# Nutrient transport in the arbuscular mycorrhizal symbiosis: the regulation of nutrient transporters in *Rhizophagus irregularis* and its host plants *Populus trichocarpa* and *Sorghum bicolor*

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## Abbreviations

| | |
|---|---|
| AM | arbuscular mycorrhiza |
| AMT | ammonium transporter |
| C | carbon |
| CBP | cap binding protein |
| CMN | common mycorrhizal network |
| DCL1 | DICER-LIKE1 |
| DDL | DAWDLE |
| ER | endoplasmic reticulum |
| ERM | extraradical mycelium |
| HATS | High-affinity transport system |
| HEN1 | HUA ENHANCER 1 |
| HYL1 | DRB HYPONASTIC leaves |
| IPS1 | INDUCED PHOSPHATE STARVATION 1 |
| IRM | intraradical mycelium |
| LATS | Low-affinity transport system |
| Mep/AMT | methylammonium/ammonium permease |
| MIR genes | miRNA genes |
| miRNA | micro RNA |
| MST | monosaccharide transporter |
| Myc-LCO | mycorrhizal lipochito oligosaccharides |
| N | nitrogen |
| NLA | NITROGEN LIMITATION ADAPTATION |
| P | phosphorus |
| PAM | periarbuscular membrane |
| PAS | periarbuscular space |
| PHF1 | PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR 1 |
| PHR1 | PHOSPHATE STARVATION RESPONSE REGULATOR 1 |
| PHS | Pi:$H^+$ symporter |
| Pi | inorganic phosphorus |
| Pol II | DNA-dependent RNA polymerase II |
| PT | phosphate transporter |
| SDN | small RNA degrading nucleases |
| SUMO | SMALL UBIQUITIN MODIFIER |
| SUT | sugar transporter |
| TAG | triacyglycerides |

## Summary

In natural and agricultural ecosystems, arbuscular mycorrhizal (AM) fungi play a major role in plant nutrition. In AM symbiosis, the AM fungi extract mineral nutrients from the substrate and transfer them to the host plant. Inside the roots of the host plant, the intraradical hyphae form tree like structures (arbuscules) where the nutrients are released to the plant fungal interface. In return, the AM fungi receive carbohydrates from the plants. Specialized transport systems enable nutrient uptake from the substrate and translocation across membranes. As main components of organic molecules, phosphorus (P), nitrogen (N) and carbon (C) are of particular importance for symbiotic nutrient exchanges. This work is focused on a range of genes that encode proteins contributing to transport molecules (P, N and C nutrients) across cellular membranes in the plants *Populus trichocarpa* (poplar) and *Sorghum bicolor* (sorghum), and in the AM fungus *Rhizophagus irregularis*.

In the AM fungus *R. irregularis* (formerly *Glomus intraradices*), we identified and characterized a novel functional ammonium transporter (AMT), GintAMT3. Quantification of transcript abundances in the extraradical mycelium (ERM) and the intraradical mycelium (IRM) during symbiosis with poplar and sorghum revealed that GintAMT3 was highly expressed in the IRM of AM roots. Phylogenetic analysis showed further, that the six glomeromycotan AMTs share high sequence similarity, but are distinct to AMTs of other fungal phyla. To functionaly analyze GintAMT3, we expressed GintAMT3 in a yeast deletion mutant devoid of all AMTs. The heterologous expression revealed that GintAMT3 is a low affinity transporter. Heterologous expression of GFP tagged GintAMT3 in yeast showed that GintAMT3 is localized in the plasma membrane and the vacuolar membrane. Further, we could show that expression of GinAMT3 is dependent on the N nutrition status and the fungal C status. Taken together, our data suggested that GintAMT3 is the main export carrier for ammonium at the arbuscular site.

Using mRNA sequencing, we could show that low N availability significantly increased gene expression of the AM fungus, including genes involved in cell growth and membrane biogenesis as well as genes involved in signaling and metabolic processes. High abundances of genes related to N metabolism, including glutamine synthase, aminotransferase, AMTs as well as arginases, indicated a high turnover rate of N in the symbiotic root tissue. Depending on P availability, gene expression of AM phosphate transporters (PT) and AMT changed. Induction of PT and AMT under low-P availability indicated that the AM fungus transfers more nutrients to the host plant.

Further, we identified amino acids transporters and $H^+$/oligopeptide transporters specifically induced in mycorrhizal poplar roots, indicating that amino acids are transferred between the AM fungus and the plant.

In poplar, we found that root colonization and low-N conditions resulted in the down-regulation of defense gene expression, suggesting that the plant stimulated symbiotic interactions with the AM fungus. We showed that root colonization specifically induced expression of known and newly identified PT and AMT in poplar and sorghum. Specific induction of nutrient transporters upon starvation strongly indicated that they are essential components of a functional symbiosis and suggested they are located in AM roots. Furthermore, root colonization suppressed the expression of genes involved in P starvation response, indicating that root colonization efficiently alleviated P stress of the plant. Moreover, we could show that the annual sorghum is more dependent on the AM fungus than the perennial poplar, but also that more P and possibly also more N is transferred from the AM fungus to the host plant. Non-mycorrhized sorghum accumulated similar quantities of P as AM sorghum under conditions, in which only the AM fungus had access to the P source. Poplar on the other hand accumulated less P in AM plants. In addition, we observed that a subset of poplar Pht1 transporters was regulated independently on the AM fungus, but depending on the P availability of the substrate.

To deepen our understanding about symbiotic C exchange, we made transcriptome analysis and qRT-PCR to investigate the role of carbohydrate transporters in AM symbiosis between *R. irregularis* and, poplar and sorghum, respectively. In *R. irregularis*, the monosaccharide transporter GintMST2 was specifically induced in the IRM independently on the nutrient condition. Interestingly, we observed the down-regulation of many carbohydrate transporters in AM roots of poplar and sorghum. However, in poplar, we identified one carbohydrate transporter, which might be involved in symbiotic C transfer. In conclusion, our data on C transport suggested that carbohydrates are taken from the plant by the AM fungus instead of actively transferred to the fungus by the host plant.

Taken together, the data summarized in my thesis add to our understanding of nutrient transport in AM symbiosis under different environmental conditions and help elucidating the underlying mechanisms. Regarding climate changes and resources shortening, a precise understanding of the efficiency of AM symbiosis may help to increase the efficiency of sustainable agriculture.

# 1. General Introduction

## 1.1 **Mycorrhizal symbiosis**

The term symbiosis describes the mutually beneficial interaction of two dissimilar organisms from distinct species. The term has been originally introduced by Franck in 1877 as a neutral term describing the mere coexistence of two organisms in the same environment and the circumstance that they potentially profited from each other (Franck, 1877). A few years later, de Bary (1887) expanded the definition of symbiosis to comprise any coexistence with interactions ranging from mutualistic over commensalistic to parasitic (Smith and Read, 2008). One of the most widespread symbiosis is the mycorrhizal symbiosis (Smith and Read, 2008). In 1885, Franck discovered that some tree species were associated with fungal mycelium and suggested that these fungi do not only provide a nutritional benefit to the plant, but rather provide the complete nutrition of the trees (Franck, 1885). This type of fungal association with plants he named "mycorrhiza", a combination of the Greek words mykes, meaning fungus, and rhiza, meaning root (Read, 2001). In his first experiments, Franck showed that mycorrhizal symbiosis promoted plant growth and therefore had a beneficial rather than a pathogenic effect on the plant. In addition, he proposed that the fungal symbiont might also extract certain nutrients such as N from soil organic matter (Read, 2001). The number of findings on the functioning of mycorrhizal interactions has considerably increased to more than 3000 scientific publications in the last 10 years (Pubmed, 2016).

Mycorrhizal fungi are a heterogeneous group of fungal species that are spread over several taxa (*Ascomycota, Basidiomycota, Glomeromycota* and *Zygomycota*). Fossil findings of plants from the Devonian era suggests that plants have already associated with mycorrhizal fungi over 400 million years ago. These findings suggests that mycorrhizal fungi might have enabled the development of land plants by colonizing the rudimentary root system of the plants by extracting nutrients from the soil and providing them to the plant (Remy *et al.*, 1994; Harrison, 1999; Smith and Read, 2008). Non-mycorrhizal plants are believed to have evolved only about 100 million years ago (Brundrett, 2002). The occurrence of non-mycorrhizal plants within various plant clades suggests that these plants lost the capability to form mycorrhizas secondarily during their evolutionary development (Paszkowski, 2006).

About 90% of the known land-dwelling plant species are mycorrhized and although mycorrhizal fungi can spend part of their life cycle as free-living organisms, they will preferably associate with roots of higher plants if given the possibility (Bonfante and Genre, 2010). Mycorrhizal associations

have been recorded in liverworts (*Marchantiophyta*), hornworts (*Anthocerophyta*), mosses (*Bryophyta*), ferns (*Pteridophyta*), lycopods (*Tracheophyta*) and in higher plants (*Spermatophyta*) (Bago *et al.*, 2000; Smith and Read, 2008). There is one other, unique type of mycorrhizal symbiosis formed between cyanobateria and the fungal species *Geosiphon pyriformis*, which belongs to the order of *Glomerales* (Schüßler et al., 2001).

In the mycorrhizal symbiosis, the fungus supplies the host plant with mineral nutrients, including N, P and water. In exchange, the fungus receives carbohydrates (in form of monosaccharides) from the plants (Figure1.1) (Parniske, 2008; Doidy *et al.*, 2012). In such symbioses, plants can receive nearly 90% of their N and P supply via their fungal symbionts. Due to the resulting increase in nutritional supply, the plants gain in fitness, have a better growth performance and an improved disease resistance (Karandashov and Bucher, 2005; Parniske, 2008; Smith and Read, 2008; Tatry *et al.*, 2009).

The heterogeneous group of mycorrhizal fungi can be divided into two functional categories, ecto- and endo-mycorrhiza. This categorization refers to the manner of proliferation of the fungus when it is associated with the roots of its host. Ectomycorrhizal fungi are most commonly found in temperate forests associated with trees and shrubs. In the ectomycorrhizal symbiosis the ectomycorrhizal fungi grow on short side-roots of the host plant where the hyphae form a densely packed mantle. These sheathing mantles cover the root tips completely. Highly branched hyphae are growing in between the epidermal and cortical root cells forming the so-called Hartig net, which is the site of nutrient exchange. Some ectomycorrhizal fungi form so called arbutoid and monotropoid mycorrhizas, which form intracellular hyphal complexes in addition of the Hartig net (Read, 2001). The majority of ectomycorrhizas are formed by *Basidiomycota*, whereas also *Ascomycota* and species of the *Zygomycota* can from ectomycorrhizas. Ectomycorrhizal fungi reproduce sexually and their fruiting bodies can be epigeous (above ground) or hypogeous (below ground). The hyphae of *Basidiomycetes* and *Ascomycetes* are septated, meaning that the hyphal continuum is divided into distinct cells by a perforated membrane allowing the flux of cytoplasm and nutrients into the neighboring compartment. The hyphae of the *Zygomycetes* are only septated when gametes are formed or to separate from dead hyphae (Read, 2001).

In the endomycorrhizal symbiosis, the fungal symbiont grows inside the root of the host plant and form intracellular structures. One type of endomycorrhizal symbiosis is the ericoid mycorrhiza, formed by *Ascomycota* associated with plant species of the orders *Ericales* and *Diapensiales*. Ericoid mycorrhizal fungi are non-obligate symbionts living in acidic and nutrient-poor soils, typical habitats for the aforementioned host plant species. The ericoides have a loose mycelium and form

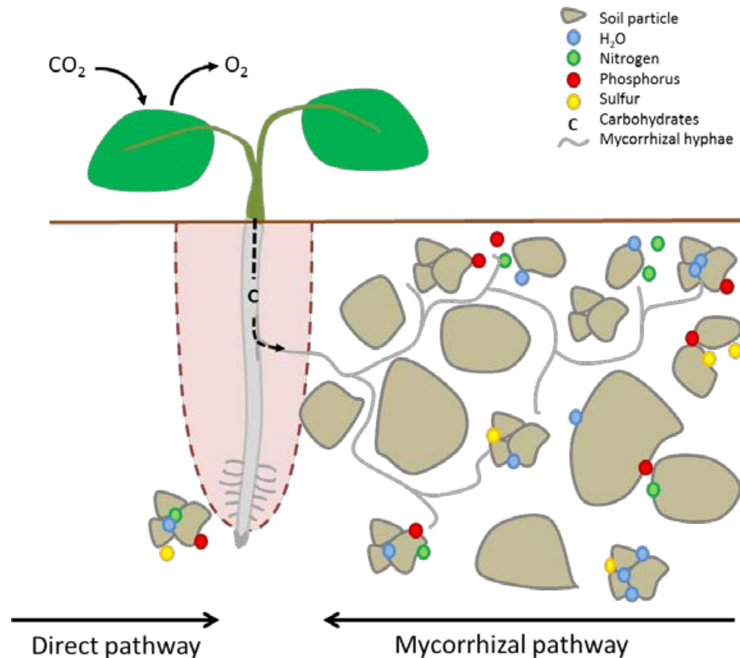


**Figure 1.1** To fulfill their life cycle, plants need mineral nutrients and water ($H_2O$). Plants can take up mineral nutrient from the rhizosphere (direct pathway), inducing a depletion zone (pink area) around the roots where nutrients become rare. To improve nutrient supply, the majority of land plants forms symbioses with mycorrhizal fungi. In the mycorrhizal pathway, the mycorrhizal symbiont supplies the plant with mineral nutrients. With their elaborate hyphal network foraging the soil, the mycorrhizal fungi can take up nutrients that are out of reach or not accessible to plants. In exchange to their services, the mycorrhizal fungi receive essential carbohydrates from the host plant.

hyphal coils within the epidermal cells when colonizing the root hair of a plant. After 8-12 weeks, the host cell and the fungal structures deteriorate (Read, 2001).

Another type of endomycorrhizal fungi is the orchid mycorrhiza. Only members of the *Basidiomycota* associate with *Orchidaceae* for which seed colonization by fungi is essential for germination and development. After successful germination, the orchid mycorrhizas are retained in the root cortex or at the base stem. As the orchids matures, it transfers less carbohydrates to the plant but still receives nutrients from the fungus. However, the exact mechanisms of upholding this unequal trade are yet unknown (Selosse and Roy, 2009; Bougoure *et al.*, 2014).

The most widespread endomycorrhizal symbiosis is formed by the ubiquitous group of arbuscular mycorrhizal (AM) fungi. The AM fungi belong to the phylum of *Glomeromycota* and associate with 70-90% of land plant species (>200’000 plant species) including hornworts, liverworts, lycopods and ferns. Woody *Gymnosperms* and several *Angiosperms* are able to form mycorrhizal symbiosis with AM fungi as well. Even though the species diversity of the *Glomeromycota* is quite low (about 230 species described up to date), they have a broad host range compared to ectomycorrhizal fungi (Schüßler and Walker, 2010; Oehl *et al.*, 2011). Contrary

to ectomycorrhizal fungi, the AM fungi grow into the cells of the inner root cortex of the plants where they form highly branched, tree-like structures called arbuscules, where nutrients are exchanged between partners. The arbuscules are surrounded by a plant-derived periarbuscular membrane (PAM) of the host cells and remain existent for only a couple of days (Karandashov and Bucher, 2005; Parniske, 2008; Tatry *et al.*, 2009; Bonfante and Genre, 2010).

## 1.2 **The arbuscular mycorrhizal fungus**

As obligate symbionts, the biotrophic AM fungi are completely dependent on a photoautotrophic partner in order to complete their live cycle and to reproduce. With their elaborated hyphal network, the fungi are able to access nutrients from a huge soil volume. One cubic centimeter of soil can contain up to 100 meters of hyphae (Parniske, 2008). The hyphal diameter ranges between 3 - 4 µm, enabling the fungi to explore pores and particles of the soil, which are not accessible to plant roots. With their extended network, AM fungi can transport nutrients over long distances to the site of nutrient exchanges within the plant (Smith and Read, 2008).

The mycelium of the AM fungi is characteristically aseptated and contains multiple nuclei in the cytoplasm (coencytic). As for reproduction, no sexual cycle has been observed so far. However, several AM fungal species undergo hyphal fusion (anastomosis), which allows the flow of cytoplasmic material between the individuals. As also nuclei are transferred during the anastomosis event, it is assumed that this sort of genetic exchange might have evolutionary influence on the AM fungi (Chagnon, 2014). To complete their life cycle, the asexual AM fungi form spores in the ERM and some species even in the IRM. These spores can reach up to 500 µm in diameter, which is exceptionally large in comparison to spores of ectomycorrhizal fungi which measure only a few micrometers in diameter (Parniske, 2008; Smith and Read, 2008). Until 1993, about 150 AM fungal species had been described through spore-based taxonomy. Subsequent molecular analyses later led to a re-organization of the taxonomy, but not to the discovery of many new species (Fitter, 2005; Smith and Read, 2008; Schüßler and Walker, 2010). Phylogenetic analyses of the small ribosomal subunit placed all AM fungi into the monophyletic group of the *Glomeromycota,* which might share a common ancestor with the *Ascomycota* and *Basidiomycota*.

With respect to the evolution of land plants, a discussion about a major role of mycorrhizal fungi in colonization of the continent by plants is currently ongoing. Interestingly, and in support of such a hypothesis, arbuscular mycorrhizas did not undergo any major diversification since their appearance approximately 460 million years ago, as evidenced by comparison of fossil with

modern AM species (Redecker *et al.*, 2000; Schüßler *et al.*, 2001; Karandashov and Bucher, 2005; Parniske, 2008).

The low species diversity of AM fungi, coupled with their broad host range and evolutionary conservation, led to the assumption that AM fungi are generalists exhibiting only low host-specificity and a high adaptability towards environmental changes. However, there are numerous AM fungal species that were described from field-collected specimens. Some but not all of these species could be cultured in pot cultures. As of this reason their phylogenetic position could not be assessed by molecular analysis (Schüßler and Walker, 2010). This, in turn, indicates that contrary to the hypothesis above, AM fungi might exhibit a restricted host range and are therefore rather specialists (Parniske, 2008).

### 1.2.1 Common mycorrhizal networks

Studies about fungal species-diversity on AM plants revealed that some plants can be colonized by up to 20 different AM fungal species at the same time. Although taxonomic compositions of communities vary, certain fungal-plant combinations seem to be more common for one plant species compared to another (Bever *et al.*, 1996; Helgason *et al.*, 2002; Fitter, 2005; Parniske, 2008; Smith and Read, 2008; Davison *et al.*, 2011). When two or more plants of the same or different species are connected via a mycorrhizal network consisting of one or more different fungal species, they are connected via the so-called common mycorrhizal network (CMN). Within this network, nutrients can be transferred between the individual plants. Apart from soluble nutrients, also C can be transported from one plant to another via this mycorrhizal network (Lerat *et al.*, 2002; Simard and Durall, 2004).

Increased transfer of N and P from the AM fungus to the plant has been shown to be rewarded with increased C supply from the plant to the fungus (Kiers *et al.*, 2011; Fellbaum *et al.*, 2012). Another study showed that nutrient allocation from the AM fungi to the plant is related to the C strength of the plant. AM fungi preferentially allocated nutrients to non-shaded host plants which were able to transfer more C to the mycorrhizal fungi (Fellbaum *et al.*, 2014). Evidence of unequal trade of nutrients between AM fungus and different plants were highlighted in a microcosm study from Walder *et al.* (2012) where sorghum ($C_4$ plant) and flax ($C_3$ plant) grew together either with *Glomus intraradices or Glomus mosseae*. When the plants were grown in monoculture, the plants received asymmetric amount of N and P from the AM fungus. While sorghum received more P from *G. mosseae*, flax received more N from *G. mosseae*. However, the amount of transferred C, is the same. In mixed culture, *G. intraradices* invested 94% of its P and 80% of its N supply in flax and

the remaining 6% and 20%, respectively in sorghum. In exchange, *G. intraradices* received 70% of its C from sorghum and only 30% from flax. However ,when grown together with *G. mosseae,* both plants received about 50% of the nutrients from the fungus while still investing the same amount of C into the fungal network (Walder *et al.*, 2012). Hence, it seems that the fungus adapts the transfer of nutrients to the culture condition, whereas the plant does not transfer similar amount of C, whatever the culture condition.

It has also been reported that belowground community composition has substantial influence on aboveground species diversity (van der Heijden *et al.*, 1998). Growth competition experiments revealed that not every plant benefits from a common mycorrhizal network to the same extent. Depending on the fungal network and plant community composition, some plants show reduced competitiveness in terms of biomass production compared to being grown with the fungus alone (Scheublin *et al.*, 2007; Van Der Heijden and Horton, 2009).

Besides the reported mutualistic relationships, a mycorrhizal network is also an attractant for mycoheterotrophic plants, which are achlorophyllous or have a reduced photosynthetic activity and are thus entirely or partly dependent on external C supply via the fungi (Selosse *et al.*, 2006). These C-dependent plants parasitize the network. Yet, other plant individuals connected to the CMN seem not to be overly affected by this parasitism (Van Der Heijden and Horton, 2009). How exactly C is transferred via the mycorrhizal network has not been fully uncovered. It is possible that C atoms are transferred in the form of amino acids such as glutamate and glutamine by which also N can be transferred (Selosse *et al.*, 2006; Van Der Heijden and Horton, 2009).

### 1.2.2 Development of mycorrhizal symbiosis

**Establishing contacts**

When AM fungi are not associated with plants, they reside in the soil in form of dormant spores. During the asymbiotic stage, the AM fungal spores show a limited hyphal growth. Once a suitable host is present, hyphal growth is stimulated through strigolactones present in the root exudates. The fungus then switches to the pre-symbiotic stage, which includes extensive hyphal branching and an altered mitochondrial activity of the hyphae (Bouwmeester *et al.*, 2003; Akiyama *et al.*, 2005; Bucher, 2007).

Studies performed in *Medicago truncatula* revealed that AM fungi exude diffusible signals, possibly induced by strigolactones. These signals, called Myc (for mycorrhization) factors, were shown to stimulate lateral root formation and to induce the expression of a gene (MtENOD11, encoding a proline-rich cell wall protein) also induced by Nod factors (Kosuta *et al.*, 2003). Nod (for

nodulation) factors are key elements of a bacterial root endosymbiosis. The induction of the Nod signaling pathway is essential for the establishment of the mutualistic symbiosis between rhizobacteria and legumes. Interestingly, treatment of plants with Nod factors increased lateral root formation and also consequently AM root colonization. These likeness between the two root endosymbioses indicate similarities in the signaling mechanisms for the establishments of symbiotic interactions (Oláh *et al.*, 2005). Indeed, many similarities in root nodule formation during the formation of rhizobial symbiosis and the induction of mycorrhizal symbiosis have been reported. At least seven genes were identified to be essential for both types of symbioses. For example, genes involved in early Nod factor signaling, such as the leucine-rich repeat receptor-like kinase (LjSYMRK/MsNORK/MtDMI2) and plastid ion channels (LjCASTOR/LjPOLLUX/MtDMI1) have been shown to be important for appressoria formation and fungal root penetration. Moreover, defects in calcium-and calmodulin dependent protein kinases (MtCCamK/MtDMI3) and CYCLOPS - a protein of unknown function that interacts with CCamK – were found to have an impact on arbuscule development. Based on these findings and the fact that AM symbiosis has existed longer than rhizobial symbiosis, it is assumed that the interaction of plants with AM fungi led to the establishment of a symbiotic signaling pathway which laid the basis for the rhizobium-legume symbioses (Catoira *et al.*, 2000; Harrison, 2005; Paszkowski, 2006; Parniske, 2008).

**Arbuscule development**

Following the pre-symbiotic phase, fungal hyphae continue interacting with their host through chemical crosstalk and prepare the formation of the endosymbiotic organogenesis.

Once the contact with the root surface is established, the hyphae form a hyphopodium (also called appressorium), which serves as the entry point for the fungal hyphae (Figure 1.2). About 4-5 hours after hyphopodium formation, there is a shift of the nucleus from the underlying plant cell towards the hyphal entry point. To enable the AM fungus to grow towards the root cortical cells, a hollow tube is formed through which the fungal hyphae can grow through. This so-called prepenetration apparatus (PPA) is made of cytoskeletal microtubules and microfilaments and forms a cytoplasmic bridge across the vacuole. Only after the tube has been finished the hypha is allowed to grow through it enabling symbiotic colonization of root cortex tissue including the formation of intraradical arbuscules, vesicles (storage organ) and the production of spores by the ERM (Paszkowski, 2006; Bucher, 2007; Parniske, 2008).

When the fungus enters a root cortical cell, the developing arbuscule is still surrounded by the plant plasma membrane, which is then called the periarbuscular membrane (PAM). The space

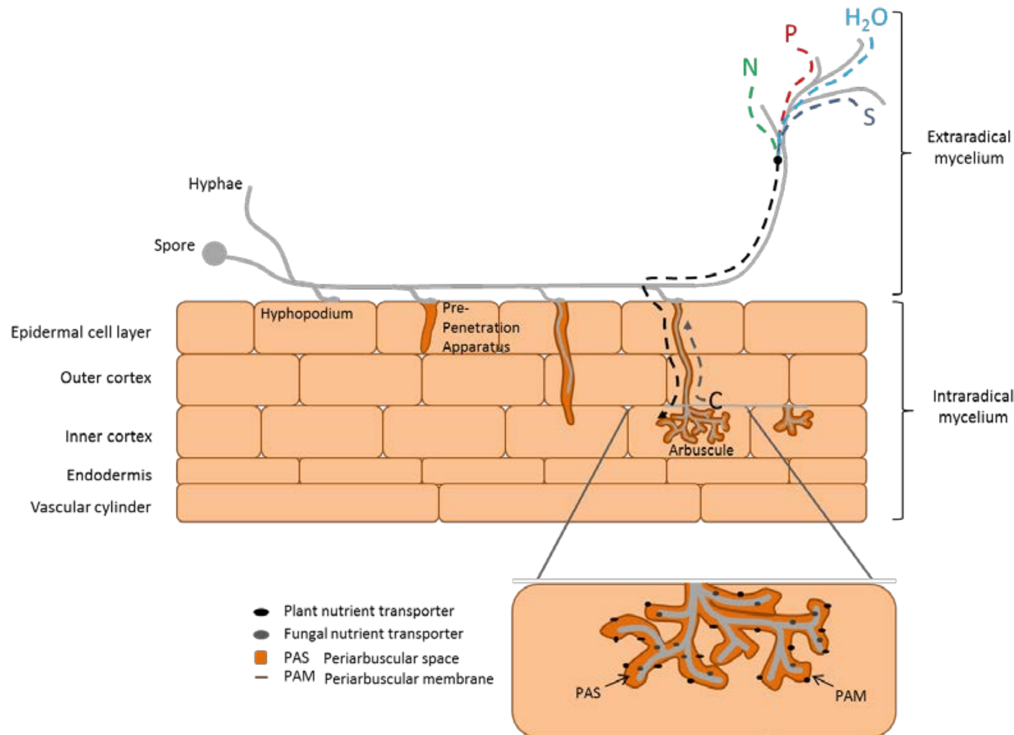


**Figure 1.2 Formation of the arbuscular mycorrhizal (AM) symbiosis** Root exudates stimulate hyphal growth. At the root surface, the hyphae form so called hyphodia which are the entry points for the fungi. The plant prepares fungal colonization by formation of a hollow tube (prepenetration apparatus) through which the fungal hyphae can grow. Inside the root cortical cells the AM fungus forms arbuscules, which are still surrounded by the plant-derived periarbuscular membrane (PAM). The space formed in between the fungal hyphae and the PAM is the periarbuscular space. Embedded in the PAM of the fungal hyphae forming the arbuscule are nutrient transporters which allow the exchange of nutrients between the two symbionts.

between the PAM and the fungal plasma membrane is the periarbuscular space (PAS) where the exchange of nutrients and carbohydrates takes place. To enable these exchanges, specialized transporters are located in both membranes (Figure1.2). The regulatory processes that rules the expression of these transporters, however, remains yet unclear.

The development of arbuscules takes 2 - 4 days. It is estimated that a complete arbuscular life cycle takes 7 - 10 days depending on the plant-fungal associations, after this time they collapse and degenerate (Parniske, 2008; Pumplin and Harrison, 2009). According to their morphological behavior within the root, the AM fungi can be further divided into the Arum and Paris type, which are named after two plant species in which these morphologies could be described approximately 100 years ago (*Paris quadrifolia* and *Arum maculatum*) (Karandashov and Bucher, 2005). The Paris type forms thick, coiled intracellular hyphae with fine-branched arbuscules, while the Arum type grows forming fine, heavily branched arbuscules. However, there are also several species exhibiting an intermediate form between both types (Karandashov and Bucher, 2005).

In the absence of a potential host, the hyphal germ tube protruding from the spore withdraws and tries to meet a host in another direction. Because hyphal growth is limited by the C storage within the spore, the germination process can take place only a limited number of times. In *Gigaspora gigantea*, for example, repeated germination attempts have been observed up to ten times (Koske, 1981; Paszkowski, 2006).

## 1.3 **Phosphate**

### 1.3.1 Phosphorus in soil

Apart from nitrogen (N) and sulfur, phosphorus (P) is the most essential mineral nutrient in plants contributing to approximately 0.2% of a plant´s dry weight (Schachtman *et al.*, 1998). P is multifunctional compound of many regulatory and metabolic processes. Involved in signaling cascades and the regulation of enzyme activity, it also plays a role in post-translational modification. As constituent of adenosine triphosphate, it is an important carrier for chemical energy used in metabolic processes for the regulation of reactions. P is also a constituent of phospholipids and nucleic acids as some of the most important requirements for life (Karandashov and Bucher, 2005; Chiou *et al.*, 2006; Branscheid *et al.*, 2010). While the P concentrations in living plant cells ranges from 1 to 10mM, the P concentration in soil water is 10'000 fold lower (Rausch and Bucher, 2002; Ai *et al.*, 2009; Branscheid *et al.*, 2010). In soil, the freely available P is taken up by the plant in form of inorganic P (Pi) as ions of orthophosphoric acid. The uptake is highly restricted because the negatively charged P is rapidly sequestered by cations, clay and organic substances in the soil (Poirier and Bucher, 2002; Aung *et al.*, 2006; Chiou *et al.*, 2006; Javot *et al.*, 2007; Tatry *et al.*, 2009). The high sequestration rate by these compounds and the fact that the diffusion rate of free available P is only $10^{-12}$ to $10^{-15} m^2 s^{-1}$ renders P highly immobile in soil and thus barely accessible to the plant (Schachtman *et al.*, 1998; Rausch and Bucher, 2002; Ai *et al.*, 2009). Studies concerned with pH dependent P uptake showed that the pH holds a key role for the uptake-efficiency of plant P transport systems. Uptake rates were highest at pH 4.5 and pH 6 when $H_2PO_4^-$ was the predominant form of P, leading to the assumption that $H_2PO_4^-$ is taken up through the plasma membrane (Schachtman *et al.*, 1998; Rausch and Bucher, 2002).

### 1.3.2 Phosphorus uptake in plants

To overcome P deficiency, plants have developed different biochemical and morphological strategies including P-partitioning and -recycling processes, changing of root structures, secretion of phosphomonoesterases and organic acids such as maleic acid and citric acid into the

rhizosphere. Among these strategies are also the formation of symbioses with mycorrhizal fungi and other microbial symbionts (Bieleski, 1973; Rausch and Bucher, 2002; Javot *et al.*, 2007; Loth-Pereda *et al.*, 2011).

To sustain a constant flux of mineral nutrients and other compounds, specialized transport systems were developed by the plant. As mentioned above, the concentration of P within the cell can be up to 10 mM whereas P concentration in the rhizosphere is generally less than 10 µM and can drop to submicromolar level at the soil/root interface (Schachtman *et al.*, 1998; Poirier and Bucher, 2002). Together with the strong negative charge in the plasmalemma, there is a strong electrochemical gradient and concentration gradient, which needs to be overcome. In order to facilitate the P uptake, there are specialized membrane-spanning proteins able to transport P across the membrane via $H^+$-symport. At the expense of ATP a membrane-integral ATPases transfer protons from the intracellular to the extracellular site, creating a proton concentration gradient and a proton electrochemical potential across the membrane. The proton movement along the electrochemical gradient and the concentration gradient facilitates Pi up take against the Pi concentration gradient (Schachtman *et al.*, 1998; Smith *et al.*, 2000; Poirier and Bucher, 2002; Karandashov and Bucher, 2005).

The first studies on plant PTs were performed with the *Arabidopsis thaliana* PTs, AtPT1 and AtPT2, which were expressed in the yeast deletion mutant PHO84 devoid of a high affinity Pi: $H^+$symporter (Muchhal *et al.*, 1996). Many PTs have since been described in other plants (Smith *et al.*, 1997; Liu *et al.*, 1998; Chiou *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Versaw and Harrison, 2002; Javot *et al.*, 2007; Ai *et al.*, 2009; Loth-Pereda *et al.*, 2011).

To attend to the plant's needs to distribute nutrients within the whole plant, transport systems are necessary to allow nutrient movements inside cells and to more distant parts and organs. Inside the cells, P needs to be transported between the cytoplasm and plastids or mitochondria, where it is used in photoxidative and oxidative phosphorylation (Versaw and Harrison, 2002). There is a constant flow of P in and out of the vacuole which functions as a storage and allows to uphold a certain cytoplasmic threshold of P (Bieleski, 1973; Versaw and Harrison, 2002). Under P-sufficient conditions, up to 95% of the plant total P supply can be located in the vacuoles (Bieleski and Ferguson, 1983; Rausch and Bucher, 2002).

To distribute P from the root hairs to shoots, P is loaded into the xylem. Since the P concentration can reach up to 7mM in the xylem, an active transport system is needed (Schachtman *et al.*, 1998). Under P-deficient conditions, P can be remobilized for example from senescing leaves to be translocated from shoots to the roots via the phloem (Versaw and Harrison, 2002; Loth-Pereda *et*

*al.*, 2011); it is worth mentioning that P in xylem is transported solely as Pi whereas big amounts of organically bound P are transported in the phloem sap (Schachtman *et al.*, 1998).

### 1.3.3 Phosphate transport across membranes

To overcome differences in P concentration between membrane potential and soil solution, nutrients are translocated by an active transport mechanism. Transporters can be characterized by their affinity ($K_m$) and maximum transport rate ($V_{max}$) to their substrate. Kinetic studies in the yeast *Saccharomyces cerevisiae* uncovered two high-affinity transport systems, PHO84 and PHO89, which enable a proton coupled P uptake across the cell membrane: PHO84, is a Pi: $H^+$ symporter and PHO89 is a $Na^+$ dependent P transporter (Bun-Ya *et al.*, 1991; Martinez and Persson, 1998). As mentioned previously, the first plant PT was identified in *A. thaliana* due to partial sequence similarity to PHO84 (Muchhal *et al.*, 1996). Then, many proton-coupled symporters were identified in the following years e.g. in *Solanum tuberosum* (StPT1 and StPT2) (Leggewie et al., 1997), *M. truncatula* (MtPT1 and MtPT2) (Liu *et al.*, 1998) and tomato (Daram *et al.*, 1998; Liu *et al.*, 1998). *A. thaliana* alone has nine PTs of which four are expressed in the root epidermis (Javot *et al.*, 2007). The kinetics found for plant PTs were similar to those of yeast and were assumed to be the result of two different uptake systems: a high-affinity uptake system (HATS) that can be induced, saturated and is active in the µM range and a low-affinity uptake system (LATS) that is permanently expressed and active in the mM range and (Schmidt *et al.*, 1992; Chiou *et al.*, 2001).
As mentioned previously, the first PTs in plants were found in *A. thaliana.* Due to partial sequence similarity to PHO84, the two first fungal PTs were found in *Neurospora crassa* (Versaw, 1995) and *Glomus versiforme* (Harrison and van Buuren, 1995; Smith *et al.*, 1997).

**Phosphate Transporter categories**

Plants PTs can be categorized into the three subfamilies Pht1, Pht2 and Pht3. Pht1 transporters belong to the Pi:$H^+$symporter (PHS) family - a sub family of the major facilitator superfamily of membrane proteins (Pao *et al.*, 1998). Pht1 transporters share high sequence similarity (up to 85% at the amino acid level) and are located at the plasma membrane. Computational prediction revealed a conserved secondary structure consisting of 12 transmembrane (TM) helices with two subdomains of six TM segments, which are linked via a large hydrophilic loop between TM6 and TM7 protruding into the cytoplasm together with the N and C termini. The two subdomains surround a hydrophilic cavity containing the substrate-binding site (Daram *et al.*, 1999; Harrison *et al.*, 2002; Rausch and Bucher, 2002; Karandashov and Bucher, 2005). Most of the currently known plant PTs belongs to the PHS, which are located in the plasma membrane and are either

exclusively or predominantly expressed in roots. Under P-deficient conditions, most of the Pht1 transporters are induced (Bucher, 2007; Javot *et al.*, 2007; Loth-Pereda *et al.*, 2011).

Apart from the Pht1 family, two other families, Pht2 and Pht3, have been identified. Members of the Pht2 family are located in the plastids and their structure is similar, but not equal, to the one of the Pht1 family members. They form a large hydrophilic loop between the eighth and ninth TM domains and a long hydrophilic N terminus including the plastid signal sequence (Poirier and Bucher, 2002; Rausch and Bucher, 2002; Javot *et al.*, 2007). In chloroplasts, P can be transported via four types of P translocators: a triose phosphate:P translocator, a PEP:phosphate translocator, a Glc-6P:P translocator and a Xylolose-5-phosphate:P translocator. The predominantly used transporter is the triose-phosphate/phosphate translocator, which exchanges every translocated P with one molecule of triose-phosphate or glycerate-3-phosphate in the opposite direction, making this way of transport about 1000 times slower than the other counter-exchange mechanisms. Under P-limited conditions, P concentration in the cytosol is low leading to a P depletion in the stroma, to an accumulation of chloroplastic metabolites and to a stop in starch production (Rausch and Bucher, 2002; Javot *et al.*, 2007).

As the main energy-transduction site in plants, mitochondria carry out important steps in the photorespiratory pathway. For this task, the uptake of P in the oxidative phosphorylation of ADP to ATP is essential. Mitochondrial PTs belong to the Pht3 family, which are located in the inner membrane and catalyze most of the P influx, most likely as homodimers. Pht3 transporters are also known as mitochondrial phosphate carrier, which exhibit a similar structure as the members of the mitochondrial carriers with six TM domains split into pairs of two. Based on mammalian and yeast homologues (Ferreira *et al.*, 1989; Wohlrab and Briggs, 1994), Pht3 transporters were predicted to function as $Pi{:}H^+$ symporter or as $Pi{:}OH^-$ antiporter able to catalyze P-P exchange (Takabatake *et al.*, 1999; Javot *et al.*, 2007).

### 1.3.4 Symbiotic phosphorus exchange

Depending on the plant-fungal association, the plant receives either a small percentage or all of its Pi demand via its fungal partner (Paszkowski, 2006; Javot *et al.*, 2007). It has been shown that the contribution of AM fungi to total P uptake in plants correlates with soil P availability. Decreased P availability resulted in increased root colonization as well as an increased P supply of the plant via the AM fungus. But, even though the AM fungi dominated total P uptake, the plants did not exceed total P uptake when mycorrhized nor did they necessarily benefited from increased growth

rates. The effect of the AM fungi remained rather hidden, the P taken up directly by plant was replaced by the P taken up from the AM fungus (Smith *et al.*, 2011).

In the mycorrhizal pathway, Pi is taken up via fungal PTs at the hyphal soil interface and translocated into the fungal cytoplasm (Figure 1.3). If the fungal need of Pi is satisfied, excess Pi is transported to the vacuole and incorporated into poly-P (Ezawa *et al.*, 2002). So far, only three PTs have been described in AM fungi (GvPT, GiPT and GmosPT) (Harrison and van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001; Benedetto *et al.*, 2005) and two in ectomycorrhizal fungi (HcPT1 and HcPT2) (van Aarle *et al.*, 2007; Tatry *et al.*, 2009). All currently described PTs in fungi are high affinity Pi:$H^+$ symporters of the major facilitator superfamily similar to plant PTs (Javot *et al.*, 2007; Tatry *et al.*, 2009). Harrison and Buuren (1995) were the first to demonstrate that GvPT is a high affinity Pi:$H^+$ transporter following Michaelis Menten kinetics with an apparent $K_m$ of 18 µM. Further analyses showed that this transporter, together with the other two transporters (GiPT and GmosPT) were mainly expressed in the ERM. The authors proposed that these transporters might have a major role in the uptake of external P.

When P accumulates in the extraradical hyphae, it is incorporated into poly-P, a linear polymer of three to thousands of Pi connected by high-energy phosphate hydrate bonds. The poly-P is stored in the vacuole to maintain cytoplasmic P concentration low (Javot *et al.*, 2007). The P is then transported from the ERM to the IRM in form of poly-P granules inside vesicles. In the IRM, it is hydrolyzed to enable the translocation of Pi into the PAS (Figure1.3). The exact mechanism of the poly-P breakdown is yet unknown (Javot *et al.*, 2007).

Once in the PAS, nutrients can be taken up by plant uptake transporters (symbiotic nutrient uptake). For P uptake, there are specialized transporters only expressed during root colonization and localized in the PAM. The transcriptional induction of PTs were described for several plant species: *S. tuberosum* (StPT3) (Rausch *et al.*, 2001), *M. truncatula* (MtPT4) (Harrison *et al.*, 2002), *Oryza sativa* (OsPT11, LePT4/StPT4) and *Lycopersicum esculentum* (LePT5/StPT5) (Nagy *et al.*, 2005), *Triticum aestivum* (TaPT1) and *Zea mays* (ZmPT6) (Glassop *et al.*, 2005), and *Populus trichocarpa* (PtPT10) (Loth-Pereda *et al.*, 2011). The first AM-inducible PT, StPT3, was described in *S. tuberosum* by Rausch *et al.* (2001) and encoded a high-affinity transporter ($K_m$=64µM). With a StPT3 promoter-GUS construct, it could be shown that this transporter was specifically expressed in arbuscule-containing cells.

Through immunolocalization, Harrison *et al.* (2002) demonstrated the position of MtPT4 to be around the fine branches of the arbuscules at the PAM of major arbuscules, but not at the arbuscular trunk. Activity depends on the pH of the arbuscular apoplast, which is about pH 4.2.

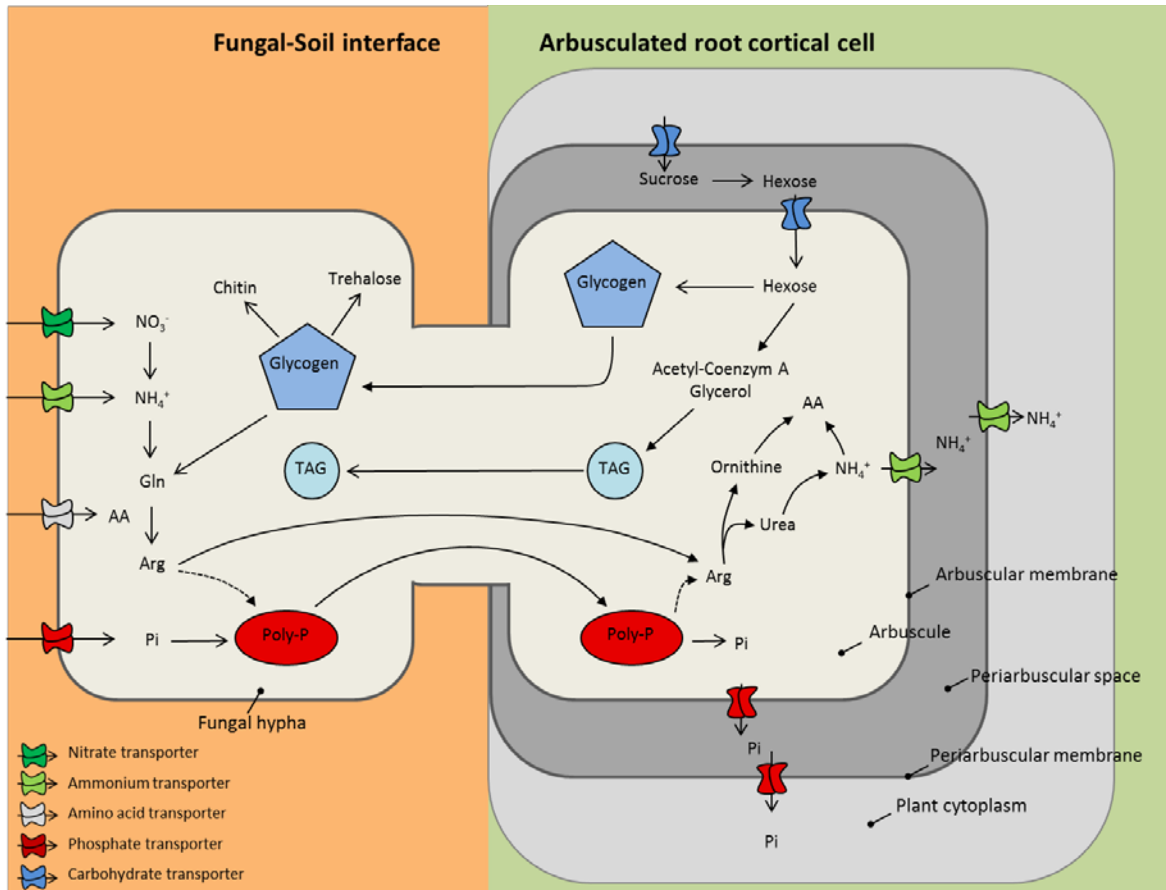


**Figure 1.3 Symbiotic nutrient exchanges in the arbuscular mycorrhizal (AM) symbiosis.** The AM fungus extracts nutrients from the soil with the help of specialized nutrient transporters. Nitrogen (N) is preferentially taken up as inorganic nitrate and ammonium by the extraradical hyphae, assimilated into glutamine and further metabolized to arginine. Arginine is the main transport form of N in the fungal hyphae and is used to transport N to the host plant. In the arbuscule, ammonium is released and transferred to the periarbuscular spaces (PAS). All nutrients transferred by the AM fungus are released to the PAS where they are available to the plant. Plant transporters located in the periarbuscular membrane (PAM), which takes up the nutrients from the PAS. The inorganic phosphorus (Pi) taken up by the fungus is incorporated into negatively charged poly-P; it is assumed that arginine binds to the poly-P for transport. In the arbuscules Pi is released and transported to the PAS. From the host plant the AM fungus receives carbohydrates, which are incorporated triacylglycerides (TAG) and glycogen. These metabolites serve for storage or can be further metabolized to synthesize chitin or other metabolites.

Measurements of the transport activity of MtPT4 showed that it was highest at pH 4.0. This optimal pH is consistent with the proton-cotransport mechanisms shared by the PHS-family of Pi transporters. Contrary to expectations, this transporter has with a Km value of 493/668 µM a low affinity for Pi (Harrison *et al.*, 2002; Pumplin and Harrison, 2009). If the plant is MtPT4 deficient, the arbuscules collapse earlier and poly-P accumulates in the arbuscules (Javot *et al.*, 2007). All AM-inducible transporters identified so far belong to the Pht1 subfamily which are $Pi{:}H^+$ symporters (Javot *et al.*, 2007). As mentioned previously, the indirect driving force for $Pi{:}H^+$ symporters are $H^+$-ATPases, which build up a proton gradient across the membrane and facilitates

Pi uptake by proton-coupled symport. In *M. truncatula*, it could be shown that a specifically induced $H^+$-ATPase, MtHA1, is crucial for MtPT4 activity and therefore for a functional symbiosis. MtHA1 is located at the PAM around the fine branches of the arbuscules. Inactivation of MtHA1 by knockout or knockdown resulted in small truncated arbuscules and in an increased apoplastic pH of cells harbouring the malformed arbuscules (Krajinski *et al.*, 2002; Krajinski *et al.*, 2014).

## 1.4 **Nitrogen**

Nitrogen (N) is one of the most important nutrients for life, as it is a constituent of amino acids and nucleic acids. But, in soils N is not homogeneously distributed and is a limiting nutrient in many terrestrial ecosystems (Vitousek and Howarth, 1991; Courty *et al.*, 2015). In soils, N is available to plants as inorganic N in from of nitrate ($NO_3^-$) and ammonium ($NH_4^+$). But often, N is bound in soil organic matter, where it is not directly accessible to plants. By depolymerization of the organic matter, monomers and amino acids are released and further metabolized by microbes and fungi (Jackson *et al.*, 2008). Heterotrophic microorganisms mineralize the organic monomers and release $NH_4^+$. $NH_4^+$ is an energy-rich source for ammonia-oxidizing microbes, which convert the $NH_4^+$ to nitrite and then to $NO_3^-$ (nitrification). Under anaerobic conditions, denitrifying bacteria use $NO_3^-$ as an electron acceptor. Bacteria reduce nitrate to nitric oxide (NO) and nitrous oxide ($N_2O$) and finally release elemental nitrogen ($N_2$) (Gödde and Conrad, 2000). Plants take up N preferentially as $NH_4^+$ and $NO_3^-$. The uptake of N in form of amino acids or other monomers is negligible in temperate zones, but plays only a role in extreme cold regions where mineralization processes are limited or in extremely N-poor ecosystems (Schimel and Chapin, 1996; Hodge and Storer, 2015). In plants and AM fungi, it was shown that the preferred N source is $NH_4^+$ as it can be directly assimilated into the GS/GOGAT pathway and incorporated into glutamine (Hodge and Storer, 2015). Subsequently, N is incorporated into other amides and amino acids such as alanine, asparagine and arginine.

### 1.4.1 Ammonium transporters

In plants as well as in fungi, ammonium is translocated via specialized ammonium transporters (AMTs). AMTs are present in all three domains of life, Eukaryota, Eubacteria and Archaea. The first ammonium transporters were identified in *S. cerevisiae* (ScMep1; Marini *et al.*, 1994) and in *A. thaliana* (AtAMT1;1; Ninnemann *et al.*, 1994). Direct uptake measurements of methylamine in yeast mutants allowed the characterization of these transporters as two high affinity transporters. As these transporters take up ammonium and the radioactive labeled methylamine, they are also called methylammine/ ammonium permease (Mep/AMT). Since their characterization, many

additional AMTs have been described in *S. cerevisiae* (ScMep2 and ScMep3; Marini *et al.*, 1997), *A. thaliana* (AtAMT1;2, AtAMT1;3, AtAMT2; Gazzarrini *et al.*, 1999; Sohlenkamp *et al.*, 2000), *Lotus japonicus* (LjAMT1;1, LjAMT1;2 and LjAMT1;3; Salvemini *et al.*, 2001; D'Apuzzo *et al.*, 2004), *O. sativa* (OsAMT1;1, OsAMT1;2 and OsAMT1;3; Sonoda *et al.*, 2003), *Geosiphon pyriformis* (GpAMT1, GpAMT2, GpAMT3; Ellerbeck *et al.*, 2013), *Rhizophagus irregularis* (formerly *Glomus intraradices,* GiAMT1 and GiAMT2; López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011), *Hebeloma cylindrosporum* (HcAMT1, HcAMT2, HcAMT3; Javelle *et al.*, 2001; Javelle *et al.*, 2003) and other organisms including *Azospirillum brasilense and Synechocystis* sp. PCC 6803 (AbAMTB and SyAMT1, SyAMT2, SyAMT3; Montesinos *et al.*, 1998; Van Dommelen *et al.*, 1998).

AMTs belong to the Mep/AMT family and are membrane-bound pore-forming units (Marini *et al.*, 1997). The majority of the Mep/AMT transporter family can form pores consisting of 11 transmembrane helices with an extracellular N-terminus and an intracellular C-terminus. *In-silico* analyses revealed that some bacterial AMTs are able to form a $12^{th}$ transmembrane helix, which results in a transporter with intracellular N- and C-termini. This additional transmembrane helix does not seem to have a functional benefit for the transporter activity, but might be involved in protein folding processes during insertion into the plasma membrane. The amino-acid length of AMTs ranges from 400 to 450 amino acids, while some members have a C-terminal extension which increases the transporter length up to 600 amino acids (Thomas *et al.*, 2000).

Physiological studies of AMTs revealed that they follow biphasic kinetics. The functional activity of the transporters depends on the substrate concentration and the pH of the environment. High-affinity transport systems (HATS) exhibit strong activity in the micromolar range and follow saturation kinetics (Ullrich *et al.*, 1984; Wang *et al.*, 1994) while low-affinity transport systems (LATS) act in the millimolar range (Fried *et al.*, 1965; Vale *et al.*, 1988; Wang *et al.*, 1993; Shelden *et al.*, 2001). In general, transporters are either HATS or LATS but there are exceptions, which exhibit both affinities, as it is the case for AtAMT1;2 in *A. thaliana* (Shelden *et al.*, 2001), the potassium transporter AtKUP1 (Fu and Luan, 1998) and the nitrate transporter CHL-1 (Liu *et al.*, 1999). The structural basis of these transporters is unknown, but it was proposed that they might be regulated by the substrate by allosterical or post-translational modification (Shelden *et al.*, 2001).

### 1.4.2 Ammonium uptake and exchange in the arbuscular mycorrhizal symbiosis

It was assumed that AM fungi play only a minor role in nitrogen acquisition because the inorganic forms of N can be transported via mass-flow since they are highly mobile. It was assumed that

fungal hyphae and plant roots would take up N with the same efficiency (Marschner and Dell, 1994; Hodge *et al.*, 2010; Smith and Smith, 2011). However, several studies directly testing the contribution of AM fungi to plant N supply revealed that N uptake of the host plant via mycorrhizal fungi partners can reach 42% (Frey and Schüepp, 1993; Mäder *et al.*, 2000; Govindarajulu *et al.*, 2005). Depending on N resources in the soil and the symbiotic partner, AM fungi transfer smaller or larger fractions of valuable ions, not always compensating the amount of received C (Walder *et al.*, 2012; Näsholm *et al.*, 2013).

In soils, inorganic N sources ($NH_4^+$ and $NO_3^-$) are the most common N sources, but ERM of AM fungi can take up N also from organic sources including small peptides and amino acids (Bago *et al.*, 1996; Hawkins *et al.*, 2000; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005). It is assumed that in the membrane of the ERM are permeases to facilitate amino acid uptake from the environment (Cappellazzo *et al.*, 2008) and that ERM are able to take up N from complex soil organic matter (Leigh *et al.*, 2009; Hodge *et al.*, 2010).

As stated above, $NH_4^+$ is the preferred N source. However, in well aerated soils $NO_3^-$ is more abundant and in contrast to $NH_4^+$, it needs to be reduced to nitrite and $NH_4^+$ first before it can be assimilated to the GS/GOGAT pathway (Johansen *et al.*, 1996; Marzluf, 1996; Bago *et al.*, 2001; Breuninger *et al.*, 2004; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005).

In AM fungi, most of the absorbed N is incorporated into arginine, which accounts for more than 90% of all available amino acids in the ERM (Govindarajulu *et al.*, 2005). Arginine is then translocated to the IRM at the arbuscular side where it is cleaved by arginases to urea and ornithine. The urea is then further cleaved by urease to release $NH_4^+$ in the urea cycle (Figure 1.3). The free $NH_4^+$ ions are then released into the PAS where they are ready to be taken up by plant ammonium transporters (AMTs) (Bago *et al.*, 2001; Govindarajulu *et al.*, 2005; Cruz *et al.*, 2007; Tian *et al.*, 2010). For the transport of arginine from the ERM to the IRM, it is thought that arginine binds to the negatively-charged polyphosphate within the vacuole and is then translocated together with the polyphosphate (Martin, 1985; Govindarajulu *et al.*, 2005).

So far, five AMTs were identified in AM fungi. The first two transporters have been described in *Rhizophagus irregularis* (GiAMT1, GiAMT2). They encode two high affinity transporters that were expressed in the IRM and the ERM, meaning that both transporters are possibly involved in ammonium uptake in the ERM and in ammonium export into the PAS (López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011; Pérez-Tienda *et al.*, 2012). The other three AMTs were found in *Geosiphon pyriformis* (GpAMT1, GpAMT2, GpAMT3) which forms symbiosis with cyanobacteria (Ellerbeck *et al.*, 2013).

On the plant site, some AMTs were found to be specifically induced upon root colonization. Their activity could be assigned to mycorrhized cortical cells. Such transporters were identified in *Lotus japonicus* (LjAMT2;2) (Guether *et al.*, 2009), *M. truncatula* (predicted AMT: IMGAG|1723.m00046) (Gomez *et al.*, 2009), (MtAMT2;3) (Breuillin-Sessoms *et al.*, 2015), *Glycine max* (GmAMT1;4, GmAMT3;1, GmAMT4;1, and GmAMT4;4) (Kobae *et al.*, 2010), *Populus trichocarpa* (PtrAMT1;2) (Couturier *et al.*, 2007) and S*orghum bicolor* (SbAMT3;1, SbAMT4) (Koegel *et al.*, 2013).

## 1.5 **Carbohydrate transfer in the arbuscular mycorrhizal symbiosis**

It is known that C is transferred from plants to AM fungi. However, the mechanisms underlying C transfer remain largely unknown (Doidy *et al.*, 2012). In AM colonized roots, apoplastic acid invertases and sucrose synthases are induced, which suggested that in colonized root sugars are provided from the plant in form sucrose that is cleaved into the monosaccharides glucose and fructose (Helber *et al.*, 2011; Casieri *et al.*, 2013). In *R. irregularis*, it has been shown that hexoses can be taken up by the IRM of AM (Helber *et al.*, 2011). In the IRM, hexoses are converted to glycogen and trehalose. A similar uptake of hexoses has not been observed in the ERM (Shachar-Hill *et al.*, 1995; Solaiman and Saito, 1997). After addition of $^{13}$C-labeleded substrate, substantial amounts of labeled triacylglycerides (TAGs) were found, which suggested that most of the hexoses taken up were used for lipid synthesis and C storage (Pfeffer *et al.*, 1999). Later on, it was shown that glycogen and TAGs are then transported to the ERM (Figure 1.3) (Olsson *et al.*, 1995; Bago *et al.*, 2003).

Recently, sugar transporters, which may play an important role in the C transfer, were identified. The first identified sugar transporter in an AM fungus was the $H^+$/glucose transporter GpMST1 of *G. pyriformis,* which has an affinity for glucose, mannose, galactose and fructose (Schüßler *et al.*, 2006). Helber *et al.* (2011) identified the three additional monosaccharide transporters (MSTs), GiMST2, GiMST3 and GiMST4 in *G. intraradices.* Of these, GiMST2 is a high-affinity $H^+$/glucose and xylose transporter expressed in arbuscules and the IRM. Host-induced silencing of GiMST2 resulted in malformed arbuscules and the down-regulation of the AM-inducible PT MtPT4 of *M. truncatula* (Helber *et al.*, 2011). These findings demonstrate that GiMST2 is a crucial component for C exchange and establishment of the symbiosis.

As photoautotrophic organisms, plants are able to fix C from carbon dioxide and incorporate it into sugars during photosynthesis in the mesophyll cells. From there, the energy-rich molecules are transported to sink tissue and organs for activities such as growth processes or maintenance of

cellular metabolism, or to storage organelles. Furthermore, sugars are not only used as energy supply but also play a crucial role in plant signaling processes (Rolland *et al.*, 2006).

In plants, sugar transporters (SUTs) belong to the major facilitator superfamily that show a 12 transmembrane topology and are predicted to be $H^+$/sugar symporters (Doidy *et al.*, 2012). By phylogenetic analyses, SUTs could be assigned into five distinct clades. SUT1 and SUT3 members are functional orthologues, which facilitate the distribution of sucrose from source to sink by loading and unloading the phloem. Thereby are SUT1 transporters dicot-specific, and the SUT3 transporters are monocot-specific. The SUT2 are sugar sensors or transporters. Members of the SUT4 clade were found to be localized intracellularly whereas in the transporters of the SUT5 clade, only one member has been characterized so far (Doidy *et al.*, 2012).

In recent years, also plant MSTs have been identified. The MST family is large; for example, the *M. truncatula* and *A. thaliana* genomes comprise more than 50 gene sets (Doidy *et al.*, 2012). Some members of the MSTs were characterized, but many more need to be described. Further, a new class of sugar transporters, named SWEETS has been identified (Chen *et al.*, 2010). The SWEET family comprises fewer members than the MST family. It was shown that SWEETs are bidirectional transporters, which can transport glucose and are crucial for pollen viability (Chen *et al.*, 2010). A possible role of the SWEETs in symbiotic interactions was indicated in *M. truncatula,* where the SWEET MtN3 was highly induced upon rhizobia infection (Gamas *et al.*, 1996; Doidy *et al.*, 2012). Chen *et al.* (2010) suggested that SWEET transporters might also be targets of pathogens as a bacterial effector was found to bind directly to a SWEET promoter. By altering sugar efflux, the pathogens might gain a nutritional benefit and having an impact on plant immunity (Chen *et al.*, 2010).

## 1.6 **Micro RNAs in mycorrhizal symbiosis and phosphorus stress**

### **1.6.1 microRNAs**

MicroRNAs (miRNA) are endogenous, non-coding 21 nucleotide long RNA molecules, which are negative regulators of gene expression in animals and plants. miRNAs are transcribed from miRNA genes (MIR genes) by DNA-dependent RNA polymerase II (Pol II). The resulting 5’capped and 3’ polyadenylated primary miRNA precursors (pri-miRNA) can be up to 3kb in size, contain several introns and might be stabilized by DAWDLE (DDL), an RNA-binding protein (Figure 1.4). These extremely long pri-miRNAs are further processed in nuclear dicing bodies by DICER-LIKE1 (DCL1), a ribonuclease III, involving the interaction with DRB HYPONASTIC LEAVES 1 (HYL1), C2H2 Zn-finger protein SERRATE (SE) and of cap-binding proteins (CBP) CBP20 and CBP80 to result in

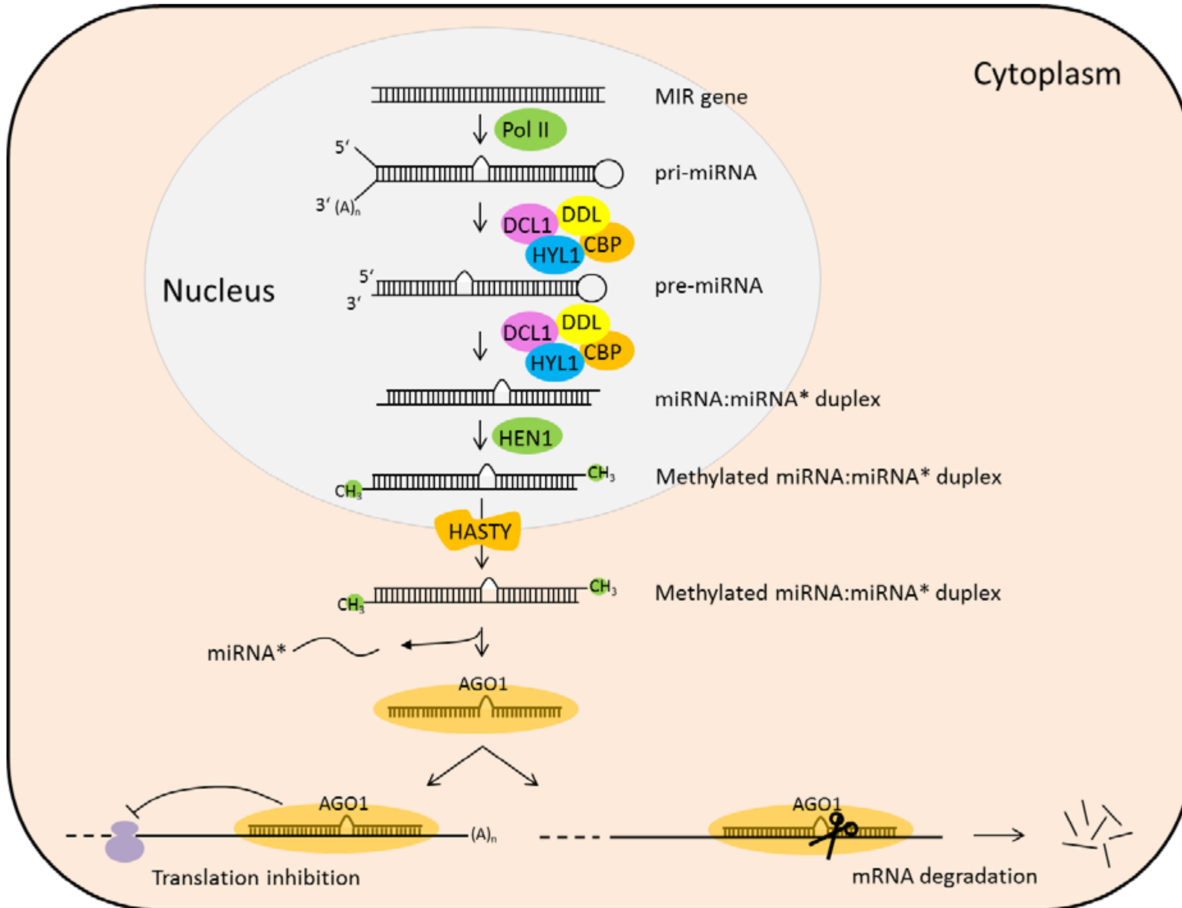


**Figure 1.4 Schematic representation of miRNA biogenesis pathway in plants.** MIR genes are transcribed by DNA-dependent RNA polymerase II (Pol II). Pri-miRNAs fold back and form a hairpin structure, which is spliced and processed further by the DCL1, HYL, DDL, CBP complex. The resulting miRNA:miRNA* duplex is methylated by HEN1. Through HASTY-dependent or HASTY-independent transport the miRNA:miRNA* duplex is transported from the nucleus to the cytoplasm where usually the miRNA strand incorporates into the AGO1 complex. Binding to mRNA leads to translation inhibition or degradation of the target mRNA. Inspired by Yang and Li (2012).

miRNA/miRNA* duplexes. Following the 2'-O-methylation by HUA ENHANCER1 (HEN1) at the 3'end, the duplexes are exported from the nucleus through a HASTY-dependent or –independent transport system (Park *et al.*, 2005; Vazquez *et al.*, 2010). In the cytosol, the mature miRNA strand and less commonly the RNA* strand is incorporated in AGO protein complexes (Figure 1.4). Of these, AGO1 is the most common one guiding the cleavage of mRNA as well as translational inhibition of target-transcripts in the middle of the miRNA/target duplex. (Chiou *et al.*, 2006; German *et al.*, 2008; Vazquez *et al.*, 2010; Branscheid *et al.*, 2011; Devers *et al.*, 2011; Lauressergues *et al.*, 2012).

There are also feedback-regulatory steps in miRNA synthesis. Vaucheret *et al.* (2004) and Xie *et al.* (2003) demonstrated that DCL1 and AGO1 activity could be inhibited by miR162 and miR168.

Moreover, Ramachandran and Chen (2008) showed the existence of SMALL RNA-DEGRADING NUCLEASEs (SDN) that degrade mature miRNA species.

### 1.6.2 Role of miRNAs in mycorrhization

To increase mycorrhizal symbiosis, plants exudate strigolactones from their roots into the soil. These strigolactones are known to stimulate spore germination and hyphal branching (Akiyama *et al.*, 2005; Besserer *et al.*, 2006). One important player in this process is NSP2, a member of the GRAS transcription factor family. NSP2 is predominantly involved in the synthesis of strigolactones in *M. truncatula* and *O. sativa.* The absence of strigolactones in mtnsp2 mutants has a negative effect on AM root colonization(Liu *et al.*, 2011). NSP2 also plays a role in Nod factor perception in rhizobial symbiosis formation and is therefore an important part of the common symbiosis signaling pathway (Kaló *et al.*, 2005; Heckmann *et al.*, 2006; Murakami *et al.*, 2007; Kouchi *et al.*, 2010). During root colonization, the AM fungi secrete lipochito-oligosaccharides (Myc-LCO) and other mycorrhizal components, which stimulate lateral root growth and increase colonization (Lauressergues *et al.*, 2012).

Bazin *et al.* (2013) discovered miR396 species that were mainly expressed in the transition zone of root tips (miR396b/a). miR396 is known to suppress post-transcriptional regulation of several members of the growth regulating family, thus controlling growth and development of leaves and stems (Bazin *et al.*, 2013). miR396 species affect the developmental processes and their overexpression lead to a decreased root colonization level, a reduced expression of six growth-regulating factor genes (MtGFR) and two basic helix-loop-helix 79 (bHLH79)-like target genes.

Also, miR393 was found to have an effect to bacterial resistance in plants (Navarro *et al.*, 2006; Vidal *et al.*, 2010; Si-Ammour *et al.*, 2011; Windels and Vazquez, 2011). Recently Etemadi *et al.* (2014) detected that the expression level of miR393 negatively correlates with AM root colonization. Overexpression of miR393 leads to a strong reduction of root colonization. The discovery of further miRNA participating in mycorrhizal interaction is expected and will provide interesting insights in the functioning of symbiotic communication between plants and fungi.

### 1.6.3 Phosphorus starvation-induced signaling in plants

**Induction of phosphate transporters and phosphate starvation induced signaling cascade**

To increase root P uptake, Pht1 transporters are activated by the MYB transcription factor PHOSPHATE STARVATION RESPONSE REGULATOR1 (PHR1). PHR1 targets a P1BS-like (PHR1-binding sequence) element (GnATATnC), which is a *cis*-regulatory element situated in the promoter region

of PHT1 genes (Rubio *et al.*, 2001). PHR1 itself is regulated by a SMALL UBIQUITIN MODIFIER (SUMO) E2 ligase (AtSIZ1) which is also involved in several other Pi starvation responses (Miura *et al.*, 2005). Sumoylation is a post-translational modification that requires a covalent attachment of SUMO to a protein. In *A. thaliana*, SUMO AtSIZ1 localizes in the nucleus and an AtSIZ1 deficiency leads to strong Pi starvation responses (Bucher, 2007; Liu *et al.*, 2014).
In the endoplasmic reticulum (ER), PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1 (PHF1) facilitates the release of several Pht1 transporters. PHF1 is required for correct targeting of Pht1 transporters to the plasma membrane (Bayle *et al.*, 2011). Upon PHF1 phosphorylation, the Pht1 transporters are retained in the ER (Liu *et al.*, 2014).

Upon P starvation, PHR1 also induces miR399 expression in shoots (Bari *et al.*, 2006; Chiou *et al.*, 2006). It could be shown that miR399 expression anti correlates negatively with P nutrition by interacting with a negative regulator of Pht1 transporters. In P-deplete conditions, miR399 is increased whereas upon P-repletion the miR399 expression level is strongly reduced. Grafting experiments in *Arabidopsis* and tobacco demonstrated long-distance movements of miR399 from shoots to the roots (Lin *et al.*, 2008). The downward movement suggests that miR399 is transported via the phloem sap or is expressed in the companion cells, from where it is transported to the root vascular cylinder to target PHO2 transcripts for degradation (Fujii *et al.*, 2005). Simultaneously, PHR1 induces two non-coding RNAs are induced, AT4 and INDUCED PHOSPHATE STARATION1 (IPS1) which antagonize the effects of miR399 (Aung *et al.*, 2006; Bari *et al.*, 2006). AT4 and IPS1 contain a sequence motif that is complementary to miR399 (target mimicry), but the pairing is interrupted by a loop at the cleavage site which leads to scavenging of miR399 species (Franco-Zorrilla *et al.*, 2007).

**Negative regulator of phosphate transporters**

Depending on the P nutrition status of the plant, Pht1 transporters are post-translationally modified by PHO2 and NLA (NITROGEN LIMITATION ADAPTATION). NLA is a ubiquitin E3 conjugating enzyme that targets Pht1 for ubiquitination, leading to clathrin-dependent endocytosis and vacuolar degradation (Lin *et al.*, 2013). Under P-limited conditions, NLA is targeted by miR827 at the SPX-domain (a hydrophilic domain at the N-termini of Pi responsive proteins, Secco *et al.*, 2012) and therefore inhibits the degradation of the plasma membrane incorporated Pht1 PTs (Kant *et al.*, 2011; Hackenberg *et al.*, 2013; Lin *et al.*, 2013; Liu *et al.*, 2014).

PHO2 (formerly UBC24) encodes an E2 ubiquitin conjugating enzyme and was found to be a critical component for plant Pi nutrition (Aung *et al.*, 2006). PHO2 has multiple target sites for miR399 in the 5' untranslated region (Fujii *et al.*, 2005). PHO2 is located in the endomembrane system where it ubiquitinates Pht1 and PHO1 proteins, which leads to their degradation (Liu *et al.*, 2012; Huang *et al.*, 2013). In P sufficient conditions, miR399 overexpression mutants degrade PHO2 transcripts, which leads to an increased P uptake and transport to the leaves (Aung *et al.*, 2006; Lin *et al.*, 2008; Pant *et al.*, 2008). In *A. thaliana*, it was shown that PHO2 regulates the two PTs AtPht1;8 and AtPht1;9 (Bari *et al.*, 2006). In roots of wild type plants, AtPht1;8 and AtPht1;9 were induced under low-P condition and repressed in high-P condition. In *pho2* mutants the expression of these transporters remained high in Pi- replete condition, which leads to an increased P uptake and translocation activity in the compared to the wild type plants, and an over-accumulation of Pi in shoots (Delhaize and Randall, 1995; Dong *et al.*, 1998).

**PHO1: a protein involved in xylem loading**

PHO1 is involved in the loading of P out of the stelar cells into the xylem. It is mainly expressed in root stelar cells and in the lower part of the hypocotyls. Even though PHO1 shares no homology with other Pi transporters it contains a SPX-domain (Poirier *et al.*, 1991; Hamburger *et al.*, 2002; Secco *et al.*, 2012). It was suggested that PHO1 defines a new class of proteins or represents a subunit of a protein complex (Hamburger *et al.*, 2002). Expression of PHO1 leaf mesophyll protoplasts leads to a rapid export of Pi which gave evidence that PHO1 is a P exporter (Arpat *et al.*, 2012). Further, it was demonstrated that there are two transcription factors directly involved in the regulation of PHO1. In high Pi conditions, WRKY6 and WRKY42 are bound to W-boxes in the promoter region of PHO1 inhibiting its transcription. But in Pi deficiency, both transcription inhibitors are released by a 26S proteasome-mediated proteolysis allowing the transcription of PHO1 which results in facilitated Pi loading into xylem and translocation of Pi from roots to the shoots (Chen *et al.*, 2009).

## 1.7 **Model organisms**

### ***1.7.1 Populus trichocarpa***

*Populus trichocarpa* (black cottonwood), an angiosperm tree, is native to temperate ecosystems with broad adaptive and genetic variability. The fast growing tree is widely used for wood and fiber production. In research it is model species to study secondary xylem development, dormancy and responses to environmental stress (Kelleher *et al.*, 2007). Its fast growth, vegetative

propagation, small genome size (485 megabases), simple transformability and a fully sequenced genome and its ability to form symbioses with ectomycorrhizal and endomycorrhizal fungi at the same time make *P. trichocarpa* an optimal model species for perennial plants and symbiotic interactions (Brunner *et al.*, 2004; Tuskan *et al.*, 2006).

