoskeleton structure is required, as for instance during meiosis. We also found that the expression of a meiosis associated gene, the *male sterility 5 (MS5)*, important for male fertility (Glover et al. 1998) was decreased due to B deficiency.

Transcriptional factors and hormones

Regarding transcription related processes, we detected the differential expression of five transcription factors during B deficiency. A putative *ovule development protein* (*ODP*), a RING-H2 finger protein, *ATL1R*, and a nuclear PHD finger, *OBE1*, were up-regulated due to B deficiency, while DBB1a and WRKY53 were down regulated.

Three of these transcription factors, ODP, OBE1 and WRKY53, have been associated to both biotic and abiotic stress and to hormonal responses. It has been reported that *ODP and OBE1 genes* are up-regulated by cytokinins (Feng et al 2005) and auxins, respectively (Saiga et al. 2008), and that *WRKY53* is down-regulated by jasmonic acid (Thomas et al. 2009). Concerning this last, we also observed that B deficiency caused the down-regulation of another gene related with jasmonic acid biosynthesis, CYP96A4, which is involved in the metabolism of oxylipins (Benveniste et al. 2006). These observations suggest that B deficiency may have some involvement with auxins, cytokinins and jasmonate.

Another gene involved with hormone biosynthesis, *short-chain dehydrogenase/reductase 1 (SDR1)*, was up-regulated due to B deficiency. SDR1 participates in ABA biosynthesis (Lee et al. 2007) and was described to function as a molecular link between nutrient signalling and plant hormone biosynthesis (Cheng et al. 2002).

The literature contains little information concerning B and hormones. In relation to auxin, there are contradictory reports on the effect of B deficiency on auxin levels (Blevins and Lukaszewski 1998, Wang et al. 2006). Regarding cytokinins, it was shown by Wang and colleagues (2006) that their levels were decreased in the apexes of B-deficient pea.

Of the other two transcription factors affected by B deficiency, a defect in the DBB1a protein was recently described to cause abnormal floral development in Arabidopsis (Wang et al. 2009). Thus, the decreased expression of *DBB1a* that we observed under B deficiency might be related with the described abnormal development of the plant reproductive structures caused by B deficiency (Brown et al. 2002). Finally, the transcription factor *ATL1R*, whose gene was up-regulated upon B deficiency, is related with RNA-binding proteins, thus being important for RNA processing (Wang et al. 2008).

Miscellaneous

We observed that the *proline oxidase* gene was induced by B deficiency. This gene encodes an enzyme involved in glutamate biosynthetic processes and proline catabolic processes. However, in *L. albus* we detected an increase in proline content during B deficiency (Chapter 4). We also observed that *AtNAP2*, which encodes an ABC transporter, was down-regulated by B deficiency. ABC transporters are responsible for the transport of a broad range of substances across membranes (Marin et al. 2006) and might therefore be involved in the plant adaptation to B deficiency.

CONCLUSIONS

A transcriptional analysis of *Arabidopsis thaliana* grown under B deficiency for 2 and 4 days was performed. Thirteen seven genes, which demonstrated similar differentially regulation after both 2 and 4 days of B deficiency were selected for discussion. In agreement with the well-documented importance of B in the cell wall structure, we found evidence for alteration in transcription of genes related with cell wall biosynthesis (9). In addition, genes related with transcriptional factors and hormones (7) were also seen to be affected by B deficiency. Interestingly, we find alterations in genes related with sulphur metabolism (9), indicating an important, novel link between sulphur metabolism and B deficiency.

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SUPPLEMENTARY DATA

Supplementary Table 5.1. Genes differentially expressed 2 days (102) and 4 days (208) after B suppression and genes differentially expressed 2 days (2,975) and 4 days (270) after Ca suppression, organized according to the fold change (FC) observed in the Affymetrix ATH1 geneChip array.

Probe Set[1]	AGI ID[2]	Curatory Summary	FC
2 days after B suppression			
252269_at	At3g49580	expressed protein	15.64
259842_at	At1g73600	phosphoethanolamine N-methyltransferase 3, putative (NMT3)	7
248844_s_at	At5g46900	protease inhibitor/seed storage/lipid transfer protein	6.81
259813_at	At1g49860	glutathione S-transferase, putative	6.17
255689_at	At4g00670	remorin	5.75
261684_at	At1g47400	expressed protein	5.72
249752_at	At5g24660	expressed protein	5.38
249867_at	At5g23020	2-isopropylmalate synthase 2 (IMS2)	5.02
260745_at	At1g78370	glutathione S-transferase, putative	4.85
250661_at	At5g07030	aspartyl protease	4.76
248139_at	At5g54970	expressed protein	4.62
266395_at	At2g43100	aconitase C-terminal domain-containing protein	4.44
262978_at	At1g75780	tubulin beta-1 chain (TUB1)	4.26
249836_at	At5g23420	high mobility group (HMG1/2)	4.22
249167_at	At5g42860	expressed protein	4.22
256125_at	At1g18250	thaumatin, putative	4.16
265121_at	At1g62560	flavin-containing monooxygenase	3.98
261913_at	At1g65860	flavin-containing monooxygenase	3.93
258675_at	At3g08770	lipid transfer protein 6 (LTP6)	3.89
262717_s_at	At1g16410	cytochrome P450	3.73
255517_at	At4g02290	glycosyl hydrolase family 9 protein	3.67
251524_at	At3g58990	aconitase C-terminal domain-containing protein	3.66
250892_at	At5g03760	glycosyl transferase family 2 protein	3.64
264262_at	At1g09200	histone H3	3.63
254789_at	At4g12880	plastocyanin-like domain-containing protein	3.49
263941_at	At2g35870	At2g35870	3.48
258851_at	At3g03190	glutathione S-transferase, putative	3.43
258470_at	At3g06035	expressed protein	3.32
263431_at	At2g22170	lipid-associated	3.3
247651_at	At5g59870	histone H2A, putative	3.28
247268_at	At5g64080	protease inhibitor/seed storage/lipid transfer protein	3.28
252442_at	At3g46940	deoxyuridine 5'-triphosphate nucleotidohydrolase	3.25
259681_at	At1g77760	nitrate reductase 1 (NR1)	3.24
253040_at	At4g37800	xyloglucan:xyloglucosyl transferase, putative	3.16
250434_at	At5g10390	histone H3	3.15
254862_at	At4g12030	bile acid:sodium symporter	3.14
255874_at	At2g40550	expressed protein	3.13
261330_at	At1g44900	DNA replication licensing factor, putative	3.07

245196_at	At1g67750	pectate lyase	3.07
267590_at	At2g39700	expansin, putative (EXP4)	3.06
245343_at	At4g15830	expressed protein	3.05
247192_at	At5g65360	histone H3	3.04
266223_at	At2g28790	osmotin-like protein, putative	3.03
263628_at	At2g04780	fasciclin-like arabinogalactan-protein (FLA7)	3.03
257021_at	At3g19710	branched-chain amino acid transaminase (BCAT4)	2.99
261080_at	At1g07370	proliferating cell nuclear antigen 1 (PCNA1)	2.97
249916_at	At5g22880	histone H2B, putative	2.94
252911_at	At4g39510	cytochrome P450	2.92
258859_at	At3g02120	hydroxyproline-rich glycoprotein	2.88
262232_at	At1g68600	expressed protein	2.88
248891_at	At5g46280	DNA replication licensing factor, putative	2.8
258480_at	At3g02640	expressed protein	2.79
260902_at	At1g21440	mutase	2.76
263882_at	At2g21790	ribonucleotide reductase, putative	2.76
247812_at	At5g58390	peroxidase, putative	2.74
262109_at	At1g02730	cellulose synthase	2.72
256237_at	At3g12610	DNA-damage-repair/toleration protein, putative (DRT100)	2.71
265656_at	At2g13820	protease inhibitor/seed storage/lipid transfer protein	2.71
261921_at	At1g65900	expressed protein	2.67
252148_at	At3g51280	male sterility MS5, putative	2.64
263535_at	At2g24970	expressed protein	2.63
254687_at	At4g13770	cytochrome P450	2.61
261309_at	At1g48600	phosphoethanolamine N-methyltransferase 2, putative (NMT2)	2.56
253340_s_at	At4g33260	WD-40 repeat	2.56
254119_at	At4g24780	pectate lyase	2.56
264377_at	At2g25060	plastocyanin-like domain-containing protein	2.55
267175_s_at	At2g37620	actin 3 (ACT3)	2.55
249866_at	At5g23010	2-isopropylmalate synthase 3 (IMS3)	2.47
264061_at	At2g27970	cyclin-dependent kinase, putative	2.38
246962_s_at	At5g24800	bZIP transcription factor	-2.71
266267_at	At2g29460	glutathione S-transferase, putative	-2.86
266590_at	At2g46240	IQ domain-containing protein	-2.96
260015_at	At1g67980	caffeoyl-CoA 3-O-methyltransferase, putative	-2.98
247095_at	At5g66400	dehydrin (RAB18)	-3
259982_at	At1g76410	zinc finger (C3HC4-type RING finger)	-3.08
250580_at	At5g07440	glutamate dehydrogenase 2 (GDH2)	-3.08
260662_at	At1g19540	isoflavone reductase, putative	-3.13
266799_at	At2g22860	phytosulfokines 2 (PSK2)	-3.18
260248_at	At1g74310	heat shock protein 101 (HSP101)	-3.2
266841_at	At2g26150	heat shock transcription factor	-3.25
266693_at	At2g19800	expressed protein	-3.49
264280_at	At1g61820	glycosyl hydrolase family 1 protein	-3.54

256965_at	At3g13450	branched-chain alpha-keto acid dehydrogenase E1 beta subunit (DIN4)	-3.61
266294_at	At2g29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI)	-3.69
263118_at	At1g03090	3-methylcrotonyl-CoA carboxylase 1 (MCCA)	-3.78
260900_s_at	At1g21400	2-oxoisovalerate dehydrogenase, putative	-3.8
252570_at	At3g45300	isovaleryl-CoA-dehydrogenase (IVD)	-3.82
261395_at	At1g79700	ovule development protein, putative	-3.83
251755_at	At3g55790	expressed protein	-4.13
252515_at	At3g46230	17.4 kDa class I heat shock protein (HSP17.4-CI)	-4.57
262047_at	At1g80160	lactoylglutathione lyase / glyoxalase I	-4.82
252415_at	At3g47340	asparagine synthetase 1 (ASN1)	-4.92
264524_at	At1g10070	branched-chain amino acid transaminase 2 (BCAT2)	-4.95
260668_at	At1g19530	expressed protein	-5.03
260741_at	At1g15045	glutamine amidotransferase-related	-5.45
262307_at	At1g71000	DNAJ heat shock N-terminal domain-containing protein	-5.59
261957_at	At1g64660	Cys/Met metabolism pyridoxal-phosphate-dependent enzyme	-6.67
253829_at	At4g28040	nodulin MtN21	-6.88
264777_at	At1g08630	L-allo-threonine aldolase-related	-7.2
250351_at	At5g12030	II heat shock protein 17.6A (HSP17.7-CII)	-7.98
264514_at	At1g09500	cinnamyl-alcohol dehydrogenase	-8.54
254001_at	At4g26260	expressed protein	-9.29
262325_at	At1g64160	disease resistance-responsive / dirigent	-12.87
4 days after B suppression			
261177_at	At1g04770	male sterility MS5	3.13
265665_at	At2g27420	cysteine proteinase, putative	3.09
249752_at	At5g24660	expressed protein	2.7
255604_at	At4g01080	expressed protein	2.6
250533_at	At5g08640	flavonol synthase 1 (FLS1)	2.43
258133_at	At3g24500	ethylene-responsive transcriptional coactivator, putative	2.32
256999_at	At3g14200	DNAJ heat shock N-terminal domain-containing protein	2.21
248676_at	At5g48850	male sterility MS5	2.2
247447_at	At5g62730	proton-dependent oligopeptide transport (POT)	2.14
260248_at	At1g74310	heat shock protein 101 (HSP101)	2.11
264898_at	At1g23205	invertase/pectin methylesterase inhibitor	2.11
254343_at	At4g21990	5'-adenylylsulfate reductase (APR3)	2.05
255284_at	At4g04610	5'-adenylylsulfate reductase (APR1)	2.03
264931_at	At1g60590	polygalacturonase, putative / pectinase, putative	2
261907_at	At1g65060	4-coumaroyl-CoA synthase 3 (4CL3)	1.97
248607_at	At5g49480	sodium-responsive calcium-binding protein (ACP1)	1.94
264331_at	At1g04130	tetratricopeptide repeat (TPR)-containing protein	1.9
263374_at	At2g20560	DNAJ heat shock	1.88
248448_at	At5g51190	AP2 domain-containing transcription factor, putative	1.87
249837_at	At5g23480	expressed protein	1.86
261953_at	At1g64440	UDP-glucose 4-epimerase, putative	1.85
258560_at	At3g06020	expressed protein	1.84

260955_at	At1g06000	UDP-glucoronosyl/UDP-glucosyl transferase	1.83
266841_at	At2g26150	heat shock transcription factor	1.82
255511_at	At4g02075	zinc finger (C3HC4-type RING finger)	1.82
252661_at	At3g44450	expressed protein	1.82
265892_at	At2g15020	expressed protein	1.81
256518_at	At1g66080	expressed protein	1.81
264745_at	At1g62180	5'-adenylylsulfate reductase 2, chloroplast (APR2)	1.81
263739_at	At2g21320	zinc finger (B-box type)	1.81
265121_at	At1g62560	flavin-containing monooxygenase	1.81
261913_at	At1g65860	flavin-containing monooxygenase	1.78
245777_at	At1g73540	MutT/nudix	1.77
267053_s_at	At2g38390	peroxidase 22 (PER22)	1.75
248190_at	At5g54120	calcium-binding EF hand	1.74
253382_at	At4g33040	glutaredoxin	1.74
248191_at	At5g54130	calcium-binding EF hand	1.74
252958_at	At4g38620	myb family transcription factor (MYB4)	1.74
247877_at	At5g57740	zinc finger (C3HC4-type RING finger)	1.7
249774_at	At5g24150	squalene epoxidase 1,1 (SQP1,1)	1.7
252363_at	At3g48460	GDSL-motif lipase/hydrolase	1.69
266720_s_at	At2g46790	timing of CAB expression 1-like protein, putative	1.68
247543_at	At5g61600	ethylene-responsive element-binding	1.67
245025_at	atpF	atpF	1.67
263477_at	At2g31790	UDP-glucoronosyl/UDP-glucosyl transferase	1.66
258830_at	At3g07090	expressed protein	1.66
259432_at	At1g01520	myb family transcription factor	1.65
266015_at	At2g24190	short-chain dehydrogenase/reductase (SDR)	1.65
254231_at	At4g23810	WRKY family transcription factor	1.65
257421_at	At1g12030	expressed protein	1.65
266278_at	At2g29300	tropinone reductase, putative	1.65
244934_at	ndhG	ndhG	1.64
259580_at	At1g28030	oxidoreductase, 2OG-Fe(II) oxygenase	1.63
258505_at	At3g06530	BAP28-related	1.62
258979_at	At3g09440	heat shock cognate 70kDa protein 3 (HSC70-3)	1.62
261597_at	At1g49780	U-box domain-containing protein	1.62
255622_at	At4g01070	UDP-glucoronosyl/UDP-glucosyl transferase	1.62
258003_at	At3g29030	expansin, putative (EXP5)	1.62
263150_at	At1g54050	heat shock protein (HSP17.4-CIII)	1.61
263122_at	At1g78510	solanesyl diphosphate synthase (SPS)	1.6
259364_at	At1g13260	DNA-binding protein RAV1	1.6
266219_at	At2g28880	para-aminobenzoate (PABA) synthase	1.6
249288_at	At5g41050	expressed protein	1.6
248353_at	At5g52320	cytochrome P450, putative	1.59
262416_at	At1g49390	oxidoreductase, 2OG-Fe(II) oxygenase	1.58
266299_at	At2g29450	glutathione S-transferase (103-1A)	1.57
247323_at	At5g64170	dentin sialophosphoprotein-related	1.57

251744_at	At3g56010	expressed protein	1.55
262656_at	At1g14200	zinc finger (C3HC4-type RING finger)	1.55
248964_at	At5g45340	cytochrome P450	1.55
245524_at	At4g15920	nodulin MtN3	1.55
254878_at	At4g11660	heat shock transcription factor 7 (HSTF7)	1.54
250315_at	At5g12130	integral membrane TerC	1.54
258687_at	At3g07860	expressed protein	1.54
260696_at	At1g32520	expressed protein	1.54
260385_at	At1g74090	sulfotransferase	1.54
250758_at	At5g06000	eukaryotic translation initiation factor 3G, putative (eIF3g)	1.53
258468_at	At3g06070	expressed protein	1.53
255028_at	At4g09890	expressed protein	1.53
252483_at	At3g46600	scarecrow transcription factor	1.53
267336_at	At2g19310	expressed protein	1.52
252011_at	At3g52720	carbonic anhydrase	1.52
261754_at	At1g76130	alpha-amylase, putative	1.52
254971_at	At4g10380	major intrinsic protein (MIP)	1.52
257710_at	At3g27350	expressed protein	1.51
266447_at	At2g43290	calmodulin-like protein (MSS3)	1.51
261911_at	At1g80750	60S ribosomal protein L7 (RPL7A)	1.51
258111_at	At3g14630	cytochrome P450, putative	1.51
256442_at	At3g10930	expressed protein	1.51
257323_at	orf294	orf294	1.51
261139_at	At1g19700	homeobox-leucine zipper	1.5
260380_at	At1g73870	zinc finger (B-box type)	1.5
247678_at	At5g59520	zinc transporter (ZIP2)	1.5
254795_at	At4g12990	expressed protein	1.5
247367_at	At5g63290	coproporphyrinogen oxidase-related	1.49
252478_at	At3g46540	clathrin assembly protein-related	1.49
249415_at	At5g39660	Dof-type zinc finger domain-containing protein	1.49
262911_s_at	At1g59860	17.8 kDa class I heat shock protein (HSP17.8-CI)	1.49
254146_at	At4g24260	endo-1,4-beta-glucanase, putative	1.49
247780_at	At5g58770	dehydrodolichyl diphosphate synthase, putative (DPS)	1.48
261084_at	At1g07440	tropinone reductase, putative	1.48
259736_at	At1g64390	endo-1,4-beta-glucanase, putative	1.47
253313_at	At4g33870	1.11.1.7: peroxidase, putative	1.47
261914_at	At1g65870	disease resistance-responsive	1.46
248304_at	At5g53180	polypyrimidine tract-binding protein, putative	1.46
251492_at	At3g59280	signaling molecule-related	1.46
253061_at	At4g37610	TAZ zinc finger	1.46
249583_at	At5g37770	calmodulin-related protein 2, touch-induced (TCH2)	1.45
246502_at	At5g16240	acyl-(acyl-carrier-protein) desaturase, putative	1.45
264850_at	At2g17340	pantothenate kinase-related	1.45
265339_at	At2g18230	inorganic pyrophosphatase (PPA)	1.45
246884_at	At5g26220	ChaC-like	1.45

256245_at	At3g12580	heat shock protein 70, putative (HSP70)	1.44
250099_at	At5g17300	myb family transcription factor	1.44
249247_at	At5g42310	pentatricopeptide (PPR) repeat-containing protein	1.44
248799_at	At5g47230	ethylene-responsive element-binding factor 5 (ERF5)	1.44
247293_at	At5g64510	expressed protein	1.44
248270_at	At5g53450	protein kinase	1.44
246597_at	At5g14760	L-aspartate oxidase	1.43
248028_at	At5g55620	expressed protein	1.43
254850_at	At4g12000	expressed protein	1.43
249770_at	At5g24110	WRKY family transcription factor	1.43
250256_at	At5g13650	elongation factor	1.43
249344_at	At5g40770	prohibitin	1.43
264519_at	At1g10000	expressed protein	1.43
252606_at	At3g45010	serine carboxypeptidase III, putative	1.42
262693_at	At1g62780	expressed protein	1.42
265327_at	At2g18210	expressed protein	1.42
249063_at	At5g44110	ABC transporter	1.41
249575_at	At5g37670	15.7 kDa class I-related small heat shock protein-like (HSP15.7-CI)	1.41
248153_at	At5g54250	cyclic nucleotide-gated channel (CNGC4)	1.41
267069_at	At2g41010	VQ motif-containing protein	1.41
247467_at	At5g62130	Per1-like protein-related	1.41
260653_at	At1g32440	pyruvate kinase, putative	1.41
260266_at	At1g68520	zinc finger (B-box type)	1.4
261081_at	At1g07350	transformer serine	1.4
267235_at	At2g43940	expressed protein	1.4
261166_s_at	At1g34570	expressed protein	1.4
250667_at	At5g07090	40S ribosomal protein S4 (RPS4B)	1.4
262170_at	At1g74940	senescence-associated protein-related	1.39
267305_at	At2g30070	potassium transporter (KUP1)	1.38
267063_at	At2g41120	expressed protein	1.38
253638_at	At4g30470	cinnamoyl-CoA reductase-related	1.37
262483_at	At1g17220	translation initiation factor IF-2, chloroplast, putative	1.36
251800_at	At3g55510	expressed protein	1.36
245993_at	At5g20700	senescence-associated protein-related	1.35
253922_at	At4g26850	expressed protein	1.34
255908_s_at	At1g18010	expressed protein	1.34
255749_at	At1g31970	DEAH box helicase, putative	1.34
250832_at	At5g04950	nicotianamine synthase, putative	1.33
246308_at	At3g51820	chlorophyll synthetase, putative	1.29
259230_at	At3g07780	expressed protein	-1.28
257350_x_at	At2g19040	rapid alkalinization factor (RALF)	-1.29
248766_at	At5g47580	expressed protein	-1.34
248279_at	At5g52910	timeless	-1.35
251996_at	At3g52840	beta-galactosidase, putative	-1.36

261467_at	At1g28520	expressed protein	-1.36
245696_at	At5g04190	phytochrome kinase substrate-related	-1.38
267202_s_at	At2g31030	oxysterol-binding	-1.4
252088_at	At3g52100	expressed protein	-1.4
264501_at	At1g09390	GDSL-motif lipase	-1.41
245947_at	At5g19530	spermine	-1.42
264021_at	At2g21200	auxin-responsive protein, putative	-1.42
246178_s_at	At5g28430	hypothetical protein	-1.43
247374_at	At5g63190	MA3 domain-containing protein	-1.44
255281_at	At4g04970	callose synthase, putative	-1.44
253660_at	At4g30140	GDSL-motif lipase	-1.45
257964_at	At3g19850	phototropic-responsive NPH3	-1.46
259163_at	At3g01490	protein kinase, putative	-1.46
252751_at	At3g43430	zinc finger (C3HC4-type RING finger)	-1.49
261567_at	At1g33055	expressed protein	-1.51
266571_at	At2g23830	vesicle-associated membrane protein, putative (VAMP)	-1.52
264264_at	At1g09250	expressed protein	-1.54
254197_at	At4g24040	trehalase, putative	-1.55
261957_at	At1g64660	Cys/Met metabolism pyridoxal-phosphate-dependent enzyme	-1.56
261395_at	At1g79700	ovule development protein, putative	-1.59
256469_at	At1g32540	zinc finger protein, putative	-1.61
260914_at	At1g02640	glycosyl hydrolase	-1.61
249384_at	At5g39890	expressed protein	-1.61
249383_at	At5g39860	bHLH protein	-1.62
252068_at	At3g51440	strictosidine synthase	-1.62
261026_at	At1g01240	expressed protein	-1.65
247284_at	At5g64410	oligopeptide transporter (OPT)	-1.67
263118_at	At1g03090	3-methylcrotonyl-CoA carboxylase 1 (MCCA)	-1.67
249527_at	At5g38710	proline oxidase, putative	-1.68
264846_at	At2g17850	senescence-associated	-1.69
252173_at	At3g50650	scarecrow-like transcription factor 7 (SCL7)	-1.69
264529_at	At1g30820	CTP synthase, putative	-1.69
247474_at	At5g62280	expressed protein	-1.72
259982_at	At1g76410	zinc finger (C3HC4-type RING finger)	-1.73
259977_at	At1g76590	zinc-binding	-1.73
264788_at	At2g17880	DNAJ heat shock protein, putative	-1.74
260221_at	At1g74670	gibberellin-responsive protein, putative	-1.77
266693_at	At2g19800	expressed protein	-1.78
264467_at	At1g10140	expressed protein	-1.78
250327_at	At5g12050	expressed protein	-1.8
263096_at	At2g16060	non-symbiotic hemoglobin 1 (HB1)	-1.85
245276_at	At4g16780	homeobox-leucine zipper protein 4 (HAT4)	-1.87
254001_at	At4g26260	expressed protein	-1.88
245353_at	At4g16000	expressed protein	-1.94
265481_at	At2g15960	expressed protein	-1.97

260900_s_at	At1g21400	2-oxoisovalerate dehydrogenase, putative	-1.97
245076_at	At2g23170	auxin-responsive GH3	-2.03
266814_at	At2g44910	homeobox-leucine zipper protein 4 (HB-4)	-2.13
267461_at	At2g33830	dormancy	-2.39
253423_at	At4g32280	auxin-responsive AUX/IAA	-2.63
250662_at	At5g07010	sulfotransferase	-2.75
264524_at	At1g10070	branched-chain amino acid transaminase 2 (BCAT2)	-3.01
2 days after Ca suppression			
256593_at	At3g28510	AAA-type ATPase	17.76
245035_at	At2g26400	acireductone dioxygenase (ARD/ARD')	17.19
267345_at	At2g44240	expressed protein	13.7
259925_at	At1g75040	pathogenesis-related protein 5 (PR-5)	12.45
260116_at	At1g33960	avirulence induced gene (AIG1)	10.99
253301_at	At4g33720	pathogenesis-related protein, putative	10.61
266385_at	At2g14610	pathogenesis-related protein 1 (PR-1)	10.37
263852_at	At2g04450	MutT/nudix	9.79
255341_at	At4g04500	protein kinase	9.34
246401_at	At1g57560	myb transcription factor (MYB50)	8.29
254975_at	At4g10500	oxidoreductase, 2OG-Fe(II) oxygenase	7.88
259388_at	At1g13420	sulfotransferase	7.54
257099_s_at	At3g24982	leucine-rich repeat, 5' fragment	7.4
251625_at	At3g57260	glycosyl hydrolase 17 protein	7.38
257591_at	At3g24900	disease resistance	7.3
255689_at	At4g00670	remorin	7.27
251612_at	At3g57950	expressed protein	7.19
249890_at	At5g22570	WRKY transcription factor	7.12
249814_at	At5g23840	MD-2-related lipid recognition domain-containing protein	7.01
257100_at	At3g25010	disease resistance	6.99
263539_at	At2g24850	aminotransferase, putative	6.55
265058_s_at	At1g52040	myrosinase-binding protein, putative (F-ATMBP)	5.92
251928_at	At3g53980	protease inhibitor/seed storage/lipid transfer protein (LTP)	5.86
254828_at	At4g12550	protease inhibitor/seed storage/lipid transfer protein (LTP)	5.86
251673_at	At3g57240	beta-1,3-glucanase (BG3)	5.81
266989_at	At2g39330	jacalin lectin	5.77
249812_at	At5g23830	MD-2-related lipid recognition domain-containing protein	5.75
255940_at	At1g20380	prolyl oligopeptidase, putative	5.72
248728_at	At5g48000	cytochrome P450	5.65
255912_at	At1g66960	lupeol synthase, putative	5.6
255254_at	At4g05030	heavy-metal-associated domain-containing protein	5.54
248844_s_at	At5g46900	protease inhibitor/seed storage/lipid transfer protein (LTP)	5.39
263161_at	At1g54020	myrosinase-associated protein, putative	5.33
262838_at	At1g14960	major latex protein-related	5.31
262516_at	At1g17190	glutathione S-transferase, putative	5.31
260869_at	At1g43800	acyl-(acyl-carrier-protein) desaturase, putative	5.29
255807_at	At4g10270	wound-responsive	5.26

252269_at	At3g49580	expressed protein	5.23
248227_at	At5g53820	expressed protein	5.16
249364_at	At5g40590	DC1 domain-containing protein	5.14
259507_at	At1g43910	AAA-type ATPase	5.13
249438_at	At5g40010	AAA-type ATPase	5.04
257774_at	At3g29250	short-chain dehydrogenase/reductase (SDR)	5.03
254385_s_at	At4g21830	methionine sulfoxide reductase domain-containing protein	4.94
248169_at	At5g54610	ankyrin repeat	4.94
260117_at	At1g33950	avirulence induced gene (AIG1)	4.93
259655_at	At1g55210	disease resistance response protein-related	4.85
246825_at	At5g26260	meprin and TRAF homology domain-containing protein	4.78
250764_at	At5g05960	protease inhibitor/seed storage/lipid transfer protein (LTP)	4.77
246125_at	At5g19875	expressed protein	4.76
258218_at	At3g18000	phosphoethanolamine N-methyltransferase 1 (NMT1)	4.75
252938_at	At4g39190	expressed protein	4.75
254907_at	At4g11190	disease resistance-responsive	4.73
263096_at	At2g16060	non-symbiotic hemoglobin 1 (HB1)	4.72
259550_at	At1g35230	arabinogalactan-protein (AGP5)	4.69
260130_s_at	At1g66280	beta-glucosidase (PSR3.2)	4.65
259813_at	At1g49860	glutathione S-transferase, putative	4.61
263594_at	At2g01880	purple acid phosphatase (PAP7)	4.6
263854_at	At2g04430	MutT/nudix	4.56
254271_at	At4g23150	protein kinase	4.56
266376_at	At2g14620	xyloglucan:xyloglucosyl transferase, putative	4.56
248727_at	At5g47990	cytochrome P450	4.51
250062_at	At5g17760	AAA-type ATPase	4.51
247755_at	At5g59090	subtilase	4.5
250942_at	At5g03350	legume lectin	4.46
249096_at	At5g43910	pfkB-type carbohydrate kinase	4.43
267567_at	At2g30770	cytochrome P450 71A13, putative (CYP71A13)	4.41
266336_at	At2g32270	zinc transporter (ZIP3)	4.34
255630_at	At4g00700	C2 domain-containing protein	4.27
249167_at	At5g42860	expressed protein	4.27
249867_at	At5g23020	2-isopropylmalate synthase 2 (IMS2)	4.26
256833_at	At3g22910	calcium-transporting ATPase, plasma membrane-type, putative (ACA13)	4.24
249491_at	At5g39130	germin-like protein, putative (GER2)	4.24
256368_at	At1g66800	cinnamyl-alcohol dehydrogenase	4.22
247337_at	At5g63660	plant defensin-fusion protein, putative (PDF2.5)	4.19
255110_at	At4g08770	peroxidase, putative	4.18
252605_s_at	At3g45070	sulfotransferase	4.16
249743_at	At5g24540	glycosyl hydrolase 1 protein	4.15
248889_at	At5g46230	expressed protein	4.09
248048_at	At5g56080	nicotianamine synthase, putative	4.09
253608_at	At4g30290	xyloglucan:xyloglucosyl transferase, putative	4.06

260462_at	At1g10970	metal transporter, putative (ZIP4)	4.06
245555_at	At4g15390	transferase	4.05
259681_at	At1g77760	nitrate reductase 1 (NR1)	4.02
257066_at	At3g18280	protease inhibitor/seed storage/lipid transfer protein (LTP)	4.01
246149_at	At5g19890	peroxidase, putative	4
259173_at	At3g03640	glycosyl hydrolase 1 protein	3.96
256596_at	At3g28540	AAA-type ATPase	3.96
264953_at	At1g77120	alcohol dehydrogenase (ADH)	3.94
260904_at	At1g02450	NIM1-interacting protein 1 (NIMIN-1)	3.93
249203_at	At5g42590	cytochrome P450 71A16, putative (CYP71A16)	3.89
266383_at	At2g14580	pathogenesis-related protein, putative	3.84
256265_at	At3g12220	serine carboxypeptidase S10	3.84
250646_at	At5g06720	peroxidase, putative	3.82
260713_at	At1g17615	disease resistance protein (TIR-NBS class), putative	3.81
248062_at	At5g55450	protease inhibitor/seed storage/lipid transfer protein (LTP)	3.81
256289_s_at	At3g12230	serine carboxypeptidase S10	3.8
248139_at	At5g54970	expressed protein	3.77
264998_at	At1g67330	expressed protein	3.76
261986_s_at	At1g33720	cytochrome P450, putative	3.69
261763_at	At1g15520	ABC transporter	3.69
251232_at	At3g62780	C2 domain-containing protein	3.68
257101_at	At3g25020	disease resistance	3.67
250455_at	At5g09980	expressed protein	3.66
259065_at	At3g07520	glutamate receptor (GLR1.4)	3.65
259559_at	At1g21240	wall-associated kinase, putative (WAK3)	3.64
265050_at	At1g52070	jacalin lectin	3.62
259040_at	At3g09270	glutathione S-transferase, putative	3.62
266363_at	At2g41250	haloacid dehalogenase-like hydrolase	3.62
246855_at	At5g26280	meprin and TRAF homology domain-containing protein	3.61
250661_at	At5g07030	aspartyl protease	3.57
257763_s_at	At3g23110	disease resistance	3.57
257473_at	At1g33840	hypothetical protein	3.54
266415_at	At2g38530	nonspecific lipid transfer protein 2 (LTP2)	3.54
248178_at	At5g54370	late embryogenesis abundant protein-related	3.53
253413_at	At4g33020	metal transporter, putative (ZIP9)	3.52
256431_s_at	At3g11010	disease resistance	3.51
249752_at	At5g24660	expressed protein	3.51
258377_at	At3g17690	cyclic nucleotide-binding transporter 2 (CNBT2)	3.5
265161_at	At1g30900	vacuolar sorting receptor, putative (AtELP6)	3.49
254361_at	At4g22212	expressed protein	3.47
251970_at	At3g53150	UDP-glucoronosyl/UDP-glucosyl transferase	3.46
248330_at	At5g52810	ornithine cyclodeaminase	3.46
249004_at	At5g44570	hypothetical protein	3.45
259276_at	At3g01190	peroxidase 27 (PER27)	3.44
255575_at	At4g01430	nodulin MtN21	3.43

