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Nitrogen-source related effects on drought
stress response in *Medicago truncatula*

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List of Abbreviations

AAA ⁺	ATPase associated with various cellular activities
ABA	abscisic acid
ACC	acetyl-CoA carboxylase
ACN	acetonitrile
ANOVA	analysis of variance
BCKDC	branched chain α -keto acid dehydrogenase complex
CHL P	geranylgeranyl reductase (unit P of procaryote chlorophyll synthase)
CHR	chalcone reductase
CID	collision induced dissociation
clp	chloroplast
db	database
ED	density of epidermal pavement cells
ESI	electrospray ionisation
EST	expressed sequence tag
FA	formic acid
FqFm	photosystem II operating efficiency
Glu1P	glucose-1-phosphate
g_s	stomatal conductance
IAA	indole acetic acid
IPP	isopentenyl diphosphate
MEP/DOXP	2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate
MIPS	myo-inositol-1-phosphate synthase
MS	mass spectrometry
MS/MS	tandem mass spectrometry
mt	mitochondrial
MtGI	Medicago truncatula Gene Index
N-fed	nitrate fertilized
OGDC	2-oxoglutarate dehydrogenase complex
PC	principal component
PCA	principal component analysis

List of Tables

PDC	pyruvate dehydrogenase complex
PPFD	photosynthetically active photon flux density
prec	precursor
ProMEX	protein mass spectra extraction
PSII	photosystem II
R	isotope ratio
rpm	runs per minute
RWC	relative water content
SAM	S-adenosylmethionine
SC	spectral count
SD	stomatal density
SE	standard error
SHMT	serine hydroxymethyltransferase
SI	stomatal index
SPEC	solid phase extraction cartridges
Sym	symbiotic
TC	tentative consensus
TCA	tricarmonic acid

1. Introduction

1.1. The legume family and the model organism *Medicago truncatula*

Legumes evolved during the early Tertiary period, approximately 60 million years ago [28]. Today they are ubiquitous elements of most of the world's biomes in the form of annuals, shrubs, vines, lianas, trees and even some aquatic life-forms. Third to Orchidaceae and Asteraceae, the Leguminosae constitute with 20 000 plant species [29] one of the largest angiosperm families. The family counts 41 domesticated crop species [19] whereof the majority is found within the Papilionoideae subfamily, namely within the hologalegia and millettoid/phaseoloid clades which group the 'cool-season or temperate legumes' and the 'warm season or tropical legumes', respectively. Crop legumes play a central role in human health and nutrition. Their secondary metabolites are used for medical purposes and their protein rich seeds contribute to balanced diets especially in developing countries. Because of their ability to establish root nodules with nitrogen-fixing soil bacteria, legumes are of special interest for sustainable agriculture.

Only 25% of the species within the basal subfamily Caesalpinioideae, and 90% in the Mimosoideae and Papilionoideae are nodule forming [20]. Lacking fossil evidence, we do not know when legumes began to associate with nodule-inducing bacteria, collectively called rhizobia. Their *nod* genes are induced after recognition of mostly flavonoid root exudates, leading to the expression of bacterial Nod-factors which stimulate further molecular communication. This results in the formation of infection threads by which rhizobia enter root tissues, the ultimate organogenesis of nodules and the differentiation of bacteria into bacterioids surrounded by a plant-derived membrane. The microsymbiont subsequently converts highly unreactive atmospheric N₂ into a reduced form, amenable to the host's metabolism. The reaction of N₂ to ammonia catalyzed by bacterial nitrogenase has a high energy demand and requires an environment of low oxygen concentration. Both prerequisites are provided by the plant host.

The establishment of *Arabidopsis thaliana* as the first plant model organism, more than three decades ago, has been facilitating the investigation of complex biological processes. The development of the model legumes *Lotus japonicus* and *M. truncatula* started in the late 1990ies, primarily aiming at the study of plant-microbe interactions. Their genomes are now almost completely sequenced.

Medicago truncatula Gaertn. is an important forage legume and has been additionally chosen as a model representative for cold season grain legumes, such as pea *Pisum sativum*, faba bean *Vicia faba*, lentil *Lens culinaris*, chickpea *Cicer arietinum*, and others. *M. truncatula* combines convenient attributes for a plant model species: it is easily transformable and cultivable, its autogamy results in abundant seed production, the genome is relatively small with ~500Mb (compared to 5000Mb in pea [39]), it has a short reproductive cycle and it features high phenotypic plasticity. Up to date the *M. truncatula* genome is nearly

1. Introduction

completely sequenced and the DFCI Medicago Gene Index comprises 268 712 expressed sequence tags, which is an important condition for the straight-forward functional annotation of protein sequences.

1.2. Mass spectrometry-based proteomics

Sessile organisms adapt to the environment through biochemical processes: signals are transduced and perceived, the response is mediated via gene expression. Completed genome sequences and the outcome of numerous sequencing projects facilitate the analysis of gene products. Gene expression is addressed on two levels: the RNA and the protein level. Studying the dynamics of RNA (transcriptomics) provides information about the relative amounts of RNA, which are not necessarily proportional to levels of the encoded proteins [2]. This is all the more evident since proteins are present at a high dynamic range and are underlying mechanisms of synthesis, degradation and postranslational modifications. Thus studying proteomes is much closer to cellular function and plays a key role in our understanding of metabolic networks. A proteome is defined as the total set of proteins expressed by a genome in a cell, tissue or organism [49, 53]. Since proteins are biochemically much more diverse than RNA, their large-scale identification and quantification requires analytical methods capable of dealing with their chemical properties and huge differences in protein concentration.

Mass spectrometry, in combination with prefractionation and separation techniques, such as 2DE and HPLC-shotgun, are the most common and efficient tools at present. Since the invention of soft ionization methods MALDI (Matrix Assisted Laser Desorption) and ESI (Electrospray ionization) it became possible to ionize larger molecules, such as peptides, making them amenable to MS analyses. MS instrumentation has substantially evolved during the past decade. Fractionation techniques (such as collision induced dissociation, CID) furnish additional criteria for compound identification. Mass analyzers became faster and highly accurate allowing for the determination of accurate mass ion-molecules.

Protein quantification is indispensable for the assessment of protein function and dynamics. Various approaches exist: quantification via stable-isotope labeling (metabolic, chemical or standard peptide spiking) and label-free techniques, like spectral counting (SC). The method of choice, depends on the initial scientific question. SC method allows for quantification within a high dynamic range (3 orders of magnitude [54, 41]). Its major advantage lies in its robust high throughput properties. A typical shotgun proteomics analysis of tryptic peptides yields two types of mass spectra: full scan spectra of all peptides eluting at a given time-point and CID fragment spectra (MS/MS) of a selection of peptides presenting usually the highest peaks in the survey scan. SC refers to the number of MS/MS spectra. In a database dependent, approach the number of MS/MS spectra of all peptides attributable to one protein. In a database independent approach, the number of MS/MS spectra per peptide. In this case, the number of fragment spectra are assigned to the peptide precursor ions by mass accuracy precursor alignment (MAPA, [21]). Liu *et al.* [30] demonstrated the correlation of SC and protein concentration. So far it has already been used in several studies, including the analysis of the *M. truncatula* nodule proteome [52]. In the present study the SC method allowed for relative quantification of over-all protein abundance changes within the *M. truncatula* shoot proteome in response to drought.

1.3. General objectives of this work

Nitrogen is a principal constituent of life in the form of amino acids, proteins and DNA. It is abundant in the atmosphere, but gaseous N_2 is highly unreactive and not amenable to most organisms. Since the invention of the Haber-Bosch process, mankind invested in promoting plant productivity by the application of synthetic N fertilizers. Today, a considerable part (over 40%) of the global human population depends upon synthetic N fertilizers [12]. The harmful effects of agricultural intensification on natural ecosystems and human health are now being intensively investigated [15, 14, 18] and novel strategies to assure global nutrition by more efficient and less energy-costly means are required. In addition, crop productivity is exposed to changing environmental conditions, claiming plant breeders to focus on tolerance to diverse abiotic stresses.

Foreseeable future climatic changes will lead to a rising atmospheric CO_2 concentration along with an increase in temperature. Thus, among abiotic stresses major attention should be given to drought. Elevated CO_2 levels and increased temperature may enhance plant production and efficiency in N_2 -fixing symbiosis. Interestingly, experiments on drought stress in symbiotic compared to nitrogen-fed plants showed an enhanced stress tolerance in nodulated common bean [31], soybean [25] and the temperate forage legume alfalfa [1] compared to nitrate-fed individuals. However, a full understanding of the interactive regulatory mechanisms between plant and bacteroids towards increased stress tolerance has not been accomplished so far.

Deciphering the role of N-source in drought stress responsiveness and the molecular mechanisms governing this differential control of water relations during drought is a fundamental question in plant physiology with important consequences on agricultural systems. To address this issue an integrative physiological and proteomic approach will be used, subdivided into the following specific goals:

- (i) Physiological analyses of *M. truncatula* grown under different N sources subjected to progressive drought.
- (ii) Large-scale mass spectrometry based comparative proteome analysis
- (iii) Metabolic modeling based on results and (public available) genomic database information.

For the first time, evidence for the different N-nutrition dependent proteome response to drought and the enhanced tolerance in symbiotic *M.truncatula* will be presented here. In addition, an attempt to unravel the regulatory relevant mechanisms is given.

2. Material and Methods

2.1. Experimental setup

The experimental setup was configured according to Larrainzar *et al.* [27]. Fertilized (N-fed) and symbiotic plants were growing for seven weeks. Three timepoints were defined for the majority of the analyses: one day before the drought stress treatment (referred to as “day 0”), control measurements were performed; after three days (“day 3”) and after six days (“day 6”) of water withdrawal, stressed plants and their respective controls were analyzed and harvested. Table 2.1 gives an overview of the measurements and their reciprocal time-points. Figure summarized the idioms used and illustrates the setup on a time-scale.

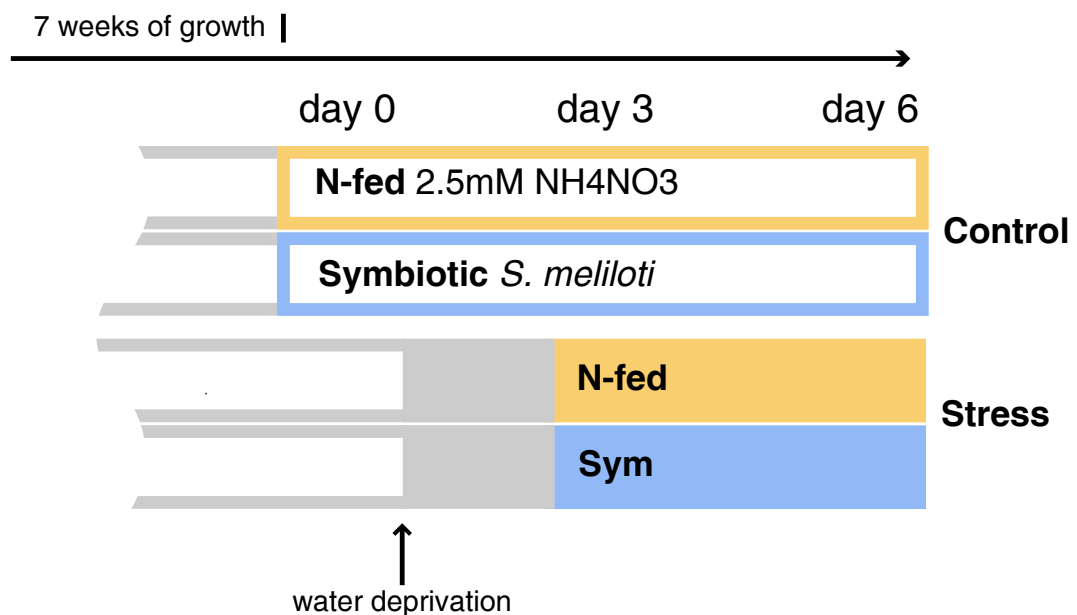


Figure 2.1.: Schematic of the experimental setup. The type of N-source is noted within the rectangles. The period of examinations is colored. Stress conditions are indicated by filled, control conditions by unfilled rectangles.

2.2. Cultivation

Medicago truncatula 'Jemalong A17'

Seeds were scarified with sulfuric acid, surface-sterilized with 14% sodium hypochlorite and pre-germinated onn 0.8% agar plates, stored for two days at 4 °C, two days in darkness at

Table 2.1.: Types of measurements and their timepoints.

	day 0			day 3			day6
substrate RWC		x	x	x	x	x	x
stomatal density	x			x			x
stomatal conductance	x			x			x
PSII operating efficiency	x			x			x
root/shoot ratio							x
$\delta^{15}N$							x
$\delta^{13}C$							x
shoot proteome							x

room temperature and one day in light. The seedlings were then set into 800 ml pots with a mixture of vermiculite:perlite (5:2, v:v) as inert substrate. Plants were grown in a climatic chamber under controlled environmental conditions similar to previous studies [27, 51, 26], in order to facilitate comparison of results (14/10 h day/night periode, 22/16 °C temperature, 50/60% relative humidity, 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD). During the first week plants were watered with Evans nutrient solution [13] containing 0.5 mmol NH_4NO_3 . During the second and third week plants received 2.5 mmol NH_4NO_3 . After 21 days of growth, plants were provided with nitrogen-free nutrient solution in order to facilitate nodule formation. One subset was inoculated twice with *S. meliloti* 2011 on days 23 and 25, the remainder of the subset was provided again with 2.5 mmol NH_4NO_3 . After 49 days of growth a randomly chosen set of plants was exposed to drought, while the rest was kept as controls.

Sinorhizobium meliloti strain 2011

S. meliloti was cultivated and stored on TY-agar medium (see A.4). For inoculation of plants with the bacterium, a TY medium-solution was prepared in which *S. meliloti* grew for two days in an incubator at 20 °C and 120 rpm (innova44, New Brunswick Scientific, USA) . To each pot 1 mL was added. A second milliliter per pot of a freshly prepared bacterial solution was added two days later in order to ensure the development of root nodules.

2.3. Stomatal density and index

For stomata printing the terminal leaflet of the youngest fully developed leaf emerging from the main axis was used. Imprints were performed as described before [32]. Four circular areas with an diameter of 0.25 mm were chosen (two at the basis and two at the tip of the lamina) and investigated with respect to their number of stomata and epidermal cells. Stomatal index was calculated as follows [38]:

$$SI = \frac{SD}{ED + SD} \cdot 100 \quad (2.1)$$

where SD is the stomatal density and ED is the density of epidermal cells. Those analyses of stomatal distribution were undertaken by Anne-Mette Hanak, Department Molecular Systems Biology, University of Vienna.

2.4. Stomatal conductance

Stomatal conductance g_s was measured 3h after the onset of the photoperiod with a steady state porometer (PMR-4, PP Systems, Hitchin, UK) connected to the EGM-4 gas monitor serving as data logger. About 0.5 cm² of terminal leaflets of fully expanded leaves were placed into the a cuvette. Records were taken after ~20 seconds, when an equilibrium was established. The inlet air flow rate was kept constant at 75 mL/min. The porometer then measures the air humidity of inlet and outlet air flow, air temperature and the PPFD reaching the leaf. From these parameters g_s was calculated.

2.5. Chlorophyll fluorometry

Measurements were performed with the MINI-head version of the IMAGING-PAM chlorophyll fluorometer M-series (Heinz Walz GmbH, Effeltrich, Germany). Primary chlorophyll fluorescence parameters were assessed by a saturation pulse method. Of each plant the second leaf of the first branch (m1n2, according to [6]) was analyzed assuring that the compared leaves are of approximately the same age and developmental stage. Sequentially augmenting PPFDs were applied to assess primary chlorophyll fluorescence parameters (F'_m, F') of light-adapted leaves. Those served to calculate the PSII operating efficiency as a ratio of the quenched fluorescence and the maximal fluorescence from light adapted leaves.

$$F'_q/F'_m = \frac{F'_m - F'}{F'_m} = \Delta F/F'_m = \Phi_{PSII} \quad (2.2)$$

For each sample three areas of interest were defined. The ImagingWin v2.32 program then calculated the average F'_m, F' value for each area which were subsequently exported for statistical analyses.

2.6. Nitrogen and carbon isotopic signatures

Nitrogen and carbon isotopic signatures were assayed as described before [50]. Lyophilized root and shoot material was grind with a ball mill (Retsch, MM400, Haan, Germany), 1.5 mg to 2 mg were filled into tin capsules and analyzed for carbon and nitrogen concentration and stable isotope ratios ($\delta^{15}N$, $\delta^{13}C$). This was done by continuous flow Elemental Analyzer (EA 1110, CE Instruments, Milan, Italy) interfaced via ConFlo III (Thermo Finnigan) with a isotope ratio mass spectrometer (Delta^{PLUS}, Finnigan MAT, Bremen, Germany) at the Stable Isotope Laboratory for Environmental Research, University of Vienna. The abundance of ^{15}N was expressed as parts per thousand relative to N_2 in the atmosphere:

$$\delta^{15}N = 1000 * \left(\frac{R_{sample}}{R_{atmosphere}} - 1 \right) \quad (2.3)$$

with $R_{atmosphere} = 0.0036$ R and R_{sample} being the ratio of measured ^{15}N atoms to the total number of N atoms in the sample. $\delta^{13}C$ was calculated in the same way using the international standard Vienna Pee Dee Belemnite. Samples were measured against CO_2 reference gas which was before calibrated to IAEA-CH-6 and IAEA-CH-7 reference material (International Atomic Energy Agency, Vienna, Austria).

2.7. Leaf protein extraction and MS analysis

Protein extraction

Six hours after onset of the light periode, three biological replicates for every condition (N-fed and symbiotic controls, N-fed and symbiotic drought stressed plants) were harvested, and directly quenched in liquid nitrogen. *M. truncatula* shoot material ground with mortar and pestle under constant addition of liquid nitrogen. 200 mg were homogenized at 4 °C with 1 mL ice-cold lysis-buffer (HEPES 50 mmol, EDTA 1 mmol, KCl 11 mmol, MgCl₂ 2 mmol, pH 7.8) and centrifuged (10000g, 20 min, 4 °C). The supernatant precipitated over night with 5 volumes of ice-cold acetone with 0.5% β -mercaptoethanol. The pellet itself was homogenized in 1 mL urea-buffer (Urea 8 mol, HEPES 50 mmol, pH 7.8) and centrifuged (4000g, 10 min 4 °C). Hardly soluble and membrane proteins in the supernatant were precipitated as mentioned above. Centrifugation (4000g, 15 min, 4 °C) acetone was decanted and the pellet of precipitated proteins was washed with 2 mL ice-cold methanol, 0.5% β -mercaptoethanol. After anew centrifugation (4000g, 10 min, 4 °C) the air dried pellet was dissolved in 500 μ L urea-buffer. Then protein concentration was determined via Bradford protein assay ([5]).

Protein digestion and desalting

Protein extracts dissolved in urea buffer were first digested for 5 h at 37 °C with the sequencing grade endoproteinase Lys-C (1:100, vol/vol, Roche, Mannheim, Germany) cutting peptide bonds C-terminally at lysine. After dilution with trypsin buffer (10% ACN, 50mM AmBic, 2mM CaCl₂) to a final concentration of 2M urea, the second digestion step was performed using Poroszyme immobilized trypsin beads (8h at 37 °C). Samples were desalted using solid phase extraction cartridges (SPEC) C18 columns (Varian) according to the manufacturer's manual and subsequently concentrated to complete dryness in a speed-vac.

nanoESI LC-MS/MS

Every protein extract was analyzed twice (2 technical, 3 biological replicates, soluble and membrane fraction). Digested protein pellets were dissolved in 5% acetonitrile (ACN), 0.1% formic acid (FA). 50 μ g of protein were loaded on a monolithic column with an inner diameter of 0.1 mm and a lenght of 100 mm (Chromolith, Merck, Darmstadt, Germany).

The column was coupled, using an UHPLC system (Eksigent) to an Orbitrap LTQ XL mass spectrometer (Thermo Electron, Bremen, Germany). Peptides were eluted during a 150min gradient from 90% solvent A (0.1% formic acid in water) to 80% solvent B (80% acetonitrile, 0.1% formic acid in water) with 400 nL/min.

The eluting peptides were analyzed with an acquisition cycle containing six different scan events. An Orbitrap survey MS scan with a resolving power of 30000 over the mass range from 400 to 1800 m/z was succeeded by five data-dependent MS/MS scans in the fast scanning linear ion trap, in which the five most abundant survey scan peptides were selected for CID using normalized collision energy of 35%.

2.8. Data mining

Database dependent analysis of MS data

For the database (db) dependent analysis the search algorithm Sequest combined with the MS data mining software Proteome discoverer (Thermo Electron, Bremen, Germany) were used. The resulting spectra were searched against a genomic database translated in all six reading frames (*Medicago truncatula* Gene Index, release 10.0) using the SEQUEST search algorithm.

Estimation of the quality of protein identifications, involves a search against a decoy database. Any match to such a shuffled database is considered as a false positive peptide identification. In this way statistical confidence measures can be assigned to every peptide.

Only protein identifications were used fulfilling stringent quality criteria. For this analysis high peptide confidence (resulting in a false positive rate less than 1% for the whole data set) and an assignment of at least two different peptides per protein were required.

Database independent analysis of MS data (MAPA)

The mass accuracy dependent peptide alignment (MAPA) has been explained in detail by Hoehenwarter *et al.* [21]. Here the technique is summarized. The Thermo Xcalibur software stores mass spectra obtained by LTQ-Orbitrap measurements in .RAW-files. Those were converted in .mzXML-files using the ReAdW program version 4.3.0 from the Institute for Systems Biology (Seattle, WA, USA, <http://www.sourceforge.net/projects/sashimi/files>). With the ProtMAX application (downloadable at <http://promex.mpimp-golm.mpg.de/>, [21]) mass precursor alignment was performed in MSn mode resulting in a matrix of spectral counts for every m/z (rounded to the second decimal) within the respective measurement. This approach has been used here for a more detailed analysis that enabled the distinct detection of two similar isoforms of regulatory relevance (see section 4.2.3).

Statistical analysis

The identified proteins were subsequently assembled to a matrix containing the spectral counts of each sample. Missing values were replaced by 1. Since spectral counts are discrete numbers they are not normally distributed. In order to make the data set amenable to statistical analyses requiring normal distribution, the data were log10 transformed.

Exclusion of all protein identifications presenting SCs in less than three (out of six) replicates resulted in a 353x24-dimensional data matrix. As biological variables are generally somehow correlated, the single data points are not equally distributed within this highly dimensional space [40]. Consequently, it is possible to reduce the number of dimensions (i.e. of variables) by maintaining as much variance as possible. The principal component analysis (PCA) transforms a d-dimensional sample vector to a vector of lower dimensionality (k). The first two principal components enclose a two-dimensional subspace explaining the highest variance of the data set. Additionally the loadings or weights which are the elements of the transformation vector, give information about the contribution of the original variable to a corresponding PC. Thus we can state how important one variable is for the separation of samples along a PC. As long as the biological question is related to the highest variance in the data set, PCA is an appropriate means to extract important variables [40]. In case influences other than those related to the initial question are more important,

2. Material and Methods

unsupervised methods, like Independent Component Analysis, are more suitable, because they ignore the experimental setup.