### ***1.7.2 Sorghum bicolor***

*Sorghum bicolor* is a C4 plant and belongs to the family of *Poaceae.* The annual plant is a widely spread agricultural crop cultivated for food, feed, fiber and fuel. It has a high drought tolerance and is therefore cultivated in semi-arid and tropical regions. The major countries cultivating this agricultural crop are America, Africa and Asia. The United States is the top (http://faostat3.fao.org/ browse/Q/QC/E). Its genome is fully sequenced and annotated and is about 730 megabases (Paterson *et al.*, 2009). Its relative small genome size, quick growth and the ability to form a symbiosis with AM fungi make it a good model for AM fungi-plant interactions.

### ***1.7.3 Rhizophagus irregularis***

*Rhizophagus irregularis*, is the most intensively studies AM fungal species. Formerly, it was known as *Glomus intraradices* but phylogenetic analyses of the complete internal transcribed space region and partial analysis of the small and large subunit of the nuclear ribosomal DNA regions could assign *G. intraradices* to two clades. One is the *G. intraradices* FL208 and Mucl49419 and the other is *R. irregularis* which included DAOM197198 and BEG195 (Stockinger *et al.*, 2009). The genome of the strain DAOM197198 has been sequenced. *R. irregularis* efficiently takes up mineral nutrients such as Pi, N, and sulfur from the substrate. It has a relative broad host range and is known to colonize rapidly model legumes, i.e. *Lotus japonicus, M. truncatula, P. trichocarpa and S. bicolor* which made it a model species to dissect the molecular mechanisms of AM symbiosis (Tisserant *et al.*, 2013).

# 2 Aims of the thesis

The aim of my thesis is the investigation of the plant and AM nutrient transporters depending on nutrient availability and the symbiotic partner. In a context of climate change and resource shortening, the development of sustainable agriculture strategies is an important issue to resolve. In AM symbiosis, the plant as well as the AM fungus benefit from the plant-fungal interaction. By characterizing the efficiency of symbiotic nutrient exchange depending on nutrient availability in different plant-fungal combinations, we can contribute to increase our knowledge on the functioning of AM symbiosis in changing environmental conditions.

N and P are the major mineral nutrients restricting plant and fungal fitness. In AM symbiosis, the AM fungus provides these macronutrients and receives in return the essential carbohydrates from the plant. However, knowledge about the mechanisms of symbiotic nutrient from the fungus to the plant and particularly about C transport from the plant to the fungus is limited. Therefore, I concentrated on the assessment of the effects of P and N availability on transporter expression linked to P and N uptake as well on possible carbohydrate transporters in the tree plant *P. trichocarpa* and the cereal plant *S. bicolor* when interacting with the fast growing AM fungus *R. irregularis*.

N is a constituent of amino acids and nucleic acids and is therefore essential for life. Recently, the importance of AM fungi in plant N nutrition became more evident. In contrast to plants, AM fungi are able to take up nutrients also from organic sources. The increased substrate range for the AM fungi is a beneficial attribute from the sustainable agriculture can profit from.

Up to date, only five AMTs were identified in AM fungi. By *in silico* analysis of the genome of *R. irregularis*, I identified a novel AMT, characterized its physiological properties and assessed the possible localization of this transporter (Chapter 3).

To analyze the effects of root colonization and N availability on plant gene expression, poplar plants were grown under different N nutrition in mycorrhizal or non-mycorrhizal conditions. I analyzed the transcriptome of colonized and non-colonized poplar roots, as well as the transcriptome of the AM fungus inside the colonized poplar roots by Illumina sequencing in combination with qRT-PCR. As the transfer of N and P is a major role of the AM symbiosis, I also assessed the effects of N availability on plant and mycorrhizal AMTs and PTs. Moreover, to gain further insight into AM nutrient transfer, the expression of AM fungal nutrient transporters inside the host plant was analyzed (Chapter 4).

To separate the effects of P availability and of the AM fungus on plant nutrient transporter expression, I set-up a sophisticated tripartite compartment system. In this system, a common

mycorrhizal network between *R. irregularis* and the two plants *P. trichocarpa* and *S. bicolor* was established. The plant compartments were separated by meshes from each other, which allowed the fungus to grow into the neighboring compartments but not the plants. The third compartment remained accessible for the AM fungus only. The AM plants received fertilizer without P. High-P or low-P fertilizer was applied in the third compartment where only the AM fungus had access to. Like that, the plants received P only by the AM fungus. As control, non-mycorrhizal *P. trichocarpa* and *S. bicolor* plants received high-P or low-P fertilizer directly into their root compartment. This system enabled the expression analysis of AM nutrient transporters in the IRM and the ERM. By combining qRT-PCR and Illumina sequencing of the transcriptome I investigated the effects of P availability on the expression of plant and AM fungal nutrient transporters. Applied methods enabled identification of transporters that have not been investigated previously in the context of AM symbiosis (Chapter 5).

# 3 GintAMT3 – a low-affinity ammonium transporter of the arbuscular mycorrhizal *Rhizophagus irregularis*

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## 3.1 **Abstract**

Nutrient acquisition and transfer are essential steps in the arbuscular mycorrhizal (AM) symbiosis, which is formed by the majority of land plants. Mineral nutrients are taken up by AM fungi from the soil and transferred to the plant partner. Within the cortical plant root cells the fungal hyphae form tree-like structures (arbuscules) where the nutrients are released to the plant-fungal interface, *i.e.* to the periarbuscular space, before being taken up by the plant. In exchange, the AM fungi receive valuable carbohydrates from the plant host. Besides the well-studied uptake of phosphorus (P), the uptake and transfer of nitrogen (N) plays a crucial role in this mutualistic interaction. In the AM fungus *Rhizophagus irregularis* (formerly called *Glomus intraradices*), two ammonium transporters (AMT) were previously described, namely GintAMT1 and GintAMT2. Here, we report the identification and characterization of a newly identified *R. irregularis* AMT, GintAMT3. Phylogenetic analyses revealed high sequence similarity to previously identified AM fungal AMTs and a clear separation from other fungal AMTs. Topological analysis indicated GintAMT3 to be a membrane bound pore forming protein, and GFP tagging showed it to be highly expressed in the intraradical mycelium (IRM) of a fully established AM symbiosis. Expression of GintAMT3 in yeast successfully complemented the yeast AMT triple deletion mutant (*MAT*a *ura3 mep1*Δ *mep2*Δ::*LEU2 mep3*Δ::*KanMX2*). GintAMT3 is characterized as a low affinity transport system with an apparent $K_m$ of 1.8 mM and a $V_{max}$ of 240 $nmol^{-1}$ $min^{-1}$ $10^8$ $cells^{-1}$, which is regulated by substrate concentration and carbon supply.

## 3.2 **Introduction**

Nitrogen is an essential, often limiting, macronutrient for plants. Since the availability of nitrogen (N) in form of ammonium ($NH_4^+$) or nitrate ($NO_3^-$) in the environment is quite low, plants have evolved different strategies to overcome this problem. Under natural conditions 70-90% of land plant species are associated with nearly ubiquitous arbuscular mycorrhizal (AM) fungi, which can increase nutrient and water supply of their host. This goes along with improved plant fitness, growth and disease resistance. In exchange, the fungal partners receive up to 20% of the photosynthates from the plant (Pearson and Jakobsen, 1993; Graham, 2000; Smith and Read, 2008). Previously it has been assumed that arbuscular mycorrhizal fungi (AMF) play only a minor role in N nutrition of their host plant. However, several studies testing the contribution of AM fungi to plant N supply revealed that N uptake of the host plant via mycorrhizal uptake pathway can reach 42% (Mäder *et al.*, 2000).

Several studies showed that inorganic $NO_3^-$ and $NH_4^+$ (Bago *et al.*, 1996; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005) or small peptides and amino acids (organic form) (Hawkins *et al.*, 2000) can be absorbed from the soil by extraradical hyphae of AMF. There is also some weak evidence that AMF can absorb N from complex organic matter (Leigh *et al.*, 2009; Hodge *et al.*, 2010) and that they take up amino acids from the environment by the expression of amino acid permeases in the extraradical mycelium (Cappellazzo *et al.*, 2008). Although fungi and plants use many different resources to obtain N, it has been demonstrated that $NH_4^+$ often is the primary N source (Villegas *et al.*, 1996; Hawkins *et al.*, 2000; Toussaint *et al.*, 2004). Assimilation of $NH_4^+$ through the glutamine synthase / glutamate synthase (GS/GOGAT) pathway is energetically less costly compared to the reduction and assimilation of $NO_3^-$ (Johansen *et al.*, 1996; Marzluf, 1996; Bago *et al.*, 2001; Breuninger *et al.*, 2004; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005).

Once absorbed, most of the inorganic N taken up by the AMF is assimilated and incorporated into arginine, constituting more than 90% of total free amino acids available in the extraradical mycelium (ERM). The arginine is translocated to the intraradical mycelium (IRM) (Govindarajulu *et al.*, 2005; Cruz *et al.*, 2007), where it is perhaps bound to the negatively charged polyphosphate in the fungal vacuole, forming a link between nitrogen and phosphorus transport (Martin, 1985; Govindarajulu *et al.*, 2005). In the arbuscule, arginine is metabolized by arginase and urease in the urea cycle, and the free $NH_4^+$ is released into the periarbuscular space where it is taken up by the plant host (Bago *et al.*, 2001; Govindarajulu *et al.*, 2005; Tian *et al.*, 2010).

For a long time it was not clear whether specialized transporters function in the AM symbiotic N exchange. Since the discovery of the first ammonium transporters in *Saccharomyces cerevisiae* (Marini *et al.*, 1994) and *Arabidopsis thaliana* (Ninnemann *et al.*, 1994) several such transporters were characterized in plants (Gazzarrini *et al.*, 1999; Sohlenkamp *et al.*, 2000; Couturier *et al.*, 2007; Guether *et al.*, 2009), fungi (Javelle *et al.*, 1999; Javelle *et al.*, 2003; Javelle *et al.*, 2003; López-Pedrosa *et al.*, 2006; Lucic *et al.*, 2008; Pérez-Tienda *et al.*, 2011; Ellerbeck *et al.*, 2013) and other organisms (Van Dommelen *et al.*, 1998; Mayer *et al.*, 2006). The so-called high-affinity transporter systems (HATS) operate in the micromolar range, exhibit saturation kinetics, and the uptake of ammonia leads to depolarization of the transmembrane electrical potential (Ullrich *et al.*, 1984; Wang *et al.*, 1994). In contrast, low-affinity transporter systems (LATS) are highly active in the millimolar range (Fried *et al.*, 1965; Vale *et al.*, 1988; Wang *et al.*, 1993; Shelden *et al.*, 2001).

Physiological studies in plant roots and the AMF *Rhizophagus irregularis* have revealed that uptake systems for ammonium and nitrate follow biphasic kinetics with respect to external substrate

concentrations (Pérez-Tienda *et al.*, 2011). The first AMF ammonium transporter, characterized from *R. irregularis* (syn. *Glomus irregularis*, formerly named *Glomus intraradices*), GintAMT1, is a high affinity transporter (López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011). Using immunolocalization and expression analysis of microdissected cells, it was shown that GintAMT1 and a second ammonium transporter, GintAMT2 (Pérez-Tienda *et al.*, 2012), were both expressed in the extra- and intraradical mycelium, participating in the uptake of $NH_4^+$ from the soil solution and possibly in retrieval of $NH_4^+$ leaking out during fungal metabolism at the symbiotic interface. Since then, three related ammonium transporters (GpyrAMT1, GpyrAMT2, GpyrAMT3) were characterized from the glomeromycotan fungus *Geosiphon pyriformis*, which forms a symbiosis with the cyanobacterium *Nostoc* (Ellerbeck *et al.*, 2013).

On the plant side, the expression of several mycorrhiza inducible AMTs could be specifically assigned to arbuscule-colonized cortical cells. Such transporters were identified in *Lotus japonicus* (LjAMT2;2) (Guether *et al.*, 2009), *Medicago truncatula* (predicted AMT: IMGAG|1723.m00046) (Gomez *et al.*, 2009), *Glycine max* (GmAMT1;4, GmAMT3;1, GmAMT4;1, and GmAMT4;4) (Kobae *et al.*, 2010) and S*orghum bicolor* (SbAMT3;1, SbAMT4) (Koegel *et al.*, 2013). The discovery of specialized transporters at the symbiotic interface was an important step to gain more insight into the symbiotic N transfer.

Here we report the discovery, biochemical characterization and localization of GintAMT3, a new AMF ammonium transporter from *R. irregularis,* which is expressed primarily in the IRM and encodes a low affinity ammonium transporter.

## 3.3 **Material and methods**

### 3.3.1 Plant growth conditions for expression analysis

Experiments were performed with sorghum (*Sorghum bicolor* (L.) Moench), cv Pant-5. This cultivar is closely related to BTx623, the sorghum cultivar used for genome sequencing (Paterson *et al.*, 2009). Seeds of cv Pant-5 were kindly provided by sorghum breeders of I.G.F.R.I. (CCS Agriculture University of Hissar, Haryana, India) and G. B. Pant University of Agriculture and Technology (Pantanagar, Uttaranchal, India). Seeds were surface-sterilized (10 min in 2.5% KClO) and then rinsed with sterile deionized water several times for 1 d and soaked in sterile deionized water overnight. Seeds were pre-germinated on autoclaved sand at 25°C for 24 h and then grown in the dark at room temperature for 72 h. The fungal isolate *Rhizophagus irregularis* BEG-75 (Botanical Institute, Basel, Switzerland) was propagated by trap cultures as previously described (Oehl *et al.*,

2004). To establish AM symbiosis, pregerminated seeds were individually inoculated in compartmented microcosms (Koegel *et al.*, 2013), where one plant and one hyphal compartment are connected, but separated by two 21 µm nylon meshes and an air gap in between. The air gap was created by placing two 5 mm plastic meshes between the two 21 µm nylon meshes. The two compartments were filled with sterile (120°C, 20 min) growth substrate consisting of a mixture of zeolithe (Symbion, Czech Republic) and sand (1 : 1 v/v). About 100 spores were added to the mixture. For the controls (non-mycorrhizal plants), the same amount of autoclaved inoculum was added to the mixture. To correct for possible differences in microbial communities, each pot received 1 ml of filtered washing of AMF inoculum. Plants were grown in a glasshouse with day : night temperatures of c. 28°C : 15°C. Plants were watered twice a week during experiments. From the first week on, 8 ml of modified Hoagland solution was applied weekly. Three different Hoagland solutions, modified after Gamborg and Wetter (1975), were prepared to obtain different N sources or N concentrations : -N, 1x $NO_3^-$ and 1x $NH_4^+$ (Koegel *et al.*, 2013).

*Populus trichocarpa* (derived from cuttings, clone 10174, Orléans, France) grew together with *S. bicolor,* in a tripartite compartment system, in a zeolithe: sand substrate (1:1; w:w). Thereby, single compartments were separated by 21 µm and 3 mm meshes to allow AMF hyphae but no plant root growth in between the compartments. Plants were inoculated with 1 ml liquid inocula of *R. irregularis,* isolate BEG75 (InoculumPlus, Dijon, France), in 0.01 M citrate buffer (pH 6) containing about 110 spores/ml. Plants were fertilized once a week with 10 ml of Hoagland solution without phosphorus. From the 22$^{nd}$ week on, when all plants showed Pi depletion as indicated by anthocyan accumulation, 10 ml Hoagland solution containing either low Pi ([Pi] = 28 µM) or high Pi ([Pi] = 560 µM) concentration was applied in the compartment for the ERM for 9 weeks. As a control both plant species were grown separately in a single compartment, receiving the fertilizer directly to their root systems.

### 3.3.2 *Rhizophagus irregularis* monoxenic cultures under different N treatments

*R. irregularis* monoxenic cultures were established in bi-compartmental Petri dishes to allow separating the root compartment from the hyphal compartment (St-Arnaud *et al.*, 1996; Fortin *et al.*, 2002). Cultures were started on M medium (Chabot *et al.*, 1992) by placing an explant of *Agrobacterium rhizogenes* transformed-carrot (*Daucus carota*) roots colonized with the AMF in the root compartment. Petri dishes were incubated in the dark at 24°C until the hyphal compartment, which contained M medium without sucrose (M-C medium), was profusely colonized by the fungus (approximately 6 weeks). The content of the hyphal compartment was then removed and

replaced by liquid M-C medium (15 ml) containing either 3.2 mM $NO_3^-$ (high N) or in a modified M media containing 0.8 mM $NO_3^-$ (low N). The mycelium then colonized this medium over the subsequent 2 weeks. At this point, the medium was removed and replaced by fresh liquid M-C medium without $NO_3^-$. The time of medium exchange was referred as time 0 for the N starvation treatment, and mycelia were harvested 2 and 7 days later. For the N re-supply experiments, mycelia grown in low N media and N-starved for 48 h were supplemented with different N sources and concentrations (3 mM or 30 µM nitrate or ammonium, or 5 mM glutamine) or water (control plates). The ERM were harvested 24 h later. For treatments with acetate or the inhibitor of glutamine synthetase, methionine sulphoximine (MSX), the N-starved mycelia (grown in the low N media for 2 weeks and for 2 d in a N-free media) were supplied with 4 mM acetate or 2.5 mM MSX, respectively, together with 3 mM ammonium sulphate. In all experiments, mycelia were collected with forceps, rinsed with sterilized water, dried with sterilized filter paper, immediately frozen in liquid N and stored at -80°C until used. All treatments were independently repeated four times.

### 3.3.3 Root colonization measurements

A subsample of fresh roots was immersed in 10% KOH and stored in the fridge at 4°C overnight. At the next day the roots were rinsed under the tap and immersed in 2% HCl for 1 h at room temperature. Afterwards the roots were rinsed under the tap, immersed in 0.05% trypan blue and stored in the fridge at 4°C overnight. The next day the trypan blue was removed, roots were rinsed with tap water and immersed in lactic-acid glycerol water for destaining. Total root colonization was measured using the grid line intersection method as described by Brundrett *et al.* (1984). Differences between means of variables were assessed by t-test ($p \leq 0.5$), using Microsoft Excel 2010.

### 3.3.4 *In-silico* analysis

The sequencing, assembly, and annotation of the *R. irregularis* genome was described in (Tisserant *et al.*, 2012). All *R. irregularis* sequences are available at the Joint Genome Institute (JGI) website (http://genome.jgi-psf.org/Sorbi1/Sorbi1.home.html) and at GenBank / European Molecular Biology Laboratory (EMBL) / DNA Data Bank of Japan (DDBJ). Using BLAST search and the INTER-PRO domains (IPR018047 and IPR001905) at the JGI website, we identified gene models coding for putative AMTs in the draft genome. Gene prediction at the JGI was performed using gene predictors (FGENESH, and GENEWISE), and gene models were selected by the JGI annotation pipeline (Tisserant *et al.*, 2012). Selection of the AMT models was based on expressed sequence

tag (EST) support, completeness, and homology to a curated set of proteins. The putative homologs detected were characterized based on conserved domains, identities, and e-values in comparison with fungal AMT sequences available at the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) and UNIPROT (http://expasy.org/) (Figure 1).

Signal peptides were predicted with SignalP 3.0 (http://www. cbs.dtu.dk/services/SignalP/) and subcellular location with TargetP 1.1 (http://www.cbs.dtu.dk/services/TargetP/). Conserved protein domains were analyzed using prosite (http://us.expasy.org/prosite) and InterProScan (http://www.ebi.ac.uk/InterPro- Scan).

Full-length amino acid sequences of fungal ammonium transporters were retrieved using BLAST (http://blast.ncbi.nlm.nih.gov) and the JGI (http://jgi.doe.gov/) webpage. Sequence alignments were performed with the ClustalW2 package. For phylogenetic analyses, the alignments were imported into the Molecular Evolutionary Genetics Analyses software (MEGA), version 5.05 (Tamura *et al.*, 2011). Neighbour-joining (NJ) method was applied with the Poisson correction model, the pairwise deletion option and bootstrap test with 1,000 replicates.

A two-dimensional model was generated with Protter – visualize proteoforms (Omasits *et al.*, 2013) and a 3D model was calculated via SWISS-MODEL (http://swissmodel.expasy.org/), based on 2b2hA, an ammonium transporter from *Archaeoglobus fulgidus*, AMT-1 (Figure S1).

### 3.3.5 Sampling, RNA isolation and quantitative reverse transcription-PCR

RNA extraction and cDNA synthesis were performed as described previously (Courty *et al.*, 2009). Primers used as controls or for analysis had an efficiency ranging between 90 and 110%. Plant parts were harvested separately and the extraradical mycelium (ERM) was extracted from the substrate by immersing the substrate in water and harvesting the floating mycelium with a 32 µm sieve. Mycelium was snap frozen in liquid nitrogen and stored at -80°C. Plant roots were carefully washed under tap water to remove all soil adhering to the roots. Three subsamples of 100 mg of fresh roots were snap-frozen and stored at -80°C for further gene expression analysis by qRT-PCR.

cDNAs were obtained using the iScript™cDNA Synthesis Kit (BIO RAD Laboratories, Paolo Alto, CA, United States). For quantification a two-step quantitative RT-PCR (qRT-PCR) approach was used. Gene specific primers were designed in Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and amplify 3.1 (http://engels.genetics.wisc.edu/amplify). Target gene expression was normalized to the expression of the transcription elongation factor TEF1$\alpha$ in *R. irregularis.* qRT-PCRs were run in a 7500 real-time PCR systems (Applied Biosystems) using the following settings: 95°C for 3 min and then 40 cycles of 95°C for 30 s, 60°C for 1 min and 72°C for

30 s. For each transporter three biological and three technical replicates (n=9) per treatment were conducted.

### 3.3.6 Isolation of GintAMT3 and functional expression in yeast

Full-length doubled-stranded cDNA was synthesized from RNA of the ERM using the SMARTer™ cDNA Synthesis Kit (Clontech, United States, Canada). GintAMT3 (JGI Protein ID: 218175; JGI Transcript ID: 218287; NCBI accession number: KU933909) was then amplified using the primer pair GintAMT3_fl_Fwd / GintAMT3_fl_Rev (Table S1). Full-length GintAMT3 was cloned into pDR196 using the Gateway technology (Invitrogen), as described previously (Wipf *et al.*, 2003), resulting in the pDR196-GintAMT3 plasmid construct. pDR196-GintAMT3 and as a control the empty vector were transformed into the *Saccharomyces cerevisae* strain 31019b (*MAT*a *ura3 mep1*Δ *mep2*Δ::*LEU2 mep3*Δ::*KanMX2*) (Marini *et al.*, 1997) as described by Dohmen *et al.* (1991). Transformants were selected on SD media lacking uracil and further transferred on yeast nitrogen base (YNB-N) glucose media without ammonium and amino acids supplemented with $NH_4Cl$ as the sole nitrogen source (1 mM and 3 mM). Sequence identities and integrities were verified by sequencing.

### 3.3.7 [$^{14}$C]-Methylamine-HCl uptake assay

Initial [$^{14}$C]methylamine uptake rates (American Radiolabeled Chemicals, Inc., St. Louis, USA) for amino acids were measured as described previously (Marini *et al.*, 1997). Single colonies were grown in liquid YNB-N supplemented with 6% glucose and 500 µg/mL L-proline to logarithmic phase and were centrifuged at an $OD_{600}$ of 0.5 to 0.8. Cells were washed twice in sterile water and resuspended in 50 mM $KH_2PO_4$ buffer pH 5, to a final $OD_{600}$ of 5. Before the uptake measurements an aliquot of yeast cells was supplemented with 20 mM glucose, incubated at 30°C for 5 min at 1,000 rpm. To start the reaction an equal amount of pre-warmed $KH_2PO_4$ buffer containing 15 kBq of [$^{14}$C]methylamine and unlabelled methylamine (0-15 mM) was added. Cells were incubated at 30°C, 1,000 rpm and 45 µl subsamples were taken after 1, 2, 3 and 4 min, diluted in 5 ml $KH_2PO_4$/sorbitol buffer, separated from the incubation buffer on glass fibre filters (Whatman), and washed twice with the same buffer. Radioactivity retained on the filter was assayed by liquid scintillation spectrometry (Packard).

### 3.3.8 Expression analysis at the cellular level by laser capture microdissection

*Sorghum* roots were washed with tap water to remove the substrate. Pieces of 10–15 mm were cut with a razor blade from differentiated regions of the mycorrhizal and non-mycorrhizal roots.

The root segments were embedded in OCT (EMS, Delta Microscopies Aygues-Vives, France) and then frozen at -23°C. 40 µm thin sections were cut with a Cryocut (Cryocut 1800 Leica), and the cuts were placed on Fisher Probe-On slides (Fisher Scientific, Ilkirch, France). The sections were washed and fixed as follows: 3 min 70% EtOH, 30 min DEPC $H_2O$, 2 min 100% EtOH. The slides were then dried for 20 min at 37°C on a warming plate and kept at -80°C before use.

An Arcturus XT microdissection system (Applied Biosystems, Foster City, CA, USA) was used to collect the cells from the mycorrhizal and non-mycorrhizal root sections. Eight replicates of two different cell types were collected: arbuscule-containing cells (ARBs), and cortical cells from non-mycorrhizal roots (Cs). A total of 5,000–15,000 cells were cut out for each sample. RNA from collected cells was extracted using the Arcturus Pico Pure RNA isolation Kit (Excilone, Applied Biosystems, Foster City, CA, USA), with an in-column DNase treatment following manufacturer's instructions. Quantity and quality of the extracted RNAs were verified using a bioanalyzer with RNA pico chips (Agilent, Santa Clara, CA, USA). Synthesis of cDNA and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis was done as previously described using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA), starting with 100 pg RNA.

## 3.4 **Results**

### 3.4.1 *In silico* analysis of GintAMT3

Based on the high conservation of amino acid sequences, a consensus signature for AMTs has been defined corresponding to Prosite PDOC00937, InterPro IPR001905 and Pfam 00909. The *ab initio* annotation and subsequent automated BLAST and INTERPRO searches of the *R. irregularis* draft genome sequence (Tisserant et al., 2012) identified three gene models containing these conserved AMT domains, from which two were already characterized, namely *GintAMT1* (López-Pedrosa *et al.*, 2006) and *GintAMT2* (Pérez-Tienda *et al.* (2012). The length of the nucleotide sequence of *GintAMT3* is 1,798 bp. The coding exon sequence (1,365 bp) was confirmed by EST alignment and cDNA sequencing, and it is interrupted by four short introns of 92 bp, 130 bp, 125 bp, and 86 bp length, typical of *R. irregularis* (Tisserant et al., 2012).

Comparisons between cDNA and genomic sequences of the *R. irregularis* AMT genes revealed 1, 3 and 4 introns for *GintAMT1*, *GintAMT2* and *GintAMT3*, respectively. Their positions are conserved between the genes, whenever present in more than one gene (Figure 1A). Location of intron 2 is even conserved in all six AMT genes of *R. irregularis* and *G. pyriformis*, indicating its presence in a common ancestral gene before these glomeromycotan species split. This is remarkable as the two

AMF are distantly related and probably have separated more than 400 million years ago (Schüßler *et al.*, 2001). A comparison with an AMT gene of the basidiomycete *Ustilago maydis*, *UmUMP2*, revealed an intron in a different position, 60 base pairs further downstream between codons for two other highly conserved residues, a glycine and an asparagine residue. Thus, the position of intron number 2 is conserved among glomeromycotan AMT genes but, based on present data, also appears to be specific for this phylum. The 6 encoded proteins show high levels of amino acid identity and similarity (Figure 1B).

The introns 1 and 4 are conserved between *GintAMT2* and *GintAMT3*, suggesting recent gene duplication. Intron 3 only exists in *GintAMT3*. Also the positions of predicted transmembrane domains (TMDs, green rectangles in Figure 1A) are highly conserved between the AMF AMTs.

A phylogenetic analysis was performed to compare the protein sequences of the glomeromycotan AMTs with the ones from other fungi. This analysis revealed a close relationship of the six glomeromycotan AMTs, and a clear homology with one AMT family of the Ascomycetes, represented by SpAMT1 (Figure 2). For the non-glomeromycotan AMTs, we observed a clear separation of the AMTs according to their affinities, with the exception of the *S. cerevisiae* high-affinity transporter ScMEP2, which is more closely related to the low affinity *S. cerevisiae* AMTs than to its orthologues in other fungi (Figure 2).

### 3.4.2 Root colonization depending on N and P conditions

After 30 weeks of growth, symbioses between *R. irregularis* and the two host plants, poplar and sorghum, were well established (Table S2). Root hyphal colonization rates ranged between 79-93% and were not significantly different (n=7). In sorghum, three times more arbuscules were found in the low Pi treatment as compared to the high Pi treatment, while no significant differences were found in poplar. In the three months old sorghum plants, set under three different N conditions, hyphal colonization ranged between 94% and 99% (n=4) (Table S2).

### 3.4.3 Yeast complementation, GFP localization and ammonium uptake

The putative transporter gene *GintAMT3* was tested for complementation of the yeast *mep1-3*Δ mutant (strain MLY131a/α, Lorenz and Heitman (1998)) in comparison with the already known AMT genes. Cells were transformed with variants of the plasmid pDR196*sfi* containing the different AMT genes or a stuffer gene (a part of a human aldolase gene without ORF) cloned into the *SfiI* sites. The genes were constitutively expressed under the *PMA1* promoter. All three transporter genes of *R. irregularis* at least partly restored the ammonium uptake capability in yeast, as proven by their capability to restore growth of the *mep1-3*Δ mutant on medium

containing 50 µM $(NH_4)_2SO_4$ as sole nitrogen source (Figure 3). *GintAMT1* complemented more efficiently the mutant phenotype than GintAMT2 and GintAMT3, demonstrated by larger colonies in a successive 5x dilution series on medium containing 50 µM $(NH_4)_2SO_4$ as sole nitrogen source (Figure 3).

To test if the different complementation efficiencies observed by the different AMTs could be due to an incorrect protein localization in the heterologous system, we cloned *GintAMT1*, *GintAMT2* and *GintAMT3* to the 5' end of a green fluorescent protein (GFP) coding gene into the expression vector pDR196*sfi* and transformed the yeast *mep1-3*Δ mutant with these constructs, resulting in the expression of C-terminal GFP-tagged AMT fusion proteins in yeast cells. The localization of these fusion proteins was performed with a Leica SP5 confocal laser-scanning microscope (CLSM, Figure 4). All tagged proteins were localized to the plasma membrane in *S. cerevisiae* (Figure 4). Additionally, we observed vacuolar or perinuclear membrane localization for some of them indicative of an endoplasmic reticulum localization, most probably as an overexpression artefact (Figure 4). All tagged transporters behaved like the untagged versions (not shown), either complementing the growth defect of the yeast mutant (GintAMT1-GFP, GintAMT2-GFP, GintAMT3-GFP) or not (soluble GFP).

### 3.4.4 Ammonium removal assay

To measure the different ammonium transport capacities of the transporters, ammonium removal assays according to Ellerbeck et al. (2013) were performed. In this experimental setup, dense yeast cultures ($OD_{600}$ = 2) were incubated in relatively high ammonium concentrations (1 mM) for several hours and the remaining ammonium in the medium was measured at distinct time points (after 10, 30, 60, 120, 180, 240 and 300 min). Therefore, no kinetics but overall ammonium uptake can be measured. The results of the removal assays confirmed the yeast complementation assays. The 3 AMTs of *R. irregularis* transported ammonium to a varying but always lower extent than ScMEP1 (Figure 5). GintAMT2 and GintAMT3 showed lower ammonium removal activity in these experiments (Figure 5) than GintAMT1, supporting the results from the complementation assays on plate (Figure 3).

### 3.4.5 *GintAMT* expression levels

Regulation of *GintAMT3* gene expression by N starvation was assessed in the ERM of *R. irregularis* developed in monoxenic cultures in M-C medium (standard or high N) or in a modified medium containing reduced N (low N), and then incubated for different periods of time in a N-free M medium. *GintAMT3* transcript levels increased when the fungus was exposed to the N-free

medium. When the fungus was grown in the low-N media, *GintAMT3* up-regulation was observed 2d after N deprivation, while in the ERM grown in the high N medium *GintAMT3* up-regulation was observed 5 d later (Figure 6A).

To further investigate the effect of N on *GintAMT3* transcript levels, we also determined whether the addition of different N sources to the N-deprived mycelia had an effect on its expression (Figure 6B). Relative to the N-deprived ERM, *GintAMT3* transcript levels significantly decreased 24 h after the addition of 3 mM $NH_4^+$. Feeding the mycelium with nitrate, glutamine or 30 µM $NH_4^+$ did not significantly change *GintAMT3* gene expression, although a slight decrease was observed after the addition of 3 mM nitrate or glutamine.

The effect of the glutamine synthetase inhibitor methionine sulphoximine (MSX) on the expression levels of the three *R. irregularis* AMT genes was also tested. For this purpose, the N-deprived ERM was incubated for 24 h in the presence of 2.5 mM MSX in the $NH_4^+$ re-supplementation media. Under these conditions, $NH_4^+$ should be accumulated and glutamine should be depleted. MSX caused a down-regulation of *GintAMT3* gene expression, but did not have any effect on *GintAMT1* and *GintAMT2* transcript levels (Figure 7). To determine if transcription of the *R. irregularis* AMT genes were affected by carbon supply, *GintAMT*s gene expression was assessed in the N-deprived ERM supplemented with $NH_4^+$ and acetate, a carbon source taken up and assimilated by the ERM (Pfeffer *et al.*, 1999). Relative to the N-deprived mycelium, supplying the ERM with ammonium and acetate induced down-regulation of the three *GintAMT*s, with the strongest and statistically significant effect for *GintAMT3* (Figure 7).

Expression of all three *R. irregularis* AMT was assessed in ERM and IRM when the fungus was associated with poplar and sorghum. In this experimental set-up the fungus had either access to a low Pi source or a high Pi source. The expression level for the high affinity transporter GintAMT1 was low and similar in the ERM and in the IRM, independently of the Pi availability. GintAMT2 was strongly expressed in the ERM and IRM, independently of Pi availability (Figure S2). Expression level of *GintAMT3* was far higher in the IRM than in the ERM. *GintAMT3* was significantly more strongly expressed under high Pi conditions compared to low-Pi in the IRM (Figure 8A). Expression patterns of all three transporters were the same in both plant species. When we measured gene expression of *GintAMT3* in laser-microdissected arbusculated cells we did not observe significant differences between high Pi and low Pi condition (Figure 8B). Moreover, *GintAMT3* expression was at least twice as high in the IRM as compared to the ERM, independent of the N source (Figure 9).

### 3.4.6 [$^{14}$C]methylamine uptake assay

Functional expression of *GintAMT3* in the yeast triple mutant revealed it to be a low affinity transporter with an apparent $K_m$ of 1.8 mM and a $V_{max}$ of 240 $nmol^{-1}$ $min^{-1}$ $10^8$ $cells^{-1}$. We observed a steep increase in methylamine uptake until reaching a plateau at about 6 mM. However, increasing the amount of supplied methylamine showed that GintAMT3 is still able to take up methylamine at a steady pace (Figure 10).

## 3.5 Discussion

In the AM symbiosis, the main role of the AM fungal partners is the acquisition of mineral nutrients from the soil, in the ERM, and the transfer of these nutrients to the IRM and from there, by way of the periarbuscular space, to the plant. Though P is the most-often named mineral nutrient in this context, N can be a limiting factor for plant growth as well, and the N delivered by AMFs may play an important role for plant growth and health. According to current knowledge, AMF take up N in the ERM, preferentially in form of ammonium, metabolize it to arginine in the GS/GOGAT (glutamine synthetase / glutamine oxoglutarate aminotransferase) pathway and in the urea cycle, and transport it to the IRM in the form of arginine (Casieri et al., 2013). At the plant fungal interface (in the arbuscule), ammonia is thought to be released from arginine through the action of arginase and urease and then transported to the plant. For the plant partner, it has been shown already that the expression of certain AMTs is specifically upregulated in arbuscule-containing cells, and that that these plant AMTs reside in the periarbuscular membrane. However, not much is known yet about the localization and regulation of the fungal AMTs involved in this process. In our study, we describe a new functional ammonium transporter, GintAMT3, of *R. irregularis*, and we try to characterize its role in the symbiotic N transfer.

### 3.5.1 AMF ammonium transporters: a separated phylogenetic group

Sequence homology analysis revealed high intraspecific and interspecific sequence conservation of GintAMT3 to the two already known AMTs of *R. irregularis* (López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011) and the three AMTs previously identified in *Geosiphon pyriformis* (Ellerbeck *et al.*, 2013) (Figure 1). All six glomeromycotan AMTs shared high sequence similarity and the 11 TM helices of AMTs. Positioning of the intron sequences showed further, that the glomeromycotan AMT genes are highly conserved. The closest homologue to GintAMT3 is GintAMT2, which shared 80% of sequence similarity. The additional intron sequences in the GintAMT2 and GintAMT3 genes suggested a recent gene duplication event.

Phylogenetic analysis of ammonium transporters from Ascomycota, Basidiomycota, Zygomycota and Glomeromycota revealed that the six AMTs from Glomeromycota species (3 from *R. irregularis*) clustered separately from the HATS and LATS of Ascomycota and Basidiomycota (Figure 2), indicating a distinct AMT evolution in these fungal phyla. Note that some of the AMTs of Ascomycota and Basidiomycota have been identified in species forming ectomycorrhizas, such as *Tuber borchii* (Montanini *et al.*, 2002), *Hebeloma cylindrosporum* (Javelle *et al.*, 2001; Javelle *et al.*, 2003; Javelle *et al.*, 2003), *Amanita muscaria* (Willmann *et al.*, 2007), and *Laccaria bicolor* (Lucic *et al.*, 2008).

### 3.5.2 GintAMT3 is a low affinity transporter system

Both a low-affinity (LATS) and a high-affinity transport system (HATS) have already been described in *R. irregularis* (Pérez-Tienda *et al.*, 2012). GintAMT1 has been characterized as a HATS with an apparent $K_m$ of 26µM (López-Pedrosa *et al.*, 2006). The kinetics of the second AMT, GintAMT2, could not be determined by methylammonium uptake assay (Pérez-Tienda *et al.*, 2011), but qRT-PCR measurements revealed that GintAMT2 is constitutively expressed under N-limiting conditions, suggesting a role in ammonium retention rather than in ammonium uptake (Pérez-Tienda *et al.*, 2011). We characterized GintAMT3 as a LATS with an apparent $K_m$ of 1.8 mM and a $V_{max}$ of 240 $nmol^{-1}$ $min^{-1}$ $10^8$ $cells^{-1}$. In our experiments, expression of GintAMT3 is dependent on the N nutritional status of the AM fungus but independent from the provided N source under N limiting conditions. Severe N stress induced expression of GintAMT3 independently of the supplied N source and abundance of GintAMT3 transcript decreased within a few days. These results indicate the existence of unknown regulatory mechanisms involved in transcriptional or post-transcriptional regulation of AMTs in AMF. Further, we could show that GintAMT3 expression is not only dependent on N nutrition status but also on fungal carbon status, indicating a tight connection to symbiotic interactions. A similar observation was reported in *Hebeloma cylindrosporum*, a Basidiomycota fungus forming ectomycorrhizal symbiosis (Javelle et al., 2003b).

Using a compartmented system, we analysed fungal nutrient transporterts in the ERM and IRM when associated with *Sorghum bicolor*. Independently of the N source, the expression level of GintAMT3 in the IRM was significantly more than two fold induced compared to the ERM. As P is also a major nutrient transferred by the AM fungus to the plant, we assessed the effect of P availability on GintAMT3 expression in the ERM and IRM when *R. irregularis* was associated with *S. bicolor* or with poplar, and found an induction of GintAMT3 by P in the IRM. The high expression of GintAMT3 in the IRM indicates that it might be located in the arbuscules.

Microdissection of *S. bicolor* roos revealed indeed that GintAMT3 is expressed in the symbiotic root tissue, and specifically in arbuscule-containing cells. Heterologous expression of GFP tagged GintAMT3 in yeast revealed localization of the AMT in the plasma membrane and vacuolar membrane. Given that current experimental evidence supports a role for AMT proteins in ammonium uptake (Khademi *et al.*, 2004; Lamoureux *et al.*, 2010) and that ammonium is the N form taken up by the plant at the arbuscular interface (Govindarajulu et al., 2005; Tian et al., 2010), expression of AMT genes in the arbuscules indicates that there might exist a competition between the plant and the fungus for N that is present in the interfacial apoplast (Guether et al., 2009a). As it was proposed for the high-affinity transporters GintAMT1 and GintAMT2, the high expression of GintAMT3 in the arbuscules also suggests a role for this transporter in ammonium retrieval from the periarbuscular space, but in situations where the ammonium concentrations are high. Additionally to its incorporation in metabolism, the vacuolar localization of GintAMT3 indicated that ammonium could be stored in vacuoles to maintain low cytoplasmic ammonium concentrations as shown in yeast (Soupene *et al.*, 2001) or plants (von Wirén *et al.*, 2000; Loqué *et al.*, 2005), or in intracellular vesicles (Chalot *et al.*, 2006). Studies on ammonium/methylammonium transporters (AMT/MEP) of enteric bacteria have shown that these transporters function as ammonia channels. Ammonium is deprotonated at the channel entrance and ammonia is transported through it. The transport through the channel is energy-independent and bidirectional (Soupene *et al.*, 1998; Soupene *et al.*, 2002; Khademi *et al.*, 2004). Therefore, it might also be possible that GintAMT3 function as a bidirectional transporter for import and export of ammonium from the vacuole. Furthermore, it is also possible that GintAMT3 functions as an export carrier for ammonium from the arbuscules to the periarbuscular space. However, to assess possible bidirectional transport properties of GintAMT3, patch clamp measurements are necessary. Knockdown of GintAMT3 by host induced gene silencing and virus induced genes silencing could illustrate the importance of this transporter for a functional symbiosis (Helber *et al.*, 2011).

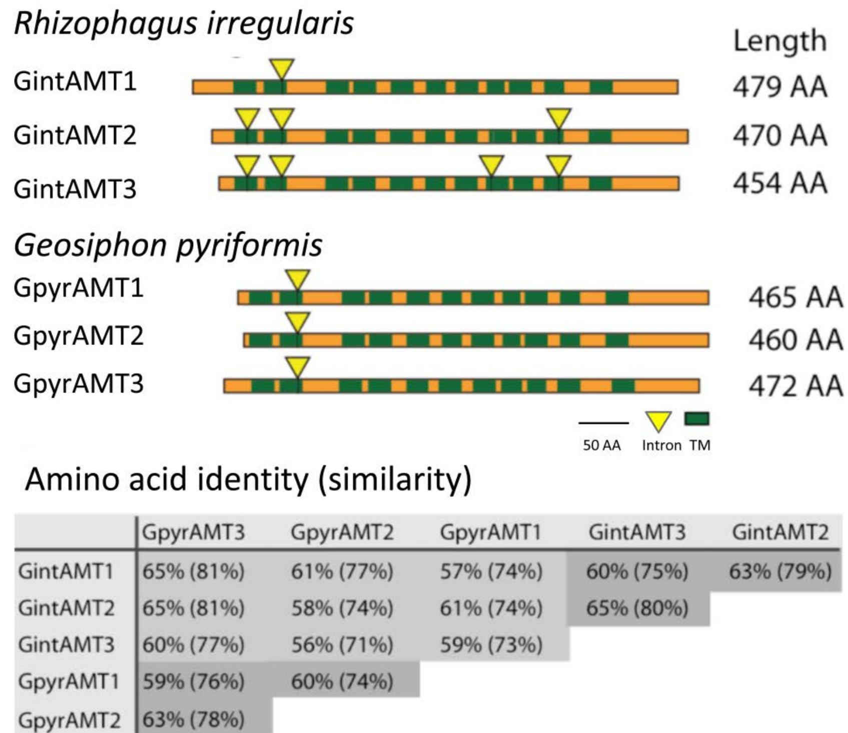
## 3.6 **Conclusion**

Here, we demonstrate that GintAMT3 encodes a functional low affinity transporter. We show that it is localized in the fungal membrane, and that it is expressed in the ERM and IRM of colonized poplar and sorghum plants. Increased expression in the IRM under high-P conditions indicates further that more ammonium is transferred when the AM fungus has increased access to a P source.

## 3.7 Acknowledgments

This project was supported by the Swiss National Science Foundation (grant no. PZ00P3_136651 to P-E.C., grant No. 127563 to T.B.) the Conseil Régional de Bourgogne PARI AGREE grant to D.W and the Spanish Ministry of Economy and Competitivity (Projects AGL2012-35611 and AGL2015-67098-R).

## 3.8 **Figures**




| | GpyrAMT3 | GpyrAMT2 | GpyrAMT1 | GintAMT3 | GintAMT2 |
|---|---|---|---|---|---|
| GintAMT1 | 65% (81%) | 61% (77%) | 57% (74%) | 60% (75%) | 63% (79%) |
| GintAMT2 | 65% (81%) | 58% (74%) | 61% (74%) | 65% (80%) | |
| GintAMT3 | 60% (77%) | 56% (71%) | 59% (73%) | | |
| GpyrAMT1 | 59% (76%) | 60% (74%) | | | |
| GpyrAMT2 | 63% (78%) | | | | |

**Figure 3.1 Topologies of glomeromycotan AMTs and AMT genes and their relationship.** (A): Transmembrane domain (TMD) topology and intron localization of the six glomeromycotan AMTs. Green boxes indicate TMD positions, yellow triangles mark intron positions. Both are highly conserved, while N and C termini differ in length and are less conserved. (B) Reciprocal BLAST (Altschul et al., 1997) analysis (Blosum62 matrix) revealed a high conservation on sequence level between the 6 transporters. They share at least 56% AA identity and 71% AA similarity. Intra species comparisons are marked in dark grey.

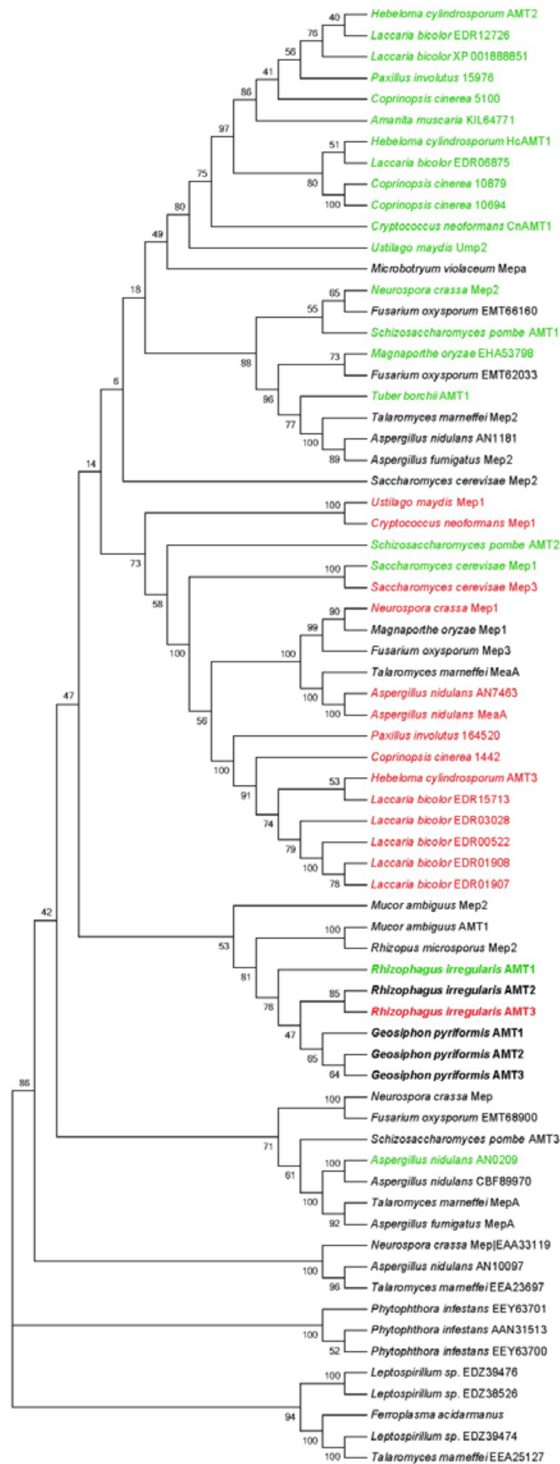



**Figure 3.2. Phylogenetic tree ("NJ Bootstrap Consensus Tree") of fungal Mep/AMT proteins.** Transporter names or accession numbers are indicated. Bootstrap values are derived from 1000 replications. High-affinity transport systems are highlighted in green and low affinity transporter are highlighted in red. Glomeromycotan AMTs are highlighted in bold. Phylogenetic tree was constructed using MEGA6.06 package (Tamura et al., 2013). Gene names, protein IDs and bootstapping values are indicated. Sequences obtained from the JGI databank: Aspergillus nidulans AMT (AN7463), AMT (AN0209), AMT (AN10097), AMT (AN1181); Coprinopsis cinerea AMT (1442), AMT (5100), AMT (10879), AMT (10694); Rhizophagus irregularis AMT1 (337025), AMT2 (314209), AMT3 (21817); Paxillus involutus AMT (164520), AMT (15976), AMT (KIJ11108). Sequences obtained from the NCBI databank: Aspergillus fumigatus Mep2 (EAL90420), MepA (EAL91508); Amanita muscaria (KIL64771); Cryptococcus neoformans Mep1 (XP_566614), AMT1 (XP_567361); Ferroplasma acidarmanus (WP_019841313); Fusarium oxysporum AMT (EMT62033), AMT (EMT68900), AMT (EMT66160), Mep3 (EMT61925); Geosyphon pyriformis AMT1 (AGO45860), AMT2 (AGO45861), AMT3 (AGO45862); Hebeloma cylindrosporum AMT1 (AAM21926), AMT2 (AAK82416), AMT (AAK82417); Laccaria bicolor AMT (EDR12726), AMT (EDR06875), AMT (EDR03028), AMT (EDR01908), AMT (EDR01907), AMT (EDR00522), AMT (EDR15713), AMT (XP_001888851); Leptospirillum sp. AMT (EDZ39474), AMT (EDZ39476), AMT (EDZ38526); Magnaporthe oryzae AMT (EHA53798), Mep1 (EHA48931); Microbotryum violaceum Mepa (AAD40955); Neurospora crassa Mep1 (EAA35174), Mep2 (EAA32441), Mep (KHE86570), Mep (EAA33119); Mucor ambiguous AMT1 (GAN10886), Mep2 (GAN10300); Phytophthora infestans AMT (AAN31513), AMT (EEY53846), AMT (EEY63701), AMT (EEY63700); Rhizopus microspores putative Mep2 (CEJ04454); Saccharomyces cerevisae Mep1 (P40260), Mep2 (P41948), Mep3 (P53390); Schizosacchromyces pombe AMT1 (NP_588424), AMT2 (CAB65815), AMT3 (P53390); Talaromyces marneffei MepA (EEA28528), MeaA (EEA28073), Mep2 (EEA20421), putative AMT (EEA25127), putative AMT (EEA23697); Tuber borchii AMT1 (AAL11032); Ustilago maydis Mep1 (KIS67424), UMP2 (KIS66151).

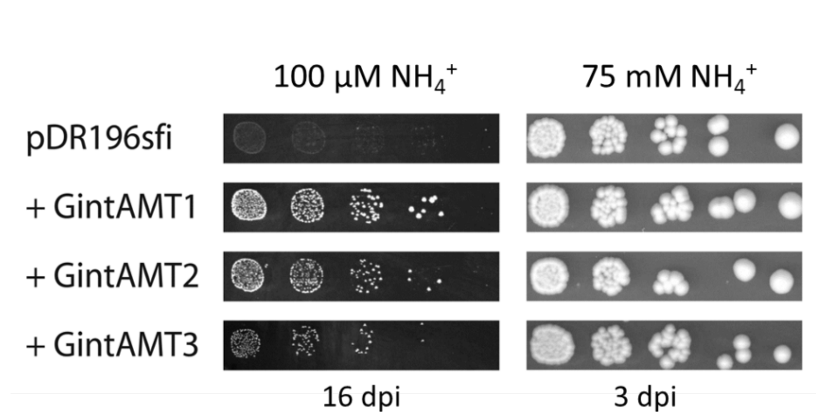



**Figure 3.3. Complementation of ammonium uptake deficiency in a yeast triple mutant by glomeromycotan AMTs.** Ammonium uptake-deficient yeast cells (*mep1-3*Δ) were transformed with an expression vector containing various AMT genes under control of the strong *PMA1* promoter. Five-fold dilution series of the transformants were incubated either on minimal medium containing 50 µM $(NH_4)_2SO_4$ (= 100 µM $NH_4^+$) as sole nitrogen source for 16 days (left panel) or on synthetic complete medium (containing roughly 37.5 mM $(NH_4)_2SO_4$) for 3 days (right panel). *Rhizophagus irregularis* AMTs are able to partly complement the growth deficiency of the Δ*mep1-3* yeast mutant on low $NH_4^+$ concentrations (100 µM, left panel).

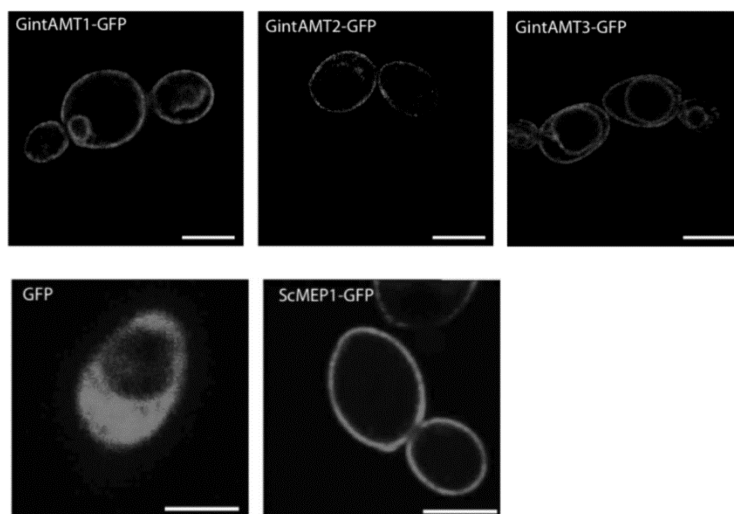



**Figure 3.4. Localization of GFP-tagged AMTs in *S. cerevisiae.*** C-terminal GFP-tagged versions of ScMEP1, GintAMT1, GintAMT2 and GintAMT3 as well as soluble GFP were cloned into pDR196sfi and expressed in *S. cerevisiae* under control of the *PMA1* promoter. The cells were grown to logarithmic growth phase and the localization of the fusion proteins was estimated by confocal microscopy. All three *R. irregularis* AMTs tagged with GFP are localized at the plasma membrane (PM) like ScMEP1-GFP (lower center panel), the positive control. Soluble GFP is localized to the cytoplasm of *S. cerevisiae* (lower left panel). Additional vacuolar membrane localization is visible for GintAMT3-GFP (upper right panel). GintAMT1-GFP (upper left panel) seems to show additional nuclear membrane localization. Bars are 2.5 µm.

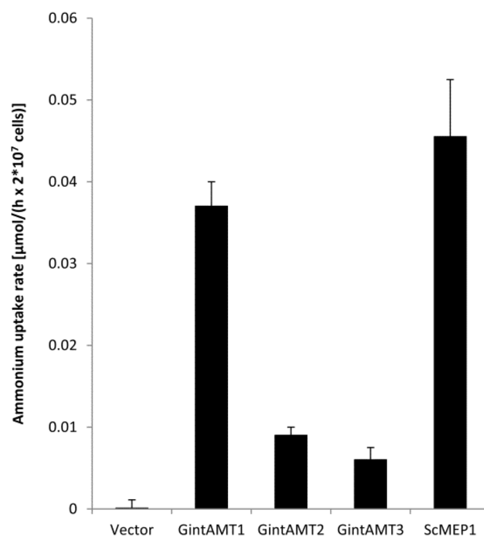



**Figure 3.5. Quantification of ammonium uptake in yeast cells expressing glomeromycotan AMTs**. Yeast cells expressing the stated genes from the plasmid pDR196sfi were grown over night in synthetic complete medium lacking uracil (HC-U), washed and cultured in liquid medium containing a starting concentration of 2 mM ammonium. Samples were taken after 10, 30, 60, 120, 180, 240 and 300 minutes, and residual ammonium was determined. Yeast cells expressing *ScMEP1* and *GintAMT1* took up ammonium quite rapidly. *GintAMT2* and *GintAMT3* expressing cells imported ammonium at a slower rate, but clearly above background level (“Vector”). Bars show average of 3-4 experiments and standard deviation.

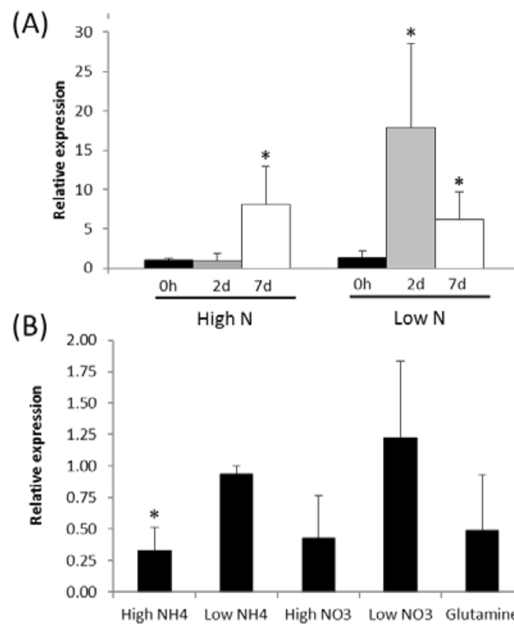



**Figure 3.6. Effect of N availability on *Gint*AMT3 gene expression**. (A) Real-time RT-PCR analysis of *GintAMT3* mRNA levels in the ERM of *R. irregularis* developed in liquid M-C medium in the presence of either 3.2 mM (High N) or 0.8 mM nitrate (Low N) and exposed for different periods of time to a N-free medium. (B) Effect of N addition to the N-starved mycelium on *GintAMT3* expression. Gene expression was analysed by real-time RT-PCR in ERM grown in 25% N media, maintained for 48 h in a N-free media (Control) and exposed for 24 h to 3 mM (High) or 30 µM (Low) of $NH_4^+$ or $NO_3^-$, or 5 mM glutamine. Control plates were supplemented with $H_2O$. Data were calibrated by the expression values obtained for the gene encoding the EF1α. Error bars represent SE of the mean of three independent experiments. *: statistically significant ($p<0.05$) in comparison to the respective control value.

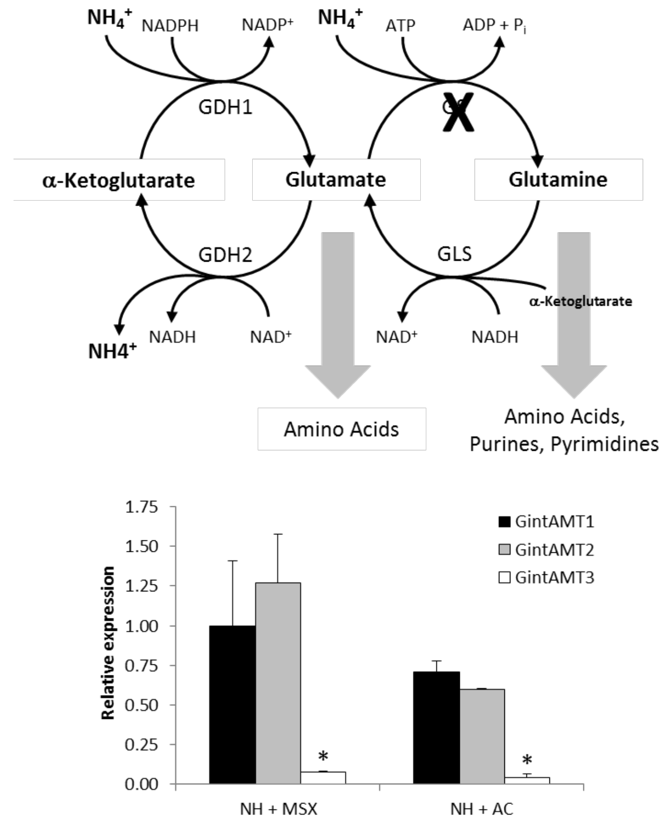



**Figure 3.7. Effect of MSX and acetate on *GintAMTs* gene expression**. Gene expression was analyzed by real time RT-PCR in the N-deprived mycelia (NH) and supplied for 24 h to 3 mM $NH_4^+$ plus 2.5 mM MSX (NH + MSX), or 3 mM $NH_4^+$ plus 4 mM acetate (NH + AC). Data were calibrated by the expression values obtained for the gene encoding the EF1α. Error bars represent SE of the mean of three independent experiments. *: statistically significant ($p$<0.05) in comparison to the respective control value.

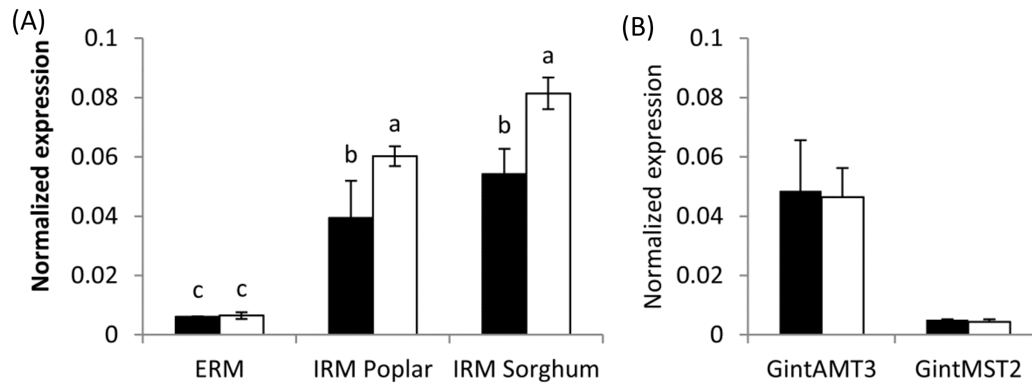



**Figure 3.8**. **Quantification of *GintAMT3* under phosphate stress.** Gene expression was measured by quantitative polymerase chain reaction in the ERM and IRM of (A) inoculated P. trichocarpa and S. bicolor and in (B) microdissected arbusculated cells in S. bicolor. (A) The sorghum and poplar plants grew in a tripartite compartment system where only the fungus had access to the high phosphorous source (open bars) or low phosphorous source (closed bars). Differences between ERM and IRM were tested with a one-way ANOVA. Data were calibrated by the expression values obtained for the gene encoding the transcription elongation factor TEF1α. Values are means of nine replicates, error bars represent SD. Difference between treatments were tested with a one-way ANOVA. Lower case letters indicate significant difference (Tukey's t-test; $p<0.05$). (B) Inoculated S. bicolor grew in a two-partite compartment system where only the fungus had access to the high phosphorous (open bars) or low phosphorous (closed bars) source. Arbusculated cells were laser microdissected and transcript abundances of GintAMT3 and GintMST2 (monosaccharide transporter essential for functional symbiosis, Helber et al. (2011)) as a positive control were measured by qPCR. Data were calibrated by the expression values obtained for the gene encoding the transcription elongation factor TEF1α. Values are means of six replicates, error bars represent SD. Difference between treatments was tested with Tukey's t-test ($p<0.05$).

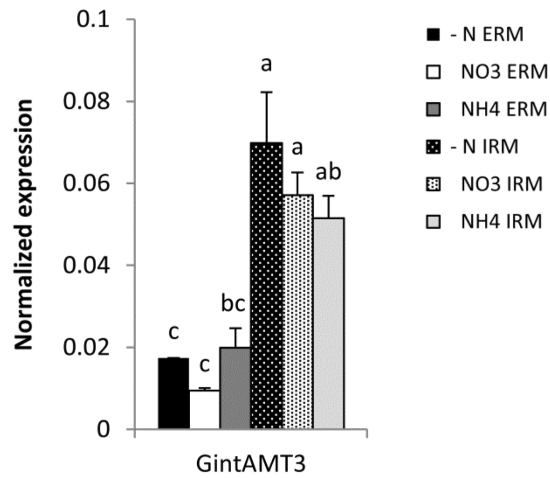



**Figure 3.9. Quantification of *GintAMT3* expression in *R. irregularis* by qPCR**. Inoculated S. bicolor grew in a two-partite compartment system where only the fungus had access to the second compartment. In this system only the fungus had access to the applied nutrients. Hyphal compartments received either Hoagland solution containing no nitrogen source (-N), or nitrate ($NO_3$) or ammonium ($NH_4$) as the sole nitrogen source. Gene expression of *GintAMT3* was measured in the ERM and IRM. Data were calibrated by the expression values obtained for the gene encoding the transcription elongation factor TEF1α. Values are means of nine replicates, error bars represent SD. Difference between treatments were tested with a one-way ANOVA. Lower case letters indicate significant difference (Tukey's t-test; $p<0.05$).

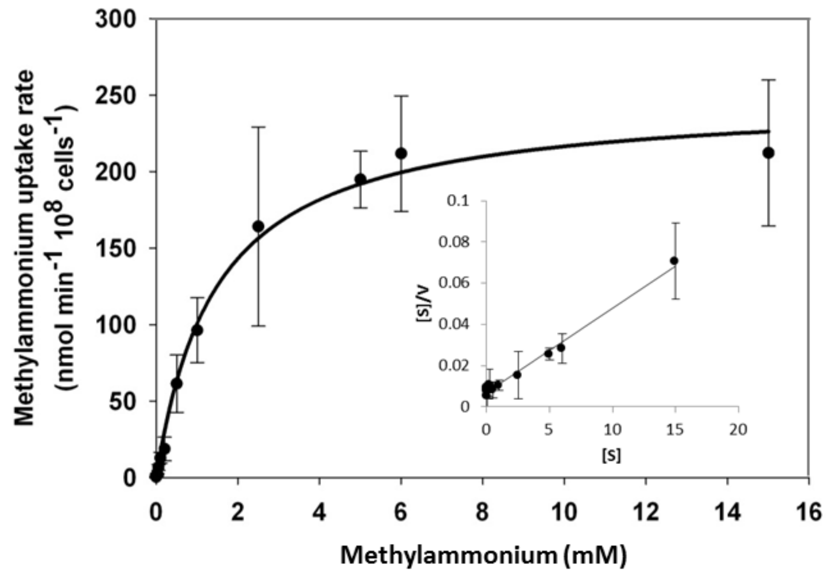



**Figure 3.10. Biochemical characterization of *GintAMT3***. Heterologous expression of pDR196-GintAMT3 in yeast strain 31019b (*MAT*a *ura3 mep1*Δ *mep2*Δ::*LEU2 mep3*Δ::*KanMX2*) (Marini et al., 1997). [$^{14}$C]methylamine uptake rates were measured at pH 5 at different substrate concentrations. Inset: Hanes-Woolf plot.

## 3.9 Supplementary figures and tables

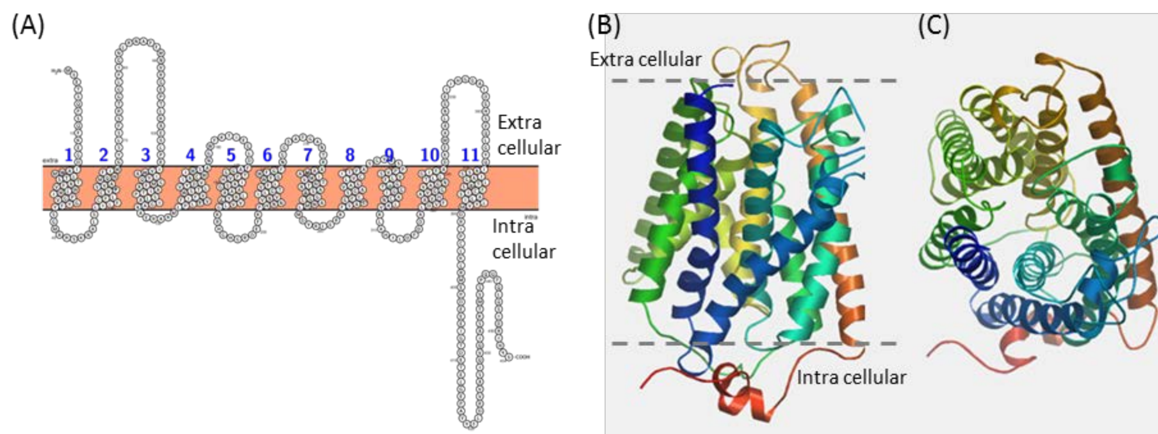



**Figure S3.1. Predicted 2D (A) and 3D topology (B and C) of GintAMT3.** Models were constructed using Protter – visualize proteoforms (Omasits et al., 2013) and SWISS-MODEL (Benkert *et al.*, 2011). 3D model shows potential tertiary structure of GintAMT3 when incorporated into the membrane (B) and from the top from the extracellular side to the intracellular side (C).

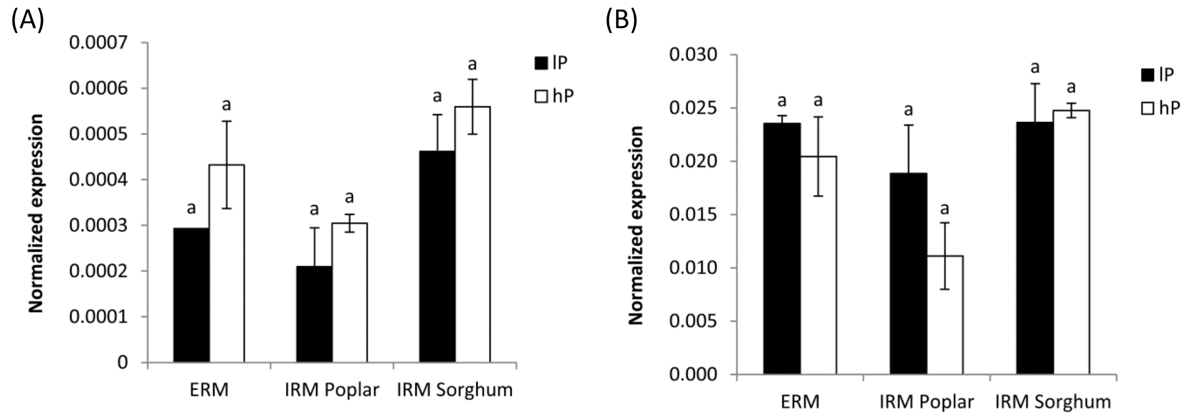



**Figure S3.2 Quantification of *GintAMT1* (A) and *GintAMT2* (B) transkripts under phosphate stress**. Gene expression was measured by quantitative polymerase chain reaction in the ERM and ORM of inoculated *P. trichocarpa* and *S. bicolor.* ) The sorghum and poplar plants grew in a tripartite compartment system where only the fungus had access to the high phosphorous source (open bars) or low phosphorous source (closed bars). Differences between ERM and IRM were tested with a one-way ANOVA. Data were calibrated by the expression values obtained for the gene encoding the transcription elongation factor TEF1α. Values are means of nine replicates, error bars represent SD. Difference between treatments were tested with a one-way ANOVA. Lower case letters indicate significant difference (Tuckey HSD; $p<0.05$).

**Table S3.1 Primer list**

| **Primer list for Gene amplification** | | |
|---|---|---|
| GintAMT3f | qPCR | GGG CTT GAC TTT GCT GGT |
| GintAMT3r | qPCR | TTC GTC CCT TCC ATG ACC |
| GintAMT3_fl_Fwd | Full length | TTTTCTTTTTCTCCCCCAAGA |
| GintAMT3_fl_Rev | Full length | AAATTAAATAAATGCGAGTGATAGAAA |
| attB1_GintAMT3_fwd | Cloning site | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCTTATGATAAAAAATGTCAG |
| attB2_GintAMT3_rev | Cloning site | GGGGACCACTTTGTACAAGAAAGCTGGGTACACTTTTTTAAGAATAATG |

**Table S3.2**. **Mycorrhizal colonization.** Hyphal and arbuscular colonization rates in different treatments were compared by one-way ANOVA. Lowercase letters indicate significant differences between treatments ($p$<0.05).

| **Mean percentage colonization** | | | | | |
|---|---|---|---|---|---|
| **Plant** | **Treatment** | **Hyphae (%)** | | **Arbuscule (%)** | |
| | | **Mean** | **SD** | **Mean** | **SD** |
| **Poplar** | **low Pi** | 79.43[a] | 9.98 | 33.57[a] | 7.68 |
| | **high Pi** | 87.29[a] | 6.29 | 33.57[a] | 8.48 |
| **Sorghum** | **low Pi** | 93.71[a] | 5.47 | 15.57[a] | 6.65 |
| | **high Pi** | 93.14[a] | 5.37 | 5.43[b] | 4.58 |
| **Sorghum** | **N** | 97.00 | 0.82 | 40.25 | 7.89 |
| | **NO3** | 98.75 | 0.50 | 35.50 | 11.68 |
| | **NH4** | 94.00 | 0.82 | 46.25 | 2.36 |

# 4 Transcriptome analysis of the *Populus trichocarpa* – *Rhizophagus irregularis* mycorrhizal symbiosis: regulation of plant and fungal transportomes, and repression of plant defense genes under nitrogen starvation

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## 4.1 **Abstract**

Nutrient transfer is a key element of the arbuscular mycorrhizal (AM) symbiosis. Valuable mineral nutrients are transferred from the AM fungus to the plant increasing its fitness and productivity, and in exchange, the AM fungus receives carbohydrates as energy source from the plant. Here, we analyzed the transcriptome of the *Populus trichocarpa – Rhizophagus irregularis* symbiosis using RNA-sequencing of non-mycorrhizal or mycorrhizal fine roots, with a focus on the effect of nitrogen (N) starvation. In *R. irregularis*, we identified 1015 differentially expressed genes, whereby N starvation led to a general induction of gene expression. Genes of the functional classes cell growth, membrane biogenesis and genes encoding structural components were highly abundant. Interestingly, N starvation also led to a general induction of fungal transporters, indicating increased nutrient demand upon N starvation. In non-mycorrhizal *P. trichocarpa* roots, 888 genes were differentially expressed under N starvation. Among the 304 up-regulated genes, most were involved in metabolic processes including secondary metabolite synthesis, while the down-regulated genes included many defense-related genes. Interestingly, mycorrhization resulted in a general down-regulation of defense-related genes suggesting the plant to foster the symbiotic relationship. Mycorrhization specifically induced expression of three ammonium transporters and one phosphate transporter, independently of the N conditions, corroborating the hypothesis that these transporters are important for symbiotic nutrient exchange. In conclusion, our data establish a framework of gene expression in the two symbiotic partners under high-N and low-N conditions.

## 4.2 **Introduction**

Arbuscular mycorrhizal (AM) fungi belong to the order of Glomerales and form mutualistic symbiosis with about 80% of land plant species. With their complex and extensive hyphal network they are able to extract mineral nutrients and water from the soil and make them available to the plant symbiont. In exchange, the plant supplies the AM fungus with photosynthates (Smith and Read, 2008). In AM symbiosis, the extracted nutrients are taken up by the extraradical mycelium (ERM), incorporated into transport molecules and transferred to the intraradical mycelium (IRM). Inside the plant cortical cells the IRM forms tree like structure (arbuscules) which are surrounded by the plant derived periarbuscular membrane and are the site of nutrient exchange (Bonfante and Genre, 2010). In addition to the nutritional benefit, it was reported that plants gain in fitness, have increased growth rates and an improved disease resistance (Smith and Read, 2008).