257673_at	At3g20370	meprin and TRAF homology domain-containing protein	3.42
253684_at	At4g29690	type I phosphodiesterase	3.38
257592_at	At3g24954	leucine-rich repeat , 5' fragment	3.37
252607_at	At3g44990	xyloglucan:xyloglucosyl transferase, putative	3.36
255516_at	At4g02270	pollen Ole e 1 allergen and extensin	3.35
248676_at	At5g48850	male sterility (MS5)	3.35
249732_at	At5g24420	glucosamine	3.35
254741_s_at	At4g13900	pseudogene, similar to NL0D	3.34
266743_at	At2g02990	ribonuclease 1 (RNS1)	3.34
256781_at	At3g13650	disease resistance response protein-related	3.34
261684_at	At1g47400	expressed protein	3.33
257952_at	At3g21770	peroxidase 30 (PER30)	3.32
260623_at	At1g08090	high-affinity nitrate transporter (ACH1)	3.32
250798_at	At5g05340	peroxidase, putative	3.32
260568_at	At2g43570	chitinase, putative	3.32
266142_at	At2g39030	GCN5-related N-acetyltransferase (GNAT)	3.32
252421_at	At3g47540	chitinase, putative	3.31
264958_at	At1g76960	expressed protein	3.31
262671_at	At1g76040	calcium-dependent protein kinase, putative	3.29
250724_at	At5g06330	hairpin-responsive protein, putative (HIN1)	3.29
252888_at	At4g39210	glucose-1-phosphate adenylyltransferase large subunit 3 (APL3)	3.29
254232_at	At4g23600	coronatine-responsive tyrosine aminotransferase	3.28
247717_at	At5g59320	lipid transfer protein 3 (LTP3)	3.27
267121_at	At2g23540	GDSL-motif lipase/hydrolase	3.26
249836_at	At5g23420	high mobility group (HMG1/2)	3.25
255111_at	At4g08780	peroxidase, putative	3.25
259609_at	At1g52410	caldesmon-related	3.24
254820_s_at	At4g12510	protease inhibitor/seed storage/lipid transfer protein (LTP)	3.23
261335_at	At1g44800	nodulin MtN21	3.22
264567_s_at	At1g05250	peroxidase, putative	3.22
262427_s_at	At1g47600	glycosyl hydrolase family 1 protein	3.22
254265_s_at	At4g23140	receptor-like protein kinase 5 (RLK5)	3.22
254805_at	At4g12480	protease inhibitor/seed storage/lipid transfer protein (LTP)	3.21
257363_at	At2g45760	BON1-associated protein (BAP1)-related	3.21
250445_at	At5g10760	aspartyl protease	3.21
247314_at	At5g64000	3'(2'),5'-bisphosphate nucleotidase	3.2
245689_at	At5g04120	phosphoglycerate	3.2
261157_at	At1g34510	peroxidase, putative	3.2
253298_at	At4g33560	expressed protein	3.18
247210_at	At5g65020	annexin 2 (ANN2)	3.17
263153_s_at	At1g54010	myrosinase-associated protein, putative	3.16
253667_at	At4g30170	peroxidase, putative	3.16
252060_at	At3g52430	phytoalexin-deficient 4 protein (PAD4)	3.15
259403_at	At1g17745	D-3-phosphoglycerate dehydrogenase (3-PGDH)	3.14

245257_at	At4g14640	calmodulin-8 (CAM8)	3.14
267546_at	At2g32680	disease resistance	3.13
256933_at	At3g22600	protease inhibitor/seed storage/lipid transfer protein (LTP)	3.13
245889_at	At5g09480	hydroxyproline-rich glycoprotein	3.12
250689_at	At5g06610	expressed protein	3.12
256647_at	At3g13610	oxidoreductase, 2OG-Fe(II) oxygenase	3.11
266967_at	At2g39530	integral membrane protein, putative	3.11
266292_at	At2g29350	tropinone reductase, putative	3.1
253687_at	At4g29520	expressed protein	3.1
264648_at	At1g09080	luminal binding protein 3 (BiP-3)	3.09
263063_s_at	At2g18140	peroxidase, putative	3.08
257421_at	At1g12030	expressed protein	3.08
250832_at	At5g04950	nicotianamine synthase, putative	3.07
247684_at	At5g59670	leucine-rich repeat protein kinase, putative	3.07
251422_at	At3g60540	sec61beta	3.06
261562_at	At1g01750	actin-depolymerizing factor, putative	3.06
255622_at	At4g01070	UDP-glucoronosyl/UDP-glucosyl transferase	3.05
249052_at	At5g44420	plant defensin protein, putative (PDF1.2a)	3.04
251045_s_at	At5g02360	pseudogene, CHP-rich zinc finger protein, putative	3.04
258470_at	At3g06035	expressed protein	3.02
257432_at	At2g21850	DC1 domain-containing protein	3.01
249599_at	At5g37990	S-adenosyl-L-methionine:carboxyl methyltransferase	3
264262_at	At1g09200	histone H3	2.99
266223_at	At2g28790	osmotin-like protein, putative	2.98
253073_at	At4g37410	cytochrome P450, putative	2.96
250302_at	At5g11920	glycosyl hydrolase family 32 protein	2.96
247765_at	At5g58860	cytochrome P450 86A1 (CYP86A1)	2.96
261443_at	At1g28480	glutaredoxin	2.96
249061_at	At5g44550	integral membrane	2.95
254606_at	At4g19030	major intrinsic protein (MIP)	2.95
255795_at	At2g33380	calcium-binding RD20 protein (RD20)	2.95
248848_at	At5g46520	disease resistance protein (TIR-NBS-LRR class), putative	2.95
247318_at	At5g63990	3'(2'),5'-bisphosphate nucleotidase, putative	2.94
257185_at	At3g13100	ABC transporter	2.94
252511_at	At3g46280	protein kinase-related	2.94
259870_at	At1g76780	expressed protein	2.93
259553_x_at	At1g21310	proline-rich extensin-like	2.92
249576_at	At5g37690	GDSL-motif lipase/hydrolase	2.91
267496_at	At2g30550	lipase class 3	2.9
251419_at	At3g60470	hypothetical protein	2.9
253998_at	At4g26010	peroxidase, putative	2.9
254190_at	At4g23885	expressed protein	2.89
246481_s_at	At5g15960	stress-responsive protein (KIN1)	2.89
246228_at	At4g36430	peroxidase, putative	2.88
254326_at	At4g22610	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.88

265049_at	At1g52060	jacalin lectin	2.88
249195_s_at	At5g42500	disease resistance-responsive	2.88
265658_at	At2g13810	aminotransferase class I and II	2.87
247268_at	At5g64080	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.86
266070_at	At2g18660	expansin (EXPR3)	2.86
247965_at	At5g56540	arabinogalactan-protein (AGP14)	2.86
256125_at	At1g18250	thaumatin, putative	2.85
266271_at	At2g29440	glutathione S-transferase, putative	2.85
251060_at	At5g01820	CBL-interacting protein kinase 14 (CIPK14)	2.84
249051_at	At5g44390	FAD-binding domain-containing protein	2.84
254603_at	At4g19050	mob1/phocein	2.83
247477_at	At5g62340	invertase/pectin methylesterase inhibitor	2.83
250433_at	At5g10400	histone H3	2.83
250517_at	At5g08260	serine carboxypeptidase S10	2.83
259721_at	At1g60890	phosphatidylinositol-4-phosphate 5-kinase	2.83
262050_at	At1g80130	expressed protein	2.83
250434_at	At5g10390	histone H3	2.82
257139_at	At3g28890	leucine-rich repeat	2.82
261125_at	At1g04990	zinc finger (CCCH-type)	2.82
265102_at	At1g30870	cationic peroxidase, putative	2.82
254387_at	At4g21850	methionine sulfoxide reductase domain-containing protein	2.82
258675_at	At3g08770	lipid transfer protein 6 (LTP6)	2.82
245401_at	At4g17670	senescence-associated protein-related	2.81
260955_at	At1g06000	UDP-glucoronosyl/UDP-glucosyl transferase	2.81
260068_at	At1g73805	calmodulin-binding protein	2.81
258930_at	At3g10040	expressed protein	2.8
248248_at	At5g53120	spermidine synthase, putative	2.79
248729_at	At5g48010	pentacyclic triterpene synthase, putative (ATPEN1)	2.79
258859_at	At3g02120	hydroxyproline-rich glycoprotein	2.78
253842_at	At4g27860	integral membrane	2.78
252618_at	At3g45140	lipoxygenase (LOX2)	2.78
251457_s_at	At3g60160	ABC transporter	2.78
245385_at	At4g14020	rapid alkalinization factor (RALF)	2.78
262939_s_at	At1g79530	glyceraldehyde 3-phosphate dehydrogenase, putative	2.77
253024_at	At4g38080	hydroxyproline-rich glycoprotein	2.77
248823_s_at	At5g46960	invertase/pectin methylesterase inhibitor	2.77
265943_at	At2g19570	cytidine deaminase (CDD)	2.76
254226_at	At4g23690	disease resistance-responsive	2.76
255591_at	At4g01630	expansin, putative (EXP17)	2.74
253879_s_at	At4g27570	glycosyltransferase	2.74
251065_at	At5g01870	lipid transfer protein, putative	2.73
251418_at	At3g60440	expressed protein	2.73
261606_at	At1g49570	peroxidase, putative	2.73
253060_at	At4g37710	VQ motif-containing protein	2.72
252448_at	At3g47050	glycosyl hydrolase 3 protein	2.71

251226_at	At3g62680	proline-rich	2.71
263535_at	At2g24970	expressed protein	2.7
258080_at	At3g25930	universal stress protein (USP)	2.7
249046_at	At5g44400	FAD-binding domain-containing protein	2.7
248309_at	At5g52540	expressed protein	2.69
256829_at	At3g22850	expressed protein	2.69
259910_at	At1g72700	haloacid dehalogenase-like hydrolase	2.69
265837_at	At2g14560	expressed protein	2.69
266358_at	At2g32280	expressed protein	2.69
259632_at	At1g56430	nicotianamine synthase, putative	2.69
254252_at	At4g23310	receptor-like protein kinase, putative	2.68
257715_at	At3g12750	zinc transporter (ZIP1)	2.68
254736_at	At4g13820	disease resistance	2.67
252487_at	At3g46660	UDP-glucoronosyl/UDP-glucosyl transferase	2.67
259640_at	At1g52400	glycosyl hydrolase family 1 protein	2.66
258942_at	At3g09960	calcineurin-like phosphoesterase	2.66
251952_at	At3g53650	histone H2B, putative	2.66
254044_at	At4g25820	endo-xyloglucan transferase (XTR9)	2.66
247594_at	At5g60800	heavy-metal-associated domain-containing protein	2.66
249545_at	At5g38030	MATE efflux	2.66
250172_at	At5g14330	expressed protein	2.65
256489_at	At1g31550	GDSL-motif lipase, putative	2.65
260884_at	At1g29240	expressed protein	2.65
256569_at	At3g19550	expressed protein	2.65
253657_at	At4g30110	ATPase E1-E2 type	2.65
264005_at	At2g22470	arabinogalactan-protein (AGP2)	2.64
248968_at	At5g45280	pectinacetylesterase, putative	2.64
256911_at	At3g24090	hexosephosphate aminotransferase, putative (GLCN6P)	2.64
263536_at	At2g25000	WRKY transcription factor	2.64
262275_at	At1g68710	haloacid dehalogenase-like hydrolase	2.63
263250_at	At2g31390	pfkB-type carbohydrate kinase	2.63
264386_at	At1g12000	pyrophosphate-fructose-6-phosphate 1-phosphotransferase beta subunit, putative	2.62
262281_at	At1g68570	proton-dependent oligopeptide transport (POT)	2.62
247192_at	At5g65360	histone H3	2.61
252403_at	At3g48080	lipase class 3	2.61
253334_at	At4g33360	terpene cyclase	2.61
255342_at	At4g04510	protein kinase	2.61
262504_at	At1g21750	protein disulfide isomerase, putative	2.61
255406_at	At4g03450	ankyrin repeat	2.6
246055_at	At5g08380	alpha-galactosidase, putative	2.6
251739_at	At3g56170	Ca(2+)-dependent nuclease	2.59
246842_at	At5g26731	expressed protein	2.59
247727_at	At5g59490	haloacid dehalogenase-like hydrolase	2.59
260745_at	At1g78370	glutathione S-transferase, putative	2.59

247151_at	At5g65640	basic helix-loop-helix (bHLH)	2.58
266364_at	At2g41230	expressed protein	2.58
260560_at	At2g43590	chitinase, putative	2.57
266776_at	At2g29070	ubiquitin fusion degradation UFD1	2.56
257714_at	At3g27360	histone H3	2.56
255842_at	At2g33530	serine carboxypeptidase S10	2.55
257295_at	At3g17420	protein kinase	2.55
251372_at	At3g60520	expressed protein	2.55
260582_at	At2g47200	expressed protein	2.55
255595_at	At4g01700	chitinase, putative	2.55
264832_at	At1g03660	expressed protein	2.55
252417_at	At3g47480	calcium-binding EF hand	2.54
248692_s_at	At4g15070	DC1 domain-containing protein	2.54
265962_at	At2g37460	nodulin MtN21	2.54
247651_at	At5g59870	histone H2A, putative	2.53
265117_at	At1g62500	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.53
257064_at	At3g18260	reticulon (RTNLB9)	2.53
254166_at	At4g24190	shepherd protein (SHD)	2.53
260392_at	At1g74030	enolase, putative	2.52
250702_at	At5g06730	peroxidase, putative	2.52
266581_at	At2g46140	late embryogenesis abundant protein, putative / LEA protein, putative	2.5
259120_at	At3g02240	expressed protein	2.5
251840_at	At3g54960	thioredoxin	2.5
256736_at	At3g29410	terpene synthase/cyclase	2.5
256252_at	At3g11340	UDP-glucoronosyl/UDP-glucosyl transferase	2.49
266395_at	At2g43100	aconitase C-terminal domain-containing protein	2.49
248499_at	At5g50400	calcineurin-like phosphoesterase	2.49
251287_at	At3g61820	aspartyl protease	2.49
252921_at	At4g39030	enhanced disease susceptibility 5 (EDS5)	2.48
251298_at	At3g62040	haloacid dehalogenase-like hydrolase	2.48
254791_at	At4g12910	serine carboxypeptidase S10	2.48
250286_at	At5g13320	auxin-responsive GH3	2.47
255340_at	At4g04490	protein kinase	2.47
246390_at	At1g77330	1-aminocyclopropane-1-carboxylate oxidase, putative	2.47
254718_at	At4g13580	disease resistance-responsive	2.47
246524_at	At5g15860	expressed protein	2.46
253103_at	At4g36110	auxin-responsive protein, putative	2.46
245738_at	At1g44130	nucellin protein, putative	2.46
265354_at	At2g16700	actin-depolymerizing factor 5 (ADF5)	2.46
251517_at	At3g59370	expressed protein	2.46
261167_at	At1g04980	thioredoxin	2.46
265025_at	At1g24575	expressed protein	2.46
264071_at	At2g27920	serine carboxypeptidase S10	2.45
260234_at	At1g74460	GDSL-motif lipase/hydrolase	2.45

247618_at	At5g60280	lectin protein kinase	2.45
264886_at	At1g61120	terpene synthase/cyclase	2.45
256560_s_at	At3g31415	terpene synthase/cyclase	2.45
252495_at	At3g46770	transcriptional factor B3	2.45
266270_at	At2g29470	glutathione S-transferase, putative	2.45
261020_at	At1g26390	FAD-binding domain-containing protein	2.44
263882_at	At2g21790	ribonucleoside-diphosphate reductase small chain, putative / ribonucleotide reductase, putative	2.44
260077_at	At1g73620	thaumatin-like protein, putative / pathogenesis-related protein, putative	2.44
267590_at	At2g39700	expansin, putative (EXP4)	2.44
265659_at	At2g25440	leucine-rich repeat	2.44
254229_at	At4g23610	expressed protein	2.44
262978_at	At1g75780	tubulin beta-1 chain (TUB1)	2.44
246197_at	At4g37010	caltractin, putative	2.44
266464_at	At2g47800	glutathione-conjugate transporter (MRP4)	2.44
253697_at	At4g29700	type I phosphodiesterase	2.44
265048_at	At1g52050	jacalin lectin	2.43
255874_at	At2g40550	expressed protein	2.43
261363_at	At1g41830	multi-copper oxidase type I	2.43
261999_at	At1g33800	expressed protein	2.43
247600_at	At5g60890	receptor-like protein kinase (ATR1) (MYB34)	2.43
263941_at	At2g35870	At2g35870	2.43
253113_at	At4g35985	senescence/dehydration-associated protein-related	2.42
264846_at	At2g17850	senescence-associated	2.42
246652_at	At5g35190	proline-rich extensin-like	2.41
266669_at	At2g29750	UDP-glucoronosyl/UDP-glucosyl transferase	2.41
265656_at	At2g13820	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.4
259228_at	At3g07720	kelch repeat-containing protein	2.4
256962_at	At3g13560	glycosyl hydrolase family 17 protein	2.4
254789_at	At4g12880	plastocyanin-like domain-containing protein	2.4
249474_s_at	At5g39190	germin-like protein, putative	2.4
261269_at	At1g26690	emp24/gp25L/p24	2.39
264260_at	At1g09210	calreticulin 2 (CRT2)	2.39
253416_at	At4g33070	pyruvate decarboxylase, putative	2.39
254740_s_at	At4g13890	glycine hydroxymethyltransferase, putative	2.39
264263_at	At1g09155	SKP1 interacting partner 3-related	2.39
255127_at	At4g08300	nodulin MtN21	2.39
246991_at	At5g67400	peroxidase 73 (PER73) (P73) (PRXR11)	2.38
262646_at	At1g62800	aspartate aminotransferase, cytoplasmic isozyme 2 / transaminase A (ASP4)	2.37
256712_at	At2g34020	calcium-binding EF hand	2.37
265572_at	At2g28210	carbonic anhydrase	2.37
252661_at	At3g44450	expressed protein	2.37
260179_at	At1g70690	kinase-related	2.37
258037_at	At3g21230	4-coumaroyl-CoA synthase, putative (4CL)	2.37

259878_at	At1g76790	O-methyltransferase family 2 protein	2.37
257844_at	At3g28480	oxidoreductase, 2OG-Fe(II) oxygenase	2.36
265464_at	At2g37080	myosin heavy chain-related	2.36
249916_at	At5g22880	histone H2B, putative	2.36
249600_s_at	At5g38000	NADP-dependent oxidoreductase, putative	2.36
253228_at	At4g34630	expressed protein	2.36
248820_at	At5g47060	senescence-associated protein-related	2.36
256428_at	At3g11080	disease resistance	2.36
260693_at	At1g32450	proton-dependent oligopeptide transport (POT)	2.36
258751_at	At3g05890	hydrophobic protein (RCI2B)	2.36
262832_s_at	At1g14870	expressed protein	2.35
254243_at	At4g23210	protein kinase	2.35
267472_at	At2g02850	plastocyanin-like domain-containing protein	2.35
250596_at	At5g07780	formin homology 2 domain-containing protein	2.35
261080_at	At1g07370	proliferating cell nuclear antigen 1 (PCNA1)	2.35
258217_at	At3g17990	phosphoethanolamine N-methyltransferase 1 (NMT1)	2.35
259723_at	At1g60960	metal transporter, putative (IRT3)	2.35
267053_s_at	At2g38390	peroxidase 22 (PER22)	2.34
260784_at	At1g06180	myb family transcription factor	2.34
261366_at	At1g53100	glycosyltransferase family 14 protein	2.34
246230_at	At4g36710	scarecrow transcription factor	2.34
264144_at	At1g79320	latex abundant protein, putative (AMC5)	2.34
267388_at	At2g44450	glycosyl hydrolase family 1 protein	2.34
258894_at	At3g05650	disease resistance	2.34
248971_at	At5g45000	Toll-Interleukin-Resistance (TIR) domain-containing protein	2.33
248263_at	At5g53370	pectinesterase	2.33
250344_at	At5g11930	glutaredoxin	2.33
265053_at	At1g52000	jacalin lectin	2.32
245315_at	At4g14800	20S proteasome beta subunit D2 (PBD2)	2.32
264044_at	At2g22480	phosphofructokinase	2.32
267457_at	At2g33790	pollen Ole e 1 allergen and extensin	2.32
261692_at	At1g08450	calreticulin 3 (CRT3)	2.32
261305_at	At1g48470	glutamine synthetase, putative	2.32
257500_s_at	At1g73300	serine carboxypeptidase S10	2.32
260941_at	At1g44970	peroxidase, putative	2.31
254832_at	At4g12490	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.31
256216_at	At1g56340	calreticulin 1 (CRT1)	2.31
246532_at	At5g15870	glycosyl hydrolase 81 protein	2.31
262930_at	At1g65690	harpin-induced protein-related	2.3
256543_at	At1g42480	expressed protein	2.3
254894_at	At4g11840	phospholipase D gamma 3 (PLDGAMMA3)	2.3
254085_at	At4g24960	ABA-responsive protein (HVA22d)	2.3
253268_s_at	At4g34135	UDP-glucoronosyl/UDP-glucosyl transferase	2.3
260919_at	At1g21520	expressed protein	2.3
247009_at	At5g67600	expressed protein	2.29

251182_at	At3g62600	DNAJ heat shock	2.29
259757_at	At1g77510	protein disulfide isomerase, putative	2.29
249306_at	At5g41400	zinc finger (C3HC4-type RING finger)	2.29
267307_at	At2g30210	laccase, putative	2.29
249375_at	At5g40730	arabinogalactan-protein (AGP24)	2.28
264968_at	At1g67360	rubber elongation factor (REF)	2.28
253040_at	At4g37800	xyloglucan:xyloglucosyl transferase, putative	2.28
250438_at	At5g10580	expressed protein	2.28
261576_at	At1g01070	nodulin MtN21	2.28
260408_at	At1g69880	thioredoxin, putative	2.28
253627_at	At4g30650	hydrophobic protein, putative	2.28
245688_at	At1g28290	pollen Ole e 1 allergen and extensin	2.27
261240_at	At1g32940	subtilase	2.27
259251_at	At3g07600	heavy-metal-associated domain-containing protein	2.27
249477_s_at	At5g38930	germin-like protein, putative	2.27
247604_at	At5g60950	phytochelatin synthetase-related	2.27
255587_at	At4g01480	inorganic pyrophosphatase, putative (soluble)	2.27
248252_at	At5g53250	arabinogalactan-protein, putative (AGP22)	2.27
245151_at	At2g47550	pectinesterase	2.26
266596_at	At2g46150	expressed protein	2.26
263963_at	At2g36080	DNA-binding protein, putative	2.26
251368_at	At3g61380	expressed protein	2.26
264537_at	At1g55610	protein kinase	2.26
264579_at	At1g05205	expressed protein	2.26
249934_at	At5g22410	peroxidase, putative	2.26
261921_at	At1g65900	expressed protein	2.26
257153_at	At3g27220	kelch repeat-containing protein	2.26
250778_at	At5g05500	pollen Ole e 1 allergen and extensin	2.26
252098_at	At3g51330	aspartyl protease	2.25
255517_at	At4g02290	glycosyl hydrolase family 9 protein	2.25
255632_at	At4g00680	actin-depolymerizing factor, putative	2.25
251304_at	At3g61990	O-methyltransferase family 3 protein	2.24
257644_at	At3g25780	allene oxide cyclase, putative	2.24
256243_at	At3g12500	basic endochitinase	2.24
250059_at	At5g17820	peroxidase 57 (PER57)	2.24
267432_at	At2g35020	UTP--glucose-1-phosphate uridylyltransferase	2.24
267483_at	At2g02810	UDP-galactose/UDP-glucose transporter	2.24
245944_at	At5g19520	mechanosensitive ion channel domain-containing protein	2.24
256927_at	At3g22550	senescence-associated protein-related	2.24
260046_at	At1g73800	calmodulin-binding protein	2.24
262717_s_at	At1g16410	cytochrome P450	2.24
249910_at	At5g22630	prephenate dehydratase	2.23
256989_at	At3g28580	AAA-type ATPase	2.23
253238_at	At4g34480	glycosyl hydrolase family 17 protein	2.23
252450_s_at	At3g47090	leucine-rich repeat transmembrane protein kinase, putative	2.22

245932_at	At5g09290	3'(2'),5'-bisphosphate nucleotidase, putative	2.21
250625_at	At5g07340	calnexin, putative	2.21
250633_at	At5g07460	peptide methionine sulfoxide reductase, putative	2.21
254914_at	At4g11290	peroxidase, putative	2.21
245196_at	At1g67750	pectate lyase	2.21
251063_at	At5g01850	protein kinase, putative	2.21
265682_at	At2g24390	avirulence-responsive protein-related	2.21
248941_s_at	At5g45460	expressed protein	2.21
246373_at	At1g51860	leucine-rich repeat protein kinase, putative	2.21
248021_at	At5g56500	chaperonin, putative	2.2
254630_at	At4g18360	(S)-2-hydroxy-acid oxidase, peroxisomal, putative	2.2
261330_at	At1g44900	DNA replication licensing factor, putative	2.2
262507_at	At1g11330	S-locus lectin protein kinase	2.2
249273_at	At5g41850	expressed protein	2.2
265695_at	At2g24490	replication protein, putative	2.2
252437_at	At3g47380	invertase	2.2
266827_at	At2g22920	serine carboxypeptidase S10	2.2
262616_at	At1g06620	2-oxoglutarate-dependent dioxygenase, putative	2.19
256012_at	At1g19250	flavin-containing monooxygenase (FMO)	2.19
259185_at	At3g01550	triose phosphate/phosphate translocator, putative	2.19
252596_at	At3g45330	lectin protein kinase	2.19
267595_at	At2g32990	glycosyl hydrolase family 9 protein	2.19
261979_at	At1g37130	nitrate reductase 2 (NR2)	2.18
252200_at	At3g50280	transferase	2.18
254390_at	At4g21940	calcium-dependent protein kinase, putative (CDPK)	2.18
259105_at	At3g05500	rubber elongation factor (REF)	2.18
247912_at	At5g57480	AAA-type ATPase	2.17
262137_at	At1g77920	bZIP family transcription factor	2.17
250435_at	At5g10380	zinc finger (C3HC4-type RING finger)	2.17
246157_at	At5g20080	NADH-cytochrome b5 reductase, putative	2.17
248861_at	At5g46700	senescence-associated protein, putative	2.17
260553_at	At2g41800	expressed protein	2.17
260405_at	At1g69930	glutathione S-transferase, putative	2.17
262694_at	At1g62790	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.17
245302_at	At4g17695	myb family transcription factor (KAN3)	2.16
263715_at	At2g20570	golden2-like transcription factor (GLK1)	2.16
251668_at	At3g57010	strictosidine synthase	2.16
260902_at	At1g21440	mutase	2.15
260648_at	At1g08050	zinc finger (C3HC4-type RING finger)	2.15
256935_at	At3g22570	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.15
252562_s_at	At3g45930	histone H4	2.14
257761_at	At3g23090	expressed protein	2.14
249441_at	At5g39730	avirulence-responsive protein-related	2.14
255578_at	At4g01450	nodulin MtN21	2.14
249188_at	At5g42830	transferase	2.14

246272_at	At4g37150	esterase, putative	2.14
248891_at	At5g46280	DNA replication licensing factor, putative	2.14
246948_at	At5g25130	cytochrome P450	2.14
249979_s_at	At5g18860	inosine-uridine preferring nucleoside hydrolase	2.14
254735_at	At4g13810	disease resistance	2.14
258023_at	At3g19450	cinnamyl-alcohol dehydrogenase (CAD)	2.13
248118_at	At5g55050	GDSL-motif lipase/hydrolase	2.13
258813_at	At3g04060	no apical meristem (NAM)	2.13
254283_s_at	At4g22870	leucoanthocyanidin dioxygenase, putative	2.13
262664_at	At1g13970	expressed protein	2.13
249058_at	At5g44510	disease resistance protein (TIR-NBS-LRR class), putative	2.12
245746_at	At1g51070	basic helix-loop-helix (bHLH)	2.12
247791_at	At5g58710	peptidyl-prolyl cis-trans isomerase, putative	2.12
247586_at	At5g60660	major intrinsic protein (MIP)	2.12
251370_at	At3g60450	expressed protein	2.12
247494_at	At5g61790	calnexin 1 (CNX1)	2.12
245267_at	At4g14060	major latex protein-related	2.12
265260_at	At2g43000	no apical meristem (NAM)	2.11
262666_at	At1g14080	xyloglucan fucosyltransferase, putative (FUT6)	2.11
252058_at	At3g52470	harpin-induced (HIN1)	2.11
254819_at	At4g12500	protease inhibitor/seed storage/lipid transfer protein (LTP)	2.1
260028_at	At1g29980	expressed protein	2.1
245343_at	At4g15830	expressed protein	2.1
259410_at	At1g13340	expressed protein	2.1
253276_at	At4g34050	caffeoyl-CoA 3-O-methyltransferase, putative	2.1
260051_at	At1g78210	hydrolase, alpha/beta fold	2.1
266356_at	At2g32300	uclacyanin I	2.1
263552_x_at	At2g24980	proline-rich extensin-like	2.1
257708_at	At3g13330	expressed protein	2.09
259385_at	At1g13470	expressed protein	2.09
249276_at	At5g41880	DNA primase small subunit family	2.09
260083_at	At1g63220	C2 domain-containing protein	2.09
261285_at	At1g35720	annexin 1 (ANN1)	2.09
264382_at	At2g25110	MIR domain-containing protein	2.09
245956_s_at	At5g28540	luminal binding protein 1 (BiP-1)	2.09
249214_at	At5g42720	glycosyl hydrolase family 17 protein	2.09
260601_at	At1g55910	metal transporter, putative (ZIP11)	2.08
254909_at	At4g11210	disease resistance-responsive	2.08
266780_at	At2g29110	glutamate receptor (GLR2.8)	2.08
264543_at	At1g55780	heavy-metal-associated domain-containing protein	2.08
247293_at	At5g64510	expressed protein	2.08
263628_at	At2g04780	fasciclin-like arabinogalactan-protein (FLA7)	2.08
250083_at	At5g17220	glutathione S-transferase, putative	2.08
252911_at	At4g39510	cytochrome P450	2.08
267605_at	At2g32920	thioredoxin	2.08

264859_at	At1g24280	glucose-6-phosphate 1-dehydrogenase, putative	2.08
251400_at	At3g60420	expressed protein	2.07
248908_at	At5g45800	leucine-rich repeat transmembrane protein kinase, putative	2.07
259534_at	At1g12290	disease resistance protein (CC-NBS-LRR class), putative	2.07
258473_s_at	At3g02610	acyl-(acyl-carrier-protein) desaturase, putative	2.07
247812_at	At5g58390	peroxidase, putative	2.07
254500_at	At4g20110	vacuolar sorting receptor, putative (AtELP3)	2.07
249021_at	At5g44820	expressed protein	2.07
245575_at	At4g14760	M protein repeat-containing protein	2.07
260146_at	At1g52770	phototropic-responsive NPH3	2.07
266941_at	At2g18980	peroxidase, putative	2.07
246145_at	At5g19880	peroxidase, putative	2.07
247265_at	At5g64550	loricrin-related	2.07
257173_at	At3g23810	adenosylhomocysteinase, putative	2.07
261500_at	At1g28400	expressed protein	2.06
259198_at	At3g03610	phagocytosis and cell motility protein ELMO1-related	2.06
248100_at	At5g55180	glycosyl hydrolase family 17 protein	2.06
249417_at	At5g39670	calcium-binding EF hand	2.06
265853_at	At2g42360	zinc finger (C3HC4-type RING finger)	2.06
261930_at	At1g22440	alcohol dehydrogenase, putative (ADH)	2.06
258160_at	At3g17820	glutamine synthetase (GS1)	2.05
256512_at	At1g33265	expressed protein	2.05
260386_at	At1g74010	strictosidine synthase	2.05
262881_at	At1g64890	integral membrane transporter	2.05
246429_at	At5g17450	heavy-metal-associated domain-containing protein	2.05
259548_at	At1g35260	Bet v I allergen	2.05
261485_at	At1g14360	UDP-galactose/UDP-glucose transporter, putative	2.04
261224_at	At1g20160	subtilase	2.04
254915_s_at	At4g11310	cysteine proteinase, putative	2.04
264577_at	At1g05260	peroxidase 3 (PER3)	2.04
257154_at	At3g27210	expressed protein	2.03
248192_at	At5g54140	IAA-amino acid hydrolase, putative (ILL3)	2.03
267261_at	At2g23120	expressed protein	2.03
261826_at	At1g11580	pectin methylesterase, putative	2.03
260731_at	At1g17500	haloacid dehalogenase-like hydrolase	2.03
252442_at	At3g46940	deoxyuridine 5'-triphosphate nucleotidohydrolase family	2.03
250142_at	At5g14650	polygalacturonase, putative	2.03
250472_at	At5g10210	expressed protein	2.02
261806_at	At1g30510	ferredoxin-NADP(+) reductase, putative	2.02
259489_at	At1g15790	expressed protein	2.02
254392_at	At4g21600	bifunctional nuclease, putative	2.02
256803_at	At3g20960	cytochrome P450	2.02
250449_at	At5g10830	embryo-abundant protein-related	2.02
267380_at	At2g26170	thromboxane-A synthase, putative	2.02
262133_at	At1g78000	sulfate transporter (Sultr1;2)	2.02