Fold-change was calculated as relative changes in protein abundance compared to controls. The denominator is the median SC of controls since it is less affected by outliers than the arithmetic mean.

$$\log_2 \text{foldchange} = \log_2 \left(\frac{SC_{\text{drought}}}{\text{median}(SC_{\text{control}})} \right) \quad (2.4)$$

The log2 transformation was performed simply to obtain symmetric values around zero instead of asymmetric values around 1 (like 0.5 and 2 for a two-fold change). The log10 transformed data set was used for assessing the significance level of drought responsive changes in protein abundance by t-test statistics.

Results of the physiological measurements were examined by two-way analysis of variance (ANOVA) to assess the statistical relevance of drought and nitrogen source effects. The number of replicates is indicated in tables and figures, respectively.

3. Results

3.1. Physiological measurements

Stomatal distribution. Examination of the stomatal distribution revealed similarity of N-fed and symbiotic plants concerning stomatal density and stomatal index under both, control and drought conditions. Interestingly, the stomatal densities and indices on both sides of the leaf differed significantly (see table A.1). Irrespective of nitrogen source conditions, stomatal density was about 25% higher on the adaxial than on the abaxial leaf surface (figure 3.1).

Stomatal conductance. In order to assess leaf-side specific differences in gas exchange, we first measured the stomatal conductance (g_s) on both surfaces. Since g_s did not differ between the surfaces, neither during water deficit nor during control conditions, we subsequently examined the abaxial g_s exclusively. Also on the level of gas exchange there was no detectable relevant difference attributable to the type of nitrogen source in controls (table A.2). At the onset of water deficit g_s decreased significantly in both sets of plants compared to the respective controls. Resulting in a 65% decrease of g_s after 6 days of water withholding.

Substrate relative water content. To assay any quantitative differences in transpiration, we determined the change in substrate RWC gravimetrically. Under all N source conditions the same rate of decrease in substrate relative water content (RWC) was observed. Already on day 3 the decrease is significant with respect to the initial RWC. At the end of the experiment RWC was decreased by 70%. The RWC of N-fed *M. truncatula* pots decreased slightly more rapidly without being statistically significant (figure A.1, page I).

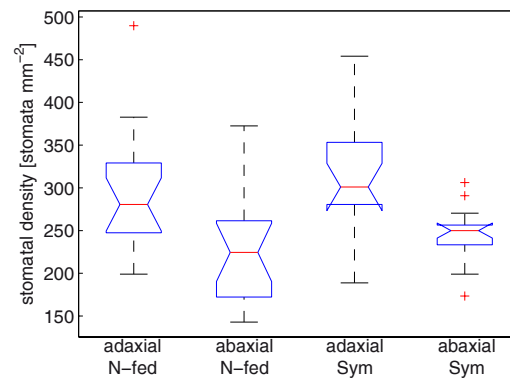


Figure 3.1.: Adaxial and abaxial stomatal density of N-fed and symbiotic *M. truncatula* leaves (n=17)

Chlorophyll fluorescence parameters. Rapid light induction curves were performed, i.e. leaves were exposed to sequentially augmenting photosynthetically active photon flux densities (PPFD) to allow for an estimation of their photosynthetic performance. The efficiency

3. Results

Table 3.1.: Effect of day 6 drought on naturally abundant stable isotope concentrations and root/shoot ratios in N-fed and symbiotic plants. Values represent mean $\pm$ standard error. Two-way ANOVA results are represented as follows: N stands for an effect of nitrogen source, D for an effect of drought and I for an effect attributed to an interaction of N and D. Triple asterisk indicate p-values <0.001 , double asterisk <0.01 and a single asterisk <0.05 .

	N-fed plants		symbiotic plants		ANOVA		
	Control	Drought	Control	Drought	N	D	I
$\delta^{15}N$ shoot	3.46 $\pm$ 0.12	2.90 $\pm$ 0.49	-0.37 $\pm$ 0.04	-0.25 $\pm$ 0.11	***	ns	ns
$\delta^{15}N$ root	0.77 $\pm$ 0.69	1.33 $\pm$ 0.18	-0.21 $\pm$ 0.2	-0.27 $\pm$ 0.08	**	ns	ns
$\delta^{13}C$ shoot	33.51 0.19	32.18 0.19	33.24 0.1	32.57 0.13	ns	***	*
$\delta^{13}C$ root	32.38 $\pm$ 0.38	31.03 $\pm$ 0.29	31.86 $\pm$ 0.34	31.56 $\pm$ 0.26	ns	**	ns
root/shoot ratio	0.63 $\pm$ 0.10	1.01 $\pm$ 0.21	0.81 $\pm$ 0.1	1.25 $\pm$ 0.06	*	**	ns

at which light energy absorbed by PSII is used to drive photochemistry (F'_q/F'_m) declines in N-fed plants during the drought treatment, while symbiotic plants had photosystem II operating efficiencies comparable to controls. The PPFD at which F'_q/F'_m began to decrease significantly in drought stressed N-fed plants corresponded to light conditions in the climatic chamber.

Root/shoot ratio. Nodulated plants presented a 30% higher root/shoot ratio than N-fed plants. In response to drought, root total dry weight compared to shoot dry weight increased in both sets similarly (table 3.1).

$\delta^{15}N$ signatures. In symbiotic plants $\delta^{15}N$ values were slightly negative. Furthermore, the ^{15}N concentrations in symbiotic root and shoot tissues are similar. There are no pronounced changes induced by 6 days of water deprivation. On the contrary, plants relying on synthetic NH_4NO_3 contained in the nutrient solution have positive and much higher $\delta^{15}N$ values (table 3.1 and figure A.3, page II). The ^{15}N isotope is accumulated to a greater extend in shoots compared to roots. The difference in ^{15}N organ-specific concentrations gets smaller under drought stress condition, because the concentration in shoots drops and the concentration in roots rises.

$\delta^{13}C$ signatures. Plants respond to drought with a significant increase of $\delta^{13}C$ in all tissues. Notably, symbiotic $\delta^{13}C$ values were in general higher, indicating distinct rates of ^{13}C discrimination (table 3.1 and figure A.4, page II).

3.2. Proteomic analyses

After shoot protein extraction of day 6 drought stressed and control plants, two different fractions were obtained. One crude extract contained soluble proteins and the other contained less soluble (membrane) proteins. Both fractions were analyzed via liquid chromatography coupled to tandem-mass spectrometry. 48 measurements resulted in approximately 400000 recorded spectra. The MS/MS spectra were compared to the predicted spectra of the six-frame translated *Medicago truncatula* Gene Index, release 10.0 genomic database using the SEQUEST algorithm [11]. By applying stringent quality criteria (high-confidence and at least two different peptides per protein), we identified 604

proteins. The identified spectra will be publicly available within the ProMEX database (<http://www.promex.pph.univie.ac/promex/> [22]).

The number of spectra that could be assigned to peptides constituting one protein (i.e. spectral count) was used for relative quantification, as described previously [27]. All proteins presenting SC in more than three of the six replicates were used for quantification and the subsequent data analysis.

As shown in figure 3.2, Principal component analysis revealed a clear clustering of the four different sets of plants. Principal component 1 (PC1), the component of highest variance, allows for discrimination of N-fed from symbiotic controls. PC2 contains valuable information to distinguish between stressed and control plants. Furthermore, N-fed and symbiotic drought stressed plants are also separated along this axis.

The separation of N-fed and symbiotic drought stressed plants is clear but minor compared to the separation of controls and plants exposed to water deficit. Consequently, two additional parameters were used to determine relevant proteins: the fold-change of abundance compared to control protein expression levels and the t-test significance. Proteins exhibiting an at least 2-fold significant ($p < 0.05$) change are displayed in table 3.3 for N-fed and in table 3.2 for symbiotic samples. The tables additionally contain the loadings for PC2. Boldfaced loadings rank among the 20 most important for this component. Proteins with lower loading values have on the contrary a stronger impact on the separation between the stressed N-nutritional conditions.

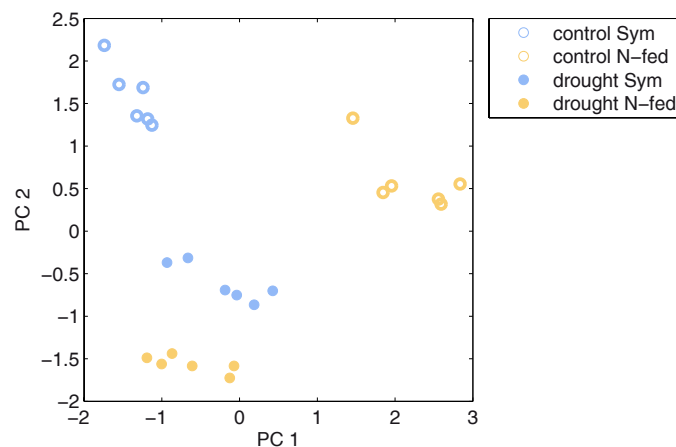


Figure 3.2.: Principal component analysis plot of label-free shotgun proteomics data. PC1 explains 23.78% and PC2 18.01% of the total variance in the data set (3 biological, 2 technical replicates).

Although the described by PC1 and PC2 variance was not very high, 57 proteins could be determined that changed significantly in response to drought, i.e. proteins with a minimum two-fold and statistically significant ($p < 0.05$, t-test) change in abundance (see tables 3.3 and 3.2). In the following some of these proteins will be described and an overview will summarize their possible roles within the metabolic network.

The database-dependent identification of plant responsiveness to drought on a protein abundance level revealed two enzymes reacting similarly in N-fed as well as in symbiotic *M.truncatula*: magnesium chelatase decreased and chalcone reductase increased in abundance relative to controls.

3. Results

Mg-chelatase inserts a Mg^{2+} ion into protoporphyrin IX. It is a heteromeric enzyme consisting of three distinct subunits of which we identified the smallest one, the CHLI 40kDa subunit. This subunit has been classified within the AAA⁺-proteins (ATPase associated with various cellular activities). The CHLI-catalyzed ATP hydrolysis was found to be determining the rate constant of the reaction yielding Mg-protoporphyrin [23]. Recently the CHLH subunit of Mg-chelatase in *A. thaliana* has been shown to be a component in retrograde signaling [33] and to counteract the transcription factor- regulated inhibition of ABA-responsive genes [42]. In *Hordeum vulgare*, however, involvement of CHLH in ABA-signaling could not be supported so far [35].

Chalcone reductase and chalcone synthase coact during the initial steps of flavonoid biosynthesis. CHR is reported to be necessary for the synthesis of 5-deoxyflavonoids. Flavonoids are well known for their role as phytoalexins and antioxidants.

Apart from Mg-chelatase and chalcone reductase, I exclusively detected distinct drought-responsive proteins in the two sets of plants. Their general stress response exhibit contrarian characters. While N-fed plants react with a general up-regulation of protein abundance level, symbiotic plants show a decrease in abundance for a great majority of the identified proteins. The following two sections comprehend these N-source specific responses to drought.

Symbiotic drought stress responsive leaf proteome (see table 3.2)

In symbiotic plants the abundances of 27 proteins were significantly altered after 6 days of drought treatment. All of them decrease in abundance, except the aforementioned CHR. The proteins can be grouped in the following functional groups: photosynthesis related, protein homeostasis (here: protein folding and degradation), amino acid metabolism, CHO metabolism, cellular development and organization, fatty acid metabolism and tricarboxylic acid (TCA) cycle. The function of some of them will be described in the following.

Photosynthesis related (3). Additionally to Mg-chelatase, I identified geranylgeranyl-reductase (CHL P) which plays a dual role during chlorophyll biosynthesis. On the one hand, it is involved in the reduction of geranylgeranyl-chlorophyll into chlorophyll a and on the other hand CHL P is part of the tetrapyrrole metabolism which plays a crucial role in the plastidial terpenoid biosynthesis, the non-mevalonate (or MEX/DOXP) pathway. In the plastid isopentenyl diphosphate (IPP), a precursor of several metabolites, is synthesized from two three-carbon compounds, pyruvate and glyceraldehyde-3-phosphate. IPP units are then isomerized producing 10-, 20- and 40-carbon compounds. CHL P catalyzes the reduction of geranylgeranyl diphosphate (20-C) to phytol diphosphate providing the phytol side chain of chlorophyll and the side chains of α -tocopherol and phylloquinone, both important antioxidant compounds. A decrease in CHL P transcript level has so far been reported in response to wounding, heat and cold stress in cucumber, wheat and peach ([34],[17]). Furthermore a significant decrease in abundance of photosystem I reaction center subunit N was stated by this experiment, which could be in direct relation to the decreased abundances of Mg-chelatase and CHL P synthesizing both constitutive moieties of chlorophyll.

Protein homeostasis (4). Four identified (precursor) proteins playing a role in protein homeostasis. One of importance in protein folding (peptidyl-prolyl cis-trans isomerase), and three involved in protein degradation: cysteine proteinase 15A, and the two subunits of Clp protease. Clp proteases are housekeeping enzymes localized in the plastid stroma.

Generally they are composed of a proteolytic system and of AAA⁺ chaperones (like the identified ClpC subunit) that target specific substrates.

Amino acid metabolism and fatty acid biosynthesis (2). SAM synthetase catalyzes the adenylation of methionine to S-adenosylmethionine (SAM) which is a methyl group donor and precursor of ethylene, nicotinamine, osmolytic polyamines and the cofactor biotin. We found biotin carboxylase which is one of four proteins constituting plastidial acetyl-CoA carboxylase. The product, malonyl-CoA is an essential precursor for the fatty acid and polyketide biosynthesis.

N-fed drought stress responsive leaf proteome (see table 3.3)

In N-fed plants the abundances of 20 identified proteins were significantly altered. 18 of them showed an increase in abundance; the log₂ fold-change values can be found in the appendix. They are grouped within the following categories: defense and signaling, free radical scavenging, photosynthesis related, cellular development and organization, TCA cycle, fatty acid and lipid metabolism and CHO metabolism. A selection of them and their metabolic relevance are noted in the following.

Defense and signaling (4). Plant endochitinases control fungal pathogens. Some of them have so far been shown to be induced on a transcriptional level by drought stress and ABA ([16],[8]) and by phosphorous deficiency on a translational level [47]. This could be related to the increased susceptibility to pathogen attack when plants are exposed to detrimental environmental conditions. O-methyltransferases confer a methyl-group mainly from SAM to various acceptor molecules. Intermediates of the phenylpropanoid pathway, flavonoids, alkaloids or simply aliphatic substrates can be methylated by such methyltransferases potentially affecting their pathogen toxicity, reactivity solubility and even compartmentation.

Free radical scavenging (3). Two significantly upregulated proteins are situated in the chloroplast: peroxyredoxin Q, attached to the thylakoid membrane, and a ferritin. Peroxyredoxin Q is an enzymatic antioxidant. Overexpression of the *Suadea salsa* gene in *Arabidopsis thaliana* conferred an increased tolerance to salt and low-temperature stress [24]. Ferritins are multimeric proteins that can accommodate thousands of iron atoms in their core. Phytoferritins are highly similar to mammalian ferritins, which function as a cellular iron repository. Nevertheless, in plants, the physiologic relevance of ferritins is still unclear. Recently a loss of function approach in *A. thaliana* indicated a relationship between these proteins and protection against oxidative damage [37]. Then, the third protein we identified in this category was a peroxisomal catalase, well-known for its H₂O₂ scavenging properties especially during photorespiration.

Photosynthesis related (3). Two chloroplast proteins, Mg-chelatase and cytochrome f, decreased in abundance while the abundance of the photorespiratory enzyme serine hydroxymethyltransferase (SHMT) increased.

CHO metabolism (1). Myo-inositol-1-phosphate synthase (MIPS) catalyzes the reaction from glucose-6-phosphate to 1-D-myo-inositol-3-phosphate. This is the rate-limiting step of myo-inositol biosynthesis, a precursor of the phosphorous storage molecule phytate and of several signal lipids, which function in diverse pathways, such as cell membrane synthesis, regulation of cell death [10] and stress response. *TCA cycle (1).* Dihydrolipoyl dehydrogenase is substantially involved in the maintenance of TCA cycle and associated glycolysis. It participates in the oxidative decarboxylation reactions catalyzed by the 2-oxoglutarate dehydrogenase complex (OGDC), the pyruvate dehydrogenase com-

3. Results

Table 3.2.: Functional annotation of drought responsive proteins (day 6) in **sybiotic** plants and their loading values for PC2 from figure 3.2. The proteins listed here experienced a significant ($p < 0.05$, t-test) and at least 2-fold change in abundance (+up / -down). PC2 loadings ranking among the 20 highest are boldfaced.

TC-Code	Acc N°	Description	PC2
Photosynthesis related			
TC154182	A9PFK1	- Photosystem I reaction centre subunit N	0.270
TC144542	Q56GA3	- Geranylgeranyl reductase	0.053
TC166820	P93162	- Magnesium-chelatase subunit chlI	0.122
Protein homeostasis			
TC171817	P25804	- Cysteine proteinase 15A precursor	0.022
TC154443		- ATP-dependent Clp protease ATP-binding subunit clpC	0.074
TC151284	Q93YH0	- Clp protease 2 proteolytic subunit precursor	0.074
TC147391	A5APT3	- Peptidyl-prolyl cis-trans isomerase	0.023
TC146297	Q41350	- Osmotin-like protein precursor	0.159
Cellular development, organization			
TC172377	A7KQH2	- Beta-tubulin	0.045
TC154149	A9XTM4	- Fasciclin-like arabinogalactan protein 19	0.105
TC148804	O24076	- Guanine nucleotide-binding protein subunit beta-like protein	0.069
CHO metabolism			
TC142159	Q9AT08	- Glucose-1-phosphate adenylyltransferase	0.119
TC162439	P53537	- Alpha-glucan phosphorylase, H isozyme	0.063
Amino acid metabolism			
TC144576	A4PU48	- S-adenosylmethionine synthetase	0.095
Fatty acid and lipid metabolism			
TC143537	O23960	- Biotin carboxylase precursor	0.180
TCA cycle			
TC143021	P51615	- NADP-dependent malic enzyme	0.005
signaling			
TC151907		- RNA-binding region RNP-1	0.110
TC144458	A9P984	- Adenosine kinase	0.089
TC155647	A7Y7E3	- Leucine-rich repeat-like protein	0.030
Secondary metabolism			
TC168983	Q41399	+ Chalcone reductase	-0.162
Unknowns			
TC142445		-	-0.162
TC147524	A7P2W0	-	0.040
TC145284	A7P8V3	-	0.030
TC148947	Q0DA88	- Os06g0669400 protein	-0.028
TC143103		-	0.021
EY478847		-	-0.014
TC155092	A7R2V3	-	0.001

plex (PDC) and the branched chain α -keto acid dehydrogenase complex (BCKDC) which degrades branched amino acids providing organic acids for the TCA-cycle.

3. Results

Table 3.3.: Functional annotation of drought responsive proteins (day 6) in **N-fed** plants and their loading values for PC2 from figure 3.2. The proteins listed here revealed a significant ($p < 0.05$, t-test) and at least 2-fold change in abundance (+up / -down). PC2 loadings ranking among the 20 highest are boldfaced.

TC-Code	Acc N°		Description	PC2
Free radical scavenging				
TC144149	Q6UBI3	+	Peroxiredoxin Q	-0.135
TC163823	O48561	+	Catalase-4	-0.025
TC146680	Q9ZP90	+	Ferritin	-0.001
Defense and signaling				
TC145968	P36907	+	Endochitinase precursor	-0.070
TC149930		+	Blue (type 1) copper domain; O-methyltransferase, family 2	-0.168
TC163347	P42654	+	14-3-3-like protein B	-0.067
TC142755	P37900	+	Heat shock 70 kDa protein, mitochondrial precursor	-0.110
Photosynthesis related				
TC154315	P93162	-	Magnesium-chelatase subunit chlI	0.151
TC144298		-	cytochrome f	0.057
TC152781	A9PL09	+	Serine hydroxymethyltransferase	0.037
Secondary metabolism				
TC168983	Q41399	+	Chalcone reductase	-0.162
TCA cycle				
TC161884	P31023	+	Dihydrolipoyl dehydrogenase	-0.013
CHO metabolism				
TC144637	Q2MJR4	+	Myo-inositol-1-phosphate synthase	-0.090
Cellular development, organization				
TC146858	O82090	+	Fiber annexin	-0.072
TC149263	P47922	+	Nucleoside diphosphate kinase 1	-0.020
Mitochondrial electron transport				
TC152948	Q41000	+	ATP synthase subunit delta, mitochondrial precursor	-0.089
Amino acid metabolism				
TC161248	Q40325	+	Aspartate aminotransferase	0.017
Fatty acid and lipid metabolism				
TC148021	P38414	+	Lipoxygenase	-0.017
Unknowns				
EV262290	A7PPK6	+		-0.009
TC157830	A7P710	+		-0.001

4. Discussion

4.1. Physiological determination of drought stress levels

The physiological measurements were conducted to assess the physiological status and extent of drought. The changes in stomatal conductance g_s , substrate RWC, root/shoot ratios, $\delta^{13}C$ signatures and with respect to controls are similar for both N-nutritional conditions, whereas chlorophyll fluorescence parameters were exclusively altered in N-fed plants.

Decreasing stomatal conductance as a result of ABA-induced closure of stomata is a well-known response to water deprivation [7], in order to diminish transpiration rates. The continuous decrease in substrate RWC shows that, in this experiment, plants are affronted with slowly increasing drought levels, simulating stress conditions under natural conditions. This is also reflected by the enhanced root growth observed. At mild water deficits, roots continue growing as long as they are preserved with photosynthates, whereas shoots stop growing when the water uptake by the root-system is insufficient for further growth [44].

$\delta^{13}C$ values increased significantly in stressed plants. This is a typical phenomenon observed in response to drought, explained by two physiochemical aspects. First, $^{13}CO_2$ diffuses more slowly within the leaf air space than $^{12}CO_2$. Second, the rate constant of Rubisco C fixation is lower for $^{13}CO_2$. Thus, Rubisco discriminates against the heavier stable isotope of C and $^{13}CO_2$ accumulates within the leaf. When stomata close, Rubisco nevertheless uses the heavier form of CO_2 and $\delta^{13}C$ consequently increases [36].

Standard parameters of drought level determination are tissue RWC and water potential. RWC was measured in this study (data not shown). Unfortunately, to calculate the correct RWC of tissues, usually many biological replicates are necessary. Here, due to high standard error, a reliable estimation of RWC and changes in response to drought are not possible.