As a constituent of amino acids – and nucleotides, nitrogen (N) is one of the most important elements for life. Due to the high mobility of nitrate and ammonium in soil, it was assumed for a long time that the depletion zone around the roots of plants diminished rather quickly. Consequently, AM fungi were expected to play only a minor role in N uptake for plants (Hodge and Storer, 2015). However, evidence is accumulating that the mycorrhizal symbiosis is also important for the plant's N nutrition (Courty *et al.*, 2015). Depending on the plant-fungal combination and soil N sources, it was demonstrated that plants can receive up to 42% of the N from the AM symbiont (Frey and Schüepp, 1993; Mäder *et al.*, 2000; Govindarajulu *et al.*, 2005; Walder *et al.*, 2012).

Ammonium seems to play a main role as an N source in the AM symbiosis; in contrast to nitrate can be directly assimilated by the GS/GOGAT pathway (Hodge and Storer, 2015): In the GS/GOGAT pathway, ammonium is incorporated into glutamine by the glutamine synthetase (GS) and further into glutamate by the glutamine oxoglutarate aminotransferase (GOGAT). In subsequent metabolic steps, N is incorporated in other amides and amino acids such as alanine, asparagine and arginine. Arginine is the most common N form transported in plants and AM fungi (Govindarajulu *et al.*, 2005; Cruz *et al.*, 2007). Stable isotope labeling experiments have shown that N is taken up by the ERM and incorporated into amino acids whereby arginine constitutes up to 90% of all labeled amino acids (Govindarajulu *et al.*, 2005). In the arbuscules, arginine is metabolized in the urea cycle and the released ammonium is translocated between the fungal plasma membrane and the periarbuscular membrane, the periarbuscular space. Translocation and uptake are accomplished by specialized nutrient transporters located in the fungal and plant membrane (Courty *et al.*, 2015; Hodge and Storer, 2015).

In plants and fungi, specialized transporters take up ammonium from the environment for internal transport and symbiotic exchange. The first described ammonium transporters (AMT) were ScMep1 (*Saccharomyces cerevisiae*, Marini et al. 1994) and AtAmt1;1 (*Arabidopsis thaliana,* Ninnemann et al. 1994), followed by many more in annual plants (Marini *et al.*, 1997; Gazzarrini *et al.*, 1999; Sohlenkamp *et al.*, 2000; Salvemini *et al.*, 2001; Sonoda *et al.*, 2003; D'Apuzzo *et al.*, 2004), perennial plants (Selle *et al.*, 2005; Couturier *et al.*, 2007) and in ectomycorrhizal (Javelle et al. 2003; Javelle et al. 2001) and AM fungi (Javelle *et al.*, 2001; Javelle *et al.*, 2003; López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011; Ellerbeck *et al.*, 2013).

In plants, nitrate is considered to be the main N source in fertile soils, and indeed, many plant nitrate transporters have been described (Miller *et al.*, 2007). Sixty transporters were identified in *A. thaliana* (Feng *et al.*, 2011), and even more in *Oryza sativa* (Araki and Hasegawa, 2006; Cai *et*

*al.*, 2008) and *Lycopersicon esculentum* (Hildebrandt *et al.*, 2002). However, in *R. irregularis,* only one nitrate transporter was identified so far (GiNT; Tian et al. 2010) and another one has been predicted (Tisserant *et al.*, 2012), indicating that nitrate uptake is less relevant in the symbiosis.

Apart from N, also phosphorus (P) is made available to plants by AM symbiosis (Karandashov and Bucher, 2005). In mycorrhizal symbiosis, the amount of P transferred from the fungus to the plant can range from a small percentage up to full nutrition of the plant (Paszkowski, 2006; Javot *et al.*, 2007). Inorganic phosphate (Pi) at the soil-hyphal interface is translocated to the fungal cytoplasm with the help of phosphate transporters and then transported into the periarbuscular space. In AM fungi only three transporters have been described so far in the three AM fungi *Glomus versiforme, R. irregularis* (formerly *Glomus intraradices)* and *Glomus mosseae* (GvPT, GintPT and GmosPt) (Harrison and van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001; Benedetto *et al.*, 2005); they all belong to the major facilitator superfamily similar to those described in plants (Javot *et al.*, 2007; Tatry *et al.*, 2009). In the periarbuscular space, Pi is taken up by the plant through $H^+$:Pi transporters that are localized at the periarbuscular membrane and are only expressed upon mycorrhization. Expression and localization of these transporters has been shown in many plant species as *Medicago truncatula* (MtPT4, Harrison et al*.* 2002) , *L. esculentum* and *Solanum tuberosum* (LePT4/StPT4, LePT5/StPT5, StPT3; Nagy et al*.* 2005) and *Populus trichocarpa* (PtPT10; Loth-Pereda et al*.* 2011).

*P. trichocarpa* is an angiosperm tree native in temperate ecosystems with broad adaptive and genetic variability that forms symbioses with ectomycorrhizal and AM fungi. So far, not much is known about the the effects of mycorrhization on N uptake by perennial plants. For *P. tremuloides*, a near relative of *P. trichocarpa,* it has been shown that it is highly capable of inorganic N uptake by high and low affinity uptake systems enabling the tree to grow in nitrate and ammonium poor or rich soils supporting the existence of specialized transporters for nutrient uptake and internal distribution (Min *et al.*, 1999; Min *et al.*, 2000). Here, we used Illumina sequencing to investigate the transcriptome of mycorrhizal and non-mycorrhizal *P. trichocarpa* (poplar), as well as the transcriptome of *R. irregularis* in poplar roots under high or low N availability. In both organisms, we analyzed the effects of N availability on gene expression in general and on the expression of N metabolism related genes. As N and P nutrition has a major role in AM symbiosis, we focused on fungal and plant ammonium and phosphate transporters. To deepen our knowledge about nutrient transport in the AM fungus, we analyzed the transportome of *R. irregularis* in the IRM.

## 4.3 **Results and Discussion**

### 4.3.1 Experimental design

AM and non-mycorrhizal plants were subjected to low-N and high-N nutrition to investigate which genes, especially transporters and genes linked to N-metabolism and transfer, were affected by the nutrient conditions. Root samples of three biological replicates per condition were sampled; RNA was extracted and sequenced (Figure 4.1). As the samples of AM plants contained IRM, our experimental set up also permitted the comparative analysis of the *R. irregularis* transcriptome in low-N and high-N conditions inside the root systems of colonized plants. We estimated AM colonization in poplar plants. Non-mycorrhizal poplar plants were not colonized. Root colonization in AM plants was significantly ($P<0.004$, student's t-test) higher in plants grown in high-N conditions (from 75 to 82%) than in low-N condition (from 45 to 59%). Consistent with previous findings, these data confirm that nutrient availability limits the AM colonization rate (Augé *et al.*, 2014). By Illumina sequencing, we got about 105 to 133 million reads per samples. About 79 to 88% of detected reads could be aligned to the genome of *P. trichocarpa*. In AM root samples, 1 to 6% of detected reads could be aligned to the genome of *R. irregularis*.
To validate the RNA sequencing data, we confirmed the expression pattern of a subset of genes by qPCR measurements. All of the eight tested genes exhibited similar gene expression patterns when analyzed by qPCR and RNA sequencing, indicating that changes of gene expression are valid and not biased by the experimental approach (Supplementary Fig. S4.1).

### 4.3.2 Gene expression analysis in *Rhizophagus irregularis* IRM

**Effects of N deficiency on *R. irregularis* gene expression in IRM**From the 30282 predicted *R. irregularis* gene models, 27030 were considered as expressed based on presence of reads. 1015 genes were differentially expressed in low-N compared to high-N conditions. One hundred and ten of these differentially expressed genes (about 0.4% of total expressed genes) exhibited expression changes of more than five-folds (Supplementary Table S4.1). Forty-eight of these highly differentially expressed genes could be identified and assigned to specific functional groups. Interestingly, virtually all differentially expressed genes were up-regulated under N deficiency, indicating that N starvation led to a general induction of gene expression within the AM fungus. It has been already observed in other organisms that N starvation induced transcriptional activity (Scheible *et al.*, 2004; Bi *et al.*, 2007; Voigt *et al.*, 2007; Krapp *et al.*, 2011).

Most of the genes with highly induced expression in the low-N condition encoded proteins involved in cellular and signaling processes as well as in metabolic processes (*i.e.* signal transduction, post-translational modification and intracellular trafficking). We also observed high representation of the functional classes cell growth and membrane biogenesis, and of genes coding for structural components. These changes may indicate that the nutrient regime activated stress-induced signaling cascades, leading to up-regulation of nutrient sensing, uptake, and transport systems as well as induction of defense mechanisms (Supplementary Table S4.1).

**Expression changes in N metabolism-related genes in *R. irregularis***The expression of most genes related to fungal N metabolism was not affected under low-N condition (Supplementary Table S4.2). Low-N condition induced expression of only seven N metabolism related genes, among them a nitrate transporter and AMTs to which we refer later. High expression of genes coding for enzymes to N metabolism (*i.e.* glutamine synthetase and glutamine oxoglutarate aminotransferase) suggested that there was a high metabolic rate of N in the IRM as proposed by Gomez *et al.* (2009) and Guether *et al.* (2009).

High expression of arginase is another indicator for a rapid turnover of N in the fungal arbuscule. Arginine is one of the main N carriers from the ERM to the IRM (Govindarajulu *et al.*, 2005; Cruz *et al.*, 2007). Interestingly, apart from transcripts coding for the GS/GOGAT pathway, we detected similar expression levels of nitrilases in low and high-N conditions. Nitrilases hydrolyze nitrile compounds into carboxylic acids and ammonia but their biological role is largely unknown. But, there is evidence that they play a role in the microbial colonization process of plants (Howden and Preston, 2009). In bacteria, it was also shown that nitrilases are involved in the conversion of indole-3-acetonitrile to indole- acetic- acid, which is involved in many physiological processes such as cell elongation, cell division, lateral root formation and tissue differentiation, all processes that might facilitate colonization of plant tissue by pathogens or mycorrhizal fungi (Pace and Brenner, 2001; Spaepen *et al.*, 2007; Kumari *et al.*, 2015; Shao *et al.*, 2015). In several ectomycorrhizal fungi, an IAA pathway was identified and the fungus-derived IAA increased hyphal growth and colonization rates (Ek *et al.*, 1983; Krause *et al.*, 2015). Taken together, the constitutive expression of most N metabolism-related genes indicated that adaptation of the fungal metabolism to N deprivation did not involve the transcript level. Instead, it may have rather involved changes in protein turnover or enzyme activity, and metabolite interconversion and reallocation.

**Differentially expressed nutrient transporters of *R. irregularis***Apart from ammonium and phosphate transporters, which we discuss below, we identified 18 additional differentially expressed transporters, all induced under low-N condition (Table 4.1). Amongst them are nitrate, ammonium, amino acid transporters and transporter of the ABC superfamily. As we analyze here only the IRM our data suggests that under N limiting conditions the AM fungus offers more N to the host plant. However, a direct link of transporter expression and the amount of actually transferred nutrients
could not be proven, yet (Walder *et al.*, 2015). It is assumed that N is transferred from the AM fungus to the host plant in form of ammonium. However, increased expression of fungal amino acid transporters may indicate an export of amino acids serving as N and S source for the plant. Further, an urea transporter, transporters actively involved in intracellular trafficking and carbohydrate transport, and a zink transporter were up-regulated in low-N conditions.

Since colonization by AMF is known to increase plant P uptake (Smith *et al.*, 2003), we also investigated the expression pattern of fungal phosphate transporters. We measured the expression level of the first characterized phosphate transporter (PT) in *R. irregularis, GiPT1* (Maldonado-Mendoza *et al.*, 2001) and of the six additional recently identified phosphate transporters (from *RiPT2* to *RiPT7*) (Walder et. al 2016). Here, the seven phosphate transporters were expressed; *GiPT1* was only marginally expressed compared to the six other transporters (Table 4.2). The significant induction of *GiPT7* in the low-N condition suggested that *GiPT7* could be involved in the transfer of P from the fungus to the plant and that the AM fungus could deliver more P. Alternatively, N-starvation may signal general nutrient deficiency resulting in the increased expression of nutrient transporters. By alleviating nutrient shortage for the plant, the AM fungus may ensure efficient return of essential carbohydrates from the plant (Olsson *et al.*, 2002; Kiers *et al.*, 2011; Fellbaum *et al.*, 2014).

### 4.3.3 Gene expression analysis in *Populus trichocarpa*

Out of the 41336 expressed sequence tags mapping to the *P. trichocarpa* genome, 946 genes (approximately 2.3 % of all expressed genes) were differentially expressed in poplar roots in the low N condition or upon mycorrhization.

**Effect of N availability on gene expression in non-mycorrhizal poplar**

In absence of a symbiotic interaction partner, 888 genes were differentially regulated by N availability in poplar roots (Supplementary Table S4.3). Among those N-deficiency induced

expression of 304 genes. One hundred and eleven of the 304 genes were assigned to functional classes of the eukaryotic orthologous groups. Most of the genes over-expressed in the low-N condition were involved in metabolic processes (*i.e.* secondary metabolite synthesis, transport and catabolism, transport of inorganic ions and transport and metabolism of carbohydrates). Within the group of secondary metabolite synthesis we observed that the expression level of many members of the cytochrome P450 and 2-oxoglutarate and Fe (II)-dependent oxygenase superfamily members were affected in low-N condition (Supplementary Table S4.3). Both gene families encode proteins involved in reduction or incorporation of oxygen (www.uniprot.org, Bolwell et al. 1994).

Further, several UDP-glycosyltransferases were up-regulated; they are known to be fundamental for the biosynthesis of natural compounds. By transferring activated sugar moieties to their acceptor molecule they are affecting the bioactivity of secondary metabolites, amino acids, phytohormones, etc., functional groups which we found over-represented when comparing low N and high N conditions using MAPMAN annotations (Fig. 4.2). Consistent with a role of glycosyltransferases in many fundamental processes, they are also presumed to be involved in stress responses (reviewed in (Ross *et al.*, 2001; Lim and Bowles, 2004; Wang, 2009). Low-N conditions further induced the expression of members of the major facilitator superfamily and ammonium and nitrate transporters to which we refer further below.

Defense related genes (*i.e.* MAP kinases and other protein kinases, ethylene forming enzymes and noduline transporters, Supplementary Table S4.3) were down-regulated in low-N condition. Consistent with previous observations (Liu *et al.*, 2003; Güimil *et al.*, 2005), the lowered defense may contribute to maintain and stimulate symbiotic interaction to improve its nutrient supply.

Further, we observed a down-regulation of the sulfur metabolism in low-N condition (Fig. 4.2). A co-regulation of S metabolism and N limitation has already been observed previously. Low-N conditions reduced accumulation of ATP sulfurylases and APS reductases, both normally induced upon sulfur starvation (Leustek *et al.*, 2000; Nocito *et al.*, 2007).

**Effect of mycorrhization on gene expression in poplar roots upon N-deficiency**

Comparison of transcript abundance in mycorrhizal *vs.* non-mycorrhizal roots in low-N conditions yielded 37 differentially expressed genes (Supplementary Table S4.4). Many of these genes encoded transcription factors involved in control of secondary metabolism and cell morphogenesis (Dubos *et al.*, 2010). Six genes were over-expressed upon mycorrhization. Two genes encoded the orthologues of the Arabidopsis gibberillic acid signaling related DELLA transcription factors RGA-

like 1. Interestingly, upon mycorrhization the RGA-like 1 was nearly 700 times overexpressed. Further, transcription factors, including one gibberillic acid signaling related GRAS transcription factors were down-regulated. Gibberillic acid is a key player in the regulation of mycorrhizal symbiosis as down-regulation of DELLA at the protein level resulted in decreased mycorrhization (Floss *et al.*, 2013; Golldack *et al.*, 2014; Gobbato, 2015). Taken together, induced expression of DELLA protein expression suggests that N-deficiency leads to induction and maintenance of symbiosis.

Interestingly, we also found a putative chitinase highly induced upon mycorrhization. Specific expression of chitinase was reported in arbusculated cells (Bonanomi *et al.*, 2001; Hogekamp *et al.*, 2011), where it is thought to contribute to the initiation of plant immunity (Tromas *et al.*, 2012). Chitinase was also up-regulated in a recent RNA-sequencing analysis on AM roots of *Lotus japonicus* (Handa *et al.*, 2015).

**Effect of mycorrhization on gene expression in poplar roots under high-N conditions**Under high-N conditions, a total of 179 genes were differentially regulated upon mycorrhization (Supplementary Table S4.5). Seventeen genes exhibited induced expression upon mycorrhization. Among them were genes encoding transcription factors, serine carboxypeptidases and interestingly also germin-like proteins and yellow stripe like (YSL) proteins. The latter two are involved in developmental processes and biotic stress responses (Lou and Baldwin, 2006; Ham *et al.*, 2012; Hofstetter *et al.*, 2013). *Pseudomonas syringae*, for instance, has been shown to secrete virulence factors into the host cell using YSL metal ion transporters (Conte and Walker, 2012; Hofstetter *et al.*, 2013). Therefore it may be possible that YSL transporters also play a role in AM colonization processes. Similarly, germin-like proteins are involved in mycorrhization and root nodule formation (Doll *et al.*, 2003; Güimil *et al.*, 2005; Puppo *et al.*, 2013).

Among the 162 down-regulated genes upon mycorrhization were genes encoding proteins with functions in carbohydrate transport and metabolism as well as in post-translational modification, protein turnover and chaperones (Supplementary Table S4.5). Consistently, MAPMAN classification-based over-representation analysis found transcripts with functions in CHO metabolism, glycolysis, TCA cycle/organic transformation over-represented upon mycorrhization under full nutrient conditions (Fig. 4.2). Down-regulation of these genes might indicate that under full nutrient conditions the plant reduced transfer of the valuable carbon as it has access to all essential nutrients by itself. It was already shown that carbon supply of the host plant triggers N transfer in mycorrhizal symbiosis and it was shown as well that the fungus rewards the plant with

the better carbon source. But in both cases, it has been assumed that the fungus is the driving factor of the symbiotic nutrient exchange (Fellbaum *et al.*, 2012; Fellbaum *et al.*, 2014).

Two phosphate starvation induced genes were also up-regulated. Under phosphate starvation, it was shown that the plant triggered expression of the microRNA species miR399 in the shoots (Bari *et al.*, 2006; Chiou *et al.*, 2006) which is then transported to the roots targeting PHO2 (an E2 ubiquitin conjugating enzyme) for degradation (Chiou and Lin, 2011). PHO2 encodes an E2 ubiquitin conjugating enzyme located in the endomembrane system targeting Pht1 members (phosphate-$H^+$-symporter) for degradation. (Bari *et al.*, 2006; Lin *et al.*, 2008). It was also shown that with these regulatory steps a class of non-coding RNA was induced, AT4 and induced phosphate starvation (Aung *et al.*, 2006; Bari *et al.*, 2006). Both mimic the target of miR399, scavenge it and lessen the phosphate starvation response (Franco-Zorrilla *et al.*, 2007). Here, we observed that high-N conditions led to increased expression of two phosphate starvation-induced genes, suggesting that the N status of the plant determines the P demand. The fact that mycorrhization reduced expression of the P starvation induced genes suggested further that the fungus alleviates phosphate stress of the plant. Specific up-regulation of mycorrhiza-inducible phosphate transporters (see section below) corroborates our hypothesis.

Consistent with the induction of S-assimilation-related genes (Fig. 4.2), we found genes involved in S metabolism down-regulated in AM plants (Supplementary Table S4.5). With respect to S metabolism, recent studies have shown that AM fungi increased sulfur supply and content of the AM plants (Casieri *et al.*, 2012; Sieh *et al.*, 2013; Giovannetti *et al.*, 2014; Gerlach *et al.*, 2015). It is thought that amino acids are transported from AM fungus to the plant, and that methionine and cysteine may cover, in this way, the plant's demand of S (Whiteside *et al.*, 2012).

The role of the regulation of defense genes in AM symbiosis needs still to be investigated. Comparative transcriptome analysis showed that AM fungi as well as pathogenic fungi induced expression of a common set of genes, showing that there are similarities in symbiotic and pathogenic infection pathways (Güimil *et al.*, 2005). It has been shown that the mycorrhization level correlated with the expression of defense related genes. Upon mycorrhization, defense related genes were shown to be either suppressed or get induced in early stages of AM colonization (Liu *et al.*, 2003). High amounts of jasmonic acid as well as up-regulation of PR family and other defense related genes led to decreased mycorrhization rates (Ruiz-Lozano *et al.*, 1999; Jung *et al.*, 2012; Gutjahr *et al.*, 2015). On the other hand, it was postulated that the AM fungus is able to bypass the immune response of the plant by suppressing or counteracting actively the immune response of the plant (Bennett *et al.*, 2009; Campos-Soriano *et al.*, 2010; Kloppholz *et al.*,

2011). In agreement with Güimil *et al.* (2005), we observed the down-regulation of the PR gene expression which might underpin the hypothesis that PR genes are directly involved in regulation of the mycorrhization rate.

**Effect of mycorrhization and N availability on poplar N metabolism**Interested in the N metabolism, we screened for differentially expressed genes in mycorrhizal and non-mycorrhizal conditions. We found differentially expressed genes in all three comparisons (*i.e.* –N+Gi vs –N-Gi, -N-Gi vs +N-Gi, +N+Gi vs +N-Gi, Fig. 4.2). In non-mycorrhizal conditions, 24 genes were differentially expressed upon N deprivation (Supplementary Table S4.6). Of those, 14 genes exhibited reduced expression. Most of these genes encoded glutamine synthetases and glutamate oxoglutarate aminotransferases and asparagine synthetases as well as one predicted AMT. On the other hand, four AMT and two possible urease accessory proteins, which are necessary for activation of ureases (Witte *et al.*, 2005) were also induced.

The comparison between mycorrhized and non-mycorrhized plants in low-N conditions revealed expression changes in only four genes (Supplementary Table S4.6), indicating that mycorrhization plays no role in the regulation of the expression of N metabolism - related genes. With respect to N uptake and transport, down-regulation of the expression of four AMTs in the low-N condition and induction of another AMT indicated that N uptake is regulated at the transcript level. While some transporters were down-regulated other transporters were induced upon N deficiency or mycorrhization (Table 4.3).

**Effect of mycorrhization and N availability on poplar ammonium and phosphate transporters**In addition to the fourteen described ammonium transporters in *P. trichocarpa* (Couturier *et al.* (2007)), we identified 6 more AMTs. By performing phylogenetic analysis, we could assign one gene to the AMT1 transporter family, three to the AMT3 family and two more genes clustered to the AMT4 transporters (Supplementary Figure S4.2). Transcripts of PtrAMT1-2, PtrAMT1-3 and PtrAMT1-4 were induced upon mycorrhization by the ectomycorrhizal fungi *Paxillus involutus* and *Amanita muscaria* (Selle *et al.*, 2005; Couturier *et al.*, 2007). However, here, PtrAMT1-2 was one of the most expressed ammonium transporters through all experimental conditions but down-regulated in mycorrhizal conditions (Table 4.3). The other two transporters were constitutively, although barely, expressed. Interestingly, three members of the AMT4 family (PtrAMT4-1, PtrAMT4-2, PtrAMT4-3) were specifically induced upon mycorrhization, independently of the N supply. Consistent with our observation, an induction of AMT4 members has been observed in several plant species (Guether *et al.*, 2009; Kobae *et al.*, 2010; Ruzicka *et al.*, 2012; Koegel *et al.*, 2013), making these genes good general markers for mycorrhiza, whatever the plant species. Specific induction of AMT4 transporters suggested that they may be specifically located at the plant-fungal interface, i,e, the periarbuscular membrane and are likely important for a functional symbiosis. Consistent with our hypothesis, it has been shown that a *M. truncatula* phosphate transporter (MtPT4) and an ammonium transporter (MtAMT2;3) were specifically expressed at the periarbusuclar membrane. Silencing of these transporters led to premature arbuscule degeneration and therefore to an insufficient symbiosis (Javot *et al.*, 2007; Breuillin-Sessoms *et al.*, 2015). However, to confirm the specific localization of the populus AMT4 transporters at the periarbuscular membrane, further expreriments need to be conducted.

Further, we found five more AMTs differentially expressed (Table 4.3). The ammonium transporters AMT2-2 and AMT3-1 were significantly induced in low-N conditions. Moreover, the newly identified Potri.013G049600.1 was induced in the non-mycorrhizal high-N condition and Potri.T000200.1 showed a similar expression pattern as AMT1-1, with induced expression in the non-mycorrhizal low-N condition.

As AM fungi are known to be important for N and P transfer to the host plant we investigated also the expression levels of plant phosphate transporters upon N starvation and mycorrhization. A comprehensive study by Loth-Pereda *et al.* (2011) already investigated the expression of twelve Pht1 phosphate transporters in poplar species. Three Pht1 transporters (*PtrPht1-1, PtrPht1-6* and *PtrPht1-11*) were down-regulated during AM symbiosis. However, *PtrPht1-10* was specifically induced in AM roots, while *PtrPht1-8*, a close homologue of *PtrPht1-10*, was not expressed at all.

They proposed that phosphate transporters of poplar have distinct roles in the acquisition and translocation of their substrate. Specific induction of transporter especially induction of mycorrhiza specific transporters enable them to extract P from deprived soils. In our experimental conditions all transporters were expressed. Specific induction of *Pht1-8* underpins the hypothesis of Loth-Pereda *et al.* (2011) that phosphate transporters of clade I are mycorrhiza-inducible (Table 4.4). Specific induction of mycorrhiza-inducible phosphate transporters has been demonstrated also in other plant species (Rausch and Bucher, 2002; Glassop *et al.*, 2005; Nagy *et al.*, 2005; Loth-Pereda *et al.*, 2011; Walder *et al.*, 2015). As shown by Harrison *et al.* (2002) the mycorrhiza-inducible MtPT4 is located at the periarbuscular membrane and is essential for the establishment of a functional symbiosis. We hypothesize that that this might also be true for Pht1-8.

Comparing high- and low-N conditions, we found the transporters Pht1-1 and Pht1-2 induced in the high-N conditions whereas Pht1-7 was induced only in the non-mycorrhizal high-N condition. By contrast, expression of Pht1-4 and Pht1-11 was increased in the low-N condition in both mycorrhizal and non-mycorrhizal poplar roots. Taken together, these data suggested that the expression of phosphate transporters depends on the N-status of the plant. High-N conditions may allow stronger expression of phosphate transporters as high-N conditions may signal a good nutritional status of the plant. Induction of the expression of phosphate transporters in low-N conditions may be explained when we assume that these transporters are involved in inter-cellular phosphate transport as nutrient deficiency generally leads to a shift in metabolic processes (Voigt *et al.*, 2007; Krapp *et al.*, 2011; Garapati *et al.*, 2015; Jost *et al.*, 2015).

## 4.4 **Conclusion**

Here, we demonstrate that N availability has significant effects on plant and mycorrhizal gene expression. In the IRM of the AM fungus, N starvation caused major changes in the expression of genes belonging to the functional categories of cell growth, membrane biogenesis and cell structural components. Moreover, the newly characterized mycorrhizal AMT and one of the newly identified mycorrhizal phosphate transporters were significantly induced upon N limitation. We hypothesize that these two transporters are key elements of AM nutrient transfer and that in the low-N condition, more N but also more phosphate is transferred to the plant symbiont.

On the plant side we found that N deficiency had significant effects on metabolic processes. Key enzymes of the GS/GOGAT pathway were down-regulated upon N limitation as well as elements involved in N translocation and transport such as AMTs, urease and arginase, suggesting that N metabolism is tightly regulated. We identified six new AMTs. Among the ammonium and

phosphate transporters, we identified three mycorrhiza-inducible AMTs and one mycorrhiza-inducible phosphate transporter that could be used as molecular markers. Specific induction of these transporters suggested a possible localization at the periarbuscular membrane, making them key elements of symbiotic nutrient exchange and essential components of a functional symbiosis. Specific induction of plant ammonium and phosphate transporters has already been shown in previous studies (Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Nagy *et al.*, 2005; Javot *et al.*, 2007; Koegel *et al.*, 2013) . These transporters were localized at the periarbuscular membrane and it was shown as well that their functionality is essential for a functional symbiosis. In the future, it will be interesting to elucidate the contribution of the transporters to nutrient transfer and nutritional status of the plant.

## 4.5 **Material and methods**

### 4.5.1 Growth conditions

*Populus trichocarpa* (derived from cuttings, clone 10174, Orléans, France) grew in an autoclaved (120°C, 20 min) quartz sand (Alsace, Kaltenhouse, Trafor AG, Basel): zeolithe (Symbion, Czech Republic) substrate (1:1, w:w) substrate ( 1:1; w:w). Plants grown under "high-N" conditions were fertilized once a week with 10 ml of Hoagland standard solution, modified after Gamborg & Wetter (1975). Plants grown under "low-N conditions" received a solution in which the ($Ca(NO_3)_2 \cdot 4H_2O$, $KNO_3$, $NH_4H_2PO_4$ and $(NH_4)_2MoO_4$ from the original solution were replaced by $CaCl_2 \cdot 2H_2O$, KCl, $KH_2PO_4$ and $Na_2MoO_4$. To analyze the fungal effect on plant nutrition under N stress plants grown simultaneously in the same conditions were inoculated with 1 ml liquid inocula of *Rhizophagus irregularis* (formerly *Glomus intraradices, abbr. Gi)*, strain BEG75 (Inoculum Plus, Dijon, France), dissolved in 0.01 M citrate buffer (pH6), containing ca. 110 spores (+N+Gi, -N+Gi). Plants grew for 12 weeks in a greenhouse at about 28:15°C day:night temperature.

### 4.5.2 Harvest and colonization measurements

Root systems were freed from substrate and washed under the tap. Primary roots and thick roots were removed. Subsamples of the fine roots were randomly taken for colonization measurements and RNA extraction. For RNA extraction, subsamples were snap frozen in liquid nitrogen and stored at -80°C.

For colonization measurements fresh root samples were immersed in 10% KOH and stored at 4°C for 18 h. Roots were rinsed and kept for 1 h at room temperature in 2% HCl. After cleaning the

root with tap water they were stained in 0.005% trypan blue (w:v in lactic-acid: glycerol: water, 1:1:1, v:v:v) at 4°C overnight. The next day the roots were rinsed with water and destained in lactic-acid:glycerol:water (1:1:1; v:v:v) for several days. Colonization was estimated by the grin line intersection method (Brundrett *et al.*, 1984). Statistics were applied using t-test ($p>0.05$) in Microsoft Excel 2010.

### 4.5.3 RNA isolation

RNA was extracted from three biological replicates per condition. Lyophilized samples were processed using the Qiagen RNeasy kit according to the manufacturer's protocol. DNA was removed using the DNA-free™ Kit, DNase Treatment and Removal Reagents (AMBION® by life technologies). Quality of total RNA was assessed by A260/280 ratios.

### 4.5.4 Data analysis and bioinformatics

Preparation of twelve libraries and 2 x 100bp Illumina HiSeq mRNA sequencing (RNA-Seq) was performed by Beckman Coulter Genomics (Danvers, MA, USA). Raw reads were trimmed for quality and aligned either to the *Rhizophagus irregularis* reference transcripts available at the JGI database (http://genome.jgi-psf.org/Gloin1/Gloin1.home.html) or to the *Populus trichocarpa* v3 reference transcripts from Phytozome 10.3 (http://phytozome.jgi.doe.gov/pz/portal.html) using CLC Genomics Workbench v7 (Supplementary Table S4.8). For mapping, the minimum length fraction was 0.9, the minimum similarity fraction 0.8 and the maximum number of hits for a read was set to 10. The unique and total mapped reads number for each transcript was determined, and then normalized to RPKM (Reads Per Kilobase of exon model per Million mapped reads). Intact pairs were counted as two, broken pairs as one. To identify differentially regulated transcripts the Baggerley test (Baggerly *et al.*, 2003) implemented in CLC Genomic workbench was applied to the data. The Baggerley test compares the proportions of counts in a group of samples against those of another group of samples. Samples are given different weights depending on total amount of counts in each sample. The weights are obtained by assuming a Beta distribution on the proportions in a group, and estimating these, along with the proportion of a binomial distribution, by the method of moments. The result is a weighted t-type test statistic. In addition Benjamini & Hochberg multiple-hypothesis testing corrections with False Discovery Rate (FDR) were used. In the current analysis transcripts with a FDR corrected p-value $<0.05$ were used.

Functional classification of differentially expressed genes in poplar was performed in the Classification SuperViewer tool (Provart and Zhu, 2003) (http://bar.utoronto.ca/) using the best corresponding Arabidopsis TAIR10 hit name.

### 4.5.5 cDNA synthesis and quantitative reverse transcription-PCR (qPCR)

cDNA synthesis was performed on RNA extracts from the same three biological replicates per condition as used for HiSeq analysis using the iScript$^{TM}$cDNA SynthesisKit (BIORAD laboratories, Paolo Alto, CA, United States). Gene-specific primers for qPCR were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and tested with amplify 3.1 (http://engels.genetics.wisc.edu/amplify). For normalization *P. trichocarpa* ubiquitin was used as a reference gene. Three biological and two technical replicates were analyzed for each gene. PCR was conducted with the following settings: initial denaturation at 95°C for 3 min, 45 cycles of at 95°C for 30 s, 60°C for 1 min and 72°C for 30 s.

### 4.5.6 Phylogenetic analysis

The neighbour-joining tree was made using amino acid sequences of AMTs of *P. trichocarpa* of more than 400 amino acids in length. Sequences were aligned using ClustalW of the MEGA6.06 package (Tamura *et al.*, 2013) with following multiple alignment parameters: gap opening penalty 15, gap extension penalty 0.3, Gonnet protein weight matrix and a delay divergent cutoff value of 30%. The phylogenetic tree was computed using the neighbor-joining method, using the Poisson correction model with pairwise deletion option. Bootstrapping was performed with 1000 replicates.

## 4.6 Acknowledgments

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## 4.7 **Figures and tables**

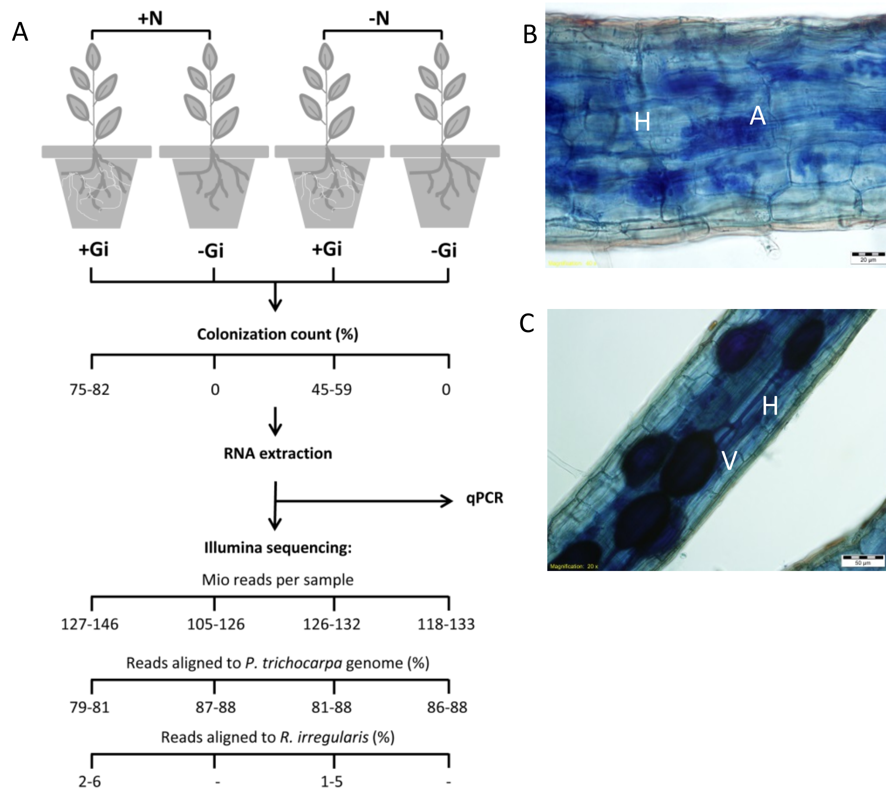



**Figure 4.1** Working model. (A) *Populus trichocarpa* cuttings were inoculated with *Rhizophagus irregularis* (+Gi) or grew without the mycorrhizal fungi (-Gi). Systems were fertilized either with standard Hoagland solution (+N) or Hoagland solution devoid of a nitrogen source (-N). Plants grew in the green house for 12 weeks. At harvest time roots were freed from substrate, snap frozen and a subsample was used for colonization count. Total RNA was extracted, mRNA sequencing was performed, data were processed and evaluated. Pictures from mycorrhizal *P. trichocarpa* roots (B and C).Mycorrhizal structures are indicated by white characters. Arbuscules, A; Hyphae, H; Vesicle, V.

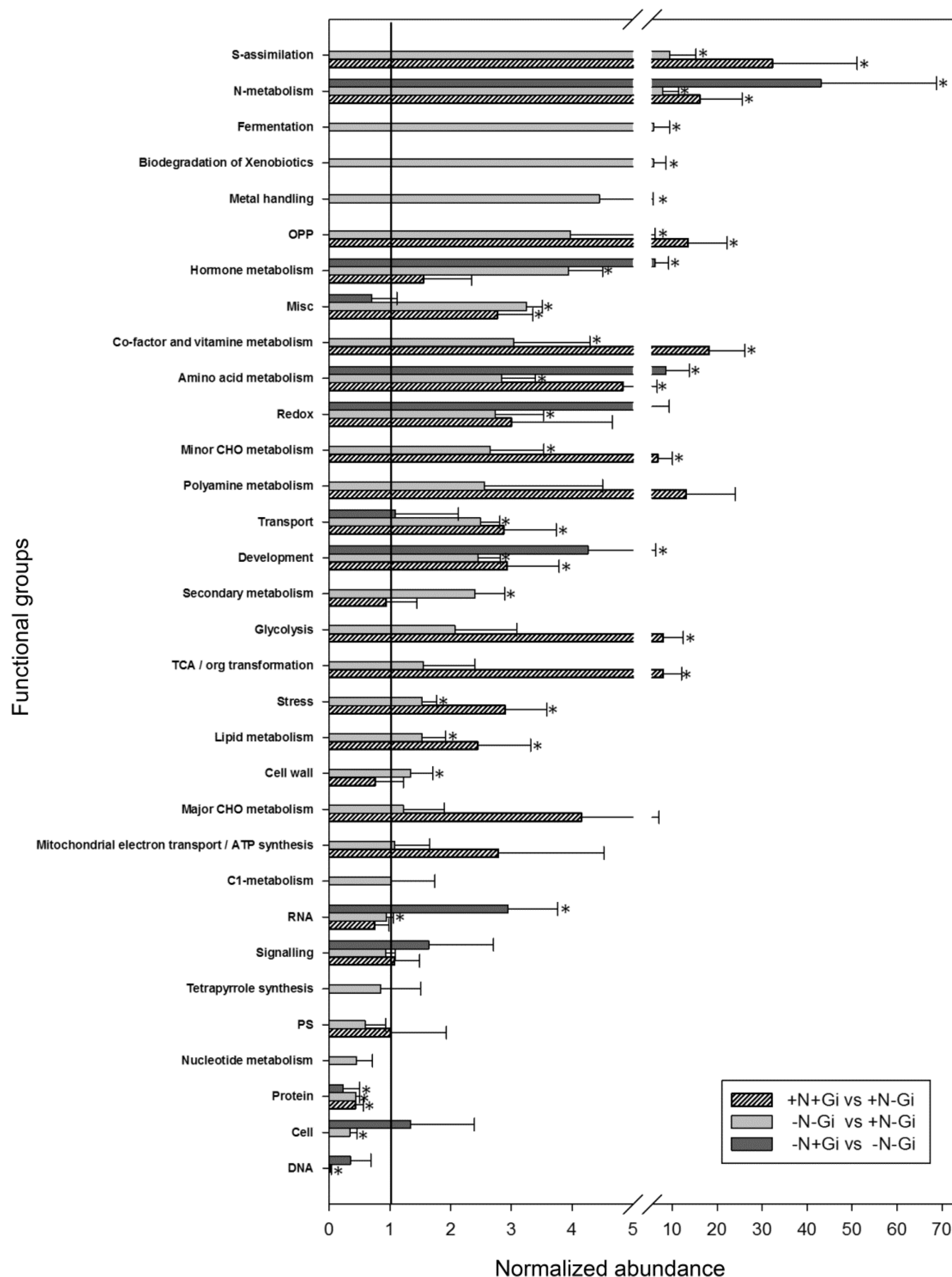



**Figure 4.2 MAPMAN-based functional classification of the poplar genes shown in Supplementary Tables S3, S4 and S5.** Poplar genes were classified to their biological processes according to their *Arabidopsis* homologue using the Classification SuperViewer tool (Provart and Zhu 2003). Classification was based on MAPMAN classes (Ath_AGI_LOCUS_TAIR10_Aug2012). Relative abundance of genes was normalized to frequency of class in the Arabidopsis reference data set. Mean and SD were calculated for 100 bootstrap repetitions. Significantly under- or over-represented sequences were labeled by an asterisk (p<0.05).

**Table 4.1 Differentially expressed transporter genes of *R. irregularis*.** Gene expressions of low-N conditions were tested against high-N condition

| | Eukaryotic orthologous groups | | | | Mean RPKM | |
|---|---|---|---|---|---|---|
| **Transcript ID** | **Defline** | **Class** | **Group** | **log2ratio** | **-N** | **+N** |
| 147773 | Protein transporter of the TRAM[†] superfamily | Intracellular trafficking, secretion, and vesicular transport | Cellular processes and signaling | 0.7 | 55 | 35 |
| 349661 | Protein transporter of the TRAM[†] superfamily | | | 1.1 | 73 | 34 |
| 339691 | Vesicular amine transporter | | | 1.2 | 110 | 49 |
| 34969 | Amino acid transporters | Amino acid transport and metabolism | Metabolism | 1.0 | 25 | 12 |
| 40376 | Amino acid transporters | | | 1.6 | 94 | 30 |
| 94248 | Urea transporter | | | 1.8 | 85 | 24 |
| 67708 | GDP-fucose transporter | Carbohydrate transport and metabolism | | 1.0 | 28 | 14 |
| 289764 | GDP-fucose transporter | | | 1.2 | 158 | 70 |
| 218287 | Ammonia permease (AMT) | Inorganic ion transport and metabolism | | 1.7 | 87 | 26 |
| 67530 | Na+/dicarboxylate, Na+/tricarboxylate and phosphate transporters | | | 1.8 | 128 | 36 |
| 30566 | Nitrate transporter(MFS*) | | | 2.5 | 40 | 7 |
| 29953 | Predicted nitrate transporter (MFS*) | | | 4.7 | 44 | 2 |
| 334075 | Predicted divalent cation transporter | | | 0.9 | 62 | 34 |
| 291068 | Predicted divalent cation transporter | | | 1.2 | 153 | 69 |
| 67368 | Putative Zn2+ transporter MSC2 (cation diffusion facilitator superfamily) | | | 0.9 | 36 | 20 |
| 286345 | Zn2+ transporter | | | 1.2 | 73 | 31 |
| 344948 | Nucleoside transporter | Nucleotide transport and metabolism | | 0.8 | 35 | 19 |
| 21303 | Transporter, ABC superfamily (Breast cancer resistance protein) | Secondary metabolites biosynthesis, transport and catabolism | | 2.5 | 20 | 4 |
| 341277 | Long-chain acyl-CoA transporter, ABC superfamily (involved in peroxisome organization and biogenesis) | General function prediction only | Poorly characterized | 0.8 | 77 | 44 |
| 9468 | Predicted transporter ADD1 (MFS*) | | | 1.9 | 18 | 5 |

*MFS, Major facilitator superfamily; [†]TRAM translocating chain-associating membrane

**Table 4.2 List of ammonium, nitrate and phosphate transporters in *R. irregularis*.** Gene expressions of low-N conditions were tested against high-N condition. Significant values were highlighted in bold (FDR corrected p-value< 0.05)

| | | Eukaryotic orthologous groups | | | | Mean RPKM | |
|---|---|---|---|---|---|---|---|
| **Name** | **Transcript ID** | **Defline** | **Class** | **Group** | **log2ratio** | **-N** | **+N** |
| GintAMT1 | 337137 | Ammonia permease (AMT) | Inorganic ion transport and metabolism | Metabolism | 1.4 | 67 | 26 |
| GintAMT2 | 314321 | Ammonia permease (AMT) | Inorganic ion transport and metabolism | Metabolism | 0.9 | 74 | 40 |
| GintAMT3 | 218287 | Ammonia permease (AMT) | Inorganic ion transport and metabolism | Metabolism | **1.7** | 87 | 26 |
| GintNT1 | 30566 | Nitrate transporter (MFS*) | Inorganic ion transport and metabolism | Metabolism | **2.5** | 40 | 7 |
| GintNT2 | 29953 | Predicted nitrate transporter (MFS*) | Inorganic ion transport and metabolism | Metabolism | **4.7** | 44 | 2 |
| RiPT1 | 345640 | Inorganic phosphate transporter | Inorganic ion transport and metabolism | Metabolism | -1.3 | 90 | 223 |
| RiPT2 | 22848 | Inorganic phosphate transporter | Inorganic ion transport and metabolism | Metabolism | -0.4 | 18 | 25 |
| RiPT3 | 7378 | Inorganic phosphate transporter | Inorganic ion transport and metabolism | Metabolism | 0.4 | 7 | 6 |
| RiPT4 | 13201 | Inorganic phosphate transporter | Inorganic ion transport and metabolism | Metabolism | -3.0 | 0 | 1 |
| RiPT5 | 346370 | Na+/Pi symporter | Inorganic ion transport and metabolism | Metabolism | 2.7 | 84 | 13 |
| RiPT6 | 49664 | Na+/Pi symporter | Inorganic ion transport and metabolism | Metabolism | -2.1 | 2 | 8 |
| RiPT7 | 67530 | Na+/tricarboxylate and phosphate transporters | Inorganic ion transport and metabolism | Metabolism | **1.8** | 128 | 36 |

*MFS, Major facilitator superfamily;

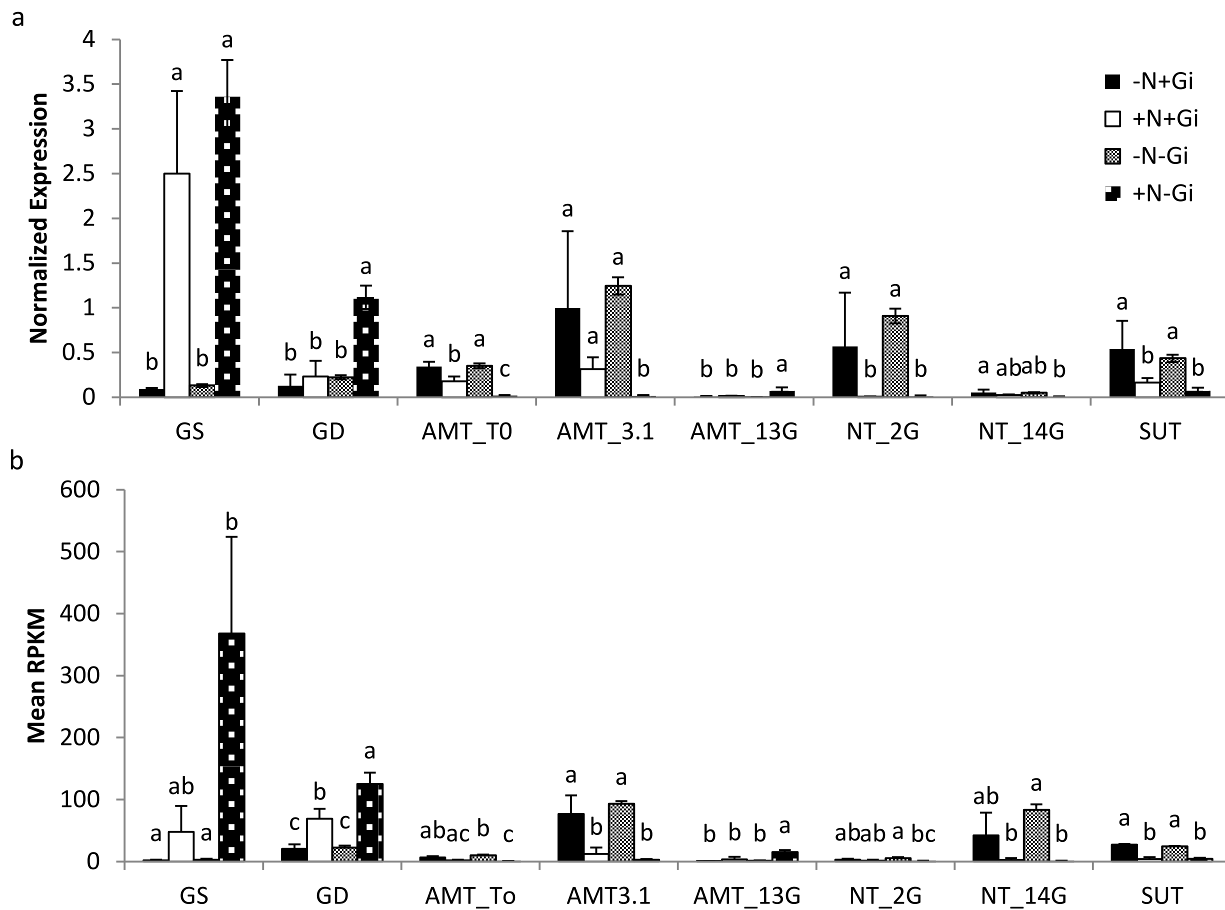
**Table 4.3 AMTs of *P. trichocarpa*.** Significant values were highlighted in bold (corrected FDR p-value< 0.05)

| Name | Transcript ID | Log2ratios | | | | | Mean RPKM | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **-N+Gi vs +N+Gi** | **-N+Gi vs -N-Gi** | **+N+Gi vs +N-Gi** | **-N-Gi vs +N-Gi** | **Myc vs NM** | **-N+Gi** | **+N+Gi** | **-N-Gi** | **+N-Gi** |
| PtrAMT1-1 | Potri.010G063500.1 | 0.3 | -0.4 | 0.2 | **0.9** | -0.2 | 26.72 | 21.65 | 36.36 | 19.25 |
| PtrAMT1-2 | Potri.019G023600.1 | 0.8 | **-0.8** | **-2.2** | -0.6 | **-1.5** | 110.36 | 62.80 | 189.95 | 289.46 |
| PtrAMT1-3 | Potri.008G173800.1 | 1.3 | 0.5 | 0.1 | 0.9 | 0.3 | 0.23 | 0.09 | 0.16 | 0.09 |
| PtrAMT1-4 | Potri.002G255100.1 | 0.9 | 2.8 | 0.1 | -1.9 | 1.2 | 0.03 | 0.01 | 0.00 | 0.01 |
| PtrAMT1-5 | Potri.002G255000.1 | 0.1 | -0.6 | 2.9 | 3.6 | 0.2 | 0.13 | 0.12 | 0.20 | 0.02 |
| PtrAMT1-6 | Potri.009G045200.1 | 0.2 | 0.0 | -1.9 | -1.7 | -1.2 | 1.20 | 1.07 | 1.21 | 3.99 |
| PtrAMT2-1 | Potri.006G102800.1 | -0.9 | 0.4 | 1.3 | 0.1 | 0.9 | 26.48 | 48.00 | 19.84 | 19.15 |
| PtrAMT2-2 | Potri.016G121400.1 | 1.6 | -0.5 | -0.3 | **1.8** | -0.4 | 10.81 | 3.69 | 15.36 | 4.39 |
| PtrAMT3-1 | Potri.001G305400.1 | **2.6** | -0.3 | 2.0 | **5.0** | -0.1 | 76.61 | 12.26 | 93.03 | 2.98 |
| PtrAMT4-1 | Potri.002G047000.1 | 0.2 | 4.1 | 4.0 | 0.1 | **4.1** | 16.91 | 15.22 | 0.98 | 0.93 |
| PtrAMT4-2 | Potri.018G033500.1 | 0.2 | 10.2 | 9.4 | -0.6 | **9.8** | 34.26 | 30.67 | 0.03 | 0.05 |
| PtrAMT4-3 | Potri.005G216000.1 | 0.0 | 7.5 | 6.3 | -1.2 | **6.8** | 51.57 | 51.56 | 0.29 | 0.67 |
| PtrAMT4-4 | Potri.T103600.1 | -0.2 | 5.2 | 6.6 | 1.1 | 5.8 | 1.11 | 1.28 | 0.03 | 0.01 |
| PtrAMT4-5 | Potri.005G106000.1 | 1.1 | 6.8 | 7.7 | 2.0 | 7.0 | 9.46 | 4.34 | 0.08 | 0.02 |
| | Potri.013G049600.1 | -2.2 | -1.0 | **-2.1** | **-3.3** | -2.0 | 0.76 | 3.40 | 1.55 | 15.06 |
| | Potri.019G000800.1 | -1.7 | -0.3 | 2.3 | 0.9 | 1.1 | 1.73 | 5.53 | 2.16 | 1.13 |
| | Potri.T000600.1 | -2.7 | -0.5 | 2.3 | 0.1 | 1.5 | 3.03 | 19.93 | 4.36 | 3.94 |
| | Potri.T000200.1 | 2.0 | -0.6 | 1.3 | **4.0** | -0.4 | 6.73 | 1.64 | 10.04 | 0.65 |
| | Potri.013G040400.1 | 0.1 | 3.9 | 6.9 | 3.1 | 4.7 | 0.94 | 0.85 | 0.06 | 0.01 |
| | Potri.006G247800.1 | 0.0 | 0.4 | 2.4 | 1.9 | 1.1 | 0.05 | 0.05 | 0.04 | 0.01 |

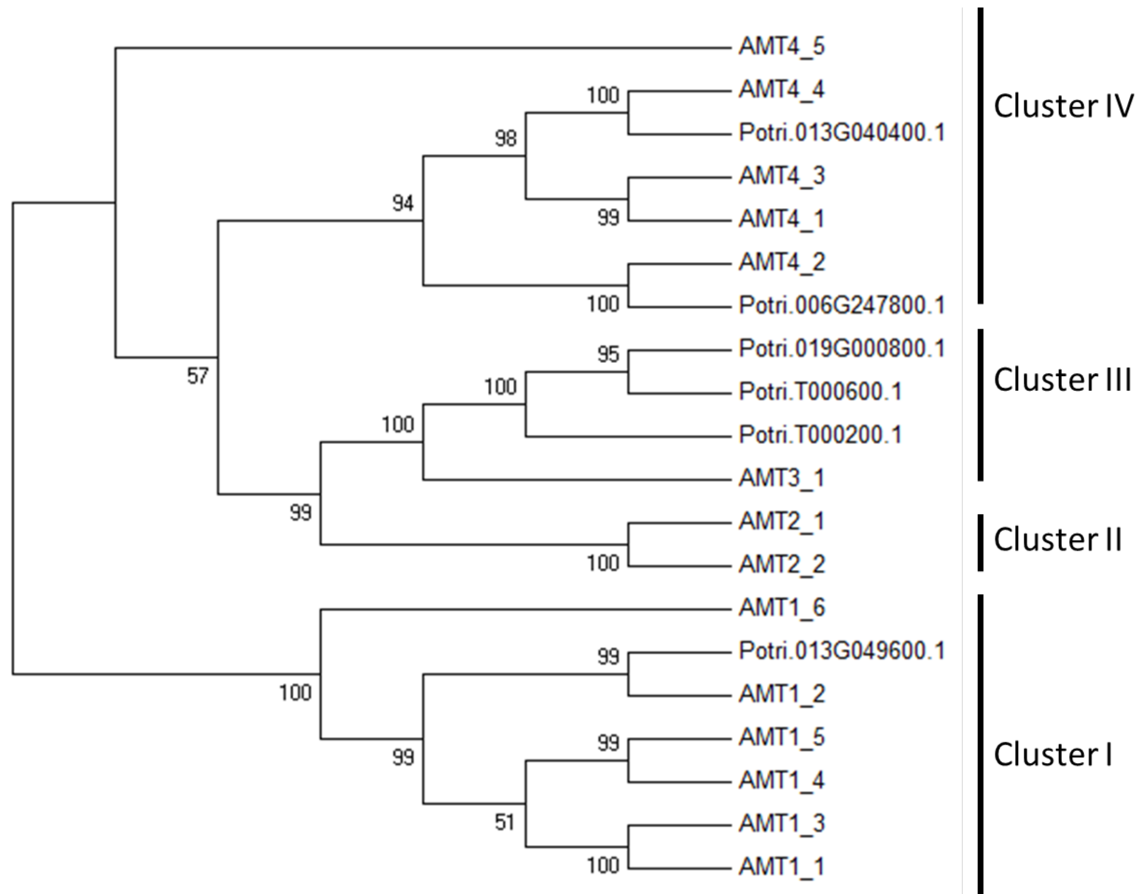
**Table 4.4 Phosphate transporter of the Pht1 family of *P. trichocarpa*.** Significant values were highlighted in bold (corrected FDR p-value< 0.05). Pht transporters belongs to different subfamilies of Pi: $H^+$ symporters : (a) cluster of the AM-inducible Pi, (b) some proteins from both monocots and dicots fall into a highly divergent subfamily ; (c) cluster of dicots Pi, according to Walder et al. (2015)

| | | Log2ratios | | | | | Mean RPKM | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Transcript ID** | **-N+Gi Vs +N+Gi** | **-N+Gi vs -N-Gi** | **+N+GI vs +N-Gi** | **-N-Gi vs +N-Gi** | **Myc vs NM** | **-N+Gi** | **+N+Gi** | **-N-Gi** | **+N-Gi** |
| PtrPht1.1 c | Potri.010G072000.1 | -2.1 | 0.2 | -2.0 | -2.3 | -1.3 | 2.06 | 2.27 | 1.77 | 8.79 |
| PtrPht1.2 c | Potri.010G071700.1 | -1.5 | -0.1 | -0.5 | **-1.5** | -0.4 | 19.40 | 38.76 | 20.59 | 56.69 |
| PtrPht1.3 c | Potri.010G071500.1 | 2.0 | -0.3 | 2.0 | 2.3 | 0.5 | 1.58 | 1.56 | 1.88 | 0.38 |
| PtrPht1.4 c | Potri.005G223500.1 | 0.1 | -0.1 | -1.2 | 0.3 | -0.5 | 67.50 | 26.78 | 73.30 | 61.34 |
| PtrPht1.5 c | Potri.002G038900.1 | -4.8 | -0.3 | -3.5 | -4.5 | -3.1 | 0.14 | 0.34 | 0.18 | 3.93 |
| PtrPht1.6 c | Potri.005G175500.1 | 2.7 | 0.6 | 0.8 | 2.0 | 0.7 | 0.03 | 0.01 | 0.02 | 0.00 |
| PtrPht1.7 c | Potri.005G223600.1 | -1.9 | -0.2 | -1.9 | **-1.7** | -1.2 | 2.51 | 2.53 | 2.79 | 9.18 |
| PtrPht1.8 a | Potri.019G061900.1 | 7.9 | 7.4 | 10.3 | 0.5 | **9.3** | 15.56 | 80.75 | 0.09 | 0.06 |
| PtrPht1.9 c | Potri.002G005500.1 | 0.2 | -0.1 | -0.6 | 0.3 | -0.3 | 3.11 | 1.79 | 3.28 | 2.74 |
| PtrPht1.10 a | Potri.015G022800.1 | 4.7 | 2.8 | 9.3 | 1.9 | 7.1 | 0.05 | 1.21 | 0.01 | 0.00 |
| PtrPht1.11 b | Potri.005G256100.1 | 1.2 | 0.4 | -1.8 | 0.8 | -0.1 | 21.60 | 2.71 | 16.91 | 9.43 |
| PtrPht1.12 c | Potri.001G318500.1 | -1.1 | -3.4 | 1.0 | 2.3 | -1.3 | 0.00 | 0.01 | 0.02 | 0.00 |

## 4.8 Supplementary figures and tables




**Supplementary Figure S4.1 Comparison of the expression of selected *P. trichocarpa* genes using qPCR and RNA-Seq**. Expression of eight *P. trichocarpa* genes involved in N metabolism and uptake or sugar transport. Transcript abundances were measured by (a) real-time polymerase chain reaction and by (b) HiSeq 2000 analysis. (a) Normalized expression values are the mean of three biological and two technical replicates. As a reference transcript ubiquitin was used. Error bars represent the standard deviation. Differences between the conditions were calculated by one-way ANOVA (Tuckey-HSD). (b) Expression values represent mean RPKM (Reads Per Kilobase of exon model per Million mapped reads) of three biological replicates. Differences between the conditions were calculated by the Baggerley's test. Lower case letters represent statistical differences (P-value<0.05). Gs, Glutamin Synthase; GD, Glutamate dehydrogenase; AMT, Ammonium transporter; NT, Nitrate transporter; SUG, Sugar transporter.




**Supplementary Fig. S4.2 Neighbour joining tree of ammonia permeases in *P.trichocarpa***. Gene names or gene ID's are indicated.

**Supplementary Table S4.1 Differentially expressed genes in *Rhizophagus irregularis of* more than 5 fold induced**. Genes expressed under low-N condition were tested against genes expressed under high-N condition.

| | | Eukaryotic orthologous groups | | | Mean RPKM | | log2ratio | |
|---|---|---|---|---|---|---|---|---|
| Transcript ID | Protein ID | Defline | Class | Group | -N | +N | -N vs +N | FDR corrected p-value |
| 152041 | 151929 | Extracellular protein SEL-1 and related proteins | Cell wall/membrane/envelope biogenesis | Cellular processes and signaling | 8 | 2 | 2.4 | 0.010 |
| 340877 | 340765 | Beta-2-glycoprotein I | Extracellular structures | Cellular processes and signaling | 61 | 11 | 2.5 | 0.008 |
| 31602 | 31490 | SNARE protein Syntaxin 1 and related proteins | Intracellular trafficking, secretion, and vesicular transport | Cellular processes and signaling | 5 | 0 | 5.5 | 0.025 |
| 82399 | 82287 | AAA+-type ATPase | Posttranslational modification, protein turnover, chaperones | Cellular processes and signaling | 29 | 1 | 4.6 | 0.006 |
| 582 | 470 | *AAA+-type ATPase* | Posttranslational modification, protein turnover, chaperones | Cellular processes and signaling | 16 | 2 | 3.0 | 0.000 |
| 23506 | 23394 | Molecular chaperones HSP70/HSC70, HSP70 superfamily | Posttranslational modification, protein turnover, chaperones | Cellular processes and signaling | 81 | 11 | 2.8 | 0.007 |
| 4943 | 4831 | Proteins containing BTB/POZ and Kelch domains, involved in regulatory/signal transduction processes | Signal transduction mechanisms | Cellular processes and signaling | 9 | 1 | 2.6 | 0.004 |
| 342832 | 342720 | Serine/threonine protein phosphatase | Signal transduction mechanisms | Cellular processes and signaling | 13 | 3 | 2.3 | 0.015 |
| 26930 | 26818 | Tyrosine kinase specific for activated (GTP-bound) p21cdc42Hs | Signal transduction mechanisms | Cellular processes and signaling | 4 | 0 | 5.3 | 0.036 |
| 55553 | 55441 | Tyrosine kinase specific for activated (GTP-bound) p21cdc42Hs | Signal transduction mechanisms | Cellular processes and signaling | 5 | 1 | 3.1 | 0.040 |
| 89203 | 89091 | Mitochondrial ribosomal protein MRP17 | Translation, ribosomal structure and biogenesis | Information storage and processing | 32 | 5 | 2.5 | 0.001 |
| 337709 | 337597 | Ubiquitin/40S ribosomal protein S27a fusion | Translation, ribosomal structure and biogenesis | Information storage and processing | 166 | 29 | 2.5 | 0.027 |
| 32456 | 32344 | Host cell transcription factor HCFC1 | Cell cycle control, cell division, chromosome partitioning | Metabolism | 242 | 40 | 2.6 | 0.012 |
| 80124 | 80012 | Uroporphyrin III methyltransferase | Coenzyme transport and Metabolism | Metabolism | 68 | 8 | 3.1 | 0.000 |
| 343957 | 343845 | Glycerol-3-phosphate dehydrogenase/dihydroxyacetone 3-phosphate reductase | Energy production and conversion | Metabolism | 44 | 8 | 2.5 | 0.000 |
| 40374 | 40262 | Ca2+/H+ antiporter VCX1 and related proteins | Inorganic ion transport and | Metabolism | 43 | 0 | 6.9 | 0.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Metabolism | | | | | |
| 234538 | 234426 | Ca2+/H+ antiporter VCX1 and related proteins | Inorganic ion transport and Metabolism | Metabolism | 31 | 6 | 2.5 | 0.003 |
| 336400 | 336288 | Ca2+/H+ antiporter VCX1 and related proteins | Inorganic ion transport and Metabolism | Metabolism | 135 | 25 | 2.5 | 0.000 |
| 51017 | 50905 | Cytochrome P450 CYP4/CYP19/CYP26 subfamilies | Lipid transport and Metabolism | Metabolism | 14 | 1 | 3.6 | 0.036 |
| 68747 | 68635 | Cytochrome P450 CYP4/CYP19/CYP26 subfamilies | Lipid transport and Metabolism | Metabolism | 11 | 2 | 2.4 | 0.008 |
| 79437 | 79325 | Cytochrome P450 CYP4/CYP19/CYP26 subfamilies | Lipid transport and Metabolism | Metabolism | 23 | 4 | 2.4 | 0.047 |
| 70904 | 70792 | Phosphatidylserine decarboxylase | Lipid transport and Metabolism | Metabolism | 79 | 13 | 2.6 | 0.000 |
| 105364 | 105252 | SAM-dependent methyltransferases | Lipid transport and Metabolism | Metabolism | 73 | 9 | 3.0 | 0.000 |
| 21303 | 21191 | Transporter, ABC superfamily (Breast cancer resistance protein) | Secondary metabolites biosynthesis, transport and catabolism | Metabolism | 20 | 4 | 2.5 | 0.001 |
| 15042 | 14930 | Predicted membrane protein | Function unknown | Poorly characterized | 63 | 10 | 2.6 | 0.044 |
| 349912 | 349800 | FOG: TPR repeat | General function prediction only | Poorly characterized | 7 | 1 | 3.0 | 0.010 |
| 350234 | 350122 | FOG: Transposon-encoded proteins with TYA, reverse transcriptase, integrase domains in various combinations | General function prediction only | Poorly characterized | 12 | 0 | 5.0 | 0.004 |
| 21299 | 21187 | FOG: Zn-finger | General function prediction only | Poorly characterized | 44 | 9 | 2.3 | 0.029 |
| 3357 | 3245 | Monodehydroascorbate/ferredoxin reductase | General function prediction only | Poorly characterized | 25 | 1 | 4.4 | 0.000 |
| 12504 | 12392 | | | | 1691 | 44 | 5.2 | 0.002 |
| 91752 | 91640 | | | | 4 | 0 | 4.9 | 0.034 |
| 29953 | 29841 | | | | 44 | 2 | 4.7 | 0.000 |
| 33479 | 33367 | | | | 8 | 0 | 4.6 | 0.000 |
| 18518 | 18406 | | | | 5 | 0 | 4.6 | 0.016 |
| 346807 | 346695 | | | | 77 | 5 | 4.1 | 0.000 |
| 345564 | 345452 | | | | 8 | 0 | 4.1 | 0.001 |
| 348890 | 348778 | | | | 47 | 3 | 4.0 | 0.000 |
| 33989 | 33877 | | | | 8 | 1 | 4.0 | 0.010 |
| 1792 | 1680 | | | | 5 | 0 | 3.9 | 0.018 |
| 339426 | 339314 | | | | 6 | 0 | 3.9 | 0.010 |
| 14465 | 14353 | | | | 6 | 0 | 3.8 | 0.005 |
| 30462 | 30350 | | | | 157 | 12 | 3.7 | 0.000 |
| 34788 | 34676 | | | | 102 | 8 | 3.6 | 0.000 |
| 10225 | 10113 | | | | 6 | 1 | 3.6 | 0.008 |

| 346392 | 346280 | 123 | 10 | 3.5 | 0.011 |
|---|---|---|---|---|---|
| 323940 | 323828 | 13 | 1 | 3.5 | 0.000 |
| 16160 | 16048 | 38 | 3 | 3.5 | 0.001 |
| 33579 | 33467 | 383 | 36 | 3.4 | 0.000 |
| 148849 | 148737 | 27 | 3 | 3.3 | 0.000 |
| 62593 | 62481 | 66 | 7 | 3.3 | 0.000 |
| 11228 | 11116 | 12 | 1 | 3.3 | 0.001 |
| 342381 | 342269 | 664 | 67 | 3.3 | 0.003 |
| 350232 | 350120 | 6 | 1 | 3.1 | 0.012 |
| 80006 | 79894 | 18 | 2 | 3.1 | 0.023 |
| 345258 | 345146 | 33 | 4 | 3.1 | 0.049 |
| 4878 | 4766 | 8 | 1 | 3.0 | 0.004 |
| 36021 | 35909 | 31 | 4 | 3.0 | 0.000 |
| 19531 | 19419 | 62 | 8 | 3.0 | 0.000 |
| 11905 | 11793 | 37 | 5 | 3.0 | 0.000 |
| 350113 | 350001 | 29 | 4 | 2.8 | 0.013 |
| 46777 | 46665 | 56 | 8 | 2.8 | 0.000 |
| 323284 | 323172 | 304 | 44 | 2.8 | 0.000 |
| 348260 | 348148 | 49 | 7 | 2.8 | 0.007 |
| 30270 | 30158 | 6 | 1 | 2.7 | 0.028 |
| 294361 | 294249 | 6 | 1 | 2.7 | 0.031 |
| 2800 | 2688 | 9 | 1 | 2.7 | 0.004 |
| 90977 | 90865 | 8 | 1 | 2.7 | 0.005 |
| 36416 | 36304 | 13 | 2 | 2.7 | 0.001 |
| 323015 | 322903 | 50 | 8 | 2.6 | 0.000 |
| 26861 | 26749 | 565 | 94 | 2.6 | 0.000 |
| 43972 | 43860 | 21 | 3 | 2.6 | 0.015 |
| 84114 | 84002 | 13 | 2 | 2.6 | 0.000 |
| 321300 | 321188 | 9 | 2 | 2.6 | 0.009 |
| 339416 | 339304 | 259 | 44 | 2.6 | 0.001 |
| 19912 | 19800 | 6 | 1 | 2.5 | 0.042 |
| 30566 | 30454 | 40 | 7 | 2.5 | 0.000 |
| 349770 | 349658 | 689 | 122 | 2.5 | 0.003 |
| 339045 | 338933 | 15 | 3 | 2.5 | 0.001 |
| 15978 | 15866 | 39 | 7 | 2.5 | 0.012 |
| 26012 | 25900 | 15 | 3 | 2.5 | 0.000 |
| 33354 | 33242 | 9 | 2 | 2.5 | 0.014 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50400 | 50288 | | | | 33 | 6 | 2.5 | 0.000 |
| 22659 | 22547 | | | | 56 | 10 | 2.5 | 0.006 |
| 349371 | 349259 | | | | 84 | 15 | 2.5 | 0.000 |
| 339530 | 339418 | | | | 25 | 5 | 2.4 | 0.000 |
| 336238 | 336126 | | | | 3218 | 593 | 2.4 | 0.000 |
| 29620 | 29508 | | | | 17 | 3 | 2.4 | 0.003 |
| 347086 | 346974 | | | | 118 | 22 | 2.4 | 0.006 |
| 148850 | 148738 | | | | 49 | 9 | 2.4 | 0.000 |
| 347645 | 347533 | | | | 9 | 2 | 2.4 | 0.012 |
| 176204 | 176092 | | | | 464 | 89 | 2.4 | 0.000 |
| 12312 | 12200 | | | | 25 | 5 | 2.4 | 0.000 |
| 337841 | 337729 | Cytochrome c oxidase, subunit I | Energy production and conversion | Metabolism | 6 | 31 | -2.4 | 0.036 |
| 64289 | 64177 | Cytochrome oxidase subunit III and related proteins | Energy production and conversion | Metabolism | 4 | 21 | -2.3 | 0.000 |
| 64223 | 64111 | NADH dehydrogenase subunits 2, 5, and related proteins | Energy production and conversion | Metabolism | 1 | 11 | -3.3 | 0.005 |
| 334389 | 334277 | NADH dehydrogenase subunits 2, 5, and related proteins | Energy production and conversion | Metabolism | 3 | 18 | -2.7 | 0.000 |
| 271872 | 271760 | NADH dehydrogenase, subunit 4 | Energy production and conversion | Metabolism | 2 | 10 | -2.4 | 0.044 |
| 83591 | 83479 | | | | 0 | 13 | -5.5 | 0.030 |
| 337939 | 337827 | | | | 0 | 8 | -4.8 | 0.001 |
| 83590 | 83478 | | | | 1 | 9 | -3.9 | 0.043 |
| 344872 | 344760 | | | | 1 | 15 | -3.7 | 0.024 |
| 89083 | 88971 | | | | 458 | 4553 | -3.3 | 0.001 |
| 329089 | 328977 | | | | 1 | 9 | -3.2 | 0.033 |
| 247407 | 247295 | | | | 313 | 2796 | -3.2 | 0.000 |
| 102847 | 102735 | | | | 247 | 2165 | -3.1 | 0.000 |
| 330818 | 330706 | | | | 2 | 17 | -3.1 | 0.002 |
| 18164 | 18052 | | | | 1 | 6 | -3.0 | 0.045 |
| 9810 | 9698 | | | | 1076 | 6392 | -2.6 | 0.001 |
| 102626 | 102514 | | | | 10048 | 51840 | -2.4 | 0.001 |
| 171552 | 171440 | | | | 13376 | 67841 | -2.3 | 0.005 |