260590_at	At1g53310	phosphoenolpyruvate carboxylase, putative (PPC1)	2.02
253227_at	At4g35030	protein kinase	2.02
253480_at	At4g31840	plastocyanin-like domain-containing protein	2.01
265561_s_at	At2g05510	glycine-rich protein	2.01
251503_at	At3g59140	ABC transporter	2.01
246522_at	At5g15830	bZIP transcription factor	2.01
255676_at	At4g00490	beta-amylase, putative	2.01
258047_at	At3g21240	4-coumarate-CoA ligase 2 (4CL2)	2.01
245175_at	At2g47470	thioredoxin	2.01
254468_at	At4g20460	NAD-dependent epimerase/dehydratase	2
247989_at	At5g56350	pyruvate kinase, putative	2
267026_at	At2g38340	AP2 domain-containing transcription factor, putative (DRE2B)	2
252077_at	At3g51720	expressed protein	2
247056_at	At5g66750	SNF2 domain-containing protein	2
261774_at	At1g76260	transducin	2
249897_at	At5g22550	expressed protein	2
252619_at	At3g45210	expressed protein	2
266613_at	At2g14900	gibberellin-regulated	1.99
266517_at	At2g35120	glycine cleavage system H protein, mitochondrial, putative	1.99
264372_at	At1g11840	lactoylglutathione lyase, putative	1.99
261309_at	At1g48600	phosphoethanolamine N-methyltransferase 2, putative (NMT2)	1.99
259472_at	At1g18910	zinc finger (C3HC4-type RING finger)	1.99
254056_at	At4g25250	invertase	1.99
261868_s_at	At1g11460	nodulin MtN21	1.99
249055_at	At5g44460	calcium-binding protein, putative	1.99
253609_at	At4g30190	ATPase 2, plasma membrane-type, putative	1.99
262286_at	At1g68585	expressed protein	1.99
247606_at	At5g61000	replication protein, putative	1.99
252712_at	At3g43800	glutathione S-transferase, putative	1.99
259447_s_at	At1g02430	ADP-ribosylation factor, putative	1.98
263584_at	At2g17040	no apical meristem (NAM)	1.98
250644_at	At5g06750	protein phosphatase (PP2C)	1.98
247945_at	At5g57150	basic helix-loop-helix (bHLH)	1.98
245998_at	At5g20830	sucrose synthase (SUS1)	1.98
265334_at	At2g18370	protease inhibitor/seed storage/lipid transfer protein (LTP)	1.98
262806_at	At1g20950	pyrophosphate-fructose-6-phosphate 1-phosphotransferase-related	1.98
260874_at	At1g21390	expressed protein	1.98
267084_at	At2g41180	sigA-binding protein-related	1.98
252537_at	At3g45710	proton-dependent oligopeptide transport (POT)	1.98
265955_at	At2g37280	ABC transporter	1.98
262788_at	At1g10690	expressed protein	1.98
256319_at	At1g35910	trehalose-6-phosphate phosphatase, putative	1.98
257151_at	At3g27200	plastocyanin-like domain-containing protein	1.97

263841_at	At2g36870	xyloglucan:xyloglucosyl transferase, putative	1.97
249469_at	At5g39320	UDP-glucose 6-dehydrogenase, putative	1.97
252010_at	At3g52740	expressed protein	1.97
265439_at	At2g21045	senescence-associated	1.97
261016_at	At1g26560	glycosyl hydrolase 1 protein	1.97
267382_at	At2g44300	lipid transfer protein-related	1.96
255065_s_at	At4g08870	arginase, putative	1.96
260076_at	At1g73630	calcium-binding protein, putative	1.96
266139_at	At2g28085	auxin-responsive	1.96
259307_at	At3g05230	signal peptidase subunit	1.96
264061_at	At2g27970	cyclin-dependent kinase, putative (CDK)	1.95
251273_at	At3g61960	protein kinase	1.95
248945_at	At5g45510	leucine-rich repeat	1.95
252984_at	At4g37990	mannitol dehydrogenase, putative (ELI3-2)	1.95
248890_at	At5g46270	disease resistance protein (TIR-NBS-LRR class), putative	1.95
264010_at	At2g21100	disease resistance-responsive protein-related	1.95
263181_at	At1g05720	selenoprotein	1.95
261904_at	At1g65040	zinc finger (C3HC4-type RING finger)	1.95
259018_at	At3g07390	auxin-induced protein (AIR12)	1.95
259596_at	At1g28130	auxin-responsive GH3	1.95
259009_at	At3g09260	glycosyl hydrolase family 1 protein	1.95
258658_at	At3g09820	adenosine kinase 1 (ADK1)	1.95
249794_at	At5g23530	expressed protein	1.95
258275_at	At3g15760	expressed protein	1.95
248322_at	At5g52760	heavy-metal-associated domain-containing protein	1.95
255240_at	At4g05530	short-chain dehydrogenase/reductase (SDR)	1.95
251633_at	At3g57460	expressed protein	1.94
261729_s_at	At1g47840	hexokinase, putative	1.94
245164_at	At2g33210	chaperonin, putative	1.94
263706_s_at	At1g31180	3-isopropylmalate dehydrogenase, chloroplast, putative	1.94
245089_at	At2g45290	transketolase, putative	1.94
263576_at	At2g17080	hypothetical protein	1.94
260254_at	At1g74210	glycerophosphoryl diester phosphodiesterase	1.94
259328_at	At3g16440	jacalin lectin	1.94
249894_at	At5g22580	expressed protein	1.94
262396_at	At1g49470	expressed protein	1.94
254521_at	At5g44810	expressed protein	1.93
248674_at	At5g48800	phototropic-responsive NPH3	1.93
267012_at	At2g39220	patatin	1.93
247030_at	At5g67210	expressed protein	1.93
258941_at	At3g09940	monodehydroascorbate reductase, putative	1.93
254818_at	At4g12470	protease inhibitor/seed storage/lipid transfer protein (LTP)	1.93
262317_at	At2g48140	protease inhibitor/seed storage/lipid transfer protein (LTP)	1.93
256942_at	At3g23290	expressed protein	1.93
245523_at	At4g15910	drought-induced protein (Di21)	1.92

245840_at	At1g58420	expressed protein	1.92
258480_at	At3g02640	expressed protein	1.92
254389_s_at	At4g21910	MATE efflux	1.92
257213_at	At3g15020	malate dehydrogenase (NAD), mitochondrial, putative	1.92
264647_at	At1g09090	respiratory burst oxidase protein B (RbohB)	1.92
249848_at	At5g23220	isochorismatase hydrolase	1.92
254932_at	At4g11120	translation elongation factor Ts (EF-Ts), putative	1.92
266566_at	At2g24040	hydrophobic protein, putative	1.91
262756_at	At1g16370	transporter-related	1.91
258089_at	At3g14740	PHD finger	1.91
258646_at	At3g08040	MATE efflux	1.91
254202_at	At4g24140	hydrolase, alpha/beta fold	1.91
253274_at	At4g34200	D-3-phosphoglycerate dehydrogenase, putative / 3-PGDH, putative	1.91
266326_at	At2g46650	cytochrome b5, putative	1.91
246093_at	At5g20550	oxidoreductase, 2OG-Fe(II) oxygenase	1.91
254690_at	At4g17830	peptidase M20/M25/M40	1.91
257755_at	At3g18760	ribosomal protein S6	1.91
266203_at	At2g02230	SKP1 interacting partner 3-related	1.9
250234_at	At5g13420	transaldolase, putative	1.9
256053_at	At1g07260	UDP-glucoronosyl/UDP-glucosyl transferase	1.9
262232_at	At1g68600	expressed protein	1.9
265204_at	At2g36650	expressed protein	1.9
249675_at	At5g35940	jacalin lectin	1.9
259758_s_at	At1g77530	O-methyltransferase family 2 protein	1.9
253191_at	At4g35350	cysteine endopeptidase, papain-type (XCP1)	1.89
248298_at	At5g53110	expressed protein	1.89
256425_at	At1g33560	disease resistance protein (CC-NBS-LRR class), putative	1.89
249178_at	At5g42890	sterol carrier protein 2 (SCP-2)	1.89
248092_at	At5g55170	small ubiquitin-like modifier 3 (SUMO)	1.89
252988_at	At4g38410	dehydrin, putative	1.89
256409_at	At1g66620	seven in absentia (SINA) protein, putative	1.89
257814_at	At3g25110	acyl-(acyl carrier protein) thioesterase	1.89
246144_at	At5g20110	dynein light chain, putative	1.89
261660_at	At1g18370	kinesin motor (NACK1)	1.89
258017_at	At3g19370	expressed protein	1.89
253622_at	At4g30560	cyclic nucleotide-regulated ion channel, putative	1.89
257056_at	At3g15350	glycosyltransferase family 14 protein	1.89
264895_at	At1g23100	10 kDa chaperonin, putative	1.89
260002_at	At1g67940	ABC transporter	1.89
259382_s_at	At3g16430	jacalin lectin	1.88
255308_at	At4g04910	AAA-type ATPase	1.88
254318_at	At4g22530	embryo-abundant protein-related	1.88
258977_s_at	At3g02020	aspartate kinase, lysine-sensitive	1.88
259247_at	At3g07570	membrane protein, putative	1.88

261481_at	At1g14260	zinc finger (C3HC4-type RING finger)	1.88
255513_at	At4g02060	prolifera protein (PRL)	1.88
262533_at	At1g17090	expressed protein	1.88
260896_at	At1g29310	protein transport protein sec61, putative	1.88
256018_at	At1g58300	heme oxygenase, putative	1.88
255243_at	At4g05590	expressed protein	1.88
254231_at	At4g23810	WRKY family transcription factor	1.88
263498_at	At2g42610	expressed protein	1.88
247333_at	At5g63600	flavonol synthase, putative	1.88
251336_at	At3g61190	BON1-associated protein 1 (BAP1)	1.88
263227_at	At1g30750	expressed protein	1.88
262913_at	At1g59960	aldo/keto reductase, putative	1.87
253990_at	At4g26160	thioredoxin	1.87
254458_at	At4g21180	DNAJ heat shock N-terminal domain-containing protein	1.87
245750_at	At1g51060	histone H2A, putative	1.87
261530_at	At1g63460	glutathione peroxidase, putative	1.87
257522_at	At3g08990	yippee	1.87
266761_at	At2g47130	short-chain dehydrogenase/reductase (SDR)	1.87
254346_at	At4g21980	autophagy 8a (APG8a)	1.87
252179_at	At3g50760	glycosyl transferase family 8 protein	1.87
258341_at	At3g22790	kinase interacting	1.87
265338_at	At2g18400	ribosomal protein L6	1.87
256451_s_at	At1g75170	SEC14 cytosolic factor	1.87
253866_at	At4g27480	glycosyltransferase family 14 protein	1.87
250978_at	At5g03080	phosphatidic acid phosphatase-related	1.87
251487_at	At3g59760	cysteine synthase, mitochondrial, putative	1.87
245052_at	At2g26440	pectinesterase	1.87
262181_at	At1g78060	glycosyl hydrolase family 3 protein	1.87
258768_at	At3g10880	hypothetical protein	1.87
266479_at	At2g31160	expressed protein	1.87
246366_at	At1g51850	leucine-rich repeat protein kinase, putative	1.87
246620_at	At5g36220	cytochrome P450 81D1 (CYP81D1)	1.86
262177_at	At1g74710	isochorismate synthase 1 (ICS1)	1.86
248932_at	At5g46050	proton-dependent oligopeptide transport (POT)	1.86
263047_at	At2g17630	phosphoserine aminotransferase, putative	1.86
252587_at	At3g45620	transducin	1.86
256890_at	At3g23830	glycine-rich RNA-binding protein, putative	1.86
265243_at	At2g43040	calmodulin-binding protein	1.86
249063_at	At5g44110	ABC transporter	1.86
259424_at	At1g13830	beta-1,3-glucanase-related	1.86
258167_at	At3g21560	UDP-glucosyltransferase, putative	1.86
263134_at	At1g78570	NAD-dependent epimerase/dehydratase	1.86
248664_at	At5g48580	FK506-binding protein 2-2 (FKBP15-2)	1.86
255284_at	At4g04610	5'-adenylylsulfate reductase (APR1)	1.86
254559_at	At4g19200	proline-rich	1.85

253992_at	At4g26060	expressed protein	1.85
251531_at	At3g58550	protease inhibitor/seed storage/lipid transfer protein (LTP)	1.85
251846_at	At3g54560	histone H2A.F/Z	1.85
260009_at	At1g67950	RNA recognition motif (RRM)-containing protein	1.85
259854_at	At1g72200	zinc finger (C3HC4-type RING finger)	1.85
253646_at	At4g29810	mitogen-activated protein kinase kinase (MAPKK)	1.85
249060_at	At5g44560	SNF7	1.85
252148_at	At3g51280	male sterility MS5, putative	1.85
252155_at	At3g50920	phosphatidic acid phosphatase-related	1.85
249581_at	At5g37600	glutamine synthetase, putative	1.85
264301_at	At1g78780	pathogenesis-related	1.85
263544_at	At2g21590	glucose-1-phosphate adenylyltransferase large subunit, putative	1.85
245293_at	At4g16660	heat shock protein 70, putative (HSP70)	1.85
267076_at	At2g41090	calmodulin-like calcium-binding protein, 22 kDa (CaBP-22)	1.85
254343_at	At4g21990	5'-adenylylsulfate reductase (APR3)	1.85
265339_at	At2g18230	inorganic pyrophosphatase (soluble) (PPA)	1.85
262658_at	At1g14220	ribonuclease T2	1.85
258040_at	At3g21190	expressed protein	1.85
258630_at	At3g02820	zinc knuckle (CCHC-type)	1.85
254824_s_at	At4g12620	replication control protein, putative	1.85
252622_at	At3g45310	cysteine proteinase, putative	1.84
251107_at	At5g01610	expressed protein	1.84
249817_at	At5g23820	MD-2-related lipid recognition domain-containing protein	1.84
247255_at	At5g64780	expressed protein	1.84
258655_at	At3g09805	isocitrate dehydrogenase, putative	1.84
261933_at	At1g22410	2-dehydro-3-deoxyphosphoheptonate aldolase, putative	1.84
256869_at	At3g26430	glycine-rich RNA-binding protein	1.84
265188_at	At1g23800	aldehyde dehydrogenase, mitochondrial (ALDH3)	1.84
257382_at	At2g40750	WRKY family transcription factor	1.84
258710_s_at	At3g09800	clathrin adaptor complex small chain	1.84
259842_at	At1g73600	phosphoethanolamine N-methyltransferase 3, putative (NMT3)	1.84
257798_at	At3g15950	DNA topoisomerase-related	1.84
262704_at	At1g16530	lateral organ boundaries domain protein 3 (LBD3)	1.84
265183_at	At1g23750	DNA-binding protein-related	1.83
252671_at	At3g44190	pyridine nucleotide-disulphide oxidoreductase	1.83
251106_at	At5g01500	mitochondrial substrate carrier	1.83
258102_at	At3g23600	dienelactone hydrolase	1.83
257466_at	At1g62840	expressed protein	1.83
264466_at	At1g10380	expressed protein	1.83
249227_at	At5g42180	peroxidase 64 (PER64)	1.83
247983_at	At5g56630	phosphofructokinase	1.83
254673_at	At4g18430	Ras-related GTP-binding protein, putative	1.83
247575_at	At5g61030	RNA-binding protein, putative	1.83

263787_at	At2g46420	expressed protein	1.83
254299_at	At4g22920	expressed protein	1.82
260551_at	At2g43510	trypsin inhibitor, putative	1.82
258437_at	At3g16560	protein phosphatase 2C-related (PP2C)	1.82
262344_at	At1g64060	respiratory burst oxidase protein F (RbohF) (RbohAp108)	1.82
259365_at	At1g13300	myb family transcription factor	1.82
248833_at	At5g47120	Bax inhibitor-1 putative	1.82
254341_at	At4g22130	protein kinase	1.82
261574_at	At1g01190	cytochrome P450, putative	1.82
249274_at	At5g41860	expressed protein	1.82
259850_at	At1g72240	expressed protein	1.82
256673_at	At3g52370	beta-Ig-H3 domain-containing protein	1.82
266334_at	At2g32380	expressed protein	1.82
253515_at	At4g31320	auxin-responsive protein, putative	1.82
266687_at	At2g19670	protein arginine N-methyltransferase, putative	1.82
253720_at	At4g29270	acid phosphatase class B	1.82
246988_at	At5g67340	armadillo/beta-catenin repeat	1.82
245874_at	At1g26250	proline-rich extensin, putative	1.82
248943_s_at	At5g45490	disease resistance protein-related	1.81
258469_at	At3g06050	alkyl hydroperoxide reductase (AhpC/TSA)	1.81
246302_at	At3g51860	cation exchanger, putative (CAX3)	1.81
253177_s_at	At4g35150	O-methyltransferase family 2 protein	1.81
266059_at	At2g40765	expressed protein	1.81
258588_s_at	At3g04120	glyceraldehyde-3-phosphate dehydrogenase (GAPC)	1.81
259575_at	At1g35320	expressed protein	1.81
249552_s_at	At5g38240	serine/threonine protein kinase, putative	1.81
247706_at	At5g59480	haloacid dehalogenase-like hydrolase	1.81
249481_at	At5g38900	DSBA oxidoreductase	1.8
249490_s_at	At5g39110	germin-like protein, putative	1.8
249494_at	At5g39050	transferase	1.8
247656_at	At5g59890	actin-depolymerizing factor 4 (ADF4)	1.8
259939_s_at	At1g71300	Vps52/Sac2	1.8
249377_at	At5g40690	expressed protein	1.8
249187_at	At5g43060	cysteine proteinase, putative	1.8
250407_at	At5g10870	chorismate mutase, cytosolic (CM2)	1.8
256766_at	At3g22231	expressed protein	1.8
245951_at	At5g19550	aspartate aminotransferase, cytoplasmic isozyme 1 (ASP2)	1.8
248333_at	At5g52390	photoassimilate-responsive protein, putative	1.8
253101_at	At4g37430	cytochrome P450 81F1 (CYP81F1)	1.8
255627_at	At4g00955	expressed protein	1.8
262615_at	At1g13950	eukaryotic translation initiation factor 5A-1 (eIF-5A-1)	1.79
261398_at	At1g79610	sodium proton exchanger, putative (NHX6)	1.79
248615_at	At5g49570	transglutaminase-like	1.79
263797_at	At2g24570	WRKY family transcription factor	1.79
254869_at	At4g11890	protein kinase	1.79

254234_at	At4g23680	major latex protein-related	1.79
264355_at	At1g03210	phenazine biosynthesis PhzC/PhzF	1.79
262081_at	At1g59540	kinesin motor protein-related	1.79
248230_at	At5g53830	VQ motif-containing protein	1.79
246600_at	At5g14930	leaf senescence-associated protein (SAG101)	1.79
263640_at	At2g25270	expressed protein	1.79
252691_at	At3g44050	kinesin motor protein-related	1.79
252309_at	At3g49340	cysteine proteinase, putative	1.79
257925_at	At3g23170	expressed protein	1.79
252094_at	At3g51260	20S proteasome alpha subunit D (PAD1)	1.79
264766_at	At1g61420	S-locus lectin protein kinase	1.79
254227_at	At4g23630	reticulon (RTNLB1)	1.79
249806_at	At5g23850	expressed protein	1.79
246511_at	At5g15490	UDP-glucose 6-dehydrogenase, putative	1.79
260605_at	At2g43780	expressed protein	1.79
250892_at	At5g03760	glycosyl transferase family 2 protein	1.79
252880_at	At4g39730	lipid-associated	1.79
263478_at	At2g31880	leucine-rich repeat transmembrane protein kinase, putative	1.78
258162_at	At3g17810	dihydroorotate dehydrogenase	1.78
253776_at	At4g28390	ADP, ATP carrier protein, mitochondrial, putative	1.78
253779_at	At4g28490	leucine-rich repeat transmembrane protein kinase, putative	1.78
263180_at	At1g05620	inosine-uridine preferring nucleoside hydrolase	1.78
251733_at	At3g56240	copper chaperone (CCH)	1.78
259360_at	At1g13310	expressed protein	1.78
264022_at	At2g21185	expressed protein	1.78
252827_at	At4g39950	cytochrome P450 79B2, putative (CYP79B2)	1.78
250236_at	At5g13450	ATP synthase delta chain, mitochondrial, putative	1.78
262324_at	At1g64170	cation/hydrogen exchanger, putative (CHX16)	1.78
266226_at	At2g28740	histone H4	1.78
259096_at	At3g04840	40S ribosomal protein S3A (RPS3aA)	1.78
265920_s_at	At2g15120	secretory protein, putative	1.78
261129_at	At1g04820	tubulin alpha-2/alpha-4 chain (TUA4)	1.78
255546_at	At4g01910	DC1 domain-containing protein	1.78
258105_at	At3g23605	UBX domain-containing protein	1.78
248236_at	At5g53870	plastocyanin-like domain-containing protein	1.78
247577_at	At5g61290	flavin-containing monooxygenase (FMO)	1.78
258984_at	At3g08970	DNAJ heat shock N-terminal domain-containing protein	1.78
248512_at	At5g50460	protein transport protein SEC61 gamma subunit, putative	1.77
258288_at	At3g23295	expressed protein	1.77
247522_at	At5g61340	expressed protein	1.77
260506_at	At1g47210	cyclin	1.77
249152_s_at	At5g43370	inorganic phosphate transporter (PHT1)	1.77
266312_at	At2g27020	20S proteasome alpha subunit G (PAG1)	1.77
263845_at	At2g37040	phenylalanine ammonia-lyase 1 (PAL1)	1.77
263441_at	At2g28620	kinesin motor protein-related	1.77

258259_s_at	At3g26840	esterase/lipase/thioesterase	1.77
247024_at	At5g66985	expressed protein	1.77
247794_at	At5g58670	phosphoinositide-specific phospholipase C (PLC1)	1.77
263183_at	At1g05570	callose synthase 1 (CALS1)	1.77
250431_at	At5g10440	cyclin	1.77
253088_at	At4g36220	cytochrome P450 84A1 (CYP84A1)	1.76
248896_at	At5g46350	WRKY family transcription factor	1.76
254233_at	At4g23800	high mobility group (HMG1/2)	1.76
257277_at	At3g14470	disease resistance protein (NBS-LRR class), putative	1.76
250540_at	At5g08580	calcium-binding EF hand	1.76
250365_at	At5g11410	protein kinase	1.76
259604_at	At1g56450	20S proteasome beta subunit G1 (PBG1)	1.76
266419_at	At2g38760	annexin 3 (ANN3)	1.76
263612_at	At2g16440	DNA replication licensing factor, putative	1.76
249726_at	At5g35480	expressed protein	1.76
250090_at	At5g17330	glutamate decarboxylase 1 (GAD 1)	1.76
246645_at	At5g35080	expressed protein	1.75
257026_at	At3g19200	hypothetical protein	1.75
259383_at	At3g16470	jacalin lectin	1.75
257334_at	orf111d	orf111d	1.75
255547_at	At4g01920	DC1 domain-containing protein	1.75
247776_at	At5g58700	phosphoinositide-specific phospholipase C	1.75
266297_at	At2g29570	proliferating cell nuclear antigen 2 (PCNA2)	1.75
260683_at	At1g17560	ribosomal protein L14	1.75
264903_at	At1g23190	phosphoglucomutase, putative	1.75
252068_at	At3g51440	strictosidine synthase	1.75
251062_at	At5g01840	ovate	1.75
245324_at	At4g17260	L-lactate dehydrogenase, putative	1.75
260325_at	At1g63940	monodehydroascorbate reductase, putative	1.75
267592_at	At2g39710	aspartyl protease	1.74
245736_at	At1g73330	protease inhibitor, putative (DR4)	1.74
264802_at	At1g08560	syntaxin-related protein KNOLLE (KN)	1.74
264969_at	At1g67320	DNA primase, large subunit family	1.74
251644_at	At3g57540	remorin	1.74
256150_at	At1g55120	beta-fructosidase, putative	1.74
265935_at	At2g19580	senescence-associated protein-related	1.74
249397_at	At5g40230	nodulin-related	1.74
250965_at	At5g03020	kelch repeat-containing F-box	1.74
250901_at	At5g03530	Ras-related GTP-binding	1.74
247962_at	At5g56580	mitogen-activated protein kinase kinase (MKK6)	1.74
262482_at	At1g17020	oxidoreductase, 2OG-Fe(II) oxygenase	1.74
262109_at	At1g02730	cellulose synthase	1.74
249809_at	At5g23910	kinesin motor protein-related	1.74
258890_at	At3g05690	CCAAT-binding transcription factor (CBF-B/NF-YA)	1.74
257678_at	At3g20420	ribonuclease III	1.74

247570_at	At5g61250	glycosyl hydrolase family 79 N-terminal domain-containing protein	1.74
265717_at	At2g03510	band 7	1.74
259366_at	At1g13280	allene oxide cyclase	1.74
264592_at	At2g17720	oxidoreductase, 2OG-Fe(II) oxygenase	1.73
249374_at	At5g40580	20S proteasome beta subunit B (PBB2) (PRCFC)	1.73
262942_at	At1g79450	LEM3 (ligand-effect modulator 3)	1.73
267175_s_at	At2g37620	actin 3 (ACT3)	1.73
253600_at	At4g30810	serine carboxypeptidase S10	1.73
248967_at	At5g45350	proline-rich	1.73
255773_at	At1g18590	sulfotransferase	1.73
251108_at	At5g01620	expressed protein	1.73
258666_at	At3g08550	elongation defective 1 protein	1.73
260503_at	At1g47250	20S proteasome alpha subunit F2 (PAF2)	1.73
252114_at	At3g51450	strictosidine synthase	1.73
259669_at	At1g52340	short-chain dehydrogenase/reductase (SDR)	1.73
261599_at	At1g49700	expressed protein	1.73
257021_at	At3g19710	branched-chain amino acid transaminase, putative (BCAT4)	1.73
266170_at	At2g39050	hydroxyproline-rich glycoprotein	1.73
254512_at	At4g20230	terpene synthase	1.73
265435_s_at	At2g21020	major intrinsic protein(MIP)	1.73
267165_at	At2g37710	lectin protein kinase, putative	1.72
264622_at	At2g17790	vacuolar protein sorting-associated protein 35 (VPS35)	1.72
254351_at	At4g22300	phospholipase	1.72
262619_at	At1g06550	enoyl-CoA hydratase	1.72
264377_at	At2g25060	plastocyanin-like domain-containing protein	1.72
264219_at	At1g60420	DC1 domain-containing protein	1.72
261368_at	At1g53070	legume lectin	1.72
253629_at	At4g30450	glycine-rich protein	1.72
247354_at	At5g63590	flavonol synthase, putative	1.72
264574_at	At1g05300	metal transporter, putative (ZIP5)	1.72
253105_at	At4g35840	zinc finger (C3HC4-type RING finger)	1.72
263892_at	At2g36890	myb family transcription factor (MYB38)	1.72
267428_at	At2g34840	coatomer protein epsilon subunit (COPE)	1.72
265097_at	At1g04020	zinc finger (C3HC4-type RING finger)	1.72
259678_at	At1g77750	30S ribosomal protein S13, chloroplast, putative	1.72
247215_at	At5g64905	expressed protein	1.72
247094_at	At5g66280	GDP-D-mannose 4,6-dehydratase, putative	1.72
256529_at	At1g33260	protein kinase	1.72
256169_at	At1g51800	leucine-rich repeat protein kinase, putative	1.71
262826_at	At1g13080	cytochrome P450	1.71
247999_at	At5g56150	ubiquitin-conjugating enzyme, putative	1.71
258913_at	At3g06450	anion exchange	1.71
266625_at	At2g35380	peroxidase 20 (PER20)	1.71
246593_at	At5g14790	expressed protein	1.71

254761_at	At4g13195	expressed protein	1.71
248528_at	At5g50760	auxin-responsive	1.71
257377_at	At2g28890	protein phosphatase 2C (PP2C)	1.71
256969_at	At3g21080	ABC transporter-related	1.71
248715_at	At5g48290	heavy-metal-associated domain-containing protein	1.71
247190_at	At5g65420	cyclin, putative	1.71
264458_at	At1g10410	expressed protein	1.7
246993_at	At5g67450	zinc finger (C2H2 type) protein 1 (AZF1)	1.7
252606_at	At3g45010	serine carboxypeptidase III, putative	1.7
262644_at	At1g62710	vacuolar processing enzyme beta	1.7
255302_at	At4g04830	methionine sulfoxide reductase domain-containing protein	1.7
263582_at	At2g17120	peptidoglycan-binding LysM domain-containing protein	1.7
253203_at	At4g34710	arginine decarboxylase 2 (SPE2)	1.7
252677_at	At3g44320	nitrilase 3 (NIT3)	1.7
267439_at	At2g19060	GDSL-motif lipase/hydrolase	1.7
266418_at	At2g38750	annexin 4 (ANN4)	1.7
246092_at	At5g20500	glutaredoxin, putative	1.7
256875_at	At3g26330	cytochrome P450	1.7
246203_at	At4g36610	hydrolase, alpha/beta fold	1.7
245253_at	At4g15440	hydroperoxide lyase (HPL1)	1.7
258371_at	At3g14410	transporter-related	1.7
266928_at	At2g45790	eukaryotic phosphomannomutase	1.7
259102_at	At3g11660	harpin-induced (HIN1)	1.7
260030_at	At1g68880	bZIP transcription factor	1.7
259735_at	At1g64405	expressed protein	1.7
253361_at	At4g33100	expressed protein	1.69
259937_s_at	At1g71330	ABC transporter	1.69
254850_at	At4g12000	expressed protein	1.69
259671_at	At1g52290	protein kinase	1.69
259928_at	At1g34380	5'-3' exonuclease	1.69
257365_x_at	At2g26020	plant defensin-fusion protein, putative (PDF1.2b)	1.69
264028_at	At2g03680	expressed protein	1.69
254380_at	At4g21865	expressed protein	1.69
245200_at	At1g67850	expressed protein	1.69
249659_s_at	At5g36710	expressed protein	1.69
256131_at	At1g13600	bZIP transcription factor	1.69
249011_at	At5g44670	expressed protein	1.69
263431_at	At2g22170	lipid-associated	1.69
262161_at	At1g52600	signal peptidase, putative	1.69
266244_at	At2g27740	expressed protein	1.69
265740_at	At2g01150	zinc finger (C3HC4-type RING finger)	1.69
259852_at	At1g72280	endoplasmic reticulum oxidoreductin 1 (ERO1)	1.69
253172_at	At4g35060	heavy-metal-associated domain-containing protein	1.69
252188_at	At3g50860	clathrin adaptor complex small chain	1.69
254093_at	At4g25110	latex-abundant (AMC2)	1.69