However, using the experimental settings and results of previous studies [27, 26], demonstrating a more than 2-fold decrease of nodule water potential on day 6, in combination with the results from the present study, I deduce that the plants were exposed to a drought stress level which can be classified as severe.

The only difference in response to drought observed between the two N-nutritional conditions, was the significant decrease in PSII operating efficiency F'_q/F'_m . This effect, suggesting a severer drought level in N-fed plants, will be explained in section 4.2.2.

4.2. Integrative physiological and proteomic comparison between *M. truncatula* plants grown under different N-sources

4.2.1. Phenotyping: Similarities and differences of the leaf depending on N-source

In this study, N-source related differences have been detected that are involved in the formation of molecular phenotypes. Our $\delta^{15}N$ data approve that this experiment's N-fed

4. Discussion

and symbiotic plants have different source N pools with distinct $\delta^{15}\text{N}$ signatures. Plants solely relying on nitrogen derived from fixation of atmospheric N_2 are known to have $\delta^{15}\text{N}$ signatures close to that of the atmosphere [43, 46, 48], which is by definition 0‰ (see equation (2.3), page 17). Plants relying on NO_3^- or NH_4^+ from the soil solution present higher $\delta^{15}\text{N}$ values, especially when the source of N is ^{15}N enriched (like organic fertilizers). NH_4NO_3 is an artificial fertilizer synthesized by acid-base reaction of ammonia with nitric acid. Although atmospheric N_2 is used to produce synthetic ammonia via the Haber-Bosch process and this ammonia is again used to produce nitric acid (by the Oswald-process), the NH_4NO_3 used in our case to feed plants devoid of nodules is ^{15}N enriched compared to the atmosphere. For a more detailed discussion of the observed differences, the $\delta^{15}\text{N}$ value of the NH_4NO_3 -solution used as N pool remains to be determined.

Notably, our data show a tendency towards $\sim 2\text{‰}$ increased $\delta^{13}\text{C}$ signatures in symbiotic control tissues. Since g_s and SD were similar for N-fed and symbiotic plants, this increase depicts a likely influence of nitrogen nutrition on carbon metabolism. When dealing with naturally abundant C isotope accumulation in plants, we only take discrimination rates into consideration and not C fixation rates. This evidence for a ^{13}C discrimination promoting effect of nitrogen fertilizer has not been studied so far. In case it persists under field conditions, this could provide an interesting parameter for characterization of agricultural systems.

PCA gives us a first overview of the variance contained within the proteomic data. Figure 3.2 conveys the strong effect of nitrogen source by a clear spatial separation of controls along PC1. During steady-state conditions both plant sets had qualitatively 204 proteins in common. The strongest impact on the separation of controls can be attributed to 50 (of the total identified proteins) which were significantly differentially expressed under both N-nutrition treatments; they are listed in table 4.1. All of them present higher abundances in nodulated plants. The majority is involved in the non-photosynthetic generation of ATP and reducing power, free radical scavenging, signaling and in cellular development. These results may indicate a higher stress level of symbiotic plants under control conditions. The reasons for this observation is not deducible from the available information. On the basis of our current knowledge, there seem to be two possible explanations related to N nutritional state and symbiosis-induced effects that may cause these results. Terpolilli *et al* [45] demonstrated that the symbiosis between *M. truncatula* and *S. meliloti* is suboptimal, and the host experiences N deficiency when relying solely on the fixation of atmospheric N_2 . In addition, symbiosis could cause stress due to different source-sink relations, but also due to infection per se.

During early nodulation events a plethora of signaling cascades is stimulated involving the establishment of nodules on the microsymbiont side and the systemic control of nodulation by the host, included the production of cell elongation promoting phytohormones like IAA and ethylene [9]. In this experiment, enzymes synthesizing the precursors for both plant hormones were found to be of importance for a clear separation of controls relying on different N-sources: 6-phosphogluconate dehydrogenase of the oxidative pentose phosphate pathway and SAM synthetase (table 4.1). This is a likely reason for higher root/shoot ratio observed in nodulated plants under steady state conditions.

The absence of significant differences in photosynthesis related proteins (except for one photorespiratory enzyme) could explain why F_q'/F_m' is similar in nodulated and in N-fed plants, although their metabolic differences seem to be substantial. Under optimal steady-state conditions, especially in the beginning of the light period, plants process the incident

light energy without excessive production of ROS.

Taken together, these results suggest that the different N-sources studied here cause strong alterations of the plants' metabolism; more precisely, that symbiotic plants had higher levels of proteins considered to be part of a general stress reactions.

4.2.2. Differential response to drought and evidence for increased tolerance of symbiotic plants

Determination of drought-induced changes in stomatal conductance g_s , $\delta^{13}C$ and root/shoot ratios revealed similarities between the two N-nutritional conditions, whereas differences in photosynthetic parameters and on the proteomic level were substantial (see figures in table 4.1, figures 4.2 and 4.3) .

On day 6 of drought, there were already detectable photoinhibitory effects in N-fed plants. The data suggests that N-fed plants are less efficient in reoxidizing excited PSII compared to their controls and to symbiotic plants grown under optimal as well as adverse conditions. Thus, they are more prone to damage by reactive oxygen species since their photosynthetic electron transport chain is highly reduced. This relates well to the changes observed on protein level. N-fed plants showed significant increases in abundance of anti-oxidative enzymes and of SHMT which is part of the photorespiratory cycle, known to protect against ROS. Symbiotic *M. truncatula*, on the contrary, did not exhibit any changes related to the free radical scavenging system, nor did F_q'/F_m' change. This is explained by a higher level of enzymes involved in non-photosynthetic energy generation. Atkin and Macherel [3] postulated the hypothesis that mitochondrial respiration is essentially involved in drought stress tolerance mechanisms. According to them, ATP is transported into the chloroplasts in order to compensate for the impairment of photophosphorylation. Compared to N-fed plants, their symbiotic counterparts had higher expression levels of enzymes directly involved in the synthesis of extra-plastidial ATP and reducing-equivalents, which remained constant in response to drought. These findings suggest an enhanced drought tolerance of symbiotic plants and provide evidence for the molecular mechanisms involved. The initial higher level of enzymes being part of the cellular anti-oxidative system and of non-photosynthetic electron transport could serve as a buffering system in order to preserve cellular functioning under adverse water deficit conditions.

4.2.3. Putative marker and future evaluation strategies

In the past, several studies aimed at the identification of molecular stress response maker. It became obvious, that a stress response is a concerted interaction of different processes involving a number of different molecular marker. Systems biology allows for a more holistic and unbiased overview of the molecular level, enabling the identification of complex regulatory relevant mechanisms of known and novel interaction partners. Using spectral counts to analyze relative changes in protein abundance, several putative protein marker could be identified (tables 3.2, 3.3, 4.1). Interestingly, many of them have already been identified or even been verified separately in different mostly transcriptional based studies. However, here the identification and functional grouping of different putative or already evaluated marker together with the identity of new candidates is described.

Besides the identification of putative key enzymes, the separation of similar isoforms may be important to unambiguously distinguish the regulatory relevant form. The application

4. Discussion

of MAPA allows for the better resolution of peptides relevant for the change of a protein abundance [21]. In the following, an example will illustrate this approach.

Closeup: two isoforms of Mg-chelatase

As stated before, the abundance of Mg-chelatase is significantly decreased after six days of water deprivation in both plant sets. We detected two isoforms of Mg-chelatase and interestingly their abundances were differentially affected according to our db-dependent analysis of peptide spectra.

Protein isoforms usually have multiple peptides in common and differ only in a few isoform-specific peptides. It is impossible to determine the exact origin of unspecific peptides occurring in multiple proteins. Thus, a potential change in concentration of isoforms has to be characterized by comparison of isoform-specific peptide abundance levels. To overcome this problem one first has to identify isoform-specific peptides and then find out how many spectral counts are attributed to their respective ion m/z values. This is the principle of db-independent approaches, i.e. MS data analysis without prior peptide identification. Here we used mass accuracy precursor alignment (MAPA, [21]), extracting for every m/z (rounded to the second decimal) the number of ions that were selected for CID. The resulting SC of the isoform-specific peptides are represented in figure 4.1. Contrarian to the results attained by db-dependent relative quantification, only one isoform (TC166820) is clearly decreased in abundance by drought under both N-nutritional conditions. Mg-chelatase has recently attracted much attention in both retrograde and ABA signaling research, to which our finding could contribute valuable information, especially when the data are attested via absolute quantification.

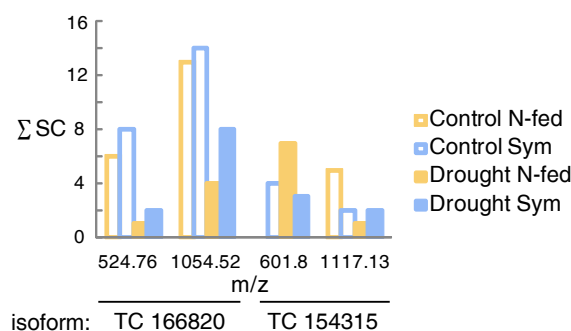


Figure 4.1.: Spectral counts of isoform-specific peptide ions of TC166820 and TC154315 Mg-chelatase

Outlook

The findings presented here, indicating stress alleviation in symbiotic *M. truncatula*, need to be further evaluated in a systems biology context.

Low abundance proteins, often key-regulatory enzymes, should be assessed by absolute quantification and the analysis of mutants. This study provides the basis for such targeted analyses, pointing at the pathways of special interest: the central carbon metabolism (glycolysis, TCA cycle and oxidative pentose phosphate pathway) and the photorespiratory

pathway.

Furthermore, the data may be complemented by metabolic analyses which will provide valuable insights into the non-enzymatic anti-oxidative system, the accumulation of osmolytes or even phytohormone levels. This could elucidate the possible impetus of phytohormones on the observed difference in root/shoot ratios, but also in the signaling pathways during the early stages of water deprivation.

Then, two major questions arise: (i) Do the stated differences between N-fed and symbiotic plants under steady-state conditions persist at other time-points (day 0 and 3) and how do they evolve on a circadian time-scale? The disruption of the circadian clock induced by cold-stress has already been demonstrated on a transcript level, questioning the use of diurnal controls [4]. Whether N-source also alters the expression of clock-genes has to be taken into consideration and remains to be determined. (ii) Is the stress syndrome in symbiotic plants due to N-starvation or resulting from symbiotic-induced effects? This question is to be addressed on two levels. The analysis of N-fed plants grown under limited N-supply (1 mmolKNO₃) and the examination of *M. truncatula* plants inoculated with *S. medicae* WSM419, a bacterial strain reported to perform better than *S. meliloti* [45].

Finally, the comprehensive analysis of roots, as the organs perceiving first water deficit, will contribute to essential advances in this research.

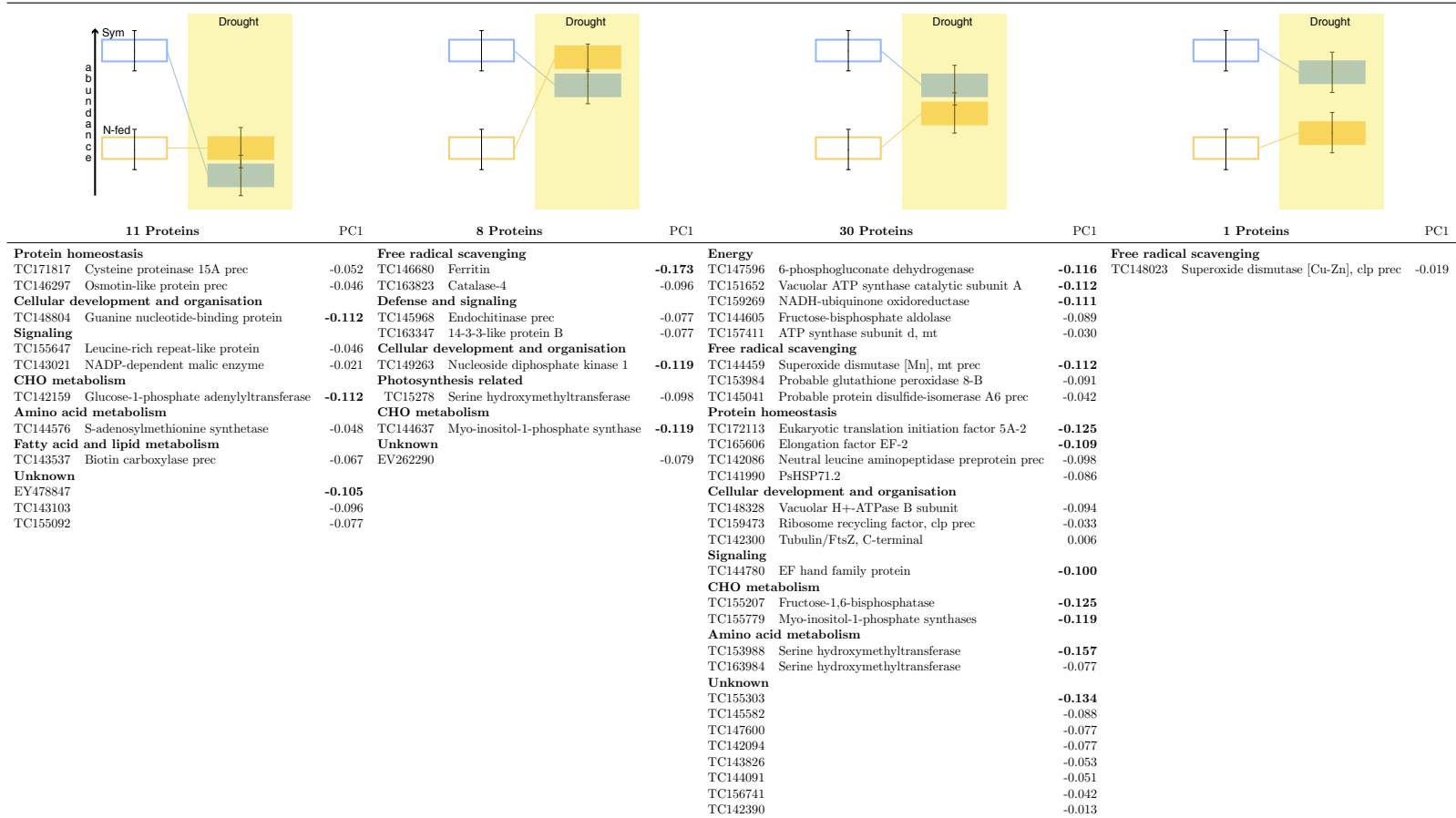


Table 4.1.: Significantly differentially expressed proteins in controls and a schematic representation of their four distinct drought responsive abundance alterations. PC1 loadings ranking among the 20 highest are boldfaced.

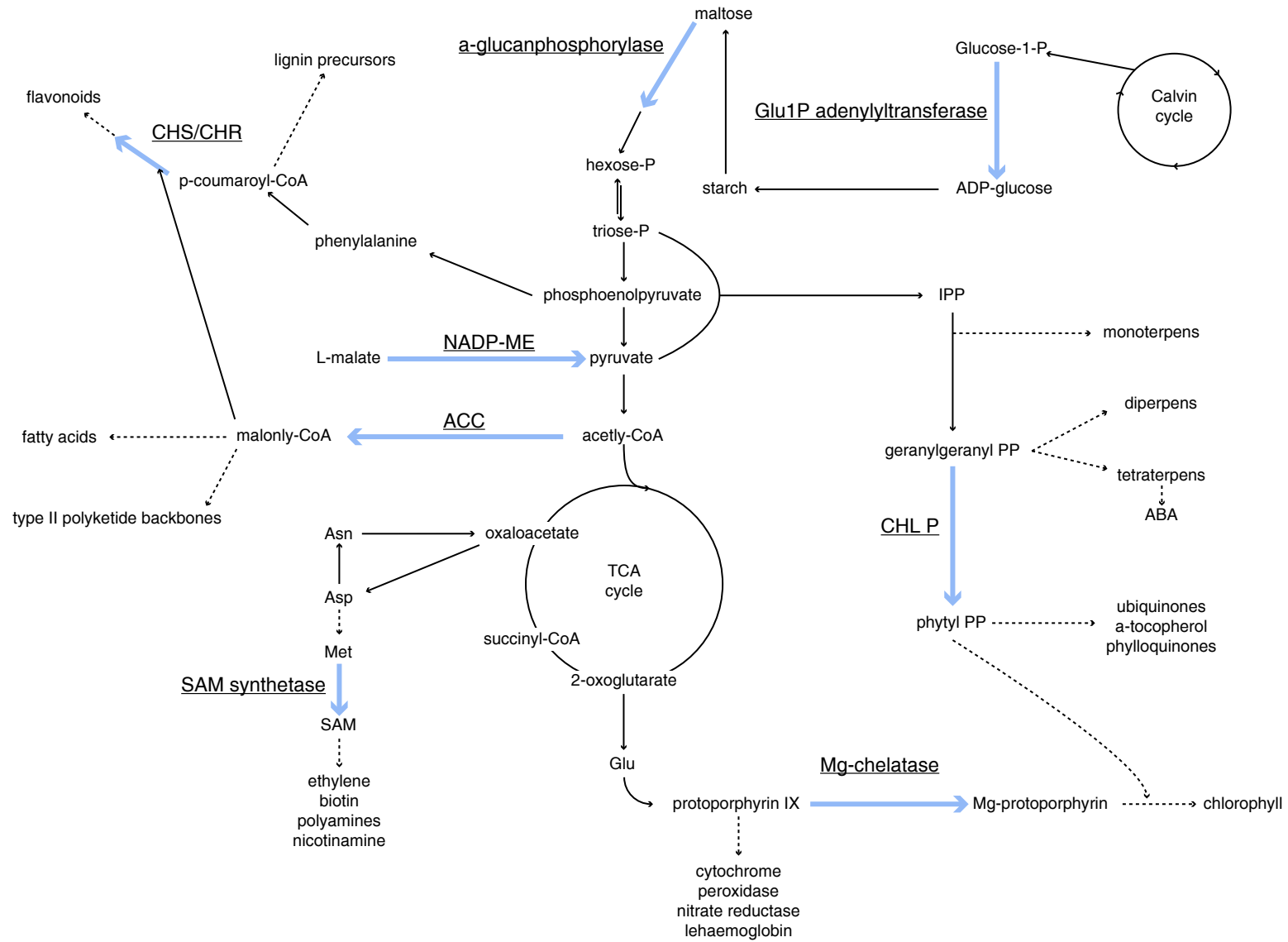
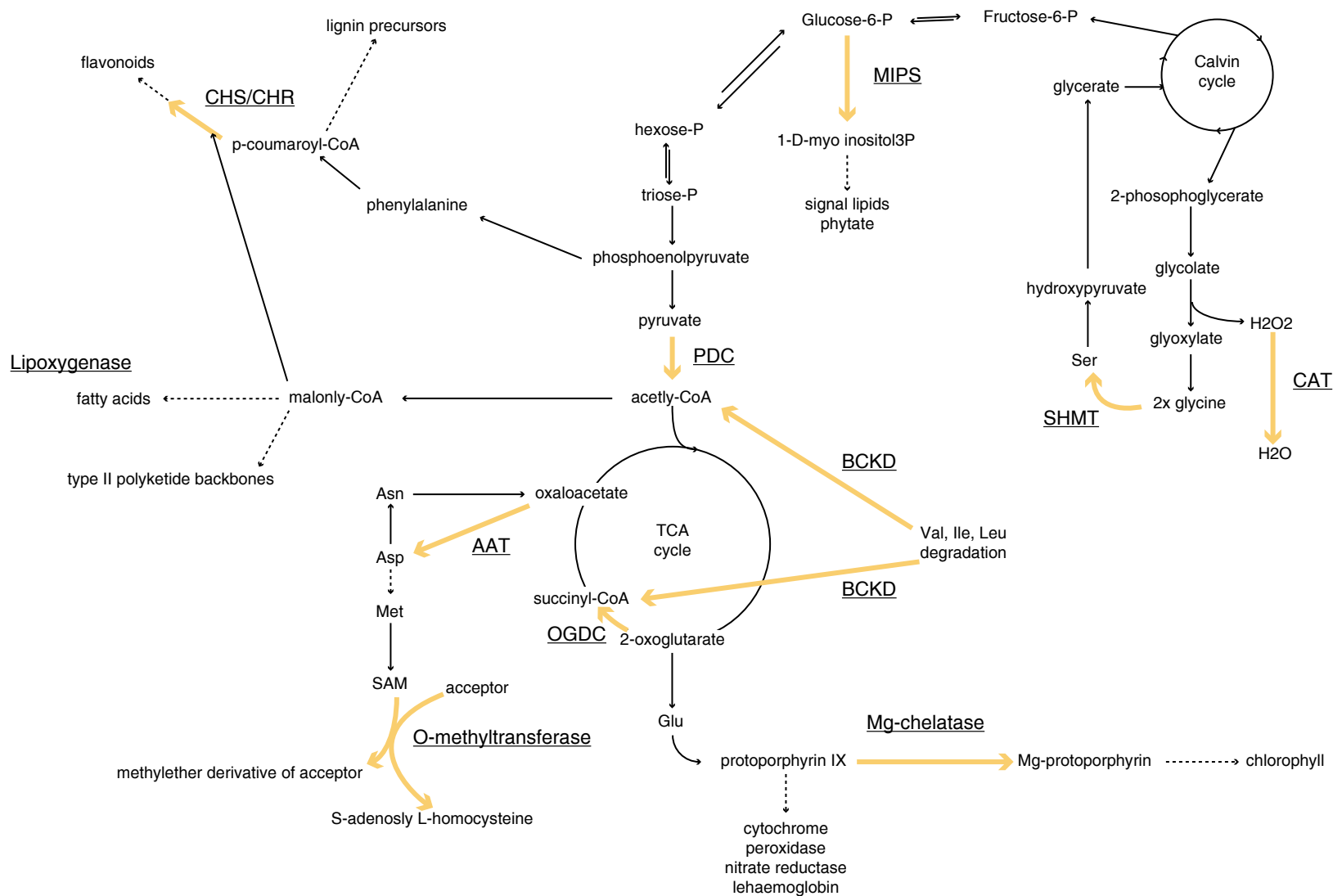


Figure 4.2.: Metabolic context of significantly affected proteins in symbiotic *M. truncatula* on day 6

Figure 4.3.: Metabolic context of significantly affected proteins in N-fed *M. truncatula* on day 6

5. Summary

Changing environmental conditions and the damage of natural ecosystems by intense and conventional agriculture will be the major issues to address in the decades to come. Two synergistic strategies should be adopted. Firstly, the mitigation of environmental pollution. Secondly, the adaptation of crop plants and cultivation methods to adverse climatic conditions, especially to the consequences of global warming.

Legume research is promising to address both, mitigation and adaptation. Legumes are productive even under nitrogen-limiting conditions and some species are known for their relatively high drought tolerance. Previous studies performed on legumes reported nitrogen-source dependent drought stress alleviation. When the plants relied on nitrogen originating from the symbiosis with diazotrophic bacteria, they exhibited better tolerance to drought compared to plants allocated with nitrogen fertilizer. The underlying mechanisms still remain to be determined.