**Supplementary Table S4.2** List of expressed nitrogen-metabolism related genes in R. irregularis.

| Transcript ID | Protein ID | IPR | Eukaryotic orthologous groups | | | Mean RPKM | | log2 ratio | FDR Corrected p-value |
|---|---|---|---|---|---|---|---|---|---|
| | | | Defline | Class | Group | -N | +N | | |
| 29953 | 29841 | Predicted nitrate Transporter ; Major facilitator superfamily | | | | 44 | 2 | 4.7 | 0.000 |
| 30566 | 30454 | Major facilitator superfamily MFS-1 ; Nitrate transporter | | | | 40 | 7 | 2.5 | 0.000 |
| 218287 | 218175 | Rh-like protein/ammonium transporter ; Ammonium transporter ; Rh-like protein/ammonium transporter | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM | 87 | 26 | 1.7 | 0.000 |
| 346723 | 346611 | Carbonic anhydrase, eukaryotic | Carbonic anhydrase | General function prediction only | POORLY CHARACTERIZED | 147 | 75 | 1.0 | 0.000 |
| 346745 | 346633 | Orn/DAP/Arg decarboxylase 2 ; Ornithine decarboxylase; Alanine racemase/group IV decarboxylase, C-terminal | Ornithine decarboxylase | Amino acid transport and metabolism | METABOLISM | 84 | 48 | 0.8 | 0.022 |
| 342688 | 342576 | Nitrogenase component 1, conserved site | | | | 127 | 74 | 0.8 | 0.006 |
| 341050 | 340938 | Aminotransferase, class I and II ; Pyridoxal phosphate-dependent transferase, major region | Kynurenine aminotransferase, glutamine transaminase K | Amino acid transport and metabolism | METABOLISM | 90 | 55 | 0.7 | 0.014 |
| 18008 | 17896 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 1 | 0 | | 0.840 |
| 74648 | 74536 | Cytochrome b5 ; | Cytochrome b5 | Energy production and conversion | METABOLISM | 12 | 0 | | 0.366 |
| 46771 | 46659 | Major facilitator superfamily MFS-1 ; MFS general substrate transporter | | | | 71 | 2 | 4.8 | 0.157 |
| 82708 | 82596 | Flavoprotein pyridine nucleotide cytochrome reductase ; NADH:cytochrome b5 reductase (CBR) ; Oxidoreductase FAD/NAD(P)-binding ; Oxidoreductase FAD-binding region | NADH-cytochrome b-5 reductase | Coenzyme transport and metabolism | METABOLISM | 43 | 2 | 4.7 | 0.166 |
| 74649 | 74537 | Eukaryotic molybdopterin oxidoreductase ; molybdopterin binding ; Moybdenum cofactor oxidoreductase, dimerisation ; molybdopterin binding ; Oxidoreductase, Immunoglobulin E-set | Sulfite oxidase, molybdopterin-binding component | Energy production and conversion | METABOLISM | 10 | 1 | 2.9 | 0.644 |
| 34060 | 33948 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 2 | 1 | 2.1 | 0.514 |
| 34166 | 34054 | Anthranilate synthase component I and chorismate binding protein | | | | 63 | 20 | 1.7 | 0.499 |
| 217252 | 217140 | Arginase, subgroup ; Ureohydrolase ; Immunoglobulin/major histocompatibility complex, conserved site ; | Arginase | Amino acid transport and metabolism | METABOLISM | 357 | 122 | 1.5 | 0.203 |

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|---|---|---|---|---|---|---|---|---|---|
| 336101 | 335989 | Cytochrome b5 ; Cytochrome b5 | Cytochrome b5 | Energy production and conversion | METABOLISM | 555 | 209 | 1.4 | 0.063 |
| 337137 | 337025 | Rh-like protein/ammonium transporter ; Ammonium transporter ; Ammonium transporter | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM | 67 | 26 | 1.4 | 0.374 |
| 13758 | 13646 | 2-nitropropane dioxygenase, NPD | | | | 56 | 24 | 1.3 | 0.063 |
| 337094 | 336982 | Amidohydrolase 2 ; Glutamine synthetase, catalytic region ; Glutamine synthetase, beta-Grasp | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM | 30 | 13 | 1.2 | 0.332 |
| 36537 | 36425 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase ; | | | | 14 | 6 | 1.2 | 0.307 |
| 2856 | 2744 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 1 | 1 | 1.1 | 0.927 |
| 40603 | 40491 | FAD dependent oxidoreductase ; TonB box, conserved site | Possible oxidoreductase | General function prediction only | POORLY CHARACTERIZED | 22 | 11 | 1.1 | 0.160 |
| 340984 | 340872 | Carbonic anhydrase, eukaryotic ; Carbonic anhydrase, eukaryotic ; | Carbonic anhydrase | General function prediction only | POORLY CHARACTERIZED | 209 | 105 | 1.0 | 0.622 |
| 1394 | 1282 | Glutamine synthetase, catalytic region | | | | 1 | 0 | 0.9 | 0.980 |
| 17992 | 17880 | Glutamine synthetase, catalytic region ; | | | | 2 | 1 | 0.9 | 0.871 |
| 35355 | 35243 | ; Asparaginase/glutaminase ; Ankyrin ; | Asparaginase | Amino acid transport and metabolism | METABOLISM | 64 | 35 | 0.9 | 0.645 |
| 314321 | 314209 | Rh-like protein/ammonium transporter ; Ammonium transporter | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM | 74 | 40 | 0.9 | 0.054 |
| 87380 | 87268 | Asparaginase/glutaminase ; | Asparaginase | Amino acid transport and metabolism | METABOLISM | 12 | 7 | 0.8 | 0.889 |
| 324826 | 324714 | Glutamine amidotransferase class-I, C-terminal | Predicted glutamine synthetase | Nucleotide transport and metabolism | METABOLISM | 80 | 47 | 0.8 | 0.110 |
| 350231 | 350119 | Aminotransferase class-III ; Pyridoxal phosphate-dependent transferase, major region | Ornithine aminotransferase | Amino acid transport and metabolism | METABOLISM | 112 | 66 | 0.8 | 0.840 |
| 36518 | 36406 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase | | | | 6 | 4 | 0.7 | 0.683 |
| 340703 | 340591 | Anthranilate synthase component I and chorismate binding protein; C-terminal, N-terminal; | Isochorismate synthase | Amino acid transport and metabolism | METABOLISM | 56 | 34 | 0.7 | 0.712 |
| 350050 | 349938 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 84 | 51 | 0.7 | 0.803 |
| 16640 | 16528 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 6 | 4 | 0.7 | 0.966 |

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| 20690 | 20578 | Glutamine synthetase, catalytic region ; Glutamine synthetase, beta-Grasp | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM | 922 | 573 | 0.7 | 0.729 |
| 86144 | 86032 | Peptidase T2, asparaginase 2 ; Phosphopantetheine attachment site | Asparaginase | Amino acid transport and metabolism | METABOLISM | 29 | 18 | 0.7 | 0.364 |
| 87927 | 87815 | Carbonic anhydrase, eukaryotic | Carbonic anhydrase | General function prediction only | POORLY CHARACTERIZED | 19 | 12 | 0.7 | 0.909 |
| 335443 | 335331 | Cytochrome b5 ; Eukaryotic molybdopterin oxidoreductase ; Oxidoreductase, molybdopterin binding ; Moybdenum cofactor oxidoreductase, dimerisation ; Immunoglobulin E-set | Sulfite oxidase, molybdopterin-binding component | Energy production and conversion | METABOLISM | 71 | 44 | 0.7 | 0.374 |
| 335569 | 335457 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase ; Protein of unknown function UPF0012, conserved site ; | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM | 58 | 37 | 0.7 | 0.259 |
| 32312 | 32200 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 12 | 8 | 0.7 | 0.904 |
| 72014 | 71902 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; O-acetylhomoserine/O-acetylserine sulfhydrylase ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 14 | 9 | 0.7 | 0.835 |
| 338383 | 338271 | Orn/DAP/Arg decarboxylase 2 ; Ornithine decarboxylase; Alanine racemase/group IV decarboxylase, C-terminal | Ornithine decarboxylase | Amino acid transport and metabolism | METABOLISM | 35 | 22 | 0.6 | 0.062 |
| 334081 | 333969 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase ; | | | | 41 | 27 | 0.6 | 0.840 |
| 273162 | 273050 | Urease accessory protein UreD | | | | 23 | 16 | 0.6 | 0.840 |
| 345461 | 345349 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Cystathionine beta-lyase, eukaryotic; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 47 | 33 | 0.5 | 0.074 |
| 334993 | 334881 | Maf-like protein | Predicted nucleic acid-binding protein ASMTL | Cell cycle control, cell division, chromosome partitioning | METABOLISM | 45 | 31 | 0.5 | 0.076 |
| 341461 | 341349 | Urease, gamma subunit region ; Urease, beta subunit, alpha subunit ; Urease alpha-subunit, N-terminal; Amidohydrolase 1 ; Urease, gamma/beta/alpha type ; Metal-dependent hydrolase, composite ; | | | | 114 | 81 | 0.5 | 0.831 |
| 260975 | 260863 | Glutamine synthetase, catalytic region ; Glutamine synthetase, beta-Grasp | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM | 73 | 52 | 0.5 | 0.217 |
| 95709 | 95597 | Aminotransferase class-III ; Pyridoxal phosphate-dependent transferase, major region | Acetylornithine aminotransferase | Amino acid transport and metabolism | METABOLISM | 4 | 2 | 0.5 | 0.900 |
| 38749 | 38637 | Glycine cleavage T-protein, N-terminal ; C-terminal barrel | Aminomethyl transferase | Amino acid transport and | METABOLISM | 89 | 64 | 0.5 | 0.642 |

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| | | | | metabolism | | | | |
| 72617 | 72505 | Cobalamin (vitamin B12) biosynthesis CobW-like ; [NiFe]-hydrogenase/urease maturation factor, Ni(2+)-binding GTPase ; Urease accessory protein UreG | | | | 124 | 93 | 0.4 | 0.866 |
| 94698 | 94586 | Aminotransferase class-III ; Ornithine aminotransferase ; Pyridoxal phosphate-dependent transferase, major region | Ornithine aminotransferase | Amino acid transport and metabolism | METABOLISM | 553 | 421 | 0.4 | 0.881 |
| 32311 | 32199 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 5 | 4 | 0.4 | 0.939 |
| 345489 | 345377 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase ; Protein of unknown function UPF0012, conserved site ; | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM | 197 | 155 | 0.3 | 0.840 |
| 345281 | 345169 | Asparagine synthase | Asparagine synthase | Amino acid transport and metabolism | METABOLISM | 12 | 10 | 0.3 | 0.887 |
| 36306 | 36194 | | Splicing coactivator SRm160/ 300, subunit SRm300 | RNA processing and modification | INFORMATION STORAGE AND PROCESSING | 11 | 9 | 0.3 | 0.926 |
| 42927 | 42815 | Ankyrin | Ankyrin | Cell wall/membrane/envelope biogenesis | CELLULAR PROCESSES AND SIGNALING | 26 | 22 | 0.3 | 0.966 |
| 229730 | 229618 | | Asparaginase | Amino acid transport and metabolism | METABOLISM | 13 | 11 | 0.2 | 0.881 |
| 32424 | 32312 | Anthranilate synthase component I and chorismate binding protein ; NUC156 | Uncharacterized conserved protein similar to ATP/ GTP-binding protein | General function prediction only | POORLY CHARACTERIZED | 22 | 19 | 0.2 | 0.840 |
| 31421 | 31309 | Glutamine amidotransferase, class-II ; | Asparagine synthase (glutamine-hydrolyzing) | Amino acid transport and metabolism | METABOLISM | 27 | 23 | 0.2 | 0.989 |
| 81791 | 81679 | Urease accessory protein UreF | | | | 19 | 17 | 0.2 | 0.974 |
| 6845 | 6733 | Phosphatidylinositol 3- and 4-kinase, catalytic ; Phosphoinositide 3-kinase accessory region PIK ; Phosphoinositide 3-kinase, C2 ; Phosphatidylinositol 3-kinase, Vps34 type ; Phosphoinositide 3-kinase accessory region PIK ; Phosphatidylinositol 3- and 4-kinase, catalytic ; Transketolase, central region; Armadillo-type fold ; C2 calcium/lipid-binding region, CaLB ; Protein kinase-like | Phosphatidylinositol 3-kinase VPS34, involved in signal transduction | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 21 | 19 | 0.2 | 0.969 |
| 346990 | 346878 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase ; | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM | 30 | 27 | 0.2 | 0.982 |
| 67661 | 67549 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma- | Amino acid transport and metabolism | METABOLISM | 18 | 16 | 0.2 | 0.988 |

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|---|---|---|---|---|---|---|---|---|---|
| | | | synthases | | | | | | |
| 9779 | 9667 | SPla/RYanodine receptor SPRY ; BTB/POZ ; B302, (SPRY)-like ; Nitrogenase component 1, conserved site ; BTB/POZ fold | | | | 4 | 3 | 0.1 | 1.000 |
| 74461 | 74349 | Glutamate/phenylalanine/leucine/valine dehydrogenase, C-terminal; NAD-dependent ; NAD(P)-binding | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM | 36 | 34 | 0.1 | 1.000 |
| 93473 | 93361 | Aminotransferase class-III ; Acetylornithine and succinylornithine aminotransferase ; Pyridoxal phosphate-dependent transferase, major region | Acetylornithine aminotransferase | Amino acid transport and metabolism | METABOLISM | 36 | 34 | 0.1 | 1.000 |
| 9755 | 9643 | SPla/RYanodine receptor SPRY ; BTB/POZ ; B302, (SPRY)-like ; Nitrogenase component 1, conserved site ; BTB/POZ fold | | | | 3 | 3 | 0.1 | 1.000 |
| 80133 | 80021 | Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase ; NAD+ synthase ; Glutamine-dependent NAD(+) synthetase, GAT region ; NAD+ synthase ; | Predicted NAD synthase, contains CN hydrolase domain | Coenzyme transport and metabolism | METABOLISM | 51 | 52 | 0.0 | 1.000 |
| 3932 | 3820 | Glutamine amidotransferase superfamily ; Anthranilate synthase component II/delta crystallin ; Carbamoyl phosphate synthase, GATase region ; Glutamine amidotransferase class-I, C-terminal ; Glutamine amidotransferase of anthranilate synthase ; Glutamine amidotransferase, class I, active site | Para-aminobenzoate (PABA) synthase ABZ1 | Translation, ribosomal structure and biogenesis | INFORMATION STORAGE AND PROCESSING | 2 | 2 | -0.1 | 1.000 |
| 340291 | 340179 | Indole-3-glycerol phosphate synthase, central region ; Glutamine amidotransferase superfamily ; Anthranilate synthase component II/delta crystallin ; Carbamoyl phosphate synthase, GATase region ; Glutamine amidotransferase class-I, C-terminal ; Indole-3-glycerol phosphate synthase ; N-(5'phosphoribosyl)anthranilate isomerase (PRAI) ; Anthranilate synthase, component II, fungi ; Glutamine amidotransferase of anthranilate synthase ; Ribulose-phosphate binding barrel | Anthranilate synthase component II | Amino acid transport and metabolism | METABOLISM | 24 | 26 | -0.1 | 1.000 |
| 346590 | 346478 | Glutamine amidotransferase superfamily ; Anthranilate synthase component II/delta crystallin ; Carbamoyl phosphate synthase, GATase region ; Aspartate/ornithine carbamoyltransferase ; eukaryotic ; Glutamine amidotransferase class-I, C-terminal ; Asp/Orn-binding region ; Carbamoyl phosphate synthase, large subunit, N-terminal ; Carbamoyl phosphate synthase, small subunit, N-terminal ; MGS-like, ATP-binding ;, oligomerisation ; Aspartate carbamoyltransferase, eukaryotic ; Metal-dependent hydrolase, composite PreATP-grasp-like fold ; | Multifunctional pyrimidine synthesis protein CAD (includes carbamoyl-phophate synthetase, aspartate transcarbamylase, and glutamine amidotransferase) | General function prediction only | POORLY CHARACTERIZED | 7 | 8 | -0.1 | 1.000 |
| 31871 | 31759 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; | Cystathionine beta-lyases/ | Amino acid transport and | METABOLISM | 2 | 2 | -0.2 | 1.000 |

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|---|---|---|---|---|---|---|---|---|---|
| | | Pyridoxal phosphate-dependent transferase, major region | cystathionine gamma-synthases | metabolism | | | | | |
| 349018 | 348906 | Anthranilate synthase component I and chorismate binding protein ; Anthranilate synthase component I and chorismate binding protein ; Chorismate binding, C-terminal ; N-terminal ; Anthranilate synthase component I and chorismate binding protein | Para-aminobenzoate (PABA) synthase ABZ1 | Translation, ribosomal structure and biogenesis | INFORMATION STORAGE AND PROCESSING | 17 | 20 | -0.3 | 0.951 |
| 347853 | 347741 | Carbonic anhydrase ; prokaryotic-like, conserved site ; | Predicted carbonic anhydrase involved in protection against oxidative damage | Inorganic ion transport and metabolism | METABOLISM | 246 | 304 | -0.3 | 0.975 |
| 12064 | 11952 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 3 | 4 | -0.3 | 1.000 |
| 13789 | 13677 | Glutamine synthetase, catalytic region ; Glutamine synthetase, beta-Grasp ; | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM | 171 | 226 | -0.4 | 0.953 |
| 309659 | 309547 | Glutamate/phenylalanine/leucine/valine dehydrogenase ; dimerisation region ; NAD(P)-binding Glutamate/phenylalanine/leucine/valine dehydrogenase, C-terminal ; | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM | 27 | 35 | -0.4 | 0.908 |
| 337340 | 337228 | FAD-dependent pyridine nucleotide-disulphide oxidoreductase ; Adrenodoxin reductase ; Pyridine nucleotide-disulphide oxidoreductase, class-II ; Glutamine amidotransferase, class-II ; Glutamate synthase, alpha subunit, C-terminal ; Glutamate synthase, central-C ; Glutamate synthase, central-N ; FAD-dependent pyridine nucleotide-disulphide oxidoreductase ; eukaryotic ; NADH/NADPH, small subunit 1 ; Alpha-helical ferredoxin alpha subunit, C-terminal | Glutamate synthase | Amino acid transport and metabolism | METABOLISM | 41 | 55 | -0.4 | 0.881 |
| 344992 | 344880 | Glutamine amidotransferase, class-II ; Asparagine synthase ; Asparagine synthase, glutamine-hydrolyzing | Asparagine synthase (glutamine-hydrolyzing) | Amino acid transport and metabolism | METABOLISM | 125 | 170 | -0.4 | 0.840 |
| 337238 | 337126 | Glutamate/phenylalanine/leucine/valine dehydrogenase, C-terminal ;dehydrogenase, dimerisation region ; Glutamate/phenylalanine/leucine/valine dehydrogenase ; NAD(P)-binding | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM | 48 | 76 | -0.7 | 0.898 |
| 334582 | 334470 | Delta crystallin ; Fumarate lyase ; Fumarate lyase ; Argininosuccinate lyase ; L-Aspartase-like | Argininosuccinate lyase | Amino acid transport and metabolism | METABOLISM | 42 | 68 | -0.7 | 0.898 |
| 324437 | 324325 | Aspartate/ornithine carbamoyltransferase ; Ornithine carbamoyltransferase ; Asp/Orn-binding region ; | Ornithine carbamoyltransferase OTC/ | Amino acid transport and metabolism | METABOLISM | 35 | 67 | -0.9 | 0.813 |

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|---|---|---|---|---|---|---|---|---|---|
| | | Aspartate/ornithine carbamoyltransferase, carbamoyl-P binding ; | ARG3 | | | | | | |
| 348151 | 348039 | Carbonic anhydrase ; prokaryotic-like, conserved site ; Carbonic anhydrase | Predicted carbonic anhydrase involved in protection against oxidative damage | Inorganic ion transport and metabolism | METABOLISM | 17 | 34 | -1.0 | 0.893 |
| 324596 | 324484 | Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 0 | 1 | -1.0 | 0.944 |
| 249989 | 249877 | Cys/Met metabolism, pyridoxal phosphate-dependent enzyme ; Pyridoxal phosphate-dependent transferase, major region | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM | 0 | 1 | -1.2 | 0.892 |
| 324842 | 324730 | | | | | 0 | 1 | -1.5 | 0.910 |
| 340995 | 340883 | Carbamoyl phosphate synthase, large subunit, N-terminal, ATP-binding ; Carbamoyl phosphate synthetase, large subunit, oligomerisation ; glutamine-dependent ; ATP-grasp fold ; oligomerisation ; PreATP-grasp-like fold | Multifunctional pyrimidine synthesis protein CAD (includes carbamoyl-phophate synthetase, aspartate transcarbamylase, and glutamine amidotransferase) | General function prediction only | POORLY CHARACTERIZED | 26 | 93 | -1.9 | 0.732 |
| 333861 | 333749 | Argininosuccinate synthase ; | Argininosuccinate synthase | Amino acid transport and metabolism | METABOLISM | 35 | 149 | -2.1 | 0.725 |
| 342881 | 342769 | Glutamine amidotransferase superfamily ; Anthranilate synthase component II/delta crystallin ; Carbamoyl phosphate synthase, GATase region ; Glutamine amidotransferase class-I, C-terminal ; Carbamoyl phosphate synthase, small subunit, N-terminal ; Carbamoyl phosphate synthase, small subunit, N-terminal | Multifunctional pyrimidine synthesis protein CAD (includes carbamoyl-phophate synthetase, aspartate transcarbamylase, and glutamine amidotransferase) | General function prediction only | POORLY CHARACTERIZED | 41 | 244 | -2.6 | 0.780 |

**Supplementary Table S4.3 Differentially expressed genes of non-mycorrhizal *P. trichocarpa*.** Expression of non-mycorrhized genes under low-N condition were tested against non-mycorrhized samples under high-N condition.

| Transcript ID | best arabidopsis TAIR10 hit defline | Eukaryotic orthologous groups | | | Mean RPKM | | log2 ratio -N-Gi vs +N-Gi | FDR corrected p-value |
|---|---|---|---|---|---|---|---|---|
| | | Defline | Class | Group | -N-Gi - | +N-Gi - | | |
| 009G118800.1 | Glucose-1-phosphate adenylyltransferase family protein | similar to ADP-glucose pyrophosphorylase large subunit. [ORG:Fragaria x ananassa]; [ co-ortholog (1of2) of AAB91467, At4g39210, At2g21590, JE0133, AAB91463, T08027, BAC66692, CAA65541, AAS00542, T06495, ] | Cell wall/membrane/envelope biogenesis | CELLULAR PROCESSES AND SIGNALING | 445.3 | 69.4 | 2.7 | 0.000 |
| 004G092500.1 | carboxyesterase 18 | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 12.5 | 3.7 | 1.8 | 0.003 |
| 002G147600.1 | NAD(P)-binding Rossmann-fold superfamily protein | similar to dihydroflavonol 4-reductase family; similar to dihydrokaempferol 4-reductase family; similar to dihydroflavonol 4-reductase (SP:P51102); similar to vestitone reductase (Medicago sativa; similar to GI:973249); [ co-ortholog (1of3) of At2g45400, | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 4.7 | 0.5 | 3.3 | 0.017 |
| 010G101700.1 | DNAse I-like superfamily protein | | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING | 21.8 | 2.2 | 3.3 | 0.000 |
| 003G113300.1 | beta vacuolar processing enzyme | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 165.6 | 64.2 | 1.4 | 0.003 |
| 001G450700.1 | brassinosteroid-responsive RING-H2 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 39.2 | 6.6 | 2.6 | 0.000 |
| 016G000600.1 | Eukaryotic aspartyl protease family protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 12.3 | 1.7 | 2.8 | 0.000 |
| 006G232900.1 | gamma vacuolar processing enzyme | similar to asparaginyl endopeptidase (VmPE-1). [ORG:Vigna mungo]; [ co-ortholog (2of2) of At2g25940, CAB42655, BAA76744, At4g32940, CAB17078, At3g20210, P49044, CAA84383, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 867.6 | 69.3 | 3.6 | 0.000 |
| 018G059400.1 | gamma vacuolar processing enzyme | similar to asparaginyl endopeptidase (VmPE-1). [ORG:Vigna mungo]; [ co-ortholog (1of2) of At2g25940, CAB42655, BAA76744, At4g32940, CAB17078, At3g20210, P49044, CAA84383, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 29.8 | 5.6 | 2.4 | 0.000 |
| 016G086400.1 | Nucleotide-diphospho-sugar transferases superfamily protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 11.6 | 1.4 | 3.1 | 0.004 |
| 013G062000.1 | protein kinases;ubiquitin-protein ligases | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 5.3 | 0.4 | 3.7 | 0.007 |
| 004G055900.1 | senescence-associated gene 12 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 5.0 | 0.1 | 6.5 | 0.031 |
| 006G226900.1 | Thioredoxin superfamily protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 62.9 | 4.0 | 4.0 | 0.005 |
| 018G063300.1 | Thioredoxin superfamily protein | similar to peroxiredoxin Q. [ORG:Populus balsamifera subsp. trichocarpa x Populus deltoides]; [ co-ortholog (2of2) of At3g26060, AAS46230, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 122.5 | 43.4 | 1.5 | 0.000 |
| 005G052800.1 | calmodulin 8 | similar to calmodulin-like protein 6a. [ORG:Medicago truncatula]; [ co-ortholog (5of7) of AAA34238, AAD10277, AAM81199, AAA34237, AAK25753, BAA96448, AAM81200, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 27.6 | 2.9 | 3.2 | 0.000 |
| 007G032300.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 6.8 | 1.3 | 2.4 | 0.011 |
| 015G134500.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 74.1 | 14.8 | 2.3 | 0.000 |
| 011G131200.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 32.7 | 18.1 | 0.9 | 0.024 |
| 006G232700.1 | Protein phosphatase 2C family protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 9.9 | 1.1 | 3.1 | 0.000 |
| 006G088900.1 | receptor lectin kinase | | Signal transduction mechanisms | CELLULAR PROCESSES | 4.2 | 0.6 | 2.9 | 0.044 |

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| | | | | AND SIGNALING | | | | |
| 018G133100.1 | homeobox from Arabidopsis thaliana | | Transcription | INFORMATION STORAGE AND PROCESSING | 9.1 | 0.3 | 4.9 | 0.000 |
| 003G144300.1 | myb domain protein 12 | | Transcription | INFORMATION STORAGE AND PROCESSING | 26.9 | 5.1 | 2.4 | 0.001 |
| 006G221800.1 | myb domain protein 4 | | Transcription | INFORMATION STORAGE AND PROCESSING | 48.2 | 6.5 | 2.9 | 0.000 |
| 001G336700.1 | myb domain protein 40 | | Transcription | INFORMATION STORAGE AND PROCESSING | 15.3 | 2.9 | 2.4 | 0.000 |
| 001G266000.1 | nuclear factor Y, subunit A1 | | Transcription | INFORMATION STORAGE AND PROCESSING | 79.5 | 21.6 | 1.9 | 0.000 |
| 014G022800.1 | D-3-phosphoglycerate dehydrogenase | | Amino acid transport and metabolism | METABOLISM | 63.3 | 10.8 | 2.6 | 0.000 |
| 005G093200.1 | glutamine synthase clone F11 | similar to glutamine synthetase. [ORG:Glycine falcata]; [ co-ortholog (7of8) of AAM71227, AAR05531, AAP51252, AAR05559, AAR05552, AAM71209, AAM71231, AAR05581, AAR05506, AAP51256, AAO41677, AAO41668, AAC37356, AAM71193, AAM71233, AAR05523, AAO41662, AAR05 | Amino acid transport and metabolism | METABOLISM | 268.4 | 45.4 | 2.6 | 0.000 |
| 002G129500.1 | Major facilitator superfamily protein | | Amino acid transport and metabolism | METABOLISM | 83.4 | 0.9 | 6.6 | 0.000 |
| 004G229000.1 | Major facilitator superfamily protein | proton-dependent oligopeptide transport (POT) family protein; [ co-ortholog (2of2) of At1g59740, ] | Amino acid transport and metabolism | METABOLISM | 5.2 | 0.6 | 3.1 | 0.013 |
| 001G027100.1 | Major facilitator superfamily protein | | Amino acid transport and metabolism | METABOLISM | 4.6 | 0.7 | 2.7 | 0.034 |
| 014G036200.1 | Major facilitator superfamily protein | | Amino acid transport and metabolism | METABOLISM | 5.6 | 0.9 | 2.6 | 0.018 |
| 014G022900.1 | N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein | similar to L-asparaginase. [ORG:Glycine max]; [ co-ortholog (1of2) of At3g16150, AAM23265, ] | Amino acid transport and metabolism | METABOLISM | 653.6 | 103.0 | 2.7 | 0.000 |
| 002G064000.1 | Pyridoxal phosphate (PLP)-dependent transferases superfamily protein | similar to alliinase. [ORG:Malus x domestica]; [ ortholog of At1g34040,AAQ54504,At1g34060,] | Amino acid transport and metabolism | METABOLISM | 97.0 | 11.4 | 3.1 | 0.000 |
| 004G019700.1 | beta glucosidase 46 | glycosyl hydrolase family 1 protein; similar to beta-glucosidase (GI:3820531) (Pinus contorta); similar to beta-glucosidase GI:804655 from (Hordeu; [ co-ortholog (1of6) of At1g61810, At1g61820, ] | Carbohydrate transport and metabolism | METABOLISM | 19.2 | 2.9 | 2.7 | 0.000 |
| 004G019800.1 | beta-glucosidase 47 | glycosyl hydrolase family 1 protein; similar to dalcochinin 8\'\'-O-beta-glucoside beta-glucosidase precursor (GI:6118076) (Dalbergia cochinchinensis; [ ortholog of At4g21760,] | Carbohydrate transport and metabolism | METABOLISM | 18.2 | 0.8 | 4.5 | 0.002 |
| 006G173500.1 | Deoxyxylulose-5-phosphate synthase | | Carbohydrate transport and metabolism | METABOLISM | 51.1 | 3.6 | 3.8 | 0.000 |
| 004G019900.1 | glucose-6-phosphate/phosphate translocator 2 | | Carbohydrate transport and metabolism | METABOLISM | 25.1 | 2.9 | 3.1 | 0.016 |
| 001G455000.1 | NOD26-like intrinsic protein 5;1 | | Carbohydrate transport and metabolism | METABOLISM | 107.3 | 14.9 | 2.8 | 0.000 |
| 001G347300.1 | phosphoenolpyruvate (pep)/phosphate translocator 2 | | Carbohydrate transport and metabolism | METABOLISM | 8.3 | 1.6 | 2.4 | 0.004 |
| 006G239700.1 | tonoplast intrinsic protein 4;1 | similar to Probable aquaporin TIP4.1 (Tonoplast intrinsic protein 4.1) (Epsilon- tonoplast intrinsic protein) (Epsilon-TIP).; [ ortholog of At2g25810,] | Carbohydrate transport and metabolism | METABOLISM | 1141.3 | 170.0 | 2.7 | 0.000 |
| 009G095500.1 | UDP-glucosyl transferase 84B1 | similar to putative glucosyltransferase. [ORG:Phaseolus lunatus]; [ co-ortholog (15of18) of Q9ZSK5, P56725, AAM09516, CAD28205, BAB86925, AAD51778, AAM09513, AAD04166, AAM09514, AAM09517, ] | Carbohydrate transport and metabolism | METABOLISM | 5.9 | 0.3 | 4.5 | 0.002 |
| 005G073800.1 | UDP-glucosyl transferase 85A2 | | Carbohydrate transport and metabolism | METABOLISM | 10.2 | 1.1 | 3.2 | 0.000 |
| 006G023600.1 | UDP-glucosyl transferase 85A2 | | Carbohydrate transport and metabolism | METABOLISM | 22.2 | 2.8 | 3.0 | 0.000 |
| 016G021500.1 | UDP-glucosyl transferase 85A3 | | Carbohydrate transport and metabolism | METABOLISM | 6.3 | 1.0 | 2.7 | 0.011 |
| 014G175000.1 | UDP-glycosyltransferase 74 F1 | similar to putative glucosyltransferase. [ORG:Phaseolus lunatus]; [ co-ortholog (11of18) of Q9ZSK5, P56725, AAM09516, CAD28205, BAB86925, AAD51778, AAM09513, AAD04166, AAM09514, AAM09517, ] | Carbohydrate transport and metabolism | METABOLISM | 9.2 | 2.0 | 2.2 | 0.004 |
| 012G034100.1 | UDP-Glycosyltransferase superfamily protein | | Carbohydrate transport and metabolism | METABOLISM | 17.4 | 2.6 | 2.8 | 0.000 |
| 017G077400.1 | UDP-Glycosyltransferase superfamily protein | | Carbohydrate transport and metabolism | METABOLISM | 5.7 | 0.9 | 2.7 | 0.016 |
| 009G095400.1 | UDP-Glycosyltransferase superfamily protein | | Carbohydrate transport and metabolism | METABOLISM | 69.6 | 44.1 | 0.7 | 0.001 |
| 006G045300.1 | ubiquitin extension protein 1 | | Cell cycle control, cell division, chromosome partitioning | METABOLISM | 4.7 | 0.5 | 3.2 | 0.019 |
| 004G090600.1 | geranylgeranyl | | Coenzyme transport | METABOLISM | 27.0 | 1.3 | 4.4 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| | pyrophosphate synthase 1 | | and metabolism | | | | | |
| 003G204600.1 | ADP/ATP carrier 2 | | Energy production and conversion | METABOLISM | 22.4 | 4.4 | 2.4 | 0.000 |
| 005G069800.1 | aldehyde dehydrogenase 3I1 | | Energy production and conversion | METABOLISM | 5.5 | 0.1 | 5.2 | 0.002 |
| 001G305400.1 | ammonium transporter 2 | | Inorganic ion transport and metabolism | METABOLISM | 93.0 | 3.0 | 5.0 | 0.000 |
| T000200.1 | ammonium transporter 2 | | Inorganic ion transport and metabolism | METABOLISM | 10.0 | 0.6 | 4.0 | 0.000 |
| 001G293400.1 | APS kinase | | Inorganic ion transport and metabolism | METABOLISM | 51.7 | 23.4 | 1.1 | 0.000 |
| 016G043100.1 | autoinhibited Ca(2+)-ATPase, isoform 4 | | Inorganic ion transport and metabolism | METABOLISM | 5.9 | 0.5 | 3.5 | 0.004 |
| 008G083600.1 | Cation efflux family protein | | Inorganic ion transport and metabolism | METABOLISM | 5.5 | 0.5 | 3.3 | 0.008 |
| 006G093200.1 | Ctr copper transporter family | | Inorganic ion transport and metabolism | METABOLISM | 42.8 | 2.6 | 4.0 | 0.000 |
| 017G141600.1 | Divalent ion symporter | | Inorganic ion transport and metabolism | METABOLISM | 9.4 | 1.5 | 2.7 | 0.001 |
| 009G135300.1 | farnesylated protein 6 | | Inorganic ion transport and metabolism | METABOLISM | 13.0 | 1.4 | 3.3 | 0.010 |
| 014G088000.1 | ferric reduction oxidase 2 | similar to ferric-chelate reductase. [ORG:Pisum sativum]; [ ortholog of AAU94355,At1g01580,AAR15416,AAU94356,AAK95654,] | Inorganic ion transport and metabolism | METABOLISM | 11.1 | 1.3 | 3.1 | 0.000 |
| 003G125600.1 | heavy metal atpase 5 | similar to Potential copper-transporting ATPase 3 (EC 3.6.3.4).; [ co-ortholog (1of3) of At1g63440, ] | Inorganic ion transport and metabolism | METABOLISM | 43.5 | 3.2 | 3.7 | 0.000 |
| 001G105800.1 | heavy metal atpase 5 | | Inorganic ion transport and metabolism | METABOLISM | 4.5 | 0.4 | 3.6 | 0.016 |
| 005G120200.1 | Heavy metal transport/detoxification superfamily protein | similar to heavy-metal-associated domain-containing protein; similar to low similarity to farnesylated protein GMFP5 (Glycine max)(GI:4097571); [ co-ortholog (1of2) of At5g26690, At3g05920, ] | Inorganic ion transport and metabolism | METABOLISM | 31.4 | 1.6 | 4.3 | 0.000 |
| 001G452400.1 | Heavy metal transport/detoxification superfamily protein | similar to copper chaperone (CCH)-related; [ ortholog of At2g18196,] | Inorganic ion transport and metabolism | METABOLISM | 29.4 | 3.9 | 2.9 | 0.000 |
| 005G181100.1 | NRAMP metal ion transporter 6 | similar to root-specific metal transporter. [ORG:Malus baccata]; [ co-ortholog (1of2) of AAU00158, ] | Inorganic ion transport and metabolism | METABOLISM | 4.7 | 0.6 | 3.0 | 0.024 |
| 005G181000.1 | NRAMP metal ion transporter 6 | | Inorganic ion transport and metabolism | METABOLISM | 23.4 | 2.9 | 3.0 | 0.000 |
| 015G117900.1 | zinc transporter 10 precursor | similar to root iron transporter protein. [ORG:Pisum sativum]; [ co-ortholog (1of2) of AAC17441, At1g31260, AAR08416, ] | Inorganic ion transport and metabolism | METABOLISM | 221.4 | 20.3 | 3.4 | 0.001 |
| 008G012800.1 | 3-oxo-5-alpha-steroid 4-dehydrogenase family protein | | Lipid transport and metabolism | METABOLISM | 74.3 | 3.0 | 4.6 | 0.000 |
| 013G134800.1 | alpha/beta-Hydrolases superfamily protein | | Lipid transport and metabolism | METABOLISM | 10.0 | 1.5 | 2.7 | 0.000 |
| 015G148500.1 | AMP-dependent synthetase and ligase family protein | | Lipid transport and metabolism | METABOLISM | 5.5 | 0.8 | 2.8 | 0.015 |
| 001G049100.1 | camelliol C synthase 1 | | Lipid transport and metabolism | METABOLISM | 5.2 | 1.0 | 2.4 | 0.038 |
| 001G007700.1 | PHYTOENE SYNTHASE | | Lipid transport and metabolism | METABOLISM | 39.2 | 1.1 | 5.2 | 0.000 |
| 010G105400.1 | Sec14p-like phosphatidylinositol transfer family protein | SEC14 cytosolic factor family protein; phosphoglyceride transfer family protein; similar to phosphatidylinositol/phosphatidylcholine transfer protein SP:P24280 (Saccharomyces cerevisiae (Baker\'s\'yeast ; similar to SEC14 cy; [ ortholog of At1g14820,] | Lipid transport and metabolism | METABOLISM | 9.0 | 0.4 | 4.5 | 0.000 |
| 006G119500.1 | permease, cytosine/purines, uracil, thiamine, allantoin family protein | | Nucleotide transport and metabolism | METABOLISM | 8.7 | 1.3 | 2.8 | 0.001 |
| 010G200900.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 2.9 | 0.0 | 7.3 | 0.040 |
| 010G201000.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 17.8 | 0.2 | 6.2 | 0.000 |
| 014G073700.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | similar to probable gibberellin 20-oxidase - garden pea. [ORG:Pisum sativum]; [ co-ortholog (3of6) of AAB67838, T06533, AAC49758, T09664, CAA51744, T09675, T11849, AAB64345, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 3.5 | 0.2 | 4.5 | 0.030 |
| 002G159500.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 21.6 | 2.4 | 3.1 | 0.000 |
| 005G097900.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | similar to oxidoreductase; 2OG-Fe(2) oxygenase family protein; similar to SP\|P10967\|1 aminocyclopropane-1-carboxylate-oxidase homolog Protein (E8 (Lycopersicon) (esculentum) ; similar to desacetoxyvindoline-4-hydroxylase (Catharanthus roseus) GI:2352812; | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 14.1 | 2.0 | 2.8 | 0.019 |
| 002G078600.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | similar to 1-aminocyclopropane-1-carboxylate oxidase; putative; similar to ACC oxidase; putative; similar to 1-aminocyclopropane-1-carboxylate oxidase GI:3386565 from (Sorghum bicolor); [ co-ortholog (2of2) of At1g77330, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 178.1 | 28.4 | 2.6 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| 002G039600.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 6.6 | 1.1 | 2.6 | 0.009 |
| T170500.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | similar to oxidoreductase; 2OG-Fe(2) oxygenase family protein; similar to desacetoxyvindoline 4-hydroxylase (Catharanthus roseus)(GI:1916643); similar to flavonol synthase 1 (SP\|Q96330); [ co-ortholog (1of2) of At3g13610, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 5.5 | 1.0 | 2.5 | 0.026 |
| 001G176500.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 40.7 | 7.9 | 2.4 | 0.000 |
| 007G104800.1 | ABC-2 type transporter family protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 9.3 | 1.7 | 2.5 | 0.001 |
| 001G265900.1 | carotenoid cleavage dioxygenase 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 93.0 | 7.7 | 3.6 | 0.000 |
| 014G056800.1 | carotenoid cleavage dioxygenase 7 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 30.8 | 1.9 | 4.0 | 0.000 |
| 006G238500.1 | carotenoid cleavage dioxygenase 8 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 75.9 | 5.6 | 3.8 | 0.000 |
| 001G118200.1 | Copper amine oxidase family protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 32.6 | 4.5 | 2.9 | 0.000 |
| 010G139400.1 | Cytochrome P450 superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 19.7 | 3.2 | 2.6 | 0.000 |
| 007G040200.1 | Cytochrome P450 superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 18.8 | 3.1 | 2.6 | 0.000 |
| 001G118500.1 | cytochrome P450, family 706, subfamily A, polypeptide 6 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 86.4 | 12.6 | 2.8 | 0.000 |
| 001G365100.1 | cytochrome P450, family 71, subfamily B, polypeptide 34 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 6.0 | 0.2 | 4.6 | 0.001 |
| 001G362600.1 | cytochrome P450, family 71, subfamily B, polypeptide 34 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 3.7 | 0.3 | 3.7 | 0.033 |
| 007G074900.1 | cytochrome P450, family 71, subfamily B, polypeptide 34 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 17.6 | 3.0 | 2.5 | 0.000 |
| 011G001400.1 | cytochrome P450, family 716, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 4.1 | 0.1 | 4.9 | 0.012 |
| 011G137800.1 | cytochrome P450, family 716, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 21.3 | 1.5 | 3.8 | 0.000 |
| 001G002800.1 | cytochrome P450, family 716, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 65.6 | 6.1 | 3.4 | 0.000 |
| 001G003000.1 | cytochrome P450, family 716, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 106.5 | 10.0 | 3.4 | 0.000 |
| 018G149300.1 | cytochrome P450, family 716, subfamily A, polypeptide 1 | similar to cytochrome P450 family; similar to simialr to taxane 13-alpha-hydroxylase (Taxus cuspidata) GI:17148242; [ ortholog of At5g36140,At5g36130,At5g36110,] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 14.2 | 1.8 | 3.0 | 0.000 |
| 015G138900.1 | cytochrome P450, family 76, subfamily C, polypeptide 6 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 10.9 | 2.1 | 2.3 | 0.001 |
| 005G029800.1 | cytochrome P450, family 76, subfamily G, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 43.6 | 1.1 | 5.3 | 0.000 |
| 005G030100.1 | cytochrome P450, family 76, subfamily G, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 77.3 | 7.8 | 3.3 | 0.003 |
| 001G083900.1 | cytochrome P450, family 78, subfamily A, polypeptide 6 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 30.2 | 1.4 | 4.4 | 0.000 |
| 014G037300.1 | cytochrome P450, family 82, subfamily C, polypeptide 4 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 8.0 | 1.0 | 2.9 | 0.003 |
| 014G037500.1 | cytochrome P450, family 82, subfamily C, polypeptide 4 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 9.9 | 1.4 | 2.8 | 0.017 |
| 012G071300.1 | cytochrome P450, family 88, subfamily A, polypeptide 3 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 449.9 | 14.4 | 5.0 | 0.000 |
| 012G071200.1 | cytochrome P450, family 88, subfamily A, polypeptide 3 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 86.1 | 6.1 | 3.8 | 0.000 |
| 006G248500.1 | pleiotropic drug resistance 12 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 49.4 | 1.4 | 5.1 | 0.000 |
| 002G188900.1 | pleiotropic drug resistance 5 | | Secondary metabolites biosynthesis, transport | METABOLISM | 3.2 | 0.1 | 5.2 | 0.032 |

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|---|---|---|---|---|---|---|---|---|
| 002G189100.1 | pleiotropic drug resistance 9 | | and catabolism Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 7.7 | 0.1 | 6.1 | 0.000 |
| 006G266400.1 | EamA-like transporter family | | Function unknown | POORLY CHARACTERIZED | 30.9 | 12.8 | 1.3 | 0.021 |
| 012G005100.1 | Major facilitator superfamily protein | similar to expressed protein in Arabidopsis thaliana; similar to 11 transmembrane domain containing protein; [ co-ortholog (1of2) of At1g18000, At1g18010, ] | Function unknown | POORLY CHARACTERIZED | 3.9 | 0.4 | 3.5 | 0.032 |
| 006G230800.1 | N-MYC downregulated-like 1 | | Function unknown | POORLY CHARACTERIZED | 37.1 | 10.0 | 1.9 | 0.000 |
| 012G130600.1 | 2 iron, 2 sulfur cluster binding | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At5g51720, ] | General function prediction only | POORLY CHARACTERIZED | 12.5 | 2.8 | 2.2 | 0.009 |
| 006G155500.1 | alpha/beta-Hydrolases superfamily protein | similar to hydrolase; alpha/beta fold family protein; similar to low similarity to 3-oxoadipate enol-lactone hydrolase (Pseudomonas sp. B13) GI:17736948; similar to B-ketoadipate enol-lactone hydrolase (Bradyrhizobium japonicum) GI:2239060; [ ortholog of | General function prediction only | POORLY CHARACTERIZED | 122.6 | 4.6 | 4.7 | 0.000 |
| 006G272800.1 | FAD-dependent oxidoreductase family protein | | General function prediction only | POORLY CHARACTERIZED | 248.5 | 151.9 | 0.7 | 0.002 |
| 011G022400.1 | indole-3-butyric acid response 1 | | General function prediction only | POORLY CHARACTERIZED | 27.7 | 3.6 | 2.9 | 0.000 |
| 013G027800.1 | Major facilitator superfamily protein | similar to sugar transporter; putative; similar to ERD6 protein ((Arabidopsis thaliana)) GI:3123712; sugar-porter family protein; similar to GI:14585701; similar to sugar trans; [ co-ortholog (2of2) of At1g08920, ] | General function prediction only | POORLY CHARACTERIZED | 24.4 | 4.5 | 2.4 | 0.000 |
| 011G087200.1 | mannose-1-phosphate guanylyltransferase (GDP)s;GDP-galactose:mannose-1-phosphate guanylyltransferases; GDP-galactose:glucose-1-phosphate guanylyltransferases; GDP-galactose:myoinositol-1-phosphate guanylyltransferases; glucose-1-phosphate guanylyltransferase | similar to VITAMIN C DEFECTIVE 2; [ co-ortholog (2of2) of At5g55120, At4g26850, ] | General function prediction only | POORLY CHARACTERIZED | 303.6 | 56.9 | 2.4 | 0.000 |
| 017G120500.1 | MATE efflux family protein | | General function prediction only | POORLY CHARACTERIZED | 8.6 | 1.5 | 2.5 | 0.003 |
| 016G074000.1 | NAD(P)-binding Rossmann-fold superfamily protein | similar to putative short-chain alcohol dehydrogenase. [ORG:Cucumis sativus]; [ co-ortholog (1of2) of BAB21571, ] | General function prediction only | POORLY CHARACTERIZED | 79.1 | 3.8 | 4.4 | 0.000 |
| 012G105600.1 | NAD(P)-binding Rossmann-fold superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 22.5 | 1.8 | 3.7 | 0.000 |
| 006G206900.1 | NAD(P)-binding Rossmann-fold superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 35.6 | 6.4 | 2.5 | 0.006 |
| 015G021900.1 | Nodulin MtN3 family protein | | General function prediction only | POORLY CHARACTERIZED | 61.2 | 8.1 | 2.9 | 0.000 |
| 001G355500.1 | Nodulin MtN3 family protein | | General function prediction only | POORLY CHARACTERIZED | 13.4 | 1.8 | 2.9 | 0.000 |
| 004G050400.1 | O-methyltransferase family protein | similar to 6a-hydroxymaackiain methyltransferase (EC 2.1.1.-) - garden pea. [ORG:Pisum sativum]; [ co-ortholog (18of20) of O24529, O22308, AAM23004, CAH05089, AAC49926, CAH05087, CAH05078, O22309, CAH05079, AAC49856, 1FP2_A, CAH05085, CAD29556, CAD29459, | General function prediction only | POORLY CHARACTERIZED | 13.1 | 0.9 | 3.8 | 0.000 |
| 004G050500.1 | O-methyltransferase family protein | similar to 6a-hydroxymaackiain methyltransferase (EC 2.1.1.-) - garden pea. [ORG:Pisum sativum]; [ co-ortholog (12of20) of O24529, O22308, AAM23004, CAH05089, AAC49926, CAH05087, CAH05078, O22309, CAH05079, AAC49856, 1FP2_A, CAH05085, CAD29556, CAD29459, | General function prediction only | POORLY CHARACTERIZED | 12.9 | 2.1 | 2.6 | 0.000 |
| 004G008100.1 | Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein | | General function prediction only | POORLY CHARACTERIZED | 14.9 | 2.6 | 2.5 | 0.000 |
| 014G041000.1 | Protein of unknown function (DUF607) | | General function prediction only | POORLY CHARACTERIZED | 29.8 | 5.8 | 2.4 | 0.000 |
| 006G070000.1 | Zinc-binding dehydrogenase family protein | | General function prediction only | POORLY CHARACTERIZED | 8.4 | 0.9 | 3.3 | 0.000 |
| 001G225100.1 | 4-(cytidine 5\'-phospho)-2-C-methyl-D-erithritol kinase | | | | 13.2 | 2.5 | 2.4 | 0.000 |

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|---|---|---|---|---|---|---|
| 003G142800.1 | ABL interactor-like protein 2 | | 16.7 | 4.3 | 1.9 | 0.000 |
| 013G112300.1 | Adenine nucleotide alpha hydrolases-like superfamily protein | | 40.9 | 3.4 | 3.6 | 0.000 |
| 001G085900.1 | Aluminium activated malate transporter family protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At4g00910, ] | 22.6 | 1.0 | 4.6 | 0.000 |
| 002G174600.1 | Aluminium activated malate transporter family protein | | 15.4 | 2.8 | 2.5 | 0.000 |
| 005G208500.1 | aluminum-activated, malate transporter 12 | | 7.2 | 0.7 | 3.3 | 0.002 |
| 016G043000.1 | autoinhibited Ca(2+)-ATPase, isoform 4 | | 5.5 | 0.7 | 3.0 | 0.012 |
| 006G249500.1 | Barwin-related endoglucanase | | 5.7 | 1.0 | 2.6 | 0.020 |
| 006G037600.1 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein | | 92.9 | 10.7 | 3.1 | 0.000 |
| 005G071100.1 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein | | 4.5 | 0.6 | 2.9 | 0.034 |
| 018G141800.1 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein | | 33.4 | 5.2 | 2.7 | 0.000 |
| 007G015200.1 | B-box type zinc finger family protein | | 36.5 | 3.9 | 3.2 | 0.000 |
| 004G019400.1 | beta glucosidase 46 | | 31.8 | 5.3 | 2.6 | 0.000 |
| 005G211800.1 | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein | | 7.2 | 1.0 | 2.8 | 0.004 |
| 003G111400.1 | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein | | 134.7 | 22.1 | 2.6 | 0.047 |
| 002G050300.1 | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein | protease inhibitor/seed storage/lipid transfer protein (LTP) family protein; [ co-ortholog (1of3) of At2g48130, ] | 9.6 | 1.7 | 2.5 | 0.013 |
| T055500.1 | Calcium-binding EF-hand family protein | | 4.3 | 0.4 | 3.3 | 0.026 |
| 005G241700.1 | Calcium-dependent lipid-binding (CaLB domain) family protein | similar to C2 domain-containing protein; [ co-ortholog (2of2) of At1g20080, ] | 119.8 | 48.9 | 1.3 | 0.010 |
| 018G042600.1 | carotenoid cleavage dioxygenase 1 | | 66.7 | 0.9 | 6.3 | 0.000 |
| 001G265800.1 | carotenoid cleavage dioxygenase 1 | | 28.3 | 3.4 | 3.0 | 0.000 |
| 002G066600.1 | cellulose synthase A9 | | 30.4 | 5.0 | 2.6 | 0.000 |
| 002G226300.1 | CLAVATA3/ESR-RELATED 20 | | 7.4 | 1.9 | 2.0 | 0.020 |
| 010G067700.1 | Cyclopropane-fatty-acyl-phospholipid synthase | | 36.5 | 0.8 | 5.6 | 0.000 |
| 010G067600.1 | Cyclopropane-fatty-acyl-phospholipid synthase | | 4.0 | 0.1 | 5.2 | 0.012 |
| 014G019200.1 | cytochrome B5 isoform D | | 107.9 | 20.6 | 2.4 | 0.016 |
| 001G363900.1 | cytochrome P450, family 71, subfamily B, polypeptide 34 | | 3.7 | 0.2 | 4.4 | 0.024 |
| 016G061000.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 31.5 | 11.4 | 1.5 | 0.000 |
| 010G137300.1 | DNA glycosylase superfamily protein | | 6.2 | 1.0 | 2.6 | 0.012 |
| 012G130900.1 | Embryo-specific protein 3, (ATS3) | | 39.5 | 7.8 | 2.3 | 0.017 |
| 017G085300.1 | expansin A5 | similar to expansin S1 precursor. [ORG:Cucumis sativus]; [ co-ortholog (2of10) of BAD00016, T10079, CAC19183, CAD19044, BAC05513, AAR09169, AAB37746, AAM88862, AAM89261, CAD19043, BAD00013, CAD28984, CAC19184, AAK72874, ] | 15.3 | 1.2 | 3.7 | 0.000 |
| 001G462800.1 | FAD-binding Berberine family protein | | 21.6 | 3.1 | 2.8 | 0.000 |
| 013G059600.1 | GATA transcription factor 12 | | 18.0 | 6.1 | 1.6 | 0.002 |
| 003G071500.1 | GDSL-motif lipase 5 | | 5.5 | 0.8 | 2.9 | 0.015 |
| 012G076700.1 | Gibberellin-regulated family protein | similar to unnamed protein product. [ORG:Glycine max]; [ co-ortholog (2of2) of CAD35168, CAD35170, CAD35169, ] | 77.4 | 10.5 | 2.9 | 0.016 |

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|---|---|---|---|---|---|---|
| 010G061300.1 | glutathione S-transferase tau 5 | | 3.4 | 0.2 | 3.9 | 0.040 |
| 001G098800.1 | glycosyl hydrolase 9B14 | | 5.9 | 0.8 | 2.8 | 0.011 |
| 002G023900.1 | glycosyl hydrolase 9B5 | glycosyl hydrolase family 9 protein; similar to endo-beta-1; similar to 4-D-glucanase GI:4165132 from (Lycopersicon esculentum); [ co-ortholog (2of2) of At1g75680, At1g19940, ] | 4.8 | 0.8 | 2.5 | 0.043 |
| 005G175300.1 | GRAS family transcription factor | | 19.3 | 2.1 | 3.2 | 0.000 |
| 009G048100.1 | Heavy metal transport/detoxification superfamily protein | | 256.7 | 53.0 | 2.3 | 0.010 |
| 014G106800.1 | Homeodomain-like superfamily protein | | 71.4 | 23.6 | 1.6 | 0.000 |
| 002G180800.1 | Homeodomain-like superfamily protein | | 50.7 | 17.1 | 1.6 | 0.000 |
| 001G128100.1 | HXXXD-type acyl-transferase family protein | transferase family protein; similar to anthranilate N-hydroxycinnamoyl benzoyltransferase GI:3288180; similar to GI:2239091 from (Dianthus caryophyllus); [ ortholog of At1g31490,] | 6.7 | 0.8 | 3.2 | 0.003 |
| 002G010700.1 | HXXXD-type acyl-transferase family protein | | 62.8 | 8.2 | 2.9 | 0.000 |
| 005G045900.1 | jasmonic acid carboxyl methyltransferase | | 45.4 | 3.1 | 3.9 | 0.000 |
| 001G200700.1 | Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family | similar to At1g17620 (At1g17620/F11A6_23) mRNA; similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (3of3) of At1g17620, ] | 26.6 | 4.8 | 2.5 | 0.000 |
| 011G054200.1 | Late embryogenesis abundant protein (LEA) family protein | similar to maturation protein pPM32. [ORG:Glycine max]; [ co-ortholog (1of2) of AAD49719, At5g44310, At4g21020, ] | 6.5 | 0.7 | 3.2 | 0.004 |
| 004G173400.1 | Major facilitator superfamily protein | nodulin family protein; similar to nodulin-like protein (Arabidopsis thaliana) GI:3329368; similar to nodule-specific protein Nlj70 (Lotus japonicus) GI:3329366; [ co-ortholog (2of4) of At4g34950, At2g16660, ] | 82.2 | 24.2 | 1.8 | 0.005 |
| 001G235500.1 | myb domain protein 48 | similar to myb family transcription factor (MYB59); similar to myb family transcription factor (MYB59); similar to myb family transcription factor (MYB59); [ co-ortholog (2of2) of At3g46130, At5g59780, ] | 568.1 | 106.4 | 2.4 | 0.000 |
| 006G221200.1 | myb domain protein 5 | | 19.6 | 1.5 | 3.8 | 0.000 |
| 014G000700.1 | myb-like HTH transcriptional regulator family protein | | 4.9 | 0.7 | 2.9 | 0.023 |
| 005G205400.1 | NAC domain containing protein 42 | | 10.4 | 0.9 | 3.5 | 0.000 |
| 015G050200.1 | NAD(P)-binding Rossmann-fold superfamily protein | | 16.4 | 2.7 | 2.6 | 0.002 |
| 010G129800.1 | NAD(P)-binding Rossmann-fold superfamily protein | | 32.0 | 6.2 | 2.4 | 0.000 |
| 005G244900.1 | NEP-interacting protein 2 | | 5.7 | 0.8 | 2.8 | 0.014 |
| 018G042700.1 | nine-cis-epoxycarotenoid dioxygenase 3 | | 27.5 | 0.6 | 5.6 | 0.000 |
| 003G119600.1 | non-yellowing 1 | | 21.8 | 2.7 | 3.0 | 0.000 |
| 019G042500.1 | Nucleotide-diphospho-sugar transferases superfamily protein | | 11.6 | 3.6 | 1.7 | 0.006 |
| 006G107700.1 | ovate family protein 2 | | 13.8 | 2.7 | 2.3 | 0.039 |
| 003G138800.1 | PA-domain containing subtilase family protein | | 5.0 | 0.6 | 3.0 | 0.019 |
| 015G144800.1 | PAR1 protein | | 9.1 | 0.6 | 4.0 | 0.000 |
| 009G040000.1 | PAR1 protein | | 16.2 | 2.9 | 2.5 | 0.000 |
| 007G144100.1 | Pectin lyase-like superfamily protein | | 6.8 | 0.7 | 3.2 | 0.003 |
| 017G037900.1 | Peroxidase superfamily protein | | 48.5 | 5.4 | 3.2 | 0.004 |
| 011G027300.1 | Peroxidase superfamily protein | | 69.4 | 13.0 | 2.4 | 0.032 |
| 015G120200.1 | phloem protein 2-A10 | similar to lectin. [ORG:Glycine max]; [ ortholog of CAB44031,At1g31200,T10250,CAB71030,BAA09704,At1g10155,] | 23.6 | 3.8 | 2.6 | 0.000 |
| 002G060100.1 | phy rapidly regulated 2 | similar to expressed protein in Arabidopsis thaliana; similar to predicted proteins; similar to Arabidopsis thaliana; [ co-ortholog (2of2) of At3g58850, At2g42870, ] | 4.6 | 0.7 | 2.7 | 0.038 |
| 003G048100.1 | phytochrome-associated protein 1 | | 65.5 | 12.3 | 2.4 | 0.000 |
| 009G148900.1 | PHYTOSULFOKINE 3 PRECURSOR | similar to Putative phytosulfokines 6 precursor (AtPSK6) (AtPSK3_2) [Contains: Phytosulfokine-alpha-like (PSK-alpha-like) (Phytosulfokine-a-like); similar to Phytosulfokine-beta (PSK-beta) (Phytosulfokine-b)].; [ co-ortholog (1of2) of At3g44735, ] | 79.6 | 6.4 | 3.6 | 0.000 |

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|---|---|---|---|---|---|---|
| 012G014500.1 | Plant invertase/pectin methylesterase inhibitor superfamily | | 17.1 | 2.5 | 2.8 | 0.000 |
| 007G133400.1 | Plant regulator RWP-RK family protein | | 29.6 | 5.6 | 2.4 | 0.000 |
| 010G179500.1 | Plant stearoyl-acyl-carrier-protein desaturase family protein | | 57.7 | 3.0 | 4.3 | 0.000 |
| 019G051200.1 | PLATZ transcription factor family protein | | 81.7 | 13.7 | 2.6 | 0.000 |
| 006G237100.1 | Polyketide cyclase/dehydrase and lipid transport superfamily protein | | 37.1 | 4.1 | 3.2 | 0.000 |
| 010G145400.1 | Protein kinase superfamily protein | | 13.7 | 2.6 | 2.4 | 0.000 |
| 011G122700.1 | Protein of unknown function (DUF1218) | similar to Expressed protein in Arabidopsis thaliana; [ co-ortholog (2of4) of At4g27435, At3g15480, At1g52910, ] | 120.4 | 23.8 | 2.3 | 0.000 |
| 015G096900.1 | Protein of unknown function (DUF579) | | 10.0 | 1.2 | 3.1 | 0.000 |
| 001G056300.1 | Protein of unknown function (DUF579) | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At1g27930, ] | 8.4 | 1.8 | 2.2 | 0.006 |
| 002G092900.1 | Protein of unknown function (DUF581) | similar to senescence-associated protein-related; similar to senescence-associated protein SAG102 (GI:22331931) (Arabidopsis thaliana); [ co-ortholog (2of2) of At1g78020, At1g22160, ] | 81.1 | 7.6 | 3.4 | 0.000 |
| 003G085700.1 | Protein of unknown function (DUF581) | similar to senescence-associated protein-related; similar to senescence-associated protein SAG102 (GI:22331931) (Arabidopsis thaliana); [ co-ortholog (2of2) of At4g17670, At5g47060, ] | 37.2 | 3.9 | 3.2 | 0.000 |
| 007G089200.1 | Protein of unknown function (DUF581) | similar to senescence-associated protein-related; similar to senescence-associated protein SAG102 (GI:22331931) (Arabidopsis thaliana); [ co-ortholog (2of2) of At4g39795, ] | 398.1 | 63.6 | 2.6 | 0.000 |
| 001G148700.1 | Protein of unknown function (DUF581) | similar to senescence-associated protein-related; similar to senescence-associated protein SAG102 (GI:22331931) (Arabidopsis thaliana); [ co-ortholog (1of2) of At4g17670, At5g47060, ] | 132.9 | 22.4 | 2.6 | 0.004 |
| 009G092400.1 | Protein of unknown function (DUF620) | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At3g19540, At1g49840, ] | 4.9 | 0.8 | 2.7 | 0.032 |
| 011G129900.1 | Protein of unknown function, DUF538 | | 5.1 | 0.5 | 3.4 | 0.028 |
| 005G011200.1 | Protein of unknown function, DUF538 | similar to expressed protein in Arabidopsis thaliana; similar to DUF538; [ ortholog of At1g09310,] | 184.3 | 38.1 | 2.3 | 0.000 |
| 006G048800.1 | Protein of unknown function, DUF617 | | 16.0 | 1.8 | 3.1 | 0.000 |
| T087500.1 | Putative glycosyl hydrolase of unknown function (DUF1680) | | 12.2 | 1.9 | 2.7 | 0.000 |
| 003G206000.1 | Putative glycosyl hydrolase of unknown function (DUF1680) | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At5g12950, At5g12960, ] | 14.9 | 2.4 | 2.7 | 0.000 |
| 004G212900.1 | Putative membrane lipoprotein | | 96.3 | 13.7 | 2.8 | 0.000 |
| 003G172100.1 | Pyridoxal phosphate (PLP)-dependent transferases superfamily protein | | 15.1 | 2.5 | 2.6 | 0.000 |
| 005G228000.1 | RAD-like 6 | similar to myb family transcription factor; [ co-ortholog (2of2) of At1g19510, ] | 8.3 | 0.2 | 5.4 | 0.000 |
| 002G188100.1 | RAD-like 6 | | 64.0 | 5.3 | 3.6 | 0.000 |
| 002G035000.1 | RAD-like 6 | similar to myb family transcription factor; [ co-ortholog (1of2) of At1g19510, ] | 10.0 | 1.7 | 2.6 | 0.007 |
| 010G199100.1 | Regulator of chromosome condensation (RCC1) family protein | | 14.2 | 1.0 | 3.9 | 0.000 |
| 012G140800.1 | Remorin family protein | remorin family protein; similar to C-terminal region; [ co-ortholog (2of2) of At3g48940, At5g23750, ] | 437.2 | 18.6 | 4.6 | 0.000 |
| 010G143500.1 | RGA-like 2 | | 3.7 | 0.3 | 3.7 | 0.032 |
| 017G122300.1 | RING/FYVE/PHD zinc finger superfamily protein | | 13.7 | 2.3 | 2.6 | 0.020 |
| 018G042400.1 | RING/U-box superfamily protein | | 4.1 | 0.4 | 3.3 | 0.033 |
| 003G173100.1 | Rubber elongation factor protein (REF) | similar to Hypothetical protein At1g67360.; [ co-ortholog (2of2) of At1g67360, ] | 394.7 | 126.4 | 1.6 | 0.005 |
| 007G021300.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | 34.5 | 1.7 | 4.4 | 0.000 |
| 017G121800.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | 37.7 | 4.9 | 2.9 | 0.000 |
| 012G052400.1 | S-adenosyl-L-methionine- | | 144.6 | 22.3 | 2.7 | 0.015 |

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|---|---|---|---|---|---|---|
| | dependent methyltransferases superfamily protein | | | | | |
| 003G070900.1 | SAUR-like auxin-responsive protein family | | 12.5 | 2.3 | 2.5 | 0.000 |
| T082800.1 | Serine protease inhibitor, potato inhibitor I-type family protein | | 4.5 | 0.5 | 3.2 | 0.024 |
| 006G088500.1 | Serine protease inhibitor, potato inhibitor I-type family protein | | 12.1 | 2.1 | 2.5 | 0.000 |
| 015G068700.1 | serine/threonine protein kinase 2 | similar to CsPK5. [ORG:Cucumis sativus]; [ co-ortholog (3of3) of BAA82167, ] | 5.5 | 1.1 | 2.4 | 0.032 |
| 003G131600.1 | SPIRAL1-like1 | | 34.2 | 5.4 | 2.7 | 0.005 |
| 015G098900.1 | Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein | | 11.5 | 2.1 | 2.4 | 0.000 |
| 002G052100.1 | Terpenoid cyclases/Protein prenyltransferases superfamily protein | similar to ent-kaurene synthase A (EC 2.5.1.-) - garden pea. [ORG:Pisum sativum]; [ co-ortholog (1of2) of At4g02780, AAB58822, BAC76429, AAD04293, AAD04292, T06783, BAC54040, BAA95612, ] | 77.1 | 0.8 | 6.6 | 0.000 |
| 008G082700.1 | Terpenoid cyclases/Protein prenyltransferases superfamily protein | | 24.2 | 1.2 | 4.4 | 0.000 |
| 005G210300.1 | Terpenoid cyclases/Protein prenyltransferases superfamily protein | | 11.8 | 0.8 | 3.9 | 0.000 |
| 006G068200.1 | Tetratricopeptide repeat (TPR)-like superfamily protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of4) of At5g20190, At1g80130, ] | 59.7 | 14.5 | 2.0 | 0.017 |
| 018G130100.1 | Tetratricopeptide repeat (TPR)-like superfamily protein | | 47.2 | 16.3 | 1.5 | 0.000 |
| 006G213000.1 | Thioredoxin family protein | | 16.8 | 4.3 | 2.0 | 0.004 |
| 001G203000.1 | Tic22-like family protein | | 11.5 | 0.2 | 5.7 | 0.000 |
| 009G136200.1 | uclacyanin 1 | | 9.9 | 1.6 | 2.6 | 0.001 |
| 009G110200.1 | Uncharacterised protein family (UPF0497) | similar to integral membrane protein; putative; similar to 4 transmembrane domain containing protein; [ ortholog of At1g49405,] | 15.2 | 2.8 | 2.4 | 0.000 |
| 001G442300.1 | Uncharacterised protein family (UPF0497) | | 70.8 | 14.9 | 2.2 | 0.000 |
| 008G095500.1 | ureide permease 2 | | 9.1 | 1.8 | 2.3 | 0.003 |
| 001G002400.1 | WRKY DNA-binding protein 51 | | 4.4 | 0.6 | 2.8 | 0.037 |
| 001G082400.1 | YELLOW STRIPE like 1 | | 5.1 | 0.8 | 2.6 | 0.029 |
| 018G043300.1 | | | 5.9 | 0.1 | 6.1 | 0.001 |
| 019G126800.1 | | | 5.0 | 0.1 | 6.0 | 0.045 |
| 007G041500.1 | | | 5.1 | 0.2 | 4.9 | 0.004 |
| 014G153200.1 | | | 10.3 | 0.4 | 4.7 | 0.000 |
| 004G104600.1 | | | 15.8 | 0.7 | 4.5 | 0.000 |
| 002G046500.1 | | | 31.9 | 2.1 | 3.9 | 0.000 |
| 001G287500.1 | | | 4.9 | 0.4 | 3.7 | 0.010 |
| 018G042300.1 | | | 4.8 | 0.4 | 3.6 | 0.012 |
| 016G098300.1 | | | 25.0 | 2.3 | 3.4 | 0.000 |
| 010G138700.1 | | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At1g69050,] | 9.8 | 1.0 | 3.3 | 0.000 |
| 001G265700.1 | | | 9.1 | 0.9 | 3.3 | 0.000 |
| 012G031900.1 | | | 7.3 | 0.8 | 3.2 | 0.002 |
| 004G015900.1 | | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At4g21920, ] | 13.1 | 1.5 | 3.1 | 0.000 |
| 005G071200.1 | | | 5.3 | 0.6 | 3.1 | 0.012 |
| 006G248300.1 | | similar to expressed protein in Arabidopsis thaliana; similar to expressed protein in Arabidopsis thaliana; [ ortholog of At2g25625,] | 11.0 | 1.3 | 3.1 | 0.004 |
| 002G240300.1 | | | 6.2 | 0.7 | 3.1 | 0.005 |
| 007G089100.1 | | | 31.5 | 3.7 | 3.1 | 0.000 |
| 009G051100.1 | | | 14.1 | 1.8 | 2.9 | 0.000 |
| 019G084500.1 | | | 9.6 | 1.4 | 2.8 | 0.000 |
| 007G082600.1 | | | 18.3 | 2.6 | 2.8 | 0.010 |
| 012G018000.1 | | | 4.3 | 0.6 | 2.8 | 0.043 |
| 016G084000.1 | | | 8.4 | 1.3 | 2.7 | 0.002 |
| 013G061900.1 | | | 6.7 | 1.0 | 2.7 | 0.007 |
| 007G041400.1 | | | 15.9 | 2.4 | 2.7 | 0.000 |
| 002G034300.1 | | | 7.4 | 1.1 | 2.7 | 0.004 |
| 014G119900.1 | | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At2g47360, ] | 7.9 | 1.2 | 2.7 | 0.003 |
| 012G102100.1 | | | 16.9 | 2.6 | 2.7 | 0.000 |
| 009G104500.1 | | | 5.5 | 0.9 | 2.7 | 0.019 |
| 001G255700.1 | | | 13.9 | 2.2 | 2.7 | 0.000 |
| 016G040400.1 | | | 14.0 | 2.2 | 2.7 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| 014G069900.1 | | | | | 28.3 | 4.5 | 2.7 | 0.000 |
| 014G000300.1 | | | | | 8.1 | 1.3 | 2.6 | 0.003 |
| 013G153100.1 | | similar to hypothetical protein; [ co-ortholog (1of2) of At1g06980, At2g30230, ] | | | 9.9 | 1.7 | 2.5 | 0.001 |
| 004G153200.1 | | | | | 149.3 | 25.8 | 2.5 | 0.000 |
| 019G087800.1 | | | | | 27.5 | 4.8 | 2.5 | 0.000 |
| 008G150300.1 | | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At5g19340, At3g05980, ] | | | 8.6 | 1.5 | 2.5 | 0.002 |
| 001G400200.1 | | | | | 18.0 | 3.1 | 2.5 | 0.000 |
| 004G068900.1 | | | | | 5.6 | 1.0 | 2.5 | 0.024 |
| 004G146800.1 | | | | | 5.1 | 0.9 | 2.5 | 0.034 |
| 002G206700.1 | | | | | 26.5 | 4.8 | 2.5 | 0.000 |
| 005G003600.1 | | similar to hypothetical protein; [ co-ortholog (1of2) of At5g26770, At1g09470, At1g09483, At3g05830, ] | | | 5.2 | 1.0 | 2.4 | 0.036 |
| 007G091900.1 | | | | | 12.4 | 2.3 | 2.4 | 0.000 |
| 010G062600.1 | | | | | 14.4 | 2.8 | 2.4 | 0.000 |
| T030700.1 | | | | | 10.2 | 2.0 | 2.3 | 0.001 |
| 004G069000.1 | | | | | 97.0 | 19.1 | 2.3 | 0.000 |
| 010G168000.1 | | | | | 36.1 | 7.1 | 2.3 | 0.000 |
| 002G196900.1 | | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At4g02090, ] | | | 35.6 | 7.1 | 2.3 | 0.023 |
| 001G025100.1 | | | | | 100.3 | 21.1 | 2.2 | 0.000 |
| 001G093100.1 | | | | | 15.4 | 4.0 | 2.0 | 0.000 |
| 013G102300.1 | | | | | 17.7 | 4.6 | 1.9 | 0.000 |
| 003G052900.1 | | similar to hypothetical protein; [ co-ortholog (1of2) of At3g24600, ] | | | 19.4 | 7.2 | 1.4 | 0.001 |
| 017G085800.1 | | | | | 10.6 | 3.9 | 1.4 | 0.029 |
| 002G177700.1 | | | | | 21.8 | 10.8 | 1.0 | 0.013 |
| 009G104100.1 | alpha/beta-Hydrolases superfamily protein | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.3 | 6.6 | -4.5 | 0.042 |
| 009G104000.1 | alpha/beta-Hydrolases superfamily protein | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.1 | 8.2 | -6.0 | 0.005 |
| 001G032400.1 | carboxyesterase 17 | similar to hsr203J homolog. [ORG:Pisum sativum]; [ co-ortholog (3of3) of BAA85654, ] | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.2 | 8.7 | -2.8 | 0.011 |
| 001G256400.1 | NAD(P)-binding Rossmann-fold superfamily protein | similar to cinnamyl-alcohol dehydrogenase; similar to putative (CAD); similar to cinnamyl-alcohol dehydrogenase; similar to Eucalyptus gunnii (GI:1143445); similar to apple tree; similar to PIR:T16995; [ co-ortholog (1of2) of At5g19440, ] | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 16.6 | 211.4 | -3.7 | 0.011 |
| 014G159100.1 | Phosphotyrosine protein phosphatases superfamily protein | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.5 | 57.5 | -5.2 | 0.000 |
| T091700.1 | Tautomerase/MIF superfamily protein | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 34.7 | 174.1 | -2.3 | 0.002 |
| 013G055000.1 | annexin 8 | | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING | 0.7 | 8.4 | -3.6 | 0.000 |
| 008G110800.1 | EXS (ERD1/XPR1/SYG1) family protein | | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING | 2.6 | 21.4 | -3.1 | 0.000 |
| 010G132300.1 | EXS (ERD1/XPR1/SYG1) family protein | EXS family protein; ERD1/XPR1/SYG1 family protein; similar to PHO1 protein (Arabidopsis thaliana) GI:20069032; [ ortholog of At1g68740,] | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING | 2.3 | 18.8 | -3.1 | 0.000 |
| 004G091300.1 | AAA-ATPase 1 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 3.6 | 33.4 | -3.2 | 0.000 |
| 015G067400.1 | AAA-ATPase 1 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.6 | 5.7 | -3.2 | 0.012 |
| 004G091500.1 | AAA-ATPase 1 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.6 | 6.3 | -3.5 | 0.003 |
| 004G012700.1 | AAA-ATPase 1 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 9.2 | 495.5 | -5.8 | 0.006 |
| 004G012500.1 | AAA-ATPase 1 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 3.2 | 357.6 | -6.8 | 0.032 |
| 018G042200.1 | Ankyrin repeat family protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.6 | 6.7 | -3.5 | 0.002 |
| 001G043100.1 | Chaperone DnaJ-domain superfamily | similar to DNAJ heat shock N-terminal domain-containing protein; similar to SP\|Q05646\|Chaperone protein dnaJ Erysipelothrix | Posttranslational modification, protein | CELLULAR PROCESSES | 9.3 | 130.0 | -3.8 | 0.000 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | protein | rhusiopathiae ; similar to SP\|P45555 Chaperone protein dnaJ (HSP40) Staphylococcus aureus; similar to J8 mRNA; similar to nuclear g | turnover, chaperones | AND SIGNALING | | | |
| 012G038800.1 | Chaperone DnaJ-domain superfamily protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 4.9 | 80.7 | -4.1 | 0.013 |
| 002G074600.1 | DNAJ heat shock N-terminal domain-containing protein | similar to DNAJ heat shock N-terminal domain-containing protein; similar to SP\|P39101\|CAJ1 protein Saccharomyces (cerevisiae ; [ co-ortholog (1of2) of At1g77020, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.7 | 16.0 | -2.6 | 0.011 |
| 018G014700.1 | Eukaryotic aspartyl protease family protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.4 | 12.5 | -2.4 | 0.021 |
| 013G070300.1 | Eukaryotic aspartyl protease family protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.7 | 4.3 | -2.7 | 0.046 |
| 018G014900.1 | Eukaryotic aspartyl protease family protein | similar to nucleoid DNA-binding protein. [ORG:Malus x domestica]; [ co-ortholog (3of9) of AAQ54564, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 1.5 | 12.0 | -3.0 | 0.000 |
| 008G203100.1 | Eukaryotic aspartyl protease family protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 1.8 | 15.4 | -3.1 | 0.000 |
| 002G054900.1 | Eukaryotic aspartyl protease family protein | similar to unnamed protein product. [ORG:Glycine max]; [ co-ortholog (1of2) of CAC17729, AAQ54572, At1g03230, At1g03220, CAE85345, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.3 | 7.2 | -4.5 | 0.000 |
| 012G118000.1 | Eukaryotic aspartyl protease family protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.0 | 6.2 | -10.5 | 0.000 |
| 016G083500.1 | Glutathione S-transferase family protein | similar to In2-1 protein. [ORG:Glycine max]; [ co-ortholog (1of2) of At5g02790, AAG34872, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 38.8 | 266.5 | -2.8 | 0.000 |
| 011G114000.1 | glutathione S-transferase TAU 19 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.9 | 704.3 | -7.9 | 0.002 |
| 011G113000.1 | glutathione S-transferase TAU 19 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.7 | 181.1 | -8.1 | 0.002 |
| 011G113400.1 | glutathione S-transferase TAU 24 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.1 | 733.0 | -8.5 | 0.003 |
| 011G113300.1 | glutathione S-transferase TAU 25 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 1.2 | 9.5 | -3.0 | 0.000 |
| 011G112900.1 | glutathione S-transferase TAU 25 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.1 | 46.4 | -4.5 | 0.000 |
| 015G042000.1 | glutathione S-transferase tau 4 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.4 | 16.7 | -5.5 | 0.031 |
| 002G254000.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 3.9 | 21.1 | -2.4 | 0.001 |
| 010G061400.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 4.2 | 31.5 | -2.9 | 0.000 |
| 010G070900.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 14.3 | 153.8 | -3.4 | 0.004 |
| T149500.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 34.3 | 481.6 | -3.8 | 0.002 |
| 016G023200.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 39.3 | 637.3 | -4.0 | 0.000 |
| 010G061200.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 6.1 | 102.7 | -4.1 | 0.000 |
| 010G061700.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein | CELLULAR PROCESSES | 22.5 | 541.9 | -4.6 | 0.020 |