250129_at	At5g16450	dimethylmenaquinone methyltransferase	1.69
250493_at	At5g09800	U-box domain-containing protein	1.69
251393_at	At3g60640	autophagy 8g (APG8g)	1.68
257535_at	At3g09490	chloroplast lumen common	1.68
247602_at	At5g60900	lectin protein kinase	1.68
262039_at	At1g80050	adenine phosphoribosyltransferase 2 (APT2)	1.68
263406_at	At2g04160	subtilisin-like protease (AIR3)	1.68
255259_at	At4g05020	NADH dehydrogenase-related	1.68
259104_at	At3g02170	expressed protein	1.68
249385_at	At5g39950	thioredoxin H-type 2 (TRX-H-2) (Gif2)	1.68
265538_at	At2g15860	expressed protein	1.68
252692_at	At3g43960	cysteine proteinase, putative	1.68
260225_at	At1g74590	glutathione S-transferase, putative	1.68
259561_at	At1g21250	wall-associated kinase 1 (WAK1)	1.68
264001_at	At2g22420	peroxidase 17 (PER17)	1.68
255879_at	At1g67000	protein kinase	1.68
264635_at	At1g65500	expressed protein	1.68
247264_at	At5g64530	no apical meristem (NAM)	1.68
249777_at	At5g24210	lipase class 3	1.67
252652_at	At3g44720	prephenate dehydratase	1.67
250299_at	At5g11910	esterase	1.67
255629_at	At4g00860	stress-related ozone-induced protein (OZI1)	1.67
256899_at	At3g24660	leucine-rich repeat transmembrane protein kinase, putative	1.67
255319_at	At4g04220	disease resistance	1.67
261410_at	At1g07610	metallothionein-like protein 1C (MT-1C)	1.67
253340_s_at	At4g33260	WD-40 repeat (FZR)	1.67
263496_at	At2g42570	expressed protein	1.67
248049_at	At5g56090	cytochrome oxidase assembly	1.67
267264_at	At2g22970	serine carboxypeptidase S10	1.67
264685_at	At1g65610	endo-1,4-beta-glucanase, putative	1.67
262180_at	At1g78050	phosphoglycerate/bisphosphoglycerate mutase	1.67
248972_at	At5g45010	DSS1/SEM1	1.67
245791_at	At1g32210	defender against cell death 1 (DAD1)	1.67
259891_at	At1g72730	eukaryotic translation initiation factor 4A, putative (eIF-4A)	1.67
259169_at	At3g03520	phosphoesterase	1.67
251761_at	At3g55700	UDP-glucoronosyl/UDP-glucosyl transferase	1.67
248320_at	At5g52720	heavy-metal-associated domain-containing protein	1.67
262131_at	At1g02900	rapid alkalinization factor (RALF)	1.67
256427_at	At3g11090	lateral organ boundaries domain (LBD21)	1.67
247692_s_at	At5g59690	histone H4	1.66
251222_at	At3g62580	expressed protein	1.66
262744_at	At1g28680	transferase	1.66
262426_s_at	At1g47630	expressed protein	1.66
265383_at	At2g16780	WD-40 repeat protein (MSI2)	1.66
265214_at	At1g05000	tyrosine specific protein phosphatase	1.66

249514_at	At5g38480	14-3-3 protein GF14 psi (GRF3)	1.66
252291_s_at	At3g49120	peroxidase 33 (PER33)	1.66
247448_at	At5g62770	expressed protein	1.66
247338_at	At5g63680	pyruvate kinase, putative	1.66
252324_at	At3g48565	protein transport protein SEC61 gamma subunit, putative	1.66
250316_at	At5g12140	cysteine protease inhibitor, putative	1.66
259936_at	At1g71220	UDP-glucose:glycoprotein glucosyltransferase, putative	1.66
258332_at	At3g16180	proton-dependent oligopeptide transport (POT)	1.66
245395_at	At4g17420	expressed protein	1.66
251661_at	At3g56950	small basic membrane integral	1.66
246073_at	At5g20180	ribosomal protein L36	1.66
246714_at	At5g28220	expressed protein	1.66
259563_s_at	At1g20590	cyclin, putative	1.66
252022_at	At3g52930	fructose-bisphosphate aldolase, putative	1.66
248732_at	At5g48070	xyloglucan:xyloglucosyl transferase, putative	1.65
259293_at	At3g11580	DNA-binding protein, putative	1.65
257705_at	At3g12760	expressed protein	1.65
256883_at	At3g26440	expressed protein	1.65
266757_at	At2g46940	expressed protein	1.65
265130_at	At1g30890	integral membrane HRF1	1.65
247740_at	At5g58940	protein kinase	1.65
250212_at	At5g13960	SET domain-containing protein (SUVH4)	1.65
266820_at	At2g44940	AP2 domain-containing transcription factor TINY, putative	1.65
264439_at	At1g27450	adenine phosphoribosyltransferase 1 (APT1)	1.65
261415_at	At1g07750	cupin	1.65
257436_s_at	At2g24650	transcriptional factor B3	1.65
262711_at	At1g16500	expressed protein	1.65
257643_at	At3g25730	AP2 domain-containing transcription factor, putative	1.65
253382_at	At4g33040	glutaredoxin	1.65
266037_at	At2g05940	protein kinase, putative	1.65
267518_at	At2g30500	kinase interacting	1.65
258270_at	At3g15650	phospholipase	1.65
248509_at	At5g50335	expressed protein	1.65
248951_at	At5g45550	mob1/phocein	1.65
263179_at	At1g05710	ethylene-responsive protein, putative	1.65
266401_s_at	At2g38620	cell divsion control protein, putative	1.65
262452_at	At1g11210	expressed protein	1.65
252549_at	At3g45860	receptor-like protein kinase, putative	1.65
259911_at	At1g72680	cinnamyl-alcohol dehydrogenase, putative (CAD)	1.64
261339_at	At1g35710	leucine-rich repeat transmembrane protein kinase, putative	1.64
260933_at	At1g02470	expressed protein	1.64
248942_at	At5g45480	expressed protein	1.64
248144_at	At5g54800	glucose-6-phosphate/phosphate translocator, putative	1.64
254759_at	At4g13180	short-chain dehydrogenase/reductase (SDR)	1.64
264669_at	At1g09630	Ras-related GTP-binding protein, putative	1.64

255499_at	At4g02730	transducin	1.64
245301_at	At4g17190	farnesyl pyrophosphate synthetase 2 (FPS2)	1.64
265510_at	At2g05630	APG8d: autophagy 8d (APG8d)	1.64
265539_at	At2g15830	expressed protein	1.64
263120_at	At1g78490	cytochrome P450	1.64
255726_at	At1g25530	lysine and histidine specific transporter, putative	1.64
258982_at	At3g08870	lectin protein kinase, putative	1.64
261258_at	At1g26640	aspartate	1.64
256426_at	At1g33420	PHD finger	1.64
253148_at	At4g35620	cyc2b: cyclin 2b (CYC2b)	1.64
265382_at	At2g16790	shikimate kinase	1.64
249092_at	At5g43710	glycoside hydrolase family 47 protein	1.64
264091_at	At1g79110	expressed protein	1.64
258898_at	At3g05740	DNA helicase (RECQl1)	1.64
250560_at	At5g08020	replication protein, putative	1.64
266802_at	At2g22900	galactosyl transferase (GMA12)	1.64
258487_at	At3g02550	lateral organ boundaries domain protein 41 (LBD41)	1.63
262408_at	At1g34750	protein phosphatase 2C, putative (PP2C)	1.63
245394_at	At4g16450	expressed protein	1.63
261177_at	At1g04770	male sterility MS5	1.63
249974_at	At5g18780	F-box	1.63
267212_at	At2g44060	late embryogenesis abundant (LEA)	1.63
260738_at	At1g14990	expressed protein	1.63
256237_at	At3g12610	DNA-damage-repair (DRT100)	1.63
246281_at	At4g36940	nicotinate phosphoribosyltransferase	1.63
257724_at	At3g18510	expressed protein	1.63
254687_at	At4g13770	cytochrome P450	1.63
246320_at	At1g16560	Per1-like	1.63
249644_at	At5g37010	expressed protein	1.63
257711_at	At3g27430	20S proteasome beta subunit B (PBB1)	1.63
253999_at	At4g26200	1-aminocyclopropane-1-carboxylate synthase, putative	1.63
260728_at	At1g48210	serine/threonine protein kinase, putative	1.63
259429_at	At1g01600	cytochrome P450, putative	1.63
258362_at	At3g14280	expressed protein	1.63
251705_at	At3g56400	WRKY family transcription factor	1.63
260023_at	At1g30040	gibberellin 2-oxidase (GA2OX2)	1.63
258067_at	At3g25980	mitotic spindle checkpoint protein, putative (MAD2)	1.63
248426_at	At5g51740	peptidase M48	1.62
251337_at	At3g60820	20S proteasome beta subunit F1 (PBF1)	1.62
261504_at	At1g71692	MADS-box protein (AGL12)	1.62
249025_at	At5g44720	molybdenum cofactor sulfurase	1.62
251886_at	At3g54260	expressed protein	1.62
247275_at	At5g64370	beta-ureidopropionase, putative	1.62
267618_at	At2g26760	cyclin, putative	1.62
251689_at	At3g56500	serine-rich protein-related	1.62

248147_at	At5g54900	RNA-binding protein 45 (RBP45), putative	1.62
264588_at	At2g17730	zinc finger (C3HC4-type RING finger)	1.62
252572_at	At3g45290	seven transmembrane MLO (MLO3)	1.62
256415_at	At3g11210	GDSL-motif lipase/hydrolase	1.62
245160_at	At2g33080	leucine-rich repeat	1.62
257176_s_at	At3g23510	cyclopropane fatty acid synthase, putative (CPA-FA)	1.62
260262_at	At1g68470	exostosin	1.62
259264_at	At3g01260	aldose 1-epimerase	1.62
255566_s_at	At4g01780	XH/XS domain-containing protein	1.62
253712_at	At4g29330	Der1-like	1.61
254833_s_at	At4g12280	copper amine oxidase, putative	1.61
258295_at	At3g23400	plastid-lipid associated protein (PAP)	1.61
254355_at	At4g22380	ribosomal protein L7Ae/L30e/S12e/Gadd45	1.61
266639_at	At2g35520	defender against cell death 2 (DAD2)	1.61
248829_at	At5g47130	Bax inhibitor-1	1.61
248200_at	At5g54160	5-hydroxyferulic acid O-methyltransferase (OMT1)	1.61
247077_at	At5g66420	expressed protein	1.61
262096_at	At1g56010	transcription activator NAC1 (NAC1)	1.61
261153_at	At1g04850	ubiquitin-associated (UBA)	1.61
246920_at	At5g25090	plastocyanin-like domain-containing protein	1.61
265913_at	At2g25625	expressed protein	1.61
263776_s_at	At2g46440	cyclic nucleotide-gated channel (CNGC3)	1.61
249801_at	At5g23580	calcium-dependent protein kinase 9 (CDPK9)	1.61
264089_at	At2g31200	actin-depolymerizing factor 6 (ADF6)	1.61
251229_at	At3g62740	glycosyl hydrolase family 1 protein	1.61
246478_at	At5g15980	pentatricopeptide (PPR) repeat-containing protein	1.61
259680_at	At1g77690	amino acid permease, putative	1.61
246580_at	At1g31770	ABC transporter	1.61
258837_at	At3g07110	60S ribosomal protein L13A (RPL13aA)	1.61
252313_at	At3g49390	RNA-binding protein, putative	1.6
248286_at	At5g52870	expressed protein	1.6
245292_at	At4g15093	catalytic LigB subunit of aromatic ring-opening dioxygenase	1.6
254262_at	At4g23480	hydroxyproline-rich glycoprotein	1.6
266873_at	At2g44740	cyclin	1.6
253987_at	At4g26270	phosphofructokinase	1.6
260908_at	At1g02580	maternal embryogenesis control protein (MEA)	1.6
267237_s_at	At2g44040	dihydrodipicolinate reductase	1.6
262444_at	At1g47480	expressed protein	1.6
257701_at	At3g12710	methyladenine glycosylase	1.6
256110_at	At1g16900	curculin-like (mannose-binding) lectin	1.6
267600_at	At2g33040	ATP synthase gamma chain, mitochondrial (ATPC)	1.6
263284_at	At2g36100	integral membrane	1.6
261025_at	At1g01225	NC domain-containing protein-related	1.6
260684_at	At1g17590	CCAAT-binding transcription factor (CBF-B/NF-YA)	1.6
256322_at	At1g54990	expressed protein	1.6

253957_at	At4g26320	arabinogalactan-protein (AGP13)	1.6
245197_at	At1g67800	copine-related	1.6
266234_at	At2g02350	SKIP3: SKP1 interacting partner 3 (SKIP3)	1.6
252763_at	At3g42725	expressed protein	1.6
261794_at	At1g16060	ovule development protein, putative	1.6
256617_at	At3g22240	expressed protein	1.6
254333_at	At4g22753	sterol desaturase	1.6
257617_at	At3g26550	DC1 domain-containing protein	1.6
246974_at	At5g24980	expressed protein	1.6
254386_at	At4g21960	peroxidase 42 (PER42)	1.6
262134_at	At1g77990	sulfate transporter	1.6
252527_at	At3g46440	NAD-dependent epimerase/dehydratase	1.59
247469_at	At5g62165	MADS-box protein (AGL42)	1.59
266416_at	At2g38710	AMMECR1 family	1.59
250550_at	At5g07870	transferase	1.59
250324_at	At5g12040	carbon-nitrogen hydrolase	1.59
246968_at	At5g24870	zinc finger (C3HC4-type RING finger)	1.59
257148_at	At3g27240	cytochrome c1, putative	1.59
256255_at	At3g11280	myb family transcription factor	1.59
248835_at	At5g47250	disease resistance protein (CC-NBS-LRR class), putative	1.59
259116_at	At3g01350	proton-dependent oligopeptide transport (POT)	1.59
249465_at	At5g39720	avirulence-responsive protein-related	1.59
263785_at	At2g46390	expressed protein	1.59
259599_at	At1g28110	serine carboxypeptidase S10	1.59
264581_at	At1g05210	expressed protein	1.59
253804_at	At4g28230	expressed protein	1.59
260530_at	At2g47320	peptidyl-prolyl cis-trans isomerase cyclophilin-type	1.59
254216_at	At4g23710	vacuolar ATP synthase subunit G 2 (VATG2)	1.59
245911_at	At5g19690	oligosaccharyl transferase STT3 subunit	1.59
261835_at	At1g16050	expressed protein	1.59
251535_at	At3g58540	expressed protein	1.59
259701_at	At1g77770	expressed protein	1.59
263595_at	At2g01890	purple acid phosphatase, putative	1.59
259964_at	At1g53680	glutathione S-transferase, putative	1.59
245463_at	At4g17030	expansin-related	1.59
266298_at	At2g29590	thioesterase	1.58
254532_at	At4g19660	ankyrin repeat	1.58
265244_at	At2g43020	amine oxidase	1.58
257072_at	At3g14220	GDSL-motif lipase	1.58
256682_at	At3g52200	dihydrolipoamide S-acetyltransferase, putative	1.58
265170_at	At1g23730	carbonic anhydrase, putative	1.58
246042_at	At5g19440	cinnamyl-alcohol dehydrogenase, putative (CAD)	1.58
260545_at	At2g43350	glutathione peroxidase, putative	1.58
254423_at	At4g21610	zinc finger protein, putative	1.58
254328_at	At4g22570	adenine phosphoribosyltransferase, putative	1.58

259520_at	At1g12320	expressed protein	1.58
252328_at	At3g48570	protein transport protein SEC61 gamma subunit, putative	1.58
263758_s_at	At2g21260	mannose 6-phosphate reductase (M6PR)	1.58
260189_at	At1g67550	urease, putative	1.58
257228_at	At3g27890	NADPH-dependent FMN reductase	1.58
252117_at	At3g51430	strictosidine synthase, putative (YLS2)	1.58
259016_at	At3g07480	expressed protein	1.58
257797_at	At3g15940	glycosyl transferase family 1 protein	1.58
253437_at	At4g32460	expressed protein	1.58
252713_at	At3g43810	calmodulin-7 (CAM7)	1.58
262716_at	At1g16470	20S proteasome alpha subunit B (PAB1)	1.58
258414_at	At3g17380	meprin and TRAF homology domain-containing protein	1.58
248944_at	At5g45500	expressed protein	1.57
267103_at	At2g41490	dolichol phosphate N-acetylglucosamine-1-phosphate transferase (UDP-GlcNAc)	1.57
252373_at	At3g48090	disease resistance protein (EDS1)	1.57
258966_at	At3g10690	DNA gyrase subunit A	1.57
246724_at	At5g29000	myb family transcription factor	1.57
262751_at	At1g16310	cation efflux	1.57
246378_at	At1g57620	emp24	1.57
257422_at	At1g11940	expressed protein	1.57
267500_s_at	At2g45510	cytochrome P450	1.57
255633_at	At4g00585	expressed protein	1.57
261436_at	At1g07870	protein kinase	1.57
254242_at	At4g23200	protein kinase	1.57
248597_at	At5g49160	DNA (cytosine-5-)-methyltransferase (ATHIM)	1.57
261221_at	At1g19960	expressed protein	1.57
266036_s_at	At2g05840	20S proteasome alpha subunit A1 (PAA1)	1.57
247881_at	At5g57700	BNR/Asp-box repeat	1.57
260943_at	At1g45145	thioredoxin H-type 5 (TRX-H-5)	1.57
262575_at	At1g15210	ABC transporter	1.57
254958_at	At4g11010	nucleoside diphosphate kinase 3, mitochondrial (NDK3)	1.57
246029_at	At5g21090	leucine-rich repeat protein, putative	1.57
251992_at	At3g53350	myosin heavy chain-related	1.57
245623_s_at	At4g14103	F-box	1.57
251438_s_at	At3g59930	expressed protein	1.56
257252_at	At3g24170	glutathione reductase, putative	1.56
262237_at	At1g48320	thioesterase	1.56
263553_at	At2g16430	purple acid phosphatase (PAP10)	1.56
257623_at	At3g26210	cytochrome P450 71B23, putative (CYP71B23)	1.56
265341_at	At2g18360	hydrolase, alpha/beta fold	1.56
260156_at	At1g52880	no apical meristem (NAM)	1.56
245832_at	At1g48850	chorismate synthase, putative	1.56
256900_at	At3g24670	pectate lyase	1.56
254416_at	At4g21380	S-locus protein kinase, putative (ARK3)	1.56

252833_at	At4g40090	arabinogalactan-protein (AGP3)	1.56
252385_at	At3g47810	calcineurin-like phosphoesterase	1.56
249523_at	At5g38630	cytochrome B561	1.56
246061_at	At5g19320	RAN GTPase activating protein 2 (RanGAP2)	1.56
249487_at	At5g39040	ABC transporter (TAP2)	1.56
249176_at	At5g42980	thioredoxin H-type 3 (TRX-H-3)	1.56
245873_at	At1g26260	basic helix-loop-helix (bHLH)	1.56
247425_at	At5g62550	expressed protein	1.56
255590_at	At4g01610	cathepsin B-like cysteine protease, putative	1.56
255527_at	At4g02360	expressed protein	1.56
251090_at	At5g01340	mitochondrial substrate carrier	1.56
248800_at	At5g47320	30S ribosomal protein S19, mitochondrial (RPS19)	1.56
255230_at	At4g05390	ferredoxin-NADP(+) reductase, putative	1.56
252478_at	At3g46540	clathrin assembly protein-related	1.56
256674_at	At3g52360	expressed protein	1.56
261944_at	At1g64650	expressed protein	1.56
257971_at	At3g27530	vesicle tethering	1.55
259255_at	At3g07690	NAD-dependent glycerol-3-phosphate dehydrogenase	1.55
260611_at	At2g43670	glycosyl hydrolase 17	1.55
264657_at	At1g09100	26S protease regulatory subunit 6A, putative	1.55
250823_at	At5g05180	expressed protein	1.55
248576_at	At5g49810	methionine S-methyltransferase	1.55
266992_at	At2g39200	seven transmembrane MLO (MLO12)	1.55
255257_at	At4g05050	polyubiquitin (UBQ11)	1.55
267040_at	At2g34300	dehydration-responsive protein-related	1.55
255412_at	At4g02980	auxin-binding protein 1 (ABP1)	1.55
247929_at	At5g57330	aldose 1-epimerase	1.55
262378_at	At1g72830	CCAAT-binding transcription factor (CBF-B/NF-YA)	1.55
262001_at	At1g33790	jacalin lectin	1.55
254109_at	At4g25240	multi-copper oxidase type I	1.55
267181_at	At2g37760	aldo/keto reductase	1.55
255462_at	At4g02940	oxidoreductase, 2OG-Fe(II) oxygenase	1.55
259381_s_at	At3g16390	jacalin lectin	1.55
250423_s_at	At5g10610	cytochrome P450	1.55
265386_at	At2g20930	expressed protein	1.54
256118_at	At1g16970	Ku70-like protein	1.54
264708_at	At1g09740	ethylene-responsive protein, putative	1.54
249791_at	At5g23810	amino acid transporter	1.54
245428_at	At4g17570	zinc finger (GATA type)	1.54
255554_at	At4g01895	expressed protein	1.54
258188_at	At3g17800	expressed protein	1.54
263681_at	At1g26840	origin recognition complex subunit 6 (ORC6)	1.54
247403_at	At5g62740	band 7	1.54
249816_at	At5g23880	cleavage and polyadenylation specificity factor	1.54
267419_at	At2g35010	thioredoxin	1.54

266926_at	At2g46000	expressed protein	1.54
256184_at	At1g51650	ATP synthase epsilon chain, mitochondrial	1.54
263399_at	At2g31490	expressed protein	1.54
263865_at	At2g36910	multidrug resistance P-glycoprotein (PGP1)	1.53
253967_at	At4g26550	expressed protein	1.53
260257_at	At1g74340	dolichol phosphate-mannose biosynthesis regulatory protein-related	1.53
265649_at	At2g27510	ferredoxin, putative	1.53
265219_at	At2g02050	NADH-ubiquinone oxidoreductase B18 subunit, putative	1.53
257745_at	At3g29240	expressed protein	1.53
246505_at	At5g16250	expressed protein	1.53
263589_at	At2g25280	expressed protein	1.53
258489_at	At3g02520	14-3-3 protein GF14 nu (GRF7)	1.53
266752_at	At2g47000	multidrug resistant (MDR) ABC transporter, putative	1.53
254375_at	At4g21800	ATP-binding	1.53
262657_at	At1g14210	ribonuclease T2	1.53
265858_at	At2g01720	ribophorin I	1.53
258072_at	At3g26090	expressed protein	1.53
248769_at	At5g47730	SEC14 cytosolic factor, putative	1.53
266221_at	At2g28760	NAD-dependent epimerase/dehydratase	1.53
249515_at	At5g38530	tryptophan synthase-related	1.53
262277_at	At1g68650	expressed protein	1.53
259832_at	At1g69580	myb family transcription factor	1.53
252870_at	At4g39940	adenylylsulfate kinase 2 (AKN2)	1.53
248288_at	At5g52840	NADH-ubiquinone oxidoreductase-related	1.53
245619_at	At4g13990	exostosin	1.53
254921_at	At4g11300	expressed protein	1.52
262814_at	At1g11660	heat shock protein, putative	1.52
264059_at	At2g31305	expressed protein	1.52
253183_at	At4g35220	cyclase	1.52
247076_at	At5g66510	bacterial transferase hexapeptide repeat-containing protein	1.52
251847_at	At3g54640	tryptophan synthase, alpha subunit (TSA1)	1.52
262105_at	At1g02810	pectinesterase	1.52
250377_at	At5g11560	PQQ enzyme repeat-containing protein	1.52
257005_at	At3g14190	expressed protein	1.52
267153_at	At2g30860	glutathione S-transferase, putative	1.52
249627_at	At5g37510	NADH-ubiquinone dehydrogenase, mitochondrial, putative	1.52
262155_at	At1g52420	glycosyl transferase family 1 protein	1.52
250117_at	At5g16440	isopentenyl diphosphate:dimethylallyl diphosphate isomerase I (IPP1)	1.52
264787_at	At2g17840	senescence/dehydration-associated protein-related (ERD7)	1.52
247359_at	At5g63560	transferase	1.51
261723_at	At1g76200	expressed protein	1.51
257208_at	At3g14910	expressed protein	1.51
265459_at	At2g46540	expressed protein	1.51

264523_at	At1g10030	integral membrane	1.51
267239_at	At2g02510	expressed protein	1.51
246422_at	At5g17060	ADP-ribosylation factor, putative	1.51
265868_at	At2g01650	zinc finger (C2H2 type)	1.51
264003_at	At2g22475	GRAM domain-containing protein	1.51
260944_at	At1g45130	beta-galactosidase, putative	1.51
256524_at	At1g66200	glutamine synthetase, putative	1.51
251210_at	At3g62810	complex 1	1.51
260153_at	At1g52760	esterase	1.51
261086_at	At1g17460	myb family transcription factor	1.51
250994_at	At5g02490	heat shock cognate 70 kDa protein 2 (HSC70-2)	1.51
247527_at	At5g61480	leucine-rich repeat transmembrane protein kinase, putative	1.51
266457_at	At2g22780	malate dehydrogenase, glyoxysomal, putative	1.51
252361_at	At3g48490	expressed protein	1.51
249711_at	At5g35680	eukaryotic translation initiation factor 1A, putative (eIF-1A)	1.51
246214_at	At4g36990	heat shock factor protein 4 (HSF4)	1.51
265065_at	At1g03980	phytochelatin synthase 2 (PCS2)	1.51
256263_at	At3g12290	tetrahydrofolate dehydrogenase	1.5
248787_at	At5g47420	expressed protein	1.5
250448_at	At5g10820	integral membrane transporter	1.5
259256_at	At3g07680	emp24	1.5
245524_at	At4g15920	nodulin MtN3	1.5
260852_at	At1g21900	emp24	1.5
252099_at	At3g51250	senescence/dehydration-associated protein-related	1.5
259529_at	At1g12400	expressed protein	1.5
251269_at	At3g62360	expressed protein	1.5
265104_at	At1g31020	thioredoxin o (TRXO2)	1.5
253277_at	At4g34230	cinnamyl-alcohol dehydrogenase, putative (CAD)	1.5
247634_at	At5g60520	late embryogenesis abundant protein-related (LEA)	1.5
247011_at	At5g67590	NADH-ubiquinone oxidoreductase-related	1.5
252080_at	At3g51670	SEC14 cytosolic factor	1.5
255104_at	At4g08685	pollen Ole e 1 allergen and extensin	1.5
252754_at	At3g43510	copia-like retrotransposon family	1.5
267565_at	At2g30750	cytochrome P450 71A12, putative (CYP71A12)	1.5
264609_at	At1g04530	expressed protein	1.5
250820_at	At5g05160	leucine-rich repeat transmembrane protein kinase, putative	1.5
245341_at	At4g16447	expressed protein	1.5
262255_at	At1g53790	F-box	1.5
252681_at	At3g44350	no apical meristem (NAM)	1.5
247644_s_at	At5g60390	elongation factor 1-alpha	1.5
261139_at	At1g19700	homeobox-leucine zipper	1.49
266413_at	At2g38740	haloacid dehalogenase-like hydrolase	1.49
257193_at	At3g13160	pentatricopeptide (PPR) repeat-containing protein	1.49
247039_at	At5g67270	microtubule-associated EB1	1.49
257849_at	At3g13060	expressed protein	1.49

259824_at	At1g66240	copper homeostasis factor, putative (CCH)	1.49
258563_at	At3g05970	long-chain acyl-CoA synthetase (LACS6)	1.49
250676_at	At5g06320	harpin-induced (HIN1)	1.49
259014_at	At3g07320	glycosyl hydrolase family 17 protein	1.49
255459_at	At4g02890	polyubiquitin (UBQ14)	1.49
256981_at	At3g13380	leucine-rich repeat	1.49
254834_at	At4g12300	cytochrome P450	1.49
263707_at	At1g09300	metallopeptidase M24	1.49
248259_at	At5g53330	expressed protein	1.48
257612_at	At3g26600	armadillo/beta-catenin repeat	1.48
258108_at	At3g23570	dienelactone hydrolase	1.48
251186_at	At3g62790	NADH-ubiquinone oxidoreductase-related	1.48
260550_at	At2g43420	3-beta hydroxysteroid dehydrogenase/isomerase	1.48
252357_at	At3g48410	hydrolase, alpha/beta fold	1.48
259297_at	At3g05360	disease resistance	1.48
254776_at	At4g13360	enoyl-CoA hydratase	1.48
249636_at	At5g36890	glycosyl hydrolase family 1 protein	1.48
261318_at	At1g53035	expressed protein	1.48
256611_at	At3g29270	expressed protein	1.48
249860_at	At5g22860	serine carboxypeptidase S28	1.48
255827_at	At2g40600	appr-1-p processing enzyme	1.48
251688_at	At3g56480	myosin heavy chain-related	1.48
254594_at	At4g18930	cyclic phosphodiesterase	1.47
253948_at	At4g26940	galactosyltransferase	1.47
252389_at	At3g47833	expressed protein	1.47
257004_s_at	At3g14150	(S)-2-hydroxy-acid oxidase, peroxisomal, putative	1.47
254891_at	At4g11740	ara4-interacting protein, putative (SAY1)	1.47
246479_at	At5g16060	expressed protein	1.47
249161_at	At5g42790	20S proteasome alpha subunit F1 (PAF1)	1.47
259560_at	At1g21270	wall-associated kinase 2 (WAK2)	1.47
249866_at	At5g23010	2-isopropylmalate synthase 3 (IMS3)	1.47
258852_at	At3g06300	oxidoreductase, 2OG-Fe(II) oxygenase	1.47
250603_at	At5g07820	expressed protein	1.47
256179_at	At1g51710	ubiquitin-specific protease 6, putative (UBP6)	1.47
254027_at	At4g25835	AAA-type ATPase	1.47
245854_at	At5g13490	adenine nucleotide translocator 2 (ANT2)	1.47
253436_at	At4g32470	ubiquinol-cytochrome C reductase complex 14 kDa protein, putative	1.47
248088_at	At5g55070	2-oxoacid dehydrogenase	1.47
254429_at	At4g21105	expressed protein	1.47
253705_at	At4g29130	hexokinase 1 (HXK1)	1.47
262637_at	At1g06640	2-oxoglutarate-dependent dioxygenase, putative	1.46
257713_at	At3g27380	sdh2-1: succinate dehydrogenase, iron-sulphur subunit, mitochondrial (SDH2-1)	1.46
251975_at	At3g53230	cell division cycle protein 48, putative (CDC48)	1.46

251922_at	At3g54030	protein kinase	1.46
251499_at	At3g59100	glycosyl transferase family 48 protein	1.46
245501_at	At4g15620	integral membrane	1.46
253303_at	At4g33780	expressed protein	1.46
249796_at	At5g23540	26S proteasome regulatory subunit, putative	1.46
262781_s_at	At1g13060	20S proteasome beta subunit E, putative	1.46
252877_at	At4g39630	expressed protein	1.46
250775_at	At5g05460	glycosyl hydrolase family 85 protein	1.46
245085_at	At2g23350	polyadenylate-binding protein, putative (PABP)	1.46
265769_at	At2g48090	expressed protein	1.46
252246_at	At3g49730	pentatricopeptide (PPR) repeat-containing protein	1.46
249372_at	At5g40760	glucose-6-phosphate 1-dehydrogenase (ACG12)	1.46
266187_at	At2g38970	zinc finger (C3HC4-type RING finger)	1.46
258614_at	At3g02770	dimethylmenaquinone methyltransferase	1.45
262769_at	At1g13180	actin-related protein 3 (ARP3)	1.45
251076_at	At5g01970	expressed protein	1.45
252864_at	At4g39740	electron transport SCO1/SenC	1.45
257809_at	At3g27060	ribonucleoside-diphosphate reductase small chain, putative	1.45
254280_at	At4g22756	sterol desaturase	1.45
259708_at	At1g77420	hydrolase, alpha/beta fold	1.45
246189_at	At5g20910	zinc finger (C3HC4-type RING finger)	1.45
255671_at	At4g00355	expressed protein	1.45
247725_at	At5g59410	expressed protein	1.45
263663_at	At1g04410	malate dehydrogenase, cytosolic, putative	1.45
261831_at	At1g10630	ADP-ribosylation factor, putative	1.45
267055_at	At2g38360	prenylated rab acceptor (PRA1)	1.44
254083_at	At4g24920	protein transport protein SEC61 gamma subunit, putative	1.44
262481_at	At1g17080	expressed protein	1.44
249354_at	At5g40480	expressed protein	1.44
264724_at	At1g22920	COP9 signalosome subunit 5B (CSN5B)	1.44
256268_at	At3g12280	RB: retinoblastoma-related protein (RBR1)	1.44
248127_at	At5g54750	transport protein particle (TRAPP) component Bet3, putative	1.44
247257_at	At5g64760	26S proteasome regulatory subunit, putative (RPN5)	1.44
262314_at	At1g70810	C2 domain-containing protein	1.43
254275_at	At4g22670	tetratricopeptide repeat (TPR)-containing protein	1.43
265446_at	At2g37110	expressed protein	1.43
256186_at	At1g51680	4-coumaroyl-CoA synthase 1 (4CL1)	1.43
267001_at	At2g34470	urease accessory protein (UREG)	1.43
266206_at	At2g27730	expressed protein	1.43
246562_at	At5g15580	expressed protein	1.43
245891_at	At5g09220	amino acid permease 2 (AAP2)	1.43
245412_at	At4g17280	auxin-responsive	1.43
260673_at	At1g19330	expressed protein	1.43
256787_at	At3g13790	beta-fructosidase (BFRUCT1)	1.43
266874_at	At2g44760	expressed protein	1.43