The legume model *Medicago truncatula* Gaertn. presents a suitable platform for the holistic understanding of temperate grain legume biology and their atmospheric nitrogen fixing symbiosis. Here, nitrate-fed plants were compared to symbiotic plants and their differential response to drought stress were analyzed.

A whole-plant to proteome approach was used: root/shoot ratio, substrate relative water content, stomatal density and conductance, PSII operating efficiency, naturally abundant stable isotope concentration and shoot proteomes were assessed under different nitrogen-source and water supply conditions.

The physiological measurements attested the general comparability between the two systems studied. In response to drought, solely a difference in PSII operating efficiency could be determined, indicating an increased stress level in nitrate-fed plants. The shoot proteome analysis, however, revealed substantial differences in the control group between N-fed and symbiotic plants. In comparison to N-fed *M.truncatula*, its symbiotic counterpart showed increased levels of enzymatic anti-oxidants and non-photosynthetic energy generating enzymes. Furthermore, the plants exhibited differences in drought-stress responsiveness. N-fed plants reacted with a general quantitative up-regulation of drought-responsive proteins, while in symbiotic plants the opposite trend was observed.

These results led to the following hypothesis: Enhanced drought tolerance of symbiotic plants is mediated by the initial comparatively high abundance of enzymatic anti-oxidants and enzymes of the central carbon metabolism. This could convey tolerance by rapidly scavenging reactive oxygen species and by providing ATP to the chloroplast in order to overcome drought-induced impairments of photophosphorylation.

Zusammenfassung

Klimawandel und die Zerstörung natürlicher Ökosysteme durch konventionelle extensive Landwirtschaft zählen zu den wichtigsten Themenkreisen der Zukunft.

Es gilt zwei synergistische Strategien anzuwenden: Erstens, Vermeidung zusätzlicher Umweltverschmutzung. Zweitens, Anpassen von Saatgut und Anbaumethoden an ungünstige klimatische Bedingungen, im Besonderen an die Folgen der globalen Erwärmung.

Die Forschung an Leguminosen verspricht beide Ansprüche zu erfüllen. Leguminosen sind sogar unter Stickstoff limitierten Bedingungen produktiv und einige Arten sind bekannt für ihre Trockentoleranz. Studien haben gezeigt, dass die Art der Stickstoffquelle für eine Abschwächung der Trockenstresssymptome von Bedeutung ist. Die Mechanismen dahinter sind jedoch noch nicht bekannt.

Der Modellorganismus *Medicago truncatula* Gaertn. stellt ein geeignetes System dar, um die Biologie der Leguminosen und deren Symbiose mit luftstickstofffixierenden Bakterien umfassend zu studieren. Während dieser Arbeit wurden Nitrat-gedüngte mit symbiontischen Pflanzen verglichen und deren jeweilige Antwort auf Trockenstress analysiert.

Physiologische und proteomische Studien wurden durchgeführt: Wurzel/Spross Verhältnisse, der relative Wassergehalt des Substrats, stomatäre Dichte und Leitfähigkeit, PSII Effizienz, natürliche Abundanzen von stabilen Isotopen und das Spross Proteom wurden unter verschiedenen Stickstoffquellen- und Wassermangelbedingungen bestimmt.

Die generelle Vergleichbarkeit der unterschiedlich ernährten Pflanzen wurde durch physiologische Messungen bestätigt. Jene Pflanzen, die mit Nitrat gedüngt wurden, wiesen unter erheblichen Trockenstressbedingungen eine Minderung PSII Effizienz auf, wohingegen die Effizienz der symbiontischen Pflanzen unverändert blieb. Dies ist ein Hinweis auf ein erhöhtes Stressniveau der gedüngten Pflanzen.

Proteomische Analysen des Sprossmaterials ließen grundlegende Unterschiede zwischen den jeweiligen Kontrollen von gedüngten und symbiontischen Pflanzen sichtbar werden. Symbiontische *Medicago truncatula* Pflanzen hatten im Vergleich zu gedüngten Pflanzen höhere Abundanzen von enzymatischen Antioxidantien und von Enzymen der nicht-photosynthetischen Energiegewinnung. Des Weiteren, war die Art der Antwort auf Trockenstress unterschiedlich. Das Expressionslevel der auf Wasserdefizit reagierenden Proteine wurde in gedüngten Pflanzen generell erhöht, wohingegen in symbiontischen der genau gegenteilige Trend beobachtet wurde.

Diese Ergebnisse führten zur folgenden Hypothese: Die erhöhte Trockentoleranz in symbiontischen Pflanzen könnte durch die, unter Kontrollbedingungen, höhere Abundanz an enzymatischen Antioxidantien und an Enzymen des zentralen Kohlenstoffmetabolismus bedingt sein. Somit könnten die durch Trockenstress vermehrt entstehenden reaktiven Sauerstoffspezies schneller beseitigt werden und ATP dem Chloroplasten zur Verfügung gestellt werden, um dort den schädlichen Effekten einer verminderten Photophosphorylierung entgegen zu wirken.

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Bibliography

- [1] MC Antolin, J. Yoller, and M. Sanchez-Diaz. Effects of temporary drought on nitrate-fed and nitrogen-fixing alfalfa plants. *Plant Science*, 107(2):159–165, 1995.
- [2] P. Armengaud, R. Sulpice, A.J. Miller, M. Stitt, A. Amtmann, and Y. Gibon. Multi-level analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in arabidopsis roots. *Plant physiology*, 150(2):772, 2009.
- [3] O.K. Atkin and D. Macherel. The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany*, 2008.
- [4] Z. Bieniawska, C. Espinoza, A. Schlereth, R. Sulpice, D.K. Hinch, and M.A. Hannah. Disruption of the arabidopsis circadian clock is responsible for extensive variation in the cold-responsive transcriptome. *Plant physiology*, 147(1):263, 2008.
- [5] M.M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2):248–254, 1976.
- [6] B. Bucciarelli, J. Hanan, D. Palmquist, and C.P. Vance. A standardized method for analysis of medicago truncatula phenotypic development. *Plant physiology*, 142(1):207, 2006.
- [7] M.M. Chaves, J.P. Maroco, and J.S. Pereira. Understanding plant responses to drought—from genes to the whole plant. *Functional Plant Biology*, 30(3):239–264, 2003.
- [8] R.D. Chen, L.X. Yu, A.F. Greer, H. Cheriti, and Z. Tabaeizadeh. Isolation of an osmotic stress-and abscisic acid-induced gene encoding an acidic endochitinase from lycopersicon chilense. *Molecular and General Genetics MGG*, 245(2):195–202, 1994.
- [9] C. DIMKPA, T. WEINAND, and F. ASCH. Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell & Environment*, 32(12):1682–1694, 2009.
- [10] J.L. Donahue, S.R. Alford, J. Torabinejad, R.E. Kerwin, A. Nourbakhsh, W.K. Ray, M. Hernick, X. Huang, B.M. Lyons, P.P. Hein, et al. The arabidopsis thaliana myo-inositol 1-phosphate synthase1 gene is required for myo-inositol synthesis and suppression of cell death. *The Plant Cell Online*, 22(3):888, 2010.
- [11] J.K. Eng, A.L. McCormack, and J.R. Yates III. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *Journal of the American Society for Mass Spectrometry*, 5(11):976–989, 1994.
- [12] JW Erisman, A. Bleeker, J. Galloway, and MS Sutton. Reduced nitrogen in ecology and the environment. *Environmental Pollution*, 150(1):140–149, 2007.

- [13] HJ Evans. Symbiotic nitrogen fixation in legume nodules. *Research Experiences in Plant Physiology*. Springer-Verlag, New York, pages 294–310, 1981.
- [14] James N Galloway, Alan R Townsend, Jan Willem Erisman, Mateete Bekunda, Zucang Cai, John R Freney, Luiz A Martinelli, Sybil P Seitzinger, and Mark A Sutton. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, 320(5878):889–892, May 2008.
- [15] JN Galloway, FJ Dentener, DG Capone, EW Boyer, RW Howarth, SP Seitzinger, GP Asner, CC Cleveland, PA Green, EA Holland, et al. Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70(2):153–226, 2004.
- [16] W.R. Gao, X.S. Wang, Q.Y. Liu, H. Peng, C. Chen, J.G. Li, J.S. Zhang, S.N. Hu, and H. Ma. Comparative analysis of ests in response to drought stress in chickpea (*C. arietinum* L.). *Biochemical and biophysical research communications*, 376(3):578–583, 2008.
- [17] D. Giannino, E. Condello, L. Bruno, G. Testone, A. Tartarini, R. Cozza, A.M. Innocenti, M.B. Bitonti, and D. Mariotti. The gene geranylgeranyl reductase of peach (*Prunus persica* [L.] Batsch) is regulated during leaf development and responds differentially to distinct stress factors. *Journal of experimental botany*, 55(405):2063, 2004.
- [18] Nicolas Gruber and James N Galloway. An earth-system perspective of the global nitrogen cycle. *Nature*, 451(7176):293–296, Jan 2008.
- [19] J.R. Harian. Crops and man. *American Society of Agronomy*, 1992.
- [20] A.M. Hirsch, M.R. Lum, and J.A. Downie. What makes the rhizobia-legume symbiosis so special? *Plant physiology*, 127(4):1484, 2001.
- [21] W. Hoehenwarter, J.T. van Dongen, S. Wienkoop, M. Steinfath, J. Hummel, A. Erban, R. Sulpice, B. Regierer, J. Kopka, P. Geigenberger, et al. A rapid approach for phenotype-screening and database independent detection of csnp/protein polymorphism using mass accuracy precursor alignment. *Proteomics*, 8(20):4214–4225, 2008.
- [22] J. Hummel, M. Niemann, S. Wienkoop, W. Schulze, D. Steinhäuser, J. Selbig, D. Walther, and W. Weckwerth. Promex: a mass spectral reference database for proteins and protein phosphorylation sites. *BMC bioinformatics*, 8(1):216, 2007.
- [23] P.E. Jensen, LC Gibson, and C.N. Hunter. ATPase activity associated with the magnesium-protoporphyrin IX chelatase enzyme of *Synechocystis* PCC6803: evidence for ATP hydrolysis during Mg^{2+} insertion, and the MgATP-dependent interaction of the ChlI and ChlD subunits. *Biochemical Journal*, 339(Pt 1):127, 1999.
- [24] L.W. Jing, S.H. Chen, X.L. Guo, H. Zhang, and Y.X. Zhao. Overexpression of a chloroplast-located peroxiredoxin q gene, *ssprxq*, increases the salt and low-temperature tolerance of *Arabidopsis*. *Journal of Integrative Plant Biology*, 48(10):1244–1249, 2006.
- [25] E. Kirova, N. Tzvetkova, I. Vaseva, and G. Ignatov. Photosynthetic responses of nitrate-fed and nitrogen-fixing soybeans to progressive water stress. *Journal of Plant*

- Nutrition*, 31(3):445–458, 2008. cites Lodeiro et al, 2000. Don't have access to the Journal of Plant Nutrition.
- [26] E. Larrainzar, S. Wienkoop, C. Scherling, S. Kempa, R. Ladrera, C. Arrese-Igor, W. Weckwerth, and E.M. González. Carbon metabolism and bacteroid functioning are involved in the regulation of nitrogen fixation in *Medicago truncatula* under drought and recovery. *Molecular Plant-Microbe Interactions*, 22(12):1565–1576, 2009.
 - [27] E. Larrainzar, S. Wienkoop, W. Weckwerth, R. Ladrera, C. Arrese-Igor, and E.M. Gonzalez. *Medicago truncatula* root nodule proteome analysis reveals differential plant and bacteroid responses to drought stress. *Plant physiology*, 144(3):1495, 2007.
 - [28] M. Lavin, P.S. Herendeen, and M.F. Wojciechowski. Evolutionary rates analysis of leguminosae implicates a rapid diversification of lineages during the tertiary. *Systematic Biology*, 54(4):575, 2005.
 - [29] G. Lewis, B. Schrire, B. Mackinder, and M. Lock. *Legumes of the World*. Royal Botanic Gardens, Kew, 2005.
 - [30] H. Liu, R.G. Sadygov, and J.R. Yates III. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal. Chem*, 76(14):4193–4201, 2004.
 - [31] AR Lodeiro, P. González, A. Hernández, LJ Balagué, and G. Favelukes. Comparison of drought tolerance in nitrogen-fixing and inorganic nitrogen-grown common beans. *Plant science (Shannon, Ireland)*, 154(1):31, 2000.
 - [32] M. Meister and H. Bolhàr Nordenkamp. Stomata imprints: A new and quick method to count stomata and epidermis cells. *Handbook of Plant Ecophysiology Techniques*, pages 235–250, 2003.
 - [33] N. Mochizuki, J.A. Brusslan, R. Larkin, A. Nagatani, and J. Chory. Arabidopsis genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. *Proceedings of the National Academy of Sciences of the United States of America*, 98(4):2053, 2001.
 - [34] S. Mohanty, B. Grimm, and B.C. Tripathy. Light and dark modulation of chlorophyll biosynthetic genes in response to temperature. *Planta*, 224(3):692–699, 2006.
 - [35] L.N. Mueller, M.Y. Brusniak, DR Mani, and R. Aebersold. An assessment of software solutions for the analysis of mass spectrometry based quantitative proteomics data. *J. Proteome Res*, 7(01):51–61, 2008.
 - [36] S. Park. *Physicochemical and environmental plant physiology*. Elsevier, 2009.
 - [37] K. Ravet, B. Touraine, J. Boucherez, J.F. Briat, F. Gaymard, and F. Cellier. Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. *The Plant Journal*, 57(3):400–412, 2009.
 - [38] EJ Salisbury. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 216:1–65, 1928.

- [39] S. Sato, S. Isobe, and S. Tabata. Structural analyses of the genomes in legumes. *Current Opinion in Plant Biology*, 2010.
- [40] M. Scholz. *Approaches to analyse and interpret biological profile data*. PhD thesis, Max-Planck-Institute for Molecular Plant Physiology, 2006.
- [41] W.X. Schulze and B. Usadel. Quantitation in mass-spectrometry-based proteomics. *Annual Review of Plant Biology*, 61:491–516, 2010.
- [42] Y. Shang, L. Yan, Z.Q. Liu, Z. Cao, C. Mei, Q. Xin, F.Q. Wu, X.F. Wang, S.Y. Du, T. Jiang, et al. The Mg-Chelatase H subunit of Arabidopsis antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. *The Plant Cell Online*, 22(6):1909, 2010.
- [43] JI Sprent, IE Geoghegan, PW Whitty, and EK James. Natural abundance of ^{15}N and ^{13}C in nodulated legumes and other plants in the Cerrado and neighbouring regions of Brazil. *Oecologia*, 105(4):440–446, 1996.
- [44] Zeiger E. Taiz L. *Plant physiology*. Springer-Verlag Berlin Heidelberg, 2007.
- [45] J.J. Terpolilli, G.W. O’Hara, R.P. Tiwari, M.J. Dilworth, and J.G. Howieson. The model legume medicago truncatula a17 is poorly matched for n2 fixation with the sequenced microsymbiont sinorhizobium meliloti 1021. *New Phytologist*, 179(1):62–66, 2008.
- [46] JD Tjepkema, CR Schwintzer, RH Burris, GV Johnson, and WB Silvester. Natural abundance of ^{15}N in actinorhizal plants and nodules. *Plant and Soil*, 219(1):285–289, 2000.
- [47] S. Torabi, M. Wissuwa, M. Heidari, M.R. Naghavi, K. Gilany, M.R. Hajirezaei, M. Omid, B. Yazdi-Samadi, A.M. Ismail, and G.H. Salekdeh. A comparative proteome approach to decipher the mechanism of rice adaptation to phosphorous deficiency. *Proteomics*, 9(1):159–170, 2009.
- [48] W. Wanek and S.K. Arndt. Difference in $\delta^{15}\text{N}$ signatures between nodulated roots and shoots of soybean is indicative of the contribution of symbiotic N_2 fixation to plant N. *Journal of Experimental Botany*, 53(371):1109, 2002.
- [49] V.C. Wasinger, S.J. Cordwell, A. Cerpa-Poljak, J.X. Yan, A.A. Gooley, M.R. Wilkins, M.W. Duncan, R. Harris, K.L. Williams, and I. Humphery-Smith. Progress with gene-product mapping of the mollicutes: Mycoplasma genitalium. *Electrophoresis*, 16(1):1090–1094, 1995.
- [50] M. Watzka, K. Buchgraber, and W. Wanek. Natural ^{15}N abundance of plants and soils under different management practices in a montane grassland. *Soil Biology and Biochemistry*, 38(7):1564–1576, 2006.
- [51] S. Wienkoop, E. Larrainzar, M. Glinski, E.M. González, C. Arrese-Igor, and W. Weckwerth. Absolute quantification of *Medicago truncatula* sucrose synthase isoforms and N-metabolism enzymes in symbiotic root nodules and the detection of novel nodule phosphoproteins by mass spectrometry. *Journal of experimental botany*, 59(12):3307, 2008.

- [52] S. Wienkoop, E. Larrainzar, M. Niemann, E.M. Gonzalez, U. Lehmann, and W. Weckwerth. Stable isotope-free quantitative shotgun proteomics combined with sample pattern recognition for rapid diagnostics. *Journal of separation science*, 29(18):2793–2801, 2006.
- [53] M.R. Wilkins, J.C. Sanchez, K.L. Williams, and D.F. Hochstrasser. Current challenges and future applications for protein maps and post-translational vector maps in proteome projects. *Electrophoresis*, 17(5):830–838, 1996.
- [54] J.W.H. Wong, M.J. Sullivan, and G. Cagney. Computational methods for the comparative quantification of proteins in label-free lcn-ms experiments. *Briefings in bioinformatics*, 9(2):156, 2008.

A. Attachment

A.1. Physiological and proteomic supplemental data

Table A.1.: Stomatal density and stomatal index on adaxial and abaxial leaf surfaces n=17

	N-fed		Sym		ANOVA		
	adaxial	abaxial	adaxial	abaxial	N	ad/ab	I
Stomatal density	294.12±69.34	226.59±56.65	314.53±65.87	245.54±30.45	ns	***	ns
Stomatal index	0.20±0.02	0.18±0.02	0.2±0.02	0.18±0.01	ns	***	ns

Table A.2.: Stomatal conductance *gs* on days 3 and 6

	N-fed		Sym		ANOVA		
	Control	Drought	Control	Drought	N	D	I
<i>gs</i> day 3 (n=14)	548.71±311.77	197.36±84.46	409.79±95.85	236.79±71.88	ns	***	ns
<i>gs</i> day 6 (n=21)	381.52±139.02	121.52±30.32	425.95±156.23	165.71±36.15	ns	***	ns

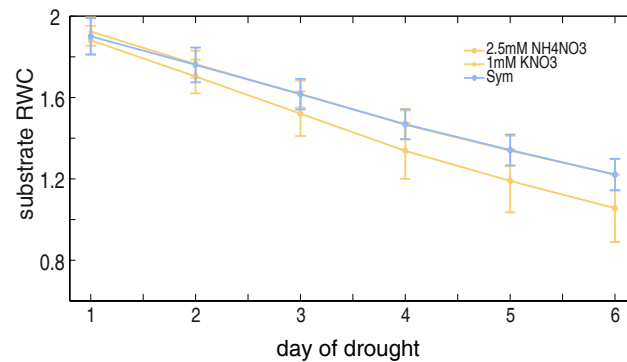


Figure A.1.: Decrease of substrate water content during the study periode(n=3).Errorbars indicate *SE*.

A. Attachment

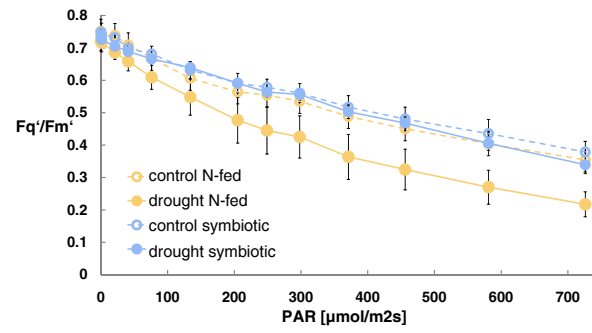


Figure A.2.: Light induction curve representing photosystem II operating efficiency ($n=9$). Errorbars indicate SE .

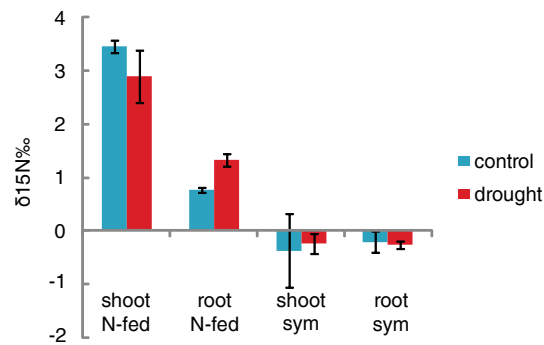


Figure A.3.: $\delta^{15}\text{N}$ signatures of day 6 shoot and root material ($n=3$). Errorbars indicate SE .

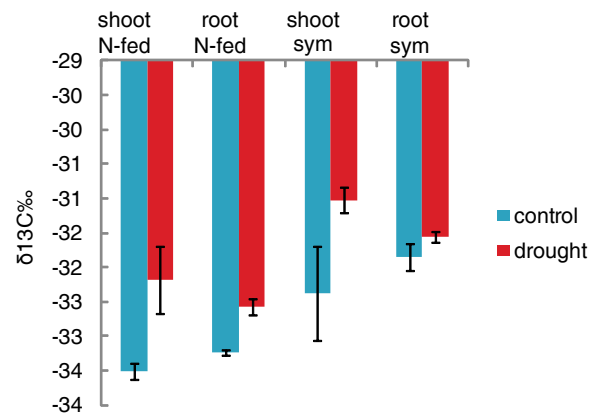


Figure A.4.: $\delta^{13}\text{C}$ signatures of day 6 shoot and root material ($n=3$). Errorbars indicate SE .

List of all 353 proteins used for statistical analyses with their full descriptions and their loading values from the PCA represented in figure 3.2.