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|---|---|---|---|---|---|---|---|---|
| | | | turnover, chaperones | AND SIGNALING | | | | |
| 010G061100.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 19.6 | 565.7 | -4.9 | 0.008 |
| 010G060900.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 25.3 | 762.0 | -4.9 | 0.013 |
| 006G024200.1 | glutathione S-transferase TAU 8 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.4 | 224.6 | -9.3 | 0.012 |
| 005G119200.1 | P-loop containing nucleoside triphosphate hydrolases superfamily protein | similar to hypothetical protein; [ co-ortholog (2of2) of At4g05380, At1g43910, At4g05340, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 12.5 | 126.6 | -3.3 | 0.000 |
| 015G031400.1 | purple acid phosphatase 17 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 6.2 | 81.7 | -3.7 | 0.000 |
| 012G042200.1 | purple acid phosphatase 17 | similar to acid phosphatase type 5 (ACP5); similar to acid phosphatase type 5 (GI:10278031) (Arabidopsis thaliana); [ co-ortholog (1of3) of At3g17790, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 3.0 | 56.4 | -4.2 | 0.000 |
| 008G139300.1 | purple acid phosphatase 3 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 3.8 | 38.2 | -3.3 | 0.000 |
| 008G139100.1 | purple acid phosphatase 3 | similar to PURPLE ACID PHOSPHATASE PRECURSOR; [ co-ortholog (1of3) of At1g25230, At2g01890, At1g14700, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.9 | 33.8 | -3.6 | 0.000 |
| 003G085800.1 | SBP (S-ribonuclease binding protein) family protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At1g32740, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 6.7 | 34.4 | -2.4 | 0.009 |
| 019G054300.1 | serine carboxypeptidase-like 20 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 9.0 | 97.9 | -3.4 | 0.006 |
| 007G134800.1 | Thioredoxin superfamily protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 8.0 | 54.9 | -2.8 | 0.018 |
| 011G058800.1 | Thioredoxin superfamily protein | similar to glutaredoxin-like protein. [ORG:Cucumis sativus]; [ co-ortholog (1of2) of AAL35982, At1g28480, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 2.2 | 15.6 | -2.8 | 0.000 |
| 017G017300.1 | Thioredoxin superfamily protein | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 14.2 | 155.4 | -3.4 | 0.000 |
| 014G134200.1 | Thioredoxin superfamily protein | glutaredoxin family protein; similar to Glutaredoxin (thioltransferase); [ co-ortholog (2of2) of At3g62950, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 0.4 | 5.3 | -3.6 | 0.007 |
| 005G118600.1 | ubiquitin-conjugating enzyme 16 | similar to UBIQUITIN-CONJUGATING ENZYME 17; [ co-ortholog (1of2) of At4g36410, At1g75440, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 29.3 | 195.8 | -2.7 | 0.000 |
| 001G378900.1 | ubiquitin-specific protease 15 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 6.4 | 79.1 | -3.6 | 0.000 |
| 018G067000.1 | 1-amino-cyclopropane-1-carboxylate synthase 7 | similar to 1-aminocyclopropene-1-carboxylate synthase 3c. [ORG:Pyrus communis]; [ co-ortholog (2of2) of BAA76388, PN0477, AAS17854, AAR38502, PN0476, AAG12247, AAF22108, AAL66201, T17018, CAA78122, CAA78123, AAF61233, AAB67989, AAS17855, BAA37134, AAR1213 | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 2.8 | 13.8 | -2.3 | 0.006 |
| 003G132300.1 | 1-aminocyclopropane-1-carboxylic acid (acc) synthase 6 | similar to 1-aminocyclopropene-1-carboxylate synthase. [ORG:Cucumis sativus]; [ co-ortholog (2of3) of S26214, AAL35745, AAL66205, S25002, BAA94599, T10513, BAA93713, CAD21840, AAF61235, CAA47474, BAB18464, BAA92351, BAA33375, P31531, AAM74939, 2019442A, A | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.0 | 7.1 | -2.9 | 0.003 |
| 009G139400.1 | ACT-like protein tyrosine kinase family protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 7.3 | 64.0 | -3.1 | 0.000 |
| 004G179100.1 | ACT-like protein tyrosine kinase family protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.1 | 66.4 | -9.6 | 0.000 |
| 004G179500.1 | ACT-like protein tyrosine kinase family protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.0 | 58.3 | -10.3 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| 002G156200.1 | Calcineurin-like metallo-phosphoesterase superfamily protein | calcineurin-like phosphoesterase family protein; [ co-ortholog (2of3) of At3g09970, At3g09960, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 12.1 | 73.9 | -2.6 | 0.033 |
| 002G156500.1 | Calcineurin-like metallo-phosphoesterase superfamily protein | calcineurin-like phosphoesterase family protein; [ co-ortholog (1of3) of At3g09970, At3g09960, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 6.4 | 51.1 | -3.0 | 0.031 |
| 006G112500.1 | calmodulin like 42 | similar to calcium-binding protein; putative; similar to SP\|Q09011\|Calcium binding-protein CAST (Solanum (tuberosum) ; [ co-ortholog (1of2) of At4g20780, At5g44460, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 8.1 | 57.9 | -2.8 | 0.000 |
| T115000.1 | calmodulin-like 38 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.2 | 6.3 | -4.9 | 0.001 |
| 014G156400.1 | chitin elicitor receptor kinase 1 | similar to LysM domain-containing receptor-like kinase 4. [ORG:Medicago truncatula]; [ co-ortholog (2of2) of AAQ73156, CAE02589, AAQ73154, At3g21630, AAQ73157, AAQ73160, CAE02591, CAE02590, AAQ73155, AAQ73159, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.8 | 7.6 | -3.3 | 0.016 |
| 011G093700.1 | Concanavalin A-like lectin protein kinase family protein | similar to lectin protein kinase; putative; similar to receptor lectin kinase 3 (Arabidopsis thaliana) gi\|4100060\|gb\|AAD00733\|; similar to protein kinase domain containing protein; similar to legume lectins alpha and beta domain containing protein; [ orth | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.6 | 11.9 | -2.9 | 0.008 |
| 011G029000.1 | cysteine-rich RLK (RECEPTOR-like protein kinase) 29 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.2 | 6.0 | -2.3 | 0.021 |
| 008G183700.1 | MAP kinase kinase 7 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.1 | 3.1 | -4.6 | 0.045 |
| 009G073200.1 | mitogen-activated protein kinase kinase kinase 13 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.9 | 7.5 | -3.1 | 0.001 |
| 004G007700.1 | mitogen-activated protein kinase kinase kinase 15 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 3.1 | 23.5 | -2.9 | 0.000 |
| 007G044800.1 | mitogen-activated protein kinase kinase kinase 19 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 3.1 | 18.0 | -2.5 | 0.002 |
| 005G139200.1 | mitogen-activated protein kinase kinase kinase 19 | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.2 | 13.7 | -3.6 | 0.000 |
| 010G107300.1 | nudix hydrolase homolog 17 | MutT/nudix family protein; similar to low similarity to SP\|Q09790 Diadenosine 5\'; similar to 5\'\'\'-P1; similar to P6-hexaphosphate hydrolase (EC 3.6.1.-) (Ap6A hydrolase) (Schizosaccharomyces pombe); [ co-ortholog (1of2) of At1g14860, At2g01670, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.4 | 41.9 | -4.9 | 0.000 |
| 003G099400.1 | phosphoribulokinase | similar to Phosphoribulokinase; similar to chloroplast precursor (EC 2.7.1.19) (Phosphopentokinase) (PRKASE) (PRK).; [ co-ortholog (2of2) of At1g32060, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 4.4 | 26.5 | -2.6 | 0.005 |
| 002G004900.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 2.3 | 13.6 | -2.5 | 0.004 |
| 002G019300.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.5 | 7.2 | -3.9 | 0.022 |
| 012G054700.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.1 | 3.7 | -5.0 | 0.019 |
| 006G072200.1 | Protein kinase superfamily protein | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 0.2 | 123.0 | -9.0 | 0.006 |
| 005G246800.1 | SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein | | Chromatin structure and dynamics | INFORMATION STORAGE AND PROCESSING | 6.5 | 49.2 | -2.9 | 0.000 |
| 005G107300.1 | SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein | | Chromatin structure and dynamics | INFORMATION STORAGE AND PROCESSING | 3.9 | 38.3 | -3.3 | 0.000 |
| 002G229200.1 | aconitase 3 | similar to aconitate hydratase; similar to cytoplasmic; putative; similar to citrate hydro-lyase/aconitase; putative; similar to SP\|P49608\|Aconitate hydratase ; similar to cytoplasmic (EC 4.2.1.3) (Citrate hydro-lyase) (Aconitase) (Cucurbita maxima); [ or | RNA processing and modification | INFORMATION STORAGE AND PROCESSING | 59.7 | 336.0 | -2.5 | 0.001 |
| 008G086700.1 | ribonuclease 1 | similar to S2-RNase. [ORG:Prunus avium]; [ co-ortholog (2of2) of BAA36387, BAD11006, AAM28172, T06435, CAG25696, AAM28160, BAC66632, AAQ73174, AAM22181, AAM28178, BAA88126, | RNA processing and modification | INFORMATION STORAGE AND PROCESSING | 0.6 | 17.2 | -4.8 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| | | BAA10892, AAM28176, AAK15072, T12075, AAK72320, AAM22180, AAL27624, 1UCG_A, AAM281 | | | | | | |
| 015G087700.1 | BTB and TAZ domain protein 1 | similar to speckle-type POZ protein-related; similar to Speckle-type POZ protein (SP:O43791) (Homo sapiens); [ co-ortholog (2of2) of At3g48360, At5g63160, ] | Transcription | INFORMATION STORAGE AND PROCESSING | 1.4 | 17.7 | -3.7 | 0.000 |
| 003G104500.1 | Homeodomain-like superfamily protein | | Transcription | INFORMATION STORAGE AND PROCESSING | 0.9 | 5.0 | -2.4 | 0.039 |
| 008G101400.1 | myb domain protein 108 | similar to GmMYB12. [ORG:Glycine max]; [ co-ortholog (1of2) of At1g48000, At3g06490, BAA81808, At5g49620, ] | Transcription | INFORMATION STORAGE AND PROCESSING | 1.1 | 6.6 | -2.6 | 0.008 |
| 002G038500.1 | myb domain protein 14 | similar to GmMYB29. [ORG:Glycine max]; [ ortholog of At2g31180,BAA81813,] | Transcription | INFORMATION STORAGE AND PROCESSING | 2.1 | 28.6 | -3.7 | 0.000 |
| 014G117000.1 | myb domain protein 2 | similar to putative MYB transcription factor [Populus x canescens]. [ORG:Populus alba x Populus tremula]; [ co-ortholog (45of51) of BAA81802, BAA81803, CAD98762, BAA81811, AAN05422, ] | Transcription | INFORMATION STORAGE AND PROCESSING | 0.2 | 4.1 | -4.2 | 0.017 |
| 013G067000.1 | myb domain protein 56 | | Transcription | INFORMATION STORAGE AND PROCESSING | 3.1 | 19.2 | -2.6 | 0.000 |
| 018G090600.1 | lysyl-tRNA synthetase 1 | | Translation, ribosomal structure and biogenesis | INFORMATION STORAGE AND PROCESSING | 18.9 | 108.2 | -2.5 | 0.002 |
| 005G098700.1 | Translation initiation factor IF6 | | Translation, ribosomal structure and biogenesis | INFORMATION STORAGE AND PROCESSING | 2.0 | 32.0 | -4.0 | 0.021 |
| 001G162800.1 | alanine aminotransferase 2 | similar to ALANINE AMINOTRANSFERAS; [ ortholog of At1g17290,] | Amino acid transport and metabolism | METABOLISM | 3.0 | 235.6 | -6.3 | 0.000 |
| 005G181500.1 | amino acid permease 3 | | Amino acid transport and metabolism | METABOLISM | 1.0 | 9.9 | -3.4 | 0.003 |
| T104400.1 | APS reductase 3 | | Amino acid transport and metabolism | METABOLISM | 11.2 | 56.9 | -2.3 | 0.000 |
| 014G143300.1 | aspartate aminotransferase 1 | | Amino acid transport and metabolism | METABOLISM | 3.1 | 20.1 | -2.7 | 0.000 |
| 006G260200.1 | aspartate aminotransferase 5 | similar to aspartate aminotransferase 2. [ORG:Canavalia lineata]; [ co-ortholog (2of2) of S46316, AAB68396, 1908424A, XNYLB, AAC12674, AAB46611, S39925, S33528, CAA04697, S39926, CAA42430, P26563, AAN76499, S39927, AAB26677, 2009357A, AAA33942, S39928, ] | Amino acid transport and metabolism | METABOLISM | 12.4 | 239.9 | -4.3 | 0.000 |
| 002G236800.1 | aspartate kinase 3 | | Amino acid transport and metabolism | METABOLISM | 4.6 | 26.6 | -2.5 | 0.004 |
| 003G083700.1 | cationic amino acid transporter 6 | | Amino acid transport and metabolism | METABOLISM | 2.5 | 24.0 | -3.2 | 0.000 |
| 001G150700.1 | cationic amino acid transporter 6 | | Amino acid transport and metabolism | METABOLISM | 1.3 | 15.3 | -3.6 | 0.000 |
| 014G086300.1 | cysteine synthase C1 | | Amino acid transport and metabolism | METABOLISM | 40.5 | 232.4 | -2.5 | 0.000 |
| 001G300900.1 | D-3-phosphoglycerate dehydrogenase | similar to D-3-phosphoglycerate dehydrogenase; putative; similar to 3-PGDH; putative; similar to phosphoglycerate dehydrogenase; similar to Arabidopsis thaliana; similar to SP:O04130; [ co-ortholog (2of2) of At4g34200, ] | Amino acid transport and metabolism | METABOLISM | 24.8 | 138.2 | -2.5 | 0.000 |
| 005G043400.1 | dehydroquinate dehydratase, putative / shikimate dehydrogenase, putative | | Amino acid transport and metabolism | METABOLISM | 1.1 | 14.9 | -3.7 | 0.018 |
| T059200.1 | glutamate decarboxylase | similar to glutamate decarboxylase 1. [ORG:Lotus corniculatus var. japonicus]; [ co-ortholog (2of3) of AAP85548, CAG30580, ] | Amino acid transport and metabolism | METABOLISM | 2.1 | 27.3 | -3.7 | 0.000 |
| 013G058300.1 | glutamate dehydrogenase 1 | similar to Glutamate dehydrogenase 1 (EC 1.4.1.3) (GDH 1).; [ co-ortholog (2of2) of At5g18170, At3g03910, ] | Amino acid transport and metabolism | METABOLISM | 22.6 | 125.4 | -2.5 | 0.000 |
| 004G085400.1 | glutamine synthase clone R1 | similar to glutamate-ammonia ligase (EC 6.3.1.2) gamma; similar to cytosolic - kidney bean. [ORG:Phaseolus vulgaris]; [ co-ortholog (2of5) of JQ0937, AAB23379, P08282, S62712, AAQ01729, AAP20795, AAB03492, AAD52008, 1211328A, P00965, AJFBQ, CAA63963, 2106 | Amino acid transport and metabolism | METABOLISM | 55.2 | 795.7 | -3.8 | 0.000 |
| 017G131100.1 | glutamine synthase clone R1 | similar to glutamate-ammonia ligase (EC 6.3.1.2) gamma; similar to cytosolic - kidney bean. [ORG:Phaseolus vulgaris]; [ co-ortholog (1of5) of JQ0937, AAB23379, P08282, S62712, AAQ01729, AAP20795, AAB03492, AAD52008, 1211328A, P00965, AJFBQ, CAA63963, 2106 | Amino acid transport and metabolism | METABOLISM | 109.1 | #### | -5.0 | 0.000 |
| 009G072900.1 | glutamine-dependent asparagine synthase 1 | similar to asparagine synthetase. [ORG:Glycine max]; [ co-ortholog (1of2) of P19251, AAC09952, At3g47340, ] | Amino acid transport and metabolism | METABOLISM | 0.7 | 35.4 | -5.7 | 0.001 |
| 001G278400.1 | glutamine-dependent asparagine synthase 1 | similar to asparagine synthetase. [ORG:Glycine max]; [ co-ortholog (2of2) of P19251, AAC09952, At3g47340, ] | Amino acid transport and metabolism | METABOLISM | 1.8 | #### | -9.1 | 0.000 |
| 010G083600.1 | homocysteine S-methyltransferase 3 | similar to Homocysteine S-methyltransferase 3 (EC 2.1.1.10) (S-methylmethionine:homocysteine methyltransferase 3) (SMM:Hcy S-methyltransferase 3) (AtHMT-3).; [ co-ortholog (1of2) of At3g22740, ] | Amino acid transport and metabolism | METABOLISM | 1.6 | 28.8 | -4.1 | 0.000 |
| 005G099600.1 | isocitrate dehydrogenase 1 | | Amino acid transport and metabolism | METABOLISM | 11.4 | 79.6 | -2.8 | 0.000 |
| 018G041600.1 | Major facilitator superfamily protein | | Amino acid transport and metabolism | METABOLISM | 4.0 | 18.5 | -2.2 | 0.000 |
| 012G011700.1 | NADH-dependent glutamate synthase 1 | similar to NADH-dependent glutamate synthase. [ORG:Medicago sativa]; [ co-ortholog (2of3) of At5g53460, AAB41904, ] | Amino acid transport and metabolism | METABOLISM | 3.1 | 367.8 | -6.9 | 0.002 |
| 004G094600.1 | Thiamine | | Amino acid transport | METABOLISM | 22.8 | 189.8 | -3.1 | 0.028 |

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|---|---|---|---|---|---|---|---|---|
| | pyrophosphate dependent pyruvate decarboxylase family protein | | and metabolism | | | | | |
| 017G083700.1 | Transmembrane amino acid transporter family protein | | Amino acid transport and metabolism | METABOLISM | 4.7 | 38.8 | -3.0 | 0.023 |
| 001G095800.1 | 6-phosphogluconate dehydrogenase family protein | 6-phosphogluconate dehydrogenase family protein; [ co-ortholog (1of2) of At1g64190, At5g41670, ] | Carbohydrate transport and metabolism | METABOLISM | 42.1 | 309.7 | -2.9 | 0.000 |
| 001G068200.1 | Aldolase-type TIM barrel family protein | similar to transaldolase; putative; [ co-ortholog (2of3) of At5g13420, ] | Carbohydrate transport and metabolism | METABOLISM | 31.2 | 167.2 | -2.4 | 0.000 |
| 012G048700.1 | don-glucosyltransferase 1 | similar to UTP-glucose glucosyltransferase. [ORG:Manihot esculenta]; [ co-ortholog (1of2) of CAA54610, BAB86932, Q40286, ] | Carbohydrate transport and metabolism | METABOLISM | 2.2 | 13.9 | -2.6 | 0.000 |
| 013G005900.1 | galactinol synthase 2 | similar to galactinol synthase. [ORG:Glycine max]; [ co-ortholog (1of3) of AAM96867, At1g09350, At1g56600, ] | Carbohydrate transport and metabolism | METABOLISM | 4.4 | 24.0 | -2.4 | 0.001 |
| 005G006800.1 | galactinol synthase 2 | similar to galactinol synthase. [ORG:Glycine max]; [ co-ortholog (2of3) of AAM96867, At1g09350, At1g56600, ] | Carbohydrate transport and metabolism | METABOLISM | 1.3 | 7.3 | -2.5 | 0.044 |
| 010G055400.1 | glyceraldehyde-3-phosphate dehydrogenase C subunit 1 | similar to glyceraldehyde 3-phosphate dehydrogenase (phosphorylating). [ORG:Pisum sativum]; [ co-ortholog (1of3) of CAH59077, CAA51675, AAD46743, AAD46755, CAH59065, CAH59071, CAH59085, AAD46759, CAH59093, CAH59089, AAD46748, At3g04120, CAH59058, AAD46753 | Carbohydrate transport and metabolism | METABOLISM | 132.5 | 1747.2 | -3.7 | 0.000 |
| 008G179300.1 | glyceraldehyde-3-phosphate dehydrogenase C subunit 1 | similar to glyceraldehyde 3-phosphate dehydrogenase (phosphorylating). [ORG:Pisum sativum]; [ co-ortholog (3of3) of CAH59077, CAA51675, AAD46743, AAD46755, CAH59065, CAH59071, CAH59085, AAD46759, CAH59093, CAH59089, AAD46748, At3g04120, CAH59058, AAD46753 | Carbohydrate transport and metabolism | METABOLISM | 2.7 | 45.0 | -4.1 | 0.009 |
| 012G106500.1 | Glycosyl hydrolase family 38 protein | | Carbohydrate transport and metabolism | METABOLISM | 1.4 | 9.6 | -2.8 | 0.000 |
| 002G236400.1 | indole-3-acetate beta-D-glucosyltransferase | similar to INDOLE-3-ACETATE BETA-D-GLUCOSYLTRANSFERASE; [ co-ortholog (1of2) of At4g14090, At4g15550, At1g05560, At1g05530, ] | Carbohydrate transport and metabolism | METABOLISM | 39.1 | 308.7 | -3.0 | 0.000 |
| 016G011000.1 | Inositol monophosphatase family protein | | Carbohydrate transport and metabolism | METABOLISM | 5.2 | 43.3 | -3.0 | 0.000 |
| 010G156300.1 | Inositol monophosphatase family protein | | Carbohydrate transport and metabolism | METABOLISM | 6.0 | 56.5 | -3.2 | 0.000 |
| 003G062400.1 | Lactoylglutathione lyase / glyoxalase I family protein | lactoylglutathione lyase family protein; glyoxalase 1 family protein; contains glyoxalase family protein; [ co-ortholog (2of2) of At1g80160, ] | Carbohydrate transport and metabolism | METABOLISM | 199.1 | #### | -2.3 | 0.000 |
| 003G109300.1 | phosphate starvation-induced gene 3 | similar to glycerol-3-phosphate transporter; putative; similar to glycerol 3-phosphate permease; putative; similar to cAMP inducible 2 protein (Mus musculus) GI:4580997; similar to glycerol-3-phosphate transporter (glycerol 3-phosphate permease) (Homo sap | Carbohydrate transport and metabolism | METABOLISM | 7.7 | 72.4 | -3.2 | 0.000 |
| 001G124200.1 | phosphate starvation-induced gene 3 | similar to glycerol-3-phosphate transporter; putative; similar to glycerol 3-phosphate permease; putative; similar to cAMP inducible 2 protein (Mus musculus) GI:4580997; similar to glycerol-3-phosphate transporter (glycerol 3-phosphate permease) (Homo sap | Carbohydrate transport and metabolism | METABOLISM | 5.0 | 101.8 | -4.3 | 0.000 |
| 005G168000.1 | Phosphoglycerate mutase family protein | similar to phosphoglycerate mutase. [ORG:Malus x domestica]; [ co-ortholog (2of2) of At1g78050, At1g22170, AAQ54516, ] | Carbohydrate transport and metabolism | METABOLISM | 36.6 | 247.8 | -2.8 | 0.000 |
| 009G121200.1 | purple acid phosphatase 10 | | Carbohydrate transport and metabolism | METABOLISM | 5.0 | 25.5 | -2.4 | 0.001 |
| 005G233400.1 | purple acid phosphatase 12 | similar to Iron(III)-zinc(II) purple acid phosphatase precursor (EC 3.1.3.2) (PAP).; [ co-ortholog (1of2) of At2g27190, ] | Carbohydrate transport and metabolism | METABOLISM | 8.5 | 58.6 | -2.8 | 0.000 |
| 001G001600.1 | Pyruvate kinase family protein | similar to Probable pyruvate kinase; similar to cytosolic isozyme (EC 2.7.1.40) (PK).; [ co-ortholog (2of2) of At5g56350, At4g26390, ] | Carbohydrate transport and metabolism | METABOLISM | 38.5 | 195.6 | -2.3 | 0.000 |
| 008G127600.1 | ribose-5-phosphate isomerase 2 | | Carbohydrate transport and metabolism | METABOLISM | 5.6 | 39.5 | -2.8 | 0.000 |
| 010G115300.1 | ribose-5-phosphate isomerase 2 | | Carbohydrate transport and metabolism | METABOLISM | 3.6 | 28.9 | -3.0 | 0.000 |
| 006G175500.1 | trehalose phosphatase/synthase 11 | glycosyl transferase family 20 protein; trehalose-phosphatase family protein; similar to trehalose-6-phosphate synthase SL-TPS/P (Selaginella lepidophylla) GI:4100325; [ ortholog of At2g18700,] | Carbohydrate transport and metabolism | METABOLISM | 4.2 | 22.4 | -2.4 | 0.000 |
| 018G097700.1 | trehalose phosphatase/synthase 11 | | Carbohydrate transport and metabolism | METABOLISM | 1.3 | 9.5 | -2.8 | 0.000 |
| 001G303600.1 | UDP-glucosyl transferase 73B3 | | Carbohydrate transport and metabolism | METABOLISM | 1.7 | 93.3 | -5.8 | 0.025 |
| 009G099000.1 | UDP-glucosyl transferase 73B3 | | Carbohydrate transport and metabolism | METABOLISM | 0.3 | 147.3 | -8.8 | 0.002 |
| 009G098400.1 | UDP-glucosyl transferase 73B5 | | Carbohydrate transport and metabolism | METABOLISM | 1.3 | 15.6 | -3.6 | 0.006 |
| 002G098400.1 | UDP-glucosyl transferase 85A2 | similar to probable UDP-glucuronosyltransferase (EC 2.4.1.-) - garden pea. [ORG:Pisum sativum]; [ ortholog of At1g22400,T06371,At1g22380,At1g22360,At1g22340,At1g22370,BAB86928,AAB99950,] | Carbohydrate transport and metabolism | METABOLISM | 0.1 | 8.6 | -7.3 | 0.000 |
| 006G048200.1 | UDP-glycosyltransferase 73B4 | similar to putative glucosyltransferase. [ORG:Phaseolus lunatus]; [ co-ortholog (4of18) of Q9ZSK5, P56725, AAM09516, CAD28205, BAB86925, AAD51778, AAM09513, AAD04166, AAM09514, AAM09517, ] | Carbohydrate transport and metabolism | METABOLISM | 0.1 | 4.0 | -4.8 | 0.043 |
| 001G030600.1 | UDP-Glycosyltransferase superfamily protein | glycosyltransferase family protein; [ ortholog of At2g22590,] | Carbohydrate transport and metabolism | METABOLISM | 1.1 | 6.5 | -2.6 | 0.008 |
| 001G281900.1 | UDP-Glycosyltransferase | | Carbohydrate transport and metabolism | METABOLISM | 1.4 | 18.9 | -3.8 | 0.001 |

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|---|---|---|---|---|---|---|---|---|
| | superfamily protein | | | | | | | |
| 016G057300.1 | UDP-Glycosyltransferase superfamily protein | similar to putative glucosyltransferase. [ORG:Phaseolus lunatus]; [ co-ortholog (5of18) of Q9ZSK5, P56725, AAM09516, CAD28205, BAB86925, AAD51778, AAM09513, AAD04166, AAM09514, AAM09517, ] | Carbohydrate transport and metabolism | METABOLISM | 0.4 | 28.6 | -6.2 | 0.001 |
| 004G179300.1 | Uridine diphosphate glycosyltransferase 74E2 | similar to putative glucosyltransferase. [ORG:Phaseolus lunatus]; [ co-ortholog (18of18) of Q9ZSK5, P56725, AAM09516, CAD28205, BAB86925, AAD51778, AAM09513, AAD04166, AAM09514, AAM09517, ] | Carbohydrate transport and metabolism | METABOLISM | 2.2 | 132.1 | -5.9 | 0.029 |
| 005G036800.1 | RING/U-box superfamily protein | | Cell cycle control, cell division, chromosome partitioning | METABOLISM | 8.7 | 46.7 | -2.4 | 0.004 |
| 006G100500.1 | Aldolase-type TIM barrel family protein | similar to Probable pyridoxin biosynthesis PDX1-like protein 3.; [ co-ortholog (1of2) of At5g01410, ] | Coenzyme transport and metabolism | METABOLISM | 20.5 | 654.6 | -5.0 | 0.001 |
| 001G310500.1 | GTP cyclohydrolase II | | Coenzyme transport and metabolism | METABOLISM | 13.5 | 68.9 | -2.4 | 0.001 |
| 017G050400.1 | GTP cyclohydrolase II | | Coenzyme transport and metabolism | METABOLISM | 1.3 | 54.7 | -5.4 | 0.002 |
| 002G240800.1 | homolog of bacterial PANC | similar to Pantoate--beta-alanine ligase (EC 6.3.2.1) (Pantothenate synthetase) (Pantoate activating enzyme).; [ ortholog of At5g48840,] | Coenzyme transport and metabolism | METABOLISM | 7.7 | 55.1 | -2.8 | 0.000 |
| 014G090500.1 | ketopantoate hydroxymethyltransferase 1 | ketopantoate hydroxymethyltransferase family protein; similar to SP\|Q9Y7B6\|3 methyl-2-oxobutanoate-hydroxymethyltransferase EC (2 1.2.11.Ketopantoate) (hydroxymethyltransferase (Emericella) (nidulans) ; [ ortholog of At2g46110,At3g61530,] | Coenzyme transport and metabolism | METABOLISM | 3.9 | 26.7 | -2.8 | 0.000 |
| 005G099900.1 | phosphoserine aminotransferase | | Coenzyme transport and metabolism | METABOLISM | 67.6 | 943.6 | -3.8 | 0.000 |
| 001G226900.1 | urophorphyrin methylase 1 | | Coenzyme transport and metabolism | METABOLISM | 7.6 | 44.0 | -2.5 | 0.000 |
| 013G102800.1 | 12-oxophytodienoate reductase 2 | | Energy production and conversion | METABOLISM | 4.6 | 158.1 | -5.1 | 0.003 |
| 013G103000.1 | 12-oxophytodienoate reductase 2 | | Energy production and conversion | METABOLISM | 2.2 | 91.3 | -5.4 | 0.006 |
| 013G102900.1 | 12-oxophytodienoate reductase 2 | | Energy production and conversion | METABOLISM | 0.3 | 45.3 | -7.2 | 0.001 |
| 001G307500.1 | FAD/NAD(P)-binding oxidoreductase family protein | | Energy production and conversion | METABOLISM | 0.2 | 9.1 | -5.4 | 0.000 |
| 001G464800.1 | FAD-binding Berberine family protein | | Energy production and conversion | METABOLISM | 1.6 | 8.6 | -2.5 | 0.002 |
| 014G161300.1 | formate dehydrogenase | | Energy production and conversion | METABOLISM | 42.8 | 1465.6 | -5.1 | 0.005 |
| 004G233800.1 | GroES-like zinc-binding alcohol dehydrogenase family protein | similar to quinone oxidoreductase-like protein. [ORG:Fragaria x ananassa]; [ co-ortholog (1of2) of AAV33454, CAA69914, T11672, ] | Energy production and conversion | METABOLISM | 67.5 | 390.6 | -2.5 | 0.001 |
| 004G112800.1 | malate dehydrogenase | similar to nodule-enhanced malate dehydrogenase. [ORG:Pisum sativum]; [ co-ortholog (3of3) of AAB99757, T09294, T06325, AAP79476, AAC28106, AAP79474, T06386, AAC24855, ] | Energy production and conversion | METABOLISM | 15.1 | 280.3 | -4.2 | 0.000 |
| 015G104400.1 | phosphate transporter 3;1 | | Energy production and conversion | METABOLISM | 1.0 | 7.0 | -2.8 | 0.004 |
| 012G105100.1 | phosphate transporter 3;1 | | Energy production and conversion | METABOLISM | 1.7 | 19.0 | -3.4 | 0.000 |
| 002G104400.1 | uncoupling protein 5 | mitochondrial substrate carrier family protein; [ co-ortholog (1of2) of At2g22500, ] | Energy production and conversion | METABOLISM | 55.8 | 154.7 | -1.5 | 0.000 |
| 013G049600.1 | ammonium transporter 1;1 | | Inorganic ion transport and metabolism | METABOLISM | 1.5 | 15.1 | -3.3 | 0.000 |
| 007G055500.1 | calcium ATPase 2 | similar to plasma membrane Ca2+-ATPase. [ORG:Glycine max]; [ ortholog of At2g22950,At4g37640,AAG28435,] | Inorganic ion transport and metabolism | METABOLISM | 2.2 | 13.3 | -2.6 | 0.020 |
| 005G251600.1 | catalase 2 | similar to catalase. [ORG:Betula pendula]; [ co-ortholog (2of2) of P25890, BAA09701, AAO17721, BAA09507, P32290, T10178, AAB88172, T06218, BAA20851, BAA04698, P48352, S46297, CAC17121, At4g35090, AAB88173, S16231, P48350, CAD42908, Q01297, AAO12509, AAP57 | Inorganic ion transport and metabolism | METABOLISM | 9.1 | 48.0 | -2.4 | 0.026 |
| 015G144200.1 | Copper transport protein family | | Inorganic ion transport and metabolism | METABOLISM | 14.2 | 74.3 | -2.4 | 0.000 |
| 002G092200.1 | Heavy metal transport/detoxification superfamily protein | | Inorganic ion transport and metabolism | METABOLISM | 1.8 | 12.2 | -2.7 | 0.000 |
| 006G124800.1 | Heavy metal transport/detoxification superfamily protein | | Inorganic ion transport and metabolism | METABOLISM | 43.7 | 519.4 | -3.6 | 0.000 |
| 006G124500.1 | Heavy metal transport/detoxification superfamily protein | similar to heavy-metal-associated domain-containing protein; [ co-ortholog (3of3) of At2g36950, ] | Inorganic ion transport and metabolism | METABOLISM | 1.9 | 23.9 | -3.6 | 0.000 |
| 017G060000.1 | selenium-binding protein 1 | similar to selenium binding protein. [ORG:Lotus corniculatus var. japonicus]; [ co-ortholog (2of2) of CAC67472, CAC67491, At3g23800, CAC67501, At4g14040, CAC67492, At4g14030, ] | Inorganic ion transport and metabolism | METABOLISM | 3.0 | 19.4 | -2.7 | 0.000 |
| 006G069500.1 | SPX domain gene 2 | | Inorganic ion transport and metabolism | METABOLISM | 3.1 | 59.1 | -4.3 | 0.000 |
| 018G131500.1 | SPX domain gene 2 | | Inorganic ion transport and metabolism | METABOLISM | 8.6 | 193.2 | -4.5 | 0.000 |
| 014G061400.1 | SPX domain gene 3 | | Inorganic ion transport and metabolism | METABOLISM | 0.5 | 57.6 | -6.9 | 0.001 |
| 002G143900.1 | SPX domain gene 3 | | Inorganic ion transport | METABOLISM | 0.0 | 5.8 | -10.7 | 0.008 |

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|---|---|---|---|---|---|---|---|---|
| | | | and metabolism | | | | | |
| 005G213500.1 | sulfate transporter 3;1 | similar to Sulfate transporter 3.2 (AST77).; [ co-ortholog (1of2) of At3g51895, At4g02700, At5g19600, ] | Inorganic ion transport and metabolism | METABOLISM | 0.4 | 33.6 | -6.6 | 0.017 |
| 002G049500.1 | sulfate transporter 3;1 | similar to Sulfate transporter 3.2 (AST77).; [ co-ortholog (2of2) of At3g51895, At4g02700, At5g19600, ] | Inorganic ion transport and metabolism | METABOLISM | 0.0 | 4.1 | -7.3 | 0.049 |
| 001G257000.1 | sulfite reductase | similar to sulfite reductase [Populus x canescens]. [ORG:Populus alba x Populus tremula]; [ co-ortholog (2of2) of AAC24584, AAQ57207, AAG59996, BAD12837, ] | Inorganic ion transport and metabolism | METABOLISM | 22.9 | 202.2 | -3.1 | 0.001 |
| 006G013900.1 | tonoplast dicarboxylate transporter | | Inorganic ion transport and metabolism | METABOLISM | 2.0 | 197.0 | -6.6 | 0.017 |
| 016G110600.1 | 3-oxo-5-alpha-steroid 4-dehydrogenase family protein | similar to 3-oxo-5-alpha-steroid 4-dehydrogenase. [ORG:Pisum sativum]; [ ortholog of At2g38050,AAL79911,] | Lipid transport and metabolism | METABOLISM | 6.5 | 61.2 | -3.2 | 0.000 |
| 007G059300.1 | acyl activating enzyme 1 | | Lipid transport and metabolism | METABOLISM | 1.1 | 14.2 | -3.7 | 0.034 |
| 015G031900.1 | alpha/beta-Hydrolases superfamily protein | similar to hydrolase; alpha/beta fold family protein; similar to monoglyceride lipase from (Homo sapiens) GI:14594904; similar to (Mus musculus) GI:2632162; similar to (Rattus norvegicus) GI:19697886; similar to alpha/beta fold family; [ co-ortholog (1of2 | Lipid transport and metabolism | METABOLISM | 0.3 | 7.4 | -4.6 | 0.019 |
| 010G057000.1 | AMP-dependent synthetase and ligase family protein | | Lipid transport and metabolism | METABOLISM | 2.0 | 17.4 | -3.1 | 0.036 |
| 001G105900.1 | AMP-dependent synthetase and ligase family protein | | Lipid transport and metabolism | METABOLISM | 22.1 | 199.2 | -3.2 | 0.016 |
| 015G144700.1 | AMP-dependent synthetase and ligase family protein | AMP-dependent synthetase and ligase family protein; similar to peroxisomal-coenzyme A synthetase (FAT2) (gi:586339) from Saccharomyces cerevisiae; similar to cDNA; similar to cDNA adenosine monophosphat; [ ortholog of At3g48990,] | Lipid transport and metabolism | METABOLISM | 21.3 | 321.7 | -3.9 | 0.015 |
| 006G036200.1 | AMP-dependent synthetase and ligase family protein | | Lipid transport and metabolism | METABOLISM | 0.5 | 34.5 | -6.2 | 0.015 |
| 013G012300.1 | phospholipase D P2 | | Lipid transport and metabolism | METABOLISM | 0.3 | 7.2 | -4.5 | 0.000 |
| 017G138900.1 | PHYTOENE SYNTHASE | | Lipid transport and metabolism | METABOLISM | 0.7 | 5.1 | -2.9 | 0.017 |
| 016G119300.1 | AMP deaminase, putative / myoadenylate deaminase, putative | similar to AMP deaminase; putative; similar to myoadenylate deaminase; putative; similar to SP\|P15274\|AMP deaminase EC (3 5.4.6.Myoadenylate) (deaminase (Saccharomyces) (cerevisiae) ; similar to AMP deaminase; putative; similar to myoadenylate; [ co-ortho | Nucleotide transport and metabolism | METABOLISM | 3.5 | 28.7 | -3.0 | 0.000 |
| 009G078200.1 | Class I glutamine amidotransferase-like superfamily protein | | Nucleotide transport and metabolism | METABOLISM | 1.8 | 60.5 | -5.1 | 0.000 |
| 018G086900.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 4.0 | 34.5 | -3.1 | 0.000 |
| 005G185000.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 0.9 | 54.1 | -5.9 | 0.001 |
| 001G378400.1 | Arabidopsis thaliana gibberellin 2-oxidase 1 | similar to dioxygenase. [ORG:Marah macrocarpus]; [ co-ortholog (2of3) of At1g78440, CAA70330, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 2.2 | 57.4 | -4.7 | 0.010 |
| 007G084400.1 | Cytochrome P450 superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 1.3 | 7.0 | -2.4 | 0.049 |
| 015G028000.1 | cytochrome P450, family 71, subfamily B, polypeptide 34 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 1.0 | 18.2 | -4.1 | 0.000 |
| 007G083900.1 | cytochrome P450, family 71, subfamily B, polypeptide 37 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 1.4 | 8.6 | -2.7 | 0.038 |
| 008G026200.1 | cytochrome P450, family 714, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 0.2 | 13.3 | -6.3 | 0.002 |
| 008G026300.1 | cytochrome P450, family 714, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 0.3 | 34.9 | -6.9 | 0.000 |
| 001G440200.1 | cytochrome P450, family 716, subfamily A, polypeptide 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 1.7 | 12.3 | -2.9 | 0.000 |
| 004G100400.1 | cytochrome P450, family 735, subfamily A, polypeptide 1 | cytochrome P450 family protein; similar to cytochrome P450 72A1 (SP:Q05047) (Catharanthus roseus); similar to member of CYP709A; [ co-ortholog (1of2) of At1g67110, At5g38450, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 2.1 | 13.1 | -2.6 | 0.000 |
| 014G037900.1 | cytochrome P450, family 82, subfamily C, polypeptide 4 | similar to putative cytochrome P450. [ORG:Glycine max]; [ co-ortholog (11of15) of T07748, T07118, AAC49188, CAA71877, T07749, AAG09208, T06523, 2209439B, O81972, O49859, CAA71876, O49858, CAA71515, Q43068, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 11.1 | 88.6 | -3.0 | 0.000 |
| 005G220700.1 | cytochrome P450, family 94, subfamily B, polypeptide 1 | similar to cytochrome P450 CYP94A1 - spring vetch. [ORG:Vicia sativa]; [ co-ortholog (3of5) of AAG33645, AAL54885, T06525, O81117, T08014, AAD10204, AAC49190, P98188, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 23.9 | 174.6 | -2.9 | 0.000 |
| 005G220900.1 | cytochrome P450, family 94, subfamily B, polypeptide 1 | similar to cytochrome P450 CYP94A1 - spring vetch. [ORG:Vicia sativa]; [ co-ortholog (1of5) of AAG33645, AAL54885, T06525, O81117, T08014, AAD10204, AAC49190, P98188, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 3.3 | 27.9 | -3.1 | 0.000 |
| 002G224100.1 | ethylene-forming | similar to 1-aminocyclopropane-1-carboxylate oxidase. [ORG:Pyrus | Secondary metabolites | METABOLISM | 8.0 | 65.7 | -3.0 | 0.024 |

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|---|---|---|---|---|---|---|---|---|
| | enzyme | pyrifolia]; [ co-ortholog (2of2) of BAA33377, AAC67234, AAD28198, At1g05010, BAD61000, BAD60999, CAD21843, AAQ10260, BAA37133, CAA49553, BAA06526, BAC53656, ] | biosynthesis, transport and catabolism | | | | | |
| 003G178200.1 | glutathione-disulfide reductase | similar to probable glutathione-disulfide reductase (EC 1.8.1.7) - garden pea. [ORG:Pisum sativum]; [ co-ortholog (2of2) of CAB66332, Q43621, At3g24170, CAA66924, T06442, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 33.2 | 232.7 | -2.8 | 0.001 |
| 014G193800.1 | GroES-like zinc-binding dehydrogenase family protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 8.4 | 234.5 | -4.8 | 0.000 |
| 012G033500.1 | multidrug resistance-associated protein 14 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 1.2 | 7.3 | -2.6 | 0.047 |
| 012G033400.1 | multidrug resistance-associated protein 14 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 6.9 | 44.6 | -2.7 | 0.038 |
| 001G362300.1 | multidrug resistance-associated protein 3 | ABC transporter family protein; similar to member of NAP subfamily; [ ortholog of At1g71330,At3g13080,] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 9.6 | 55.6 | -2.5 | 0.025 |
| 004G235600.1 | NAD(P)-binding Rossmann-fold superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 36.7 | 628.5 | -4.1 | 0.002 |
| 014G113500.1 | P-glycoprotein 21 | similar to multidrug resistant (MDR) ABC transporter; putative; similar to multidrug-resistant protein CjMDR1 GI:14715462 from (Coptis japonica); [ co-ortholog (1of7) of At2g47000, At3g62150, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 6.9 | 44.4 | -2.7 | 0.002 |
| 003G179000.1 | pleiotropic drug resistance 12 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 0.1 | 8.1 | -5.9 | 0.016 |
| 001G355100.1 | senescence-related gene 1 | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 0.9 | 25.1 | -4.8 | 0.001 |
| 001G456400.1 | Auxin efflux carrier family protein | | Function unknown | POORLY CHARACTERIZED | 5.2 | 66.8 | -3.7 | 0.002 |
| 005G099300.1 | Auxin efflux carrier family protein | auxin efflux carrier family protein; similar to auxin efflux carrier domain containing protein; auxin efflux carrier family protein; similar to auxin efflux carrier domain containing protein; auxin efflux carrier family protein; similar to auxin efflux ca | Function unknown | POORLY CHARACTERIZED | 5.4 | 145.8 | -4.8 | 0.010 |
| 001G288600.1 | pathogenesis-related gene 1 | similar to [Segment 1 of 3] Pathogenesis-related protein (PR-1) (Allergen Cuc m 3). [ORG:Cucumis melo]; [ co-ortholog (4of5) of At2g14610, At2g14580, P83834_1, At4g07820, ] | Function unknown | POORLY CHARACTERIZED | 75.1 | 637.1 | -3.1 | 0.000 |
| 007G031800.1 | PQ-loop repeat family protein / transmembrane family protein | PQ-loop repeat family protein; transmembrane family protein; similar to SP\|Q10482\|Seven transmembrane protein 1 (Schizosaccharomyces (pombe) ; [ ortholog of At4g36850,] | Function unknown | POORLY CHARACTERIZED | 23.8 | 182.6 | -2.9 | 0.000 |
| T160800.1 | receptor like protein 43 | | Function unknown | POORLY CHARACTERIZED | 1.5 | 9.0 | -2.6 | 0.004 |
| 005G190800.1 | Vacuolar iron transporter (VIT) family protein | | Function unknown | POORLY CHARACTERIZED | 7.7 | 58.6 | -2.9 | 0.005 |
| 001G161800.1 | | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At1g53760,] | Function unknown | POORLY CHARACTERIZED | 3.7 | 19.7 | -2.4 | 0.014 |
| 010G085600.1 | Acyl-CoA N-acyltransferases (NAT) superfamily protein | GCN5-related N-acetyltransferase (GNAT) family protein; similar to GNAT family; [ ortholog of At2g32020,At2g32030,] | General function prediction only | POORLY CHARACTERIZED | 3.1 | 83.0 | -4.7 | 0.017 |
| 013G115000.1 | alpha/beta-Hydrolases superfamily protein | esterase/lipase/thioesterase family protein; similar to late embryogenesis abundant protein (EMB8) GI:1350544 SP\|Q40863\|from Picea (glauca ; [ co-ortholog (1of2) of At1g34340, ] | General function prediction only | POORLY CHARACTERIZED | 2.8 | 27.3 | -3.3 | 0.000 |
| 008G118600.1 | Aluminium activated malate transporter family protein | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At2g17470,At1g25480,At1g68600,] | General function prediction only | POORLY CHARACTERIZED | 0.7 | 7.8 | -3.5 | 0.001 |
| T175200.1 | basic chitinase | | General function prediction only | POORLY CHARACTERIZED | 80.0 | 645.8 | -3.0 | 0.000 |
| 009G142200.1 | basic chitinase | | General function prediction only | POORLY CHARACTERIZED | 8.4 | 68.3 | -3.0 | 0.000 |
| 004G182000.1 | basic chitinase | similar to chitinase. [ORG:Euonymus europaeus]; [ ortholog of CAA07413,CAA40212,AAP35269,AAP35272,CAD24068,AAG23965,Q06016,AAC16010,AAM12888,AAD03820,Q06012,AAD03581,CAA40209,AAF69836,BAB40816,CAA40211,AAM12890,P36361,AAD03577,At3g12500,AAD03821,CAB97002, | General function prediction only | POORLY CHARACTERIZED | 0.4 | 45.4 | -6.7 | 0.000 |
| 004G076000.1 | Class I glutamine amidotransferase-like superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 24.9 | 188.8 | -2.9 | 0.000 |
| 004G076200.1 | Class I glutamine amidotransferase-like superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 6.6 | 63.6 | -3.3 | 0.002 |
| 009G069400.1 | CRT (chloroquine-resistance transporter)-like transporter 1 | similar to expressed protein in Arabidopsis thaliana; similar to unknown protein (pir\|\|T09909\|\|; [ ortholog of At5g12170,At5g19380,At5g12160,] | General function prediction only | POORLY CHARACTERIZED | 6.9 | 35.7 | -2.4 | 0.033 |
| 010G059600.1 | DC1 domain- | | General function | POORLY | 9.6 | 49.5 | -2.4 | 0.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | containing protein | | prediction only | CHARACTERIZED | | | | |
| 010G059500.1 | DC1 domain-containing protein | | General function prediction only | POORLY CHARACTERIZED | 7.5 | 39.8 | -2.4 | 0.000 |
| 010G059700.1 | DC1 domain-containing protein | | General function prediction only | POORLY CHARACTERIZED | 21.1 | 164.6 | -3.0 | 0.025 |
| 018G008700.1 | FAD-dependent oxidoreductase family protein | similar to Potential sarcosine oxidase (EC 1.5.3.1).; [ ortholog of At2g24580,] | General function prediction only | POORLY CHARACTERIZED | 7.8 | 66.0 | -3.1 | 0.001 |
| 001G147300.1 | Haloacid dehalogenase-like hydrolase (HAD) superfamily protein | haloacid dehalogenase-like hydrolase family protein; similar to SP\|P71447\|Beta phosphoglucomutase-EC (5 4.2.6.(Lactococcus) (lactis) ; [ co-ortholog (2of4) of At2g38740, ] | General function prediction only | POORLY CHARACTERIZED | 1.4 | 35.7 | -4.6 | 0.000 |
| 010G116900.1 | MATE efflux family protein | MATE efflux family protein; [ ortholog of At1g15170,At1g15180,] | General function prediction only | POORLY CHARACTERIZED | 3.5 | 19.3 | -2.4 | 0.030 |
| 019G086100.1 | MATE efflux family protein | | General function prediction only | POORLY CHARACTERIZED | 2.5 | 14.9 | -2.6 | 0.000 |
| 015G135600.1 | MATE efflux family protein | | General function prediction only | POORLY CHARACTERIZED | 2.7 | 16.6 | -2.6 | 0.000 |
| 008G187100.1 | MATE efflux family protein | | General function prediction only | POORLY CHARACTERIZED | 0.8 | 5.5 | -2.9 | 0.013 |
| 005G133900.1 | methyl esterase 8 | similar to Chain A; similar to K236l Mutant Of Hydroxynitrile Lyase From Hevea Brasiliensis In Complex With Acetonecyanohydrin. [ORG:Hevea brasiliensis]; [ co-ortholog (6of17) of 1SCQ_A, ] | General function prediction only | POORLY CHARACTERIZED | 0.1 | 44.1 | -9.2 | 0.000 |
| 016G035200.1 | NAD(P)-binding Rossmann-fold superfamily protein | short-chain dehydrogenase/reductase (SDR) family protein; similar to 3-oxoacyl-(acyl-carrier protein) reductase SP:P51831 from (Bacillus subtilis); [ ortholog of At2g17845,At3g55290,At3g55310,At3g46170,] | General function prediction only | POORLY CHARACTERIZED | 17.1 | 91.2 | -2.4 | 0.030 |
| 013G059000.1 | NAD(P)-binding Rossmann-fold superfamily protein | short-chain dehydrogenase/reductase (SDR) family protein; similar to short-chain type dehydrogenase/reductase SP:Q08632 (Picea abies); [ ortholog of At4g13180,] | General function prediction only | POORLY CHARACTERIZED | 1.9 | 14.1 | -2.9 | 0.008 |
| 016G073900.1 | NAD(P)-binding Rossmann-fold superfamily protein | similar to alcohol dehydroge. [ORG:Phaseolus lunatus]; [ co-ortholog (2of13) of AAK83035, T11579, BAA13541, AAF98270, AAK83036, BAC53872, ] | General function prediction only | POORLY CHARACTERIZED | 0.2 | 5.2 | -4.5 | 0.004 |
| 006G207100.1 | NAD(P)-binding Rossmann-fold superfamily protein | similar to putative short-chain alcohol dehydrogenase. [ORG:Cucumis sativus]; [ co-ortholog (2of2) of BAB21571, ] | General function prediction only | POORLY CHARACTERIZED | 2.1 | 116.2 | -5.8 | 0.010 |
| 005G106100.1 | NAD(P)-binding Rossmann-fold superfamily protein | similar to alcohol dehydroge. [ORG:Phaseolus lunatus]; [ co-ortholog (13of13) of AAK83035, T11579, BAA13541, AAF98270, AAK83036, BAC53872, ] | General function prediction only | POORLY CHARACTERIZED | 0.0 | 2.8 | -8.1 | 0.043 |
| 016G102100.1 | NAD(P)-linked oxidoreductase superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 20.8 | 170.3 | -3.0 | 0.000 |
| 003G195900.1 | NADPH:quinone oxidoreductase | similar to NADPH:QUINONE OXIDOREDUCTASE; [ co-ortholog (3of3) of At3g27890, ] | General function prediction only | POORLY CHARACTERIZED | 0.1 | 61.1 | -9.6 | 0.008 |
| 004G152300.1 | polyol/monosaccharide transporter 5 | | General function prediction only | POORLY CHARACTERIZED | 1.5 | 8.1 | -2.4 | 0.004 |
| 005G127800.1 | Protein of unknown function (DUF607) | similar to hypothetical protein; similar to DUF607; [ co-ortholog (2of2) of At4g36820, At2g23790, ] | General function prediction only | POORLY CHARACTERIZED | 0.6 | 8.1 | -3.8 | 0.042 |
| 003G034600.1 | Pyridoxal phosphate phosphatase-related protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of3) of At1g73010, At1g17710, ] | General function prediction only | POORLY CHARACTERIZED | 2.0 | 104.9 | -5.7 | 0.001 |
| 008G196800.1 | Pyridoxal phosphate phosphatase-related protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of3) of At1g73010, At1g17710, ] | General function prediction only | POORLY CHARACTERIZED | 1.4 | 677.9 | -9.0 | 0.000 |
| 005G066200.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 4.7 | 58.7 | -3.6 | 0.000 |
| 006G042200.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | similar to embryo-abundant protein-related; similar to embryo-abundant protein (Picea glauca) GI:1350531; [ ortholog of At2g41380,] | General function prediction only | POORLY CHARACTERIZED | 0.6 | 712.6 | -10.1 | 0.001 |
| T163900.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 0.0 | 62.3 | -10.9 | 0.002 |
| 001G061100.1 | senescence-related gene 3 | | General function prediction only | POORLY CHARACTERIZED | 3.0 | 24.8 | -3.0 | 0.000 |
| 001G325200.1 | senescence-related gene 3 | similar to SENESCENCE-RELATED GENE 3; [ ortholog of At5g43300,At3g02040,] | General function prediction only | POORLY CHARACTERIZED | 10.0 | 180.2 | -4.2 | 0.000 |
| 017G063500.1 | senescence-related gene 3 | | General function prediction only | POORLY CHARACTERIZED | 4.6 | 109.7 | -4.6 | 0.000 |
| 006G109600.1 | serine/threonine | similar to Serine/threonine-protein kinase AtPK19 (EC 2.7.1.37) | General function | POORLY | 9.0 | 76.8 | -3.1 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| | protein kinase 2 | (Ribosomal- protein S6 kinase homolog).; [ co-ortholog (2of2) of At3g08720, ] | prediction only | CHARACTERIZED | | | | |
| 006G025400.1 | short-chain dehydrogenase-reductase B | similar to 2; similar to 4-dienoyl-CoA reductase-related; similar to low similarity to peroxisomal 2; similar to 4-dienoyl-CoA reductase (Homo sapiens) GI:9967554; [ ortholog of At2g07640,At1g36580,At3g12800,] | General function prediction only | POORLY CHARACTERIZED | 11.8 | 77.4 | -2.7 | 0.001 |
| 018G006300.1 | Zinc finger protein 622 | zinc finger (C2H2 type) family protein; similar to C2H2 type; similar to C2H2 zinc finger protein FZF mRNA; [ co-ortholog (2of2) of At2g24500, At4g31420, ] | General function prediction only | POORLY CHARACTERIZED | 4.5 | 26.3 | -2.6 | 0.044 |
| 008G010600.1 | zinc induced facilitator-like 1 | | General function prediction only | POORLY CHARACTERIZED | 8.6 | 190.5 | -4.5 | 0.036 |
| 005G185600.1 | ACT domain repeat 3 | similar to ACT domain containing protein; similar to low similarity to uridylyltransferase SP\|P56884 from Rhizobium meliloti; similar to ACT domain containing protein; similar to low similarity to uridylyltransferase SP\|P56884 from Rhizobium meliloti; sim | | | 4.0 | 21.4 | -2.4 | 0.000 |
| 017G051800.1 | Aldolase-type TIM barrel family protein | | | | 4.3 | 80.9 | -4.2 | 0.000 |
| 008G024100.1 | alkaline/neutral invertase | | | | 1.1 | 8.0 | -2.9 | 0.001 |
| 012G001500.1 | alternative oxidase 1A | | | | 6.5 | 75.8 | -3.5 | 0.019 |
| 012G001600.1 | alternative oxidase 1A | | | | 4.5 | 126.5 | -4.8 | 0.001 |
| 003G103900.1 | alternative oxidase 1D | | | | 8.1 | 173.4 | -4.4 | 0.000 |
| 001G403000.1 | Aluminium induced protein with YGL and LRDR motifs | similar to expressed protein in Arabidopsis thaliana; similar to auxin down-regulated protein ARG10 (Vigna radiata) GI:2970051; similar to wali7 (aluminum-induced protein) (Triticum aestivum) GI:451193; [ ortholog of At3g15450,At4g27450,] | | | 1.0 | 25.9 | -4.7 | 0.013 |
| 001G422300.1 | Aminotransferase-like, plant mobile domain family protein | | | | 0.4 | 5.3 | -3.6 | 0.007 |
| 006G043400.1 | Ankyrin repeat family protein / BTB/POZ domain-containing protein | ankyrin repeat family protein; similar to BTB/POZ domain-containing protein; [ co-ortholog (1of2) of At3g57130, At2g41370, ] | | | 2.0 | 13.2 | -2.8 | 0.000 |
| 015G015800.1 | AP2/B3-like transcriptional factor family protein | | | | 0.9 | 5.2 | -2.5 | 0.029 |
| 004G163300.1 | arginine decarboxylase 2 | similar to Arginine decarboxylase (ARGDC) (ADC). [ORG:Glycine max]; [ ortholog of At2g16500,At4g34710,T06593,Q39827,AAN74941,] | | | 19.5 | 155.0 | -3.0 | 0.000 |
| 008G137700.1 | ARM repeat superfamily protein | | | | 0.5 | 4.4 | -3.1 | 0.027 |
| 008G074500.1 | arogenate dehydrogenase | | | | 0.8 | 13.7 | -4.2 | 0.002 |
| T058300.1 | Auxin efflux carrier family protein | | | | 2.6 | 18.1 | -2.8 | 0.006 |
| 019G052800.1 | Auxin efflux carrier family protein | similar to putative auxin efflux carrier protein 9. [ORG:Medicago truncatula]; [ co-ortholog (2of2) of AAT48629, ] | | | 2.6 | 19.1 | -2.9 | 0.010 |
| 010G055300.1 | B12D protein | | | | 19.5 | 104.7 | -2.4 | 0.000 |
| 011G065500.1 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein | | | | 3.4 | 40.1 | -3.6 | 0.000 |
| 008G113400.1 | basic leucine-zipper 42 | similar to bZIP. [ORG:Phaseolus acutifolius]; [ co-ortholog (1of2) of AAK01953, AAK25822, ] | | | 16.0 | 84.3 | -2.4 | 0.000 |
| 016G057600.1 | beta-1,3-glucanase 1 | | | | 0.4 | 14.9 | -5.1 | 0.015 |
| 017G040800.1 | beta-amylase 5 | similar to Chain A; similar to Crystal Structure Of Soybean Beta-Amylase Mutant (M51t) With Increased Ph Optimum. [ORG:Glycine max]; [ ortholog of At4g15210,O64407,BAA20453,1Q6D_A,] | | | 18.9 | 101.7 | -2.4 | 0.000 |
| 002G197200.1 | beta-xylosidase 2 | | | | 1.8 | 7.5 | -2.0 | 0.021 |
| 010G085300.1 | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein | | | | 2.2 | 19.1 | -3.1 | 0.000 |
| 014G078700.1 | BON association protein 2 | similar to BON ASSOCIATION PROTEIN 1-LIKE; similar to BON ASSOCIATION PROTEIN 2; [ co-ortholog (2of2) of At2g45760, ] | | | 2.9 | 38.7 | -3.7 | 0.000 |
| 002G155300.1 | BON association protein 2 | similar to BON ASSOCIATION PROTEIN 1-LIKE; similar to BON ASSOCIATION PROTEIN 2; [ co-ortholog (1of2) of At2g45760, ] | | | 1.8 | 52.8 | -4.8 | 0.000 |
| 004G136000.1 | BURP domain-containing protein | | | | 0.2 | 7.4 | -4.9 | 0.000 |
| 004G136700.1 | BURP domain-containing protein | | | | 1.0 | 36.7 | -5.2 | 0.000 |
| 004G136900.1 | BURP domain-containing protein | | | | 2.2 | 86.4 | -5.3 | 0.000 |
| 001G235800.1 | C2H2 and C2HC zinc fingers superfamily protein | zinc finger (C2H2 type) family protein; similar to C2H2 type; [ ortholog of At3g53600,At2g37430,] | | | 2.0 | 37.1 | -4.2 | 0.000 |
| 009G027700.1 | C2H2-type zinc finger family protein | similar to RESPONSIVE TO HIGH LIGHT 41; [ ortholog of At5g59820,] | | | 0.9 | 34.8 | -5.2 | 0.000 |
| 004G122900.1 | Calcium-binding EF-hand family protein | | | | 32.6 | 172.7 | -2.4 | 0.000 |
| 006G226500.1 | calmodulin-binding family protein | calmodulin-binding family protein; calmodulin-binding family protein; calmodulin-binding family protein; [ co-ortholog (1of2) of At4g33050, At2g26190, ] | | | 9.7 | 64.9 | -2.7 | 0.000 |
| 006G226400.1 | calmodulin-binding | | | | 2.6 | 19.5 | -2.9 | 0.000 |

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|---|---|---|---|---|---|---|
| 004G135300.1 | family protein catalytic LigB subunit of aromatic ring-opening dioxygenase family | | 7.1 | 46.9 | -2.7 | 0.001 |
| 003G130500.1 | CCT motif family protein | similar to hypothetical protein; [ co-ortholog (1of2) of At5g41380, At1g63820, ] | 2.7 | 30.4 | -3.5 | 0.007 |
| 002G020800.1 | Chaperone DnaJ-domain superfamily protein | | 4.3 | 27.9 | -2.7 | 0.000 |
| 002G020700.1 | Chaperone DnaJ-domain superfamily protein | | 0.2 | 5.9 | -4.6 | 0.001 |
| 015G024200.1 | chitinase A | similar to Chain A; similar to Hevamine Mutant D125aE127AY183F IN COMPLEX WITH TETRA-Nag. [ORG:Hevea brasiliensis]; [ ortholog of S57992,S57649,At5g24090,S57475,1KR0_A,CAA07608,1KQZ_A,] | 0.9 | 11.2 | -3.7 | 0.000 |
| T149600.1 | cinnamyl-alcohol dehydrogenase | | 7.7 | 88.1 | -3.5 | 0.017 |
| 011G063700.1 | CLAVATA3/ESR-RELATED 1 | | 0.1 | 28.4 | -8.1 | 0.000 |
| 011G096800.1 | CLAVATA3/ESR-RELATED 1 | | 0.5 | 219.7 | -8.7 | 0.000 |
| 013G119100.1 | CLAVATA3/ESR-RELATED 4 | similar to CLAVATA3/ESR-RELATED 3; [ co-ortholog (1of2) of At2g31081, At1g06225, ] | 1.2 | 89.2 | -6.2 | 0.000 |
| 004G053700.1 | CLAVATA3/ESR-RELATED 4 | | 0.0 | 15.6 | -9.2 | 0.002 |
| 019G091100.1 | CLAVATA3/ESR-RELATED 4 | similar to CLAVATA3/ESR-RELATED 3; [ co-ortholog (2of2) of At2g31081, At1g06225, ] | 0.2 | 198.1 | -9.8 | 0.000 |
| 019G090800.1 | CLAVATA3/ESR-RELATED 5 | similar to CLAVATA3/ESR-RELATED 4; [ ortholog of At2g31083,At2g31085,] | 1.3 | 287.3 | -7.8 | 0.000 |
| 001G376200.1 | CLAVATA3/ESR-RELATED 6 | | 0.0 | 4.6 | -8.4 | 0.008 |
| 011G096900.1 | CLAVATA3/ESR-RELATED 6 | | 0.0 | 25.5 | -11.1 | 0.000 |
| 017G108900.1 | conserved peptide upstream open reading frame 10 | | 12.8 | 73.3 | -2.5 | 0.000 |
| 001G378700.1 | Copper transport protein family | | 0.1 | 8.7 | -6.4 | 0.000 |
| 013G130500.1 | Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein | | 1.4 | 7.8 | -2.4 | 0.010 |
| 008G026100.1 | Cysteine proteinases superfamily protein | | 3.3 | 17.7 | -2.4 | 0.000 |
| 015G048700.1 | D-aminoacyl-tRNA deacylases | | 20.1 | 144.2 | -2.8 | 0.008 |
| 004G230000.1 | Disease resistance protein (TIR-NBS-LRR class) family | | 1.0 | 10.3 | -3.3 | 0.020 |
| 003G216400.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 9.6 | 104.6 | -3.4 | 0.000 |
| 003G134600.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 4.8 | 56.0 | -3.5 | 0.000 |
| 003G134700.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 1.1 | 15.4 | -3.8 | 0.000 |
| 003G134800.1 | Disease resistance-responsive (dirigent-like protein) family protein | similar to pathogenesis-related protein. [ORG:Pisum sativum]; [ co-ortholog (5of6) of AAF25371, P13240, AAD25355, AAB18669, 1604467C, T06433, AAF25372, AAA33662, ] | 0.2 | 64.5 | -8.6 | 0.000 |
| 011G110000.1 | D-mannose binding lectin protein with Apple-like carbohydrate-binding domain | curculin-like (mannose-binding) lectin family protein; similar to low similarity to Ser/Thr protein kinase (Zea mays) GI:2598067; [ co-ortholog (3of3) of At1g78860, At1g78850, ] | 1.6 | 40.5 | -4.6 | 0.000 |
| 011G110200.1 | D-mannose binding lectin protein with Apple-like carbohydrate-binding domain | | 0.7 | 17.2 | -4.7 | 0.000 |
| 001G219100.1 | Duplicated homeodomain-like superfamily protein | | 0.9 | 7.3 | -3.0 | 0.002 |
| 009G143800.1 | early nodulin-related | | 3.2 | 16.6 | -2.4 | 0.000 |
| 019G063700.1 | ENTH/ANTH/VHS superfamily protein | similar to Putative clathrin assembly protein At1g33340.; [ ortholog of At1g33340,] | 0.1 | 5.3 | -5.8 | 0.002 |
| 011G057000.1 | erf domain protein 9 | | 8.3 | 48.6 | -2.5 | 0.000 |
| 007G138100.1 | erf domain protein 9 | | 47.2 | 288.3 | -2.6 | 0.001 |
| 002G039100.1 | ethylene response factor 1 | | 4.5 | 23.8 | -2.4 | 0.000 |
| 013G045200.1 | ethylene response factor 1 | | 0.7 | 6.3 | -3.3 | 0.004 |

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|---|---|---|---|---|---|---|
| 008G166200.1 | ethylene response factor 1 | | 0.6 | 6.5 | -3.4 | 0.003 |
| 011G061700.1 | ethylene response factor 1 | | 2.4 | 26.9 | -3.5 | 0.000 |
| 010G072300.1 | ethylene response factor 1 | similar to ETHYLENE RESPONSE FACTOR 1; [ ortholog of At3g23240,] | 0.0 | 7.4 | -7.9 | 0.000 |
| 004G051700.1 | ethylene responsive element binding factor 1 | | 0.8 | 11.9 | -3.9 | 0.000 |
| 001G154100.1 | ethylene responsive element binding factor 1 | | 0.4 | 7.7 | -4.1 | 0.000 |
| 001G154200.1 | ethylene responsive element binding factor 5 | similar to Ethylene responsive element binding factor 5 (AtERF5).; [ co-ortholog (1of2) of At5g47230, At4g17490, ] | 5.8 | 52.6 | -3.2 | 0.014 |
| 001G147200.1 | expansin-like B1 | | 0.3 | 15.7 | -5.5 | 0.004 |
| 002G070100.1 | extensin 3 | | 7.8 | 39.0 | -2.3 | 0.017 |
| 005G190100.1 | extensin 4 | | 0.6 | 5.9 | -3.2 | 0.005 |
| 019G130200.1 | extra-large GTP-binding protein 2 | | 0.2 | 4.0 | -4.6 | 0.015 |
| 011G162800.1 | FAD-binding Berberine family protein | | 2.2 | 22.7 | -3.4 | 0.022 |
| 019G063400.1 | FAD-binding Berberine family protein | | 0.6 | 12.1 | -4.4 | 0.000 |
| 009G108900.1 | Family of unknown function (DUF716) | | 29.9 | 332.4 | -3.5 | 0.000 |
| 001G008900.1 | Family of unknown function (DUF716) | | 0.0 | 17.4 | -9.8 | 0.035 |
| 006G267000.1 | F-box family protein | | 2.2 | 11.3 | -2.4 | 0.002 |
| 004G034400.1 | F-box family protein | F-box family protein; similar to late embryogenesis abundant protein GI:1350540 from (Picea glauca); [ co-ortholog (1of2) of At1g61340, ] | 3.4 | 30.9 | -3.2 | 0.000 |
| 004G202500.1 | ferredoxin 3 | similar to ferredoxin; putative; similar to non-photosynthetic ferredoxin from Citrus sinensis (GI:1360725); similar to Ferredoxin; similar to root R-B2 from Raphanus sativus (SP\|P14937); [ co-ortholog (1of2) of At2g27510, ] | 26.1 | 151.6 | -2.5 | 0.000 |
| 013G065100.1 | GDSL lipase 1 | similar to 50 kDa protein. [ORG:Hevea brasiliensis]; [ co-ortholog (2of4) of AAP41849, At5g40990, At1g53990, AAR98518, At3g14225, At1g53940, ] | 4.1 | 24.7 | -2.6 | 0.023 |
| 017G134600.1 | GDSL lipase 1 | | 0.7 | 11.6 | -4.1 | 0.007 |
| 018G088500.1 | GDSL-like Lipase/Acylhydrolase superfamily protein | | 0.4 | 5.0 | -3.7 | 0.009 |
| 010G236800.1 | GDSL-like Lipase/Acylhydrolase superfamily protein | | 0.7 | 9.5 | -3.8 | 0.000 |
| 002G083800.1 | GDSL-like Lipase/Acylhydrolase superfamily protein | | 0.0 | 3.4 | -6.7 | 0.030 |
| 001G191400.1 | GDSL-like Lipase/Acylhydrolase superfamily protein | similar to putative proline-rich protein APG isolog. [ORG:Cicer arietinum]; [ ortholog of CAB81548,At3g16370,] | 0.0 | 11.2 | -7.9 | 0.000 |
| 017G134700.1 | GDSL-motif lipase 3 | | 0.1 | 4.8 | -5.9 | 0.004 |
| 008G192600.1 | glucosyl transferase family 8 | | 4.3 | 33.0 | -2.9 | 0.002 |
| 003G126900.1 | glutamate-cysteine ligase | | 33.2 | 250.5 | -2.9 | 0.029 |
| T017000.1 | glutamine dumper 1 | | 2.7 | 9.8 | -1.9 | 0.007 |
| 017G107300.1 | glutamine dumper 3 | | 3.0 | 33.9 | -3.5 | 0.000 |
| 017G107200.1 | glutamine dumper 3 | | 0.3 | 32.5 | -6.6 | 0.000 |
| 011G113200.1 | glutathione S-transferase TAU 23 | | 2.6 | 117.6 | -5.5 | 0.002 |
| 009G163700.1 | Glycosyl hydrolase superfamily protein | | 12.2 | 100.3 | -3.0 | 0.000 |
| T167100.1 | Glycosyl hydrolase superfamily protein | | 0.7 | 36.7 | -5.6 | 0.000 |
| 010G142800.1 | Glycosyl hydrolase superfamily protein | similar to Glucan endo-1; similar to 3-beta-glucosidase; similar to basic vacuolar isoform precursor ((1->3)-beta-glucan endohydrolase) ((1->3)-beta-glucanase) (Beta-1; similar to 3-endoglucanase). [ORG:Hevea brasiliensis]; [ ortholog of P52407,At3g57270, | 14.7 | 744.4 | -5.7 | 0.000 |
| 002G027000.1 | glyoxal oxidase-related protein | similar to glyoxal oxidase-related; similar to glyoxal oxidase precursor (Phanerochaete chrysosporium) gi\|1050302\|gb\|AAA87594\|; [ ortholog of At1g75620,] | 0.1 | 4.3 | -4.9 | 0.010 |
| 006G020000.1 | Heavy metal transport/detoxification superfamily protein | | 1.9 | 43.3 | -4.5 | 0.015 |
| 009G110800.1 | hemoglobin 1 | | 4.9 | 859.3 | -7.5 | 0.000 |
| 014G132500.1 | high affinity K+ transporter 5 | | 3.4 | 27.0 | -3.0 | 0.002 |
| 005G134600.1 | Homeodomain-like superfamily protein | | 3.0 | 88.8 | -4.9 | 0.000 |
| 006G097500.1 | HXXXD-type acyl-transferase family protein | transferase family protein; [ co-ortholog (1of2) of At5g01210, ] | 1.6 | 48.2 | -4.9 | 0.000 |
| 005G024500.1 | Hypoxia-responsive | hypoxia-responsive family protein; [ co-ortholog (2of3) of | 27.4 | 256.0 | -3.2 | 0.000 |