262918_at	At1g65000	expressed protein	1.42
253052_at	At4g37310	cytochrome P450, putative	1.42
263919_at	At2g36470	expressed protein	1.42
260003_at	At1g68100	IAA-alanine resistance protein 1, putative	1.42
255728_at	At1g25500	choline transporter-related	1.42
255236_at	At4g05520	calcium-binding EF hand	1.42
248760_at	At5g47570	expressed protein	1.42
253642_at	At4g29960	expressed protein	1.42
250553_at	At5g07960	expressed protein	1.42
250471_at	At5g10170	inositol-3-phosphate synthase, putative	1.42
258391_at	At3g15420	expressed protein	1.42
255596_at	At4g01720	WRKY family transcription factor	1.42
255261_s_at	At4g05110	equilibrative nucleoside transporter, putative (ENT6)	1.42
254053_s_at	At4g25300	oxidoreductase, 2OG-Fe(II) oxygenase	1.42
245202_at	At1g67720	leucine-rich repeat	1.42
262366_at	At1g72890	disease resistance protein (TIR-NBS class), putative	1.41
258457_at	At3g22422	imidazoleglycerol-phosphate dehydratase 1 (IGPD1)	1.41
267355_at	At2g39900	LIM domain-containing protein	1.41
262946_at	At1g79390	expressed protein	1.41
255291_at	At4g04700	calcium-dependent protein kinase, putative (CDPK)	1.41
251034_at	At5g02040	prenylated rab acceptor (PRA1)	1.41
261227_at	At1g20200	26S proteasome regulatory subunit S3, putative (RPN3)	1.41
254462_at	At4g20150	expressed protein	1.4
252955_at	At4g38630	26S proteasome regulatory subunit S5A (RPN10)	1.4
263224_at	At1g30580	expressed protein	1.4
252027_at	At3g52850	vacuolar sorting receptor, putative	1.4
246824_at	At5g26990	drought-responsive	1.4
260274_at	At1g80460	glycerol kinase, putative	1.4
259978_at	At1g76540	cell division control protein, putative	1.4
252723_at	At3g43520	expressed protein	1.4
264249_at	At1g78920	vacuolar-type H+-translocating inorganic pyrophosphatase (AVPL1)	1.39
263419_at	At2g17220	protein kinase, putative	1.39
247080_at	At5g66140	20S proteasome alpha subunit D2 (PAD2)	1.39
247521_at	At5g61450	2-phosphoglycerate kinase-related	1.39
263986_at	At2g42790	citrate synthase, glyoxysomal, putative	1.39
263314_at	At2g05760	xanthine/uracil permease	1.39
261087_at	At1g17350	auxin-induced-related	1.38
250863_at	At5g04750	F1F0-ATPase inhibitor protein, putative	1.38
251828_at	At3g55070	expressed protein	1.38
248778_at	At5g47940	expressed protein	1.38
261862_at	At1g50410	SNF2 domain-containing protein	1.38
260887_at	At1g29160	Dof-type zinc finger domain-containing protein	1.38
257431_at	At2g36360	kelch repeat-containing protein	1.38
267189_at	At2g44180	methionyl aminopeptidase, putative	1.38

265965_at	At2g37500	arginine biosynthesis protein ArgJ family	1.38
257444_at	At2g12550	ubiquitin-associated (UBA)	1.38
265343_at	At2g22570	isochorismatase hydrolase	1.38
264506_at	At1g09560	germin-like protein (GLP4)	1.38
249095_at	At5g43900	myosin heavy chain (MYA2)	1.38
250406_at	At5g10810	enhancer of rudimentary protein, putative	1.37
264303_s_at	At1g78870	ubiquitin-conjugating enzyme, putative	1.37
251954_at	At3g53670	expressed protein	1.37
248276_at	At5g53550	transporter, putative	1.37
266553_at	At2g46170	reticulon (RTNLB5)	1.37
260772_at	At1g49050	aspartyl protease	1.37
251932_at	At3g54010	peptidyl-prolyl cis-trans isomerase, putative (PAS1-D)	1.37
265912_at	At2g25570	expressed protein	1.36
261144_s_at	At1g19660	wound-responsive	1.36
265738_at	At2g01350	quinolinate phosphoribosyl transferase	1.36
256042_at	At1g07220	expressed protein	1.36
247971_at	At5g56730	peptidase M16	1.36
257634_s_at	At3g26170	cytochrome P450 71B19, putative (CYP71B19)	1.35
257893_at	At3g17000	ubiquitin-conjugating enzyme, putative	1.35
257926_at	At3g23280	zinc finger (C3HC4-type RING finger)	1.35
253317_at	At4g33960	expressed protein	1.35
247330_at	At5g63510	bacterial transferase hexapeptide repeat-containing protein	1.35
266090_at	At2g38000	chaperone protein dnaJ-related	1.35
245046_at	At2g26510	xanthine/uracil permease	1.35
264871_at	At1g24180	pyruvate dehydrogenase E1 component alpha subunit, putative	1.35
250193_at	At5g14540	proline-rich	1.34
259342_at	At3g03890	expressed protein	1.33
254547_at	At4g19860	lecithin:cholesterol acyltransferase (LACT)	1.32
251787_at	At3g55410	2-oxoglutarate dehydrogenase E1 component, putative	1.31
259230_at	At3g07780	expressed protein	-1.28
260127_at	At1g36320	expressed protein	-1.33
246045_at	At5g19430	zinc finger (C3HC4-type RING finger)	-1.33
262468_at	At1g50200	aminoacyl-tRNA synthetase	-1.33
251237_at	At3g62420	bZIP transcription factor	-1.33
245042_at	At2g26540	uroporphyrinogen-III synthase	-1.33
249181_at	At5g42920	expressed protein	-1.33
247103_at	At5g66610	LIM domain-containing protein	-1.33
260610_at	At2g43680	calmodulin-binding	-1.33
267000_at	At2g34310	expressed protein	-1.34
266606_at	At2g46310	AP2 domain-containing transcription factor, putative	-1.34
263688_at	At1g26920	expressed protein	-1.34
247043_at	At5g66880	serine/threonine protein kinase, putative	-1.34
263334_at	At2g03820	nonsense-mediated mRNA decay NMD3	-1.34
259768_at	At1g29390	stress-responsive protein, putative	-1.35

250385_at	At5g11520	aspartate aminotransferase (ASP3)	-1.35
253756_at	At4g28980	cyclin-dependent kinase-activating kinase 1At (CAK1)	-1.35
249138_at	At5g43070	MFP1 attachment factor, putative	-1.35
264750_at	At1g22870	protein kinase	-1.35
256765_at	At3g22200	4-aminobutyrate aminotransferase	-1.35
260800_at	At1g78240	dehydration-responsive protein-related	-1.35
261887_at	At1g80780	CCR4-NOT transcription complex protein, putative	-1.36
263750_at	At2g21530	forkhead-associated domain-containing protein	-1.36
259092_at	At3g04870	zeta-carotene desaturase (ZDS1)	-1.36
263760_at	At2g21280	expressed protein	-1.36
262882_at	At1g64900	cytochrome P450, putative	-1.36
261535_at	At1g01725	expressed protein	-1.36
267092_at	At2g38120	amino acid permease, putative (AUX1)	-1.37
262959_at	At1g54290	eukaryotic translation initiation factor SUI1, putative	-1.37
252922_at	At4g39040	expressed protein	-1.37
265413_s_at	At2g16650	expressed protein	-1.37
263053_at	At2g13440	glucose-inhibited division family A protein	-1.37
256747_at	At3g29180	expressed protein	-1.37
245572_at	At4g14720	expressed protein	-1.37
247000_at	At5g67380	casein kinase II alpha chain 1	-1.37
251845_at	At3g54540	ABC transporter	-1.37
259258_at	At3g07670	SET domain-containing protein	-1.38
251403_at	At3g60300	RWD domain-containing protein	-1.38
250705_at	At5g06340	diadenosine 5',5'''-P1,P4-tetraphosphate hydrolase, putative	-1.38
266335_at	At2g32440	ent-kaurenoic acid hydroxylase, putative	-1.38
265318_at	At2g22650	FAD-dependent oxidoreductase	-1.38
255376_x_at	At4g03790	At4g03790	-1.38
265305_at	At2g20340	tyrosine decarboxylase, putative	-1.38
264828_at	At1g03380	expressed protein	-1.38
247653_at	At5g59950	RNA and export factor-binding protein, putative	-1.38
264970_at	At1g67280	lactoylglutathione lyase, putative	-1.38
260705_at	At1g32400	senescence-associated	-1.38
258052_at	At3g16190	isochorismatase hydrolase	-1.38
260903_at	At1g02460	glycoside hydrolase family 28 protein	-1.38
258643_at	At3g08010	expressed protein	-1.38
258585_at	At3g04340	FtsH protease	-1.38
253440_at	At4g32570	expressed protein	-1.39
246611_at	At5g35330	methyl-CpG-binding domain-containing protein	-1.39
264996_at	At1g67230	expressed protein	-1.39
260510_at	At1g51580	KH domain-containing protein	-1.39
248181_at	At5g54290	cytochrome c biogenesis protein family	-1.39
257810_at	At3g27070	translocase of outer membrane 20kDa subunit 1 (TOM20-1)	-1.39
263898_at	At2g21950	SKP1 interacting partner 6 (SKIP6)	-1.39
251450_at	At3g60030	squamosa promoter-binding protein-like 12 (SPL12)	-1.39

267492_at	At2g30620	histone H1.2	-1.4
259862_at	At1g72650	myb family transcription factor	-1.4
254612_at	At4g19100	expressed protein	-1.4
266728_at	At2g03140	CAAX amino terminal protease	-1.4
263946_at	At2g36000	mitochondrial transcription termination factor-related	-1.4
245165_at	At2g33180	expressed protein	-1.4
251211_s_at	At3g62470	pentatricopeptide (PPR) repeat-containing protein	-1.4
255720_at	At1g32060	phosphoribulokinase (PRK)	-1.4
259038_at	At3g09210	KOW domain-containing transcription factor	-1.4
253834_at	At4g27800	protein phosphatase 2C PPH1 (PPH1)	-1.4
265707_at	At2g03390	uvrB/uvrC motif-containing protein	-1.4
262502_at	At1g21600	expressed protein	-1.4
258206_at	At3g14010	hydroxyproline-rich glycoprotein	-1.41
266923_at	At2g45980	expressed protein	-1.41
258602_at	At3g02750	protein phosphatase 2C (PP2C)	-1.41
254057_at	At4g25170	expressed protein	-1.41
252759_at	At3g42630	pentatricopeptide (PPR) repeat-containing protein	-1.41
263365_at	At2g20550	DNAJ chaperone C-terminal domain-containing protein	-1.41
262592_at	At1g15400	expressed protein	-1.41
261179_at	At1g04985	expressed protein	-1.41
250711_at	At5g06110	DNAJ heat shock N-terminal domain-containing protein / cell division protein-related	-1.41
249827_at	At5g23330	riboflavin biosynthesis protein-related	-1.41
259242_at	At3g33520	actin-related protein 6 (ARP6)	-1.41
249288_at	At5g41050	expressed protein	-1.41
247108_at	At5g66160	protease-associated zinc finger (C3HC4-type RING finger)	-1.41
260490_at	At1g51500	ABC transporter	-1.41
261628_at	At1g50000	hypothetical protein	-1.41
261190_at	At1g32990	ribosomal protein L11	-1.41
253355_at	At4g33380	expressed protein	-1.41
262988_at	At1g23310	glutamate:glyoxylate aminotransferase 1 (GGT1)	-1.41
245795_at	At1g32160	expressed protein	-1.42
245396_at	At4g14870	expressed protein	-1.42
259207_at	At3g09050	expressed protein	-1.42
251855_at	At3g54690	sugar isomerase (SIS) domain-containing protein / CBS domain-containing protein	-1.42
262110_at	At1g02840	pre-mRNA splicing factor SF2 (SF2) / SR1 protein	-1.42
252105_at	At3g51470	protein phosphatase 2C, putative / PP2C, putative	-1.42
261320_at	At1g53120	RNA-binding S4 domain-containing protein	-1.42
260283_at	At1g80480	PRLI-interacting factor L, putative	-1.42
257908_at	At3g25410	bile acid:sodium symporter	-1.42
261844_at	At1g15940	expressed protein	-1.42
256855_at	At3g15190	chloroplast 30S ribosomal protein S20, putative	-1.42
254764_at	At4g13250	short-chain dehydrogenase/reductase (SDR)	-1.42
245285_s_at	At4g14040	selenium-binding protein, putative	-1.42

265142_at	At1g51360	expressed protein	-1.42
262599_at	At1g15350	expressed protein	-1.43
255015_at	At4g09980	methyltransferase MT-A70	-1.43
252612_at	At3g45160	expressed protein	-1.43
261197_at	At1g12900	glyceraldehyde 3-phosphate dehydrogenase, chloroplast, putative	-1.43
259514_at	At1g12480	C4-dicarboxylate transporter	-1.43
266229_at	At2g28840	ankyrin repeat	-1.43
262235_at	At1g48350	ribosomal protein L18	-1.43
256892_at	At3g19000	oxidoreductase, 2OG-Fe(II) oxygenase	-1.43
247617_at	At5g60270	lectin protein kinase	-1.43
255829_at	At2g40540	potassium transporter, putative (KT2)	-1.43
246294_at	At3g56910	expressed protein	-1.43
246856_at	At5g26210	PHD finger	-1.43
266929_at	At2g45850	DNA-binding	-1.43
266716_at	At2g46820	expressed protein	-1.43
250430_at	At5g10460	haloacid dehalogenase-like hydrolase	-1.43
245008_at	ycf3	ycf3	-1.43
263906_at	At2g36250	chloroplast division protein FtsZ (FtsZ2-1)	-1.43
256680_at	At3g52230	expressed protein	-1.43
258161_at	At3g17930	expressed protein	-1.43
255710_at	At4g00030	plastid-lipid associated protein PAP	-1.43
253001_at	At4g38490	expressed protein	-1.43
248774_at	At5g47830	expressed protein	-1.44
266575_at	At2g24060	translation initiation factor 3 (IF-3)	-1.44
260930_at	At1g02620	GTP-binding protein (SAR1A)	-1.44
260260_at	At1g68540	oxidoreductase	-1.44
254697_at	At4g17970	expressed protein	-1.44
255217_s_at	At4g07680	pseudogene, Ulp1 protease famiy	-1.44
254504_at	At4g20030	RNA recognition motif (RRM)-containing protein	-1.44
267089_at	At2g38300	myb family transcription factor	-1.44
260898_at	At1g29070	ribosomal protein L34	-1.44
248713_at	At5g48180	kelch repeat-containing protein	-1.44
245952_at	At5g28500	expressed protein	-1.44
264977_at	At1g27090	glycine-rich protein	-1.44
264342_at	At1g12080	expressed protein	-1.44
254680_at	At4g18130	phytochrome E (PHYE)	-1.44
265732_at	At2g01300	expressed protein	-1.44
259123_at	At3g02200	proteasome	-1.44
252199_at	At3g50270	transferase	-1.44
263046_at	At2g05380	glycine-rich protein (GRP3S)	-1.44
256402_at	At3g06130	heavy-metal-associated domain-containing protein	-1.44
248838_at	At5g46800	mitochondrial carnitine	-1.44
247079_at	At5g66055	ankyrin repeat protein (AKR)	-1.44
264956_at	At1g76990	ACT domain containing protein	-1.44

267553_s_at	At2g32650	expressed protein	-1.45
262295_at	At1g27650	U2 snRNP auxiliary factor small subunit, putative	-1.45
246215_at	At4g37180	myb family transcription factor	-1.45
255413_at	At4g03140	short-chain dehydrogenase/reductase (SDR)	-1.45
246339_at	At3g44890	50S ribosomal protein L9, chloroplast (CL9)	-1.45
265247_at	At2g43030	ribosomal protein L3	-1.45
260467_at	At1g10890	F-box	-1.45
254073_at	At4g25500	arginine/serine-rich splicing factor RSP40 (RSP40)	-1.45
251810_at	At3g55250	expressed protein	-1.45
266642_at	At2g35410	33 kDa ribonucleoprotein, chloroplast, putative	-1.45
262480_at	At1g11340	S-locus lectin protein kinase	-1.45
257953_at	At3g21865	expressed protein	-1.45
261141_at	At1g19740	ATP-dependent protease La (LON)	-1.45
261196_at	At1g12860	basic helix-loop-helix (bHLH)	-1.45
258696_at	At3g09650	pentatricopeptide (PPR) repeat-containing protein	-1.45
246588_at	At5g14840	expressed protein	-1.45
261668_at	At1g18500	2-isopropylmalate synthase, putative	-1.45
246548_at	At5g14910	heavy-metal-associated domain-containing protein	-1.45
266121_at	At2g02160	zinc finger (CCCH-type)	-1.45
259160_at	At3g05410	expressed protein	-1.46
253235_at	At4g34350	LytB	-1.46
247146_at	At5g65610	expressed protein	-1.46
259991_at	At1g68040	S-adenosyl-L-methionine:carboxyl methyltransferase	-1.46
259773_at	At1g29500	auxin-responsive protein, putative	-1.46
264613_at	At1g04640	biotin/lipoate A/B protein ligase	-1.46
260044_at	At1g73655	immunophilin	-1.46
256796_at	At3g22210	expressed protein	-1.46
256021_at	At1g58270	meprin and TRAF homology domain-containing protein	-1.46
249283_at	At5g41800	amino acid transporter	-1.46
266856_at	At2g26910	ABC transporter	-1.46
262721_at	At1g43560	thioredoxin	-1.46
250786_at	At5g05540	exonuclease	-1.46
248634_at	At5g49030	tRNA synthetase class I (I, L, M and V)	-1.46
256033_at	At1g07250	UDP-glucoronosyl/UDP-glucosyl transferase	-1.46
261230_at	At1g20010	tubulin beta-5 chain (TUB5)	-1.46
260409_at	At1g69935	expressed protein	-1.46
247307_at	At5g63860	UVB-resistance protein (UVR8)	-1.46
254769_at	At4g13330	expressed protein	-1.47
254388_at	At4g21860	methionine sulfoxide reductase domain-containing protein	-1.47
246454_at	At5g16710	dehydroascorbate reductase, putative	-1.47
265415_at	At2g20890	expressed protein	-1.47
265284_at	At2g20230	expressed protein	-1.47
254188_at	At4g23920	UDP-glucose 4-epimerase, putative	-1.47
262945_at	At1g79510	expressed protein	-1.47
261218_at	At1g20020	ferredoxin-NADP(+) reductase, putative	-1.47

257789_at	At3g27020	oligopeptide transporter OPT	-1.47
252823_at	At4g40045	expressed protein	-1.47
259436_at	At1g01500	expressed protein	-1.47
258512_at	At3g06510	glycosyl hydrolase family 1 protein	-1.47
247750_at	At5g58950	protein kinase	-1.47
244996_at	rps2	rps2	-1.47
255332_at	At4g04340	early-responsive to dehydration protein-related (ERD)	-1.47
261264_at	At1g26710	expressed protein	-1.48
252694_at	At3g43630	nodulin, putative	-1.48
247972_at	At5g56740	histone acetyltransferase	-1.48
264991_s_at	At3g43400	phagocytosis and cell motility protein ELMO1-related	-1.48
246829_at	At5g26570	glycoside hydrolase starch-binding domain-containing protein	-1.48
246463_at	At5g16970	NADP-dependent oxidoreductase, putative (P1)	-1.48
257477_at	At1g10660	expressed protein	-1.48
251218_at	At3g62410	CP12 domain-containing protein	-1.48
248854_at	At5g46580	pentatricopeptide (PPR) repeat-containing protein	-1.48
249993_at	At5g18570	GTP1/OBG	-1.48
267220_at	At2g02500	expressed protein	-1.48
250763_at	At5g06060	tropinone reductase, putative	-1.48
248828_at	At5g47110	lil3 protein, putative	-1.48
246613_at	At5g35360	acetyl-CoA carboxylase, biotin carboxylase subunit (CAC2)	-1.48
253307_at	At4g33670	L-galactose dehydrogenase (L-GalDH)	-1.48
264121_at	At1g02280	GTP-binding protein (TOC33)	-1.48
248603_at	At5g49430	transducin	-1.49
245718_at	At5g04100	DNA topoisomerase II	-1.49
260205_at	At1g70700	expressed protein	-1.49
256063_at	At1g07130	OB-fold nucleic acid binding domain-containing protein	-1.49
244997_at	rpoC2	rpoC2	-1.49
261483_at	At1g14270	CAAX amino terminal protease	-1.49
254659_at	At4g18240	starch synthase-related protein	-1.49
263014_at	At1g23400	expressed protein	-1.49
247855_at	At5g58210	hydroxyproline-rich glycoprotein	-1.49
267504_at	At2g45530	zinc finger (C3HC4-type RING finger)	-1.49
255572_at	At4g01050	hydroxyproline-rich glycoprotein	-1.49
250146_at	At5g14660	peptide deformylase, chloroplast 1B (PDF1B)	-1.49
264584_at	At1g05140	membrane-associated zinc metalloprotease, putative	-1.49
263920_at	At2g36410	expressed protein	-1.49
259296_at	At3g05350	aminopeptidase P, cytosolic, putative	-1.49
248238_at	At5g53900	expressed protein	-1.49
264624_at	At1g08930	early-responsive to dehydration stress protein (ERD6)	-1.49
256502_at	At1g36730	eukaryotic translation initiation factor 5, putative (eIF-5)	-1.49
245905_at	At5g11090	serine-rich protein-related	-1.49
266624_s_at	At2g35390	ribose-phosphate pyrophosphokinase 2 (PRS2)	-1.49
263296_at	At2g38800	calmodulin-binding protein-related	-1.49
251929_at	At3g53920	RNA polymerase sigma subunit SigC (SIG3)	-1.49

246090_at	At5g20520	expressed protein	-1.49
257827_at	At3g26630	pentatricopeptide (PPR) repeat-containing protein	-1.5
260086_at	At1g63240	expressed protein	-1.5
248250_at	At5g53130	cyclic nucleotide-regulated ion channel (CNGC1)	-1.5
245354_at	At4g17600	lil3 protein	-1.5
263412_at	At2g28720	histone H2B, putative	-1.5
265139_at	At1g51310	tRNA methyl transferase	-1.5
245117_at	At2g41560	calcium-transporting ATPase 4, plasma membrane-type (ACA4)	-1.5
246007_at	At5g08410	ferredoxin-thioredoxin reductase, putative	-1.5
254760_at	At4g13200	expressed protein	-1.5
245198_at	At1g67700	expressed protein	-1.5
247163_at	At5g65685	soluble glycogen synthase-related	-1.5
264521_at	At1g10020	expressed protein	-1.5
264045_at	At2g22450	riboflavin biosynthesis protein, putative	-1.5
247826_at	At5g58480	glycosyl hydrolase family 17 protein	-1.5
262174_at	At1g74910	ADP-glucose pyrophosphorylase	-1.5
246019_at	At5g10690	pentatricopeptide (PPR) repeat-containing protein	-1.5
262104_at	At1g02910	tetratricopeptide repeat (TPR)-containing protein	-1.5
258060_at	At3g26030	serine/threonine protein phosphatase 2A (PP2A)	-1.51
248029_at	At5g55700	glycosyl hydrolase family 14 protein	-1.51
262572_at	At1g15140	oxidoreductase NAD-binding domain-containing protein	-1.51
248094_at	At5g55220	trigger factor type chaperone	-1.51
264845_at	At1g03675	thioredoxin M-type 1, chloroplast (TRX-M1)	-1.51
264640_at	At1g65680	beta-expansin, putative (EXBP2)	-1.51
262483_at	At1g17220	translation initiation factor IF-2, chloroplast, putative	-1.51
261457_at	At1g21065	expressed protein	-1.51
258071_s_at	At3g26070	plastid-lipid associated protein PAP	-1.51
255788_at	At2g33310	IAA13: auxin-responsive protein	-1.51
247095_at	At5g66400	RAB18: dehydrin (RAB18)	-1.51
261053_at	At1g01320	tetratricopeptide repeat (TPR)-containing protein	-1.51
253421_at	At4g32340	expressed protein	-1.51
255636_at	At4g00730	anthocyaninless2 (ANL2)	-1.51
246031_at	At5g21160	La domain-containing protein	-1.51
245592_at	At4g14540	CCAAT-box binding transcription factor subunit B (NF-YB)	-1.51
263705_at	At1g31190	inositol monophosphatase	-1.51
251055_at	At5g01710	expressed protein	-1.51
248140_at	At5g54980	integral membrane	-1.51
247524_at	At5g61440	thioredoxin	-1.51
267196_at	At2g30950	FtsH protease (VAR2)	-1.51
252978_at	At4g38590	glycosyl hydrolase family 35 protein	-1.51
249519_at	At5g38660	expressed protein	-1.51
248906_at	At5g46420	16S rRNA processing protein RimM family	-1.51
257611_at	At3g26580	expressed protein	-1.51
249109_at	At5g43700	auxin-responsive protein (AUX2-11)	-1.52

250649_at	At5g06690	thioredoxin	-1.52
267511_at	At2g45670	calcineurin B subunit-related	-1.52
253009_at	At4g37930	glycine hydroxymethyltransferase (SHM1)	-1.52
247543_at	At5g61600	ethylene-responsive element-binding	-1.52
263000_at	At1g54350	ABC transporter	-1.52
262127_at	At1g52550	expressed protein	-1.52
259379_at	At3g16350	myb family transcription factor	-1.52
259181_at	At3g01690	expressed protein	-1.52
266207_at	At2g27680	aldo/keto reductase	-1.52
256058_at	At1g07240	UDP-glucoronosyl/UDP-glucosyl transferase	-1.52
260730_at	At1g48030	dihydrolipoamide dehydrogenase 1, mitochondrial (MTLPD1)	-1.52
258963_at	At3g10550	expressed protein	-1.52
258708_at	At3g09580	amine oxidase	-1.52
247386_at	At5g63430	metallo-beta-lactamase	-1.52
259858_at	At1g68400	leucine-rich repeat transmembrane protein kinase, putative	-1.52
250884_at	At5g03940	signal recognition particle 54 kDa protein, chloroplast (FFC)	-1.52
246053_at	At5g08340	riboflavin biosynthesis protein-related	-1.52
265392_at	At2g20860	lipoic acid synthase (LIP1)	-1.52
261211_at	At1g12780	UDP-glucose 4-epimerase	-1.53
250058_at	At5g17870	plastid-specific ribosomal protein-related	-1.53
262503_at	At1g21670	expressed protein	-1.53
262065_at	At1g56180	expressed protein	-1.53
246154_at	At5g19940	plastid-lipid associated protein PAP-related	-1.53
266099_at	At2g38040	acetyl co-enzyme A carboxylase carboxyltransferase alpha subunit family	-1.53
258149_at	At3g18110	pentatricopeptide (PPR) repeat-containing protein	-1.53
257706_at	At3g12685	expressed protein	-1.53
259308_at	At3g05180	GDSL-motif lipase/hydrolase	-1.53
247126_at	At5g66080	protein phosphatase 2C (PP2C)	-1.53
261534_at	At1g01820	peroxisomal biogenesis factor 11 (PEX11)	-1.53
253858_at	At4g27600	pfkB-type carbohydrate kinase	-1.53
265790_at	At2g01170	amino acid permease	-1.53
258782_at	At3g11750	dihydroneopterin aldolase, putative	-1.53
267471_at	At2g30390	ferrochelatase II	-1.53
264328_at	At1g04100	indoleacetic acid-induced protein 10 (IAA10)	-1.53
246194_at	At4g37000	accelerated cell death 2 (ACD2)	-1.53
260638_at	At1g62390	Bem1p (PB1) domain-containing protein	-1.53
250218_at	At5g14170	SWIB complex BAF60b domain-containing protein	-1.53
262600_at	At1g15340	methyl-CpG-binding domain-containing protein	-1.53
253823_at	At4g28030	GCN5-related N-acetyltransferase (GNAT)	-1.53
261206_at	At1g12800	S1 RNA-binding domain-containing protein	-1.54
257519_at	At3g01210	RNA recognition motif (RRM)-containing protein	-1.54
246311_at	At3g51880	high mobility group protein alpha (HMGalpha)	-1.54
261507_at	At1g71720	S1 RNA-binding domain-containing protein	-1.54
255456_at	At4g02920	expressed protein	-1.54

263947_at	At2g35820	expressed protein	-1.54
253517_at	At4g31390	ABC1	-1.54
256653_at	At3g18870	mitochondrial transcription termination factor-related	-1.54
249810_at	At5g23920	expressed protein	-1.54
245532_at	At4g15110	cytochrome P450 97B3, putative (CYP97B3)	-1.54
252391_at	At3g47860	apolipoprotein D-related	-1.54
264781_at	At1g08540	RNA polymerase sigma subunit SigB (sigB)	-1.54
251193_at	At3g62910	peptide chain release factor, putative	-1.54
258797_at	At3g04730	indoleacetic acid-induced protein 16 (IAA16)	-1.54
264963_at	At1g60600	UbiA prenyltransferase	-1.54
247813_at	At5g58330	malate dehydrogenase (NADP) putative	-1.54
255078_at	At4g09010	L-ascorbate peroxidase putative	-1.54
253477_at	At4g32320	peroxidase	-1.54
261272_at	At1g26665	expressed protein	-1.54
264841_at	At1g03740	protein kinase	-1.55
263889_at	At2g37010	ABC transporter	-1.55
259523_at	At1g12500	phosphate translocator-related	-1.55
247637_at	At5g60600	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase, putative	-1.55
248404_at	At5g51460	trehalose-6-phosphate phosphatase (TPPA)	-1.55
261728_at	At1g76160	multi-copper oxidase type I	-1.55
258920_at	At3g10520	non-symbiotic hemoglobin 2 (HB2)	-1.55
251987_at	At3g53280	cytochrome P450 71B5 (CYP71B5)	-1.55
249849_at	At5g23230	isochorismatase hydrolase	-1.55
263460_at	At2g31810	acetolactate synthase small subunit, putative	-1.55
260783_at	At1g06160	ethylene-responsive factor, putative	-1.55
266402_at	At2g38780	expressed protein	-1.55
245229_at	At4g25620	hydroxyproline-rich glycoprotein	-1.55
250128_at	At5g16540	zinc finger (CCCH-type)	-1.55
244979_at	rps11	rps11	-1.55
261559_at	At1g01780	LIM domain-containing protein	-1.55
252993_at	At4g38540	monooxygenase, putative (MO2)	-1.55
261570_at	At1g01120	fatty acid elongase 3-ketoacyl-CoA synthase 1 (KCS1)	-1.56
250378_at	At5g11570	proton-dependent oligopeptide transport (POT)	-1.56
247613_at	At5g60740	ABC transporter	-1.56
255625_at	At4g01120	G-box binding factor 2 (GBF2)	-1.56
246999_at	At5g67440	phototropic-responsive NPH3	-1.56
248547_at	At5g50280	pentatricopeptide (PPR) repeat-containing protein	-1.56
259980_at	At1g76520	auxin efflux carrier	-1.56
249007_at	At5g44650	expressed protein	-1.56
267581_at	At2g41980	seven in absentia (SINA)	-1.56
258081_at	At3g26085	CAAX amino terminal protease	-1.56
258923_at	At3g10450	serine carboxypeptidase S10	-1.56
246633_at	At1g29720	protein kinase	-1.56
255866_at	At2g30350	endo/excinuclease amino terminal domain-containing protein	-1.56

246612_at	At5g35320	expressed protein	-1.56
257965_at	At3g19900	expressed protein	-1.56
248899_at	At5g46390	peptidase S41 (ctpA)	-1.56
250061_at	At5g17710	co-chaperone grpE	-1.56
261118_at	At1g75460	ATP-dependent protease La (LON) domain-containing protein	-1.56
256088_at	At1g20810	immunophilin	-1.56
255482_at	At4g02510	chloroplast outer membrane protein, putative	-1.56
252272_at	At3g49670	leucine-rich repeat transmembrane protein kinase, putative	-1.57
260431_at	At1g68190	zinc finger (B-box type)	-1.57
258494_at	At3g02450	cell division protein ftsH, putative	-1.57
258375_at	At3g17470	RelA/SpoT domain-containing protein	-1.57
255635_at	At4g00720	shaggy-related protein kinase theta (ASK8)	-1.57
250730_at	At5g06490	zinc finger (C3HC4-type RING finger)	-1.57
247139_at	At5g66090	expressed protein	-1.57
256223_at	At1g56200	expressed protein	-1.57
251120_at	At3g63490	ribosomal protein L1	-1.57
245278_at	At4g17730	syntaxin 23 (SYP23)	-1.57
257769_at	At3g23050	indoleacetic acid-induced protein 7 (IAA7)	-1.57
266971_at	At2g39580	expressed protein	-1.57
259591_at	At1g28150	expressed protein	-1.57
256655_at	At3g18890	expressed protein	-1.57
262712_at	At1g16460	mercaptopyruvate sulfurtransferase (MST2)	-1.57
245593_at	At4g14550	auxin-responsive AUX/IAA	-1.58
266120_at	At2g02070	zinc finger (C2H2 type)	-1.58
250694_at	At5g06710	homeobox-leucine zipper protein 14 (HAT14)	-1.58
264901_at	At1g23090	sulfate transporter, putative	-1.58
256728_at	At3g25660	glutamyl-tRNA(Gln) amidotransferase, putative	-1.58
258927_at	At3g10160	folylpolyglutamate synthetase (DHFS/FPGS3)	-1.58
255440_at	At4g02530	chloroplast thylakoid lumen protein	-1.58
264844_at	At1g03520	glycosyltransferase family 14 protein	-1.58
262693_at	At1g62780	expressed protein	-1.58
265673_at	At2g32090	lactoylglutathione lyase	-1.58
266882_at	At2g44670	senescence-associated protein-related	-1.58
263420_at	At2g17240	expressed protein	-1.58
258281_at	At3g26900	shikimate kinase	-1.58
246158_at	At5g19855	expressed protein	-1.58
251701_at	At3g56650	thylakoid lumenal 20 kDa protein	-1.58
251227_at	At3g62700	glutathione-conjugate transporter, putative	-1.58
264985_at	At1g27150	expressed protein	-1.58
266922_s_at	At2g45950	SKP1	-1.58
265918_at	At2g15090	fatty acid elongase, putative	-1.58
263556_at	At2g16350	F-box	-1.58
266224_at	At2g28800	chloroplast membrane protein (ALBINO3)	-1.58
252975_s_at	At4g38430	expressed protein	-1.58