Protein accession	Protein description	PC1	PC2
AC148291.22	ABE81834.1 H ⁺ -transporting two-sector ATPase, alpha/beta subunit, N-terminal_rframe1	-0.00301	-0.02292
AC149580.19	ABE88357.1 Lipoxigenase_rframe1	-0.00963	-0.0043
AL371197 homologue to UniRef100_Q9SP10 Cluster: Glycine-rich RNA binding protein; n=1; Medicago sativa	Rep: Glycine-rich RNA binding protein - Medicago sativa (Alfalfa), complete_rframe1	0.046947	0.121595
AW775375 similar to UniRef100_Q9ZP07 Cluster: Ribulose 1,5-bisphosphate carboxylase small subunit precursor; n=1; Cicer arietinum	Rep: Ribulose 1,5-bisphosphate carboxylase small subunit precursor - Cicer arietinum (Chickpea) (Garbanzo), partial (95%)_rframe1	0.009978	0.018406
BF644346 UniRef100_P05493 Cluster: ATP synthase subunit alpha, mitochondrial; n=1; Pisum sativum	Rep: ATP synthase subunit alpha, mitochondrial - Pisum sativum (Garden pea), partial (20%)_rframe3	-0.05739	0.050228
BQ150346 homologue to UniRef100_Q6V7X5 Cluster: 10 kDa photosystem II polypeptide; n=1; Trifolium pratense	Rep: 10 kDa photosystem II polypeptide - Trifolium pratense (Red clover), partial (86%)_rframe3	-0.00622	0.008563
BQ152232 homologue to UniRef100_O65194 Cluster: Ribulose bisphosphate carboxylase small chain, chloroplast precursor; n=1; Medicago sativa	Rep: Ribulose bisphosphate carboxylase small chain, chloroplast precursor - Medicago sativa (Alfalfa), partial (65%)_rframe1	-0.00075	0.038553
BQ157925 homologue to UniRef100_Q9XQR3 Cluster: Photosystem II reaction center protein H; n=1; Pisum sativum	Rep: Photosystem II reaction center protein H - Pisum sativum (Garden pea), partial (95%)_rframe1	-0.09812	-0.03345
CX520563 homologue to UniRef100_A8MS75 Cluster: Uncharacterized protein At3g54890.4; n=1; Arabidopsis thaliana	Rep: Uncharacterized protein At3g54890.4 - Arabidopsis thaliana (Mouse-ear cress), partial (64%)_rframe1	-0.03799	0.013066
CX52361 similar to UniRef100_Q9SA52 Cluster: Uncharacterized protein At1g09340, chloroplast precursor; n=1; Arabidopsis thaliana	Rep: Uncharacterized protein At1g09340, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (41%)_rframe2	-0.03225	0.039623
CX526624 similar to UniRef100_A7PY10 Cluster: Chromosome chr15 scaffold_37, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr15 scaffold_37, whole genome shotgun sequence - Vitis vinifera (Grape), partial (50%)_rframe1	-0.04063	-0.02199
CX532538 similar to UniRef100_P49231 Cluster: Profilin-1; n=1; Phaseolus vulgaris	Rep: Profilin-1 - Phaseolus vulgaris (Kidney bean) (French bean), complete_rframe3	-0.08448	-0.03
DY615543 homologue to UniRef100_Q2HTQ3 Cluster: Granulin; Peptidase C1A, papain; n=1; Medicago truncatula	Rep: Granulin; Peptidase C1A, papain - Medicago truncatula (Barrel medic), partial (38%)_rframe2	0.007554	0.052344
DY617089 similar to UniRef100_Q5QHW7 Cluster: Chloroplast thylakoid-bound ascorbate peroxidase; n=1; Vigna unguiculata	Rep: Chloroplast thylakoid-bound ascorbate peroxidase - Vigna unguiculata (Cowpea), partial (48%)_rframe3	-0.00852	0.065551
EV262290 similar to UniRef100_A7PPK6 Cluster: Chromosome chr8 scaffold_23, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr8 scaffold_23, whole genome shotgun sequence - Vitis vinifera (Grape), partial (63%)_rframe1	-0.08644	-0.00982
EY478847_rframe1	EY478847_rframe1	-0.10775	-0.01445
TC141899	Lipoxigenase_rframe2	-0.06584	-0.06388
TC141924 homologue to UniRef100_P34811 Cluster: Elongation factor G, chloroplast precursor; n=1; Glycine max	Rep: Elongation factor G, chloroplast precursor - Glycine max (Soybean), partial (92%)_rframe2	-0.0516	0.053008
TC141990 homologue to UniRef100_Q40980 Cluster: PsHSP71.2; n=1; Pisum sativum	Rep: PsHSP71.2 - Pisum sativum (Garden pea), complete_rframe1	-0.0917	0.013974
TC142086	Peptidase M17, leucyl aminopeptidase_rframe1	-0.09605	0.037823
TC142094 homologue to UniRef100_A7PNK8 Cluster: Chromosome chr8 scaffold_23, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr8 scaffold_23, whole genome shotgun sequence - Vitis vinifera (Grape), partial (89%)_rframe1	-0.08296	-2.45E-05
TC142159 homologue to UniRef100_Q9AT08 Cluster: Glucose-1-phosphate adenylyltransferase; n=1; Cicer arietinum	Rep: Glucose-1-phosphate adenylyltransferase - Cicer arietinum (Chickpea) (Garbanzo), complete_rframe2	-0.07563	0.119051
TC142251 UniRef100_A2Q5A2 Cluster: Ribulose bisphosphate carboxylase, large chain; n=1; Medicago truncatula	Rep: Ribulose bisphosphate carboxylase, large chain - Medicago truncatula (Barrel medic), complete_rframe1	0.002121	0.003355
TC142276	Aminotransferases class-I pyridoxal-phosphate-binding site_rframe3	-0.04848	0.053264
TC142300 UniRef100_A2Q5W0 Cluster: Tubulin/FtsZ, C-terminal; n=1; Medicago truncatula	Rep: Tubulin/FtsZ, C-terminal - Medicago truncatula (Barrel medic), complete_rframe1	0.007969	0.011191
TC142390 homologue to UniRef100_A7P4J8 Cluster: Chromosome chr4 scaffold_6, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr4 scaffold_6, whole genome shotgun sequence - Vitis vinifera (Grape), complete_rframe1	0.000526	0.064359
TC142445	Rhodanese-like_rframe2	0.000592	0.040949
TC142500 homologue to UniRef100_Q9LKJ2 Cluster: Phosphoglycerate kinase; n=1; Pisum sativum	Rep: Phosphoglycerate kinase - Pisum sativum (Garden pea), complete_rframe2	-0.01982	-0.00219
TC142538 homologue to UniRef100_Q2HU12 Cluster: Glycoside hydrolase, family 17; n=1; Medicago truncatula	Rep: Glycoside hydrolase, family 17 - Medicago truncatula (Barrel medic), complete_rframe2	0.021127	-0.00955

TC142539	homologue to UniRef100_P41346	Cluster: Ferredoxin-NADP reductase, chloroplast precursor; n=1; Vicia faba	Rep: Ferredoxin-NADP reductase, chloroplast precursor - Vicia faba (Broad bean), partial (95%).rframe1	-0.04039	0.013104
TC142554	homologue to UniRef100_Q8W557	Cluster: UDP-glucose pyrophosphorylase; n=1; Amorpha fruticosa	Rep: UDP-glucose pyrophosphorylase - Amorpha fruticosa, complete_rframe2	-0.06981	0.035441
TC142614	homologue to UniRef100_Q9SGT4	Cluster: Elongation factor EF-2; n=1; Arabidopsis thaliana	Rep: Elongation factor EF-2 - Arabidopsis thaliana (Mouse-ear cress), complete_rframe2	-0.07555	-0.04654
TC142639	UniRef100_O48905	Cluster: Malate dehydrogenase, cytoplasmic; n=3; Medicago	Rep: Malate dehydrogenase, cytoplasmic - Medicago sativa (Alfalfa), complete_rframe3	-0.08023	-0.00964
TC142674	UniRef100_O24080	Cluster: Peroxidase2 precursor; n=1; Medicago sativa	Rep: Peroxidase2 precursor - Medicago sativa (Alfalfa), complete_rframe1	-0.04496	0.068339
TC142709	UniRef100_Q45FF2	Cluster: Pyridoxine biosynthesis protein; n=1; Medicago truncatula	Rep: Pyridoxine biosynthesis protein - Medicago truncatula (Barrel medic), complete_rframe3	0.001842	0.047352
TC142755	homologue to UniRef100_P37900	Cluster: Heat shock 70 kDa protein, mitochondrial precursor; n=1; Pisum sativum	Rep: Heat shock 70 kDa protein, mitochondrial precursor - Pisum sativum (Garden pea), complete_rframe1	-0.03242	-0.11012
TC142771	homologue to UniRef100_P29501	Cluster: Tubulin beta-2 chain; n=1; Pisum sativum	Rep: Tubulin beta-2 chain - Pisum sativum (Garden pea), complete_rframe3	-0.05375	-0.00866
TC142835			Leucine-rich repeat, plant specific_rframe1	-0.0456	0.044442
TC142888	similar to UniRef100_Q5I2D1	Cluster: Caffeoyl CoA 3-O-methyltransferase; n=1; Betula platyphylla	Rep: Caffeoyl CoA 3-O-methyltransferase - Betula platyphylla, partial (94%).rframe2	-0.02688	0.031604
TC142910	homologue to UniRef100_O82043	Cluster: Ketol-acid reductoisomerase, chloroplast precursor; n=1; Pisum sativum	Rep: Ketol-acid reductoisomerase, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe2	-0.00448	0.009866
TC142918	homologue to UniRef100_Q96558	Cluster: UDP-glucose 6-dehydrogenase; n=1; Glycine max	Rep: UDP-glucose 6-dehydrogenase - Glycine max (Soybean), partial (56%).rframe3	-0.00443	0.0766
TC142926	UniRef100_Q6J9X6	Cluster: S-adenosylmethionine synthetase; n=2; eusids I	Rep: S-adenosylmethionine synthetase - Medicago sativa (Alfalfa), complete_rframe1	-0.09155	0.006296
TC142931	similar to UniRef100_Q3SCM6	Cluster: Cinnamoyl-CoA reductase; n=1; Acacia auriculiformis x Acacia mangium	Rep: Cinnamoyl-CoA reductase - Acacia auriculiformis x Acacia mangium, complete_rframe1	-0.07557	-0.02248
TC142934	UniRef100_Q7X9B3	Cluster: 9/13 hydroperoxide lyase; n=1; Medicago truncatula	Rep: 9/13 hydroperoxide lyase - Medicago truncatula (Barrel medic), complete_rframe2	-0.02151	-0.0444
TC142960	UniRef100_Q4J8R5	Cluster: Dehydroascorbate reductase; n=1; Medicago truncatula	Rep: Dehydroascorbate reductase - Medicago truncatula (Barrel medic), complete_rframe2	-0.04426	0.037587
TC142967	similar to UniRef100_A7NVP1	Cluster: Chromosome chr18 scaffold.1, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr18 scaffold.1, whole genome shotgun sequence - Vitis vinifera (Grape), partial (74%).rframe2	0.001374	0.004863
TC143021			Malic oxidoreductase_rframe2	-0.01838	0.005465
TC143036			ATP-binding region, ATPase-like; Four-helical cytokine_rframe1	-0.00537	0.023374
TC143053	homologue to UniRef100_P29828	Cluster: Protein disulfide-isomerase precursor; n=1; Medicago sativa	Rep: Protein disulfide-isomerase precursor - Medicago sativa (Alfalfa), partial (58%).rframe3	-0.02634	0.049686
TC143078	UniRef100_A2Q5A3	Cluster: H+-transporting two-sector ATPase, alpha/beta subunit, central region; H+-transporting two-sector ATPase, delta/epsilon subunit; n=1; Medicago truncatula	Rep: H+-transporting two-sector ATPase, alpha/beta subunit, central region; H+-transporting two-sector ATPase, delta/epsilon subunit - Medicago truncatula (Barrel medic), complete_rframe1	0.002689	0.002239
TC143080	similar to UniRef100_Q9AR81	Cluster: Germin-like protein precursor; n=1; Pisum sativum	Rep: Germin-like protein precursor - Pisum sativum (Garden pea), partial (96%).rframe2	-0.03381	-0.02989
TC143103	rframe3		TC143103_rframe3	-0.08934	0.021603
TC143106	similar to UniRef100_A7P8V3	Cluster: Chromosome chr3 scaffold.8, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr3 scaffold.8, whole genome shotgun sequence - Vitis vinifera (Grape), partial (79%).rframe3	-0.01441	-0.003
TC143125			Chlorophyll A-B binding protein_rframe1	0.002387	-0.03252
TC143176	homologue to UniRef100_Q39640	Cluster: Glycolate oxidase; n=1; Cucurbita cv. Kurokawa Amakuri	Rep: Glycolate oxidase - Cucurbita cv. Kurokawa Amakuri, complete_rframe1	-0.02069	-0.00569
TC143204	similar to UniRef100_P41127	Cluster: 60S ribosomal protein L13-1; n=1; Arabidopsis thaliana	Rep: 60S ribosomal protein L13-1 - Arabidopsis thaliana (Mouse-ear cress), complete_rframe2	-0.06934	0.063356
TC143261			Phosphoserine aminotransferase_rframe3	0.039061	-0.01973
TC143276	homologue to UniRef100_Q9XQB6	Cluster: Chlorophyll a/b-binding protein CP24; n=1; Vigna radiata var. radiata	Rep: Chlorophyll a/b-binding protein CP24 - Phaseolus aureus (Mung bean) (Vigna radiata), partial (97%).rframe2	0.013395	0.002907
TC143323	homologue to UniRef100_A5YM78	Cluster: 14-3-3-like protein; n=1; Cicer arietinum	Rep: 14-3-3-like protein - Cicer arietinum (Chickpea) (Garbanzo), complete_rframe3	-0.04038	-0.01003
TC143330	homologue to UniRef100_P34922	Cluster: Glyceraldehyde-3-phosphate dehydrogenase, cytosolic; n=1; Pisum sativum	Rep: Glyceraldehyde-3-phosphate dehydrogenase, cytosolic - Pisum sativum (Garden pea), complete_rframe1	-0.04936	0.021662
TC143340	homologue to UniRef100_Q38IW8	Cluster: Triosephosphate isomerase; n=1; Glycine max	Rep: Triosephosphate isomerase - Glycine max (Soybean), complete_rframe1	-0.03732	0.013933
TC143370	homologue to UniRef100_Q9T0N0	Cluster: 14-3-3-like protein; n=1; Pisum sativum	Rep: 14-3-3-like protein - Pisum sativum (Garden pea), complete_rframe2	0.002872	-0.03114

TC143391 UniRef100_Q2HUJ7 Cluster: V-ATPase subunit C; n=1; Medicago truncatula	Rep: V-ATPase subunit C - Medicago truncatula (Barrel medic), complete_rframe1	-0.05206	0.08012
TC143397 similar to UniRef100_Q58QP9 Cluster: Oxalic acid oxidase; n=1; Brassica napus	Rep: Oxalic acid oxidase - Brassica napus (Rape), partial (90%)_rframe2	0.022957	0.066166
TC143431 homologue to UniRef100_A7PHE4 Cluster: Chromosome chr17 scaffold_16, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr17 scaffold_16, whole genome shotgun sequence - Vitis vinifera (Grape), partial (98%)_rframe2	0.023403	0.015508
TC143437 homologue to UniRef100_A5Z2K3 Cluster: Photosystem I subunit Psd; n=1; Glycine max	Rep: Photosystem I subunit Psd - Glycine max (Soybean), partial (80%)_rframe1	0.011587	0.032824
TC143441 similar to UniRef100_Q94B07 Cluster: Gamma hydroxybutyrate dehydrogenase; n=1; Arabidopsis thaliana	Rep: Gamma hydroxybutyrate dehydrogenase - Arabidopsis thaliana (Mouse-ear cress), partial (97%)_rframe2	-0.03399	-0.04649
TC143537 homologue to UniRef100_O23960 Cluster: Biotin carboxylase precursor; n=1; Glycine max	Rep: Biotin carboxylase precursor - Glycine max (Soybean), partial (88%)_rframe1	-0.01292	0.180932
TC143554 similar to UniRef100_Q9SA52 Cluster: Uncharacterized protein Atlg09340, chloroplast precursor; n=1; Arabidopsis thaliana	Rep: Uncharacterized protein Atlg09340, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (71%)_rframe1	-0.0514	0.061498
TC143562 similar to UniRef100_P41127 Cluster: 60S ribosomal protein L13-1; n=1; Arabidopsis thaliana	Rep: 60S ribosomal protein L13-1 - Arabidopsis thaliana (Mouse-ear cress), complete_rframe1	0.015139	0.093355
TC143603 homologue to UniRef100_Q93X25 Cluster: 2-Cys peroxiredoxin; n=1; Pisum sativum	Rep: 2-Cys peroxiredoxin - Pisum sativum (Garden pea), partial (91%)_rframe1	-0.04181	0.003804
TC143644 similar to UniRef100_A7PF69 Cluster: Chromosome chr11 scaffold_13, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr11 scaffold_13, whole genome shotgun sequence - Vitis vinifera (Grape), partial (71%)_rframe3	0.00737	0.086685
TC143649 similar to UniRef100_Q940F8 Cluster: Sedoheptulose-bisphosphatase; n=1; Arabidopsis thaliana	Rep: Sedoheptulose-bisphosphatase - Arabidopsis thaliana (Mouse-ear cress), partial (94%)_rframe3	-0.0417	0.070742
TC143708 homologue to UniRef100_Q6RIB6 Cluster: Malate dehydrogenase; n=1; Glycine max	Rep: Malate dehydrogenase - Glycine max (Soybean), complete_rframe3	-0.04807	0.031532
TC143826 similar to UniRef100_A7PNN9 Cluster: Chromosome chr8 scaffold_23, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr8 scaffold_23, whole genome shotgun sequence - Vitis vinifera (Grape), partial (98%)_rframe1	-0.0401	0.051097
TC143849 homologue to UniRef100_P81406 Cluster: NADP-dependent glyceraldehyde-3-phosphate dehydrogenase; n=1; Pisum sativum	Rep: NADP-dependent glyceraldehyde-3-phosphate dehydrogenase - Pisum sativum (Garden pea), complete_rframe2	-0.01217	0.037079
TC143877 homologue to UniRef100_Q43082 Cluster: Porphobilinogen deaminase, chloroplast precursor; n=1; Pisum sativum	Rep: Porphobilinogen deaminase, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3	0.005758	0.108016
TC143946 homologue to UniRef100_P30707 Cluster: 60S ribosomal protein L9; n=1; Pisum sativum	Rep: 60S ribosomal protein L9 - Pisum sativum (Garden pea), complete_rframe2	-0.00498	-0.01165
TC143969 similar to UniRef100_A7PJQ1 Cluster: ATP-dependent Clp protease proteolytic subunit; n=1; Vitis vinifera	Rep: ATP-dependent Clp protease proteolytic subunit - Vitis vinifera (Grape), partial (73%)_rframe2	-0.0143	0.047303
TC143970 homologue to UniRef100_P49364 Cluster: Aminomethyltransferase, mitochondrial precursor; n=1; Pisum sativum	Rep: Aminomethyltransferase, mitochondrial precursor - Pisum sativum (Garden pea), complete_rframe2	-0.10025	-0.02005
TC143980 homologue to UniRef100_O22639 Cluster: Endoplasmic reticulum HSC70-cognate binding protein precursor; n=1; Glycine max	Rep: Endoplasmic reticulum HSC70-cognate binding protein precursor - Glycine max (Soybean), partial (98%)_rframe2	-0.03457	-0.03978
TC144015 homologue to UniRef100_Q3S4G9 Cluster: Enzymatic resistance protein; n=1; Glycine max	Rep: Enzymatic resistance protein - Glycine max (Soybean), complete_rframe3	-0.02902	-0.02611
TC144029	Aminotransferase, class I and II_rframe2	-0.04316	0.007696
TC144042 homologue to UniRef100_Q9FXL2 Cluster: Vf14-3-3c protein; n=1; Vicia faba	Rep: Vf14-3-3c protein - Vicia faba (Broad bean), partial (95%)_rframe3	-0.01807	-0.02332
TC144091 weakly similar to UniRef100_A7NYP6 Cluster: Chromosome chr6 scaffold_3, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr6 scaffold_3, whole genome shotgun sequence - Vitis vinifera (Grape), partial (96%)_rframe2	-0.05685	0.006418
TC144099	Porin, eukaryotic type_rframe2	-0.04549	0.029826
TC144149 similar to UniRef100_Q6UBI3 Cluster: Peroxiredoxin Q, chloroplast precursor; n=1; Suaeda salsa	Rep: Peroxiredoxin Q, chloroplast precursor - Suaeda salsa (Seepweed) (Chenopodium salsum), partial (77%)_rframe2	-0.08027	-0.13554
TC144205 homologue to UniRef100_Q96558 Cluster: UDP-glucose 6-dehydrogenase; n=1; Glycine max	Rep: UDP-glucose 6-dehydrogenase - Glycine max (Soybean), partial (78%)_rframe2	-0.01177	0.008059
TC144298 UniRef100_UPI0001596BB1 Cluster: cytochrome f; n=1; Medicago truncatula	Rep: cytochrome f - Medicago truncatula, partial (63%)_rframe3	0.021226	0.05738
TC144306 UniRef100_Q84RD9 Cluster: Adenosylhomocysteinase; n=1; Medicago truncatula	Rep: Adenosylhomocysteinase - Medicago truncatula (Barrel medic), complete_rframe3	-0.04954	0.011636
TC144347 homologue to UniRef100_P48534 Cluster: L-ascorbate peroxidase, cytosolic; n=1; Pisum sativum	Rep: L-ascorbate peroxidase, cytosolic - Pisum sativum (Garden pea), complete_rframe3	-0.05735	-0.02945
TC144388 homologue to UniRef100_Q40977 Cluster: Monodehydroascorbate reductase; n=1; Pisum sativum	Rep: Monodehydroascorbate reductase - Pisum sativum (Garden pea), complete_rframe3	-0.06169	0.017259
TC144423 homologue to UniRef100_A7Q3K1 Cluster: Chromosome chr13 scaffold_48, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr13 scaffold_48, whole genome shotgun sequence - Vitis vinifera (Grape), complete_rframe1	-0.00689	-0.02329