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|---|---|---|---|---|---|---|
| | family protein | At5g27760, At3g05550, ] | | | | |
| 002G236500.1 | indole-3-acetate beta-D-glucosyltransferase | | 25.2 | 183.3 | -2.9 | 0.001 |
| 018G122700.1 | Inorganic H pyrophosphatase family protein | | 23.3 | 129.8 | -2.5 | 0.000 |
| 018G119500.1 | Inorganic H pyrophosphatase family protein | | 15.5 | 156.8 | -3.3 | 0.000 |
| 009G084600.1 | Inositol 1,3,4-trisphosphate 5/6-kinase family protein | | 5.9 | 33.3 | -2.5 | 0.000 |
| 015G065500.1 | Integral membrane HPP family protein | | 14.6 | 83.5 | -2.5 | 0.000 |
| 019G088000.1 | Integrase-type DNA-binding superfamily protein | | 6.9 | 51.6 | -2.9 | 0.000 |
| 018G047300.1 | Integrase-type DNA-binding superfamily protein | | 4.7 | 61.5 | -3.7 | 0.036 |
| 012G134000.1 | Integrase-type DNA-binding superfamily protein | similar to AP2 domain-containing protein; similar to low similarity to DREB1B GI:3738226 from (Arabidopsis thaliana); [ ortholog of At5g52020,] | 0.6 | 9.5 | -3.9 | 0.009 |
| 002G201600.1 | Integrase-type DNA-binding superfamily protein | | 4.6 | 76.8 | -4.1 | 0.000 |
| 005G195000.1 | Integrase-type DNA-binding superfamily protein | similar to AP2 domain-containing transcription factor; putative; similar to transcription factor; [ co-ortholog (1of2) of At5g64750, ] | 0.7 | 14.4 | -4.4 | 0.003 |
| 002G065600.1 | Integrase-type DNA-binding superfamily protein | | 2.5 | 57.8 | -4.5 | 0.001 |
| 010G072400.1 | Integrase-type DNA-binding superfamily protein | | 0.2 | 5.5 | -5.2 | 0.002 |
| 006G104200.1 | Integrase-type DNA-binding superfamily protein | similar to Dehydration responsive element binding protein 2E (DREB2E protein).; [ co-ortholog (2of2) of At2g38340, ] | 0.2 | 8.2 | -5.4 | 0.007 |
| 005G223100.1 | Integrase-type DNA-binding superfamily protein | | 0.1 | 7.8 | -6.1 | 0.000 |
| 008G166000.1 | Integrase-type DNA-binding superfamily protein | | 0.1 | 10.5 | -6.3 | 0.000 |
| 010G072600.1 | Integrase-type DNA-binding superfamily protein | | 0.0 | 3.2 | -7.2 | 0.025 |
| 002G039200.1 | Integrase-type DNA-binding superfamily protein | | 0.1 | 37.6 | -8.6 | 0.014 |
| 004G067900.1 | Kunitz family trypsin and protease inhibitor protein | similar to trypsin protein inhibitor 3. [ORG:Cicer arietinum]; [ co-ortholog (2of3) of S19190, BAD04942, BAD04939, JC7311, AAB21123, B45588, P09941, P25272, P83594, JQ0968, AAB23464, 1AVX_B, TISYB, AAB26177, CAH61462, P32733, P83051, AAA32618, AAT45474, P | 49.5 | 258.8 | -2.4 | 0.000 |
| 001G309900.1 | Kunitz family trypsin and protease inhibitor protein | similar to truncated Kunitz trypsin inhibitor. [ORG:Glycine max]; [ co-ortholog (1of6) of AAK20290, ] | 0.0 | 30.9 | -10.6 | 0.000 |
| 016G046400.1 | Late Embryogenesis Abundant 4-5 | | 0.9 | 12.3 | -3.8 | 0.005 |
| 005G145500.1 | LOB domain-containing protein 38 | similar to LOB domain protein 39.; [ co-ortholog (2of2) of At5g67420, At4g37540, At3g49940, ] | 10.3 | 72.8 | -2.8 | 0.000 |
| 002G119400.1 | LOB domain-containing protein 38 | | 8.0 | 62.8 | -3.0 | 0.000 |
| T062400.1 | LOB domain-containing protein 38 | | 2.4 | 23.3 | -3.3 | 0.019 |
| 007G053600.1 | LOB domain-containing protein 38 | similar to LOB domain protein 39.; [ co-ortholog (1of2) of At5g67420, At4g37540, At3g49940, ] | 4.2 | 46.3 | -3.5 | 0.000 |
| 014G017400.1 | LOB domain-containing protein 38 | | 1.9 | 66.9 | -5.1 | 0.000 |
| 009G143300.1 | lysm domain GPI-anchored protein 2 precursor | similar to LysM-domain GPI-anchored protein 2 precursor.; [ ortholog of At2g17120,] | 0.4 | 4.4 | -3.6 | 0.025 |
| 003G105600.1 | Major facilitator superfamily protein | | 1.0 | 6.5 | -2.7 | 0.007 |
| 001G128200.1 | Major facilitator superfamily protein | similar to expressed protein in Arabidopsis thaliana; similar to Requires functional assignment.; [ co-ortholog (1of2) of At5g45275, At4g19450, ] | 0.7 | 7.7 | -3.5 | 0.008 |
| 005G112400.1 | Major facilitator superfamily protein | | 0.3 | 5.9 | -4.2 | 0.002 |
| 005G133800.1 | methyl esterase 1 | similar to Chain A; similar to K236l Mutant Of Hydroxynitrile Lyase From Hevea Brasiliensis In Complex With Acetonecyanohydrin. [ORG:Hevea brasiliensis]; [ co-ortholog (11of17) of 1SCQ_A, ] | 60.0 | 279.8 | -2.2 | 0.005 |
| 011G025400.1 | methylesterase PCR A | similar to pectin methylesterase; putative; similar to pectin methylesterase GI:1617583 from (Lycopersicon esculentum); [ ortholog of At1g11580,] | 8.3 | 145.7 | -4.1 | 0.000 |
| 006G018000.1 | microtubule-associated proteins | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At4g17220, ] | 1.3 | 15.0 | -3.6 | 0.000 |

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|---|---|---|---|---|---|---|
| | 70-5 | | | | | |
| 016G046500.1 | MLP-like protein 423 | | 0.0 | 7.8 | -8.5 | 0.000 |
| 002G105900.1 | MSCS-like 3 | | 0.3 | 6.4 | -4.4 | 0.003 |
| 010G128900.1 | myb-like transcription factor family protein | | 0.5 | 39.2 | -6.3 | 0.000 |
| 005G069500.1 | NAC (No Apical Meristem) domain transcriptional regulator superfamily protein | | 8.4 | 231.3 | -4.8 | 0.004 |
| 007G099400.1 | NAC (No Apical Meristem) domain transcriptional regulator superfamily protein | | 4.1 | 372.0 | -6.5 | 0.002 |
| 019G099900.1 | NAC domain containing protein 71 | | 3.8 | 72.1 | -4.2 | 0.017 |
| 014G019600.1 | NAD(P)-binding Rossmann-fold superfamily protein | | 91.4 | 630.5 | -2.8 | 0.003 |
| 005G017300.1 | nicotianamine synthase 3 | | 8.6 | 83.0 | -3.3 | 0.000 |
| 005G014300.1 | nicotianamine synthase 3 | | 11.9 | 134.8 | -3.5 | 0.000 |
| 010G143100.1 | nicotianamine synthase 3 | | 0.4 | 5.6 | -3.7 | 0.004 |
| 002G190800.1 | NIM1-interacting 1 | similar to NPR1/NIM1-interacting protein 1 (NIMIN-1); similar to NIMIN-1 protein (Arabidopsis thaliana) gi\|12057154\|emb\|CAC19844\|; similar to cDNA NIMIN-1 protein (nimin-1 gene)GI:12057153; [ ortholog of At1g02450,] | 1.7 | 10.9 | -2.7 | 0.000 |
| 018G099500.1 | nodulin MtN21 /EamA-like transporter family protein | | 0.4 | 7.2 | -4.2 | 0.001 |
| 016G031400.1 | nodulin MtN21 /EamA-like transporter family protein | | 4.3 | 145.8 | -5.1 | 0.000 |
| 006G177700.1 | nodulin MtN21 /EamA-like transporter family protein | | 0.1 | 7.1 | -6.6 | 0.003 |
| 010G191300.1 | ortholog of sugar beet HS1 PRO-1 2 | similar to putative Hs1pro-1-like protein. [ORG:Cicer arietinum]; [ co-ortholog (2of2) of AAG44839, At2g40000, At3g55840, AAK72104, ] | 6.3 | 125.3 | -4.3 | 0.000 |
| 001G102400.1 | osmotin 34 | | 32.6 | 797.9 | -4.6 | 0.000 |
| 001G107600.1 | osmotin 34 | similar to protein P21. [ORG:Glycine max]; [ co-ortholog (3of7) of PC1121, AAB23170, 1906370A, CAC22342, AAF13707, CAA09229, CAB36911, CAE54084, A33176, P25096, ] | 116.8 | #### | -4.7 | 0.000 |
| 001G190700.1 | oxidative stress 3 | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At5g56550,] | 0.9 | 13.1 | -3.9 | 0.000 |
| 006G094300.1 | PAR1 protein | | 1.6 | 10.3 | -2.7 | 0.000 |
| 013G041600.1 | pathogenesis-related 4 | similar to HEV1.2; similar to hevein. [ORG:Hevea brasiliensis]; [ ortholog of AAO63572,At3g04720,] | 13.5 | 284.4 | -4.4 | 0.000 |
| 005G188300.1 | pathogenesis-related family protein | | 14.0 | 63.5 | -2.2 | 0.001 |
| 001G389800.1 | pathogenesis-related family protein | | 22.7 | 121.0 | -2.4 | 0.011 |
| 005G188400.1 | pathogenesis-related family protein | similar to putative pathogenesis-related protein. [ORG:Cucumis sativus]; [ co-ortholog (2of3) of CAF33484, ] | 34.5 | 206.7 | -2.6 | 0.000 |
| 001G389400.1 | pathogenesis-related family protein | | 24.7 | 1374.1 | -5.8 | 0.003 |
| 009G082900.1 | pathogenesis-related gene 1 | similar to pathogenesis-related protein. [ORG:Pyrus pyrifolia]; [ co-ortholog (5of5) of CAB66337, At4g33720, AAD33696, AAF78528, AAM18099, ] | 0.1 | 18.1 | -7.4 | 0.043 |
| 005G240900.1 | Pathogenesis-related thaumatin superfamily protein | pathogenesis-related thaumatin family protein; similar to receptor serine/threonine kinase PR5K (Arabidopsis thaliana) GI:1235680; pathogenesis-related thaumatin family protein; similar to receptor serine/t; [ ortholog of At1g20030,] | 5.9 | 75.2 | -3.7 | 0.000 |
| 005G112700.1 | Pathogenesis-related thaumatin superfamily protein | | 1.5 | 102.8 | -6.1 | 0.000 |
| 007G134000.1 | PCF11P-similar protein 4 | | 3.4 | 30.9 | -3.2 | 0.044 |
| 002G190600.1 | Pectin lyase-like superfamily protein | glycoside hydrolase family 28 protein; polygalacturonase (pectinase) family protein; similar to polygalacturonase (Lycopersicon esculentum) GI:7381227; [ co-ortholog (2of2) of At4g01890, At1g02460, ] | 0.4 | 10.2 | -4.5 | 0.000 |
| 003G214500.1 | Peroxidase superfamily protein | similar to peroxidase (EC 1.11.1.7) - large-leaved lupine (fragment). [ORG:Lupinus polyphyllus]; [ co-ortholog (10of16) of JC4779, CAC38073, 1908234A, CAA76680, AAB41810, BAC81650, 1FHF_B, AAB06183, CAA62226, AAB28139, 1FHF_A, JC4781, AAB97734, AAO13838, | 43.9 | 239.0 | -2.4 | 0.000 |
| 002G065300.1 | Peroxidase superfamily protein | similar to peroxidase precursor. [ORG:Euphorbia characias]; [ co-ortholog (1of3) of At1g71695, AAS97959, ] | 14.9 | 85.2 | -2.5 | 0.000 |
| 006G129900.1 | Peroxidase superfamily protein | similar to Peroxidase 21 precursor (EC 1.11.1.7) (Atperox P21) (PRXR5) (ATP2a/ATP2b).; [ ortholog of At2g37130,] | 7.9 | 55.5 | -2.8 | 0.040 |
| 005G072800.1 | Peroxidase | similar to Peroxidase 17 precursor (EC 1.11.1.7) (Atperox P17) | 28.5 | 261.0 | -3.2 | 0.006 |

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|---|---|---|---|---|---|---|
| | superfamily protein | (ATP25a).; [ co-ortholog (1of2) of At2g22420, ] | | | | |
| 003G214900.1 | Peroxidase superfamily protein | | 2.8 | 31.9 | -3.5 | 0.000 |
| 012G006800.1 | Peroxidase superfamily protein | | 1.0 | 34.5 | -5.2 | 0.000 |
| 001G112100.1 | phospholipase D beta 1 | | 2.1 | 10.1 | -2.3 | 0.004 |
| T180000.1 | phospholipase D beta 1 | | 4.3 | 23.1 | -2.4 | 0.000 |
| 013G101000.1 | Phosphorylase superfamily protein | | 87.0 | 688.0 | -3.0 | 0.000 |
| 013G100800.1 | Phosphorylase superfamily protein | | 5.3 | 875.9 | -7.4 | 0.000 |
| 013G100700.1 | Phosphorylase superfamily protein | | 8.0 | 1598.8 | -7.6 | 0.000 |
| 002G224000.1 | Phosphotyrosine protein phosphatases superfamily protein | tyrosine specific protein phosphatase family protein; [ co-ortholog (2of2) of At2g32960, At1g05000, ] | 0.6 | 4.8 | -3.1 | 0.019 |
| 001G438800.1 | photosystem II subunit R | similar to polypeptide precursor of photosystem II. [ORG:Pyrus pyrifolia]; [ co-ortholog (2of2) of AAF78511, ] | 1.4 | 28.8 | -4.4 | 0.000 |
| 011G142300.1 | photosystem II subunit R | similar to polypeptide precursor of photosystem II. [ORG:Pyrus pyrifolia]; [ co-ortholog (1of2) of AAF78511, ] | 0.5 | 50.9 | -6.6 | 0.000 |
| 019G120600.1 | pinoid-binding protein 1 | | 5.8 | 58.2 | -3.3 | 0.000 |
| 013G151000.1 | pinoid-binding protein 1 | | 4.7 | 111.8 | -4.6 | 0.007 |
| 001G299500.1 | Plant basic secretory protein (BSP) family protein | plant basic secretory protein (BSP) family protein; similar to NtPRp27 (Nicotiana tabacum) GI:5360263; [ co-ortholog (1of7) of At2g15130, At2g15220, At2g15170, ] | 4.4 | 38.6 | -3.1 | 0.000 |
| 018G029100.1 | plant natriuretic peptide A | | 40.9 | 246.3 | -2.6 | 0.000 |
| 018G101600.1 | plant natriuretic peptide A | | 6.4 | 197.8 | -5.0 | 0.000 |
| 018G087400.1 | Plant protein of unknown function (DUF946) | similar to expressed protein in Arabidopsis thaliana; similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g44260, ] | 1.4 | 11.4 | -3.0 | 0.037 |
| 009G087500.1 | Plant regulator RWP-RK family protein | similar to RWP-RK domain-containing protein; similar to nodule inception protein (Lotus japonicus) GI:6448579; [ co-ortholog (2of2) of At2g17150, ] | 14.9 | 351.2 | -4.6 | 0.000 |
| 001G293300.1 | Plant regulator RWP-RK family protein | | 12.4 | 392.8 | -5.0 | 0.000 |
| 016G069400.1 | plant U-box 22 | similar to U-box domain-containing protein; similar to immediate-early fungal elicitor protein CMPG1 (Petroselinum crispum) GI:14582200; [ co-ortholog (3of3) of At2g35930, At3g52450, ] | 2.8 | 21.5 | -2.9 | 0.001 |
| 006G058600.1 | polygalacturonase inhibiting protein 1 | similar to polygalacturonase inhibiting protein. [ORG:Prunus persica]; [ co-ortholog (1of2) of AAK43425, CAF04488, AAK43460, AAK43400, AAK43471, AAK43389, AAK43414, AAK43432, BAA96450, AAK43413, AAK43457, AAK43392, AAK43452, S60713, AAK43428, AAK43468, AA | 0.1 | 8.0 | -5.9 | 0.010 |
| 003G115200.1 | Protein of unknown function (DUF1442) | | 4.7 | 50.2 | -3.4 | 0.000 |
| 004G143500.1 | Protein of unknown function (DUF1645) | similar to Calmodulin-binding protein; similar to AR781 GI:1669593 from (Arabidopsis thaliana); similar to AR781 complements pheromone receptor deficient mutant of Shizosaccharomyces pombe; [ co-ortholog (2of2) of At2g15760, ] | 0.8 | 5.9 | -2.9 | 0.011 |
| 014G034900.1 | Protein of unknown function (DUF1645) | similar to mRNA for AR781; similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g26530, ] | 2.2 | 24.1 | -3.4 | 0.000 |
| 011G053300.1 | Protein of unknown function (DUF506) | | 0.2 | 5.7 | -4.6 | 0.002 |
| 004G044300.1 | Protein of unknown function (DUF506) | | 0.3 | 8.9 | -4.8 | 0.000 |
| 013G129900.1 | Protein of unknown function (DUF506) | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At2g20670, At4g32480, ] | 2.9 | 155.3 | -5.7 | 0.016 |
| 009G144600.1 | Protein of unknown function (DUF506) | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At3g07350, ] | 0.0 | 14.6 | -8.7 | 0.000 |
| 004G184700.1 | Protein of unknown function (DUF506) | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At3g07350, ] | 0.0 | 17.1 | -11.7 | 0.000 |
| 010G171000.1 | Protein of unknown function (DUF793) | | 13.2 | 88.2 | -2.7 | 0.000 |
| 015G001300.1 | Protein of unknown function, DUF599 | | 4.6 | 121.2 | -4.7 | 0.006 |
| 009G010800.1 | Putative lysine decarboxylase family protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g28305, ] | 1.3 | 9.9 | -3.0 | 0.018 |
| 010G131500.1 | Putative membrane lipoprotein | | 2.3 | 25.9 | -3.5 | 0.000 |
| 003G120500.1 | Pyridoxal phosphate (PLP)-dependent transferases superfamily protein | similar to hypothetical protein. [ORG:Cicer arietinum]; [ co-ortholog (2of3) of At4g22980, CAG14987, At5g51920, ] | 0.2 | 4.7 | -4.3 | 0.008 |
| 012G089500.1 | quinolinate synthase | | 4.2 | 30.3 | -2.8 | 0.002 |
| 004G117800.1 | reversibly glycosylated polypeptide 2 | similar to type IIIa membrane protein cp-wap13. [ORG:Vigna unguiculata]; [ co-ortholog (4of4) of AAB61672, T11576, AAB61671, T11577, AAR13306, ] | 38.2 | 184.2 | -2.3 | 0.001 |
| 001G309600.1 | RING/U-box superfamily protein | zinc finger (C3HC4-type RING finger) family protein; similar to C3HC4 type (RING finger); [ co-ortholog (1of2) of At1g49230, ] | 1.9 | 11.5 | -2.6 | 0.022 |
| 018G098000.1 | RING/U-box superfamily protein | | 0.3 | 34.4 | -7.1 | 0.000 |
| 009G140400.1 | RmlC-like cupins | | 0.0 | 16.8 | #ZAHL! | 0.004 |

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|---|---|---|---|---|---|---|
| 017G112100.1 | superfamily protein ROTUNDIFOLIA like 21 | | 1.5 | 6.5 | -2.1 | 0.028 |
| 006G042400.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | 0.3 | 270.2 | -9.8 | 0.003 |
| 002G119300.1 | salt tolerance zinc finger | | 27.3 | 163.4 | -2.6 | 0.000 |
| 001G295500.1 | salt tolerance zinc finger | | 21.1 | 199.9 | -3.2 | 0.000 |
| T148000.1 | salt tolerance zinc finger | | 1.8 | 17.9 | -3.3 | 0.000 |
| 002G057500.1 | SAUR-like auxin-responsive protein family | | 19.4 | 97.8 | -2.3 | 0.000 |
| 009G133500.1 | Senescence/dehydration-associated protein-related | | 2.3 | 102.1 | -5.5 | 0.036 |
| 002G203500.1 | senescence-associated gene 21 | | 77.3 | 685.8 | -3.1 | 0.000 |
| 014G127700.1 | senescence-associated gene 21 | | 7.3 | 702.3 | -6.6 | 0.000 |
| 009G028300.1 | Serine protease inhibitor, potato inhibitor I-type family protein | | 109.3 | 563.7 | -2.4 | 0.000 |
| 005G026700.1 | soybean gene regulated by cold-2 | | 13.7 | 74.4 | -2.4 | 0.031 |
| 010G182500.1 | Staphylococcal nuclease homologue | similar to Ca(2+)-dependent nuclease; putative; similar to Ca(2++)-dependent nuclease (Arabidopsis thaliana) GI:7684292; similar to Ca(2+)-dependent nuclease; putative; similar to Ca(2++)-dependent nuclease (Arabidopsis thaliana) GI:7684292; [ co-ortholog | 3.7 | 21.3 | -2.5 | 0.000 |
| 011G030900.1 | Sulfite exporter TauE/SafE family protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At1g11540, At1g61740, ] | 17.7 | 93.4 | -2.4 | 0.000 |
| 002G256900.1 | Tetratricopeptide repeat (TPR)-like superfamily protein | | 0.6 | 8.0 | -3.7 | 0.000 |
| 019G013300.1 | VQ motif-containing protein | | 1.9 | 9.9 | -2.4 | 0.001 |
| 006G006300.1 | VQ motif-containing protein | similar to VQ motif-containing protein; [ co-ortholog (2of2) of At3g22160, At4g15120, ] | 0.3 | 7.5 | -4.5 | 0.000 |
| 007G006200.1 | VQ motif-containing protein | similar to VQ motif-containing protein; [ co-ortholog (1of2) of At2g22880, ] | 0.9 | 23.8 | -4.7 | 0.000 |
| 004G033300.1 | Wound-responsive family protein | | 20.3 | 103.5 | -2.4 | 0.002 |
| 019G116500.1 | Wound-responsive family protein | | 4.3 | 48.6 | -3.5 | 0.000 |
| 001G099000.1 | WRKY DNA-binding protein 27 | | 1.2 | 7.7 | -2.7 | 0.037 |
| 003G182200.1 | WRKY DNA-binding protein 40 | similar to Probable WRKY transcription factor 40 (WRKY DNA-binding protein 40).; [ co-ortholog (1of2) of At3g32090, At1g80840, ] | 7.8 | 99.5 | -3.7 | 0.000 |
| 001G044500.1 | WRKY DNA-binding protein 40 | similar to Probable WRKY transcription factor 40 (WRKY DNA-binding protein 40).; [ co-ortholog (2of2) of At3g32090, At1g80840, ] | 9.3 | 120.6 | -3.7 | 0.003 |
| 007G079800.1 | WRKY DNA-binding protein 51 | | 0.9 | 8.1 | -3.2 | 0.001 |
| 016G137900.1 | WRKY DNA-binding protein 70 | | 8.6 | 48.8 | -2.5 | 0.000 |
| 012G101000.1 | WRKY DNA-binding protein 75 | | 0.0 | 10.4 | -9.8 | 0.014 |
| T093900.1 | xylose isomerase family protein | | 10.3 | 75.2 | -2.9 | 0.002 |
| 011G138500.1 | zinc finger (AN1-like) family protein | zinc finger (AN1-like) family protein; similar to putative zinc finger protein (PMZ) mRNA; [ ortholog of At3g28210,] | 0.6 | 45.3 | -6.2 | 0.032 |
| 006G006900.1 | zinc transporter 1 precursor | similar to zinc transporter protein ZIP1. [ORG:Glycine max]; [ co-ortholog (3of4) of AAK37761, AAR08414, ] | 7.3 | 38.2 | -2.4 | 0.000 |
| 005G193900.1 | | | 45.5 | 115.6 | -1.3 | 0.001 |
| 007G093200.1 | | | 13.9 | 36.7 | -1.4 | 0.030 |
| 013G045700.1 | | | 3.6 | 11.6 | -1.7 | 0.006 |
| 019G121800.1 | | | 6.5 | 24.7 | -1.9 | 0.000 |
| 002G122400.1 | | | 1.0 | 5.3 | -2.4 | 0.041 |
| 001G194100.1 | | | 3.4 | 17.7 | -2.4 | 0.010 |
| 008G121700.1 | | | 4.0 | 21.2 | -2.4 | 0.000 |
| 005G006200.1 | | | 1.0 | 5.3 | -2.4 | 0.032 |
| 017G135300.1 | | | 5.2 | 27.8 | -2.4 | 0.002 |
| 001G058500.1 | | | 2.6 | 14.4 | -2.4 | 0.000 |
| 004G202600.1 | | | 0.9 | 4.9 | -2.5 | 0.042 |
| 010G043400.1 | | | 112.7 | 621.0 | -2.5 | 0.000 |
| 014G166900.1 | | | 2.0 | 11.2 | -2.5 | 0.008 |
| 006G050800.1 | | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At3g57450, ] | 9.8 | 56.2 | -2.5 | 0.015 |
| 013G049700.1 | | | 0.9 | 5.0 | -2.5 | 0.036 |
| 001G450000.1 | | | 85.2 | 497.4 | -2.5 | 0.000 |
| 009G038300.1 | | similar to expressed protein in Arabidopsis thaliana; similar to expression supported by MPSS; [ ortholog of | 21.5 | 126.7 | -2.6 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| | At5g59080,At5g02020,At3g46880,] | | | | |
| 001G226500.1 | | 50.5 | 305.8 | -2.6 | 0.001 |
| 018G078800.1 | | 7.2 | 44.2 | -2.6 | 0.000 |
| 008G127200.1 | | 2.3 | 14.3 | -2.6 | 0.001 |
| 010G111300.1 | similar to hypothetical protein; [ co-ortholog (1of2) of At1g23110, At1g70900, ] | 2.7 | 16.6 | -2.6 | 0.000 |
| 010G116000.1 | | 3.4 | 21.4 | -2.7 | 0.001 |
| T089100.1 | | 16.3 | 110.5 | -2.8 | 0.000 |
| 005G259100.1 | | 7.7 | 53.5 | -2.8 | 0.001 |
| 006G213800.1 | | 1.2 | 8.2 | -2.8 | 0.001 |
| 016G004500.1 | | 2.5 | 18.0 | -2.8 | 0.037 |
| 008G210400.1 | | 1.5 | 11.1 | -2.9 | 0.001 |
| 007G076600.1 | | 0.5 | 4.0 | -2.9 | 0.048 |
| T099900.1 | | 39.0 | 300.4 | -2.9 | 0.000 |
| 004G188600.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g27830, ] | 47.0 | 363.5 | -3.0 | 0.000 |
| 005G129400.1 | | 0.5 | 4.3 | -3.0 | 0.035 |
| 006G073400.1 | | 4.1 | 33.9 | -3.1 | 0.000 |
| 012G007100.1 | | 0.9 | 7.7 | -3.1 | 0.026 |
| 001G422000.1 | | 0.5 | 3.9 | -3.1 | 0.046 |
| 012G021600.1 | | 4.3 | 37.9 | -3.1 | 0.012 |
| 002G073900.1 | similar to hypothetical protein. [ORG:Populus deltoides x Populus maximowiczii]; [ co-ortholog (4of5) of CAG27628, ] | 0.7 | 6.1 | -3.2 | 0.005 |
| 017G110800.1 | | 105.0 | 966.8 | -3.2 | 0.000 |
| 018G114200.1 | | 6.1 | 57.5 | -3.2 | 0.001 |
| 001G226700.1 | | 3.0 | 29.6 | -3.3 | 0.000 |
| 008G048300.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g37380, At2g39370, ] | 0.9 | 8.8 | -3.3 | 0.006 |
| 001G449800.1 | | 6.3 | 65.1 | -3.4 | 0.000 |
| 001G449900.1 | | 9.7 | 102.5 | -3.4 | 0.000 |
| 010G196400.1 | | 0.7 | 8.6 | -3.6 | 0.000 |
| T160900.1 | | 24.8 | 296.5 | -3.6 | 0.020 |
| 009G111900.1 | | 0.7 | 8.0 | -3.6 | 0.014 |
| 001G336200.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At5g40690, ] | 13.5 | 164.4 | -3.6 | 0.001 |
| 007G098800.1 | | 0.4 | 5.2 | -3.6 | 0.008 |
| 018G006800.1 | | 0.5 | 5.8 | -3.6 | 0.004 |
| 016G041300.1 | | 7.5 | 92.8 | -3.6 | 0.000 |
| 018G072100.1 | | 22.4 | 278.1 | -3.6 | 0.000 |
| 010G218300.1 | | 0.7 | 8.8 | -3.8 | 0.004 |
| 008G134100.1 | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At1g67920,] | 0.4 | 5.7 | -3.9 | 0.003 |
| 001G226400.1 | | 5.6 | 89.9 | -4.0 | 0.000 |
| 002G100500.1 | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At1g72240,At1g22470,] | 3.2 | 56.4 | -4.1 | 0.000 |
| 018G094100.1 | | 0.5 | 8.6 | -4.2 | 0.006 |
| 001G294800.1 | | 1.7 | 30.9 | -4.2 | 0.000 |
| 002G002100.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At1g76600, At1g21010, ] | 0.5 | 9.5 | -4.2 | 0.013 |
| 004G095200.1 | | 0.7 | 12.5 | -4.2 | 0.000 |
| 016G133200.1 | similar to Expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g40475, ] | 11.8 | 221.1 | -4.2 | 0.000 |
| 008G164300.1 | | 0.3 | 5.3 | -4.4 | 0.003 |
| 002G074300.1 | | 1.4 | 27.8 | -4.4 | 0.000 |
| 005G076100.1 | | 1.5 | 33.6 | -4.5 | 0.000 |
| 001G008100.1 | | 2.8 | 68.7 | -4.6 | 0.001 |
| 001G192800.1 | | 1.0 | 25.7 | -4.7 | 0.018 |
| 003G152000.1 | | 11.9 | 313.7 | -4.7 | 0.049 |
| 008G127800.1 | similar to expressed protein in Arabidopsis thaliana; [ ortholog of At2g01300,At1g15010,] | 0.2 | 5.8 | -4.8 | 0.001 |
| 008G061700.1 | | 1.4 | 40.5 | -4.8 | 0.001 |
| 010G115100.1 | | 0.1 | 3.2 | -4.9 | 0.041 |
| 001G338100.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At5g40690, ] | 0.6 | 18.5 | -4.9 | 0.005 |
| 013G045300.1 | | 0.3 | 8.7 | -4.9 | 0.000 |
| 005G170200.1 | | 2.4 | 79.8 | -5.1 | 0.006 |
| 004G113000.1 | | 0.2 | 7.7 | -5.1 | 0.002 |
| 006G137700.1 | | 2.2 | 86.7 | -5.3 | 0.000 |
| 004G093000.1 | | 0.5 | 22.0 | -5.5 | 0.000 |
| 015G027400.1 | | 0.2 | 10.4 | -5.5 | 0.000 |
| 004G061300.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At1g29290, ] | 0.7 | 32.6 | -5.6 | 0.010 |
| 014G022300.1 | | 0.1 | 5.0 | -5.7 | 0.017 |
| 002G022400.1 | | 0.4 | 22.7 | -5.7 | 0.000 |
| 010G124700.1 | | 0.9 | 50.4 | -5.9 | 0.000 |
| 007G049500.1 | | 0.2 | 11.0 | -5.9 | 0.008 |
| T090900.1 | | 0.1 | 6.3 | -6.3 | 0.000 |
| 005G131000.1 | | 2.4 | 237.2 | -6.7 | 0.000 |
| 010G208800.1 | | 4.6 | 555.4 | -6.9 | 0.000 |
| 010G218500.1 | | 0.0 | 4.8 | -7.6 | 0.003 |
| 007G048400.1 | | 0.0 | 5.5 | -8.0 | 0.002 |
| 008G051800.1 | | 0.1 | 23.5 | -8.1 | 0.000 |
| 010G218400.1 | | 0.0 | 5.5 | -8.3 | 0.001 |
| 014G022200.1 | | 0.0 | 3.3 | -8.4 | 0.021 |
| 019G090900.1 | | 0.7 | 270.4 | -8.6 | 0.000 |

**Supplementary Table S4.4 Differentially expressed genes of *P.trichocarpa* in low-N condition**. Gene expression in mycorrhized samples was tested against those in non-mycorrhized samples.

| | | Eukaryotic orthologous groups | | | Mean RPKM | | | |
|---|---|---|---|---|---|---|---|---|
| Transcript ID | best arabidopsis TAIR10 hit defline | Defline | Class | Group | -N+Gi | -N-Gi | log2ratio | FDR corrected p-value |
| 010G101700.1 | DNAse I-like superfamily protein | TYPE I INOSITOL POLYPHOSPHATE 5-PHOSPHATASE, ARATH | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING | 8.6 | 21.8 | -1.3 | 0.004 |
| 007G117000.1 | PR5-like receptor kinase | LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 4.7 | 20.9 | -2.1 | 0.000 |
| 002G024100.1 | 5\'-3\' exoribonuclease 3 | similar to EXORIBONUCLEASE 2; | Replication, recombination and repair | INFORMATION STORAGE AND PROCESSING | 1.7 | 9.8 | -2.5 | 0.004 |
| 010G228800.1 | kow domain-containing transcription factor 1 | SUPPRESSOR OF TY 5 | Transcription | INFORMATION STORAGE AND PROCESSING | 0.6 | 5.7 | -3.2 | 0.033 |
| 014G022800.1 | D-3-phosphoglycerate dehydrogenase | Phosphoglycerate dehydrogenase. | Amino acid transport and metabolism | METABOLISM | 44.8 | 63.3 | -0.5 | 0.021 |
| 005G093200.1 | glutamine synthase clone F11 | similar to glutamine synthetase | Amino acid transport and metabolism | METABOLISM | 163.7 | 268.4 | -0.7 | 0.011 |
| 006G248500.1 | pleiotropic drug resistance 12 | ATP-BINDING CASSETTE TRANSPORTER (PDR) | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 20.6 | 49.4 | -1.3 | 0.020 |
| 001G118500.1 | cytochrome P450, family 706, subfamily A, polypeptide 6 | Cytochrome P450 CYP2 subfamily | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 43.1 | 86.4 | -1.0 | 0.013 |
| 002G159500.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | OXIDOREDUCTASE, 2OG-FE(II) OXYGENASE FAMILY PROTEIN | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 9.6 | 21.6 | -1.2 | 0.012 |
| 002G078600.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | similar to 1-aminocyclopropane-1-carboxylate oxidase; putative; similar to ACC oxidase; putative; similar to 1-aminocyclopropane-1-carboxylate oxida | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 86.0 | 178.1 | -1.0 | 0.033 |
| 006G272800.1 | FAD-dependent oxidoreductase family protein | PEROXISOMAL SARCOSINE OXIDASE | General function prediction only | POORLY CHARACTERIZED | 91.8 | 248.5 | -1.4 | 0.000 |
| 015G021900.1 | Nodulin MtN3 family protein | RAG1-ACTIVATING PROTEIN 1 | General function prediction only | POORLY CHARACTERIZED | 38.2 | 61.2 | -0.7 | 0.019 |
| 011G087200.1 | mannose-1-phosphate guanylyltransferase (GDP)s;GDP-galactose:mannose-1-phosphate | similar to VITAMIN C DEFECTIVE 2; [ co-ortholog (2of2) of At5g55120, At4g26850, ] | General function prediction only | POORLY CHARACTERIZED | 204.3 | 303.6 | -0.6 | 0.007 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | guanylyltransferases;GDP-galactose:glucose-1-phosphate guanylyltransferases;GDP-galactose:myoinositol-1-phosphate guanylyltransferases;glucose-1-phosphate guanylyltransferase | | | | | |
| 002G174600.1 | Aluminium activated malate transporter family protein | Aluminium activated malate transporter | 6.3 | 15.4 | -1.3 | 0.039 |
| 007G015200.1 | B-box type zinc finger family protein | B-box zinc finger | 9.9 | 36.5 | -1.9 | 0.000 |
| 010G209200.1 | nodulin MtN21 /EamA-like transporter family protein | EamA-like transporter family | 3.9 | 28.1 | -2.9 | 0.000 |
| 005G175300.1 | GRAS family transcription factor | GRAS domain family | 8.6 | 19.3 | -1.2 | 0.017 |
| 014G106800.1 | Homeodomain-like superfamily protein | MYB-related transcription factor LHY | 12.3 | 71.4 | -2.5 | 0.000 |
| 002G180800.1 | Homeodomain-like superfamily protein | MYB-related transcription factor LHY | 9.7 | 50.7 | -2.4 | 0.000 |
| 010G199100.1 | Regulator of chromosome condensation (RCC1) family protein | REGULATOR OF CHROMOSOME CONDENSATION-RELATED | 1.8 | 14.2 | -3.0 | 0.000 |
| 001G235500.1 | myb domain protein 48 | similar to myb family transcription factor (MYB59) | 334.3 | 568.1 | -0.8 | 0.003 |
| 018G108000.1 | actin binding | Transcription factor Abd-B, contains HOX domain | 1.4 | 7.0 | -2.4 | 0.040 |
| 007G117100.1 | Protein kinase superfamily protein | | 1.1 | 6.8 | -2.6 | 0.031 |
| 001G203000.1 | Tic22-like family protein | | 2.4 | 11.5 | -2.2 | 0.020 |
| 001G019700.1 | | | 0.5 | 5.1 | -3.4 | 0.048 |
| 001G020000.1 | | | 1.4 | 11.5 | -3.1 | 0.000 |
| 001G025100.1 | | | 16.8 | 100.3 | -2.6 | 0.000 |
| 018G055400.1 | | | 2.0 | 11.7 | -2.6 | 0.001 |
| 001G019900.1 | | | 2.6 | 14.9 | -2.5 | 0.000 |
| 001G255700.1 | | | 4.7 | 13.9 | -1.6 | 0.012 |
| 009G051100.1 | | | 5.5 | 14.1 | -1.4 | 0.030 |
| 015G100600.1 | | CHITINASE | 5.8 | 0.0 | 8.5 | 0.006 |
| 015G091200.1 | RGA-like 1 | DELLA protein | 6.7 | 0.0 | 9.4 | 0.002 |
| 009G028300.1 | Serine protease inhibitor, potato inhibitor I-type family protein | Potato inhibitor I family | 209.6 | 109.3 | 0.9 | 0.011 |
| 005G228000.1 | RAD-like 6 | **similar to myb family transcription factor; [ co-ortholog (2of2) of At1g19510, ]** | 25.6 | 8.3 | 1.6 | 0.001 |
| 009G037100.1 | C2H2 and C2HC zinc fingers superfamily protein | | 10.6 | 0.7 | 3.9 | 0.012 |
| 004G212900.1 | Putative membrane lipoprotein | | 341.8 | 96.3 | 1.8 | 0.048 |

**Supplementary Table S4.5 Differentially expressed genes of *P.trichocarpa* in high-N condition**. Gene expression mycorrhized samples were tested against those in non-mycorrhized samples.

| Transcript ID | best arabidopsis TAIR10 hit defline | Eukaryotic orthologous groups | | | Mean RPKM | | log2 ratio | FDR corrected p-value |
|---|---|---|---|---|---|---|---|---|
| | | Defline | Class | Group | +N+GI | +N-Gi | | |
| 006G198800.1 | alpha/beta-Hydrolases superfamily protein | similar to PrMC3 | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 17.8 | 2.5 | 2.9 | 0.005 |
| 006G036000.1 | serine carboxypeptidase-like 45 | SERINE CARBOXYPEPTIDASE II | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 12.7 | 0.0 | 8.4 | 0.000 |
| 004G069300.1 | YELLOW STRIPE like 7 | Sexual differentiation process protein ISP4 | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 10.4 | 0.3 | 5.2 | 0.040 |
| 018G152200.1 | alpha-galactosidase 2 | alpha-galactosidase | Carbohydrate transport and metabolism | METABOLISM | 7.2 | 1.0 | 2.9 | 0.022 |
| 011G153800.1 | | Exopolyphosphatases and related proteins | Energy production and conversion | METABOLISM | 7.8 | 0.6 | 3.7 | 0.005 |
| 008G049000.1 | alpha/beta-Hydrolases superfamily protein | similar to hydrolase; alpha/beta fold family protein; | Lipid transport and metabolism | METABOLISM | 6.4 | 0.5 | 3.6 | 0.013 |
| 006G070000.1 | Zinc-binding dehydrogenase family protein | 2-alkenal reductase. | General function prediction only | POORLY CHARACTERIZED | 26.2 | 0.9 | 4.9 | 0.000 |
| 005G226800.1 | GRAS family transcription factor | GRAS domain family | | | 8.9 | 1.1 | 3.0 | 0.022 |
| 002G010700.1 | HXXXD-type acyl-transferase family protein | Transferase family | | | 29.3 | 8.2 | 1.8 | 0.000 |
| 019G124400.1 | Kunitz family trypsin and protease inhibitor protein | Trypsin and protease inhibitor | | | 11.0 | 2.0 | 2.4 | 0.003 |
| 006G101000.1 | myb-like HTH transcriptional regulator family protein | Myb-like DNA-binding domain | | | 17.6 | 0.3 | 5.9 | 0.000 |
| 004G003100.1 | RING/U-box superfamily protein | UNCHARACTERIZED RING ZINC FINGER-CONTAINING PROTEIN | | | 13.8 | 1.7 | 3.0 | 0.000 |
| 011G163200.1 | RmlC-like cupins superfamily protein | similar to germin-like protein 1 | | | 20.1 | 0.3 | 6.1 | 0.006 |
| 014G186000.1 | | | | | 612.6 | 31.1 | 4.3 | 0.001 |
| 008G079200.1 | | | | | 12.0 | 0.9 | 3.7 | 0.012 |
| 019G121800.1 | | | | | 151.3 | 24.7 | 2.6 | 0.013 |
| 001G058500.1 | | | | | 60.2 | 14.4 | 2.1 | 0.001 |
| 009G118800.1 | Glucose-1-phosphate adenylyltransferase family protein | similar to ADP-glucose pyrophosphorylase large subunit. [ORG:Fragaria x ananassa]; [ co-ortholog (1of2) of AAB91467, At4g39210, At2g21590, JE0133, AAB91463, T08027, BAC66692, CAA65541, AAS00542, T06495, ] | Cell wall/membrane/envelope biogenesis | CELLULAR PROCESSES AND SIGNALING | 17.2 | 69.4 | -2.0 | 0.003 |
| 014G159100.1 | Phosphotyrosine protein phosphatases superfamily protein | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 9.1 | 57.5 | -2.7 | 0.000 |
| T091700.1 | Tautomerase/MIF superfamily protein | | Defense mechanisms | CELLULAR PROCESSES AND SIGNALING | 43.7 | 174.1 | -2.0 | 0.045 |
| 008G110800.1 | EXS (ERD1/XPR1/SYG1) family protein | | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING | 4.7 | 21.4 | -2.2 | 0.002 |
| 004G091300.1 | AAA-ATPase 1 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 9.3 | 33.4 | -1.8 | 0.012 |
| 011G113000.1 | glutathione S-transferase TAU 19 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 16.0 | 181.1 | -3.5 | 0.048 |
| 011G113300.1 | glutathione S-transferase TAU | | Posttranslational modification, | CELLULAR PROCESSES AND | 1.8 | 9.5 | -2.4 | 0.046 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 25 | | protein turnover, chaperones | SIGNALING | | | | |
| 010G061400.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 8.0 | 31.5 | -2.0 | 0.025 |
| 010G061200.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 25.6 | 102.7 | -2.0 | 0.020 |
| 002G254000.1 | glutathione S-transferase tau 7 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 3.2 | 21.1 | -2.7 | 0.003 |
| 005G119200.1 | P-loop containing nucleoside triphosphate hydrolases superfamily protein | similar to hypothetical protein; [ co-ortholog (2of2) of At4g05380, At1g43910, At4g05340, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 25.4 | 126.6 | -2.3 | 0.001 |
| 012G042200.1 | purple acid phosphatase 17 | similar to acid phosphatase type 5 (ACP5); similar to acid phosphatase type 5 (GI:10278031) (Arabidopsis thaliana); [ co-ortholog (1of3) of At3g17790, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 12.1 | 56.4 | -2.2 | 0.043 |
| 015G031400.1 | purple acid phosphatase 17 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 17.2 | 81.7 | -2.2 | 0.005 |
| 008G139300.1 | purple acid phosphatase 3 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 5.7 | 38.2 | -2.8 | 0.000 |
| 008G139100.1 | purple acid phosphatase 3 | similar to PURPLE ACID PHOSPHATASE PRECURSOR; [ co-ortholog (1of3) of At1g25230, At2g01890, At1g14700, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 4.2 | 33.8 | -3.0 | 0.000 |
| 005G118600.1 | ubiquitin-conjugating enzyme 16 | similar to UBIQUITIN-CONJUGATING ENZYME 17; [ co-ortholog (1of2) of At4g36410, At1g75440, ] | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 48.7 | 195.8 | -2.0 | 0.025 |
| 001G378900.1 | ubiquitin-specific protease 15 | | Posttranslational modification, protein turnover, chaperones | CELLULAR PROCESSES AND SIGNALING | 23.1 | 79.1 | -1.8 | 0.033 |
| 018G067000.1 | 1-amino-cyclopropane-1-carboxylate synthase 7 | similar to 1-aminocyclopropene-1-carboxylate synthase 3c. [ORG:Pyrus communis]; [ co-ortholog (2of2) of BAA76388, PN0477, AAS17854, AAR38502, PN0476, AAG12247, AAF22108, AAL66201, T17018, CAA78122, CAA78123, AAF61233, AAB67989, AAS17855, BAA37134, AAR1213 | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 2.6 | 13.8 | -2.4 | 0.037 |
| 006G112500.1 | calmodulin like 42 | similar to calcium-binding protein; putative; similar to SP\|Q09011\|Calcium binding-protein CAST (Solanum (tuberosum) ; [ co-ortholog (1of2) of At4g20780, At5g44460, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 13.8 | 57.9 | -2.1 | 0.024 |
| 008G160200.1 | CBL-interacting protein kinase 4 | similar to CBL-interacting protein kinase 4; [ co-ortholog (1of2) of At4g14580, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 2.3 | 13.4 | -2.5 | 0.001 |
| 011G093700.1 | Concanavalin A-like lectin protein kinase family protein | similar to lectin protein kinase; putative; similar to receptor lectin kinase 3 (Arabidopsis thaliana) gi\|4100060\|gb\|AAD00733\|; similar to protein kinase domain containing protein; similar to legume lectins alpha and beta domain containing protein; [ orth | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 1.3 | 11.9 | -3.2 | 0.032 |
| 003G099400.1 | phosphoribulokinase | similar to Phosphoribulokinase; similar to chloroplast precursor (EC 2.7.1.19) (Phosphopentokinase) (PRKASE) (PRK).; [ co-ortholog (2of2) of At1g32060, ] | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 4.2 | 26.5 | -2.7 | 0.027 |
| 007G117000.1 | PR5-like receptor kinase | | Signal transduction mechanisms | CELLULAR PROCESSES AND SIGNALING | 3.3 | 20.6 | -2.7 | 0.000 |
| 005G107300.1 | SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein | | Chromatin structure and dynamics | INFORMATION STORAGE AND PROCESSING | 11.7 | 38.3 | -1.7 | 0.016 |
| 002G229200.1 | aconitase 3 | similar to aconitate hydratase; similar to cytoplasmic; putative; similar to citrate hydro-lyase/aconitase; putative; similar to SP\|P49608\|Aconitate hydratase ; similar to cytoplasmic (EC 4.2.1.3) (Citrate hydro-lyase) (Aconitase) (Cucurbita maxima); [ or | RNA processing and modification | INFORMATION STORAGE AND PROCESSING | 80.2 | 336.0 | -2.1 | 0.030 |
| 002G038500.1 | myb domain protein 14 | similar to GmMYB29. [ORG:Glycine max]; [ ortholog of At2g31180,BAA81813,] | Transcription | INFORMATION STORAGE AND PROCESSING | 9.3 | 28.6 | -1.6 | 0.011 |
| 013G067000.1 | myb domain protein 56 | | Transcription | INFORMATION STORAGE | 4.7 | 19.2 | -2.0 | 0.000 |

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|---|---|---|---|---|---|---|---|---|
| 018G090600.1 | lysyl-tRNA synthetase 1 | | Translation, ribosomal structure and biogenesis | AND PROCESSING INFORMATION STORAGE AND PROCESSING | 24.6 | 108.2 | -2.1 | 0.038 |
| 001G162800.1 | alanine aminotransferase 2 | similar to ALANINE AMINOTRANSFERAS; [ ortholog of At1g17290,] | Amino acid transport and metabolism | METABOLISM | 61.6 | 235.6 | -1.9 | 0.030 |
| T104400.1 | APS reductase 3 | | Amino acid transport and metabolism | METABOLISM | 19.0 | 56.9 | -1.6 | 0.009 |
| 014G086300.1 | cysteine synthase C1 | | Amino acid transport and metabolism | METABOLISM | 80.5 | 232.4 | -1.5 | 0.047 |
| 001G300900.1 | D-3-phosphoglycerate dehydrogenase | similar to D-3-phosphoglycerate dehydrogenase; putative; similar to 3-PGDH; putative; similar to phosphoglycerate dehydrogenase; similar to Arabidopsis thaliana; similar to SP:O04130; [ co-ortholog (2of2) of At4g34200, ] | Amino acid transport and metabolism | METABOLISM | 27.2 | 138.2 | -2.3 | 0.000 |
| T059200.1 | glutamate decarboxylase | similar to glutamate decarboxylase 1. [ORG:Lotus corniculatus var. japonicus]; [ co-ortholog (2of3) of AAP85548, CAG30580, ] | Amino acid transport and metabolism | METABOLISM | 11.5 | 27.3 | -1.3 | 0.044 |
| 013G058300.1 | glutamate dehydrogenase 1 | similar to Glutamate dehydrogenase 1 (EC 1.4.1.3) (GDH 1).; [ co-ortholog (2of2) of At5g18170, At3g03910, ] | Amino acid transport and metabolism | METABOLISM | 69.0 | 125.4 | -0.9 | 0.003 |
| 017G131100.1 | glutamine synthase clone R1 | similar to glutamate-ammonia ligase (EC 6.3.1.2) gamma; similar to cytosolic - kidney bean. [ORG:Phaseolus vulgaris]; [ co-ortholog (1of5) of JQ0937, AAB23379, P08282, S62712, AAQ01729, AAP20795, AAB03492, AAD52008, 1211328A, P00965, AJFBQ, CAA63963, 2106 | Amino acid transport and metabolism | METABOLISM | 723.6 | 3395.9 | -2.2 | 0.005 |
| 005G099600.1 | isocitrate dehydrogenase 1 | | Amino acid transport and metabolism | METABOLISM | 19.6 | 79.6 | -2.0 | 0.027 |
| 018G041600.1 | Major facilitator superfamily protein | | Amino acid transport and metabolism | METABOLISM | 3.0 | 18.5 | -2.6 | 0.000 |
| 001G095800.1 | 6-phosphogluconate dehydrogenase family protein | 6-phosphogluconate dehydrogenase family protein; [ co-ortholog (1of2) of At1g64190, At5g41670, ] | Carbohydrate transport and metabolism | METABOLISM | 71.3 | 309.7 | -2.1 | 0.000 |
| 001G068200.1 | Aldolase-type TIM barrel family protein | similar to transaldolase; putative; [ co-ortholog (2of3) of At5g13420, ] | Carbohydrate transport and metabolism | METABOLISM | 39.6 | 167.2 | -2.1 | 0.010 |
| 013G005900.1 | galactinol synthase 2 | similar to galactinol synthase. [ORG:Glycine max]; [ co-ortholog (1of3) of AAM96867, At1g09350, At1g56600, ] | Carbohydrate transport and metabolism | METABOLISM | 5.2 | 24.0 | -2.2 | 0.011 |
| 010G055400.1 | glyceraldehyde-3-phosphate dehydrogenase C subunit 1 | similar to glyceraldehyde 3-phosphate dehydrogenase (phosphorylating). [ORG:Pisum sativum]; [ co-ortholog (1of3) of CAH59077, CAA51675, AAD46743, AAD46755, CAH59065, CAH59071, CAH59085, AAD46759, CAH59093, CAH59089, AAD46748, At3g04120, CAH59058, AAD46753 | Carbohydrate transport and metabolism | METABOLISM | 445.0 | 1747.2 | -2.0 | 0.011 |
| 016G011000.1 | Inositol monophosphatase family protein | | Carbohydrate transport and metabolism | METABOLISM | 8.5 | 43.3 | -2.4 | 0.000 |
| 010G156300.1 | Inositol monophosphatase family protein | | Carbohydrate transport and metabolism | METABOLISM | 9.9 | 56.5 | -2.5 | 0.000 |
| 003G109300.1 | phosphate starvation-induced gene 3 | similar to glycerol-3-phosphate transporter; putative; similar to glycerol 3-phosphate permease; putative; similar to cAMP inducible 2 protein (Mus musculus) GI:4580997; similar to glycerol-3-phosphate transporter (glycerol 3-phosphate permease) (Homo sap | Carbohydrate transport and metabolism | METABOLISM | 32.0 | 72.4 | -1.2 | 0.000 |
| 001G124200.1 | phosphate starvation-induced gene 3 | similar to glycerol-3-phosphate transporter; putative; similar to glycerol 3-phosphate permease; putative; similar to cAMP inducible 2 protein (Mus musculus) GI:4580997; similar to glycerol-3-phosphate transporter (glycerol 3-phosphate permease) (Homo sap | Carbohydrate transport and metabolism | METABOLISM | 29.9 | 101.8 | -1.8 | 0.009 |
| 005G168000.1 | Phosphoglycerate mutase family protein | similar to phosphoglycerate mutase. [ORG:Malus x domestica]; [ co-ortholog (2of2) of At1g78050, At1g22170, AAQ54516, ] | Carbohydrate transport and metabolism | METABOLISM | 69.6 | 247.8 | -1.8 | 0.020 |
| 003G030700.1 | purple acid phosphatase 22 | | Carbohydrate transport and | METABOLISM | 3.9 | 21.0 | -2.4 | 0.004 |

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|---|---|---|---|---|---|---|---|
| | | | metabolism | | | | |
| 001G001600.1 | Pyruvate kinase family protein | similar to Probable pyruvate kinase; similar to cytosolic isozyme (EC 2.7.1.40) (PK).; [ co-ortholog (2of2) of At5g56350, At4g26390, ] | Carbohydrate transport and metabolism | METABOLISM | 59.1 | 195.6 | -1.7 | 0.008 |
| 010G115300.1 | ribose-5-phosphate isomerase 2 | | Carbohydrate transport and metabolism | METABOLISM | 11.7 | 28.9 | -1.3 | 0.003 |
| 008G127600.1 | ribose-5-phosphate isomerase 2 | | Carbohydrate transport and metabolism | METABOLISM | 9.8 | 39.5 | -2.0 | 0.000 |
| 009G099000.1 | UDP-glucosyl transferase 73B3 | | Carbohydrate transport and metabolism | METABOLISM | 5.5 | 147.3 | -4.7 | 0.020 |
| 002G098400.1 | UDP-glucosyl transferase 85A2 | similar to probable UDP-glucuronosyltransferase (EC 2.4.1.-) - garden pea. [ORG:Pisum sativum]; [ ortholog of At1g22400,T06371,At1g22380,At1g22360,At1g22340,At1g22370,BAB86928,AAB99950,] | Carbohydrate transport and metabolism | METABOLISM | 1.3 | 8.6 | -2.7 | 0.009 |
| 009G095400.1 | UDP-Glycosyltransferase superfamily protein | | Carbohydrate transport and metabolism | METABOLISM | 14.2 | 44.1 | -1.6 | 0.009 |
| 006G100500.1 | Aldolase-type TIM barrel family protein | similar to Probable pyridoxin biosynthesis PDX1-like protein 3.; [ co-ortholog (1of2) of At5g01410, ] | Coenzyme transport and metabolism | METABOLISM | 91.0 | 654.6 | -2.8 | 0.044 |
| 001G310500.1 | GTP cyclohydrolase II | | Coenzyme transport and metabolism | METABOLISM | 20.4 | 68.9 | -1.8 | 0.043 |
| 017G050400.1 | GTP cyclohydrolase II | | Coenzyme transport and metabolism | METABOLISM | 6.4 | 54.7 | -3.1 | 0.050 |
| 002G240800.1 | homolog of bacterial PANC | similar to Pantoate--beta-alanine ligase (EC 6.3.2.1) (Pantothenate synthetase) (Pantoate activating enzyme).; [ ortholog of At5g48840,] | Coenzyme transport and metabolism | METABOLISM | 8.8 | 55.1 | -2.6 | 0.006 |
| 014G090500.1 | ketopantoate hydroxymethyltransferase 1 | ketopantoate hydroxymethyltransferase family protein; similar to SP\|Q9Y7B6\|3 methyl-2-oxobutanoate-hydroxymethyltransferase EC (2 1.2.11.Ketopantoate) (hydroxymethyltransferase (Emericella) (nidulans) ; [ ortholog of At2g46110,At3g61530,] | Coenzyme transport and metabolism | METABOLISM | 6.8 | 26.7 | -2.0 | 0.027 |
| 005G099900.1 | phosphoserine aminotransferase | | Coenzyme transport and metabolism | METABOLISM | 96.7 | 943.6 | -3.3 | 0.000 |
| 013G102900.1 | 12-oxophytodienoate reductase 2 | | Energy production and conversion | METABOLISM | 3.6 | 45.3 | -3.7 | 0.025 |
| 004G112800.1 | malate dehydrogenase | similar to nodule-enhanced malate dehydrogenase. [ORG:Pisum sativum]; [ co-ortholog (3of3) of AAB99757, T09294, T06325, AAP79476, AAC28106, AAP79474, T06386, AAC24855, ] | Energy production and conversion | METABOLISM | 48.0 | 280.3 | -2.5 | 0.009 |
| 012G105100.1 | phosphate transporter 3;1 | | Energy production and conversion | METABOLISM | 3.4 | 19.0 | -2.5 | 0.007 |
| 002G104400.1 | uncoupling protein 5 | mitochondrial substrate carrier family protein; [ co-ortholog (1of2) of At2g22500, ] | Energy production and conversion | METABOLISM | 27.1 | 154.7 | -2.5 | 0.000 |
| 013G049600.1 | ammonium transporter 1;1 | | Inorganic ion transport and metabolism | METABOLISM | 3.4 | 15.1 | -2.1 | 0.020 |
| 003G134900.1 | cation/H+ exchanger 18 | | Inorganic ion transport and metabolism | METABOLISM | 5.4 | 30.7 | -2.5 | 0.000 |
| 006G124800.1 | Heavy metal transport/detoxification superfamily protein | | Inorganic ion transport and metabolism | METABOLISM | 176.0 | 519.4 | -1.6 | 0.010 |
| 006G069500.1 | SPX domain gene 2 | | Inorganic ion transport and metabolism | METABOLISM | 16.4 | 59.1 | -1.8 | 0.024 |
| 018G131500.1 | SPX domain gene 2 | | Inorganic ion transport and metabolism | METABOLISM | 34.2 | 193.2 | -2.5 | 0.018 |
| 014G061400.1 | SPX domain gene 3 | | Inorganic ion transport and | METABOLISM | 4.1 | 57.6 | -3.8 | 0.024 |

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|---|---|---|---|---|---|---|---|---|
| | | | metabolism | | | | | |
| 002G143900.1 | SPX domain gene 3 | | Inorganic ion transport and metabolism | METABOLISM | 0.0 | 5.8 | -8.4 | 0.044 |
| 001G257000.1 | sulfite reductase | similar to sulfite reductase [Populus x canescens]. [ORG:Populus alba x Populus tremula]; [ co-ortholog (2of2) of AAC24584, AAQ57207, AAG59996, BAD12837, ] | Inorganic ion transport and metabolism | METABOLISM | 30.3 | 202.2 | -2.7 | 0.011 |
| 018G124100.1 | Voltage-gated chloride channel family protein | similar to chloride channel-like (CLC) protein; putative; similar to CLC-c; similar to At5g49890 (Arabidopsis thaliana) and chloride channel protein ClC-1 - Nicotiana tabacum; similar to PIR:T02939; [ co-ortholog (2of2) of At5g33280, ] | Inorganic ion transport and metabolism | METABOLISM | 1.5 | 8.3 | -2.4 | 0.020 |
| 013G012300.1 | phospholipase D P2 | | Lipid transport and metabolism | METABOLISM | 0.9 | 7.2 | -3.1 | 0.016 |
| 018G086900.1 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 5.1 | 34.5 | -2.8 | 0.003 |
| 005G220700.1 | cytochrome P450, family 94, subfamily B, polypeptide 1 | similar to cytochrome P450 CYP94A1 - spring vetch. [ORG:Vicia sativa]; [ co-ortholog (3of5) of AAG33645, AAL54885, T06525, O81117, T08014, AAD10204, AAC49190, P98188, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 56.9 | 174.6 | -1.6 | 0.029 |
| 005G220900.1 | cytochrome P450, family 94, subfamily B, polypeptide 1 | similar to cytochrome P450 CYP94A1 - spring vetch. [ORG:Vicia sativa]; [ co-ortholog (1of5) of AAG33645, AAL54885, T06525, O81117, T08014, AAD10204, AAC49190, P98188, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 8.5 | 27.9 | -1.7 | 0.016 |
| 014G193800.1 | GroES-like zinc-binding dehydrogenase family protein | | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 43.0 | 234.5 | -2.4 | 0.032 |
| 014G113500.1 | P-glycoprotein 21 | similar to multidrug resistant (MDR) ABC transporter; putative; similar to multidrug-resistant protein CjMDR1 GI:14715462 from (Coptis japonica); [ co-ortholog (1of7) of At2g47000, At3g62150, ] | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM | 6.2 | 44.4 | -2.8 | 0.012 |
| 009G142200.1 | basic chitinase | | General function prediction only | POORLY CHARACTERIZED | 21.1 | 68.3 | -1.7 | 0.018 |
| 006G272800.1 | FAD-dependent oxidoreductase family protein | | General function prediction only | POORLY CHARACTERIZED | 29.5 | 151.9 | -2.4 | 0.000 |
| 001G147300.1 | Haloacid dehalogenase-like hydrolase (HAD) superfamily protein | haloacid dehalogenase-like hydrolase family protein; similar to SP\|P71447\|Beta phosphoglucomutase-EC (5 4.2.6.(Lactococcus) (lactis) ; [ co-ortholog (2of4) of At2g38740, ] | General function prediction only | POORLY CHARACTERIZED | 4.0 | 35.7 | -3.1 | 0.000 |
| 008G196800.1 | Pyridoxal phosphate phosphatase-related protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of3) of At1g73010, At1g17710, ] | General function prediction only | POORLY CHARACTERIZED | 36.9 | 677.9 | -4.2 | 0.000 |
| 003G034600.1 | Pyridoxal phosphate phosphatase-related protein | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of3) of At1g73010, At1g17710, ] | General function prediction only | POORLY CHARACTERIZED | 5.2 | 104.9 | -4.3 | 0.010 |
| 005G066200.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 9.7 | 58.7 | -2.6 | 0.007 |
| T163900.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | General function prediction only | POORLY CHARACTERIZED | 5.3 | 62.3 | -3.6 | 0.042 |
| 006G042200.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | similar to embryo-abundant protein-related; similar to embryo-abundant protein (Picea glauca) GI:1350531; [ ortholog of At2g41380,] | General function prediction only | POORLY CHARACTERIZED | 31.2 | 712.6 | -4.5 | 0.019 |
| 001G325200.1 | senescence-related gene 3 | similar to SENESCENCE-RELATED GENE 3; [ ortholog of At5g43300,At3g02040,] | General function prediction only | POORLY CHARACTERIZED | 29.0 | 180.2 | -2.6 | 0.013 |
| 001G061100.1 | senescence-related gene 3 | | General function prediction only | POORLY CHARACTERIZED | 3.8 | 24.8 | -2.7 | 0.000 |
| 004G020500.1 | thiazole biosynthetic enzyme, chloroplast (ARA6) (THI1) (THI4) | | General function prediction only | POORLY CHARACTERIZED | 1.6 | 12.3 | -2.9 | 0.002 |
| 012G001600.1 | alternative oxidase 1A | | | | 11.0 | 126.5 | -3.5 | 0.013 |

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|---|---|---|---|---|---|---|
| 003G103900.1 | alternative oxidase 1D | | 27.9 | 173.4 | -2.6 | 0.015 |
| 004G163300.1 | arginine decarboxylase 2 | similar to Arginine decarboxylase (ARGDC) (ADC). [ORG:Glycine max]; [ ortholog of At2g16500,At4g34710,T06593,Q39827,AAN74941,] | 31.9 | 155.0 | -2.3 | 0.013 |
| 010G055300.1 | B12D protein | | 33.0 | 104.7 | -1.7 | 0.029 |
| 011G065500.1 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein | | 8.3 | 40.1 | -2.3 | 0.001 |
| 008G113400.1 | basic leucine-zipper 42 | similar to bZIP. [ORG:Phaseolus acutifolius]; [ co-ortholog (1of2) of AAK01953, AAK25822, ] | 27.2 | 84.3 | -1.6 | 0.015 |
| 001G235800.1 | C2H2 and C2HC zinc fingers superfamily protein | zinc finger (C2H2 type) family protein; similar to C2H2 type; [ ortholog of At3g53600,At2g37430,] | 9.3 | 37.1 | -2.0 | 0.049 |
| 006G226400.1 | calmodulin-binding family protein | | 7.3 | 19.5 | -1.4 | 0.025 |
| 006G226500.1 | calmodulin-binding family protein | calmodulin-binding family protein; calmodulin-binding family protein; calmodulin-binding family protein; [ co-ortholog (1of2) of At4g33050, At2g26190, ] | 19.9 | 64.9 | -1.7 | 0.001 |
| 003G130500.1 | CCT motif family protein | similar to hypothetical protein; [ co-ortholog (1of2) of At5g41380, At1g63820, ] | 1.8 | 30.4 | -4.1 | 0.028 |
| 011G096800.1 | CLAVATA3/ESR-RELATED 1 | | 64.7 | 219.7 | -1.8 | 0.003 |
| 008G026100.1 | Cysteine proteinases superfamily protein | | 4.3 | 17.7 | -2.1 | 0.000 |
| 002G013200.1 | Dehydrin family protein | | 6.7 | 45.9 | -2.8 | 0.006 |
| 003G134600.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 17.6 | 56.0 | -1.7 | 0.014 |
| 003G134700.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 3.8 | 15.4 | -2.0 | 0.009 |
| 003G134800.1 | Disease resistance-responsive (dirigent-like protein) family protein | similar to pathogenesis-related protein. [ORG:Pisum sativum]; [ co-ortholog (5of6) of AAF25371, P13240, AAD25355, AAB18669, 1604467C, T06433, AAF25372, AAA33662, ] | 14.5 | 64.5 | -2.2 | 0.032 |
| 003G216400.1 | Disease resistance-responsive (dirigent-like protein) family protein | | 21.9 | 104.6 | -2.3 | 0.007 |
| 010G072300.1 | ethylene response factor 1 | similar to ETHYLENE RESPONSE FACTOR 1; [ ortholog of At3g23240,] | 1.1 | 7.4 | -2.8 | 0.042 |
| 001G147200.1 | expansin-like B1 | | 1.0 | 15.7 | -4.0 | 0.045 |
| 011G113200.1 | glutathione S-transferase TAU 23 | | 11.0 | 117.6 | -3.4 | 0.048 |
| 009G110800.1 | hemoglobin 1 | | 146.1 | 859.3 | -2.6 | 0.010 |
| 006G097500.1 | HXXXD-type acyl-transferase family protein | transferase family protein; [ co-ortholog (1of2) of At5g01210, ] | 9.0 | 48.2 | -2.4 | 0.007 |
| 005G024500.1 | Hypoxia-responsive family protein | hypoxia-responsive family protein; [ co-ortholog (2of3) of At5g27760, At3g05550, ] | 48.8 | 256.0 | -2.4 | 0.013 |
| 018G122700.1 | Inorganic H pyrophosphatase family protein | | 52.9 | 129.8 | -1.3 | 0.004 |
| 018G119500.1 | Inorganic H pyrophosphatase family protein | | 36.3 | 156.8 | -2.1 | 0.002 |
| 019G088000.1 | Integrase-type DNA-binding superfamily protein | | 15.2 | 51.6 | -1.8 | 0.019 |
| 002G201600.1 | Integrase-type DNA-binding superfamily protein | | 19.4 | 76.8 | -2.0 | 0.002 |
| 005G223100.1 | Integrase-type DNA-binding | | 0.9 | 7.8 | -3.1 | 0.009 |

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|---|---|---|---|---|---|---|
| | superfamily protein | | | | | |
| 004G173400.1 | Major facilitator superfamily protein | nodulin family protein; similar to nodulin-like protein (Arabidopsis thaliana) GI:3329368; similar to nodule-specific protein Nlj70 (Lotus japonicus) GI:3329366; [ co-ortholog (2of4) of At4g34950, At2g16660, ] | 10.0 | 24.2 | -1.3 | 0.005 |
| 005G133800.1 | methyl esterase 1 | similar to Chain A; similar to K236l Mutant Of Hydroxynitrile Lyase From Hevea Brasiliensis In Complex With Acetonecyanohydrin. [ORG:Hevea brasiliensis]; [ co-ortholog (11of17) of 1SCQ_A, ] | 48.2 | 279.8 | -2.5 | 0.024 |
| 016G031400.1 | nodulin MtN21 /EamA-like transporter family protein | | 33.8 | 145.8 | -2.1 | 0.027 |
| 006G094300.1 | PAR1 protein | | 2.6 | 10.3 | -2.0 | 0.025 |
| 005G188300.1 | pathogenesis-related family protein | | 10.7 | 63.5 | -2.6 | 0.012 |
| 005G188400.1 | pathogenesis-related family protein | similar to putative pathogenesis-related protein. [ORG:Cucumis sativus]; [ co-ortholog (2of3) of CAF33484, ] | 30.6 | 206.7 | -2.8 | 0.013 |
| 005G240900.1 | Pathogenesis-related thaumatin superfamily protein | pathogenesis-related thaumatin family protein; similar to receptor serine/threonine kinase PR5K (Arabidopsis thaliana) GI:1235680; pathogenesis-related thaumatin family protein; similar to receptor serine/t; [ ortholog of At1g20030,] | 22.0 | 75.2 | -1.8 | 0.001 |
| 005G112700.1 | Pathogenesis-related thaumatin superfamily protein | | 10.3 | 102.8 | -3.3 | 0.000 |
| 012G006800.1 | Peroxidase superfamily protein | | 2.1 | 34.5 | -4.0 | 0.002 |
| T180000.1 | phospholipase D beta 1 | | 4.6 | 23.1 | -2.3 | 0.005 |
| 001G112100.1 | phospholipase D beta 1 | | 1.7 | 10.1 | -2.6 | 0.012 |
| 009G087500.1 | Plant regulator RWP-RK family protein | similar to RWP-RK domain-containing protein; similar to nodule inception protein (Lotus japonicus) GI:6448579; [ co-ortholog (2of2) of At2g17150, ] | 90.1 | 351.2 | -2.0 | 0.001 |
| 010G044100.1 | Pyruvate phosphate dikinase, PEP/pyruvate binding domain | | 19.0 | 88.4 | -2.2 | 0.000 |
| 012G089500.1 | quinolinate synthase | | 4.1 | 30.3 | -2.9 | 0.015 |
| 004G117800.1 | reversibly glycosylated polypeptide 2 | similar to type IIIa membrane protein cp-wap13. [ORG:Vigna unguiculata]; [ co-ortholog (4of4) of AAB61672, T11576, AAB61671, T11577, AAR13306, ] | 33.9 | 184.2 | -2.4 | 0.007 |
| 006G042400.1 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | | 12.0 | 270.2 | -4.5 | 0.031 |
| 002G119300.1 | salt tolerance zinc finger | | 55.3 | 163.4 | -1.6 | 0.025 |
| 002G203500.1 | senescence-associated gene 21 | | 147.2 | 685.8 | -2.2 | 0.005 |
| 014G127700.1 | senescence-associated gene 21 | | 110.0 | 702.3 | -2.7 | 0.001 |
| 009G044200.1 | Tetratricopeptide repeat (TPR)-like superfamily protein | | 10.7 | 66.4 | -2.6 | 0.003 |
| 002G256900.1 | Tetratricopeptide repeat (TPR)-like superfamily protein | | 0.5 | 8.0 | -3.9 | 0.003 |
| 007G006200.1 | VQ motif-containing protein | similar to VQ motif-containing protein; [ co-ortholog (1of2) of At2g22880, ] | 4.2 | 23.8 | -2.5 | 0.006 |
| 003G182200.1 | WRKY DNA-binding protein 40 | similar to Probable WRKY transcription factor 40 (WRKY DNA-binding protein 40).; [ co-ortholog (1of2) of At3g32090, At1g80840, ] | 13.4 | 99.5 | -2.9 | 0.010 |
| T099900.1 | | | 116.5 | 300.4 | -1.4 | 0.000 |
| 017G110800.1 | | | 285.6 | 966.8 | -1.8 | 0.010 |
| 004G188600.1 | | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g27830, ] | 100.7 | 363.5 | -1.9 | 0.008 |
| 017G135300.1 | | | 6.1 | 27.8 | -2.2 | 0.030 |

| | | | | | |
|---|---|---|---|---|---|
| 001G226400.1 | | 19.3 | 89.9 | -2.2 | 0.021 |
| 016G133200.1 | similar to Expressed protein in Arabidopsis thaliana; [ co-ortholog (2of2) of At2g40475, ] | 43.0 | 221.1 | -2.4 | 0.009 |
| 005G193900.1 | | 22.4 | 115.6 | -2.4 | 0.003 |
| 010G111300.1 | similar to hypothetical protein; [ co-ortholog (1of2) of At1g23110, At1g70900, ] | 3.1 | 16.6 | -2.4 | 0.002 |
| 007G093200.1 | | 6.3 | 36.7 | -2.5 | 0.014 |
| 013G045700.1 | | 1.9 | 11.6 | -2.6 | 0.002 |
| 015G027400.1 | | 1.5 | 10.4 | -2.8 | 0.011 |
| 005G131000.1 | | 34.7 | 237.2 | -2.8 | 0.002 |
| 016G041300.1 | | 12.9 | 92.8 | -2.8 | 0.000 |
| 010G208800.1 | | 72.8 | 555.4 | -2.9 | 0.016 |
| 013G062100.1 | | 8.1 | 66.8 | -3.0 | 0.001 |
| 002G022400.1 | | 2.7 | 22.7 | -3.1 | 0.000 |
| 001G379400.1 | | 1.3 | 11.2 | -3.1 | 0.001 |
| 010G218400.1 | | 0.6 | 5.5 | -3.3 | 0.043 |
| 001G336200.1 | similar to expressed protein in Arabidopsis thaliana; [ co-ortholog (1of2) of At5g40690, ] | 13.3 | 164.4 | -3.6 | 0.010 |
| 004G093000.1 | | 1.8 | 22.0 | -3.6 | 0.009 |
| 008G051800.1 | | 1.2 | 23.5 | -4.3 | 0.001 |
| 006G137700.1 | | 3.4 | 86.7 | -4.7 | 0.004 |