251117_at	At3g63390	expressed protein	-1.58
251122_at	At5g01020	protein kinase	-1.58
261611_at	At1g49730	protein kinase	-1.59
255193_at	At4g07400	F-box (FBL8)	-1.59
253251_at	At4g34730	ribosome-binding factor A	-1.59
261519_at	At1g71810	ABC1	-1.59
259859_at	At1g68410	protein phosphatase 2C-related	-1.59
247130_at	At5g66180	expressed protein	-1.59
244975_at	psbH	psbH	-1.59
263699_at	At1g31120	potassium transporter	-1.59
252659_at	At3g44430	expressed protein	-1.59
255830_at	At2g33340	transducin	-1.59
263980_at	At2g42770	peroxisomal membrane 22 kDa	-1.59
256115_at	At1g16880	uridylyltransferase-related	-1.59
251744_at	At3g56010	expressed protein	-1.59
257773_at	At3g29185	expressed protein	-1.59
250371_at	At5g11450	oxygen-evolving complex-related	-1.59
247216_at	At5g64860	4-alpha-glucanotransferase, putative	-1.59
246308_at	At3g51820	chlorophyll synthetase, putative	-1.59
264300_at	At1g78670	gamma-glutamyl hydrolase, putative	-1.59
245020_at	petA	petA	-1.6
267549_at	At2g32640	expressed protein	-1.6
254041_at	At4g25830	integral membrane	-1.6
247979_at	At5g56750	Ndr	-1.6
245668_at	At1g28330	dormancy-associated protein, putative (DRM1)	-1.6
255088_at	At4g09350	DNAJ heat shock N-terminal domain-containing protein	-1.6
263763_at	At2g21385	expressed protein	-1.6
257647_at	At3g25805	expressed protein	-1.6
248459_at	At5g51020	expressed protein	-1.6
252409_at	At3g47650	bundle-sheath defective protein 2 family	-1.6
247320_at	At5g64040	photosystem I reaction center subunit PSI-N, chloroplast, putative (PSAN)	-1.6
257966_at	At3g19800	expressed protein	-1.6
267061_at	At2g32480	membrane-associated zinc metalloprotease, putative	-1.6
262505_at	At1g21680	expressed protein	-1.6
259118_at	At3g01310	expressed protein	-1.6
249224_at	At5g42130	mitochondrial substrate carrier	-1.6
256530_at	At1g33290	sporulation protein-related	-1.6
267088_at	At2g38140	chloroplast 30S ribosomal protein S31 (PSRP4)	-1.6
247889_at	At5g57930	expressed protein	-1.6
245244_at	At1g44350	IAA-amino acid hydrolase 6, putative (ILL6)	-1.61
246011_at	At5g08330	TCP family transcription factor, putative	-1.61
254370_at	At4g21750	L1 specific homeobox gene (ML1)	-1.61
262539_at	At1g17200	integral membrane	-1.61
256873_at	At3g26310	cytochrome P450	-1.61

249583_at	At5g37770	calmodulin-related protein 2, touch-induced (TCH2)	-1.61
258800_at	At3g04550	expressed protein	-1.61
255077_at	At4g09150	T-complex protein 11	-1.61
258468_at	At3g06070	expressed protein	-1.61
252412_at	At3g47295	expressed protein	-1.61
263442_at	At2g28605	expressed protein	-1.61
257666_at	At3g20270	lipid-binding serum glycoprotein	-1.61
245561_at	At4g15500	UDP-glucoronosyl/UDP-glucosyl transferase	-1.61
250293_s_at	At5g13360	auxin-responsive GH3	-1.61
262089_s_at	At1g55980	expressed protein	-1.61
261081_at	At1g07350	transformer serine/arginine-rich ribonucleoprotein, putative	-1.61
260089_at	At1g73170	expressed protein	-1.61
251391_at	At3g60910	expressed protein	-1.61
265495_at	At2g15695	expressed protein	-1.62
262355_at	At1g72820	mitochondrial substrate carrier	-1.62
267477_at	At2g02710	PAC motif-containing protein	-1.62
264700_at	At1g70100	expressed protein	-1.62
258133_at	At3g24500	ethylene-responsive transcriptional coactivator, putative	-1.62
266219_at	At2g28880	para-aminobenzoate (PABA) synthase	-1.62
259316_at	At3g01175	expressed protein	-1.62
261635_at	At1g50020	expressed protein	-1.62
262884_at	At1g64720	expressed protein	-1.62
254580_at	At4g19390	expressed protein	-1.62
257895_at	At3g16950	lipoamide dehydrogenase 1 (PTLPD1)	-1.62
244950_at	cox2	cox2	-1.62
260872_at	At1g21350	expressed protein	-1.62
263726_at	At2g13610	ABC transporter	-1.62
262287_at	At1g68660	expressed protein	-1.62
251969_at	At3g53130	cytochrome P450	-1.62
265472_at	At2g15580	zinc finger (C3HC4-type RING finger)	-1.62
263374_at	At2g20560	DNAJ heat shock	-1.62
254298_at	At4g22890	expressed protein	-1.62
267294_at	At2g23670	expressed protein	-1.62
252957_at	At4g38680	cold-shock DNA-binding	-1.62
263755_at	At2g21340	salicylic acid induction deficient protein, putative	-1.63
259625_at	At1g42970	glyceraldehyde-3-phosphate dehydrogenase B, chloroplast (GAPB)	-1.63
257800_at	At3g15900	expressed protein	-1.63
256700_at	At3g52260	pseudouridine synthase	-1.63
245397_at	At4g14560	indoleacetic acid-induced protein 1 (IAA1)	-1.63
256697_at	At3g20660	organic cation transporter	-1.63
264930_at	At1g60800	leucine-rich repeat	-1.63
245449_at	At4g16870	At4g16870	-1.63
254117_at	At4g24750	expressed protein	-1.63
251328_at	At3g61600	BTB/POZ domain-containing protein	-1.63

254545_at	At4g19830	immunophilin	-1.63
263471_at	At2g31890	expressed protein	-1.63
264673_at	At1g09795	ATP phosphoribosyl transferase 2 (ATP-PRT2)	-1.63
256156_at	At3g08510	phosphoinositide-specific phospholipase C (PLC2)	-1.63
254187_at	At4g23890	expressed protein	-1.63
252325_at	At3g48560	acetohydroxy-acid synthase (ALS)	-1.63
257171_at	At3g23760	expressed protein	-1.63
246284_at	At4g36780	brassinosteroid signalling positive regulator-related	-1.63
250262_at	At5g13410	immunophilin	-1.63
262598_at	At1g15260	expressed protein	-1.64
253585_at	At4g30720	expressed protein	-1.64
256458_at	At1g75220	integral membrane protein, putative	-1.64
251330_at	At3g61550	zinc finger (C3HC4-type RING finger)	-1.64
247734_at	At5g59400	expressed protein	-1.64
244982_at	rpl14	rpl14	-1.64
249775_at	At5g24160	squalene monooxygenase 1,2 (SQP1,2)	-1.64
257288_at	At3g29670	transferase	-1.64
246509_at	At5g16715	tRNA synthetase class I (I, L, M and V)	-1.64
246654_s_at	At5g35210	peptidase M50	-1.64
251395_at	At2g45470	fasciclin-like arabinogalactan-protein (FLA8)	-1.64
264709_at	At1g09770	myb family transcription factor	-1.64
254068_at	At4g25450	ABC transporter	-1.64
258156_at	At3g18050	expressed protein	-1.64
247783_at	At5g58800	quinone reductase	-1.64
245347_at	At4g14890	ferredoxin	-1.64
263846_at	At2g36990	RNA polymerase sigma subunit SigF (sigF)	-1.64
260388_at	At1g74070	peptidyl-prolyl cis-trans isomerase cyclophilin-type	-1.64
264437_at	At1g27510	expressed protein	-1.64
250016_at	At5g18100	copper/zinc superoxide dismutase (CSD3)	-1.64
254999_at	At4g09830	expressed protein	-1.64
250867_at	At5g03880	expressed protein	-1.64
256982_at	At3g13460	expressed protein	-1.64
259822_at	At1g66230	myb family transcription factor (MYB20)	-1.65
247439_at	At5g62670	ATPase, plasma membrane-type, putative	-1.65
265321_at	At2g18280	TULP2: tubby-like protein 2 (TULP2)	-1.65
259499_at	At1g15730	PRLI-interacting factor L, putative	-1.65
261873_at	At1g11350	S-locus lectin protein kinase	-1.65
261732_at	At1g47770	hypothetical protein	-1.65
248285_at	At5g52960	expressed protein	-1.65
266608_at	At2g35500	shikimate kinase-related	-1.65
251516_s_at	At3g59310	expressed protein	-1.65
253342_at	At4g33520	metal-transporting P-type ATPase, putative (PAA1)	-1.65
251118_at	At3g63410	chloroplast inner envelope membrane protein, putative (APG1)	-1.65
250824_at	At5g05200	ABC1	-1.65

254126_at	At4g24770	RNA-binding protein cp31, putative	-1.65
261351_at	At1g79790	haloacid dehalogenase-like hydrolase	-1.65
245790_at	At1g32200	glycerol-3-phosphate acyltransferase, chloroplast (ATS1)	-1.65
246319_at	At3g56680	expressed protein	-1.65
262489_at	At1g21830	expressed protein	-1.66
257236_at	At3g15095	expressed protein	-1.66
255522_at	At4g02260	RelA/SpoT protein, putative (RSH1)	-1.66
251720_at	At3g56160	expressed protein	-1.66
250267_at	At5g12930	expressed protein	-1.66
258038_at	At3g21260	glycolipid transfer protein-related	-1.66
257485_at	At1g63580	protein kinase-related	-1.66
255779_at	At1g18650	glycosyl hydrolase 17	-1.66
261821_at	At1g11530	thioredoxin	-1.66
248104_at	At5g55250	S-adenosyl-L-methionine:carboxyl methyltransferase	-1.66
261629_at	At1g49980	UMUC-like DNA repair	-1.66
262634_at	At1g06690	aldo/keto reductase	-1.66
260685_at	At1g17650	6-phosphogluconate dehydrogenase NAD-binding domain-containing protein	-1.66
267397_at	At1g76170	expressed protein	-1.66
254105_at	At4g25080	magnesium-protoporphyrin O-methyltransferase, putative	-1.66
246005_at	At5g08415	lipoic acid synthase	-1.66
262648_at	At1g14030	ribulose-1,5 bisphosphate carboxylase oxygenase large subunit N-methyltransferase, putative	-1.66
261295_at	At1g48450	expressed protein	-1.66
250886_at	At5g04440	expressed protein	-1.66
265494_at	At2g15680	calmodulin-related protein, putative	-1.67
262190_at	At1g78030	expressed protein	-1.67
251225_at	At3g62660	glycosyl transferase family 8 protein	-1.67
251491_at	At3g59480	pfkB-type carbohydrate kinase	-1.67
257235_at	At3g15060	Ras-related GTP-binding	-1.67
262370_at	At1g73090	expressed protein	-1.67
256383_at	At1g66820	glycine-rich protein	-1.67
246069_at	At5g20220	zinc knuckle (CCHC-type)	-1.67
248502_at	At5g50450	zinc finger (MYND type)	-1.67
246748_at	At5g27730	expressed protein	-1.67
253391_at	At4g32590	ferredoxin-related	-1.67
264307_at	At1g61900	expressed protein	-1.67
267606_at	At2g26640	beta-ketoacyl-CoA synthase, putative	-1.67
256516_at	At1g66150	leucine-rich repeat protein kinase, putative (TMK1)	-1.68
256672_at	At3g52310	ABC transporter	-1.68
264371_at	At1g12090	protease inhibitor/seed storage/lipid transfer protein (LTP)	-1.68
256757_at	At3g25620	ABC transporter	-1.68
258745_at	At3g05920	heavy-metal-associated domain-containing protein	-1.68
245632_at	At1g25290	rhomboid	-1.68
256542_at	At1g42550	expressed protein	-1.68

246702_at	At5g28050	cytidine/deoxycytidylate deaminase	-1.68
265024_at	At1g24600	expressed protein	-1.68
248283_at	At5g52920	pyruvate kinase, putative	-1.68
255692_at	At4g00400	phospholipid/glycerol acyltransferase	-1.68
249785_at	At5g24300	starch synthase, putative	-1.68
249900_at	At5g22640	MORN (Membrane Occupation and Recognition Nexus) repeat-containing protein	-1.68
266045_s_at	At2g07727	cytochrome b (MTCYB)	-1.68
245948_at	At5g19540	expressed protein	-1.68
258607_at	At3g02730	thioredoxin, putative	-1.68
256792_at	At3g22150	pentatricopeptide (PPR) repeat-containing protein	-1.68
252724_at	At3g43540	expressed protein	-1.68
246110_at	At5g20140	SOUL heme-binding	-1.68
262172_at	At1g74970	ribosomal protein S9 (RPS9)	-1.68
248235_at	At5g53860	expressed protein	-1.68
246547_at	At5g14970	expressed protein	-1.68
253624_at	At4g30580	phospholipid/glycerol acyltransferase	-1.68
250369_at	At5g11300	cyclin, putative (CYC3b)	-1.69
257563_at	At3g19610	hypothetical protein	-1.69
267027_at	At2g38330	MATE efflux	-1.69
263912_at	At2g36390	starch branching enzyme class II (SBE2-1)	-1.69
247044_at	At5g66850	protein kinase	-1.69
262954_at	At1g54500	rubredoxin	-1.69
253412_at	At4g33000	calcineurin B-like protein 10 (CBL10)	-1.69
245797_at	At1g45261	defective chloroplasts and leaves protein-related (DCL)	-1.69
258472_at	At3g06080	expressed protein	-1.69
251610_at	At3g57930	expressed protein	-1.69
256324_at	At1g66760	MATE efflux	-1.7
258565_at	At3g04350	expressed protein	-1.7
248336_at	At5g52420	expressed protein	-1.7
254635_at	At4g18670	leucine-rich repeat	-1.7
262845_at	At1g14740	expressed protein	-1.7
265680_at	At2g32150	haloacid dehalogenase-like hydrolase	-1.7
257672_at	At3g20300	expressed protein	-1.7
264837_at	At1g03600	photosystem II	-1.7
255926_at	At1g22190	AP2 domain-containing transcription factor, putative	-1.7
253476_at	At4g32300	lectin protein kinase	-1.7
249978_at	At5g18850	expressed protein	-1.7
245000_at	ycf6	ycf6	-1.7
266267_at	At2g29460	glutathione S-transferase, putative	-1.71
261633_at	At1g49930	hypothetical protein	-1.71
267635_at	At2g42220	rhodanese-like domain-containing protein	-1.71
263198_at	At1g53990	GDSL-motif lipase/hydrolase	-1.71
246222_at	At4g36900	AP2 domain-containing protein RAP2.10 (RAP2.10)	-1.71
262797_at	At1g20840	transporter-related	-1.71

266319_s_at	At2g46720	fatty acid elongase 3-ketoacyl-CoA synthase, putative	-1.71
253688_at	At4g29590	expressed protein	-1.71
261924_at	At1g22550	proton-dependent oligopeptide transport (POT)	-1.71
254770_at	At4g13340	leucine-rich repeat	-1.71
246432_at	At5g17490	gibberellin response modulator, putative	-1.71
258929_at	At3g10060	immunophilin, putative	-1.71
255623_at	At4g01310	ribosomal protein L5	-1.71
260967_at	At1g12230	transaldolase, putative	-1.71
253302_at	At4g33660	expressed protein	-1.71
247525_at	At5g61380	ABI3-interacting protein 1 (AIP1)	-1.71
261577_at	At1g01080	33 kDa ribonucleoprotein, chloroplast, putative	-1.71
248669_at	At5g48730	pentatricopeptide (PPR) repeat-containing protein	-1.72
251670_at	At3g57190	peptide chain release factor, putative	-1.72
244981_at	rps8	rps8	-1.72
245761_at	At1g66890	expressed protein	-1.72
265675_at	At2g32120	heat shock protein 70 (HSP70)	-1.72
249542_at	At5g38140	histone-like transcription factor (CBF/NF-Y)	-1.72
246170_at	At5g32450	RNA recognition motif (RRM)-containing protein	-1.72
253548_at	At4g30993	expressed protein	-1.72
255617_at	At4g01330	protein kinase	-1.72
264177_at	At1g02150	pentatricopeptide (PPR) repeat-containing protein	-1.72
246159_at	At5g20935	expressed protein	-1.72
253160_at	At4g35760	expressed protein	-1.72
267644_s_at	At2g32880	meprin and TRAF homology domain-containing protein	-1.73
258269_at	At3g15690	biotin carboxyl carrier protein of acetyl-CoA carboxylase-related	-1.73
253335_at	At4g33500	protein phosphatase 2C-related	-1.73
264528_at	At1g30810	zinc finger (C5HC2 type)	-1.73
260134_at	At1g66370	myb family transcription factor (MYB113)	-1.73
245383_at	At4g17810	zinc finger (C2H2 type)	-1.73
254638_at	At4g18740	expressed protein	-1.73
245370_at	At4g16840	expressed protein	-1.73
262114_at	At1g02860	SPX domain-containing protein	-1.73
258087_at	At3g26060	peroxiredoxin Q, putative	-1.73
255982_at	At1g34000	light stress-responsive one-helix protein (OHP2)	-1.73
253758_at	At4g29060	elongation factor Ts	-1.73
249101_at	At5g43580	protease inhibitor, putative	-1.73
266483_at	At2g47910	expressed protein	-1.73
248578_at	At5g49820	expressed protein	-1.73
262202_at	At2g01110	thylakoid membrane formation protein (APG2)	-1.73
249125_at	At5g43450	2-oxoglutarate-dependent dioxygenase, putative	-1.74
248075_at	At5g55740	pentatricopeptide (PPR) repeat-containing protein	-1.74
258359_s_at	At3g14415	(S)-2-hydroxy-acid oxidase, peroxisomal, putative	-1.74
248622_at	At5g49360	glycosyl hydrolase family 3 protein	-1.74
255436_at	At4g03150	expressed protein	-1.74

252132_at	At3g50790	late embryogenesis abundant protein, putative (LEA)	-1.74
255059_at	At4g09420	disease resistance protein (TIR-NBS class), putative	-1.74
246408_at	At1g57680	expressed protein	-1.74
256440_at	At3g10970	haloacid dehalogenase-like hydrolase	-1.74
252473_s_at	At3g46610	pentatricopeptide (PPR) repeat-containing protein	-1.74
261622_at	At1g01970	pentatricopeptide (PPR) repeat-containing protein	-1.74
267130_at	At2g23390	expressed protein	-1.75
245885_at	At5g09440	phosphate-responsive protein, putative	-1.75
246200_at	At4g37240	expressed protein	-1.75
261583_at	At1g01090	pyruvate dehydrogenase E1 component alpha subunit, chloroplast	-1.75
260567_at	At2g43820	UDP-glucoronosyl/UDP-glucosyl transferase	-1.75
252859_at	At4g39780	AP2 domain-containing transcription factor, putative	-1.75
257723_at	At3g18500	nocturnin-related	-1.75
262322_at	At1g27590	expressed protein	-1.75
264158_at	At1g65260	PspA/IM30	-1.75
256595_x_at	At3g28530	expressed protein	-1.75
260465_at	At1g10910	pentatricopeptide (PPR) repeat-containing protein	-1.75
253337_at	At4g33470	histone deacetylase	-1.75
267152_at	At2g31040	ATP synthase protein I -related	-1.75
260856_at	At1g21910	AP2 domain-containing transcription factor	-1.75
248398_at	At5g51970	sorbitol dehydrogenase, putative	-1.75
260542_at	At2g43560	immunophilin	-1.75
264920_at	At1g60550	naphthoate synthase, putative	-1.75
253028_at	At4g38160	mitochondrial transcription termination factor-related	-1.75
266570_at	At2g24090	ribosomal protein L35	-1.76
263391_at	At2g11810	1,2-diacylglycerol 3-beta-galactosyltransferase, putative	-1.76
248516_at	At5g50540	expressed protein	-1.76
248585_at	At5g49640	expressed protein	-1.76
245399_at	At4g17340	major intrinsic protein (MIP)	-1.76
265974_at	At2g11260	Hypothetical protein, complete cds, clone: RAFL16-43-P18	-1.76
259965_at	At1g53670	transcription factor-related	-1.76
267005_at	At2g34460	flavin reductase-related	-1.76
264435_at	At1g10360	glutathione S-transferase, putative	-1.76
267505_at	At2g45560	cytochrome P450	-1.76
262168_at	At1g74730	expressed protein	-1.76
265966_at	At2g37220	29 kDa ribonucleoprotein, chloroplast, putative	-1.76
261666_at	At1g18440	peptidyl-tRNA hydrolase	-1.77
259970_at	At1g76570	chlorophyll A-B binding	-1.77
249383_at	At5g39860	bHLH protein	-1.77
264037_at	At2g03750	sulfotransferase	-1.77
258742_at	At3g05800	expressed protein	-1.77
265741_at	At2g01320	ABC transporter	-1.77
256698_at	At3g20680	expressed protein	-1.77
245357_at	At4g17560	ribosomal protein L19	-1.77

252563_at	At3g45970	expansin (EXPL1)	-1.77
265021_at	At1g24610	SET domain-containing protein	-1.77
266673_at	At2g29630	thiamine biosynthesis	-1.78
260015_at	At1g67980	caffeoyl-CoA 3-O-methyltransferase, putative	-1.78
245015_at	rbcL	rbcL	-1.78
266636_at	At2g35370	glycine cleavage system H protein 1 (GDCSH)	-1.78
265022_at	At1g24520	anther-specific protein agp1	-1.78
244977_at	petD	petD	-1.78
250906_at	At5g03650	starch branching enzyme class II (SBE2-2)	-1.78
253076_at	At4g36160	no apical meristem (NAM)	-1.78
267201_at	At2g31010	protein kinase	-1.78
261078_at	At1g07320	50S ribosomal protein L4, chloroplast (CL4)	-1.78
248798_at	At5g47190	ribosomal protein L19	-1.78
262850_at	At1g14920	gibberellin response modulator (GAI)	-1.78
254727_at	At4g13670	peptidoglycan-binding domain-containing protein	-1.78
265967_at	At2g37450	nodulin MtN21	-1.78
248402_at	At5g52100	dihydrodipicolinate reductase	-1.78
247034_at	At5g67260	cyclin	-1.78
255082_at	At4g09160	SEC14 cytosolic factor	-1.79
259511_at	At1g12520	superoxide dismutase copper chaperone, putative	-1.79
257794_at	At3g27050	expressed protein	-1.79
246001_at	At5g20790	expressed protein	-1.79
256836_at	At3g22960	pyruvate kinase, putative	-1.79
250668_at	At5g07020	proline-rich	-1.79
265394_at	At2g20725	CAAX amino terminal protease	-1.79
260367_at	At1g69760	expressed protein	-1.79
255540_at	At4g01800	preprotein translocase secA subunit, putative	-1.79
251820_at	At3g55040	In2-1 protein, putative	-1.79
247347_at	At5g63780	zinc finger (C3HC4-type RING finger)	-1.79
261767_s_at	At1g15500	chloroplast ADP, ATP carrier protein, putative	-1.79
255447_at	At4g02790	GTP-binding	-1.79
253537_at	At4g31560	expressed protein	-1.79
249869_at	At5g23050	acyl-activating enzyme 17 (AAE17)	-1.79
256754_at	At3g25690	hydroxyproline-rich glycoprotein	-1.79
252979_at	At4g38225	expressed protein	-1.79
256856_at	At3g15110	expressed protein	-1.79
253208_at	At4g34820	pentatricopeptide (PPR) repeat-containing protein	-1.8
251195_at	At3g62930	glutaredoxin	-1.8
262115_at	At1g02813	expressed protein	-1.8
262566_at	At1g34310	transcriptional factor B3	-1.8
258263_at	At3g15780	expressed protein	-1.8
265417_at	At2g20920	expressed protein	-1.8
264041_at	At2g03710	MADS-box protein (AGL3)	-1.8
258860_at	At3g02050	potassium transporter (KUP3)	-1.8
265724_at	At2g32100	ovate protein-related	-1.8

254794_at	At4g12970	expressed protein	-1.8
266460_at	At2g47930	hydroxyproline-rich glycoprotein	-1.8
265175_at	At1g23480	glycosyl transferase family 2 protein	-1.8
262368_at	At1g73060	expressed protein	-1.8
264424_at	At1g61740	expressed protein	-1.8
259983_at	At1g76490	3-hydroxy-3-methylglutaryl-CoA reductase 1 (HMG1)	-1.8
249899_at	At5g22620	phosphoglycerate/bisphosphoglycerate mutase	-1.8
253322_at	At4g33980	expressed protein	-1.8
264508_at	At1g09570	phytochrome A (PHYA)	-1.81
261926_at	At1g22530	SEC14 cytosolic factor	-1.81
248606_at	At5g49450	bZIP family transcription factor	-1.81
266251_s_at	At2g27540	expressed protein	-1.81
245047_at	psbA	psbA	-1.81
263674_at	At2g04790	expressed protein	-1.81
251719_at	At3g56140	expressed protein	-1.81
247100_at	At5g66520	pentatricopeptide (PPR) repeat-containing protein	-1.81
245388_at	At4g16410	expressed protein	-1.81
250503_at	At5g09820	plastid-lipid associated protein PAP	-1.81
263048_s_at	At2g05310	expressed protein	-1.81
265454_at	At2g46530	transcriptional factor B3	-1.81
246847_at	At5g26820	ferroportin-related	-1.81
244973_at	psbT	psbT	-1.81
262369_at	At1g73010	expressed protein	-1.81
249148_at	At5g43260	chaperone protein dnaJ-related	-1.82
245726_at	At1g73360	homeobox-leucine zipper	-1.82
266892_at	At2g26080	glycine dehydrogenase (decarboxylating), putative	-1.82
245925_at	At5g28770	bZIP transcription factor	-1.82
261518_at	At1g71695	peroxidase 12 (PER12)	-1.82
247880_at	At5g57780	expressed protein	-1.82
245798_at	At1g45545	hypothetical protein	-1.82
261265_at	At1g26800	zinc finger (C3HC4-type RING finger)	-1.82
250268_s_at	At5g12950	expressed protein	-1.82
257033_at	At3g19170	peptidase	-1.82
246075_at	At5g20410	1,2-diacylglycerol 3-beta-galactosyltransferase, putative	-1.82
263111_s_at	At1g65190	protein kinase	-1.82
252215_at	At3g50240	kinesin motor protein-related	-1.83
257253_at	At3g24190	ABC1	-1.83
246028_at	At5g21170	5'-AMP-activated protein kinase beta-2 subunit, putative	-1.83
264483_at	At1g77230	AMP-binding protein, putative	-1.83
244901_at	orf25	orf25	-1.83
264575_at	At1g05190	ribosomal protein L6	-1.83
262377_at	At1g73110	ribulose bisphosphate carboxylase/oxygenase activase, putative	-1.83
246237_at	At4g36390	radical SAM domain-containing protein	-1.83
261338_at	At1g44920	expressed protein	-1.83

256417_s_at	At3g11170	omega-3 fatty acid desaturase, chloroplast (FAD7)	-1.83
260840_at	At1g29050	expressed protein	-1.84
245794_at	At1g32170	xyloglucan:xyloglucosyl transferase, putative (XTR4)	-1.84
265400_at	At2g10940	protease inhibitor/seed storage/lipid transfer protein (LTP)	-1.84
251421_at	At3g60510	enoyl-CoA hydratase	-1.84
245005_at	rps14	rps14	-1.84
244965_at	ORF31	ORF31	-1.84
244936_at	ndhA	ndhA	-1.84
247201_at	At5g65220	ribosomal protein L29	-1.84
256525_at	At1g66180	aspartyl protease	-1.84
254413_at	At4g21440	myb family transcription factor (MYB102)	-1.84
258622_at	At3g02720	DJ-1	-1.84
251031_at	At5g02120	thylakoid membrane one helix protein (OHP)	-1.84
252411_at	At3g47430	peroxisomal biogenesis factor 11 (PEX11)	-1.84
247925_at	At5g57560	xyloglucan:xyloglucosyl transferase (TCH4)	-1.85
246843_at	At5g26734	expressed protein	-1.85
264280_at	At1g61820	glycosyl hydrolase family 1 protein	-1.85
263606_at	At2g16280	very-long-chain fatty acid condensing enzyme, putative	-1.85
250189_at	At5g14410	expressed protein	-1.85
266925_at	At2g45740	peroxisomal biogenesis factor 11 (PEX11)	-1.85
244940_at	rps12.2	rps12.2	-1.85
266165_at	At2g28190	copper/zinc superoxide dismutase (CSD2)	-1.85
251755_at	At3g55790	expressed protein	-1.85
248409_at	At5g51540	peptidase M3	-1.85
254553_at	At4g19530	disease resistance protein (TIR-NBS-LRR class), putative	-1.85
249876_at	At5g23060	expressed protein	-1.85
256168_at	At1g51805	leucine-rich repeat protein kinase, putative	-1.85
248242_at	At5g53580	aldo/keto reductase	-1.85
249244_at	At5g42270	FtsH protease, putative	-1.85
259193_at	At3g01480	peptidyl-prolyl cis-trans isomerase, putative	-1.85
255289_at	At4g04690	F-box (FBX15)	-1.86
245010_at	ndhJ	ndhJ	-1.86
254424_at	At4g21510	F-box	-1.86
247745_at	At5g59030	copper transporter 1 (COPT1)	-1.86
254985_x_at	At4g10580	gypsy-like retrotransposon family	-1.86
260041_at	At1g68780	leucine-rich repeat	-1.86
259839_at	At1g52190	proton-dependent oligopeptide transport (POT)	-1.86
261488_at	At1g14345	expressed protein	-1.86
266355_at	At2g01400	expressed protein	-1.86
245806_at	At1g45474	chlorophyll A-B binding protein, putative (LHCA5)	-1.86
264546_at	At1g55805	BolA-like	-1.86
250745_at	At5g05850	leucine-rich repeat	-1.87
251575_at	At3g58120	bZIP transcription factor	-1.87
266656_at	At2g25900	zinc finger (CCCH-type)	-1.87
252463_at	At3g47070	expressed protein	-1.87

261165_at	At1g34430	dihydrolipoamide S-acetyltransferase, putative	-1.87
253174_at	At4g35090	catalase 2	-1.87
248380_at	At5g51820	phosphoglucomutase, chloroplast (PGM)	-1.87
249658_s_at	At5g36700	phosphoglycolate phosphatase, putative	-1.87
247488_at	At5g61820	expressed protein	-1.87
253489_at	At4g31780	monogalactosyldiacylglycerol synthase, putative (MGD1)	-1.87
249759_at	At5g24380	transporter, putative	-1.88
266901_at	At2g34600	expressed protein	-1.88
258554_at	At3g06980	DEAD/DEAH box helicase, putative	-1.88
263873_at	At2g21860	violaxanthin de-epoxidase-related	-1.88
255943_at	At1g22370	UDP-glucoronosyl/UDP-glucosyl transferase	-1.88
247278_at	At5g64380	fructose-1,6-bisphosphatase	-1.88
256097_at	At1g13670	expressed protein	-1.88
266518_at	At2g35170	MORN (Membrane Occupation and Recognition Nexus) repeat-containing protein	-1.88
251146_at	At3g63520	neoxanthin cleavage enzyme (NCED1)	-1.88
263499_at	At2g42580	tetratricopeptide repeat (TPR)-containing protein	-1.88
264513_at	At1g09420	glucose-6-phosphate 1-dehydrogenase, putative	-1.88
247266_at	At5g64570	glycosyl hydrolase family 3 protein	-1.89
245025_at	atpF	atpF	-1.89
251142_at	At5g01015	expressed protein	-1.89
258386_at	At3g15520	peptidyl-prolyl cis-trans isomerase (TLP38)	-1.89
262397_at	At1g49380	cytochrome c biogenesis protein family	-1.89
261353_at	At1g79600	ABC1	-1.9
258025_at	At3g19480	D-3-phosphoglycerate dehydrogenase, putative	-1.9
246449_at	At5g16810	expressed protein	-1.9
267430_at	At2g34860	chaperone protein dnaJ-related	-1.9
253373_at	At4g33150	lysine-ketoglutarate reductase	-1.9
262784_at	At1g10760	starch excess protein (SEX1)	-1.9
266614_at	At2g14910	expressed protein	-1.9
263533_at	At2g24820	Rieske (2Fe-2S) domain-containing protein	-1.9
262059_at	At1g80030	DNAJ heat shock protein, putative	-1.9
249847_at	At5g23210	serine carboxypeptidase S10	-1.91
261801_at	At1g30520	acyl-activating enzyme 14 (AAE14)	-1.91
250498_at	At5g09660	malate dehydrogenase, glyoxysomal	-1.91
246427_at	At5g17400	ADP, ATP carrier protein, mitochondrial, putative	-1.91
266018_at	At2g18710	preprotein translocase secY subunit, chloroplast (CpSecY)	-1.92
265959_at	At2g37240	expressed protein	-1.92
245559_at	At4g15460	glycine-rich protein	-1.92
245701_at	At5g04140	glutamate synthase (GLU1)	-1.92
256940_at	At3g30720	expressed protein	-1.92
250940_at	At5g03310	auxin-responsive	-1.93
260201_at	At1g67600	expressed protein	-1.93
245352_at	At4g15490	UDP-glucoronosyl/UDP-glucosyl transferase	-1.93
253283_at	At4g34090	expressed protein	-1.93