TC144429	homologue to UniRef100.Q3LUM5 Cluster: Elongation factor 1-alpha; n=1; Gossypium hirsutum	Rep: Elongation factor 1-alpha - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), partial (95%)_rframe3	0.026971	-0.0509
TC144458		Adenosine kinase_rframe1	-0.02359	0.089243
TC144459	homologue to UniRef100.P27084 Cluster: Superoxide dismutase [Mn], mitochondrial precursor; n=1; Pisum sativum	Rep: Superoxide dismutase [Mn], mitochondrial precursor - Pisum sativum (Garden pea), partial (97%)_rframe2	-0.12721	-0.0566
TC144508	similar to UniRef100.A7QWX5 Cluster: Chromosome chr13 scaffold_210, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr13 scaffold_210, whole genome shotgun sequence - Vitis vinifera (Grape), partial (66%)_rframe2	0.022212	0.12559
TC144529	similar to UniRef100.Q43092 Cluster: Granule-bound starch synthase 1, chloroplast precursor; n=1; Pisum sativum	Rep: Granule-bound starch synthase 1, chloroplast precursor - Pisum sativum (Garden pea), partial (75%)_rframe2	-0.08035	0.060621
TC144542	UniRef100.Q56GA3 Cluster: Geranylgeranyl reductase; n=1; Medicago truncatula	Rep: Geranylgeranyl reductase - Medicago truncatula (Barrel medic), complete_rframe1	-0.01839	0.05319
TC144547	similar to UniRef100.A7R058 Cluster: Chromosome chr10 scaffold_297, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr10 scaffold_297, whole genome shotgun sequence - Vitis vinifera (Grape), partial (94%)_rframe1	-0.01089	0.015406
TC144576	UniRef100.A4PU48 Cluster: S-adenosylmethionine synthetase; n=2; Papilionoideae	Rep: S-adenosylmethionine synthetase - Medicago truncatula (Barrel medic), complete_rframe1	-0.10886	0.095496
TC144593	UniRef100.Q2HT97 Cluster: Heat shock protein Hsp70; n=1; Medicago truncatula	Rep: Heat shock protein Hsp70 - Medicago truncatula (Barrel medic), complete_rframe2	-0.07981	0.017714
TC144597	similar to UniRef100.A5ATB7 Cluster: Methylenetetrahydrofolate reductase; n=1; Vitis vinifera	Rep: Methylenetetrahydrofolate reductase - Vitis vinifera (Grape), partial (91%)_rframe2	-0.02165	0.015173
TC144605	homologue to UniRef100.P46257 Cluster: Fructose-bisphosphate aldolase, cytoplasmic isozyme 2; n=1; Pisum sativum	Rep: Fructose-bisphosphate aldolase, cytoplasmic isozyme 2 - Pisum sativum (Garden pea), partial (55%)_rframe1	-0.08409	0.049356
TC144622	homologue to UniRef100.P08926 Cluster: RuBisCO large subunit-binding protein subunit alpha, chloroplast precursor; n=1; Pisum sativum	Rep: RuBisCO large subunit-binding protein subunit alpha, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3	-0.01502	0.025124
TC144634	similar to UniRef100.Q5WM51 Cluster: Trypsin protein inhibitor 3; n=1; Cicer arietinum	Rep: Trypsin protein inhibitor 3 - Cicer arietinum (Chickpea) (Garbanzo), partial (87%)_rframe3	-0.05142	-0.16292
TC144637	homologue to UniRef100.Q2MJR4 Cluster: Myo-inositol-1-phosphate synthase; n=1; Glycine max	Rep: Myo-inositol-1-phosphate synthase - Glycine max (Soybean), complete_rframe3	-0.15241	-0.09025
TC144694	UniRef100.UPI0001596BA6 Cluster: photosystem II CP47 chlorophyll apoprotein; n=1; Medicago truncatula	Rep: photosystem II CP47 chlorophyll apoprotein - Medicago truncatula, partial (49%)_rframe3	0.024753	0.078999
TC144780	similar to UniRef100.Q6L4B1 Cluster: EF hand family protein; n=1; Solanum demissum	Rep: EF hand family protein - Solanum demissum (Wild potato), complete_rframe1	-0.10017	-0.00452
TC144808	UniRef100.A2Q5A8 Cluster: Aldo/keto reductase; n=1; Medicago truncatula	Rep: Aldo/keto reductase - Medicago truncatula (Barrel medic), complete_rframe2	-0.15294	0.068743
TC144947	homologue to UniRef100.P12859 Cluster: Glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor; n=1; Pisum sativum	Rep: Glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3	-0.03996	0.021623
TC145041	homologue to UniRef100.P38661 Cluster: Probable protein disulfide-isomerase A6 precursor; n=1; Medicago sativa	Rep: Probable protein disulfide-isomerase A6 precursor - Medicago sativa (Alfalfa), complete_rframe3	-0.04008	0.020398
TC145083	homologue to UniRef100.Q93XV7 Cluster: Hydroxypyruvate reductase; n=1; Bruguiera gymnorhiza	Rep: Hydroxypyruvate reductase - Bruguiera gymnorhiza, complete_rframe3	-0.05192	-0.01409
TC145098	UniRef100.A2Q5N9 Cluster: Galactose mutarotase-like; n=1; Medicago truncatula	Rep: Galactose mutarotase-like - Medicago truncatula (Barrel medic), complete_rframe1	-0.07126	0.066972
TC145158	similar to UniRef100.Q6NLE1 Cluster: At5g58250; n=1; Arabidopsis thaliana	Rep: At5g58250 - Arabidopsis thaliana (Mouse-ear cress), partial (64%)_rframe2	-0.02851	-0.00968
TC145170	homologue to UniRef100.P46256 Cluster: Fructose-bisphosphate aldolase, cytoplasmic isozyme 1; n=1; Pisum sativum	Rep: Fructose-bisphosphate aldolase, cytoplasmic isozyme 1 - Pisum sativum (Garden pea), complete_rframe1	-0.04505	0.080105
TC145241	similar to UniRef100.Q9M4S8 Cluster: Triosephosphate isomerase, chloroplast precursor; n=1; Fragaria x ananassa	Rep: Triosephosphate isomerase, chloroplast precursor - Fragaria ananassa (Strawberry), partial (87%)_rframe3	0.00147	0.005232
TC145256	similar to UniRef100.Q7X998 Cluster: MRNA-binding protein; n=1; Nicotiana tabacum	Rep: MRNA-binding protein - Nicotiana tabacum (Common tobacco), partial (82%)_rframe1	-0.05039	-0.06393
TC145284	similar to UniRef100.A7P8V3 Cluster: Chromosome chr3 scaffold_8, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr3 scaffold_8, whole genome shotgun sequence - Vitis vinifera (Grape), partial (66%)_rframe1	-0.03242	0.030001
TC145319	UniRef100.P29500 Cluster: Tubulin beta-1 chain; n=1; Pisum sativum	Rep: Tubulin beta-1 chain - Pisum sativum (Garden pea), partial (98%)_rframe2	-0.05891	-0.03604
TC145347	similar to UniRef100.A7PVK1 Cluster: Chromosome chr9 scaffold_33, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr9 scaffold_33, whole genome shotgun sequence - Vitis vinifera (Grape), partial (75%)_rframe1	-0.01029	0.020587
TC145566	similar to UniRef100.A7PKZ9 Cluster: Chromosome chr7 scaffold_20, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr7 scaffold_20, whole genome shotgun sequence - Vitis vinifera (Grape), partial (88%)_rframe1	-0.05213	-0.00395
TC145582	similar to UniRef100.A7PRN7 Cluster: Chromosome chr14 scaffold_27, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr14 scaffold_27, whole genome shotgun sequence - Vitis vinifera (Grape), partial (90%)_rframe3	-0.06682	0.059291
TC145690	homologue to UniRef100.A3F7Q3 Cluster: Benzoquinone reductase; n=2; Malvoideae	Rep: Benzoquinone reductase - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), complete_rframe2	-0.12253	-0.00606

TC145702	homologue to UniRef100_A7QBT1 Cluster: Chromosome chr1 scaffold_75, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr1 scaffold_75, whole genome shotgun sequence - Vitis vinifera (Grape), complete_rframe3	-0.03479	0.032623
TC145715	homologue to UniRef100_Q5JC56 Cluster: Malate dehydrogenase; n=1; Pisum sativum	Rep: Malate dehydrogenase - Pisum sativum (Garden pea), complete_rframe1	-0.04961	0.014183
TC145942	UniRef100_Q1SL16 Cluster: Elongation factor 1, gamma chain; Glutathione S-transferase, C- terminal; Thioredoxin-like fold; n=1; Medicago truncatula	Rep: Elongation factor 1, gamma chain; Glutathione S-transferase, C- terminal; Thioredoxin-like fold - Medicago truncatula (Barrel medic), complete_rframe2	-0.07553	0.072514
TC145943	similar to UniRef100_A7PXD5 Cluster: Chromosome chr12 scaffold_36, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr12 scaffold_36, whole genome shotgun sequence - Vitis vinifera (Grape), partial (64%)_rframe3	0.008473	-0.00312
TC145968		Glycoside hydrolase, family 19; Chitin-binding, type 1_rframe3	-0.09713	-0.07073
TC146074	UniRef100_A4ULF8 Cluster: S-adenosylmethionine synthetase; n=1; Medicago sativa subsp. falcata	Rep: S-adenosylmethionine synthetase - Medicago falcata (Sickle medic), partial (73%)_rframe3	-0.12085	0.014406
TC146112	homologue to UniRef100_O65735 Cluster: Fructose-bisphosphate aldolase, cytoplasmic isozyme; n=2; Cicer arietinum	Rep: Fructose-bisphosphate aldolase, cytoplasmic isozyme - Cicer arietinum (Chickpea) (Garbanzo), partial (98%)_rframe3	-0.09067	0.028398
TC146131	homologue to UniRef100_Q9ZTV0 Cluster: 5,10-methylenetetrahydrofolate dehydrogenase-5,10- methenyltetrahydrofolate cyclohydrolase; n=1; Pisum sativum	Rep: 5,10-methylenetetrahydrofolate dehydrogenase-5,10- methenyltetrahydrofolate cyclohydrolase - Pisum sativum (Garden pea), complete_rframe1	0.032421	0.11012
TC146234	similar to UniRef100_A7P375 Cluster: Chromosome chr1 scaffold_5, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr1 scaffold_5, whole genome shotgun sequence - Vitis vinifera (Grape), partial (25%)_rframe1	0.024753	0.078999
TC146266	UniRef100_Q2HUA3 Cluster: C2; Peptidase, cysteine peptidase active site; n=1; Medicago truncatula	Rep: C2; Peptidase, cysteine peptidase active site - Medicago truncatula (Barrel medic), complete_rframe2	-0.02116	0.009092
TC146297	weakly similar to UniRef100_Q41350 Cluster: Osmotin-like protein precursor; n=1; Solanum lycopersicum	Rep: Osmotin-like protein precursor - Solanum lycopersicum (Tomato) (Lycopersicon esculentum), partial (88%)_rframe2	0.001684	0.159891
TC146498	similar to UniRef100_Q41050 Cluster: Core protein; n=1; Pisum sativum	Rep: Core protein - Pisum sativum (Garden pea), partial (98%)_rframe3	-4.93E-32	1.23E-32
TC146522	similar to UniRef100_Q42896 Cluster: Fructokinase-2; n=1; Solanum lycopersicum	Rep: Fructokinase-2 - Solanum lycopersicum (Tomato) (Lycopersicon esculentum), partial (86%)_rframe2	-0.10265	-0.09048
TC146640	homologue to UniRef100_Q69F96 Cluster: Reversibly glycosylated protein; n=1; Phaseolus vulgaris	Rep: Reversibly glycosylated protein - Phaseolus vulgaris (Kidney bean) (French bean), partial (98%)_rframe3	0.027761	0.080739
TC146680	homologue to UniRef100_Q9ZP90 Cluster: Ferritin; n=1; Medicago sativa	Rep: Ferritin - Medicago sativa (Alfalfa), complete_rframe3	-0.18354	-0.001
TC146729	weakly similar to UniRef100_Q84VZ7 Cluster: At2g03440; n=1; Arabidopsis thaliana	Rep: At2g03440 - Arabidopsis thaliana (Mouse-ear cress), partial (31%)_rframe2	-0.06153	-0.01958
TC146858	similar to UniRef100_O82090 Cluster: Fiber annexin; n=1; Gossypium hirsutum	Rep: Fiber annexin - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), complete_rframe1	-0.10151	-0.07246
TC146906	homologue to UniRef100_P25890 Cluster: Catalase; n=1; Pisum sativum	Rep: Catalase - Pisum sativum (Garden pea), partial (45%)_rframe1	-0.06917	0.003253
TC146983	homologue to UniRef100_P16059 Cluster: Oxygen-evolving enhancer protein 2, chloroplast precursor; n=1; Pisum sativum	Rep: Oxygen-evolving enhancer protein 2, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe1	-0.05111	-0.01367
TC147000	similar to UniRef100_P29344 Cluster: 30S ribosomal protein S1, chloroplast precursor; n=1; Spinacia oleracea	Rep: 30S ribosomal protein S1, chloroplast precursor - Spinacia oleracea (Spinach), partial (92%)_rframe3	-0.0469	0.048056
TC147026	rframe1	TC147026_rframe1	-0.07681	0.001121
TC147078	similar to UniRef100_O49855 Cluster: Acid phosphatase; n=1; Glycine max	Rep: Acid phosphatase - Glycine max (Soybean), partial (85%)_rframe2	-0.01665	0.089437
TC147093	homologue to UniRef100_Q6EJD0 Cluster: 1-deoxy-D-xylulose 5-phosphate reductoisomerase; n=1; Pueraria montana var. lobata	Rep: 1-deoxy-D-xylulose 5-phosphate reductoisomerase - Pueraria lobata (Kudzu vine), partial (69%)_rframe3	-0.03851	-0.03092
TC147138	similar to UniRef100_A7PFX4 Cluster: Chromosome chr6 scaffold_15, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr6 scaffold_15, whole genome shotgun sequence - Vitis vinifera (Grape), partial (96%)_rframe3	0.003635	-0.03254
TC147215	similar to UniRef100_A7Q5Y6 Cluster: Chromosome chr14 scaffold_54, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr14 scaffold_54, whole genome shotgun sequence - Vitis vinifera (Grape), partial (83%)_rframe3	-0.03118	0.013339
TC147230	homologue to UniRef100_P29828 Cluster: Protein disulfide-isomerase precursor; n=1; Medicago sativa	Rep: Protein disulfide-isomerase precursor - Medicago sativa (Alfalfa), partial (76%)_rframe2	-0.07099	0.036371
TC147263	UniRef100_Q9SLQ6 Cluster: Actin isoform B; n=1; Mimosa pudica	Rep: Actin isoform B - Mimosa pudica (Sensitive plant), complete_rframe3	-0.04208	0.009849
TC147391	homologue to UniRef100_A5APT3 Cluster: Peptidyl-prolyl cis-trans isomerase; n=1; Vitis vinifera	Rep: Peptidyl-prolyl cis-trans isomerase - Vitis vinifera (Grape), partial (89%)_rframe1	-0.0158	0.02382
TC147524	homologue to UniRef100_A7P2W0 Cluster: Chromosome chr1 scaffold_5, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr1 scaffold_5, whole genome shotgun sequence - Vitis vinifera (Grape), partial (80%)_rframe2	0.004072	0.040576
TC147596	UniRef100_Q2HVD9 Cluster: Hydroxyacid dehydrogenase/reductase; 6-phosphogluconate dehydrogenase, C-terminal-like; n=1; Medicago truncatula	Rep: Hydroxyacid dehydrogenase/reductase; 6-phosphogluconate dehydrogenase, C-terminal-like - Medicago truncatula (Barrel medic), complete_rframe3	-0.11149	0.010362
TC147600		Ribosomal protein L10_rframe3	-0.05973	0.048999
TC147811	homologue to UniRef100_Q43791 Cluster: Peroxidase1C precursor; n=1; Medicago sativa	Rep: Peroxidase1C precursor - Medicago sativa (Alfalfa), complete_rframe3	-0.01216	-0.03748

TC147872 similar to UniRef100.Q42908 Cluster: 2,3-bisphosphoglycerate-independent phosphoglycerate mutase; n=1; Mesembryanthemum crystallinum
 TC147932 similar to UniRef100.A7NUU5 Cluster: Chromosome chr18 scaffold_1, whole genome shotgun sequence; n=1; Vitis vinifera
 TC147954 similar to UniRef100.A7P5N3 Cluster: Chromosome chr4 scaffold_6, whole genome shotgun sequence; n=1; Vitis vinifera
 TC148021
 TC148023 homologue to UniRef100.O65198 Cluster: Superoxide dismutase [Cu-Zn], chloroplast precursor; n=1; Medicago sativa
 TC148024 homologue to UniRef100.A2XLF2 Cluster: Actin-1; n=2; Oryza sativa
 TC148177 homologue to UniRef100.Q43467 Cluster: Elongation factor Tu, chloroplast precursor; n=1; Glycine max
 TC148305
 TC148322 homologue to UniRef100.A7PT66 Cluster: Chromosome chr8 scaffold_29, whole genome shotgun sequence; n=1; Vitis vinifera
 TC148328 UniRef100.A6Y950 Cluster: Vacuolar H⁺-ATPase B subunit; n=1; Medicago truncatula
 TC148488
 TC148538 homologue to UniRef100.O23755 Cluster: Elongation factor 2; n=1; Beta vulgaris
 TC148543 homologue to UniRef100.Q43437 Cluster: Photosystem II type I chlorophyll a/b-binding protein precursor; n=1; Glycine max
 TC148559 homologue to UniRef100.A7PWR3 Cluster: Chromosome chr19 scaffold_35, whole genome shotgun sequence; n=1; Vitis vinifera
 TC148676 similar to UniRef100.Q41709 Cluster: Ferritin-2, chloroplast precursor; n=1; Vigna unguiculata
 TC148688 homologue to UniRef100.A7P0Q1 Cluster: Chromosome chr19 scaffold_4, whole genome shotgun sequence; n=1; Vitis vinifera
 TC148740 homologue to UniRef100.Q43557 Cluster: Unidentified precursor; n=1; Medicago sativa
 TC148773 homologue to UniRef100.P26291 Cluster: Cytochrome b6-f complex iron-sulfur subunit, chloroplast precursor; n=1; Pisum sativum
 TC148804 UniRef100.Q24076 Cluster: Guanine nucleotide-binding protein subunit beta-like protein; n=1; Medicago sativa
 TC148818 homologue to UniRef100.A7Q7E6 Cluster: Chromosome chr18 scaffold_59, whole genome shotgun sequence; n=1; Vitis vinifera
 TC148846 homologue to UniRef100.Q8W596 Cluster: Fe-superoxide dismutase; n=1; Medicago sativa
 TC148866 homologue to UniRef100.Q43437 Cluster: Photosystem II type I chlorophyll a/b-binding protein precursor; n=1; Glycine max
 TC148869
 TC148930 similar to UniRef100.A9T0D3 Cluster: Predicted protein; n=1; Physcomitrella patens subsp. patens
 TC148947 homologue to UniRef100.Q0DA88 Cluster: Os06g0669400 protein; n=3; Oryza sativa
 TC148966 homologue to UniRef100.P08927 Cluster: RuBisCO large subunit-binding protein subunit beta, chloroplast precursor; n=1; Pisum sativum
 TC149058 similar to UniRef100.Q9SXJ6 Cluster: ATP-dependent Clp protease proteolytic subunit 3, chloroplast precursor; n=1; Arabidopsis thaliana
 TC149178 homologue to UniRef100.A9PL09 Cluster: Serine hydroxymethyltransferase; n=1; Populus tremuloides
 TC149216 weakly similar to UniRef100.A8C978 Cluster: Forisome; n=1; Medicago truncatula
 TC149263 homologue to UniRef100.P47922 Cluster: Nucleoside diphosphate kinase 1; n=1; Pisum sativum
 TC149279 similar to UniRef100.Q93X25 Cluster: 2-Cys peroxiredoxin; n=1; Pisum sativum
 TC149403 homologue to UniRef100.P14226 Cluster: Oxygen-evolving enhancer protein 1, chloroplast precursor; n=1; Pisum sativum
 TC149435 homologue to UniRef100.O04275 Cluster: ATP synthase subunit beta; n=1; Pisum sativum

Rep: 2,3-bisphosphoglycerate-independent phosphoglycerate mutase - Mesembryanthemum crystallinum (Common ice plant), partial (93%)_rframe1
 Rep: Chromosome chr18 scaffold_1, whole genome shotgun sequence - Vitis vinifera (Grape), partial (79%)_rframe1
 Rep: Chromosome chr4 scaffold_6, whole genome shotgun sequence - Vitis vinifera (Grape), complete_rframe2
 Lipoxigenase_rframe2
 Rep: Superoxide dismutase [Cu-Zn], chloroplast precursor - Medicago sativa (Alfalfa), complete_rframe1
 Rep: Actin-1 - Oryza sativa subsp. indica (Rice), complete_rframe3
 Rep: Elongation factor Tu, chloroplast precursor - Glycine max (Soybean), partial (47%)_rframe2
 H⁺-transporting two-sector ATPase, B/B subunit_rframe1
 Rep: Chromosome chr8 scaffold_29, whole genome shotgun sequence - Vitis vinifera (Grape), partial (95%)_rframe2
 Rep: Vacuolar H⁺-ATPase B subunit - Medicago truncatula (Barrel medic), complete_rframe1
 Leucine-rich repeat, plant specific_rframe2
 Rep: Elongation factor 2 - Beta vulgaris (Sugar beet), partial (32%)_rframe2
 Rep: Photosystem II type I chlorophyll a/b-binding protein precursor - Glycine max (Soybean), complete_rframe1
 Rep: Chromosome chr19 scaffold_35, whole genome shotgun sequence - Vitis vinifera (Grape), partial (87%)_rframe2
 Rep: Ferritin-2, chloroplast precursor - Vigna unguiculata (Cowpea), partial (84%)_rframe2
 Rep: Chromosome chr19 scaffold_4, whole genome shotgun sequence - Vitis vinifera (Grape), partial (76%)_rframe1
 Rep: Unidentified precursor - Medicago sativa (Alfalfa), complete_rframe1
 Rep: Cytochrome b6-f complex iron-sulfur subunit, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3
 Rep: Guanine nucleotide-binding protein subunit beta-like protein - Medicago sativa (Alfalfa), complete_rframe2
 Rep: Chromosome chr18 scaffold_59, whole genome shotgun sequence - Vitis vinifera (Grape), partial (82%)_rframe2
 Rep: Fe-superoxide dismutase - Medicago sativa (Alfalfa), complete_rframe3
 Rep: Photosystem II type I chlorophyll a/b-binding protein precursor - Glycine max (Soybean), complete_rframe3
 PDZ/DHR/GLGF; Tetratricopeptide-like helical_rframe1
 Rep: Predicted protein - Physcomitrella patens subsp. patens, partial (85%)_rframe1
 Rep: Os06g0669400 protein - Oryza sativa subsp. japonica (Rice), partial (72%)_rframe2
 Rep: RuBisCO large subunit-binding protein subunit beta, chloroplast precursor - Pisum sativum (Garden pea), partial (76%)_rframe3
 Rep: ATP-dependent Clp protease proteolytic subunit 3, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (68%)_rframe1
 Rep: Serine hydroxymethyltransferase - Populus tremuloides (Quaking aspen), partial (83%)_rframe3
 Rep: Forisome - Medicago truncatula (Barrel medic), partial (44%)_rframe1
 Rep: Nucleoside diphosphate kinase 1 - Pisum sativum (Garden pea), complete_rframe2
 Rep: 2-Cys peroxiredoxin - Pisum sativum (Garden pea), partial (86%)_rframe2
 Rep: Oxygen-evolving enhancer protein 1, chloroplast precursor - Pisum sativum (Garden pea), partial (98%)_rframe2
 Rep: ATP synthase subunit beta - Pisum sativum (Garden pea), partial (97%)_rframe2