**Supplementary Table S4.6 List of expressed N-metabolism related genes in *P. trichocarpa.***

| Transcript ID | best arabidopsis TAIR10 hit defline | Eukaryotic orthologous groups | | |
|---|---|---|---|---|
| | | Defline | Class | Group |
| Potri.001G305400.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.002G047000.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.002G255000.1 | ammonium transporter 1;1 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.002G255100.1 | ammonium transporter 1;1 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.005G054900.1 | ammonium transporter 2 | | | |
| Potri.005G106000.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.005G216000.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.006G102800.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.006G247800.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.008G173800.1 | ammonium transporter 1;1 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.009G045200.1 | ammonium transporter 1;1 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.010G063500.1 | ammonium transporter 1;1 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.013G040400.1 | ammonium transporter 2 | Ammonium transporter RHBG | Intracellular trafficking, secretion, and vesicular transport | CELLULAR PROCESSES AND SIGNALING |
| Potri.013G049600.1 | ammonium transporter 1;1 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.016G121300.1 | ammonium transporter 2 | | | |
| Potri.016G121400.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.018G033500.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.019G000800.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.019G023600.1 | ammonium transporter 1;2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.T000100.1 | ammonium transporter 2 | | | |
| Potri.T000200.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.T000600.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.T103600.1 | ammonium transporter 2 | Ammonia permease | Inorganic ion transport and metabolism | METABOLISM |
| Potri.016G036900.1 | glutamate synthase 1 | Glutamate sythase | Amino acid transport and metabolism | METABOLISM |
| Potri.007G005300.1 | N-acetyl-l-glutamate synthase 2 | Acetylglutamate kinase/ acetylglutamate synthase | Amino acid transport and metabolism | METABOLISM |
| Potri.015G017500.1 | NADH-dependent glutamate synthase 1 | Glutamate sythase | Amino acid transport and metabolism | METABOLISM |
| Potri.006G038400.1 | glutamate synthase 1 | | | |
| Potri.014G009900.1 | N-acetyl-l-glutamate synthase 2 | N-acetyltransferase | General function prediction only | POORLY CHARACTERIZED |
| Potri.014G005000.1 | N-acetyl-l-glutamate synthase 2 | Acetylglutamate kinase/ acetylglutamate synthase | Amino acid transport and metabolism | METABOLISM |
| Potri.012G011700.1 | NADH-dependent glutamate synthase 1 | Glutamate sythase | Amino acid transport and metabolism | METABOLISM |
| Potri.019G034800.1 | glutamate dehydrogenase 1 | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM |
| Potri.001G199600.1 | Amino acid dehydrogenase family protein | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM |
| Potri.001G193600.1 | Amino acid dehydrogenase family protein | | | |
| Potri.001G151200.1 | glutamate dehydrogenase 2 | | | |
| Potri.013G058300.1 | glutamate dehydrogenase 1 | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM |
| Potri.013G112400.1 | glutamate dehydrogenase 1 | | | |

**Supplementary Table S4.6** continued

| | log2ratio | | | | FDR corrected p-values | | | | Mean RPKM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Transcript ID | -N+Gi Vs +N+Gi | -N+Gi Vs -N-Gi | +N+Gi Vs +N-Gi | -N-Gi Vs +N-Gi | +N+GI vs -N+Gi | -N-Gi Vs -N+Gi | +N-Gi Vs +N+GI | +N-Gi Vs -N-Gi | -N+Gi - | +N+GI - | -N-Gi - | +N-Gi - |
| Potri.001G305400.1 | 2.6 | -0.3 | 2.0 | 5.0 | 0.031 | 1.000 | 0.961 | 0.000 | 76.6 | 12.3 | 93.0 | 3.0 |
| Potri.002G047000.1 | 0.2 | 4.1 | 4.0 | 0.1 | 1.000 | 0.989 | 0.554 | 1.000 | 16.9 | 15.2 | 1.0 | 0.9 |
| Potri.002G255000.1 | 0.1 | -0.6 | 2.9 | 3.6 | 1.000 | 1.000 | 1.000 | 1.000 | 0.1 | 0.1 | 0.2 | 0.0 |
| Potri.002G255100.1 | 0.9 | 2.8 | 0.1 | -1.9 | 1.000 | 1.000 | 1.000 | 1.000 | 0.0 | 0.0 | 0.0 | 0.0 |
| Potri.005G054900.1 | 0.6 | 0.1 | 0.3 | 0.9 | 1.000 | 1.000 | 1.000 | 0.893 | 2.8 | 1.8 | 2.6 | 1.4 |
| Potri.005G106000.1 | 1.1 | 6.8 | 7.7 | 2.0 | 1.000 | 1.000 | 0.681 | 1.000 | 9.5 | 4.3 | 0.1 | 0.0 |
| Potri.005G216000.1 | 0.0 | 7.5 | 6.3 | -1.2 | 1.000 | 1.000 | 0.688 | 1.000 | 51.6 | 51.6 | 0.3 | 0.7 |
| Potri.006G102800.1 | -0.9 | 0.4 | 1.3 | 0.1 | 0.795 | 1.000 | 0.812 | 1.000 | 26.5 | 48.0 | 19.8 | 19.2 |
| Potri.006G247800.1 | 0.0 | 0.4 | 2.4 | 1.9 | 1.000 | 1.000 | 1.000 | 1.000 | 0.1 | 0.1 | 0.0 | 0.0 |
| Potri.008G173800.1 | 1.3 | 0.5 | 0.1 | 0.9 | 1.000 | 1.000 | 1.000 | 1.000 | 0.2 | 0.1 | 0.2 | 0.1 |
| Potri.009G045200.1 | 0.2 | 0.0 | -1.9 | -1.7 | 1.000 | 1.000 | 0.701 | 0.328 | 1.2 | 1.1 | 1.2 | 4.0 |
| Potri.010G063500.1 | 0.3 | -0.4 | 0.2 | 0.9 | 1.000 | 0.284 | 1.000 | 0.002 | 26.7 | 21.7 | 36.4 | 19.3 |
| Potri.013G040400.1 | 0.1 | 3.9 | 6.9 | 3.1 | 1.000 | 1.000 | 0.983 | 1.000 | 0.9 | 0.9 | 0.1 | 0.0 |
| Potri.013G049600.1 | -2.2 | -1.0 | -2.1 | -3.3 | 0.943 | 1.000 | 0.020 | 0.000 | 0.8 | 3.4 | 1.5 | 15.1 |
| Potri.016G121300.1 | 0.3 | -0.3 | -0.3 | 0.4 | 1.000 | 1.000 | 1.000 | 1.000 | 0.5 | 0.4 | 0.6 | 0.5 |
| Potri.016G121400.1 | 1.6 | -0.5 | -0.3 | 1.8 | 0.353 | 0.965 | 1.000 | 0.000 | 10.8 | 3.7 | 15.4 | 4.4 |
| Potri.018G033500.1 | 0.2 | 10.2 | 9.4 | -0.6 | 1.000 | 1.000 | 0.688 | 1.000 | 34.3 | 30.7 | 0.0 | 0.0 |
| Potri.019G000800.1 | -1.7 | -0.3 | 2.3 | 0.9 | 0.791 | 1.000 | 0.882 | 0.914 | 1.7 | 5.5 | 2.2 | 1.1 |
| Potri.019G023600.1 | 0.8 | -0.8 | -2.2 | -0.6 | 0.971 | 0.037 | 0.001 | 0.054 | 110.4 | 62.8 | 190.0 | 289.5 |
| Potri.T000100.1 | -4.4 | -0.9 | 2.4 | -1.0 | 0.538 | 1.000 | 0.874 | 0.852 | 0.5 | 10.8 | 1.0 | 2.0 |
| Potri.T000200.1 | 2.0 | -0.6 | 1.3 | 4.0 | 0.144 | 0.934 | 1.000 | 0.000 | 6.7 | 1.6 | 10.0 | 0.6 |
| Potri.T000600.1 | -2.7 | -0.5 | 2.3 | 0.1 | 0.605 | 1.000 | 0.861 | 1.000 | 3.0 | 19.9 | 4.4 | 3.9 |
| Potri.T103600.1 | -0.2 | 5.2 | 6.6 | 1.1 | 1.000 | 0.876 | 0.864 | 1.000 | 1.1 | 1.3 | 0.0 | 0.0 |
| Potri.016G036900.1 | -1.9 | -1.0 | -2.3 | -3.2 | 1.000 | 1.000 | 0.723 | 0.166 | 0.1 | 0.5 | 0.3 | 2.4 |
| Potri.007G005300.1 | -1.1 | 0.2 | -0.5 | -1.8 | 0.430 | 1.000 | 1.000 | 0.000 | 5.1 | 10.8 | 4.4 | 15.8 |
| Potri.015G017500.1 | -1.3 | -0.7 | -1.1 | -1.6 | 0.115 | 1.000 | 0.302 | 0.010 | 6.7 | 16.1 | 10.8 | 33.6 |
| Potri.006G038400.1 | -1.2 | -1.1 | -0.8 | -0.9 | 0.323 | 0.000 | 0.284 | 0.000 | 32.1 | 76.1 | 69.4 | 134.0 |
| Potri.014G009900.1 | -1.2 | -1.6 | 0.1 | 0.5 | 1.000 | 1.000 | 1.000 | 1.000 | 0.4 | 0.8 | 1.1 | 0.8 |
| Potri.014G005000.1 | -0.3 | -0.5 | 0.4 | 0.5 | 0.886 | 1.000 | 1.000 | 0.629 | 9.1 | 11.4 | 12.5 | 8.7 |
| Potri.012G011700.1 | -4.5 | -0.6 | -2.9 | -6.9 | 0.477 | 1.000 | 0.056 | 0.002 | 2.1 | 47.9 | 3.1 | 367.8 |
| Potri.019G034800.1 | 0.0 | -0.6 | -0.5 | 0.0 | 1.000 | 0.624 | 1.000 | 1.000 | 55.1 | 56.7 | 82.4 | 81.1 |
| Potri.001G199600.1 | -0.2 | -0.4 | 0.0 | 0.3 | 0.999 | 0.954 | 1.000 | 0.979 | 11.9 | 13.4 | 15.8 | 13.1 |
| Potri.001G193600.1 | 0.4 | -0.2 | 0.2 | 0.8 | 1.000 | 1.000 | 1.000 | 1.000 | 0.3 | 0.2 | 0.3 | 0.2 |
| Potri.001G151200.1 | 0.8 | 0.4 | 0.7 | 1.1 | 0.970 | 1.000 | 1.000 | 0.723 | 3.9 | 2.2 | 2.9 | 1.4 |
| Potri.013G058300.1 | -1.8 | -0.2 | -0.9 | -2.5 | 0.000 | 1.000 | 0.003 | 0.000 | 20.2 | 69.0 | 22.6 | 125.4 |
| Potri.013G112400.1 | | | | | 1.000 | 1.000 | 1.000 | 1.000 | 0.1 | 0.0 | 0.0 | 0.0 |

| Transcript ID | best arabidopsis TAIR10 hit defline | Eukaryotic orthologous groups | | |
|---|---|---|---|---|
| | | **Defline** | **Class** | **Group** |
| Potri.012G113500.1 | glutamate dehydrogenase 2 | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM |
| Potri.015G111000.1 | glutamate dehydrogenase 2 | Glutamate/ leucine/ phenylalanine/ valine dehydrogenases | Amino acid transport and metabolism | METABOLISM |
| Potri.009G024100.1 | cofactor of nitrate reductase and xanthine dehydrogenase 2 | Molybdenum cofactor biosynthesis pathway protein | Coenzyme transport and metabolism | METABOLISM |
| Potri.005G172400.1 | nitrate reductase 2 | Sulfite oxidase, molybdopterin-binding component | Energy production and conversion | METABOLISM |
| Potri.001G230700.1 | cofactor of nitrate reductase and xanthine dehydrogenase 2 | | | |
| Potri.014G099500.1 | cofactor of nitrate reductase and xanthine dehydrogenase 3 | | | |
| Potri.002G088600.1 | nitrate reductase 1 | Sulfite oxidase, molybdopterin-binding component | Energy production and conversion | METABOLISM |
| Potri.009G101600.1 | nitrite reductase 1 | | | |
| Potri.004G140800.1 | nitrite reductase 1 | Sulfite reductase (ferredoxin) | Inorganic ion transport and metabolism | METABOLISM |
| Potri.009G116000.1 | hydroxylamine reductase | NADH:ubiquinone oxidoreductase, NDUFS8/ 23 kDa subunit | Energy production and conversion | METABOLISM |
| Potri.014G080600.1 | N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein | Asparaginase | Amino acid transport and metabolism | METABOLISM |
| Potri.002G122900.1 | N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein | Asparaginase | Amino acid transport and metabolism | METABOLISM |
| Potri.014G022900.1 | N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein | Asparaginase | Amino acid transport and metabolism | METABOLISM |
| Potri.005G145400.1 | Acetamidase/ Formamidase family protein | | | |
| Potri.007G053800.1 | Acetamidase/ Formamidase family protein | | | |
| Potri.003G179400.1 | Nitrilase/ cyanide hydratase and apolipoprotein N-acyltransferase family protein | | | |
| Potri.004G201400.1 | nitrilase-like protein 1 | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.004G199600.1 | nitrilase 4 | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.006G222900.1 | Nitrilase/ cyanide hydratase and apolipoprotein N-acyltransferase family protein | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.009G162600.1 | nitrilase-like protein 1 | | | |
| Potri.008G047100.1 | Nitrilase/ cyanide hydratase and apolipoprotein N-acyltransferase family protein | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.016G074200.1 | nitrilase 4 | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.010G214600.1 | Nitrilase/ cyanide hydratase and apolipoprotein N-acyltransferase family protein | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.006G207700.1 | nitrilase 4 | Carbon-nitrogen hydrolase | Amino acid transport and metabolism | METABOLISM |
| Potri.010G068200.1 | cyanase | | | |
| Potri.006G126800.1 | PHE ammonia lyase 1 | Phenylalanine and histidine ammonia-lyase | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM |
| Potri.010G224100.1 | PHE ammonia lyase 1 | Phenylalanine and histidine ammonia-lyase | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM |
| Potri.016G091100.1 | PHE ammonia lyase 1 | Phenylalanine and histidine ammonia-lyase | Secondary metabolites biosynthesis, transport and catabolism | METABOLISM |
| Potri.008G038200.1 | PHE ammonia lyase 1 | Phenylalanine and histidine ammonia- | Secondary metabolites biosynthesis, transport and | METABOLISM |

lyase catabolism

| Transcript ID | log2ratio | | | | FDR corrected p-values | | | | Mean RPKM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | -N+Gi Vs +N+Gi | -N+Gi Vs -N-Gi | +N+Gi Vs +N-Gi | -N-Gi Vs +N-Gi | +N+GI vs -N+Gi | -N-Gi Vs -N+Gi | +N-Gi Vs +N+GI | +N-Gi Vs -N-Gi | -N+Gi - | +N+GI - | -N-Gi - | +N-Gi - |
| Potri.012G113500.1 | -5.9 | -0.3 | 2.9 | -2.6 | 0.971 | 1.000 | 1.000 | 0.081 | 0.8 | 47.5 | 1.0 | 6.2 |
| Potri.015G111000.1 | -6.5 | 0.1 | 2.1 | -4.5 | 0.942 | 1.000 | 1.000 | 0.231 | 5.0 | 444.6 | 4.6 | 103.9 |
| Potri.009G024100.1 | -0.5 | 0.0 | -0.7 | -1.2 | 0.661 | 1.000 | 0.750 | 0.037 | 8.1 | 11.6 | 8.3 | 19.0 |
| Potri.005G172400.1 | -0.6 | 0.3 | 0.2 | -0.7 | 1.000 | 1.000 | 1.000 | 0.455 | 29.9 | 46.1 | 24.8 | 40.0 |
| Potri.001G230700.1 | -1.7 | -1.1 | 0.1 | -0.5 | 1.000 | 1.000 | 1.000 | 1.000 | 0.0 | 0.1 | 0.1 | 0.1 |
| Potri.014G099500.1 | 1.2 | 0.3 | -0.2 | 0.7 | 0.585 | 1.000 | 1.000 | 0.774 | 7.0 | 3.0 | 5.6 | 3.5 |
| Potri.002G088600.1 | 1.6 | -0.1 | -0.9 | 0.8 | 0.307 | 1.000 | 1.000 | 0.194 | 76.9 | 25.6 | 80.6 | 47.2 |
| Potri.009G101600.1 | 2.8 | -1.5 | -0.6 | 3.7 | 1.000 | 1.000 | 1.000 | 1.000 | 0.1 | 0.0 | 0.3 | 0.0 |
| Potri.004G140800.1 | -1.0 | -0.3 | -1.0 | -1.6 | 0.828 | 1.000 | 0.263 | 0.000 | 24.3 | 46.9 | 29.9 | 90.7 |
| Potri.009G116000.1 | 0.4 | 0.1 | -0.7 | -0.5 | 1.000 | 1.000 | 0.135 | 0.001 | 79.5 | 61.7 | 72.4 | 102.6 |
| Potri.014G080600.1 | -0.1 | 0.0 | 0.5 | 0.4 | 1.000 | 1.000 | 1.000 | 0.765 | 15.1 | 15.9 | 14.7 | 11.1 |
| Potri.002G122900.1 | 0.8 | 0.6 | 0.3 | 0.5 | 0.971 | 1.000 | 1.000 | 1.000 | 5.6 | 3.3 | 3.8 | 2.7 |
| Potri.014G022900.1 | 1.8 | -0.3 | 0.6 | 2.7 | 0.098 | 1.000 | 1.000 | 0.000 | 549.5 | 157.3 | 653.6 | 103.0 |
| Potri.005G145400.1 | -0.5 | 0.2 | -0.4 | -1.1 | 0.609 | 1.000 | 1.000 | 0.000 | 98.0 | 142.0 | 87.5 | 192.5 |
| Potri.007G053800.1 | -1.3 | 0.4 | 1.2 | -0.6 | 0.177 | 1.000 | 0.201 | 0.287 | 29.8 | 71.9 | 21.8 | 32.0 |
| Potri.003G179400.1 | -1.2 | -1.4 | -0.6 | -0.3 | 0.846 | 0.921 | 1.000 | 1.000 | 1.0 | 2.4 | 2.9 | 3.5 |
| Potri.004G201400.1 | 0.4 | 0.3 | -0.3 | -0.2 | 1.000 | 1.000 | 1.000 | 0.952 | 31.1 | 24.4 | 25.7 | 29.4 |
| Potri.004G199600.1 | 0.4 | 0.3 | -0.4 | -0.2 | 0.941 | 1.000 | 1.000 | 0.983 | 31.0 | 22.7 | 25.5 | 29.0 |
| Potri.006G222900.1 | -0.1 | -0.4 | -0.2 | 0.1 | 1.000 | 0.560 | 1.000 | 1.000 | 33.9 | 36.4 | 44.8 | 42.6 |
| Potri.009G162600.1 | -0.3 | -0.1 | 0.6 | 0.5 | 1.000 | 1.000 | 1.000 | 1.000 | 2.4 | 2.9 | 2.7 | 1.9 |
| Potri.008G047100.1 | 0.2 | -0.6 | -0.3 | 0.6 | 1.000 | 0.318 | 1.000 | 0.178 | 18.4 | 15.8 | 27.8 | 18.8 |
| Potri.016G074200.1 | 0.2 | -0.7 | 0.0 | 1.0 | 1.000 | 1.000 | 1.000 | 1.000 | 0.4 | 0.3 | 0.6 | 0.3 |
| Potri.010G214600.1 | 1.2 | 0.0 | -0.2 | 1.1 | 0.444 | 1.000 | 1.000 | 0.009 | 22.1 | 9.4 | 21.8 | 10.5 |
| Potri.006G207700.1 | 0.2 | -0.1 | 1.0 | 1.3 | 1.000 | 1.000 | 1.000 | 1.000 | 0.4 | 0.3 | 0.4 | 0.2 |
| Potri.010G068200.1 | 0.7 | 0.4 | -0.3 | 0.1 | 0.581 | 0.299 | 1.000 | 1.000 | 139.9 | 84.0 | 107.1 | 100.5 |
| Potri.006G126800.1 | -0.3 | 0.0 | 0.5 | 0.2 | 1.000 | 1.000 | 1.000 | 1.000 | 102.9 | 127.6 | 103.0 | 92.5 |
| Potri.010G224100.1 | 0.2 | -0.4 | 0.0 | 0.6 | 1.000 | 0.397 | 1.000 | 0.098 | 39.9 | 35.6 | 53.1 | 35.8 |
| Potri.016G091100.1 | 0.4 | -0.1 | 0.9 | 1.4 | 1.000 | 1.000 | 0.433 | 0.005 | 137.6 | 106.6 | 149.2 | 55.6 |
| Potri.008G038200.1 | -0.8 | -0.4 | 0.0 | -0.5 | 0.196 | 0.782 | 1.000 | 0.071 | 44.4 | 78.8 | 58.5 | 81.2 |

| Transcript ID | best arabidopsis TAIR10 hit defline | Eukaryotic orthologous groups | | |
|---|---|---|---|---|
| | | Defline | Class | Group |
| Potri.006G041300.1 | cystathionine beta-lyase | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM |
| Potri.016G038200.1 | cystathionine beta-lyase | Cystathionine beta-lyases/ cystathionine gamma-synthases | Amino acid transport and metabolism | METABOLISM |
| Potri.004G085400.1 | glutamine synthase clone R1 | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM |
| Potri.006G121000.1 | glutamate-ammonia ligases;catalytics;glutamate-ammonia ligases | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM |
| Potri.010G181500.1 | glutamate-ammonia ligases;catalytics;glutamate-ammonia ligases | | | |
| Potri.017G131100.1 | glutamine synthase clone R1 | Glutamine synthetase | Amino acid transport and metabolism | METABOLISM |
| Potri.001G278400.1 | glutamine-dependent asparagine synthase 1 | Asparagine synthase (glutamine-hydrolyzing) | Amino acid transport and metabolism | METABOLISM |
| Potri.009G072900.1 | glutamine-dependent asparagine synthase 1 | Asparagine synthase (glutamine-hydrolyzing) | Amino acid transport and metabolism | METABOLISM |
| Potri.010G157700.1 | Asparagine synthase family protein | Asparagine synthase | Amino acid transport and metabolism | METABOLISM |
| Potri.008G177900.1 | urease | | | |
| Potri.001G159600.1 | urease accessory protein D | | | |
| Potri.002G243700.1 | urease accessory protein G | | | |
| Potri.002G243500.1 | urease accessory protein G | | | |
| Potri.001G224400.1 | urease accessory protein F | | | |

| | **log2ratio** | | | | **FDR corrected p-values** | | | | **Mean RPKM** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Transcript ID** | **-N+Gi Vs +N+Gi** | **-N+Gi Vs -N-Gi** | **+N+Gi Vs +N-Gi** | **-N-Gi Vs +N-Gi** | **+N+GI vs -N+Gi** | **-N-Gi Vs -N+Gi** | **+N-Gi Vs +N+GI** | **+N-Gi Vs -N-Gi** | **-N+Gi -** | **+N+GI -** | **-N-Gi -** | **+N-Gi -** |
| Potri.006G041300.1 | -2.4 | 0.3 | 1.6 | -1.1 | 1.000 | 1.000 | 1.000 | 1.000 | 0.0 | 0.2 | 0.0 | 0.1 |
| Potri.016G038200.1 | -0.3 | 0.0 | 0.1 | -0.2 | 0.935 | 1.000 | 1.000 | 1.000 | 15.7 | 19.8 | 16.1 | 18.7 |
| Potri.004G085400.1 | -1.6 | 0.3 | -1.9 | -3.8 | 1.000 | 1.000 | 0.136 | 0.000 | 66.9 | 209.0 | 55.2 | 795.7 |
| Potri.006G121000.1 | 0.4 | -0.9 | -0.4 | 0.9 | 1.000 | 0.001 | 1.000 | 0.000 | 28.7 | 22.0 | 52.9 | 28.2 |
| Potri.010G181500.1 | -2.3 | -0.9 | 2.7 | 1.2 | 0.848 | 1.000 | 1.000 | 1.000 | 0.4 | 2.1 | 0.8 | 0.3 |
| Potri.017G131100.1 | -2.6 | 0.1 | -2.2 | -5.0 | 0.964 | 1.000 | 0.005 | 0.000 | 120.6 | 723.6 | 109.1 | 3395.9 |
| Potri.001G278400.1 | -7.8 | 0.7 | -0.6 | -9.1 | 0.560 | 1.000 | 1.000 | 0.000 | 3.0 | 653.9 | 1.8 | 1003.4 |
| Potri.009G072900.1 | -7.7 | 0.8 | 2.9 | -5.7 | 0.951 | 1.000 | 1.000 | 0.001 | 1.2 | 259.8 | 0.7 | 35.4 |
| Potri.010G157700.1 | 0.4 | -0.4 | -0.9 | -0.1 | 1.000 | 1.000 | 1.000 | 1.000 | 2.4 | 1.8 | 3.2 | 3.3 |
| Potri.008G177900.1 | -0.5 | -0.9 | -0.1 | 0.4 | 0.259 | 0.002 | 1.000 | 0.514 | 22.4 | 31.0 | 42.3 | 32.2 |
| Potri.001G159600.1 | 0.3 | -0.1 | 0.0 | 0.5 | 1.000 | 1.000 | 1.000 | 0.593 | 16.5 | 13.1 | 17.4 | 12.7 |
| Potri.002G243700.1 | 0.4 | 0.1 | 0.2 | 0.5 | 0.650 | 1.000 | 1.000 | 0.035 | 60.6 | 44.5 | 55.2 | 38.5 |
| Potri.002G243500.1 | 0.5 | 0.1 | 0.3 | 0.8 | 0.294 | 1.000 | 0.831 | 0.000 | 61.0 | 42.1 | 58.4 | 33.4 |
| Potri.001G224400.1 | 0.4 | 0.4 | 0.2 | 0.2 | 1.000 | 1.000 | 1.000 | 1.000 | 7.2 | 5.6 | 5.6 | 4.9 |

**Supplementary Table S4.7 qPCR primers**

| Target gene | Gene ID | Primer sequence (5' -> 3') | |
|---|---|---|---|
| | | **Forward** | **Reverse** |
| **Amonium transporter** | Potri.T000200.1 | CAAGCATGGGGATATCACAG | GATTTCTGGATCCCCTTCTC |
| | Potri.013G049600.1 | TGGGTCCATTGTTCTACGG | AAGCCACCATGCCTTGTC |
| | Potri.001G305400.1 | GCCGTGCATGGTGAAGAG | TTGATGACTTGCGCTCCA |
| **Nitrate transporter** | Potri.014G036200.1 | AGCAGGCATCAGCACAGT | TCCCTTGGCATCGTCTTC |
| | Potri.002G129500.1 | TGGTGGATGGCTGGCTAA | CCAAGATGCACAAGCCAAG |
| **Glutamate synthase** | Potri.012G011700.1 | TCTCGAGAAACACAGGATCG | GGAGGTGCACCCCATTAC |
| **Glutamate dehydrogenase** | Potri.013G058300.1 | TTGGGAAGCCTGAGATGC | GAAACGCCTTAGGGGTAGAA |
| **Sugar transporter** | Potri.013G027800.1 | TGGAGCTCAGCAGGAACA | TGAACGCCCTTTCGTCTC |
| **Ubiquitin** | Potri.015G013600.1 | GCAGGGAAACAGTGAGGAAGG | TGGACTCACGAGGACAG |

**Supplemental Table S4.8: Summary of alignment of reads.** *Rhizophagus irregularis* reference transcripts are available at the JGI database (http://genome.jgi-psf.org/Gloin1/Gloin1.home.html) or for *Populus trichocarpa* v3 reference transcripts on Phytozome 10.3 (http://phytozome.jgi.doe.gov/pz/portal.html). Alignments were performed using CLC Genomics Workbench v7.

| Sample ID | Number of reads after quality trimming | Aligned to Rhizophagus reference | % aligned | Aligned to Populus reference | % aligned | Total % aligned |
|---|---|---|---|---|---|---|
| +Gi -N | 127001274 | 1482015 | 1.2 | 111394545 | 87.7 | **88.9** |
| +Gi -N | 132764602 | 5363844 | 4.0 | 107034161 | 80.6 | **84.7** |
| +Gi -N | 126397280 | 6419409 | 5.1 | 103987366 | 82.3 | **87.3** |
| +Gi +N | 131451898 | 2880584 | 2.2 | 104161890 | 79.2 | **81.4** |
| +Gi +N | 127008400 | 3442469 | 2.7 | 103001548 | 81.1 | **83.8** |
| +Gi +N | 146589890 | 8221418 | 5.6 | 117731654 | 80.3 | **85.9** |
| -Gi -N | 133742000 | | | 117424310 | 87.8 | **87.8** |
| -Gi -N | 123856326 | | | 107615010 | 86.9 | **86.9** |
| -Gi -N | 118362950 | | | 102105199 | 86.3 | **86.3** |
| -Gi +N | 105226466 | | | 91321893 | 86.8 | **86.8** |
| -Gi +N | 126056970 | | | 109408429 | 86.8 | **86.8** |
| -Gi +N | 126530594 | | | 111423079 | 88.1 | **88.1** |

# 5 Arbuscular mycorrhizal symbiosis under phosphate stress: expression of nutrient transporters in *Populus trichocarpa, Sorghum bicolor* and *Rhizophagus irregularis*

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**Highlight**

Analysis of key nutrient transporters in mycorrhizal symbiosis upon phosphate stress identifies differences in perennial and annual plants and new mycorrhiza-inducible transporters. Furthermore, the data suggest active fungal carbohydrate uptake.

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## 5.1 **Abstract**

In arbuscular mycorrhizal (AM) symbiosis the AM fungus helps the host plant to acquire the mineral nutrients phosphorus (P) and nitrogen (N), and in return receives essential carbohydrates from the plant. Key components of nutrient uptake and exchange are specialized transporters that facilitate nutrient transport across membranes. We performed transportome analysis on the extraradical and intraradical mycelium of the AM fungus *Rhizophagus irregularis* and its host plants *Populus trichocarpa* and *Sorghum bicolor* under high and low P availability using quantitative RT-PCR and Illumina RNA sequencing. We show that mycorrhization specifically induces expression of phosphate and ammonium transporters in both plants. Furthermore, we identify new AM-inducible transporters and show that a subset of phosphate transporters is regulated independently of symbiotic nutrient exchanges. mRNA-Sequencing revealed that many carbohydrate transporters were down-regulated in *P. trichocarpa* mycorrhizal root tissue, but also that one sugar transporter and an UDP-galactose transporter possibly localized in the Golgi lumen were induced. Metabolome analysis revealed further that AM root colonization modified root primary metabolism under low and high P availability and decreased metabolite pools in general. In *R. irregularis*, a gene coding for a carbohydrate transporter was induced in the intraradical mycelium and genes coding for phosphate transporters were regulated depending on P availability. Our data further elucidate nutrient transport during AM symbiosis in two different host plants and upon nutrient stress. Moreover, our data indicate that the plant limits carbohydrate flow from shoot to the mycorrhizal roots and the fungus rather actively extracts sugars.

## 5.2 **Introduction**

Phosphorus (P), and nitrogen (N), are among the most essential nutrients for plants. As P is involved in many key metabolic processes, it can make up to 0.2% of the dry weight of a plant (Schachtman *et al.*, 1998). In living plants, the cellular P concentration ranges between 1-10mM whereas soil concentration is about 10000-times less (Rausch and Bucher, 2002; Ai *et al.*, 2009; Branscheid *et al.*, 2010). The plants take up the freely available P as inorganic P or as orthophosphoric acid, but due to its negative charge the inorganic P is rapidly sequestered by cations and organic substances in the soil (e.g. clay) and is therefore only barely accessible to plants (Poirier and Bucher, 2002; Aung *et al.*, 2006; Chiou *et al.*, 2006; Javot *et al.*, 2007; Tatry *et al.*, 2009). To circumvent nutrient deficiency a majority of land plants form symbioses with the so called arbuscular mycorrhizal (AM) fungi. In the AM symbiosis the AM fungus provides macro and

micro nutrients to the plants an in return receives essential carbohydrates from the host plant (Smith and Read, 2008). Characteristically AM fungi are considered to be generalists with a broad host range. With their hyphal network they can colonize several plant individuals, also from different species, at the same time forming a so called common mycorrhizal network (Walder *et al.*, 2012). Within this network nutrients can be transferred whereby the contribution of single individuals varies (Walder *et al.*, 2012; Fellbaum *et al.*, 2014).

With their elaborate hyphal network, the extraradical mycelium (ERM), the AM fungi are able to extract nutrients that are out of reach or not accessible to the plant (Smith and Read, 2008). This makes the mycorrhizal uptake pathway more effective than direct uptake pathway from the locally bound plant. The nutrients taken up by the ERM are incorporated into carrier molecules and transported to the hyphal network inside the host root, the intraradical mycelium (IRM). For nutrient exchanges, the fungal mycelium forms highly branched tree-like structures (arbuscules) inside the root cortical cells, which are still surrounded by the plant cell-derived periarbuscular membrane and the inter-membrane interstice, the periarbuscular space. Mineral nutrients, which are taken up by the AM fungus from the soil are released from their carrier molecules in the arbuscules, translocated to the periarbuscular space, and taken-up by plant nutrient transporters located at the plant derived periarbuscular membrane (Smith and Smith, 2011).

The extent to which plants cover their P-demand through the AM fungus ranges from only a small percentage of P-supply to complete coverage of the plant P demand (Paszkowski, 2006; Javot *et al.*, 2007). The P taken up by the ERM is incorporated into poly-P, transported to the arbuscules, hydrolyzed and translocated to the periarbuscular space (Ezawa *et al.*, 2002; Javot *et al.*, 2007). Essential key players in this process are transporters and permeases that facilitate uptake and transport of nutrients across membranes. The expression of transporters is regulated by nutrient availability. In this way, a steady and efficient translocation of nutrients adapted to given environmental conditions can be guaranteed (Smith and Smith, 2011; Courty *et al.*, 2015).

In AM fungi, only few phosphate transporters (PT) were characterized so far: one in *Glomus mosseae* (GmosPT, Benedetto *et al.* (2005) and *Glomus versiforme* (GvPT, Harrison and van Buuren (1995), and seven in *Rhizophagus irregularis* (formerly *Glomus intraradices*, RiPT1-RiPT7; Maldonado-Mendoza *et al.* (2001), Walder *et al.,* 2015). Expression of the three high affinity transporters, RiPT1, GvPT and GmosPT, is dependent on external P concentrations in the ERM (Harrison and van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001; Benedetto *et al.*, 2005). Reduced expression of GmosPT in the intraradical mycelium (IRM) suggested a concentration-dependent regulation of PTs in the symbiotic root tissue.

In plants, the family of PTs can be divided into three subfamilies. Subfamily I transporters (Pht1) are membrane bound $H^+$/P symporter driven by an $H^+$ gradient. They are a subgroup of the major facilitator superfamily to which most of the PTs known to date belong (Pao *et al.*, 1998). Subfamily II members are located in the plastids and function as antiporters (Poirier and Bucher, 2002; Rausch and Bucher, 2002; Javot *et al.*, 2007) and members of the subfamily III are located in the mitochondrial inner membrane and are predicted to function as $H^+$/P symporters or as P/$OH^-$ antiporters (Takabatake *et al.*, 1999; Javot *et al.*, 2007). In mycorrhizal plants, some Pht1 phosphate transporters are specifically induced. The first mycorrhiza-inducible PT was identified in *Solanum tuberosum* (StPT3) and was localized in arbusculated root sections (Rausch *et al.*, 2001). Then, more mycorrhiza-inducible transporters were identified in several other plants (Harrison *et al.*, 2002; Glassop *et al.*, 2005; Nagy *et al.*, 2005; Loth-Pereda *et al.*, 2011). StPT3 and MtPT4 from *Medicago truncatula* were expressed in the periarbuscular membrane only. Furthermore, it was demonstrated that MtPT4-deficient plants accumulated P as poly-P in the arbuscules, which resulted in an advanced collapse of the arbuscules and in inefficient symbiosis (Javot *et al.*, 2007; Breuillin-Sessoms *et al.*, 2015).

For N, it was long assumed that AM symbiosis plays only a minor role in plant nutrition. In the soil, N is mostly present as nitrate or ammonium that can be transported via mass flow. Therefore, it was assumed that AM fungi take up N with the same efficiency as plants (Marschner and Dell, 1994; Hodge *et al.*, 2010; Smith and Smith, 2011). But, it was shown that AM fungi can contribute up to 42% of the plant N demand (Frey and Schüepp, 1993; Mäder *et al.*, 2000; Govindarajulu *et al.*, 2005). In addition to the uptake of ammonium and nitrate, it was shown that AM fungi can extract N from organic sources (i.e. small peptides and amino acids) (Bago *et al.*, 1996; Hawkins *et al.*, 2000; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005) and possibly also from more complex organic compounds (Leigh *et al.*, 2009; Hodge *et al.*, 2010). However, in plants as well as in AM fungi, it was shown that ammonium is the preferred N source as it can be directly incorporated into the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway whereas nitrate needs to be reduced before incorporation into the GS/GOGAT pathway (Villegas *et al.*, 1996; Hawkins *et al.*, 2000; Toussaint *et al.*, 2004). In AM symbiosis, the glutamine is further metabolized into amino acids such as arginine, alanine and asparagine for transport. Studies using metabolic labelling showed that arginine was the most common amino acid in the ERM of AM fungi that incorporated the labeled N source (Govindarajulu *et al.*, 2005). Arginine is then transported from the ERM to the IRM where it is cleaved by arginases in the arbuscules. The released ammonium is transported to the periarbuscular space where it can be taken up by the

plant ammonium transporters (AMT). So far, only six transporters have been identified in AM fungi, three in *R. irregularis* GiAMT1, GintAMT2 and GintAMT3 (López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011) Calabrese *et al.*, unpublished), and three in *Geosiphon pyriformis* (GpAmt1, GpAmt2, GpAmt3; (Ellerbeck *et al.*, 2013).

In plants, the family of AMT can be divided into two subfamilies: subfamily I and subfamily II (reviewed in Courty *et al.* (2015). While members of the subfamily I were found to be mostly expressed in roots, members of the subfamily II were preferentially expressed in shoots (Couturier *et al.*, 2007). Several mycorrhiza-inducible AMTs have been identified in several plant species (Gomez *et al.*, 2009; Guether *et al.*, 2009; Kobae *et al.*, 2010), including poplar PtAMT1;2 (Selle *et al.*, 2005; Couturier *et al.*, 2007) and sorghum SbAMT3;1 and SbAMT4 (Koegel *et al.*, 2013).

In return for the mineral nutrient the AM fungi receives carbohydrates from the plants. However, researches on sugar transporter (SUT) expression in plants are not consistent. Mycorrhization caused either increased or decreased expression of SUTs in root and shoots of the host plants (Ge *et al.*, 2008; Boldt *et al.*, 2011; Doidy *et al.*, 2012). Recently, a new class of SUT, the SWEETs, was identified. These transporters were located in the plasma membrane and shown to function as bidirectional sugar uniporters (Chen *et al.*, 2010). Due to their involvement in rhizobial symbiosis it is assumed that they also play a role in other biotrophic plant symbioses such as the AM symbiosis (Gamas *et al.*, 1996; Doidy *et al.*, 2012).

In the AM fungus *R. irregularis* four carbohydrate transporters have been identified (Helber *et al.*, 2011). The monosaccharide transporter RiMST2 was the most highly expressed transporter in symbiotic tissue that could be localized at the arbuscular side but also in the IRM.

Despite accumulating knowledge about transporter expression and activity in AM symbiosis, we still lack precise understanding about the behavior of symbiosis under suboptimal environmental conditions. However, a comprehensive view of symbiosis under environmental and nutritional stress is important in times of climate change and resource shortening. Therefore, in this study, we analyzed the effects of mycorrhization and contrasting P nutrition on the transporter expression and metabolite accumulation in *Populus trichocarpa* (poplar) *and Sorghum bicolor* (sorghum) when colonized by the AM fungus *R. irregularis.* Our main focus was on the regulatory function of the mycorrhization and P concentration on the expression of the Pht1 PTs in the plant and in the AM fungus. Further we assessed the effect of the applied conditions on AMTs and carbohydrate transporters. In the AM fungus *R. irregularis* we determined expression values of PTs, AMTs and monosaccharide transporters (MST) in the ERM and in the IRM of colonized *P. trichocarpa* and *S.*

*bicolor* roots. We identified new specific mycorrhiza-inducible PTs and AMTs in poplar and sorghum. Moreover, our data allowed us to gain further insight into symbiotic carbon exchange.

## 5.3 Material and Methods

### 5.3.1 Experimental set-up

Experiments were performed with *P. trichocarpa* cuttings (clone 10174, Orléans, France) and *S. bicolor* (L.) Moench, cv Pant-5. Sorghum seeds were kindly provided by I.G.F.R.I. (CSS Agriculture University of Hissar, Haryana, India) and G.B. Pant University of Agriculture and Technology (Pantanagar, Uttaranchal, India). Seeds were surface-sterilized in 2.5% KClO for 10 min, rinsed several times with sterile deionized water and soaked o/n in sterile deionized water. Seeds were germinated in the dark at 25°C for three days. Mycorrhizal plants were fertilized with 1 ml liquid inoculum of *R. irregularis*, isolate BEG75 (Inoculum Plus, Dijon, France), in 0.01 M citrate buffer (pH 6) with about 110 spores/ml. The microcosms were set-up in tripartite compartments (mycorrhizal treatment) or single compartments (non-mycorrhizal treatments). Compartments were filled with an autoclaved (120°C, 20 min) quarz sand (Alsace, Kaltenhouse, Trafor AG, Basel): zeolithe (Symbion, Czech Republic) substrate (1:1, w:w). In the tripartite compartment system poplar cuttings were planted in the middle compartment and sorghum seedlings in the right compartment. Both plants were inoculated with *R. irregularis* to create a common mycorrhizal network and to increase poplar root colonization (Supplementary Fig. S5.1). Compartments were separated by two 21 µM meshes and one 3 mm mesh, to allow the AM fungus to grow from one compartment to the other but to avoid plant roots protruding the neighboring compartment. As control, non-inoculated poplar and sorghum plantlets grew in single compartments receiving the P containing fertilizer treatments directly to their roots. Plants were fertilized once a week with 10 ml Hoagland solution without P, until all plants showed signs of P depletion, indicated by anthocyan accumulation. From the 22$^{nd}$ week high-P (560 µM) or low-P (28 µM) containing Hoagland solution was applied to the first compartment for 9 weeks, to obtain ERM and to ensure that P was delivered via the mycorrhizal uptake pathway. Control plants received fertilizer treatment directly to their root systems.

### 5.3.2 Harvest

The ERM was extracted by aggrading the substrate with tap water and fishing it from the surface using a 32 µM mesh. These steps were repeated several times. Afterwards the cleaned ERM samples were snap frozen in liquid nitrogen and stored at -80°C.

For RNA extractions, two leaves from the top of Poplar plants and two young leaves of Sorghum plants were snap frozen in liquid nitrogen and stored at -80°C. The rest of the shoots was harvested and dried in an oven at 55°C for 4 days for total P measurement.

Roots were removed from substrate under tap water and cut into ~1 cm small pieces. Two subsamples of about 100mg were immediately frozen in liquid nitrogen and stored at -80°C. One subsample of about 100mg was taken for root colonization measurements. Remaining roots were placed in a paper bag and dried at 55°C for 3.5 days for determination of total P content.

### 5.3.3 Colonization measurements and P extraction

Roots were immersed in 10% KOH and stored at 4°C o/n. The next day, the roots were rinsed with tap water and immersed in 2% HCl for 1 hour at room temperature. Then, the roots were rinsed with tap water, immersed in 0.005% trypan blue (w:v in lactic-acid: glycerol: water, 1:1:1, v:v:v) and stored at 4°C o/n. The next day, the roots were rinsed and immersed in lactic-acid glycerol water (1:1:1, v:v:v) for destaining. Total colonization count was performed using the grid line intersection method as described by (Brundrett *et al.*, 1984). Differences between means of variables were assessed by t-test ($P \leq 0.5$), using Microsoft Excel 2010.

For determination of P concentration within in the plants, dried root and shoot samples of six biological replicates were ground using a ball mill. Up to 500mg were used for the modified Pi extraction method by Murphy and Riley (1962).

### 5.3.4 RNA extraction

Total RNA was extracted from six biological replicates per plant species and mycelium, respectively. Total RNA was extracted from lyophilized extraradical mycelia, root and leaf samples using the RNeasy Plant Mini Kit (Qiagen, Courtaboeuf, France). RNA extracts were DNase treated with the DNA-free™ Kit, DNase Treatment and Removal Reagents (AMBION® by life technologies). Total RNA was quantified with the Qbit RNA BR Assay kit and purity was estimated using the Nanodrop (ND-1000, Witec, Switzerland).

### 5.3.5 Reverse transcription and qRT-PCR

cDNAs from three biological replicates were obtained using the iScript™cDNA Synthesis Kit (BIO RAD Laboratories, Paolo Alto, CA, United States), using 200ng of total RNA per reaction. For quantification a two-step quantitative RT-PCR approach was used. Gene specific primers were designed in Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and tested as well in amplify 3.1 (http://engels.genetics.wisc.edu/amplify). Target gene expressions were

normalized to the expression of the reference gene ubiquitin in Poplar (Potri.015G013600) and Sorghum (Sb10g026870) and translation elongation factor in *R. irregularis,* respectively. All primers used are listed in Supplementary Table S5.1. qRT-PCRs were run in a 7500 real-time PCR system (Roche) using the following settings: 95°C for 3 min and then 40 cycles of 95°C for 30 s, 60°C for 1 min and 72°C for 30 s. The number of replicates comprised three biological and three technical replicates per treatment. Differences in gene expression between applied conditions were tested by a one-way ANOVA using SPSS Statistics, version 22 (IBM, Chicago, USA).

### 5.3.6 RNA sequencing and data analysis

Total RNA sequencing was done for three biological replicates per condition. Eighteen libraries were prepared and paired-end Illumina HiSeq mRNA sequencing (2x100bp RNA-Seq) was performed by Beckman Coulter Genomics France (Grenoble, France), which producing around 2x80 million reads per library in average. After quality check using FastQC, adaptor sequences were removed using FASTX-Toolkit. Only inserts of at least 30-nt were conserved for further analysis. Reads were mapped with TopHat v2.0.11 (Kim *et al.*, 2013) and bowtie2 v2.2.1 (Langmead *et al.*, 2009) on *P. trichocarpa* genome and gene annotation Ptrichocarpa_210_v3.0. Default parameters were applied but for the maximum intron length which was set to 4kb. Then, cuffquant and cuffnorm functions from Cufflinks v2.2.0 (Trapnell *et al.*, 2012) were applied to estimate reads abundance over annotations and generate tables of normalized expression. Differential analysis comparison between samples was done with DEseq2 v1.2.10 (Love *et al.*, 2014) taking into account the 3 replicates per samples.

### 5.3.7 Metabolite profiling and data analysis

For extraction of soluble metabolites about 90 mg of deep frozen poplar root and ERM samples (three biological replicates per condition) were pulverized in liquid nitrogen. Metabolite profiling was performed as described in (Dethloff *et al.*, 2014) by gas chromatography coupled to electron impact ionization/time-of-flight mass spectrometry (GC-EI/TOF-MS) using an Agilent 6890N24 gas chromatograph (Agilent Technologies, Böblingen, Germany; http://www.agilent.com). Guidelines for manually supervised metabolite identification were the presence of at least 3 specific mass fragments per compound and a retention index deviation < 1.0% (Strehmel *et al.*, 2008). For quantification purposes all mass features were evaluated for best specific, selective and quantitative representation of observed analytes. Laboratory and reagent contaminations were evaluated and removed according to non-sample control experiments. Metabolites were routinely assessed by log2-transformed relative changes expressed as response ratios (Supplemental Table

S5-S6). Statistical testing, namely 2-way analysis of variance (ANOVA) and Wilcoxon-Mann-Whitney testing of significance were performed using relative abundances or log2-transformed ratios. Statistical assessments and data visualizations were performed using the multi-experiment viewer software, MeV (Version 4.9; http://www.tm4.org/mev.html; (Saeed *et al.*, 2006)) and the Microsoft-Excel 2010 program.

## 5.4 **Results**

### 5.4.1 Colonization and P measurements

AM colonization of poplar roots was between 79% and 87% and of about 93% in sorghum roots (**Supplementary Table S5.**2). Non-AM plants were not colonized. The hyphal colonization and the percentage of vesicles were not significantly different between low-P and high-P treatments in sorghum and poplar plants. However, sorghum roots contained significantly three times more arbuscules in the low-P treatment, indicating that P starvation supported mycorrhization.

P treatment had significant effects on P content in the shoots and roots of poplar and sorghum (Supplementary Fig. S5.2). High P treatment increased P content in roots and shoot. Non-AM poplar accumulated more P than AM poplar whereas in AM sorghum, P accumulation was comparable to non-AM plants.

### 5.4.2 Regulation of phosphate transporter expression

#### Gene expression of plant Pht1 transporters

Using qRT-PCR, we measured the expression of the twelve Pht1 phosphate transporters in roots and shoots of AM and non-AM poplar plants grown in high-P and low-P conditions. PtPT8 and PtPT10 were induced in AM-roots only, suggesting an important role of these two transporters in symbiotic P uptake at the periarbuscular space (Fig. 5.1).

Low-P treatment and mycorrhization induced expression of PtPT1.1, PtPT1.2, PtPT1.4 and PtPT1.11 in roots (Fig. 5.1B). PtPT1.2, PtPT1.4 and PtPT1.11 were strongly induced in shoots, suggesting that these transporters are involved in intercellular P transfer and transport of P over long distances, respectively (Fig. 5.1A). PtPT1.6 was neither expressed in roots nor in shoots. mRNA-Seq analysis independently confirmed our results for Pht1 expression (Supplementary Fig. S5.3). Comparing qRT-PCR data with mRNA-Seq data we observed also comparable fold changes for the tested genes (Supplementary Table S5.4).

In sorghum, SbPT1.8 and SbPT1.10 were induced in AM roots (Fig. 5.2). However, SbPT1.8 was only marginally expressed in the non-AM low-P treatment, showing the same expression pattern as SbPT1.11. SbPT1.1, SbPT1.4, SbPT1.6 and SbPT1.7 were significantly induced in the non-AM low-P treatment. It seems that mycorrhization complemented sufficiently the P deficiency by increased P transfer to its host plant (Fig. 5.2). In comparison to poplar Pht1, sorghum Pht1 were more susceptible to mycorrhization than to P concentration.

**Gene expression of mycorrhizal phosphate transporters (PT)**

qRT-PCR analysis of PT in the AM fungus *R. irregularis* in the ERM and IRM revealed expression of RiPT1, RiPT3, RiPT5 and RiPT7, with highest expression values for RiPT1 (Fig. 5.3A). RiPT1 was induced in low-P treatment and significantly more expressed in the IRM. RiPT7 tended to be highly expressed in the IRM compared to the ERM and RiPT3 was lowly expressed in the ERM and induced in high-P treatment in the IRM. In Sorghum, we observed similar expression patterns except for RiPT3 and RiPT7, significantly induced in the IRM than in the ERM (Supplementary Fig. S5.4).

### 5.4.3 Nitrogen exchange

**Expression of plant AMTs**

As nitrogen is a major component of AM symbiosis, we measured the expression of three selected AMTs in poplar by qRT-PCR. While PtAMT1.1 and PtAMT1.2 were described as induced in poplar upon mycorrhization with the ectomycorrhizal fungi *Paxillus involutus* (Couturier *et al.*, 2007) and *Amanita muscaria* (Selle *et al.*, 2005), no previous expression data were available for PtAMT3.1. Interestingly, PtAMT3.1 clustered next to the three AM-inducible transporters GmAMT3.1 (Kobae *et al.*, 2010), SbAMT3.1 (Koegel *et al.*, 2013) and OsAMT3.1 (Pérez-Tienda *et al.*, 2014) (Supplementary Fig. S5.5). Here, PtAMT1.1 was induced in the AM low-P treatment and the PtAMT1.2 and PtAMT3.1 were specifically induced in the AM treatments (Fig. 5.4B). In addition, PtAMT1.2 and PtAMT3.1 were even higher expressed in the high P condition, suggesting that both AMTs play a major role in symbiotic nitrogen transfer. Higher expression of these transporters under AM high-P condition might point to an increased nitrogen transfer when the AM fungus has access to more P. In shoots, we PtAMT1.1 has a similar expression pattern as in roots. PtAMT3.1 was only marginally expressed and PtAMT1.2 was not expressed in leaves (Fig. 5.4A). mRNA-Seq confirmed our observations for AMT expression levels in the roots of Poplar (Supplementary Fig. S5.6, Supplementary Table S5.4). In a previous transcriptome study (Calabrese *et al.,* unpublished),

in which AM poplar plants were exposed to nitrogen deficiency, PtAMT4.1, PtAMT4.2 and PtAMT4.3 were AM-induced. Consistent with this previous study, we observed a specific induction of PtAMT2.2, PtAMT4.1, PtAMT4.2 and PtAMT4.3 upon mycorrhization even though these transporters were expressed at lower levels compared to PtAMT1.2 and PtAMT3.1.

In Sorghum, SbAMT3.1 was specifically induced in AM-roots, and SbAMT1.1 and SbAMT1.2 were induced in the non-AM low-P treatment (Fig. 5.5). However, SbAMT1.1 and SbAMT1.2 were nearly twice more expressed in shoots compared to roots. In roots, they were induced in AM low P treatment.

**AMTs in *R. irregularis***

Quantitative expression analysis of the three AMTs in the AM fungus revealed that the expression of GintAMT3 was significantly higher in the IRM compared to the ERM (Calabrese *et al.*, unpublished). GintAMT2 and GintAMT1 were equally expressed in the ERM and IRM in poplar and sorghum (Supplementary Table S5.3). Specific induction of GintAMT3 in the IRM might indicate a possible localization of the transporter at the arbuscular side for the transfer of ammonium to the periarbuscular side to enable ammonium uptake for the plant.

**Amino acid transporters**

By searching the transcriptome dataset for poplar transporters possibly involved in symbiotic N transfer, we identified several amino acid transporters and H+/oligopeptide symporter that were either specifically induced or repressed upon mycorrhization. (Figure 5.6). Here, root colonization highly induced expression of two $H^+$/oligopeptide transporters. AtPTR1, a PTR in *Arabidopsis* and homologue to the highly induced Potri.005G233500, was shown to transport di-/tripeptides with low selectivity. AtPTR1 is situated in the plasma membrane of vascular tissue which indicates a role in long-distance transport (Dietrich *et al.*, 2004). AtPTR3, a homologue of Potri.002G258900, was induced upon salt stress and was shown to be regulated by methyl jasmonate, salicylic acid and abscisic acid. Further AtPTR3 was induced upon inoculation of the plant with pathogens. A reduced activation of AtPTR3 in *hrpA* mutant indicated that it is a defense related gene protecting the plant against abiotic and biotic stress (Karim *et al.*, 2005; Karim *et al.*, 2006). The differential expression of these transporters upon mycorrhization may suggest a role of the transporters in N uptake but also a role in AM root colonization.

### 5.4.4 Carbon exchange

Quantitative expression analysis using qRT-PCR of five sugar transporters (SUT1 and SUT3 to SUT6) in poplar revealed that all five transporters were expressed (Fig. 5.7, ). SUT1 was only marginally expressed while SUT4 was strongly expressed in roots and shoots. While SUT1 and SUT4 were not differentially expressed upon mycorrhization, surprisingly, SUT3 was down-regulated upon mycorrhization in roots and shoots of poplar. Interestingly, SUT6 was down-regulated in shoots by mycorrhization. In sorghum, SUT1 was also down-regulated, (Fig. 5.8). In addition, we screened our transcriptome data set for other carbohydrate transporters. We found three carbohydrate transporters induced and four repressed upon mycorrhization (Figure 5.9). For symbiotic carbon transfer a carbohydrate transporter needs to be localized in the plasma membrane, which leaves only the transporter/ spinster transmembrane protein (Potri.001G286600). The UDP-galactose transporter related proteins are localized at the lumen of the Golgi cisternae (Norambuena et al., 2002). On the fungal side, the monosaccharide transporter GintMST2 was specifically induced in the IRM of mycorrhizal poplar and sorghum (Fig. 5.3). Induction of the two predicted carbohydrate transporters suggested a role in symbiotic carbohydrate transfer but functional properties still need to be determined. Downregulation the six carbohydrate transporters and the fact that the AM GintMST2 is also expressed in the IRM indicate that the AM fungus extracts the sugar from the intercellular space without the cooperation of the plant itself, turning the AM root into a sink for sugars.

### *5.4.5* Primary metabolism of poplar roots and the ERM of *R. irregularis*

To gain further insights into the changes of root primary metabolism caused by the interaction between poplar and *R. irregularis* we conducted exemplary GC-MS metabolite profiling experiments. Under our experimental conditions the ERM of *R. irregularis* was not significantly affected by P conditions (Table 5.1, Table A1 in Appendix). In the ERM we observed slight but mostly non-significant increases of organic acids, glucose, trehalose, glycine and of amino acids with branched aliphatic side chains, with leucine as the only significant increase in our experiment (Table 5.1, Table A1 in Appendix).

In contrast, non-AM low-P treatment on poplar roots increased general organic acid and amino acid pools and coincidently decreased pools of glucose-6-phosphate or fructose-6-phosphate (Table 5.2, Table A2 in Appendix). Analysis of variance indicated significant general accumulation of only few metabolites, i.e., 4-amino-butanoic acid (GABA), isoleucine, phenylalanine, serine, threonic acid, ribonic acid and arabinonic acid-1,4-lactone independently of the mycorrhizal status

(Table 5.2, Table A2 in Appendix). AM colonization ameliorated nutrient acquisition in the low-P treatment (Table 5.2, Table A2 in Appendix). In addition, mycorrhization modified the root primary metabolism under low-P and high-P and exclusively decreased metabolite pools. Thirty-eight out of 79 monitored primary metabolite pools were decreased with only one exception besides few still non-identified metabolites, namely trehalose (Table 5.2, Table A2 in Appendix). trehalose is a major storage carbohydrate of AM fungi (Bécard *et al.*, 1991). Mycorrhization not only decreased the main organic acids of the TCA cycle, e.g., malic acid, aconitic acid, 2-oxo-glutaric acid, succinic acid and fumaric acid but also many amino acids including aspartic and glutamic acid, as well as phenylalanine, glycine, serine, leucine, isoleucine and valine. In addition, mycorrhization decreased the glucose-6-phosphate, fructose-6-phosphate, *myo*-inositol and galactinol pools and additional carbohydrates including maltose.

## 5.5 **Discussion**

In this study, we described the effects of P nutrition and AM colonization on phosphate transporters, AMTs and carbohydrate transporters in poplar and sorghum, and in the ERM and IRM of the AM fungus *R. irregularis.*

### **5.5.1 Symbiotic phosphorous exchange**

In our study, we confirmed the expression of, PtPT1.10 in Poplar AM roots only (Fig. 5.1) as already reported by (Loth-Pereda *et al.*, 2011). In addition, PtPT1.8 was also expressed in AM roots only. In sorghum roots, AM colonisation induced the specific expression of SbPT1.8, SbPT1.10 and partially of SbPT1.11 (Fig. 5.2). The specific induction of PtPT1.8 and PtPT1.10 in poplar and of SbPT1.8, SbPT1.10 in sorghum upon mycorrhization strongly suggested that there is a symbiosis-dependent P uptake system. In *M. truncatula*, it has been shown that MtPT4 is specifically expressed in the periarbuscular membrane (Harrison *et al.*, 2002). The specific induction of PtPT1.8 and PtPT1.10 in poplar and of SbPT1.8, SbPT1.10 in sorghum suggested that these transporters are localized at the periarbuscular membrane (Fig. 5.10).

On the fungal side, the PTs of *R. irregularis* were expressed in the ERM and in the IRM of poplar and sorghum roots. The high affinity transporter RiPT1 was previously found to be regulated in the ERM by external P concentration (Maldonado-Mendoza *et al.*, 2001) and expressed at the arbuscular side (Fiorilli *et al.*, 2013). Here, RiPT1 was up-regulated by external low-P concentrations in the ERM but also in the IRM. High expression of RiPT1 suggests that RiPT1 is the main transporter for P uptake and symbiotic P transfer in our conditions. Maldonado-Mendoza *et*

*al.* (2001) predicted the existence of other PTs operating at high external P concentrations. Accordingly, we could show that RiPT3 and RiPT7 were expressed but not affected by the nutrient conditions in the ERM. It might be that higher P concentrations are needed to increase expression of these possible high affinity transporters. But, in the IRM RiPT3 and RiPT7 were clearly induced in sorghum and had a tendency in poplar, suggesting that they participate also in symbiotic P transfer acting as export carrier.

### 5.5.2 P-dependent regulation of PT expression

Low-P conditions induced expression of PtPT1.1, PtPT1.2, PtPT1.4 and PtPT1.11 in poplar roots, showing that expression and regulation of PTs is dependent on P availability (Fig. 5.1). In the mycorrhizal microcosm, the only P source was the AM fungal symbiont and the absence of a direct P source further increased expression of these four transporters. As we still observed, a P-dependent regulation of transporter expression, our data suggest that these PTs are regulated by external and internal P-concentrations and are regulated independently by the mycorrhizal pathway. Increased expression of PTs in low-P condition further suggests that the AM fungus supplies the host plant with more P if the fungus itself has increased access to P. By regulating these PTs independently poplar ensures a P nutrition uncoupled from the AM symbiont. Symbiosis-independent P-nutrition is necessary for perennial plants as mycorrhizal abundance varies in nature with the seasons (Courty *et al.*, 2008; Dumbrell *et al.*, 2011).

The fact that PtPT1.2, PtPT1.4 and PtPT1.11PtPT expression was also induced upon P-limiting conditions in the shoots suggests that they function in P uptake at the root-soil interface as well as in intercellular distribution and translocation of P from root to shoot. PtPT1.9 was mainly expressed in the shoot, which suggests that it is mainly responsible for P allocation in the shoots.

Sorghum on the other hand turned out to be more mycorrhiza-susceptible than poplar. Under mycorrhization PTs were equally low expressed as under a non-mycorrhizal high-P condition which shows that the AM fungus was able to cover the P needs of sorghum. Induction of PT in the shoots in the non-mycorrhizal low-P condition further showed that the plant suffered of P deficiency and therefore probably reallocated P from old leaves (source) to young leaves (sink). The stronger dependency of sorghum to the AM fungus was also indicated by P-accumulation in mycorrhizal and non-mycorrhizal sorghum (Supplementary Fig. S5.2).

### 5.5.3 Symbiotic nitrogen exchange

As symbiotic N transfer is also an important aspect of AM symbiosis we analyzed the effects of mycorrhization and P availability on plant and AM fungal AMT expression. In poplar

mycorrhization induced expression of three AMTs. Our results are supported by previous studies which showed that PtAMT1.1 and PtAMT1.2 were mycorrhiza-inducible when poplar was mycorrhizal with the ectomycorrhizal fungi *Paxillus involutes* (Couturier *et al.*, 2007) and *Amanita muscaria* (Selle *et al.*, 2005) (Fig. 5.4). In addition, we found that AMT3.1 is as well a mycorrhiza-inducible transporter in the roots which is in contrast to the data of Couturier *et al.* (2007) who detected PtAMT3;1 solely in senescing leaves. Further, increased expression of PtAMT1;2 and PtAMT3;1 suggests an increased ammonium transfer when the fungal needs of P are accomplished. Analysis of the transcriptome dataset revealed that PtAMT4;1, PtAMT4;2 and PtAMT4;3 were also induced upon mycorrhization independently from the P supply of the fungus as it was the case in our previous study where mycorrhizal poplar was set under N stress (Calabrese *et al.*, unpublished).

As there is an ongoing debate on whether amino acids as an organic N source can be taken up by AM fungi and transferred from the fungus to the plant (reviewed in Hodge and Storer (2015)) we screened the transcriptome data of poplar and identified several amino acid transporters and $H^+$/oligopeptide symporters that were either induced or repressed upon mycorrhization (Figure 5.6). Specific induction of amino acid transporters and one of the $H^+$/oligopeptide transporters indicate that amino acids are transferred from the AM fungus to the plant as an alternative N source. However, our metabolome analysis on mycorrhizal and non-mycorrhizal poplar roots showed that mycorrhization reduced the abundance of most metabolites including amino acids in the colonized roots tissue (Table 5.2, Table A2 in Appendix), suggesting high rates of metabolic turnover by the fungus or the host roots or, alternatively, transport to the shoots. Interestingly, an accumulation of relevant metabolites might be detectable in the shoots as it was demonstrated by Whiteside *et al.* (2012) for the amino acids phenylalanine, lysine, asparagine, arginine, histidine, cysteine, methionine and tryptophan with quantum dot analysis.

In the well-established mycorrhizal symbiosis described here, GintAMT3 was significantly induced in the IRM mycelium in both hosts, poplar and in sorghum (Supplementary Table S5.3), indicating a major participation of this transporter in the transfer of ammonium from the arbuscule to the periarbuscular space where it is accessible to the plant (Calabrese *et al.*, unpublished). High GinAMT2 expression levels independent of P-supply and localization in the ERM and IRM indicate that it is a low affinity transporter for ammonium. Moreover, GinAMT2 displays high sequence similarity to GintAMT3 which is a low affinity transporter (Calabrese *et al.*, unpublished). GintAMT1 on the other hand, a high affinity transporter, was expressed at low levels in the ERM and IRM independent of the P availability. Together, these findings indicate that GintAMT1 and

GintAMT2 are involved in the uptake and distribution of ammonium from the ERM to the IRM and that GintAMT3 is mainly involved in the transfer of ammonium to the host plant.

In sorghum only SbAMT3.1 was induced in mycorrhizal roots (Fig. 5.5). In contrast to Koegel *et al.* (2013) SbAMT4 was not expressed in our experimental conditions. Induced expression of SbAMT1;1 and SbAMT1;2 in the low-P condition suggests that upon sensing of nutrient stress the plant activates a general nutrient uptake program to avoid running short on one or more essential nutrients. In addition our data show that regulation of sorghum AMTs is less mycorrhiza-dependent than nutrient-dependent. Under mycorrhization the plant is supplied with sufficient P and N to keep the P and N level constant within the plant. This scenario may explain the unchanged expression of the SbAMTs. Interestingly, mycorrhization induced expression of SbAMT1.1 and SbAMT1.2 in the shoots in low-P and of AMT3.3 in high-P conditions. An increased translocation rate of ammonium from root to shoot upon mycorrhization may be a possible explanation. Moreover, SbAMT1;1 and SbAMT1;2 expression levels have a tendency to be also concentration dependent. Strong induction of SbAMT3.3 in the shoots indicated an increased ammonium flux during high P and mycorrhizal condition.

### 5.5.4 Symbiotic carbon exchange

In order to shed some light on symbiotic carbon exchange we used qRT-PCR and mRNA-Seq analysis to identify possible transporters that enable carbohydrate transport from the plant to the fungus. Interestingly, quantitative RT-PCR expression analysis and mRNA-Seq analysis revealed downregulation of carbohydrate transporters in poplar (Fig. 5.7, Figure 5.9) and sorghum (Supplementary Fig. S5.7), which might indicate that carbohydrates are actively sequestered by the fungus. But we also identified two carbohydrate transporters induced upon mycorrhization (Figure 5.9). The UDP-galactose transporters are intracellular transporters situated in the cisternae of the Golgi lumen where UDP-galactose is used for synthesis of non-cellulosic polysaccharides and glycoproteins (Norambuena et al., 2002). Only recently, it was shown that they also transport rhamnose (Rautengarten et al., 2014). In the Golgi-network and the early endosome the newly synthesized proteins are sorted either for the secretion to the plasma membrane, the extracellular matrix or for degradation (Feraru *et al.*, 2012; Brandizzi and Barlowe, 2013; McFarlane *et al.*, 2014). Induced expression of UDP-galactose therefore indicates an increased transport activity of proteins to colonized cells which goes along with increased plasma membrane synthesis. In *M. truncatula* it has been shown that arbuscule development goes along with the synthesis of the periarbuscular membrane which is distinct from the plasma membrane (Pumplin and Harrison,

2009). Further is has been shown that AM-induced transporters are specifically directed to the periarbuscular membrane and an involvement of the trans-Golgi for PT has been implicated (Pumplin et al., 2012). The other transporter induced upon mycorrhization is a sugar transporter/spinster transmembrane protein, a member of the major facilitator proteins which makes it a candidate for being localized at the cell membrane eventually stimulating carbohydrate transport to the AM symbiont.

Interesting is also that a Glucose-6-phosphate/phosphate and phosphoenolpyruvate/ phosphate antiporter was induced (Figure 5.9). Glucose-6 phosphate/phosphate antiporters are normally expressed in non-green plastids and serves as carbohydrate importer. In amyloplasts glucose-6 phosphate/phosphate antiporters are located at the site of starch synthesis (Kammerer *et al.*, 1998). Increased activity of this transporter suggests a decrease of freely available sugars.

In the AM fungi *Geosiphon pyriformis* and *Rhizophagus irregularis* several carbohydrate transporters were identified. For GpMST1 and RiMST2, it was shown that they specifically transport monosaccharides. RiMST2 was found to be expressed at the arbuscular site and in the intraradical hyphae (Schüßler *et al.*, 2006; Helber *et al.*, 2011). Helber *et al.* (2011) proposed a model in which the absorbed sugars might derive from cell wall degradation but this is contradictory to previous observations in which carbohydrate transfer varied with the source strength of the host plant (Olsson *et al.*, 2010; Fellbaum *et al.*, 2014; Zhang *et al.*, 2015). We demonstrated that RiMST2 was induced in the IRM of its host plants poplar (Fig. 5.3) and sorghum (Supplementary Fig. S5.4) and that its expression level remained unchanged by P availability. The metabolome analysis revealed further that mycorrhization significantly decreased the abundance of monosaccharides (i.e. glucose, rhamnose and ribose) in AM poplar roots. In AM fungi it has been shown that hexoses received from the host plants are transformed to glycogen, trehalose and lipids and preferentially accumulate carbon in form of triacylglycerols (Shachar-Hill *et al.*, 1995; Pfeffer *et al.*, 1999; Bago *et al.*, 2003). Our observations are consistent with these findings that monosaccharides are taken up mainly by RiMST2 from the apoplast, converted into disaccharides (i.e. trehalose) for transport and hydrolyzed in the ERM into monosaccharides, ready for direct usage or for storage reasons in from of triacylglycerols and lipids. The mycorrhization-dependent downregulation of carbohydrate transporters and monosaccharide abundance in poplar roots, along with reduced RiMST2 expression at the arbuscular site and in the IRM suggests that the fungus actively sequesters the sugars on its own demand. As sugars are also transported via the apoplastic pathway the plat has only limited possibilities to restrict carbohydrate flux towards the fungal symbiont.

Taken together our data suggest that the plant strictly regulates carbon allocation on mycorrhizal roots. With the Sugar transporter/spinster transmembrane protein a possible candidate for symbiotic carbon transfer is found. However, it is also possible that the fungus extracts sugars from the plant.

## 5.6 Conclusion

Here we demonstrated that mycorrhization leads to specific induction of a variety of transporters. As P and N are key elements of mycorrhizal symbiosis we tested the expression of PT and AMT and show that mycorrhization specifically induces expression of a selection of PT and AMTs in poplar and in sorghum. Further, we identified one carbohydrate transporter specifically induced in mycorrhizal root tissue that might be a possible candidate for symbiotic carbon exchange. By contrast, other carbohydrate transporters were downregulated upon mycorrhization indicating that the plant may not volunteer provision of carbohydrates in exchange for mineral nutrients. We further showed that some nutrient transporters are stronger expressed in the IRM compared to the ERM, which indicates that they are directly involved in nutrient transfer at the arbuscular membrane or, as in the case of MST, in nutrient uptake in the intraradical hyphae.
In agriculture, vast amounts of mineral fertilizers are applied to the field to increase crop yield. Indeed, the amount of applied fertilizer might actually exceed the plant needs for mineral nutrients. With our data we were able to show that the perennial poplar plant is less mycorrhiza-dependent than the annual sorghum. Collecting data such as ours will help to further deepen our understanding of the plant-AM symbiosis and may lead to development of a more sustainable agriculture with a reduced fertilizer input and improved adaptation to changing environmental conditions.