249691_at	At5g36170	peptide chain release factor, putative	-1.93
249685_at	At5g36120	YGGT	-1.93
267379_at	At2g26340	expressed protein	-1.93
265547_at	At2g28305	expressed protein	-1.94
247474_at	At5g62280	expressed protein	-1.94
254465_at	At4g20420	tapetum-specific protein-related	-1.94
259373_at	At1g69160	expressed protein	-1.94
254815_at	At4g12420	multi-copper oxidase, putative (SKU5)	-1.94
254642_at	At4g18810	expressed protein	-1.94
248287_at	At5g52970	thylakoid lumen 15.0 kDa protein	-1.94
249174_at	At5g42900	expressed protein	-1.94
249510_at	At5g38510	rhomboid	-1.94
260547_at	At2g43550	trypsin inhibitor, putative	-1.95
257772_at	At3g23080	expressed protein	-1.95
246122_at	At5g20380	transporter-related	-1.95
244990_s_at	orf77.1	orf77.1	-1.95
251109_at	At5g01600	ferritin 1 (FER1)	-1.95
259738_at	At1g64355	expressed protein	-1.95
253495_at	At4g31850	pentatricopeptide (PPR) repeat-containing protein	-1.95
245730_at	At1g73470	expressed protein	-1.95
244966_at	petG	petG	-1.95
249872_at	At5g23130	peptidoglycan-binding LysM domain-containing protein	-1.96
249852_at	At5g23270	sugar transporter, putative	-1.96
251461_at	At3g59780	expressed protein	-1.96
252876_at	At4g39970	haloacid dehalogenase-like hydrolase	-1.96
266509_at	At2g47940	DegP2 protease (DEGP2)	-1.96
249378_at	At5g40450	expressed protein	-1.96
251584_at	At3g58620	tetratricopeptide repeat (TPR)-containing protein	-1.96
266766_at	At2g46880	calcineurin-like phosphoesterase	-1.97
254502_at	At4g20130	ribulose-1,5 bisphosphate carboxylase	-1.97
265628_at	At2g27290	expressed protein	-1.97
250073_at	At5g17170	rubredoxin	-1.97
256149_at	At1g55110	zinc finger (C2H2 type)	-1.97
247943_at	At5g57170	macrophage migration inhibitory factor (MIF)	-1.98
246736_at	At5g27560	expressed protein	-1.98
246838_at	At5g26675	endonuclease, putative	-1.98
259292_at	At3g11560	expressed protein	-1.98
265998_at	At2g24270	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, putative	-1.98
259275_at	At3g01060	expressed protein	-1.99
253039_at	At4g37760	squalene monooxygenase, putative	-1.99
256544_at	At1g42560	seven transmembrane MLO (MLO9)	-1.99
264229_at	At1g67480	kelch repeat-containing F-box	-1.99
247709_at	At5g59250	sugar transporter	-1.99
262473_at	At1g50250	cell division protein ftsH homolog 1 (FTSH1)	-1.99

245026_at	atpH	atpH	-1.99
257831_at	At3g26710	expressed protein	-1.99
259981_at	At1g76450	oxygen-evolving complex-related	-1.99
258736_at	At3g05900	neurofilament protein-related	-1.99
247252_at	At5g64770	expressed protein	-1.99
251243_at	At3g61870	expressed protein	-1.99
262176_at	At1g74960	3-ketoacyl-ACP synthase, putative	-1.99
263352_at	At2g22080	expressed protein	-1.99
251494_at	At3g59350	serine/threonine protein kinase, putative	-1.99
260877_at	At1g21500	expressed protein	-2
258227_at	At3g15620	6-4 photolyase (UVR3)	-2
261135_at	At1g19610	plant defensin-fusion protein, putative (PDF1.4)	-2
245017_at	psaI	psaI	-2
245528_at	At4g15530	pyruvate phosphate dikinase	-2
256788_at	At3g13730	cytochrome P450, putative	-2
256423_at	At1g33540	serine carboxypeptidase S10	-2
262878_at	At1g64770	expressed protein	-2
259943_at	At1g71480	nuclear transport factor 2 (NTF2)	-2
256130_at	At1g18170	immunophilin	-2
253522_at	At4g31290	ChaC-like	-2
258114_at	At3g14660	cytochrome P450, putative	-2.01
264887_at	At1g23120	major latex protein-related / MLP-related	-2.01
253849_at	At4g28080	expressed protein	-2.01
256503_at	At1g75250	myb family transcription factor	-2.01
259658_at	At1g55370	expressed protein	-2.01
248975_at	At5g45040	cytochrome c6 (ATC6)	-2.01
251268_at	At3g62350	hypothetical protein	-2.01
265867_at	At2g01620	expressed protein	-2.01
247954_at	At5g56870	beta-galactosidase, putative / lactase, putative	-2.01
254137_at	At4g24930	thylakoid lumenal 17.9 kDa protein, chloroplast	-2.01
252181_at	At3g50685	expressed protein	-2.01
246268_at	At1g31800	cytochrome P450	-2.01
253547_at	At4g30950	omega-6 fatty acid desaturase, chloroplast (FAD6)	-2.01
257381_at	At2g37950	zinc finger (C3HC4-type RING finger)	-2.01
249002_at	At5g44520	ribose 5-phosphate isomerase-related	-2.01
247348_at	At5g63810	beta-galactosidase, putative / lactase, putative	-2.01
247261_at	At5g64460	expressed protein	-2.01
246071_at	At5g20150	SPX domain-containing protein	-2.02
264580_at	At1g05340	expressed protein	-2.02
259791_at	At1g29700	expressed protein	-2.02
258935_at	At3g10120	expressed protein	-2.02
257172_at	At3g23700	S1 RNA-binding domain-containing protein	-2.02
263761_at	At2g21330	fructose-bisphosphate aldolase, putative	-2.02
263350_at	At2g13360	serine-glyoxylate aminotransferase-related	-2.02
246226_at	At4g37200	thioredoxin	-2.02

266357_at	At2g32290	beta-amylase, putative	-2.02
255331_at	At4g04330	expressed protein	-2.02
252116_at	At3g51510	expressed protein	-2.02
253597_at	At4g30690	translation initiation factor 3 (IF-3)	-2.03
266277_at	At2g29310	tropinone reductase, putative	-2.03
263678_at	At1g04420	aldo/keto reductase	-2.03
266617_at	At2g29670	expressed protein	-2.04
247884_at	At5g57800	CER1 protein, putative (WAX2)	-2.04
245284_at	At4g14210	phytoene desaturase (PDS)	-2.04
251192_at	At3g62720	galactosyl transferase (GMA12)	-2.04
264799_at	At1g08550	violaxanthin de-epoxidase precursor, putative (AVDE1)	-2.04
255939_at	At1g12730	cell division cycle protein-related	-2.04
256514_at	At1g66130	oxidoreductase N-terminal domain-containing protein	-2.04
245349_at	At4g16690	esterase	-2.04
257168_at	At3g24430	expressed protein	-2.04
250812_at	At5g04900	short-chain dehydrogenase/reductase (SDR)	-2.04
263953_at	At2g36050	ovate protein-related	-2.04
253956_at	At4g26700	fimbrin-like protein (FIM1)	-2.04
252425_at	At3g47620	TCP family transcription factor, putative	-2.04
245011_at	psbG	psbG	-2.05
261674_at	At1g18270	ketose-bisphosphate aldolase class-II	-2.05
257547_at	At3g13000	expressed protein	-2.05
259037_at	At3g09350	beta-catenin repeat	-2.05
248395_at	At5g52120	SKP1 interacting partner 3-related	-2.05
247641_at	At5g60540	SNO glutamine amidotransferase	-2.05
249065_at	At5g44260	zinc finger (CCCH-type)	-2.06
250777_at	At5g05440	expressed protein	-2.06
248273_at	At5g53500	WD-40 repeat	-2.06
246596_at	At5g14740	carbonate dehydratase 2 (CA2)	-2.06
246958_at	At5g24690	expressed protein	-2.06
251306_at	At3g61260	DNA-binding protein	-2.06
266904_at	At2g34590	transketolase	-2.06
259161_at	At3g01500	carbonate dehydratase 1 (CA1)	-2.07
266813_at	At2g44920	thylakoid lumenal 15 kDa protein, chloroplast	-2.07
264653_at	At1g08980	amidase	-2.07
264394_at	At1g11860	aminomethyltransferase, putative	-2.07
256061_at	At1g07040	expressed protein	-2.07
244974_at	psbN	psbN	-2.08
251885_at	At3g54050	fructose-1,6-bisphosphatase, putative	-2.08
259015_at	At3g07350	expressed protein	-2.09
256914_at	At3g23880	F-box	-2.09
245440_at	At4g16680	RNA helicase, putative	-2.09
260056_at	At1g78140	methyltransferase-related	-2.09
260914_at	At1g02640	glycosyl hydrolase family 3 protein	-2.1
263597_at	At2g01870	expressed protein	-2.1

263157_at	At1g54100	aldehyde dehydrogenase, putative	-2.1
248335_at	At5g52450	MATE efflux protein-related	-2.1
261658_at	At1g50040	expressed protein	-2.1
252092_at	At3g51420	strictosidine synthase	-2.1
265742_at	At2g01290	expressed protein	-2.11
248205_at	At5g54300	expressed protein	-2.11
259849_at	At1g72190	oxidoreductase	-2.11
256786_at	At3g13740	URF 4-related	-2.11
250563_at	At5g08050	expressed protein	-2.11
258383_at	At3g15440	expressed protein	-2.12
249837_at	At5g23480	expressed protein	-2.12
251235_at	At3g62860	esterase	-2.12
258495_at	At3g02690	integral membrane	-2.12
262226_at	At1g53885	senescence-associated protein-related	-2.13
245088_at	At2g39850	subtilase	-2.13
262970_at	At1g75690	chaperone protein dnaJ-related	-2.13
257003_at	At3g14110	tetratricopeptide repeat (TPR)-containing protein	-2.13
266285_at	At2g29180	expressed protein	-2.13
247162_at	At5g65730	xyloglucan:xyloglucosyl transferase, putative	-2.14
256577_at	At3g28220	meprin and TRAF homology domain-containing protein	-2.14
251750_at	At3g55710	UDP-glucoronosyl/UDP-glucosyl transferase	-2.14
258327_at	At3g22640	cupin	-2.14
250856_at	At5g04810	pentatricopeptide (PPR) repeat-containing protein	-2.14
265768_at	At2g48020	sugar transporter, putative	-2.15
255381_at	At4g03510	zinc finger (C3HC4-type RING finger) (RMA1)	-2.15
263275_at	At2g14170	methylmalonate-semialdehyde dehydrogenase, putative	-2.15
250256_at	At5g13650	elongation factor	-2.15
264525_at	At1g10060	branched-chain amino acid transaminase 1 (BCAT1)	-2.15
253825_at	At4g28025	expressed protein	-2.15
256870_at	At3g26300	cytochrome P450	-2.15
266963_at	At2g39450	cation efflux	-2.16
262760_at	At1g10770	invertase	-2.16
255774_at	At1g18620	expressed protein	-2.16
251996_at	At3g52840	beta-galactosidase, putative	-2.16
263184_at	At1g05560	UDP-glucose transferase (UGT75B2)	-2.16
246959_at	At5g24700	expressed protein	-2.16
265569_at	At2g05620	expressed protein	-2.16
246199_at	At4g36530	hydrolase, alpha/beta fold	-2.16
250255_at	At5g13730	sigma-like factor (SIG4)	-2.16
257367_at	At2g25780	hypothetical protein	-2.17
259103_at	At3g11690	expressed protein	-2.17
264553_s_at	At1g09480	cinnamyl-alcohol dehydrogenase family (CAD)	-2.17
250580_at	At5g07440	glutamate dehydrogenase 2 (GDH2)	-2.18
245743_at	At1g51080	expressed protein	-2.18
264383_at	At2g25080	phospholipid hydroperoxide glutathione peroxidase, chloroplast	-2.18

		(GPX1)	
260522_x_at	At2g41730	expressed protein	-2.18
252353_at	At3g48200	expressed protein	-2.18
250781_at	At5g05410	DRE-binding protein (DREB2A)	-2.18
245018_at	ycf4	ycf4	-2.18
264954_at	At1g77060	mutase	-2.18
260379_at	At1g73880	expressed protein	-2.18
267247_at	At2g30170	expressed protein	-2.18
265182_at	At1g23740	oxidoreductase, zinc-binding dehydrogenase	-2.19
252950_at	At4g38690	1-phosphatidylinositol phosphodiesterase-related	-2.19
249073_at	At5g44020	acid phosphatase class B	-2.19
244932_at	psaC	psaC	-2.19
262897_at	At1g59840	expressed protein	-2.19
248962_at	At5g45680	FK506-binding protein 1 (FKBP13)	-2.19
264397_at	At1g11820	glycosyl hydrolase family 17 protein	-2.19
259603_at	At1g56500	haloacid dehalogenase-like hydrolase	-2.19
259896_at	At1g71500	Rieske (2Fe-2S) domain-containing protein	-2.19
264442_at	At1g27480	lecithin:cholesterol acyltransferase (LACT)	-2.2
251059_at	At5g01810	CBL-interacting protein kinase 15 (CIPK15)	-2.2
261388_at	At1g05385	photosystem II 11 kDa protein-related	-2.2
251784_at	At3g55330	photosystem II reaction center PsbP	-2.2
262304_at	At1g70890	major latex protein-related	-2.2
262979_s_at	At1g75610	histone H3, putative	-2.21
260106_at	At1g35420	dienelactone hydrolase	-2.21
261861_at	At1g50450	expressed protein	-2.21
246211_at	At4g36730	G-box binding factor 1 (GBF1)	-2.22
253790_at	At4g28660	photosystem II reaction centre W (PsbW)	-2.22
248449_at	At5g51110	expressed protein	-2.22
256304_at	At1g69523	UbiE/COQ5 methyltransferase	-2.23
253951_at	At4g26860	alanine racemase	-2.23
261931_at	At1g22430	alcohol dehydrogenase, putative(ADH)	-2.23
245793_at	At1g32220	expressed protein	-2.23
251143_at	At5g01220	sulfolipid synthase (SQD2)	-2.23
260014_at	At1g68010	glycerate dehydrogenase	-2.23
247931_at	At5g57040	lactoylglutathione lyase	-2.23
265073_at	At1g55480	expressed protein	-2.24
262645_at	At1g62750	elongation factor Tu	-2.24
248193_at	At5g54080	homogentisic acid oxidase (HGO)	-2.25
267497_at	At2g30540	glutaredoxin	-2.25
261793_at	At1g16080	expressed protein	-2.25
251647_at	At3g57770	protein kinase, putative	-2.25
249472_at	At5g39210	expressed protein	-2.25
251155_at	At3g63160	expressed protein	-2.26
247924_at	At5g57655	xylose isomerase	-2.26
249927_at	At5g19220	ADP-glucose pyrophosphorylase (ADG2)	-2.26

254038_at	At4g25910	nitrogen fixation protein, putative	-2.27
260969_at	At1g12240	beta-fructosidase (BFRUCT4)	-2.27
250752_at	At5g05690	cytochrome P450 90A1 (CYP90A1)	-2.27
263443_at	At2g28630	beta-ketoacyl-CoA synthase	-2.28
261536_at	At1g01790	K+ efflux antiporter, putative (KEA1)	-2.28
251230_at	At3g62750	glycosyl hydrolase family 1 protein	-2.28
261822_at	At1g11380	expressed protein	-2.29
250151_at	At5g14570	transporter, putative	-2.29
259199_at	At3g08980	signal peptidase I	-2.29
244972_at	psbB	psbB	-2.29
248419_at	At5g51550	phosphate-responsive 1	-2.3
253394_at	At4g32770	sucrose export defective 1 (SXD1)	-2.3
265511_at	At2g05540	glycine-rich protein	-2.3
263676_at	At1g09340	expressed protein	-2.3
248224_at	At5g53490	thylakoid lumenal 17.4 kDa protein	-2.3
246951_at	At5g04880	expressed protein	-2.31
251762_at	At3g55800	sedoheptulose-1,7-bisphosphatase	-2.31
260968_at	At1g12250	thylakoid lumenal protein-related	-2.31
255543_at	At4g01870	tolB protein-related	-2.32
247845_at	At5g58090	glycosyl hydrolase 17 protein	-2.33
259633_at	At1g56505	haloacid dehalogenase-like hydrolase	-2.33
245744_at	At1g51110	plastid-lipid associated protein PAP	-2.33
244995_at	atpI	atpI	-2.33
264096_at	At1g78995	expressed protein	-2.33
248657_at	At5g48570	peptidyl-prolyl cis-trans isomerase, putative	-2.34
263150_at	At1g54050	17.4 kDa class III heat shock protein (HSP17.4-CIII)	-2.34
245291_at	At4g16155	lipoamide dehydrogenase 2 (PTLPD2)	-2.34
262603_at	At1g15380	lactoylglutathione lyase	-2.35
266589_at	At2g46250	myosin heavy chain-related	-2.35
252853_at	At4g39710	FKBP-type peptidyl-prolyl cis-trans isomerase, putative	-2.35
249226_at	At5g42170	family II extracellular lipase, putative	-2.36
257315_at	At3g30775	proline oxidase, mitochondrial (POX)	-2.36
263881_at	At2g21820	expressed protein	-2.37
262162_at	At1g78020	senescence-associated protein-related	-2.37
250531_at	At5g08650	GTP-binding protein LepA, putative	-2.37
261480_at	At1g14280	phytochrome kinase, putative	-2.37
252441_at	At3g46780	expressed protein	-2.37
266426_x_at	At2g07140	F-box	-2.37
263880_at	At2g21960	expressed protein	-2.37
265703_at	At2g03430	ankyrin repeat	-2.38
258055_at	At3g16250	ferredoxin-related	-2.38
253387_at	At4g33010	glycine dehydrogenase (decarboxylating), putative	-2.38
263668_at	At1g04350	2-oxoglutarate-dependent dioxygenase, putative	-2.39
260704_at	At1g32470	glycine cleavage system H protein, mitochondrial, putative	-2.39
266767_at	At2g46910	plastid-lipid associated protein PAP	-2.39

264209_at	At1g22740	Ras-related protein (RAB7)	-2.39
256772_at	At3g13750	beta-galactosidase, putative	-2.39
252991_at	At4g38470	protein kinase	-2.4
262151_at	At1g52510	hydrolase, alpha/beta fold	-2.4
260696_at	At1g32520	expressed protein	-2.4
260978_at	At1g53540	17.6 kDa class I small heat shock protein (HSP17.6C-CI) (AA 1-156)	-2.41
266278_at	At2g29300	tropinone reductase, putative	-2.42
253281_at	At4g34138	UDP-glucoronosyl/UDP-glucosyl transferase	-2.43
265387_at	At2g20670	expressed protein	-2.43
263410_at	At2g04039	expressed protein	-2.43
260837_at	At1g43670	fructose-1,6-bisphosphatase, putative	-2.43
258972_at	At3g01920	yrdC	-2.43
256036_at	At1g07110	fructose-2,6-bisphosphatase (F2KP)	-2.43
264289_at	At1g61890	MATE efflux	-2.44
254496_at	At4g20070	peptidase M20/M25/M40	-2.44
250075_at	At5g17670	expressed protein	-2.44
250167_at	At5g15310	myb family transcription factor	-2.44
248624_at	At5g48790	expressed protein	-2.44
250910_at	At5g03720	heat shock transcription factor	-2.45
251919_at	At3g53800	armadillo/beta-catenin repeat	-2.45
246962_s_at	At5g24800	bZIP transcription factor	-2.45
252539_at	At3g45730	expressed protein	-2.46
262605_at	At1g15170	MATE efflux	-2.46
259694_at	At1g63180	UDP-glucose 4-epimerase, putative	-2.46
245007_at	psaA	psaA	-2.46
246651_at	At5g35170	adenylate kinase	-2.46
245003_at	psbC	psbC	-2.47
263142_at	At1g65230	expressed protein	-2.47
265116_at	At1g62480	vacuolar calcium-binding protein-related	-2.48
261948_at	At1g64680	expressed protein	-2.49
249230_at	At5g42070	expressed protein	-2.49
256548_at	At3g14770	nodulin MtN3	-2.5
267036_at	At2g38465	expressed protein	-2.5
245016_at	accD	accD	-2.5
252081_at	At3g51910	heat shock transcription factor	-2.51
267057_at	At2g32500	expressed protein	-2.52
246411_at	At1g57770	amine oxidase family	-2.52
261084_at	At1g07440	tropinone reductase, putative	-2.52
260284_at	At1g80380	phosphoribulokinase	-2.53
264728_at	At1g22850	expressed protein	-2.53
267262_at	At2g22990	sinapoylglucose:malate sinapoyltransferase (SNG1)	-2.54
267523_at	At2g30610	BTB/POZ domain-containing protein	-2.55
256076_at	At1g18060	expressed protein	-2.55
248491_at	At5g51010	rubredoxin	-2.55

255793_at	At2g33250	expressed protein	-2.56
253217_at	At4g34970	actin-depolymerizing factor, putative	-2.56
255962_at	At1g22335	expressed protein	-2.57
246630_at	At1g50730	expressed protein	-2.58
248566_s_at	At5g49730	ferric reductase-like transmembrane component	-2.6
246034_at	At5g08350	GRAM domain-containing protein	-2.6
246998_at	At5g67370	expressed protein	-2.6
246792_at	At5g27290	expressed protein	-2.6
249120_at	At5g43750	expressed protein	-2.6
256999_at	At3g14200	DNAJ heat shock N-terminal domain-containing protein	-2.61
259982_at	At1g76410	zinc finger (C3HC4-type RING finger)	-2.62
251036_at	At5g02160	expressed protein	-2.62
262569_at	At1g15180	MATE efflux	-2.62
258250_at	At3g15850	fatty acid desaturase	-2.62
250972_at	At5g02840	myb family transcription factor	-2.63
251157_at	At3g63140	mRNA-binding protein, putative	-2.63
253496_at	At4g31870	glutathione peroxidase, putative	-2.64
253279_at	At4g34030	3-methylcrotonyl-CoA carboxylase 2 (MCCB)	-2.64
247919_at	At5g57650	eukaryotic translation initiation factor-related	-2.65
262698_at	At1g75960	AMP-binding protein, putative	-2.66
251759_at	At3g55630	dihydrofolate synthetase/folylpolyglutamate synthetase (DHFS/FPGS4)	-2.66
261788_at	At1g15980	expressed protein	-2.66
251642_at	At3g57520	alkaline alpha galactosidase, putative	-2.67
249524_at	At5g38520	hydrolase, alpha/beta fold	-2.67
263827_at	At2g40420	amino acid transporter	-2.68
251248_at	At3g62150	multidrug resistant (MDR) ABC transporter, putative	-2.68
254561_at	At4g19160	expressed protein	-2.68
245002_at	psbD	psbD	-2.69
259914_at	At1g72640	expressed protein	-2.71
253049_at	At4g37300	expressed protein	-2.71
259707_at	At1g77490	L-ascorbate peroxidase, thylakoid-bound (tAPX)	-2.71
249875_at	At5g23120	photosystem II stability/assembly factor, chloroplast (HCF136)	-2.71
248207_at	At5g53970	aminotransferase, putative	-2.72
252924_at	At4g39070	zinc finger (B-box type)	-2.72
256015_at	At1g19150	chlorophyll A-B binding protein, putative	-2.73
259329_at	At3g16360	phosphotransfer	-2.73
251068_at	At5g01920	protein kinase	-2.73
245745_at	At1g51115	plastid-lipid associated protein PAP	-2.74
263252_at	At2g31380	salt tolerance-like protein (STH)	-2.76
258338_at	At3g16150	L-asparaginase, putative	-2.76
244933_at	ndhE	ndhE	-2.76
246510_at	At5g15410	cyclic nucleotide-gated channel (CNGC2)	-2.77
266329_at	At2g01590	expressed protein	-2.77

267035_at	At2g38400	alanine-glyoxylate aminotransferase, putative (AGT)	-2.79
260900_s_at	At1g21400	2-oxoisovalerate dehydrogenase, putative	-2.79
255012_at	At4g10030	hydrolase, alpha/beta fold	-2.8
261942_at	At1g22590	MADS-box	-2.81
254835_s_at	At4g12310	cytochrome P450, putative	-2.82
254020_at	At4g25700	beta-carotene hydroxylase	-2.82
255016_at	At4g10120	sucrose-phosphate synthase, putative	-2.88
261814_at	At1g08310	esterase	-2.88
254839_at	At4g12400	stress-inducible protein, putative	-2.89
266979_at	At2g39470	photosystem II reaction center PsbP	-2.89
266294_at	At2g29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI)	-2.91
261422_at	At1g18730	expressed protein	-2.91
262635_at	At1g06570	4-hydroxyphenylpyruvate dioxygenase (HPD)	-2.93
262911_s_at	At1g59860	17.8 kDa class I heat shock protein (HSP17.8-CI)	-2.93
254787_at	At4g12690	expressed protein	-2.95
256894_at	At3g21870	cyclin	-2.99
245876_at	At1g26230	chaperonin, putative	-2.99
260662_at	At1g19540	isoflavone reductase, putative	-3
248347_at	At5g52250	transducin	-3.01
249765_at	At5g24030	C4-dicarboxylate transporter	-3.01
247882_at	At5g57785	expressed protein	-3.02
266590_at	At2g46240	IQ domain-containing protein	-3.02
247816_at	At5g58260	expressed protein	-3.04
263133_at	At1g78450	SOUL heme-binding	-3.05
266279_at	At2g29290	tropinone reductase, putative	-3.06
260743_at	At1g15040	At1g15040	-3.1
262175_at	At1g74880	expressed protein	-3.1
258527_at	At3g06850	branched chain alpha-keto acid dehydrogenase E2 subunit (din3)	-3.11
248793_at	At5g47240	MutT/nudix	-3.11
266984_at	At2g39570	ACT domain-containing protein	-3.11
252570_at	At3g45300	isovaleryl-CoA-dehydrogenase (IVD)	-3.12
259789_at	At1g29395	stress-responsive protein, putative	-3.15
265070_at	At1g55510	2-oxoisovalerate dehydrogenase, putative	-3.16
255719_at	At1g32080	membrane protein, putative	-3.18
253161_at	At4g35770	senescence-associated protein (SEN1)	-3.24
258956_at	At3g01440	oxygen evolving enhancer 3 (PsbQ)	-3.24
254016_at	At4g26150	zinc finger (GATA type)	-3.27
261663_at	At1g18330	myb family transcription factor	-3.28
264213_at	At1g65400	disease resistance protein (TIR class), putative	-3.29
263981_at	At2g42870	expressed protein	-3.29
246195_at	At4g36410	ubiquitin-conjugating enzyme 17 (UBC17)	-3.29
251353_at	At3g61080	fructosamine kinase	-3.29
254384_at	At4g21870	26.5 kDa class P-related heat shock protein (HSP26.5-P)	-3.33
267524_at	At2g30600	BTB/POZ domain-containing protein	-3.37

245757_at	At1g35140	phosphate-responsive protein, putative	-3.38
248434_at	At5g51440	23.5 kDa mitochondrial small heat shock protein (HSP23.5-M)	-3.39
266956_at	At2g34510	expressed protein	-3.42
253197_at	At4g35250	vestitone reductase-related	-3.45
262047_at	At1g80160	lactoylglutathione lyase	-3.51
264561_at	At1g55810	uracil phosphoribosyltransferase, putative	-3.52
253943_at	At4g27030	expressed protein	-3.6
256965_at	At3g13450	branched-chain alpha-keto acid dehydrogenase E1 beta subunit (DIN4)	-3.62
261395_at	At1g79700	ovule development protein, putative	-3.63
259076_at	At3g02140	expressed protein	-3.64
248050_at	At5g56100	glycine-rich protein	-3.67
251356_at	At3g61060	F-box	-3.68
252366_at	At3g48420	haloacid dehalogenase-like hydrolase	-3.68
262288_at	At1g70760	inorganic carbon transport protein-related	-3.69
247814_at	At5g58310	hydrolase, alpha/beta fold	-3.7
267138_s_at	At2g38210	ethylene-responsive protein, putative	-3.71
264923_s_at	At1g60740	peroxiredoxin type 2, putative	-3.73
266693_at	At2g19800	expressed protein	-3.79
260668_at	At1g19530	expressed protein	-3.83
263287_at	At2g36145	expressed protein	-3.83
252415_at	At3g47340	glutamine-dependent asparagine synthetase 1 (ASN1)	-3.85
258856_at	At3g02040	glycerophosphoryl diester phosphodiesterase	-3.91
256245_at	At3g12580	heat shock protein 70, putative (HSP70)	-3.96
262612_at	At1g14150	oxygen evolving enhancer 3 (PsbQ)	-3.98
260248_at	At1g74310	heat shock protein 101 (HSP101)	-4
248377_at	At5g51720	expressed protein	-4.12
261957_at	At1g64660	Cys/Met metabolism pyridoxal-phosphate-dependent enzyme	-4.14
251196_at	At3g62950	glutaredoxin	-4.15
264524_at	At1g10070	branched-chain amino acid transaminase 2 (BCAT2)	-4.17
255298_at	At4g04840	methionine sulfoxide reductase domain-containing protein	-4.22
251428_at	At3g60140	glycosyl hydrolase family 1 protein	-4.26
266841_at	At2g26150	heat shock transcription factor	-4.27
245362_at	At4g17460	homeobox-leucine zipper protein 1 (HAT1)	-4.31
260536_at	At2g43400	electron transfer flavoprotein-ubiquinone oxidoreductase	-4.32
257670_at	At3g20340	expressed protein	-4.34
263118_at	At1g03090	3-methylcrotonyl-CoA carboxylase 1 (MCCA)	-4.35
250351_at	At5g12030	17.7 kDa class II heat shock protein 17.6A (HSP17.7-CII)	-4.51
264238_at	At1g54740	expressed protein	-4.62
267265_at	At2g22980	serine carboxypeptidase S10	-4.64
258181_at	At3g21670	nitrate transporter (NTP3)	-4.65
247478_at	At5g62360	invertase	-4.67
253966_at	At4g26520	fructose-bisphosphate aldolase	-4.69
260915_at	At1g02660	lipase class 3	-4.82