TC149483	homologue to UniRef100_P12858	Cluster: Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor; n=1; Pisum sativum	Rep: Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor - Pisum sativum (Garden pea), partial (51%)_rframe2	-0.01623	0.013046
TC149487	weakly similar to UniRef100_A7Q0F8	Cluster: Chromosome chr7 scaffold_42, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr7 scaffold_42, whole genome shotgun sequence - Vitis vinifera (Grape), partial (17%)_rframe3	0.00692	0.01105
TC149800	homologue to UniRef100_P12858	Cluster: Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor; n=1; Pisum sativum	Rep: Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3	-0.01186	0.017838
TC149890	homologue to UniRef100_Q6UJX4	Cluster: Molecular chaperone Hsp90-1; n=1; Solanum lycopersicum	Rep: Molecular chaperone Hsp90-1 - Solanum lycopersicum (Tomato) (Lycopersicon esculentum), partial (74%)_rframe2	-0.05206	-0.02356
TC149930			Blue (type 1) copper domain; O-methyltransferase, family 2; Dimerisation_rframe3	-0.09774	-0.16842
TC149938			Phosphoglycerate kinase_rframe1	-0.01668	-0.02486
TC150012	similar to UniRef100_Q9S7W1	Cluster: Chlorophyll a-b binding protein CP29.3, chloroplast precursor; n=1; Arabidopsis thaliana	Rep: Chlorophyll a-b binding protein CP29.3, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (80%)_rframe1	0.002689	0.013124
TC150229	weakly similar to UniRef100_A7Q2B1	Cluster: Chromosome chr13 scaffold_45, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr13 scaffold_45, whole genome shotgun sequence - Vitis vinifera (Grape), partial (80%)_rframe1	-0.00812	-0.02578
TC150239	homologue to UniRef100_Q01516	Cluster: Fructose-bisphosphate aldolase 1, chloroplast precursor; n=1; Pisum sativum	Rep: Fructose-bisphosphate aldolase 1, chloroplast precursor - Pisum sativum (Garden pea), partial (65%)_rframe2	-0.05557	-0.01293
TC150311	homologue to UniRef100_P14226	Cluster: Oxygen-evolving enhancer protein 1, chloroplast precursor; n=1; Pisum sativum	Rep: Oxygen-evolving enhancer protein 1, chloroplast precursor - Pisum sativum (Garden pea), partial (95%)_rframe2	0.010777	0.030818
TC150378	homologue to UniRef100_Q48906	Cluster: Malate dehydrogenase precursor; n=2; Medicago sativa	Rep: Malate dehydrogenase precursor - Medicago sativa (Alfalfa), partial (42%)_rframe3	-0.08566	-0.02563
TC150404	UniRef100_A4ULF8	Cluster: S-adenosylmethionine synthetase; n=1; Medicago sativa subsp. falcata	Rep: S-adenosylmethionine synthetase - Medicago falcata (Sickle medic), complete_rframe2	-0.10714	0.019351
TC150490	similar to UniRef100_A8VYM8	Cluster: Cytosolic fructose-1,6-bisphosphatase; n=1; Fragaria x ananassa	Rep: Cytosolic fructose-1,6-bisphosphatase - Fragaria ananassa (Strawberry), complete_rframe1	0.068525	0.12237
TC150588	homologue to UniRef100_A0EJL8	Cluster: GDP-D-mannose-3',5'-epimerase; n=1; Malpighia glabra	Rep: GDP-D-mannose-3',5'-epimerase - Malpighia glabra (Barbados cherry), partial (41%)_rframe1	-0.08448	-0.03
TC150794	similar to UniRef100_Q9AXQ2	Cluster: Mitochondrial processing peptidase beta subunit; n=1; Cucumis melo	Rep: Mitochondrial processing peptidase beta subunit - Cucumis melo (Muskmelon), partial (93%)_rframe1	-0.04367	0.006181
TC150837	homologue to UniRef100_A7PRV4	Cluster: Chromosome chr14 scaffold_27, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr14 scaffold_27, whole genome shotgun sequence - Vitis vinifera (Grape), partial (90%)_rframe1	0.032421	0.11012
TC150861	homologue to UniRef100_Q6T706	Cluster: Fructose-bisphosphate aldolase; n=1; Trifolium pratense	Rep: Fructose-bisphosphate aldolase - Trifolium pratense (Red clover), partial (88%)_rframe2	-0.04028	0.017621
TC150946	homologue to UniRef100_Q6EJD0	Cluster: 1-deoxy-D-xylulose 5-phosphate reductoisomerase; n=1; Pueraria montana var. lobata	Rep: 1-deoxy-D-xylulose 5-phosphate reductoisomerase - Pueraria lobata (Kudzu vine), partial (64%)_rframe2	-0.02398	0.00445
TC150950	UniRef100_P46259	Cluster: Tubulin alpha-1 chain; n=1; Pisum sativum	Rep: Tubulin alpha-1 chain - Pisum sativum (Garden pea), partial (98%)_rframe1	-0.01946	0.026056
TC150990	homologue to UniRef100_P08927	Cluster: RuBisCO large subunit-binding protein subunit beta, chloroplast precursor; n=1; Pisum sativum	Rep: RuBisCO large subunit-binding protein subunit beta, chloroplast precursor - Pisum sativum (Garden pea), partial (32%)_rframe1	-0.01356	0.055946
TC151014	homologue to UniRef100_A7QY53	Cluster: Chromosome chr12 scaffold_238, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr12 scaffold_238, whole genome shotgun sequence - Vitis vinifera (Grape), partial (73%)_rframe2	-4.93E-32	1.23E-32
TC151103	homologue to UniRef100_Q71EW8	Cluster: Methionine synthase; n=2; Papilionoideae	Rep: Methionine synthase - Glycine max (Soybean), complete_rframe3	-0.03005	-0.00134
TC151139	UniRef100_Q84UC1	Cluster: Glutamine synthetase; n=2; Papilionoideae	Rep: Glutamine synthetase - Medicago truncatula (Barrel medic), complete_rframe1	-0.01236	0.03516
TC151145	homologue to UniRef100_A9PL04	Cluster: Serine hydroxymethyltransferase; n=1; Populus tremuloides	Rep: Serine hydroxymethyltransferase - Populus tremuloides (Quaking aspen), partial (62%)_rframe2	-0.06941	0.018782
TC151157	UniRef100_Q2TFP2	Cluster: Tubulin A; n=1; Glycine max	Rep: Tubulin A - Glycine max (Soybean), complete_rframe1	0.002633	0.079317
TC151243	similar to UniRef100_P07689	Cluster: Ribulose biphosphate carboxylase small chain 3A, chloroplast precursor; n=2; Pisum sativum	Rep: Ribulose biphosphate carboxylase small chain 3A, chloroplast precursor - Pisum sativum (Garden pea), partial (93%)_rframe2	0.015442	0.020631
TC151284	homologue to UniRef100_Q93YH0	Cluster: Clp protease 2 proteolytic subunit precursor; n=1; Solanum lycopersicum	Rep: Clp protease 2 proteolytic subunit precursor - Solanum lycopersicum (Tomato) (Lycopersicon esculentum), partial (66%)_rframe2	-0.03683	0.074512
TC151423	homologue to UniRef100_A4ULF9	Cluster: Myo-inositol-1-phosphate synthases; n=1; Medicago sativa subsp. falcata	Rep: Myo-inositol-1-phosphate synthases - Medicago falcata (Sickle medic), partial (56%)_rframe2	-0.07272	-0.01991
TC151485	similar to UniRef100_Q6W2J5	Cluster: VDAC1.1; n=1; Lotus japonicus	Rep: VDAC1.1 - Lotus japonicus, partial (98%)_rframe3	0.008362	-0.01106
TC151490	homologue to UniRef100_P28552	Cluster: ATP synthase gamma chain, chloroplast precursor; n=1; Pisum sativum	Rep: ATP synthase gamma chain, chloroplast precursor - Pisum sativum (Garden pea), partial (98%)_rframe1	0.003706	0.083338
TC151598	homologue to UniRef100_P35100	Cluster: ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplast precursor; n=1; Pisum sativum	Rep: ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplast precursor - Pisum sativum (Garden pea), partial (48%)_rframe3	-0.01955	0.111688
TC151622	similar to UniRef100_A7NVR7	Cluster: Chromosome chr18 scaffold_1, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr18 scaffold_1, whole genome shotgun sequence - Vitis vinifera (Grape), partial (97%)_rframe1	-0.04057	0.004671
TC151652	homologue to UniRef100_Q9SM09	Cluster: Vacuolar ATP synthase catalytic subunit A; n=2; Citrus unshiu	Rep: Vacuolar ATP synthase catalytic subunit A - Citrus unshiu (Satsuma orange), partial (74%)_rframe3	-0.10894	0.004039

TC151764 similar to UniRef100_A3F7Q2 Cluster: Benzoquinone reductase; n=1; Gossypium hirsutum	Rep: Benzoquinone reductase - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), partial (87%)_rframe1	-0.0051	-0.03633
TC151877 similar to UniRef100_A7P597 Cluster: Chromosome chr4 scaffold_6, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr4 scaffold_6, whole genome shotgun sequence - Vitis vinifera (Grape), partial (79%)_rframe1	-0.03527	-0.00865
TC151907	RNA-binding region RNP-1 (RNA recognition motif)_rframe1	0.032421	0.11012
TC152101	Plant lipoxygenase; Lipase/lipoxygenase, PLAT/LH2_rframe1	-0.0262	0.038996
TC152136 homologue to UniRef100_Q41038 Cluster: Type II chlorophyll a/b binding protein from photosystem I precursor; n=1; Pisum sativum	Rep: Type II chlorophyll a/b binding protein from photosystem I precursor - Pisum sativum (Garden pea), complete_rframe3	-0.00013	-0.02736
TC152187 similar to UniRef100_A7P9H4 Cluster: Chromosome chr3 scaffold_8, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr3 scaffold_8, whole genome shotgun sequence - Vitis vinifera (Grape), partial (70%)_rframe1	-0.01358	0.019905
TC152287 similar to UniRef100_A7QT89 Cluster: Chromosome chr1 scaffold_166, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr1 scaffold_166, whole genome shotgun sequence - Vitis vinifera (Grape), partial (86%)_rframe3	-0.00089	0.001752
TC152310 similar to UniRef100_A7QF07 Cluster: Chromosome chr16 scaffold_86, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr16 scaffold_86, whole genome shotgun sequence - Vitis vinifera (Grape), partial (74%)_rframe1	-0.06167	0.095538
TC152332 homologue to UniRef100_P17067 Cluster: Carbonic anhydrase, chloroplast precursor (Carbonate dehydratase) [Contains: Carbonic anhydrase, 27 kDa isoform; Carbonic anhydrase, 25 kDa isoform]; n=1; Pisum sativum	Rep: Carbonic anhydrase, chloroplast precursor (Carbonate dehydratase) [Contains: Carbonic anhydrase, 27 kDa isoform; Carbonic anhydrase, 25 kDa isoform] - Pisum sativum (Garden pea), partial (98%)_rframe2	-0.06305	0.020487
TC152540 homologue to UniRef100_Q5QHT4 Cluster: 70 kDa heat shock cognate protein 1; n=1; Vigna radiata	Rep: 70 kDa heat shock cognate protein 1 - Vigna radiata, complete_rframe1	-0.08566	0.000758
TC152702 similar to UniRef100_P36212 Cluster: 50S ribosomal protein L12-3, chloroplast precursor; n=1; Arabidopsis thaliana	Rep: 50S ribosomal protein L12-3, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (76%)_rframe3	-0.00077	0.056529
TC152714 similar to UniRef100_Q32904 Cluster: Chlorophyll a-b binding protein 3, chloroplast precursor; n=1; Pisum sativum	Rep: Chlorophyll a-b binding protein 3, chloroplast precursor - Pisum sativum (Garden pea), partial (93%)_rframe2	-0.02988	0.015357
TC152781 homologue to UniRef100_A9PL09 Cluster: Serine hydroxymethyltransferase; n=1; Populus tremuloides	Rep: Serine hydroxymethyltransferase - Populus tremuloides (Quaking aspen), complete_rframe1	-0.08986	0.037721
TC152879 homologue to UniRef100_Q9SM09 Cluster: Vacuolar ATP synthase catalytic subunit A; n=2; Citrus unshiu	Rep: Vacuolar ATP synthase catalytic subunit A - Citrus unshiu (Satsuma orange), partial (49%)_rframe3	-0.08372	0.034668
TC152948	H ⁺ -transporting two-sector ATPase, delta/epsilon subunit_rframe1	-0.02864	-0.08998
TC153020 homologue to UniRef100_Q9SQL2 Cluster: Chlorophyll a-b binding protein P4, chloroplast precursor; n=1; Pisum sativum	Rep: Chlorophyll a-b binding protein P4, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3	-0.00313	-0.01735
TC153031 homologue to UniRef100_P08927 Cluster: RuBisCO large subunit-binding protein subunit beta, chloroplast precursor; n=1; Pisum sativum	Rep: RuBisCO large subunit-binding protein subunit beta, chloroplast precursor - Pisum sativum (Garden pea), partial (63%)_rframe2	-0.03845	0.024146
TC153097 UniRef100_A2Q2V1 Cluster: ATP-citrate lyase/succinyl-CoA ligase; n=1; Medicago truncatula	Rep: ATP-citrate lyase/succinyl-CoA ligase - Medicago truncatula (Barrel medic), complete_rframe1	0.011943	0.100529
TC153152 homologue to UniRef100_O81221 Cluster: Actin; n=1; Gossypium hirsutum	Rep: Actin - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), complete_rframe1	-0.05231	0.016458
TC153385 UniRef100_A7QZH1 Cluster: Chromosome chr7 scaffold_275, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr7 scaffold_275, whole genome shotgun sequence - Vitis vinifera (Grape), partial (82%)_rframe1	-0.01621	0.069356
TC153480 similar to UniRef100_A7PDS7 Cluster: Chromosome chr11 scaffold_13, whole genome shotgun sequence; n=3; Vitis vinifera	Rep: Chromosome chr11 scaffold_13, whole genome shotgun sequence - Vitis vinifera (Grape), partial (91%)_rframe1	-0.07097	0.052357
TC153502 similar to UniRef100_O04945 Cluster: Enoyl-ACP reductase precursor; n=1; Nicotiana tabacum	Rep: Enoyl-ACP reductase precursor - Nicotiana tabacum (Common tobacco), partial (89%)_rframe2	-0.03239	-0.11738
TC153548 UniRef100_UPI0001596BB7 Cluster: ATP synthase CF1 alpha subunit; n=1; Medicago truncatula	Rep: ATP synthase CF1 alpha subunit - Medicago truncatula, partial (43%)_rframe3	0.036125	-0.01263
TC153554 homologue to UniRef100_Q6RIB7 Cluster: Enolase; n=1; Glycine max	Rep: Enolase - Glycine max (Soybean), complete_rframe2	-0.05503	0.005049
TC153721 similar to UniRef100_Q0PN10 Cluster: Glutathione S-transferase; n=1; Caragana korshinskii	Rep: Glutathione S-transferase - Caragana korshinskii, complete_rframe3	-0.05625	0.00594
TC153829 homologue to UniRef100_Q41219 Cluster: Dihydrolipoyl dehydrogenase; n=1; Glycine max	Rep: Dihydrolipoyl dehydrogenase - Glycine max (Soybean), partial (51%)_rframe3	-0.07071	-0.00748
TC153930	Glycoside hydrolase, family 17; von Willebrand factor, type A_rframe1	-0.04011	0.016724
TC153963 homologue to UniRef100_P35100 Cluster: ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplast precursor; n=1; Pisum sativum	Rep: ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplast precursor - Pisum sativum (Garden pea), partial (42%)_rframe1	-0.05385	0.082169
TC153984 similar to UniRef100_Q5U583 Cluster: Probable glutathione peroxidase 8-B; n=1; Xenopus laevis	Rep: Probable glutathione peroxidase 8-B - Xenopus laevis (African clawed frog), partial (6%)_rframe-2	-0.09389	-0.01583
TC153988 homologue to UniRef100_Q45FE6 Cluster: Serine hydroxymethyltransferase; n=1; Medicago truncatula	Rep: Serine hydroxymethyltransferase - Medicago truncatula (Barrel medic), partial (49%)_rframe2	-0.15257	0.015445
TC153991 similar to UniRef100_A7P275 Cluster: Chromosome chr19 scaffold_4, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr19 scaffold_4, whole genome shotgun sequence - Vitis vinifera (Grape), partial (66%)_rframe3	-0.0149	-0.00308
TC154064	Pyridoxal-5-phosphate-dependent enzyme, beta subunit_rframe3	-0.0546	0.068478
TC154149 similar to UniRef100_A9XTM4 Cluster: Fasciclin-like arabinogalactan protein 19; n=1; Gossypium hirsutum	Rep: Fasciclin-like arabinogalactan protein 19 - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), partial (71%)_rframe1	-0.00971	0.105441

TC154182	Photosystem I reaction centre subunit N_rframe3	0.012956	0.270692
TC154302 similar to UniRef100_A7PT29 Cluster: Chromosome chr8 scaffold_29, whole genome shotgun sequence; n=2; Vitis vinifera	Rep: Chromosome chr8 scaffold_29, whole genome shotgun sequence - Vitis vinifera (Grape), partial (97%)_rframe2	-0.01597	0.001457
TC154315 similar to UniRef100.P93162 Cluster: Magnesium-chelatase subunit chlI, chloroplast precursor; n=1; Glycine max	Rep: Magnesium-chelatase subunit chlI, chloroplast precursor - Glycine max (Soy-bean), partial (66%)_rframe2	-0.02235	0.151381
TC154443 homologue to UniRef100.P35100 Cluster: ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplast precursor; n=1; Pisum sativum	Rep: ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplast precursor - Pisum sativum (Garden pea), partial (71%)_rframe3	-0.0326	0.074686
TC154538 similar to UniRef100.Q2HU12 Cluster: Glycoside hydrolase, family 17; n=1; Medicago truncatula	Rep: Glycoside hydrolase, family 17 - Medicago truncatula (Barrel medic), partial (53%)_rframe1	-0.11569	0.010373
TC154744	Transketolase, C-terminal-like_rframe2	-0.03819	0.052997
TC155092 similar to UniRef100_A7R2V3 Cluster: Chromosome undetermined scaffold_457, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome undetermined scaffold_457, whole genome shotgun sequence - Vitis vinifera (Grape), partial (69%)_rframe1	-0.07681	0.001121
TC155108 UniRef100_A9YWS0 Cluster: Serine-hydroxymethyltransferase; n=1; Medicago truncatula	Rep: Serine-hydroxymethyltransferase - Medicago truncatula (Barrel medic), complete_rframe2	-0.07788	-0.01639
TC155207 homologue to UniRef100.Q94FA7 Cluster: Fructose-1,6-bisphosphatase; n=1; Pisum sativum	Rep: Fructose-1,6-bisphosphatase - Pisum sativum (Garden pea), partial (88%)_rframe1	-0.12629	-0.01339
TC155303 similar to UniRef100_A7QIZ0 Cluster: Chromosome chr2 scaffold_105, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr2 scaffold_105, whole genome shotgun sequence - Vitis vinifera (Grape), partial (59%)_rframe3	-0.12297	0.031139
TC155647 similar to UniRef100_A7Y7E3 Cluster: Leucine-rich repeat-like protein; n=1; Stylosanthes hamata	Rep: Leucine-rich repeat-like protein - Stylosanthes hamata (Caribbean stylo), partial (55%)_rframe3	-0.03619	0.030469
TC155685 similar to UniRef100.Q9SA52 Cluster: Uncharacterized protein At1g09340, chloroplast precursor; n=1; Arabidopsis thaliana	Rep: Uncharacterized protein At1g09340, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (47%)_rframe3	-0.02431	0.074333
TC155694 UniRef100.Q1SKX2 Cluster: Chaperone DnaK; n=1; Medicago truncatula	Rep: Chaperone DnaK - Medicago truncatula (Barrel medic), complete_rframe1	-0.02684	0.035117
TC155779 UniRef100_A4ULF9 Cluster: Myo-inositol-1-phosphate synthases; n=1; Medicago sativa subsp. falcata	Rep: Myo-inositol-1-phosphate synthases - Medicago falcata (Sickle medic), partial (79%)_rframe2	-0.1147	-0.0022
TC156129 UniRef100.O04999 Cluster: Glutamine synthetase; n=2; Medicago truncatula	Rep: Glutamine synthetase - Medicago truncatula (Barrel medic), complete_rframe2	-0.01242	0.033918
TC156149	AAA ATPase, central region; Homeodomain-like_rframe2	0.001805	0.015894
TC156152 similar to UniRef100.P20115-2 Cluster: Isoform 2 of P20115 ; n=1; Arabidopsis thaliana	Rep: Isoform 2 of P20115 - Arabidopsis thaliana (Mouse-ear cress), partial (98%)_rframe1	0.019729	0.062662
TC156165 similar to UniRef100_A7PCN3 Cluster: Chromosome chr17 scaffold_12, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr17 scaffold_12, whole genome shotgun sequence - Vitis vinifera (Grape), partial (65%)_rframe2	-0.02331	-0.03181
TC156211 homologue to UniRef100.Q04918 Cluster: Chlorophyll a/b-binding protein precursor; n=1; Pisum sativum	Rep: Chlorophyll a/b-binding protein precursor - Pisum sativum (Garden pea), partial (97%)_rframe1	-0.02823	-0.02581
TC156383 UniRef100.Q39445 Cluster: Tubulin beta chain; n=1; Cicer arietinum	Rep: Tubulin beta chain - Cicer arietinum (Chickpea) (Garbanzo), partial (98%)_rframe2	-0.08429	0.00493
TC156600 similar to UniRef100_A7NYV0 Cluster: Chromosome chr6 scaffold_3, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr6 scaffold_3, whole genome shotgun sequence - Vitis vinifera (Grape), partial (43%)_rframe3	0.047114	0.01632
TC156718 similar to UniRef100_A7PQI2 Cluster: Chromosome chr6 scaffold_25, whole genome shotgun sequence; n=2; Vitis vinifera	Rep: Chromosome chr6 scaffold_25, whole genome shotgun sequence - Vitis vinifera (Grape), partial (96%)_rframe3	0.03362	0.060696
TC156735 homologue to UniRef100_A7QUM8 Cluster: Chromosome chr10 scaffold_179, whole genome shotgun sequence; n=2; Vitis vinifera	Rep: Chromosome chr10 scaffold_179, whole genome shotgun sequence - Vitis vinifera (Grape), partial (57%)_rframe1	-0.08448	-0.03
TC156741 homologue to UniRef100_A7PT66 Cluster: Chromosome chr8 scaffold_29, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr8 scaffold_29, whole genome shotgun sequence - Vitis vinifera (Grape), partial (65%)_rframe2	-0.03983	0.009986
TC156803 similar to UniRef100_A7NVJ4 Cluster: Chromosome chr18 scaffold_1, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr18 scaffold_1, whole genome shotgun sequence - Vitis vinifera (Grape), partial (75%)_rframe3	0.013677	0.096795
TC156889 similar to UniRef100_A7Q228 Cluster: Ribosomal protein L1; n=1; Vitis vinifera	Rep: Ribosomal protein L1 - Vitis vinifera (Grape), partial (57%)_rframe3	0.02728	0.043497
TC156968 similar to UniRef100_A7QJI8 Cluster: Chromosome chr8 scaffold_106, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr8 scaffold_106, whole genome shotgun sequence - Vitis vinifera (Grape), complete_rframe1	-0.04405	0.0214
TC157090 homologue to UniRef100.Q9SM60 Cluster: Phosphoglucomutase, cytoplasmic; n=1; Pisum sativum	Rep: Phosphoglucomutase, cytoplasmic - Pisum sativum (Garden pea), complete_rframe1	-0.04734	0.098474
TC157092 similar to UniRef100.Q41119 Cluster: Peptidyl-prolyl cis-trans isomerase; n=1; Phaseolus vulgaris	Rep: Peptidyl-prolyl cis-trans isomerase - Phaseolus vulgaris (Kidney bean) (French bean), complete_rframe1	-0.05815	-0.01809
TC157261 similar to UniRef100.Q02758 Cluster: ATP synthase delta chain, chloroplast precursor; n=1; Pisum sativum	Rep: ATP synthase delta chain, chloroplast precursor - Pisum sativum (Garden pea), partial (78%)_rframe1	-0.02557	0.012786
TC157277 weakly similar to UniRef100.A8HX52 Cluster: Predicted protein; n=1; Chlamydomonas reinhardtii	Rep: Predicted protein - Chlamydomonas reinhardtii, partial (5%)_rframe3	-0.08448	-0.03
TC157411 similar to UniRef100.Q9FT52 Cluster: ATP synthase subunit d, mitochondrial; n=1; Arabidopsis thaliana	Rep: ATP synthase subunit d, mitochondrial - Arabidopsis thaliana (Mouse-ear cress), complete_rframe2	-0.0176	0.043905