249337_at	At5g41080	glycerophosphoryl diester phosphodiesterase	-4.86
254783_at	At4g12830	hydrolase, alpha/beta fold	-4.92
260741_at	At1g15045	glutamine amidotransferase-related	-5.03
250199_at	At5g14180	lipase	-5.21
246251_at	At4g37220	stress-responsive protein, putative	-5.23
254001_at	At4g26260	expressed protein	-5.26
265111_at	At1g62510	protease inhibitor/seed storage/lipid transfer protein (LTP)	-5.52
262307_at	At1g71000	DNAJ heat shock N-terminal domain-containing protein	-5.96
264777_at	At1g08630	L-allo-threonine aldolase-related	-6.42
264514_at	At1g09500	cinnamyl-alcohol dehydrogenase family (CAD)	-7.02
253971_at	At4g26530	fructose-bisphosphate aldolase, putative	-7.93
253829_at	At4g28040	nodulin MtN21	-8.18
256527_at	At1g66100	thionin, putative	-8.35
4 days after Ca suppression			
264400_at	At1g61800	glucose-6-phosphate/phosphate translocator, putative	4.36
267147_at	At2g38240	oxidoreductase, 2OG-Fe(II) oxygenase	3.36
252265_at	At3g49620	2-oxoacid-dependent oxidase, putative (DIN11)	2.75
266720_s_at	At2g46790	pseudo-response regulator, putative	2.53
252661_at	At3g44450	expressed protein	2.49
249364_at	At5g40590	DC1 domain-containing protein	2.46
252827_at	At4g39950	cytochrome P450 79B2, putative (CYP79B2)	2.44
258586_s_at	At3g04320	trypsin and protease inhibitor	2.36
260130_s_at	At1g66280	beta-glucosidase (PSR3.2)	2.36
260557_at	At2g43610	glycoside hydrolase family 19 protein	2.3
253608_at	At4g30290	xyloglucan:xyloglucosyl transferase, putative	2.29
254361_at	At4g22212	expressed protein	2.2
256999_at	At3g14200	DNAJ heat shock N-terminal domain-containing protein	2.17
265665_at	At2g27420	cysteine proteinase, putative	2.08
246490_at	At5g15950	adenosylmethionine decarboxylase	2.06
247755_at	At5g59090	subtilase	2.02
258133_at	At3g24500	ethylene-responsive transcriptional coactivator, putative	1.91
265221_s_at	At2g02010	glutamate decarboxylase, putative	1.9
260248_at	At1g74310	heat shock protein 101 (HSP101)	1.89
254550_at	At4g19690	iron-responsive transporter (IRT1)	1.89
253382_at	At4g33040	glutaredoxin	1.89
266841_at	At2g26150	heat shock transcription factor	1.87
245627_at	At1g56600	galactinol synthase, putative	1.87
252563_at	At3g45970	expansin (EXPL1)	1.84
262656_at	At1g14200	zinc finger (C3HC4-type RING finger)	1.84
256518_at	At1g66080	expressed protein	1.79
256619_at	At3g24470	TMS membrane (TDE)	1.79
265892_at	At2g15020	expressed protein	1.78
258830_at	At3g07090	expressed protein	1.78
258419_at	At3g16670	expressed protein	1.77
247573_at	At5g61160	transferase	1.77

252679_at	At3g44260	CCR4-NOT transcription complex protein, putative	1.74
266271_at	At2g29440	glutathione S-transferase, putative	1.73
252984_at	At4g37990	mannitol dehydrogenase, putative (ELI3-2)	1.72
260134_at	At1g66370	myb family transcription factor (MYB113)	1.72
248732_at	At5g48070	xyloglucan:xyloglucosyl transferase, putative	1.7
248138_at	At5g54960	pyruvate decarboxylase, putative	1.69
264787_at	At2g17840	senescence/dehydration-associated protein-related (ERD7)	1.68
258259_s_at	At3g26840	esterase	1.67
255773_at	At1g18590	sulfotransferase	1.67
252114_at	At3g51450	strictosidine synthase	1.67
258418_at	At3g16660	expressed protein	1.65
267411_at	At2g34930	disease resistance	1.65
245734_at	At1g73480	hydrolase, alpha/beta fold	1.63
253162_at	At4g35630	phosphoserine aminotransferase, chloroplast (PSAT)	1.62
252958_at	At4g38620	myb family transcription factor (MYB4)	1.61
256178_s_at	At1g51780	IAA-Ala hydrolase 3 (IAR3)	1.61
253398_at	At4g32810	dioxygenase-related	1.6
247877_at	At5g57740	zinc finger (C3HC4-type RING finger) / ankyrin repeat	1.6
247452_at	At5g62430	Dof-type zinc finger domain-containing protein	1.59
265897_at	At2g25680	expressed protein	1.58
251154_at	At3g63110	cytokinin synthase (IPT3)	1.58
266326_at	At2g46650	cytochrome b5, putative	1.58
259518_at	At1g20510	4-coumaroyl-CoA synthase	1.57
252952_at	At4g38710	glycine-rich protein	1.56
253405_at	At4g32800	AP2 domain-containing transcription factor TINY, putative	1.53
248271_at	At5g53420	expressed protein	1.52
252255_at	At3g49220	pectinesterase	1.52
255700_at	At4g00200	DNA-binding	1.52
260712_at	At1g17550	protein phosphatase 2C-related	1.51
248190_at	At5g54120	calcium-binding EF hand	1.5
266609_at	At2g35510	WWE domain-containing protein	1.5
266553_at	At2g46170	reticulon (RTNLB5)	1.5
251234_s_at	At2g47650	NAD-dependent epimerase/dehydratase	1.49
254563_at	At4g19120	early-responsive to dehydration stress protein (ERD3)	1.49
260830_at	At1g06760	histone H1, putative	1.49
256529_at	At1g33260	protein kinase	1.49
251704_at	At3g56360	expressed protein	1.47
250500_at	At5g09530	hydroxyproline-rich glycoprotein	1.47
248469_at	At5g50820	no apical meristem (NAM)	1.47
265359_at	At2g16720	myb family transcription factor	1.47
251791_at	At3g55500	expansin, putative (EXP16)	1.47
257335_at	orf105b	orf105b	1.46
267112_at	At2g14750	adenylylsulfate kinase 1 (AKN1)	1.46
256645_at	At3g24250	glycine-rich protein	1.45
261749_at	At1g76180	dehydrin (ERD14)	1.45

248272_at	At5g53485	importin beta-2, putative	1.45
248270_at	At5g53450	protein kinase	1.44
259894_at	At1g71430	expressed protein	1.43
260342_at	At1g69280	expressed protein	1.41
250096_at	At5g17190	expressed protein	1.41
256022_at	At1g58360	amino acid permease I (AAP1)	1.39
259366_at	At1g13280	allene oxide cyclase	1.38
260494_at	At2g41820	leucine-rich repeat transmembrane protein kinase, putative	1.37
267561_at	At2g45590	protein kinase	-1.3
261049_at	At1g01430	expressed protein	-1.32
267135_at	At2g23430	kip-related protein 1 (KRP1)	-1.32
245269_at	At4g14500	expressed protein	-1.32
245313_at	At4g15420	PRLI-interacting factor K	-1.33
256659_at	At3g12020	kinesin motor protein-related	-1.33
265675_at	At2g32120	heat shock protein 70 (HSP70)	-1.34
261802_at	At1g30550	expressed protein	-1.35
250107_at	At5g15330	SPX domain-containing protein	-1.35
244977_at	petD	petD	-1.35
260554_at	At2g41790	peptidase M16	-1.35
264060_at	At2g27980	expressed protein	-1.36
257618_at	At3g24720	protein kinase	-1.36
260364_at	At1g70560	alliinase C-terminal domain-containing protein	-1.38
247980_at	At5g56860	zinc finger (GATA type)	-1.38
247713_at	At5g59330	At5g59330	-1.38
258544_at	At3g07040	disease resistance protein RPM1 (CC-NBS-LRR class), putative	-1.39
255382_at	At4g03430	pre-mRNA splicing factor-related	-1.4
253703_at	At4g29910	origin recognition complex subunit 5-related (ORC5)	-1.4
257205_at	At3g16520	UDP-glucoronosyl/UDP-glucosyl transferase	-1.4
266348_at	At2g01450	mitogen-activated protein kinase, putative (MPK17)	-1.41
258931_at	At3g10010	HhH-GPD base excision DNA repair	-1.41
246327_at	At1g16670	protein kinase	-1.41
264649_at	At1g09060	transcription factor jumonji (jmjC) domain-containing protein	-1.41
252972_at	At4g38840	auxin-responsive protein, putative	-1.41
256457_at	At1g75230	HhH-GPD base excision DNA repair	-1.42
266606_at	At2g46310	AP2 domain-containing transcription factor, putative	-1.43
250063_at	At5g17880	disease resistance protein (TIR-NBS-LRR class), putative	-1.43
265868_at	At2g01650	zinc finger (C2H2 type)	-1.43
261791_at	At1g16170	expressed protein	-1.43
263133_at	At1g78450	SOUL heme-binding	-1.43
262394_at	At1g49510	expressed protein	-1.43
246975_at	At5g24890	expressed protein	-1.43
254032_at	At4g25940	epsin N-terminal homology (ENTH) domain-containing protein	-1.43
257935_at	At3g25440	group II intron splicing factor CRS1-related	-1.44

250257_at	At5g13770	pentatricopeptide (PPR) repeat-containing protein	-1.44
250549_at	At5g07860	transferase	-1.44
264867_at	At1g24150	formin homology 2 domain-containing protein / FH2 domain-containing protein	-1.44
248106_at	At5g55100	SWAP (Suppressor-of-White-APricot)	-1.45
244990_s_at	orf77.1	orf77.1	-1.45
258647_at	At3g07870	F-box	-1.45
249074_at	At5g44080	bZIP transcription factor	-1.45
259560_at	At1g21270	wall-associated kinase 2 (WAK2)	-1.46
252504_at	At3g46590	telomere repeat-binding protein, putative	-1.46
248873_at	At5g46450	disease resistance protein (TIR-NBS-LRR class), putative	-1.46
258351_at	At3g17700	cyclic nucleotide-binding transporter 1 (CNGC20)	-1.46
253369_at	At4g33210	F-box (FBL15)	-1.46
266502_at	At2g47720	expressed protein	-1.46
249341_at	At5g41170	pentatricopeptide (PPR) repeat-containing protein	-1.46
245506_at	At4g15700	glutaredoxin	-1.46
263305_at	At2g01930	expressed protein	-1.46
252126_at	At3g50950	disease resistance protein (CC-NBS-LRR class), putative	-1.46
265023_at	At1g24440	expressed protein	-1.47
262129_at	At1g52500	formamidopyrimidine-DNA glycolase (MMH-1)	-1.47
256193_at	At1g30200	F-box	-1.47
258297_at	At3g23325	splicing factor, putative	-1.47
266361_at	At2g32450	calcium-binding EF hand	-1.47
257751_at	At3g18690	VQ motif-containing protein	-1.47
245821_at	At1g26270	phosphatidylinositol 3- and 4-kinase	-1.47
259582_at	At1g28060	small nuclear ribonucleoprotein (snRNP)	-1.47
267357_at	At2g40000	expressed protein	-1.48
259822_at	At1g66230	myb family transcription factor (MYB20)	-1.48
260770_at	At1g49200	zinc finger (C3HC4-type RING finger)	-1.48
250398_at	At5g11000	expressed protein	-1.49
262922_at	At1g79420	expressed protein	-1.5
256790_at	At3g16857	two-component responsive regulator	-1.5
264700_at	At1g70100	expressed protein	-1.5
250177_at	At5g14420	copine-related	-1.5
250004_at	At5g18750	DNAJ heat shock N-terminal domain-containing protein	-1.51
256542_at	At1g42550	expressed protein	-1.51
246283_at	At4g36860	LIM domain-containing protein	-1.51
258350_at	At3g17510	CBL-interacting protein kinase 1 (CIPK1)	-1.51
251321_at	At3g61460	zinc finger (C3HC4-type RING finger) (BRH1)	-1.51
260431_at	At1g68190	zinc finger (B-box type)	-1.52
254016_at	At4g26150	zinc finger (GATA type)	-1.52
266230_at	At2g28830	armadillo/beta-catenin repeat	-1.53
257858_at	At3g12920	expressed protein	-1.54
256262_at	At3g12150	expressed protein	-1.54
264200_at	At1g22650	beta-fructofuranosidase, putative	-1.55

255563_at	At4g01740	DC1 domain-containing protein	-1.55
264040_at	At2g03730	ACT domain-containing protein (ACR5)	-1.55
249319_s_at	At5g40880	WD-40 repeat (ZFWD4)	-1.55
254318_at	At4g22530	embryo-abundant protein-related	-1.56
250821_at	At5g05190	expressed protein	-1.56
265908_at	At4g00270	DNA-binding storekeeper protein-related	-1.57
264339_at	At1g70290	trehalose-6-phosphate synthase, putative	-1.57
251641_at	At3g57470	peptidase M16	-1.57
247413_at	At5g63020	disease resistance protein (CC-NBS-LRR class), putative	-1.57
262552_at	At1g31350	F-box	-1.57
261052_at	At1g01440	extra-large G-protein-related	-1.57
249694_at	At5g35790	glucose-6-phosphate 1-dehydrogenase (APG1)	-1.57
266882_at	At2g44670	senescence-associated protein-related	-1.58
256914_at	At3g23880	F-box	-1.58
252250_at	At3g49790	expressed protein	-1.58
249485_at	At5g39020	protein kinase	-1.58
267614_at	At2g26710	cytochrome P450, putative	-1.59
263106_at	At2g05160	zinc finger (CCCH-type)	-1.59
262801_at	At1g21010	expressed protein	-1.59
248888_at	At5g46240	inward rectifying potassium channel (KAT1)	-1.6
251621_at	At3g57700	protein kinase, putative	-1.6
249384_at	At5g39890	expressed protein	-1.6
257053_at	At3g15210	ethylene-responsive element-binding factor 4 (ERF4)	-1.6
260734_at	At1g17600	disease resistance protein (TIR-NBS-LRR class), putative	-1.6
260303_at	At1g70520	protein kinase	-1.6
258668_at	At3g08780	expressed protein	-1.61
256487_at	At1g31540	disease resistance protein (TIR-NBS-LRR class), putative	-1.62
267289_at	At2g23770	protein kinase	-1.62
254553_at	At4g19530	disease resistance protein (TIR-NBS-LRR class), putative	-1.62
245362_at	At4g17460	homeobox-leucine zipper protein 1 (HAT1)	-1.62
246180_at	At5g20840	phosphoinositide phosphatase	-1.62
259332_at	At3g03830	auxin-responsive protein, putative	-1.64
246215_at	At4g37180	myb family transcription factor	-1.65
250012_x_at	At5g18060	auxin-responsive protein, putative	-1.65
253075_at	At4g36150	disease resistance protein (TIR-NBS-LRR class), putative	-1.65
254573_at	At4g19420	pectinacetylesterase	-1.65
266803_at	At2g28930	protein kinase (APK1b)	-1.66
252167_at	At3g50560	short-chain dehydrogenase/reductase (SDR)	-1.66
257950_at	At3g21780	UDP-glucoronosyl/UDP-glucosyl transferase	-1.66
249606_at	At5g37260	myb family transcription factor	-1.67
246275_at	At4g36540	basic helix-loop-helix (bHLH)	-1.67
260362_at	At1g70530	protein kinase	-1.68
266313_at	At2g26980	CBL-interacting protein kinase 3 (CIPK3)	-1.68
253181_at	At4g35180	amino acid transporter	-1.68
255602_at	At4g01026	expressed protein	-1.69

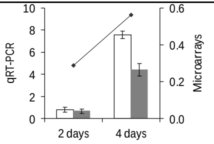
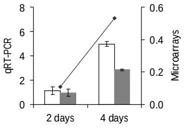
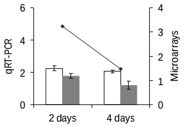
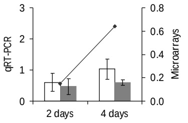
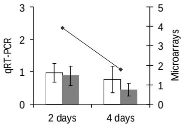
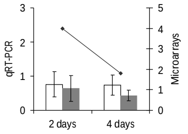
245005_at	rps14	rps14	-1.69
245493_at	At4g16380	At4g16380	-1.71
259784_at	At1g29450	auxin-responsive protein, putative	-1.71
249923_at	At5g19120	expressed protein	-1.71
253493_at	At4g31820	phototropic-responsive NPH3	-1.71
252862_at	At4g39830	L-ascorbate oxidase, putative	-1.72
252040_at	At3g52060	expressed protein	-1.72
264445_at	At1g27290	expressed protein	-1.73
257516_at	At1g69040	ACT domain containing protein (ACR4)	-1.74
253626_at	At4g30640	F-box (FBL19)	-1.75
260567_at	At2g43820	UDP-glucoronosyl/UDP-glucosyl transferase	-1.76
259076_at	At3g02140	expressed protein	-1.76
266259_at	At2g27830	expressed protein	-1.77
253298_at	At4g33560	expressed protein	-1.77
257764_at	At3g23010	disease resistance	-1.78
259879_at	At1g76650	calcium-binding EF hand	-1.79
259977_at	At1g76590	zinc-binding	-1.79
256789_at	At3g13672	seven in absentia (SINA)	-1.79
262399_at	At1g49500	expressed protein	-1.8
267238_at	At2g44130	kelch repeat-containing F-box	-1.81
258906_at	At3g06380	F-box	-1.82
256098_at	At1g13700	glucosamine	-1.83
252088_at	At3g52100	expressed protein	-1.83
245181_at	At5g12420	expressed protein	-1.84
258487_at	At3g02550	lateral organ boundaries domain protein 41 (LBD41)	-1.84
250860_at	At5g04770	amino acid permease	-1.85
257153_at	At3g27220	kelch repeat-containing protein	-1.85
249383_at	At5g39860	bHLH protein	-1.87
245566_at	At4g14610	At4g14610	-1.88
259787_at	At1g29460	auxin-responsive protein, putative	-1.9
266140_at	At2g28120	nodulin	-1.9
265387_at	At2g20670	expressed protein	-1.92
254931_at	At4g11460	protein kinase	-1.93
259790_s_at	At1g29430	auxin-responsive	-1.94
252234_at	At3g49780	phytosulfokines 3 (PSK3)	-1.96
265342_at	At2g18300	basic helix-loop-helix (bHLH)	-1.97
267115_s_at	At2g32540	cellulose synthase	-1.97
264836_at	At1g03610	expressed protein	-1.99
256948_at	At3g18920	zinc finger (C3HC4-type RING finger)	-2.01
267093_at	At2g38170	calcium exchanger (CAX1)	-2.02
266695_at	At2g19810	zinc finger (CCCH-type)	-2.02
264788_at	At2g17880	DNAJ heat shock protein, putative	-2.04
265276_at	At2g28400	expressed protein	-2.04
259015_at	At3g07350	expressed protein	-2.05
264846_at	At2g17850	senescence-associated	-2.07

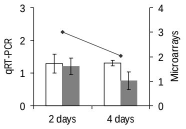
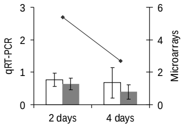
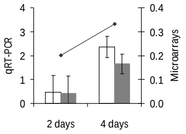
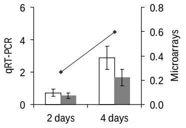
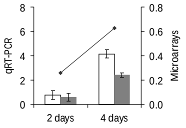
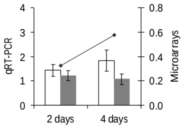
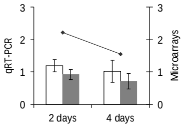
254027_at	At4g25835	AAA-type ATPase	-2.07
260126_at	At1g36370	glycine hydroxymethyltransferase, putative	-2.1
255807_at	At4g10270	wound-responsive	-2.2
259466_at	At1g19050	two-component responsive regulator	-2.21
247524_at	At5g61440	thioredoxin	-2.3
266072_at	At2g18700	glycosyl transferase family 20 protein	-2.36
250327_at	At5g12050	expressed protein	-2.44
251039_at	At5g02020	expressed protein	-2.49
248606_at	At5g49450	bZIP family transcription factor	-2.5
252367_at	At3g48360	speckle-type POZ protein-related	-2.53
249862_at	At5g22920	zinc finger (C3HC4-type RING finger)	-2.58
265067_at	At1g03850	glutaredoxin	-2.61
263302_at	At2g15190	pseudogene, Ulp1 protease family	-2.66
258930_at	At3g10040	expressed protein	-2.83
261567_at	At1g33055	expressed protein	-3.24
266814_at	At2g44910	homeobox-leucine zipper protein 4 (HB-4)	-3.95
245276_at	At4g16780	homeobox-leucine zipper protein 4 (HAT4)	-4.71

[1] Affymetrix microarray ATH1

[2] From the TAIR8 (The Arabidopsis Information Resource) database (http://www.arabidopsis.org)

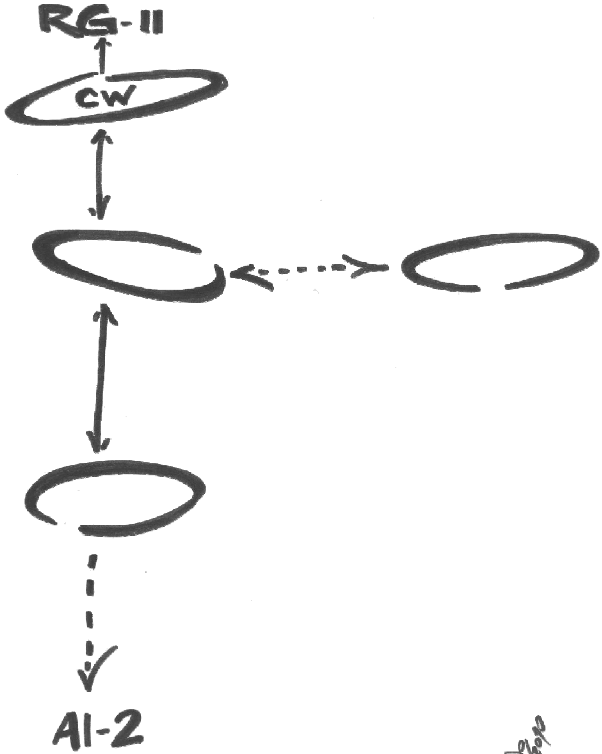
Supplementary Table 5.2. Genes selected to be validated by RT-qPCR. The thirteen genes selected were differentially expressed 2 and 4 days after B deficiency. The primers used, amplicons specifications [E(%), efficiency; AL(nt) amplicon length; Ta(ºC) annealing temperature] and relative expression are described.

AGI ID	**5'→3' Primer Sequences (Forward/Reverse)**			**Relative expression** Affymetrix ATH1 microarray (◆), RT-qPCR using two house-keeping genes: UBC10 (□) and PP2A (■).
	E(%)	**AL (nt)**	**Ta (ºC)**	
Housekeeping genes				
At1g13320	TAACGTGGCCAAAATGATGC GTTCTCCACAACCGCTTGGT			
	90.6	105	55	
At5g53300	ATGGGTCCTTCAGAGAGTCCT CTTGGTCCTAAAGGCCACCT			
	94.8	105	60	
Cell wall biosynthesis				
At2g19800	CTTGTTGAACATTTTGTTCCTGA CAAAACCTCCATCCAACACC			
	90.8	91	59	
At4g26260	ATCATAAGATACCATTCCTTTTACCC TGTCTTCCTCGTTCATAAGATGG			
	102.4	73	59	
At1g64390	GCTACTACTCACTCACGCCTATCA CGGTAGTATTTCTGGGCAACA			
	105.0	96	61	
Sulphur metabolism				
At1g64660	ACCGTGCTCAACCTCAGC GTTGCAGCATCACTGACGATA			
	101.7	103	61	
At1g65860	GTTGCTCATATTCCTGGGATAAA AATTTCCGATCACCACCACT			
	105.6	109	62	
At1g62560	GAGTTCCTGATCCATTCGAAAA CACCACTCGCAAAATTTCCT			
	96.9	60	61	

At4g04610	CTGTGAAGATGGCAATGTCTG ACCTCAACGAACCAATTTGC			
	92.0	114	61	
At5g24660	AGAAGCGGAGGAGCGTCT CGAGCCTGGTCTAAAGATTCTG			
	102.4	66	61	
Branched-chain amino acid (BCAA) catabolism				
At1g10070	TCACAAATTATGCGCCAGTT CGAGATAAAGAACGTCTGAAAACC			
	102.9	75	61	
At1g03090	AGAGACATGGGTGATAAAAGTGC TATCCAGGCACGAGAGGAAC			
	108.4	71	55	
Transcription				
At1g79700	ATCAAAATACAGAGGCGTTGC TCTTGCGTGGCGTATGTTC			
	98.6	111	61	
At1g76410	TCGTGGAGTGTGCTATCTGTTT GCAACACCCTAAGCTCGTCT			
	105.2	60	62	
Unknown				
At4g15920	GCCGTTTTGGTTGTCGTT ACACAAACCCCACTCCATTT			
	97.1	110	61	

CHAPTER 6

GENERAL DISCUSSION AND FUTURE PERSPECTIVES



GENERAL DISCUSSION

The aim of this Thesis was to increment knowledge of the role of B in higher plant metabolism. For this purpose, long-term effects of B deficiency were studied in *Lupinus albus* and early effects were studied in *Arabidopsis thaliana* using distinct approaches. The experimental work provided clear evidence of B requirements during plant growth and development, not only by the marked morphological changes observed, but also by the significant alterations detected in the plant metabolism.

Long-term B deficiency effects

The marked alterations observed in the leaves and roots of *Lupinus albus* after 4 weeks of withholding B, gave indications on the long-term B deficiency responses. The analysis of the metabolite content of *Lupinus albus* plants revealed minor changes in the content of sugars and absence of variation in malate (Chapter 4). The fact that malate is considered to be the ultimate product of glycolysis (Schulze et al. 2002) seems to indicate that the central carbohydrate metabolism is being little affected by B deficiency. Furthermore, in the study of the root proteome it was observed the *de novo* expression of UDP-glucose pyrophosphorylase, that is part of an alternative biochemical pathway for sucrose degradation (Chapter 3) and some ATPases were suppressed. Therefore, there appears to be an activation of alternative energy sources that will permit the carbon flow to continue under stressful conditions with a reduced ATP demand. Indeed, pyrophosphate is known to be produced in large amounts in the plant cells as a by-product of anabolic reaction and its preferential utilization as an energy donor is particularly important when cellular ATP pools become diminished during stress (e. g. cold, anoxia, phosphate starvation) (Stitt 1998). Such a metabolic flexibility that helps plants to survive stress thus appears to be operating under B deficiency.

Concomitant with the changes observed in energy metabolism we observed marked shifts in the expression of proteins related with several metabolic processes, such as those associated with the cell wall, protein and amino acid metabolism, defence responses and cytoskeleton biosynthesis. Considering the cell wall, proteins related with the synthesis of extracellular matrix polysaccharides were suppressed, supporting the observation that B deficiency causes the decreased expression of several cell wall modifying enzymes (Camacho-Cristóbal et al. 2008).

Proteins related to protein folding and proteolytic processes were markedly affected by B deficiency, some being suppressed and others expressed *de novo*. This observation points out that a shift in protein metabolism had occurred. It has been suggested that a repression of protein degradation is a cellular response to stress in order to reduce ATP demand (Geigenberger 2003). It is not directly evident if the altered free amino acid contents (Chapter 4) could somehow be related with the described alterations in protein metabolism, and/or if they could act as signalling molecules.

Several proteins related with cytoskeleton biosynthesis were affected by B deficiency. For instance, tubulins and actins were expressed *de novo* (Chapter 3). The additional increased content of the branched-chain amino acids (Chapter 4), possibly related with cytoskeleton biosynthesis (Kimball and Jefferson 2006, Rhoads and Wu 2009), also suggests an involvement of B with cytoskeleton biosynthetic processes.

The long-term effects just discussed could result from both direct and indirect stress responses to B deficiency, and indirect effects could have a high contribution to the overall metabolic responses. However, a certain degree of specificity was suggested from studies of the leaf apoplast (Chapter 2), where the majority of the responsive proteins that were commonly responsive to both B and water-deficit showed different patterns of expression in the two stress regimes.

Several protein isoforms were observed to be responsive to B deficiency in both leaf apoplast and root proteomes (Chapters 2 and 3). The differential expression of different protein isoforms were already found to have some relevance in fungal infection and symbiosis studies in *Medicago truncatula* (Salzer et al. 2000) and could play a determinant role in the ability of the plant to cope with stressful conditions. Amino acids could also be involved in regulatory processes (Kimball and Jefferson 2006), as referred to above for the branched-chain amino acids. Glycine, that was the only amino acid to show a strong negative effect due to B deficiency, was proposed to have a connection with free levels of Ca^{2+} in the cytosol, important for plant cell signalling processes (Dubos et al. 2003, Hepler 2005).

Short-term B deficiency effects

In *Arabidopsis thaliana* plants subjected to short-term B deficiency, no marked morphological effects were observed, although evident metabolic alterations were detected at the level of the transcriptome (Chapter 5). Altered expression of genes related with the cell wall were observed, which is in accordance with a previous report of down-regulation of cell wall related genes in short-term B-deficient roots of Arabidopsis (Camacho-Cristóbal et al. 2008). The other relevant results we obtained concern branched-chain amino acids, sulphur metabolism and transcription-related processes. The increased expression of branched-chain amino acid transferase gene was also recently reported by Kasajima et al. (2010) in B-deficient wild-type *Arabidopsis thaliana* plants. The increased expression of this gene could be related with impaired cytoskeleton biosynthesis since in animal systems, branched-chain amino acids were described to stimulate muscle protein synthesis (Rhoads and Wu 2009). Furthermore, increased levels of actin and tubulin with altered polymerization patterns have been reported in Arabidopsis roots in response to short-term B deficiency (Yu et al. 2001, 2003).

Concerning sulphur metabolism, we observe that the three genes of 5'-adenylylsulfate reductase (APR) are repressed in response to B deficiency. APR is a key enzyme of the sulphate assimilation pathway and the three genes constitute the entire family so far described (Gutierrez-Marcos et al. 1996, Vauclare et al. 2002). Hence, B deficiency seems to trigger responses similar to sulphate excess (Saito 2004).

Of the five genes involved in transcriptional processes, which were differentially expressed in B-deficient plants, three of them are associated with hormonal responses (e. g. auxin, cytokinin and jasmonic acid). Considering that there are as yet few studies relating B deficiency and plant hormones, this observation could be a starting point for further studies.

A plausible hypothesis for B participation in plant metabolism

Several hypotheses on the participation of B in plant metabolism have been proposed (Brown et al. 2002, Goldbach and Wimmer

2007, Bonilla et al. 2009). A few authors have proposed a participation of B in binding *cis*-diol molecules. However, the cell contains high abundance of many molecules possessing this chemical characteristic, including sugar moieties such as mannose, apiose, galactose (cell wall related), ribose (a major component of ribonucleic acids and nucleotides), glycoproteins and glycolipids (minor component of membranes) and other molecules like serine or threonine (e. g. proteins), which are also capable of forming stable B complexes (Loomis and Durst 1992, Brown et al. 2002). Considering that B is a relatively scarce micronutrient in plants and that about 90% of the B is covalently linked to the cell wall structure (Martini and Thellier 1993), the amount of B available for forming potential alternative interactions with compounds other than RG-II is severely limited. It is difficult to imagine how the loss of such a minor and non-specific interaction may lead to the observed symptoms of B deficiency and to alterations in specific areas of metabolism.

However, although the specific cellular mechanisms remain unknown, there is a large body of empirical evidence that B is specifically attached to RG-II during the biosynthesis of extracellular matrix components, implying the operation of highly specific targeting mechanisms. The demonstrable importance of B in other areas of metabolism, including that in animal systems which are devoid of cell walls, at least allows us to consider the possibility of additional mechanisms operating for the targeted delivery of B to other important acceptor molecules.

For instance, boronic acids and B containing peptides are known to be inhibitors of serine proteases (Kettner and Shenvi 1984, Nakamura et al. 2009). Serine proteases have been described as modulators of a high number of metabolic events in plants (Antão and Malcata 2005), including the infection process of plant cells during symbiosis (Ribeiro et al. 1995) and in a defence process that comprises an oxidative burst (Yano et al. 1999). The putative role of serine proteases in differentiation of specialized plant tissues was also proposed to be mediated by signalling processes associated with secondary cell wall synthesis (Groover and Jones 1999).

Some of metabolic processes affected by serine proteases are common to the processes affected by B depletion (Chapter 1), suggesting that at least part of the plant response to B deficiency may be mediated by B effects on serine proteases. This is an avenue of research which would be interesting to explore further.

Conclusions

In conclusion, the alteration of cytoskeleton biosynthetic processes appears to be a major result from both short- and long-term B deficiency.

Long-term effects of B deficiency on other areas of metabolism are also clearly observable. However, these almost certainly include those which are not directly affected by B deficiency, but result from down-stream events which are epistatic to earlier responses. These might include the later shift to alternative energy pathway, or other epistatic effects from the activation of signal pathways, including those involving, for instance ABA and jasmonate.

However, changes to the biosynthesis of the cytoskeleton might occur as a more immediate result to alterations in cell wall structure; whether this occurs as a result of perturbations in the cell wall–plasmalemma-cytoskeleton continuum, or due to other types of B interactions with the cytoskeleton remains to be elucidated. Such B participation in higher plant metabolism is consistent with the observation of higher B requirements in the early phases of animal development (Rowe and Eckhert 1999) and reproductive stages in plants (Behrendt and Zoglauer 1996), where the cytoskeleton provides an essential structure during cell division processes (Gunning and Hardham 1982).

In addition to the observed changes in cell wall metabolism that corroborate the described participation of B in this structure, an important novel link between sulphur metabolism and B deficiency was also proposed with the short-term B deficiency studies in *A. thaliana*.

FUTURE PERSPECTIVES

Although analytical instruments and procedures have steadily improved during the last decade, further progress greatly depends on the development of new methodologies with a higher capability for analytical imaging of B isotopes (^{10}B and ^{11}B) at physiological concentrations in plant tissues, in order to identify B-binding ligands. Promising biological tools are also available for an attempt to identify additional functions for B that may include for instance, the use of mutants available for *Arabidopsis thaliana* plants. Considering the previous discussion, it would seem interesting to assess if knock-out mutants in cytoskeleton components would have an ameliorated response to extra B supply, or if the over-expression of APR, the key enzyme in sulphur assimilation metabolism, would reduce symptoms of B deficiency.

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