TC157536 homologue to UniRef100_Q24470 Cluster: Lipoygenase; n=1; Pisum sativum
 TC157830 similar to UniRef100_A7P710 Cluster: Chromosome chr9 scaffold_7, whole genome shotgun sequence; n=1; Vitis vinifera
 TC157864 UniRef100_Q9BAE0 Cluster: Cell division protease ftsH homolog, chloroplast precursor; n=1; Medicago sativa
 TC158016 similar to UniRef100_Q6QPJ4 Cluster: Methionine sulfoxide reductase A; n=1; Populus trichocarpa x Populus deltoides
 TC158096 homologue to UniRef100_P29501 Cluster: Tubulin beta-2 chain; n=1; Pisum sativum
 TC158157 homologue to UniRef100_P16048 Cluster: Glycine cleavage system H protein, mitochondrial precursor; n=9; Pisum
 TC158194 homologue to UniRef100_Q6RJY3 Cluster: 40S ribosomal protein S17; n=1; Capsicum annuum
 TC158354 homologue to UniRef100_O48906 Cluster: Malate dehydrogenase precursor; n=2; Medicago sativa
 TC158504 homologue to UniRef100_Q43594 Cluster: Tubulin beta-1 chain; n=3; Oryza sativa
 TC158568 UniRef100_Q2HU12 Cluster: Glycoside hydrolase, family 17; n=1; Medicago truncatula
 TC158878 homologue to UniRef100_O22636 Cluster: Poly(A) polymerase; n=1; Pisum sativum
 TC158962 similar to UniRef100_A7PZU0 Cluster: Chromosome chr15 scaffold_40, whole genome shotgun sequence; n=1; Vitis vinifera
 TC159269 similar to UniRef100_Q43644 Cluster: NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial precursor; n=1; Solanum tuberosum
 TC159281 homologue to UniRef100_A7Q613 Cluster: Chromosome chr14 scaffold_54, whole genome shotgun sequence; n=2; Vitis vinifera
 TC159419 weakly similar to UniRef100_Q9T076 Cluster: Early nodulin-like protein 2 precursor; n=2; Arabidopsis thaliana
 TC159446 homologue to UniRef100_A7QGQ2 Cluster: Chromosome chr12 scaffold_93, whole genome shotgun sequence; n=1; Vitis vinifera
 TC159455 similar to UniRef100_Q41122 Cluster: Proline-rich protein precursor; n=1; Phaseolus vulgaris
 TC159473 similar to UniRef100_Q9M1X0 Cluster: Ribosome recycling factor, chloroplast precursor; n=1; Arabidopsis thaliana
 TC160649 similar to UniRef100_A0S5Z5 Cluster: Dehydroascorbate reductase; n=1; Sesamum indicum
 TC161068 homologue to UniRef100_Q8S4X2 Cluster: Ribulose-5-phosphate-3-epimerase; n=1; Pisum sativum
 TC161205 homologue to UniRef100_P45960 Cluster: Tubulin beta-4 chain; n=2; Oryza sativa Japonica Group
 TC161248 UniRef100_Q40325 Cluster: Aspartate aminotransferase; n=1; Medicago sativa
 TC161515 homologue to UniRef100_P26969 Cluster: Glycine dehydrogenase [decarboxylating], mitochondrial precursor; n=1; Pisum sativum
 TC161557
 TC161750 UniRef100_A2Q5A3 Cluster: H⁺-transporting two-sector ATPase, alpha/beta subunit, central region; H⁺-transporting two-sector ATPase, delta/epsilon subunit; n=1; Medicago truncatula
 TC161884 homologue to UniRef100_P31023 Cluster: Dihydrolipoyl dehydrogenase, mitochondrial precursor; n=1; Pisum sativum
 TC161936 similar to UniRef100_A7PNA3 Cluster: Chromosome chr1 scaffold_22, whole genome shotgun sequence; n=1; Vitis vinifera
 TC162040 similar to UniRef100_A7NYJ8 Cluster: Chromosome chr6 scaffold_3, whole genome shotgun sequence; n=2; Vitis vinifera
 TC162439 homologue to UniRef100_P53537 Cluster: Alpha-glucan phosphorylase, H isozyme; n=1; Vicia faba
 TC162949 homologue to UniRef100_P45621 Cluster: Glutamate-1-semialdehyde 2,1-aminomutase, chloroplast precursor; n=1; Glycine max

Rep: Lipoygenase - Pisum sativum (Garden pea), complete_rframe1 0.001125 0.031867
 Rep: Chromosome chr9 scaffold_7, whole genome shotgun sequence - Vitis vinifera (Grape), partial (85%)_rframe2 -0.05126 -0.00104
 Rep: Cell division protease ftsH homolog, chloroplast precursor - Medicago sativa (Alfalfa), complete_rframe3 -0.01388 0.054467
 Rep: Methionine sulfoxide reductase A - Populus jackii (Balm of Gilead) (Populus trichocarpa x Populusdeltoides), partial (56%)_rframe1 -4.93E-32 1.23E-32
 Rep: Tubulin beta-2 chain - Pisum sativum (Garden pea), complete_rframe2 -0.05293 0.038335
 Rep: Glycine cleavage system H protein, mitochondrial precursor - Pisum sativum (Garden pea), complete_rframe2 -0.06178 -0.00728
 Rep: 40S ribosomal protein S17 - Capsicum annuum (Bell pepper), partial (86%)_rframe1 0.052062 -0.08012
 Rep: Malate dehydrogenase precursor - Medicago sativa (Alfalfa), partial (94%)_rframe2 0.008618 0.031481
 Rep: Tubulin beta-1 chain - Oryza sativa subsp. japonica (Rice), complete_rframe1 -0.01419 -0.01191
 Rep: Glycoside hydrolase, family 17 - Medicago truncatula (Barrel medic), partial (67%)_rframe3 0.011192 0.029461
 Rep: Poly(A) polymerase - Pisum sativum (Garden pea), partial (83%)_rframe2 -0.03271 0.030077
 Rep: Chromosome chr15 scaffold_40, whole genome shotgun sequence - Vitis vinifera (Grape), partial (82%)_rframe1 -0.07681 0.001121
 Rep: NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial precursor - Solanum tuberosum (Potato), partial (66%)_rframe2 -0.11137 -0.00168
 Rep: Chromosome chr14 scaffold_54, whole genome shotgun sequence - Vitis vinifera (Grape), partial (98%)_rframe3 -0.01544 0.025395
 Rep: Early nodulin-like protein 2 precursor - Arabidopsis thaliana (Mouse-ear cress), partial (35%)_rframe2 0.001811 -0.06995
 Rep: Chromosome chr12 scaffold_93, whole genome shotgun sequence - Vitis vinifera (Grape), partial (80%)_rframe3 -0.03639 0.018277
 Rep: Proline-rich protein precursor - Phaseolus vulgaris (Kidney bean) (French bean), partial (46%)_rframe3 -0.01672 -0.05922
 Rep: Ribosome recycling factor, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress), partial (70%)_rframe2 -0.02978 0.018886
 Rep: Dehydroascorbate reductase - Sesamum indicum (Oriental sesame) (Gingelly), complete_rframe2 -0.02146 0.032013
 Rep: Ribulose-5-phosphate-3-epimerase - Pisum sativum (Garden pea), complete_rframe2 0.017271 -0.01547
 Rep: Tubulin beta-4 chain - Oryza sativa subsp. japonica (Rice), complete_rframe2 -0.04067 0.020455
 Rep: Aspartate aminotransferase - Medicago sativa (Alfalfa), partial (80%)_rframe2 0.049419 0.017548
 Rep: Glycine dehydrogenase [decarboxylating], mitochondrial precursor - Pisum sativum (Garden pea), complete_rframe2 -0.08107 0.006804
 AAA ATPase, central region; Homeodomain-like_rframe1 0.01102 0.001538
 Rep: H⁺-transporting two-sector ATPase, alpha/beta subunit, central region; H⁺-transporting two-sector ATPase, delta/epsilon subunit - Medicago truncatula (Barrel medic), partial (54%)_rframe3 -0.01269 -0.03819
 Rep: Dihydrolipoyl dehydrogenase, mitochondrial precursor - Pisum sativum (Garden pea), partial (80%)_rframe1 -0.05481 -0.01364
 Rep: Chromosome chr1 scaffold_22, whole genome shotgun sequence - Vitis vinifera (Grape), partial (63%)_rframe1 -0.00079 0.077415
 Rep: Chromosome chr6 scaffold_3, whole genome shotgun sequence - Vitis vinifera (Grape), partial (49%)_rframe1 -0.01606 -0.03355
 Rep: Alpha-glucan phosphorylase, H isozyme - Vicia faba (Broad bean), partial (68%)_rframe1 -0.01869 0.06308
 Rep: Glutamate-1-semialdehyde 2,1-aminomutase, chloroplast precursor - Glycine max (Soybean), partial (92%)_rframe2 -0.0394 0.048803

TC163013 UniRef100_O04998 Cluster: Glutamine synthetase; n=2; Medicago truncatula	Rep: Glutamine synthetase - Medicago truncatula (Barrel medic), partial (66%)_rframe3	-0.00299	-0.00918
TC163347 homologue to UniRef100_P42654 Cluster: 14-3-3-like protein B; n=1; Vicia faba	Rep: 14-3-3-like protein B - Vicia faba (Broad bean), complete_rframe3	-0.09755	-0.06714
TC163459 homologue to UniRef100_Q9SM59 Cluster: Phosphoglucosomutase, chloroplast precursor; n=1; Pisum sativum	Rep: Phosphoglucosomutase, chloroplast precursor - Pisum sativum (Garden pea), partial (78%)_rframe3	-0.03833	0.047337
TC163626 similar to UniRef100_Q5QJB6 Cluster: Harpin binding protein 1; n=1; Glycine max	Rep: Harpin binding protein 1 - Glycine max (Soybean), partial (80%)_rframe3	-0.01696	-0.0209
TC163823 homologue to UniRef100_O48561 Cluster: Catalase-4; n=1; Glycine max	Rep: Catalase-4 - Glycine max (Soybean), partial (73%)_rframe1	-0.11077	-0.02558
TC163858 UniRef100_UP10001596BB7 Cluster: ATP synthase CF1 alpha subunit; n=1; Medicago truncatula	Rep: ATP synthase CF1 alpha subunit - Medicago truncatula, partial (29%)_rframe3	0.014504	0.031332
TC163984 UniRef100_Q45FE6 Cluster: Serine hydroxymethyltransferase; n=1; Medicago truncatula	Rep: Serine hydroxymethyltransferase - Medicago truncatula (Barrel medic), complete_rframe1	-0.10718	-0.09499
TC164554 homologue to UniRef100_P00288 Cluster: Plastocyanin; n=1; Vicia faba	Rep: Plastocyanin - Vicia faba (Broad bean), partial (88%)_rframe1	-0.01456	0.001199
TC164637 similar to UniRef100_Q9AT39 Cluster: SHOOT1 protein; n=1; Glycine max	Rep: SHOOT1 protein - Glycine max (Soybean), partial (30%)_rframe3	0.034427	-0.04695
TC164927 UniRef100_Q5MGA8 Cluster: Heat shock protein 70; n=1; Medicago sativa	Rep: Heat shock protein 70 - Medicago sativa (Alfalfa), complete_rframe2	-0.07191	0.037576
TC165236 homologue to UniRef100_O04300 Cluster: Alpha-1,4-glucan-protein synthase [UDP-forming]; n=1; Pisum sativum	Rep: Alpha-1,4-glucan-protein synthase [UDP-forming] - Pisum sativum (Garden pea), partial (95%)_rframe1	-0.0344	0.070658
TC165432 similar to UniRef100_P46280 Cluster: Elongation factor Tu, chloroplast precursor; n=1; Glycine max	Rep: Elongation factor Tu, chloroplast precursor - Glycine max (Soybean), partial (73%)_rframe3	-0.01037	0.029051
TC165605 homologue to UniRef100_P16059 Cluster: Oxygen-evolving enhancer protein 2, chloroplast precursor; n=1; Pisum sativum	Rep: Oxygen-evolving enhancer protein 2, chloroplast precursor - Pisum sativum (Garden pea), partial (93%)_rframe1	-0.03955	0.019355
TC165606 homologue to UniRef100_Q9SGT4 Cluster: Elongation factor EF-2; n=1; Arabidopsis thaliana	Rep: Elongation factor EF-2 - Arabidopsis thaliana (Mouse-ear cress), complete_rframe3	-0.12362	-0.01017
TC165709 similar to UniRef100_Q7Y1T5 Cluster: Oxygen-evolving enhancer protein 3; n=1; Pisum sativum	Rep: Oxygen-evolving enhancer protein 3 - Pisum sativum (Garden pea), partial (89%)_rframe2	-0.03027	-0.0148
TC166093 homologue to UniRef100_A7R4C2 Cluster: Chromosome undetermined scaffold.648, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome undetermined scaffold.648, whole genome shotgun sequence - Vitis vinifera (Grape), partial (61%)_rframe2	-0.01498	-0.0031
TC166118 similar to UniRef100_A5ATB7 Cluster: Methylenetetrahydrofolate reductase; n=1; Vitis vinifera	Rep: Methylenetetrahydrofolate reductase - Vitis vinifera (Grape), partial (30%)_rframe1	-0.03446	0.041083
TC166440 similar to UniRef100_Q0PWS5 Cluster: Chloroplast pigment-binding protein CP26; n=1; Nicotiana tabacum	Rep: Chloroplast pigment-binding protein CP26 - Nicotiana tabacum (Common tobacco), partial (89%)_rframe1	0.00065	-0.01561
TC166515 homologue to UniRef100_P29828 Cluster: Protein disulfide-isomerase precursor; n=1; Medicago sativa	Rep: Protein disulfide-isomerase precursor - Medicago sativa (Alfalfa), partial (50%)_rframe3	-0.00746	0.013749
TC166820 homologue to UniRef100_P93162 Cluster: Magnesium-chelatase subunit chlI, chloroplast precursor; n=1; Glycine max	Rep: Magnesium-chelatase subunit chlI, chloroplast precursor - Glycine max (Soybean), partial (73%)_rframe3	-0.00508	0.122889
TC167139 UniRef100_Q40345 Cluster: Isocitrate dehydrogenase [NADP], chloroplast precursor (Oxalosuccinate decarboxylase) (IDH) (NADP(+)-specific ICDH); n=1; Medicago sativa	Rep: Isocitrate dehydrogenase [NADP], chloroplast precursor (Oxalosuccinate decarboxylase) (IDH) (NADP(+)-specific ICDH) - Medicago sativa (Alfalfa), partial (98%)_rframe2	-0.02682	0.008355
TC167473 similar to UniRef100_O65194 Cluster: Ribulose biphosphate carboxylase small chain, chloroplast precursor; n=1; Medicago sativa	Rep: Ribulose biphosphate carboxylase small chain, chloroplast precursor - Medicago sativa (Alfalfa), partial (97%)_rframe2	0.016172	0.029007
TC167667 homologue to UniRef100_Q8W0Q7 Cluster: Methionine synthase protein; n=1; Sorghum bicolor	Rep: Methionine synthase protein - Sorghum bicolor (Sorghum) (Sorghum vulgare), partial (51%)_rframe2	-0.02759	0.015657
TC167788 similar to UniRef100_O65194 Cluster: Ribulose biphosphate carboxylase small chain, chloroplast precursor; n=1; Medicago sativa	Rep: Ribulose biphosphate carboxylase small chain, chloroplast precursor - Medicago sativa (Alfalfa), partial (93%)_rframe1	-0.00256	0.025135
TC167914 homologue to UniRef100_P93681 Cluster: Phosphoribulokinase; n=1; Pisum sativum	Rep: Phosphoribulokinase - Pisum sativum (Garden pea), complete_rframe2	-0.026	0.007003
TC168018 UniRef100_O48904 Cluster: Malate dehydrogenase precursor; n=1; Medicago sativa	Rep: Malate dehydrogenase precursor - Medicago sativa (Alfalfa), complete_rframe2	-0.06883	-0.07213
TC168053 similar to UniRef100_A7PIQ8 Cluster: Chromosome chr13 scaffold.17, whole genome shotgun sequence; n=1; Vitis vinifera	Rep: Chromosome chr13 scaffold.17, whole genome shotgun sequence - Vitis vinifera (Grape), partial (97%)_rframe1	-0.00014	0.014551
TC168860 homologue to UniRef100_P27520 Cluster: Chlorophyll a-b binding protein 215, chloroplast precursor; n=2; Pisum sativum	Rep: Chlorophyll a-b binding protein 215, chloroplast precursor - Pisum sativum (Garden pea), complete_rframe3	-0.02857	0.014455
TC168923 similar to UniRef100_P47923 Cluster: Nucleoside diphosphate kinase 2, chloroplast precursor (Nucleoside diphosphate kinase II) (NDK II) (NDP kinase II) (NDPK II) [Contains: Nucleoside diphosphate kinase 2 high molecular weight; Nucleoside diphosphate kinase 2 low molecular weight] - Pi	Rep: Nucleoside diphosphate kinase 2, chloroplast precursor (Nucleoside diphosphate kinase II) (NDK II) (NDP kinase II) (NDPK II) [Contains: Nucleoside diphosphate kinase 2 high molecular weight; Nucleoside diphosphate kinase 2 low molecular weight] - Pi	-0.09023	-0.0562
TC168983	Aldo/keto reductase_rframe2	-0.04984	-0.16201

TC169370 UniRef100_O81391 Cluster: Chlorophyll a/b binding protein; n=1; Medicago sativa	Rep: Chlorophyll a/b binding protein - Medicago sativa (Alfalfa), complete_rframe3	-0.01588	-0.03346
TC169859 homologue to UniRef100_Q41698 Cluster: Cysteine proteinase precursor; n=1; Vicia sativa	Rep: Cysteine proteinase precursor - Vicia sativa (Spring vetch) (Tare), partial (88%)_rframe2	-0.05985	0.040414
TC171155 homologue to UniRef100_O65194 Cluster: Ribulose bisphosphate carboxylase small chain, chloroplast precursor; n=1; Medicago sativa	Rep: Ribulose bisphosphate carboxylase small chain, chloroplast precursor - Medicago sativa (Alfalfa), partial (97%)_rframe3	-0.00744	0.023798
TC171817 homologue to UniRef100_P25804 Cluster: Cysteine proteinase 15A precursor; n=1; Pisum sativum	Rep: Cysteine proteinase 15A precursor - Pisum sativum (Garden pea), complete_rframe1	-0.04781	0.022642
TC172113 UniRef100_Q945F4 Cluster: Eukaryotic translation initiation factor 5A-2; n=1; Medicago sativa	Rep: Eukaryotic translation initiation factor 5A-2 - Medicago sativa (Alfalfa), complete_rframe2	-0.09188	0.094871
TC172377 homologue to UniRef100_A7KQH2 Cluster: Beta-tubulin; n=1; Eucalyptus grandis	Rep: Beta-tubulin - Eucalyptus grandis (Flooded gum), partial (30%)_rframe2	0.008296	0.04569

A.2. Formulations

Table A.4.: TY-0.8% Agar Medium

Chemical	Quantity [g]
Peptone/Tryptone	8
Yeast extract	1
NaCl	2.8
Glucose	0.5
Agar agar Kobe 1	4
qs ddH ₂ O to 500ml	

Table A.5.: TY Medium

Chemical	Quantity [g]
Peptone/Tryptone	2.5
Yeast extract	1.5
CaCl ₂	0.5
qs ddH ₂ O to 500ml	

A.3. Curriculum vitae

Christiana Elisabeth Staudinger

Education

1995-2003	Stiftsgymnasium Kremsmünster, Austria Matura
2003-2005	Lycée Polyvalent Elisa Lemonnier Paris, France BTS Brevet de Technicien Supérieur Esthétique Cosmétique
2005-continued	University of Vienna, Austria Diploma-study of Biology, specialized in botany, focus physiology and phytochemistry

Employment

2009-2010	University of Vienna, Department of Molecular Systems Biology Tutor for the practical course "Metabolomics"
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Conference presentations

06/2010	Austrian Society of Plant Biology Conference. Illmitz, Austria Poster: Evidence for a differential nitrogen nutrition regulated drought stress response mechanism observed in <i>Medicago truncatula</i>
06/2010 Zaragoza, Spain	National Meeting of the Spanish Society of Nitrogen Fixation. Oral communication: Evidence for a differential nitrogen nutrition regulated drought stress response mechanism observed in <i>Medicago truncatula</i>
09/ 2010	European Nitrogen Fixation Conference. Geneva, Switzerland Poster: Nitrogen-source regulated drought tolerance in <i>Medicago truncatula</i>

Honors and Awards

06/2003	Matura passed with distinction
06/2005	BTS centralized diploma examination passed with distinction
03/2010	Science Day Award of the Vienna Ecology Center