## 5.7 Acknowledgement

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## 5.8 **Figures**

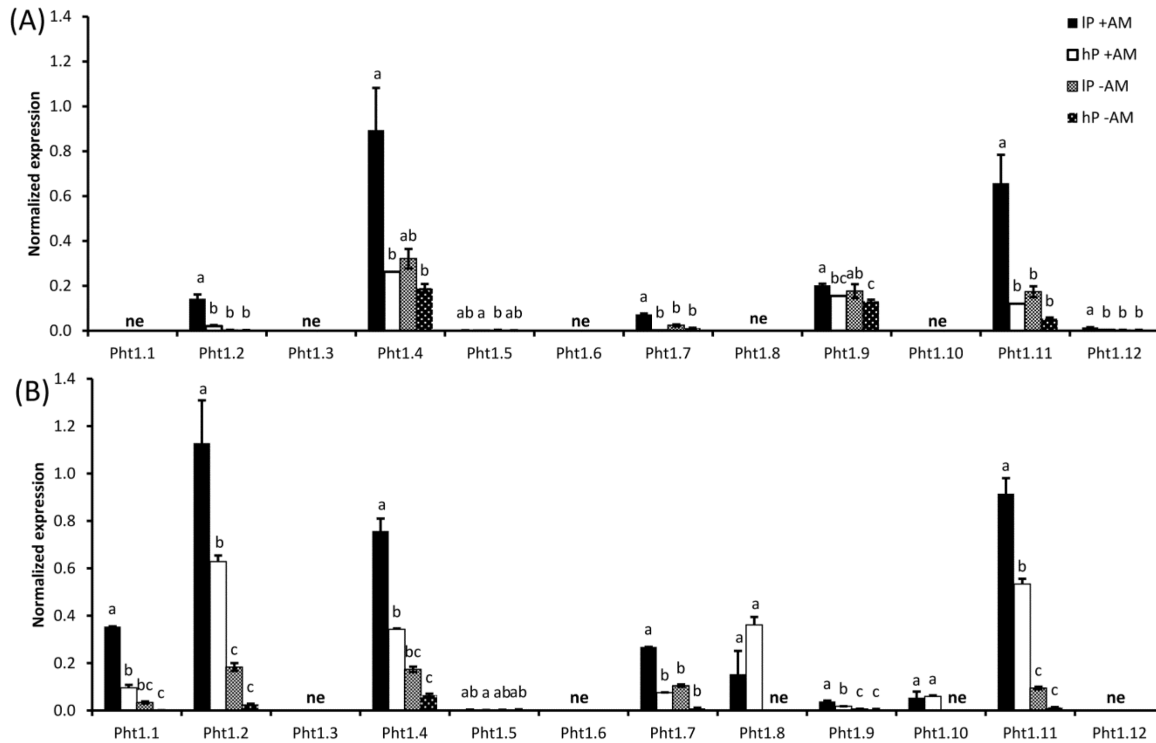



**Fig. 5.1. Quantification by qRT-PCR of the transcript abundances of Pht1 genes in *P. trichocarpa.*** Quantification of transcript levels of the 12 Pht1 transporter genes in the shoots (A) and roots (B) in *P. trichocarpa* when mycorrhized (+AM) or not mycorrhized (-AM) with *R. irregularis* under low-P (lP) and high-P (hP) conditions. Transporter Pht1.3, Pht1.6 and Pht1.12 were not expressed; Pht1.8 and Pht1.10 only when the plant was mycorrhized and Pht1.1 was root specific. Values are the means of three biological and three technical replicates. Error bars represent the SE. Ubiquitin was used as reference transcript. Statistical analysis was performed by analysis of variance (ANOVA) for each gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$). Lower case letters indicate statistical differences.

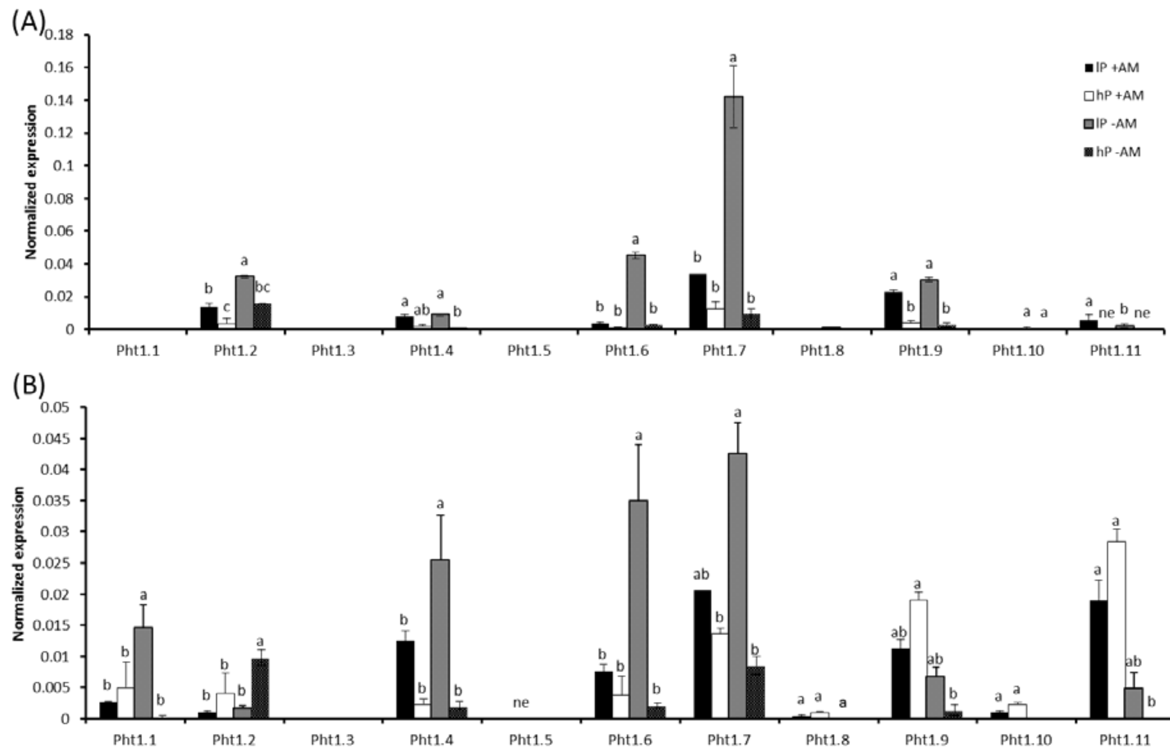



**Fig. 5.2. Quantification by qRT-PCR of the transcript abundances of Pht1 phosphate transporter genes in *S. bicolor*.** Quantification of transcript levels of the 11 Pht1 transporter genes in the shoots (A) and roots (B) of *S. bicolor* when mycorrhized (+AM) or not (-AM) by the AM fungus *R.irregularis* in low-P (lP) and high-P (hP) conditions. Transporter Pht1.3 and Pht1.5 were not expressed; Pht1.10 only in roots when mycorrhized and only in shoots when plant was not mycorrhized and Pht1.1 was root specific. Values are the means of three biological and three technical replicates. Error bars represent the SE. Ubiquitin was used as reference transcript. Statistical analysis was performed by analysis of variance (ANOVA) for each gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$).

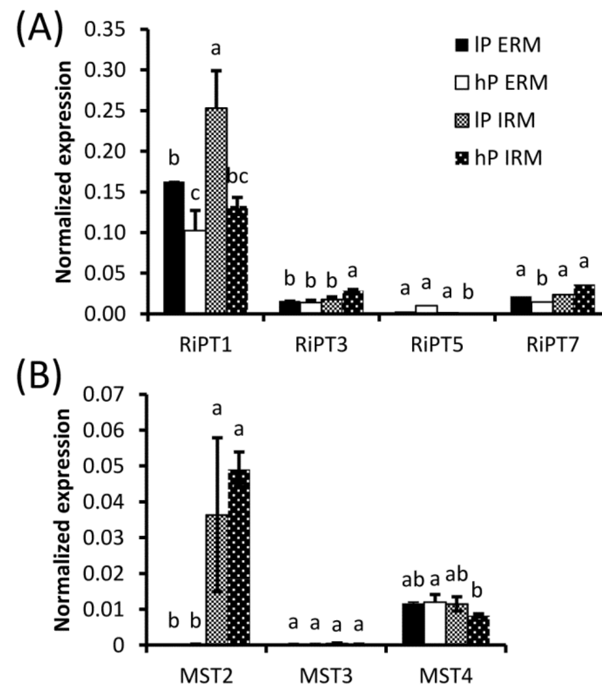



**Fig. 5.3. Quantification by qRT-PCR of the transcript abundances of phosphate (A) and monosaccharide transporters (B) in *R. irregularis.*** Quantification of transcript levels of transporters in the ERM and in the IRM of the host plant poplar under high (hP) and low (lP) availability of P. Values are means of three biological and three technical replicates. Error bars represent the SE. Translational elongation factor was used as a reference transcript. Statistical analysis was performed by analysis of variance for each gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$). Lower case letters indicate statistical differences.

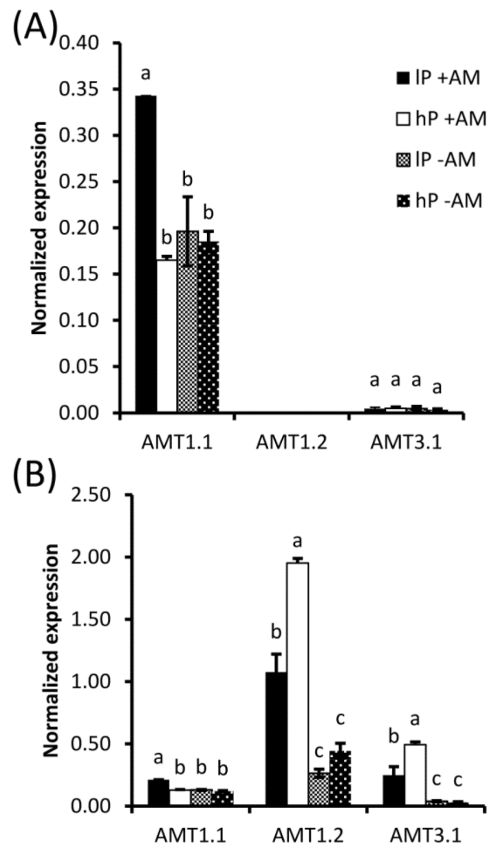



**Fig. 5.4. Quantification by qRT-PCR of the transcript abundances** of the three ammonium transporters AMT1.1, AMT1.2 and AMT3.1 in the shoot (A) and root (B) of mycorrhized (+AM) and non-mycorrhized (-AM) *P.trichocarpa* under low P (lP) and high P (hP) condition. Values are the means of three biological replicates and three technical replicates, each. Error bars represent the SE. Ubiquitin was used as reference transcript. Statistical analysis was performed by analysis of variance (ANOVA) per gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$). Lower case letters indicate statistical differences.

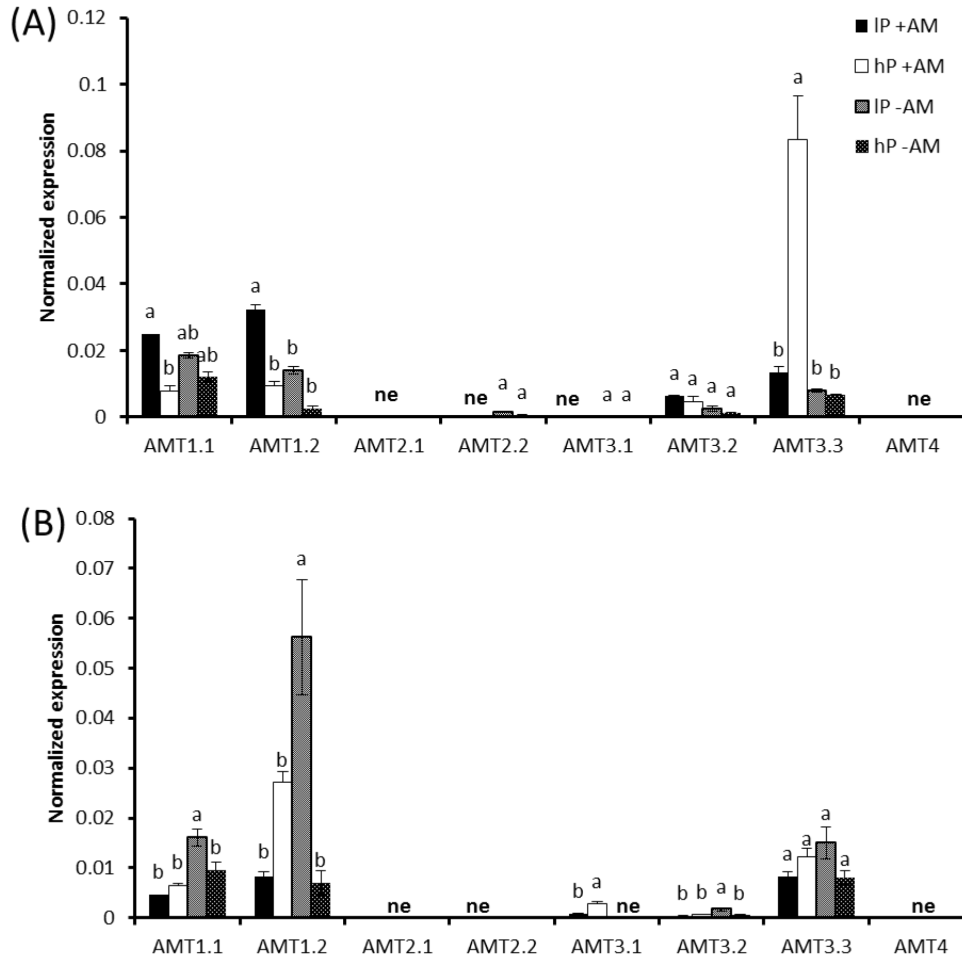



**Fig. 5.5. Quantification by qRT-PCR of the transcript abundances** of ammonium transporters in *S. bicolor*. Transcript abundances were measured in shoots (A) and roots (B) of *S. bicolor* when mycorrhized (+AM) or not (-AM) by the AM fungus *R.irregularis* in low-P (lP) and high-P (hP) conditions. Values are the means of three biological replicates and three technical replicates, each. Error bars represent the SE. Ubiquitin was used as reference transcript. Statistical analysis was performed by analysis of variance (ANOVA) per gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$).

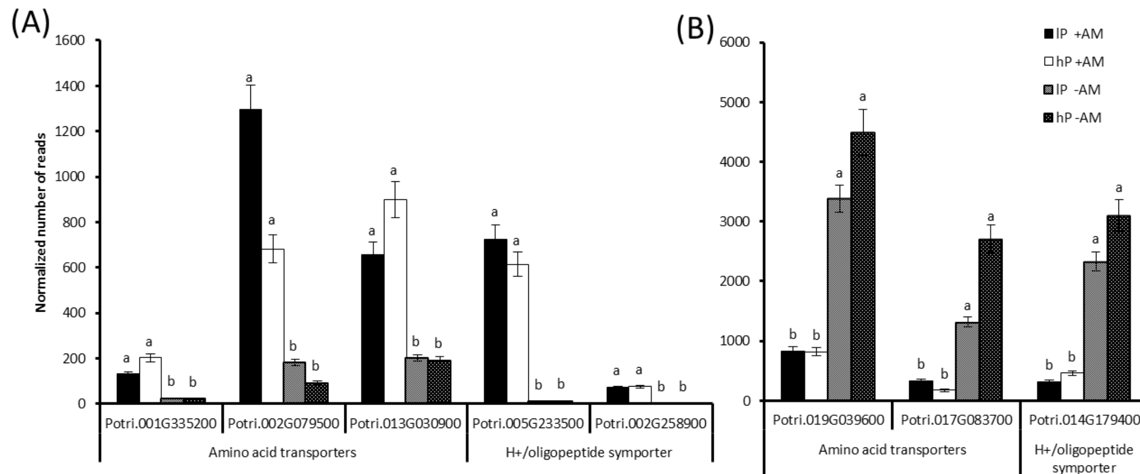



**Figure 5.6 Quantification by mRNA-Sequencing of transcript abundances of highly AM-induced and repressed amino acid transporter and H+/oligopeptide symporters in in the roots of *P. trichocarpa*.** Transcript abundances were quantified in mycorrhized (+AM) and non-mycorrhized (-AM) roots in high-P (hP) and low-P (lP) treatment. Significant differences were estimated per gene using the Wald test. Lower case letters indicate significant differences in transcript abundances (p-value < 0.05). Numbers of reads in the three biological replicates per condition were normalized per gene using cufflink and cuffquant.

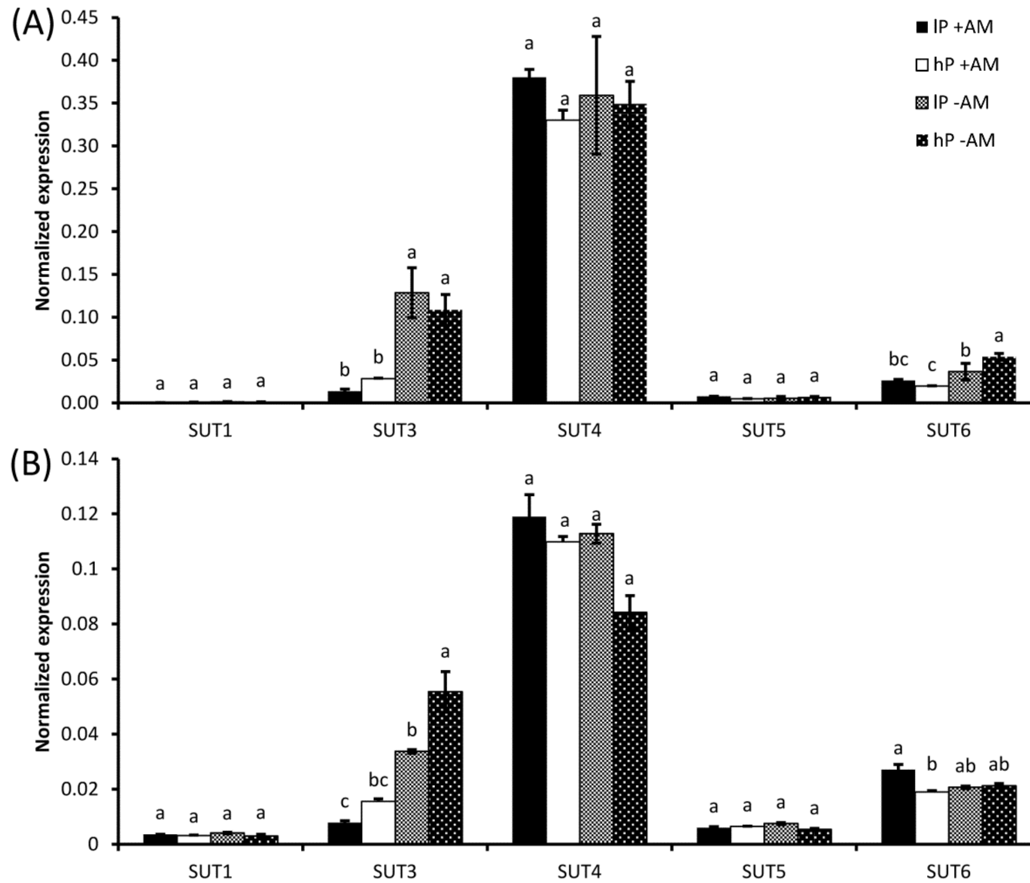



**Fig. 5.7. Quantification by qRT-PCR of the transcript abundances** of five sugar transporters (SUT) in the shoots (A) and roots (B) of mycorrhized (+AM) and non-mycorrhized (-AM) *P.trichocarpa* under low P (lP) and high P (hP) condition. Values are the means of three biological replicates and three technical replicates, each. Error bars represent the SE. Ubiquitin was used as reference transcript. Statistical analysis was performed by analysis of variance (ANOVA) per gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$). Lower case letters indicate statistical differences.

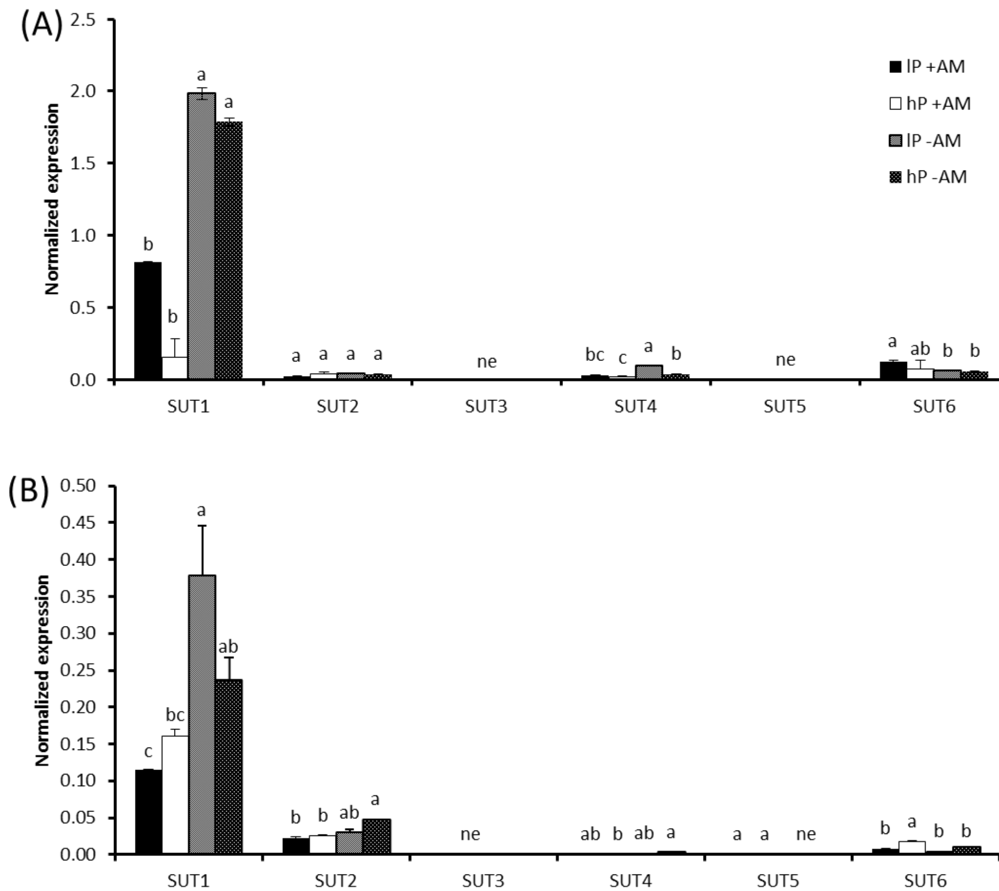



**Fig. 5.8. Quantification by qRT-PCR of the transcript abundances of 6 sugar transporters (SUT) in *S. bicolor.*** Transcript abundances were measured in shoots (A) and roots (B) when mycorrhized (+AM) or not (-AM) by the AM fungus *R. irregularis* in low-P (lP) and high-P (hP) conditions. Values are the means of three biological replicates and three technical replicates, each. Error bars represent the SE. Ubiquitin was used as reference transcript. Statistical analysis was performed by analysis of variance (ANOVA) per gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$).

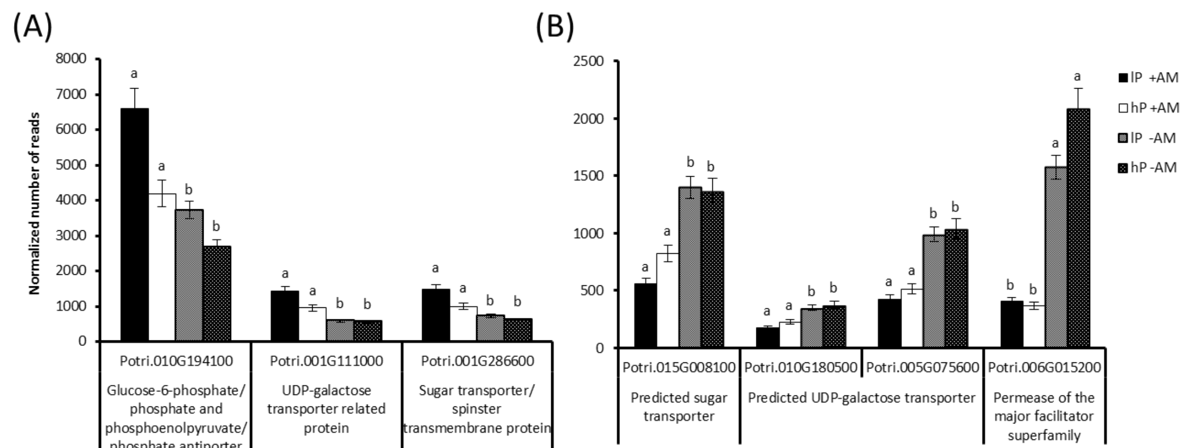



**Figure 5.9 Quantification by mRNA-Sequencing of transcript abundances of AM-induced and repressed carbohydrate transporters in in the roots of *P. trichocarpa*.** Transcript abundances were quantified in mycorrhized (+AM) and non-mycorrhized (-AM) roots in high-P (hP) and low-P (lP) treatment. Significant differences were estimated per gene using the Wald test. Lower case letters indicate significant differences in transcript abundances (p-value < 0.05). Numbers of reads in the three biological replicates per condition were normalized per gene using cufflink and cuffquant.

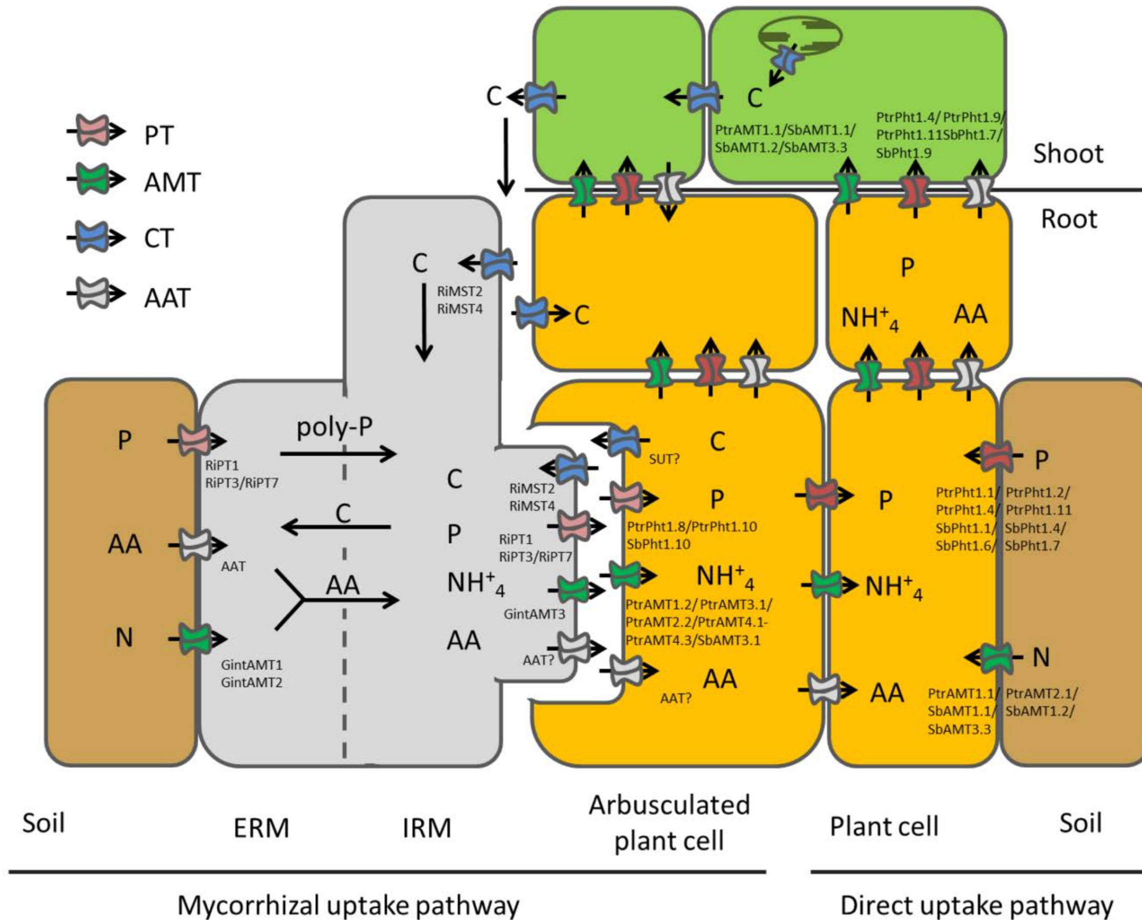



**Fig. 5.10. Schematic representation of the mycorrhizal nutrient uptake pathway and the direct nutrient uptake pathway in our model systems poplar and sorghum when colonized by *R. irregularis*.** In the direct uptake pathway (right hand side) nutrients, i.e. phosphate (P) and nitrogen (N) are taken up from the rhizosphere by phosphate transporters (PT) and ammonium transporters (AMT) and are transported to the shoot. In symbiotic interaction, the mycorrhizal fungus partially takes over nutrition of the plant. Nutrients are taken up by specialized transporters in the extraradical mycelium (ERM) and are further transported to the intraradical mycelium (IRM) where they are transferred to the periarbuscular space, to be taken up by plant transporters. N has been suggested to be additionally taken up from the soil in form of amino acids (AA) by predicted amino acid transporters (AAT). In exchange for the transfer of the mineral nutrients the mycorrhizal fungus is rewarded with essential carbohydrates from the plant. As some transporters were specifically induced by mycorrhization a possible localization at the periarbuscular membrane was assumed for the plant transporters. High induction of mycorrhizal transporters in the intraradical mycelium (IRM) compared to the extraradical mycelium (ERM) suggest that these transporters are mainly involved in nutrient exchange at the symbiotic interface.

**Table 5.1 Relative abundances of metabolites detected in the ERM of *R. irregularis*.** Data was log transformed and tested by Wilcoxon rank sum test ($p<0.05$, n=3) in MeV v4.9 (http://www.tm4.org/mev.html). Significant p-values are highlighted in bold.

| Class | Name | log2 ratio lP vs hP | p-value |
|---|---|---|---|
| Acids | Aconitic acid, cis- | -0.53 | 0.275 |
| Acids | Benzoic acid | 0.02 | 0.827 |
| Acids | Benzoic acid, 4-hydroxy- | 0.04 | 0.827 |
| Acids | Citric acid | 0.25 | 0.827 |
| Acids | Fumaric acid | 0.36 | 0.513 |
| Acids | Isocitric acid | 0.32 | 0.513 |
| Acids | Lactic acid | -0.34 | 0.275 |
| Acids | Malic acid | 0.60 | 0.275 |
| Acids | Pyruvic acid | 0.11 | 0.275 |
| Acids | Quinic acid | -1.88 | 0.513 |
| Acids | Succinic acid | 0.26 | 0.827 |
| Alcohols | Benzylalcohol | -0.18 | 0.513 |
| Amino Acids | Aspartic acid | -0.24 | 0.513 |
| Amino Acids | Glutamic acid | -0.44 | 0.513 |
| Amino Acids | Glycine | 0.80 | 0.275 |
| Amino Acids | Isoleucine | 0.63 | 0.513 |
| Amino Acids | Leucine | 0.19 | **0.050** |
| Amino Acids | Lysine | 0.11 | 0.564 |
| Amino Acids | Ornithine | -1.28 | 0.127 |
| Amino Acids | Phenylalanine | -0.12 | 0.827 |
| Amino Acids | Pyroglutamic acid | -0.06 | 0.513 |
| Amino Acids | Serine | 0.19 | 0.827 |
| Amino Acids | Valine | 0.62 | 0.513 |
| N- Compounds | Ethanolamine | 0.15 | 0.275 |
| N- Compounds | Putrescine | 0.14 | 0.513 |
| N- Compounds | Pyridine, 2-hydroxy- | 0.02 | 0.827 |
| Phenylpropanoids | Caffeic acid, trans- | -0.03 | 1.000 |
| Phosphates | Phosphoric acid | 0.25 | 0.513 |
| Phosphates | Phosphoric acid monomethyl ester | -0.06 | 0.827 |
| Polyols | Arabitol | -0.07 | 0.827 |
| Polyols | Glycerol | -0.24 | 0.513 |
| Polyols | Inositol, myo- | 0.05 | 0.827 |
| Polyols | Mannitol | -0.92 | 0.513 |
| Sugar Conjugates | Galactinol | -2.29 | 0.248 |
| Sugar Conjugates | Salicin | -4.25 | 1.000 |
| Sugars | Glucose | 0.41 | 0.127 |
| Sugars | Glucose, 1,6-anhydro-, beta- | 0.11 | 0.513 |
| Sugars | Rhamnose | 0.16 | 0.513 |
| Sugars | Ribose | -0.32 | 0.564 |
| Sugars | Sucrose | -0.06 | 0.827 |
| Sugars | Trehalose, alpha,alpha'- | 0.39 | 0.127 |

**Table 5.2 Relative abundances of metabolites detected in poplar roots. Abundances were measured in the mycorrhized (+AM) and non-mycorrhized (-AM) poplar roots under high (hP) and low (lP) P availability.** Data was log transformed and tested by y 2-way analysis of variance (ANOVA) (p<0.05, n=3) in MeV v4.9 (http://www.tm4.org/mev.html). Significant p-values are highlighted in bold.

| Class | Name | log2 ratios | | | | | 2-way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| | | +AM vs -AM | lP+AM vs hP+AM | lP-AM vs hP -AM | lP-AM vs hP -AM | hP +AM vs hP-AM | Effect of mycorrhization | Effect of p-availability | Effect of interaction |
| Acids | Aconitic acid, cis- | -1.62 | -0.01 | -0.17 | -1.54 | -1.69 | **0.006** | 0.863 | 0.934 |
| Acids | Benzoic acid | -0.63 | -0.18 | 0.72 | -1.03 | -0.13 | **0.013** | 0.182 | **0.034** |
| Acids | Benzoic acid, 3,4-dihydroxy- | -0.16 | -0.09 | 0.13 | -0.27 | -0.05 | 0.750 | 0.839 | 0.602 |
| Acids | Benzoic acid, 4-hydroxy- | 0.09 | -0.10 | 0.83 | -0.32 | 0.62 | 0.767 | 0.426 | 0.162 |
| Acids | Citric acid | 0.46 | 0.06 | 0.65 | 0.20 | 0.78 | 0.054 | 0.125 | 0.172 |
| Acids | Fumaric acid | -0.87 | 0.25 | 0.34 | -0.92 | -0.82 | **0.038** | 0.325 | 0.814 |
| Acids | Glutaric acid, 2-hydroxy- | -1.59 | -0.28 | 0.58 | -2.00 | -1.14 | **0.003** | 0.546 | 0.420 |
| Acids | Glutaric acid, 2-oxo- | -1.70 | 0.18 | 1.64 | -2.21 | -0.75 | **0.009** | 0.111 | 0.180 |
| Acids | Glutaric acid, 3-hydroxy-3-methyl- | -0.87 | -0.37 | 0.80 | -1.41 | -0.24 | 0.065 | 0.549 | 0.199 |
| Acids | Isocitric acid | 0.57 | 0.18 | 0.86 | 0.29 | 0.97 | 0.054 | 0.084 | 0.190 |
| Acids | Lactic acid | -0.74 | -0.68 | 1.36 | -1.65 | 0.39 | 0.083 | 0.389 | **0.013** |
| Acids | Malic acid | 0.07 | -0.21 | 0.72 | -0.35 | 0.57 | 0.958 | 0.412 | 0.135 |
| Acids | Malic acid, 2-isopropyl- | ND | ND | 1.04 | ND | ND | ND | ND | ND |
| Acids | Malic acid, 2-methyl- | ND | ND | 0.85 | ND | ND | ND | ND | ND |
| Acids | Pyruvic acid | 0.31 | 0.05 | 0.29 | 0.20 | 0.44 | 0.147 | 0.400 | 0.454 |
| Acids | Quinic acid | -0.15 | -0.79 | 0.92 | -0.98 | 0.72 | 0.895 | 0.723 | 0.092 |
| Acids | Shikimic acid | -2.32 | -0.73 | 0.82 | -3.08 | -1.53 | **0.000** | 0.658 | **0.008** |
| Acids | Succinic acid | -0.85 | 0.04 | 0.72 | -1.14 | -0.46 | **0.032** | 0.212 | 0.552 |
| Acids | Vanillic acid | -0.29 | 0.18 | 0.51 | -0.44 | -0.10 | 0.353 | 0.258 | 0.631 |
| Alcohols | Benzylalcohol | -0.37 | -0.09 | 0.95 | -0.81 | 0.23 | 0.479 | 0.180 | 0.104 |
| Amino Acids | Aspartic acid | -4.52 | 0.82 | 0.59 | -4.43 | -4.66 | **0.000** | 0.134 | 0.845 |
| Amino Acids | Butanoic acid, 4-amino- | -3.03 | 0.68 | 0.51 | -2.96 | -3.13 | **0.000** | **0.032** | 0.572 |
| Amino Acids | Glutamic acid | -2.87 | 1.27 | -0.85 | -1.88 | -4.01 | **0.001** | 0.574 | 0.090 |
| Amino Acids | Glycine | -0.92 | 0.44 | 0.63 | -0.99 | -0.80 | **0.023** | 0.112 | 0.736 |
| Amino Acids | Isoleucine | -1.75 | 0.51 | 0.66 | -1.81 | -1.67 | **0.000** | **0.041** | 0.873 |
| Amino Acids | Leucine | -1.25 | -0.06 | 0.34 | -1.44 | -1.04 | **0.001** | 0.483 | 0.306 |
| Amino Acids | Phenylalanine | -3.55 | 0.76 | 0.65 | -3.51 | -3.61 | **0.000** | **0.048** | 0.964 |
| Amino Acids | Pyroglutamic acid | -1.45 | 0.24 | -0.05 | -1.31 | -1.60 | **0.001** | 0.532 | 0.762 |
| Amino Acids | Serine | -1.09 | 0.45 | 0.58 | -1.14 | -1.02 | **0.000** | **0.014** | 0.765 |
| Amino Acids | Valine | -2.32 | -0.09 | 0.78 | -2.71 | -1.83 | **0.000** | 0.224 | 0.118 |
| Aromatic | Catechol | -1.33 | 1.43 | 1.85 | -1.43 | -1.00 | 0.245 | 0.276 | 0.598 |
| N- Compounds | Ethanolamine | -0.05 | -0.16 | -0.02 | -0.13 | 0.02 | 0.927 | 0.722 | 0.594 |
| N- Compounds | Phenol, 2-amino- | -1.28 | 0.26 | 0.87 | -1.53 | -0.91 | **0.019** | 0.242 | 0.306 |
| N- Compounds | Putrescine | 0.19 | 0.02 | 0.19 | 0.11 | 0.27 | 0.362 | 0.499 | 0.616 |
| N- Compounds | Pyridine, 2-hydroxy- | -0.08 | -0.33 | 0.34 | -0.41 | 0.25 | 0.803 | 0.872 | 0.161 |
| Phenylpropanoids | Caffeic acid, cis- | 0.15 | 0.03 | 0.91 | -0.21 | 0.66 | 0.298 | 0.070 | 0.075 |
| Phenylpropanoids | Caffeic acid, trans- | -0.05 | 0.06 | 1.14 | -0.48 | 0.60 | 0.666 | 0.056 | 0.070 |
| Phenylpropanoids | Cinnamic acid, 4-hydroxy-, trans- | ND | ND | 0.60 | ND | -0.47 | ND | ND | ND |
| Phenylpropanoids | Epicatechin | -0.14 | -0.16 | 0.60 | -0.50 | 0.26 | 0.668 | 0.323 | 0.138 |
| Phenylpropanoids | Ferulic acid, trans- | -0.12 | 0.10 | 0.94 | -0.47 | 0.37 | 0.703 | 0.066 | 0.121 |
| Phenylpropanoids | Quinic acid, 3-caffeoyl-, cis- | 1.01 | 0.16 | 0.07 | 1.05 | 0.96 | 0.102 | 0.745 | 0.572 |
| Phenylpropanoids | Quinic acid, 3-caffeoyl-, trans- | 0.80 | 0.13 | 0.42 | 0.68 | 0.96 | 0.155 | 0.487 | 0.347 |
| Phosphates | Fructose-6-phosphate | -1.85 | -0.51 | -0.22 | -2.01 | -1.73 | **0.003** | 0.420 | 0.806 |
| Phosphates | Glucose-6-phosphate | -2.22 | -1.10 | -0.43 | -2.64 | -1.97 | **0.000** | 0.065 | 0.453 |
| Phosphates | myo-Inositol-phosphate | ND | ND | -1.11 | -1.17 | ND | ND | ND | ND |
| Phosphates | Phosphoric acid | -3.48 | 0.45 | -2.86 | -1.22 | -4.54 | **0.042** | 0.634 | 0.468 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phosphates | Phosphoric acid monomethyl ester | -2.35 | -0.39 | -0.05 | -2.53 | -2.20 | **0.001** | 0.648 | 0.676 |
| Polyhydroxy Acids | Arabinonic acid-1,4-lactone | -0.18 | 0.51 | 1.57 | -0.52 | 0.53 | 0.914 | **0.001** | **0.041** |
| Polyhydroxy Acids | Galactaric acid | -1.18 | -0.25 | 0.81 | -1.66 | -0.60 | **0.011** | 0.455 | 0.209 |
| Polyhydroxy Acids | Galactonic acid | -1.45 | -0.63 | 0.64 | -2.08 | -0.81 | **0.002** | 0.963 | 0.100 |
| Polyhydroxy Acids | Gluconic acid | -1.14 | -0.22 | 1.22 | -1.73 | -0.29 | **0.018** | 0.217 | 0.139 |
| Polyhydroxy Acids | Glyceric acid | -1.62 | -0.28 | 0.20 | -1.87 | -1.38 | **0.000** | 0.900 | 0.238 |
| Polyhydroxy Acids | Gulonic acid | -0.36 | 0.04 | 0.62 | -0.61 | -0.03 | 0.425 | 0.257 | 0.311 |
| Polyhydroxy Acids | Lyxonic acid-1,4-lactone | -0.11 | 0.30 | 0.91 | -0.35 | 0.26 | 0.772 | 0.080 | 0.292 |
| Polyhydroxy Acids | Ribonic acid | -1.15 | 0.42 | 0.51 | -1.19 | -1.10 | **0.001** | **0.048** | 0.686 |
| Polyhydroxy Acids | Saccharic acid | -1.77 | 0.01 | 1.37 | -2.30 | -0.94 | **0.001** | 0.065 | **0.039** |
| Polyhydroxy Acids | Threonic acid | -2.04 | 0.50 | 1.05 | -2.24 | -1.69 | **0.000** | **0.019** | 0.292 |
| Polyols | Arabitol | -1.19 | -0.20 | 0.26 | -1.42 | -0.97 | **0.004** | 0.722 | 0.471 |
| Polyols | Galactitol | -0.16 | 0.54 | -0.46 | 0.33 | -0.67 | 0.507 | 0.853 | 0.143 |
| Polyols | Glycerol | 0.17 | -0.86 | -0.12 | -0.27 | 0.48 | 0.927 | 0.307 | 0.475 |
| Polyols | Inositol, myo- | -2.84 | 0.98 | 0.26 | -2.56 | -3.28 | **0.000** | 0.123 | 0.422 |
| Polyols | Mannitol | -0.23 | 0.26 | -0.86 | 0.39 | -0.74 | 0.896 | 0.895 | 0.468 |
| Sugar Conjugates | Galactinol | -2.34 | 0.70 | -0.99 | -1.45 | -3.14 | **0.010** | 0.829 | 0.391 |
| Sugar Conjugates | Salicin | -0.94 | 0.72 | 1.58 | -1.21 | -0.35 | 0.193 | 0.137 | 0.275 |
| Sugar Conjugates | Salicylic acid-glucopyranoside | -2.48 | -0.06 | -1.13 | -1.84 | -2.90 | **0.038** | 0.913 | 0.998 |
| Sugars | Arabinose | -1.21 | -0.18 | 0.38 | -1.48 | -0.92 | **0.008** | 0.776 | 0.575 |
| Sugars | Fructose | 0.65 | 0.30 | >0.01 | 0.79 | 0.49 | 0.300 | 0.769 | 0.986 |
| Sugars | Galactose | 0.22 | -0.38 | 0.64 | -0.26 | 0.75 | 0.984 | 0.813 | 0.615 |
| Sugars | Glucose | 0.18 | 0.23 | -0.23 | 0.41 | -0.05 | 0.728 | 0.791 | 0.941 |
| Sugars | Glucose, 1,6-anhydro-, beta- | -0.75 | -0.13 | 0.70 | -1.13 | -0.29 | **0.001** | 0.076 | **0.018** |
| Sugars | Maltose | -1.35 | 0.36 | 0.85 | -1.55 | -1.05 | **0.006** | 0.139 | 0.622 |
| Sugars | Mannose | 0.05 | -0.68 | -0.03 | -0.32 | 0.33 | 0.800 | 0.791 | 0.866 |
| Sugars | Raffinose | -1.59 | 1.23 | -4.14 | 2.12 | -3.25 | 0.602 | 0.766 | 0.173 |
| Sugars | Rhamnose | -1.00 | -0.04 | 0.48 | -1.24 | -0.72 | **0.009** | 0.674 | 0.351 |
| Sugars | Ribose | -1.96 | 0.13 | 0.89 | -2.28 | -1.52 | **0.001** | 0.258 | 0.258 |
| Sugars | Sucrose | 0.10 | -0.02 | 0.11 | 0.04 | 0.17 | 0.534 | 0.669 | 0.658 |
| Sugars | Trehalose, alpha,alpha'- | 1.20 | -0.49 | -0.18 | 1.03 | 1.33 | **0.046** | 0.883 | 0.841 |
| Sugars | Xylose | -0.50 | -0.12 | 0.94 | -0.95 | 0.10 | 0.639 | 0.526 | 0.363 |
| Sugars | Xylulose | ND | ND | 1.14 | -2.36 | ND | ND | ND | ND |

## 5.9 **Supplementary Information**

**Supplementary Methods S1**

**Phylogenetic analysis**

Neighbour joining trees were conducted on amino acid sequences of plant Pht1 phosphate transporters and ammonium transporters. Using the MEGA6.06 package (Tamura *et al.*, 2013) sequences were aligned by ClustalW with the alignment parameters: gap opening penalty 15, gap extension penalty 0.3m Gonnet protein weight matrix and a delayed divergent cutoff value of 25%. Phyolgentic trees were made using the Poisson correction model with pairwise deletion. Bootstrap values are derived from 1000 replications. Sequences for phylogenetic analysis of plant ammonium transporters are derived based on DNA sequences as filed in the UniProt data base.

The accession numbers or gene models of UniProt data base were used: PtrAMT1;1 (B9HSW3), PtrAMT1;2 (B9IPE2), PtrAMT1;3 (B9HKW8), PtrAMT1;4 (B9GRB5), PtrAMT1;5 (B9GRB4), PtrAMT1;6 (B9HP47), PtrAMT2;1, (B9HCZ0), PtrAMT2;2 (B9IGE2), PtrAMT3;1 (B9GHA5), PtrAMT4;1 (B9GS88), PtrAMT4;2 (B9IKS2), PtrAMT4;3 (B9H8E7), PtrAMT4;4 (B9I5F0), PtrAMT4;5 (B9MX92). *Arabidopsis thaliana* (at NCBI): AtAMT1;1 (At4g13510), AtAMT1;2 (At1g64780), AtAMT1;3 (At3g24300), AtAMT1;4 (At4g28700), AtAMT1;5 (At3g24290), AtAMT2;1 (At2g38290), *Lycopersicon esculentum:* LeAMT1;1 (P58905), LeAMT1;2 (O04161), LeAMT1;3 (Q9FVN0), *Lotus japonicus:* LjAMT1;1 (Q9FSH3), LjAMT1;2 (Q7Y1B9), LjAMT1;3 (Q70KK9), LjAMT2;1 (Q93X02), *Oryza sativa:* OsAMT1;1 (Q7XQ12), OsAMT1;2 (Q6K9G1), OsAMT1;3 (Q6K9G3), OsAMT2;1 (Q84KJ7), OsAMT2;2 (Q8S230), OsAMT2;3 (Q8S233), OsAMT3;1 (Q84KJ6), OsAMT3;2 (Q851M9), OsAMT3;3 (Q69T29), OsAMT4;1 (Q10CV4), *Brassica napus:* BnAMT1;2 (Q9FUH7), *Populus tremula x tremuloides:* PttAMT1;2 (Q5K411), *Triticum aestivum:* TaAMT1;1 (Q6QU81), TaAMT1;2 (Q6QU80), TaAMT2;1 (Q6T8L6), *Nitrosomona europeae:* NeAMT/Rh1 (Q82X47), *Escherichia coli:* EcAMTB (P69681). Sequences from the *S. bicolor* genome (v1.1) database were: SbAMT1;1 (Sb06g022230), SbAMT1;2 (Sb04g026290), SbAMT2;1 (Sb09g023030), SbAMT2;2 (Sb03g038840), SbAMT3;1 (Sb03g041140), SbAMT3;2 (Sb01g001970), SbAMT3;3 (Sb04g022390), SbAMT4 (Sb01g008060). Sequences derived from Phytozome 6.0 database: *Glycine max*: GmAMT1.1 (Glyma20g21030.1), GmAMT1;2 (Glyma10g26690.1), GmAMT1;3 (Glyma10g31080.1), GmAMT1;4 (Glyma10g31110.1), GmAMT1;5 (Glyma1031130.1), GmAMT1;6 (Glyma20g36390.1), GmAMT2;1 (Glyma07g18670.1), GmAMT2;2 (Glyma18g43540.1), GmAMT2;3 (Glyma01g30920.1), GmAMT3;1 (Glyma05g33010.1), GmAMT4;1 (Glyma09g41810.1), GmAMT4;2 (Glyma20g00680.1), GmAMT4;3

(Glyma19g43380.1), GmAMT4;4 (Glyma02g04960.1), GmAMT4;5 (Glyma02g16200), GmAMT4;6 (Glyma10g03600.1).

**Supporting Material Reference**

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729

## 5.10 Supplementary Tables and Figures

**Supplementary Table S5.1 Primer used for qRT-PCR in *P. trichocarpa, S. bicolor and R. irregularis***

| Name | Sequence (5´-> 3´) | Transcript ID/ Protein ID | Reference |
|---|---|---|---|
| PtPht1.1f | GCGATTCACGAGGTTTTTCA | Potri.010G072000 | Loth-Pereda *et al*. (2011) |
| PtPht1.1r | GGCGAAGAAAAAGGTCAACG | | |
| PtPht1.2f | CACAGACCGAACGAAGACTG | Potri.010G071700 | Loth-Pereda *et al*. (2011) |
| PtPht1.2r | ATCACACTGAAGCCATCCTAGC | | |
| PtPht1.3f | ACCACCGAATTGGCTTCG | Potri.010G071500 | Loth-Pereda *et al*. (2011) |
| PtPht1.3r | ATGGCAAGTAGACCTCAATCTCG | | |
| PtPht1.4f | GATCTTCCCTGCAAGGTTAAGG | Potri.005G223500 | Loth-Pereda *et al*. (2011) |
| PtPht1.4r | TCCTTGTGCGGGCTCTG | | |
| PtPht1.5f | GGACCCAATGCCACCAC | Potri.002G038900 | Loth-Pereda *et al*. (2011) |
| PtPht1.5r | CTTGGCCTGGTCCTGATTC | | |
| PtPht1.6f | ATCGCCAGGGCTCAATTC | Potri.005G175500 | Loth-Pereda *et al*. (2011) |
| PtPht1.6r | CTATTATTGCGCCAGCCTTC | | |
| PtPht1.7f | CGAATTGGATTCGTGGTTATG | Potri.005G223600 | Loth-Pereda *et al*. (2011) |
| PtPht1.7r | CCTTGTCCTGGTCCTGAGC | | |
| PtPht1.8f | GATAGGATCGGAAGGTTCATAATC | Potri.019G061900 | Loth-Pereda *et al*. (2011) |
| PtPht1.8r | GGTGACCACCACAGAAATCC | | |
| PtPht1.9f | GATCACCTACAATTTCTTCTG | Potri.002G005500 | |
| PtPht1.9r | GGTGAACTAGGAGTAATCAGATAC | | |
| PtPht1.10f | TGCTAATGCTTGGTGCTTG | Potri.015G022800 | Loth-Pereda *et al*. (2011) |
| PtPht1.10r | GATGAACATGGCACGAGATG | | |
| PtPht1.11f | GCCACCCAAAGACTTCTGTC | Potri.005G256100 | |
| PtPht1.11r | TTCAATCATCTGATAGGACACCA | | |
| PtPht1.12f | GGAAACCATGTGGGAGTGC | Potri.001G318500 | |
| PtPht1.12r | CTGGCCAGCTAAAGTTCCAC | | |
| PtrAMT1.1f | TTCGCAAGCTCAAGCTC | Potri.010G063500 | Selle *et al*. (2005) |
| PtrAMT1.1r | AATATGAGGTCCCTCTTAGACG | | Couturier *et al*. (2007) |
| PtrAMT1.2f | GCCTGCGAAGAACACCTC | Potri.019G023600 | |
| PtrAMT1.2r | GCATCAAACTTGATCACACATTG | | Couturier *et al*. (2007) |
| PtrAMT3.1f | GCCGTGCATGGTGAAGAG | Potri.001G305400 | |
| PtrAMT3.1r | TTGATGACTTGCGCTCCA | | |
| PtrSUT1f | CCCACCAGTAGTAGTGC | Potri.013G115200 | |
| PtrSUT1r | CCAGTCACTAGTCTTGGAAGG | | |
| PtrSUT3f | CTGGTGCTGGCCAAGG | Potri.019G085800 | |
| PtrSUT3r | GGCATCCCAAGGTCCAC | | |
| PtrSUT4f | AGGCAGGGTGAGGAGGAT | Potri.002G106900 | |
| PtrSUT4r | AGCGACACGACCTTCCAG | | |
| PtrSUT5f | GCGGTGGCCAAGGATT | Potri.008G148100 | |
| PtrSUT5r | GAACGAAGGCTGGTATA | | |
| PtrSUT6f | GGTCTACAGTCAGAGAGATGGTTC | Potri.010G093600 | |
| PtrSUT6r | AGCGTTCTGCGCGCTTCT | | |
| PtUBI f | GCAGGGAAACAGTGAGGAAGG | Potri.015G013600 | |
| PtUBI r | TGGACTCACGAGGACAG | | |
| SbPht1.1f | GGCCAAGGTGCTCAAGAAG | Sobic.001G502000 | Walder *et al*. (2015) |
| SbPht1.1b | GGAGGAACTGCACCGAGAAG | | |
| SbPht1.2f | ACTAAGCAGCAGCCTCCGTA | Sobic.006G027300 | Walder *et al*. (2015) |

| | | | |
|---|---|---|---|
| SbPht1.2b | AAGCCACAAGGAAACCATTG | | |
| SbPht1.3f | TACTCGCGTATGAACATGCC | Sobic.001G513400 | Walder *et al*. (2015) |
| SbPht1.3b | TCCTCCTTATTGCCGATGTC | | |
| SbPht1.4f | GGCGCCGTCGTACCAGGACAA | Sobic.001G234900 | Walder *et al*. (2015) |
| SbPht1.4b | GAGCGCCGCCGGGATGGT | | |
| SbPht1.5f | GAGAATCTGGACGAGATCAC | Sobic.001G502100 | Walder *et al*. (2015) |
| SbPht1.5b | CAGGTTCTGGCTGTAGTAGG | | |
| SbPht1.6f | CAAGCTCGGCCGTAAGAAGG | Sobic.007G164400 | Walder *et al*. (2015) |
| SbPht1.6b | GCCAGAAGCGGAAGAAGCAC | | |
| SbPht1.7f | GGACACCAGCAAGGACAAC | Sobic.001G234800 | Walder *et al*. (2015) |
| SbPht1.7b | CGCGATGGAGCAGATGAC | | |
| SbPht1.8f | GCAGCGAGGCCAATGAGACT | Sobic.002G116100 | Walder *et al*. (2015) |
| SbPht1.8b | TTGGCTCCGGTAGGAAGCAG | | |
| SbPht1.9f | GAGGACGAGCCGTTCAAGAG | Sobic.006G026900 | Walder *et al*. (2015) |
| SbPht1.9b | CGCGACGGAGAAGAAGTACC | | |
| SbPht1.10f | CACCATGTGCTGGTTACTTC | Sobic.006G026800 | Walder *et al*. (2015) |
| SbPht1.10b | GATAATCGCCTGAGTACGTG | | |
| SbPht1.11f | CGTGGTTCCTTCTGGACATA | Sobic.003G243400 | Walder *et al*. (2015) |
| SbPht1.11b | TCTCGAACACCTCCTTGAGT | | |
| SbSUT1f | GTGCTCCTGTAATCTTTGTGTCC | Sb01g045720 | |
| SbSUT1r | ACTATACTGCACATTGATTGATCG | | |
| SbSUT2f | GCACATGCATTGAATGAACC | Sb04g038030 | |
| SbSUT2r | TTCGCATTTGGAAATTCCTC | | |
| SbSUT3f | GGCCGGATCAAACAAGAT | Sb01g022430 | |
| SbSUT3r | GGCATTGCGAAGGAATGA | | |
| SbSUT4f | CGATCCATGATGATGTCCAG | Sb08g023310 | |
| SbSUT4r | GTTCCAGGCCTTGCTGTC | | |
| SbSUT5f | CCCGTAGTGTTGCGGAGTC | Sb04g023860 | |
| SbSUT5r | CCAATGGATCGGAAAATAAAG | | |
| SbSUT6f | GCACAACAGCACAAAGAAGG | Sb07g028120 | |
| SbSUT6r | AGGCAGAAGAGGCTGAGATG | | |
| SbAMT1;1f | GCTGTGGTTCGGCTGGTA | Sb06g022230 | Koegel *et al*. (2013) |
| SbAMT1;1r | GGACTTGAGGATGGTGGTGAA | | |
| SbAMT1;2f | TCCATTGCTCCTCGTTGC | Sb09g023030 | Koegel *et al*. (2013) |
| SbAMT1;2r | GGCTTTGCTCCCTCTTCC | | |
| SbAMT2;1f | TCCCGCCCGCCTACAGCT | Sb09g023030 | Koegel *et al*. (2013) |
| SbAMT2;1r | GTCACCATTCAGCTGTAG | | |
| SbAMT2;2f | GCGGCTTCCTCTACCAGTG | Sb03g038840 | Koegel *et al*. (2013) |
| SbAMT2;2r | CCTCTCCCTGTCGCTCTTC | | |
| SbAMT3;1f | GGCCTCGTCTGCATCACT | Sb03g041140 | Koegel *et al*. (2013) |
| SbAMT3;1r | GGGTGTCGTCCACTTGCT | | |
| SbAMT3;2f | CCGCACGCACTCTATCTGTA | Sb01g001970 | Koegel *et al*. (2013) |
| SbAMT3;2r | TCGCTGCTTATTGGGGTTAG | | |
| SbAMT3.3f | CGTCATTGCCTGGAACATC | Sb04g022390 | Koegel *et al*. (2013) |
| SbAMT3.3r | AGCATCATCCCCGATAAGC | | |
| SbAMT4f | CGAACAACATTCTCCTGACG | Sb01g008060 | Koegel *et al*. (2013) |
| SbAMT4r | CCCGAACACGAAGCAGTC | | |

| | | | | |
|---|---|---|---|---|
| SbUBIf | CAAGGAGTGCCCCAACAC | Sb10g026870 | | Koegel *et al*. (2013) |
| SbUbir | TGGTAGGCGGGTAAAGCAAA | | | |
| GintAMT1f | TGTGTCAGCATTGTCTTCAGT | 337137/ | 337025 | López-Pedrosa *et al*. ( 2006) |
| GintAMT1r | GGCAAGTGCGGGTGTAATAG | | | |
| GintAMT2f | GTGCCAATGCCGCTAACA | 314321/ | 314209 | Pérez-Tienda *et al*. (2011) |
| GintAMT2r | GCCAGAACAGAATCCCAAAG | | | |
| GintAMT3f | GGG CTT GAC TTT GCT GGT | 218287/ | 218175 | |
| GintAMT3r | TTC GTC CCT TCC ATG ACC | | | |
| RiPT1f | ATGGGTTTCGCTGTCCTCAC | 345640/ | 345528 | |
| RiPT1r | CCCCTGGAACGATGAATG | | | |
| RiPT3 | CGGCGGTGATTATCCTCT | 7378/ | 7266 | |
| RiPT3 | GCCGCCATCATTGCTC | | | |
| RiPT5f | GGCGCGAATACGTCAGAA | 346370/ | 346258 | |
| RiPT5r | GCTGCAACACCAACACCA | | | |
| RiPT7 | GATCCTTGGACTGGAACAC | 67530/ | 67418 | |
| RiPT7r | GCGATGACTCCCATATCACC | | | |
| RiMST2f | GGCAGGATATTTGTCTGATAG | 341721/ | 341609 | Helber *et al*. (2011) |
| RiMST2r | GCAATAACTCTTCCCGTATAC | | | |
| RiMST3f | ATTCTCGATTCTTGGTGCATC | 34584/ | 34472 | Helber *et al.* (2011) |
| RiMST3r | ATACGCCAGCAACGACTC | | | |
| RiMST4f | TAGCTACATTTGCTATTGGTTTAG | 155358/ | 155246 | Helber *et al.* (2011) |
| RiMST4r | CCCTAACTTCCAAAAATAATGAAC | | | |

**Supplementary Table S5.2.** Percentage of colonized *P. trichocarpa* and *S. bicolor* roots colonized by *R. irregularis.* Statistical analysis was performed on six biological replicates by T.TEST ($p<0.05$) for each plant species. Significant differences are indicated by lower case letters.

| Plant | Treatment | Hyphae (%) | Arbuscule (%) | Vesicle (%) |
|---|---|---|---|---|
| *P. trichocarpa* | low Pi | 79 ± 10 [a] | 34 ± 8 [a] | 10 ± 8 [a] |
| | high Pi | 87 ± 6 [a] | 34 ± 8 [a] | 9 ± 4 [a] |
| *S. bicolor* | low Pi | 94 ± 5 [a] | 16 ± 7 [a] | 37 ± 11 [a] |
| | high Pi | 93 ± 5 [a] | 5 ± 5 [b] | 43 ± 17 [a] |

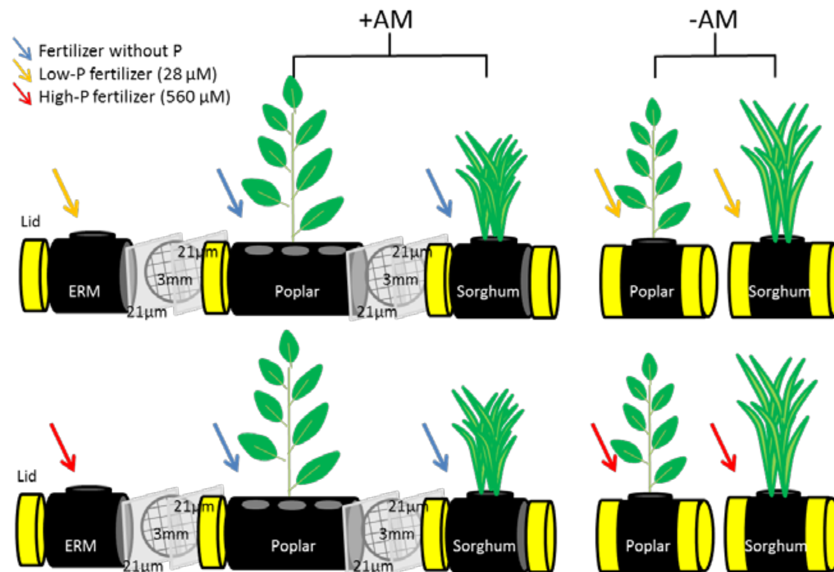
**Supplementary Table S5.3. Quantification by qRT-PCR of the transcript abundances of ammonium transporters in *R. irregularis*.** Transcript abundances were measured under low (lP) and high (hP) P availability. Transcript abundances of the three transporters GintAMT1, GintAMT2, GintAM3 were measured in the extra-radical mycelium (ERM) and in the intraradical mycelium (IRM) when associated to the host plants poplar and sorghum. Values are means of three biological and three technical replicates. Translational elongation factor was used as a reference transcript. Statistical differences between ERM and IRM was tested by analysis of variance for each gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$). Significant differences are indicated by lower case letters (Calabrese *et al.,* unpublished).

| | | ***GintAMT1*** | ***GintAMT2*** | ***GintAMT3*** |
|---|---|---|---|---|
| ***R. irregularis*** | **lP ERM** | 0.0003 ± 4E-07 [a] | 0.0235 ± 0.001 [a] | 0.0061 ± 0.000 [c] |
| | **hP ERM** | 0.0004 ± 1E-04 [a] | 0.0205 ± 0.004 [a] | 0.0065 ± 0.001 [c] |
| ***P. trichocarpa*** | **lP IRM** | 0.0002 ± 8E-05 [a] | 0.0188 ± 0.005 [a] | 0.0394 ± 0.013 [b] |
| | **hP IRM** | 0.0003 ± 2E-05 [a] | 0.0111 ± 0.003 [a] | 0.0603 ± 0.003 [a] |
| ***S. bicolor*** | **lP IRM** | 0.0005 ± 8E-05 [a] | 0.0236 ± 0.004 [a] | 0.0542 ± 0.008 [b] |
| | **hP IRM** | 0.0006 ± 6E-05 [a] | 0.0248 ± 0.001 [a] | 0.0814 ± 0.005 [a] |

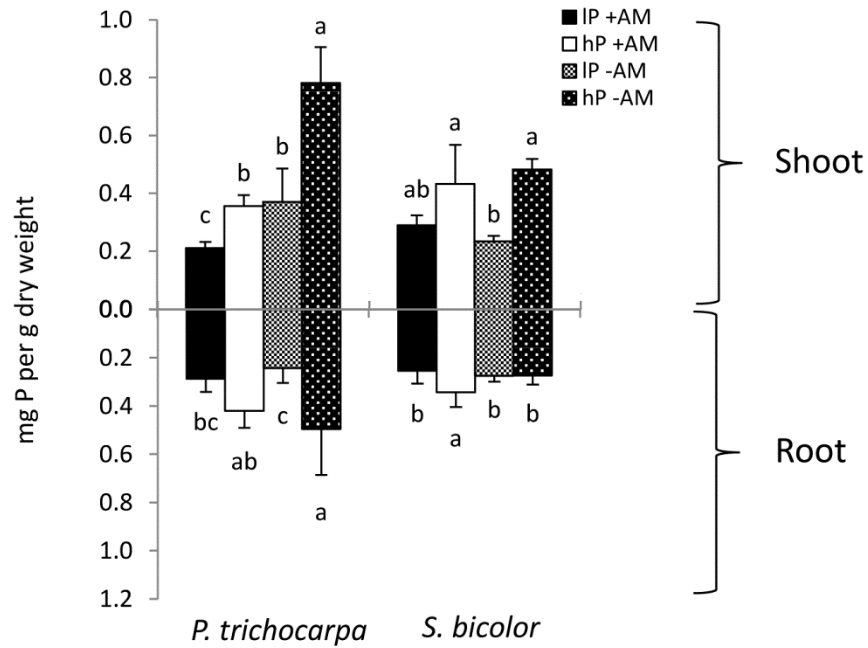
**Supplementary Table S5.4** Fold changes of transcript abundances of Pht1 phosphate transporters, sugar transporters (SUTs) and ammonium transporters (AMTs) in *P. trichocarpa*. Transcript abundances of the 18 genes were quantified in mycorrhized (+AM) and non-mycorrhized (-AM) roots in high-P (hP) and low-P (lP) treatment by qRT-PCR and mRNA-Sequencing (mRNA-Seq).

| Gene ID | Name | Fold change by mRNA-Seq | | | | Fold change by qRT-PCR | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | lP-AM vs hP-AM | hP+AM vs hP-AM | lP+AM vs hP+AM | lP+AM vs lP-AM | lP-AM vs hP-AM | hP+AM vs hP-AM | lP+AM vs hP+AM | lP+AM vs lP-AM |
| Potri.010G072000 | Pht1.1 | 30.4 | 65.2 | 1.4 | 3.0 | 53.5 | 152.3 | 3.7 | 10.6 |
| Potri.010G071700 | Pht1.2 | 6.4 | 13.3 | 1.7 | 3.4 | 7.2 | 24.6 | 1.8 | 6.2 |
| Potri.010G071500 | Pht1.3** | 1.5 | 1.3 | 0.8 | 0.7 | | | | |
| Potri.005G223500 | Pht1.4 | 2.6 | 3.2 | 1.9 | 2.3 | 2.7 | 5.3 | 2.2 | 4.4 |
| Potri.002G038900 | Pht1.5 | 0.4 | 0.2 | 1.5 | 0.8 | 0.6 | 0.5 | 2.1 | 1.7 |
| Potri.005G175500 | Pht1.6* | | | | | | | | |
| Potri.005G223600 | Pht1.7 | 4.8 | 2.6 | 3.1 | 1.6 | 12.3 | 8.8 | 3.6 | 2.6 |
| Potri.019G061900 | Pht1.8*** | 3.6 | 745.0 | 1.2 | 242.5 | | | 0.4 | |
| Potri.002G005500 | Pht1.9 | 1.6 | 3.7 | 1.6 | 3.7 | 1.2 | 3.4 | 2.2 | 6.2 |
| Potri.015G022800 | Pht1.10*** | 2.3 | 587.8 | 2.0 | 501.6 | | | 0.9 | |
| Potri.005G256100 | Pht1.11 | 9.6 | 26.3 | 1.4 | 3.8 | 7.4 | 41.6 | 1.7 | 9.7 |
| Potri.001G318500 | Pht1.12* | | | | | | | | |
| Potri.013G115200 | SUT1 | 0.9 | 0.6 | 0.9 | 0.6 | 1.4 | 1.0 | 1.2 | 0.9 |
| Potri.019G085800 | SUT3 | 0.7 | 0.3 | 0.7 | 0.3 | 0.6 | 0.3 | 0.5 | 0.2 |
| Potri.002G106900 | SUT4 | 1.0 | 0.9 | 0.8 | 0.7 | 1.3 | 1.3 | 1.1 | 1.1 |
| Potri.008G148100 | SUT5 | 1.2 | 0.8 | 0.7 | 0.5 | 1.4 | 1.2 | 0.9 | 0.8 |
| Potri.010G093600 | SUT6 | 0.8 | 1.0 | 1.0 | 1.1 | 1.0 | 0.9 | 1.4 | 1.3 |
| Potri.010G063500 | AMT1.1 | 1.3 | 1.5 | 1.2 | 1.4 | 1.1 | 1.1 | 1.7 | 1.6 |
| Potri.019G023600 | AMT1.2 | 0.8 | 3.9 | 0.5 | 2.2 | 0.6 | 4.4 | 0.6 | 4.1 |
| Potri.001G305400 | AMT3.1 | 1.3 | 11.2 | 0.4 | 3.3 | 1.3 | 16.3 | 0.5 | 6.4 |

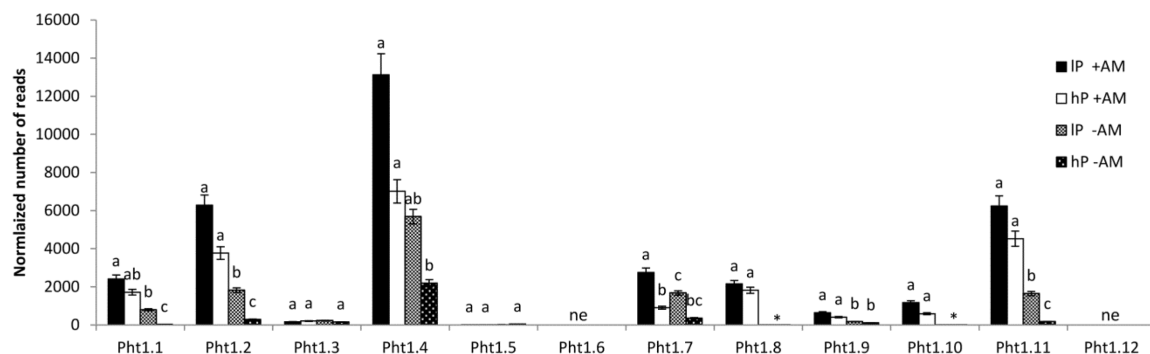
* Pht1.6 and Pht1.12 were not expressed
** Pht1.3 only detected by RNA-Seq in all four conditions, but not by qPCR
*** Pht1.8 and Pht1.10 were only detected in +AM condition by qPCR. By RNA-Seq less than 10 reads were recorded in –AM condition



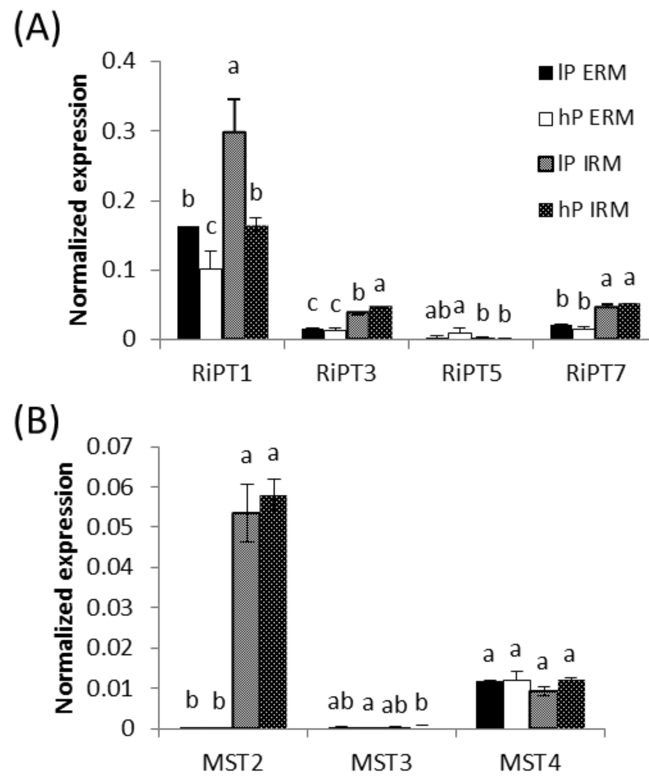

**Supplementary Fig. S5.1. Experimental growth systems.** For a deeper understanding of symbiotic P transport microcosms with tripartite compartments (mycorrhizal treatment; +AM) or single compartments (non-mycorrhizal treatments; -AM) were set-up. All compartments contained a mixture of sand: zeolithe (1:1; w:w). In the mycorrhizal treatments a *P. trichocarpa* (poplar) cutting was planted in the middle compartment and *S. bicolor* (sorghum) seedlings were planted in the right compartment to create a common mycorrhizal network and to increase poplar root colonization. Both poplar and sorghum were inoculated with the AM fungus *R. irregularis* and were fertilized once a week with Hoagland solution without phosphorus (P). To obtain extraradical mycelium (ERM), P containing Hoagland solution (low-P, 28 µM; high-P, 560 µM) was applied in a third compartment where only the mycorrhizal fungus had access to it. In the non-mycorrhizal treatment poplar cuttings and sorghum seedling grew separately from each other in a single compartment. The control plants received the P containing fertilizer directly into their root compartment.



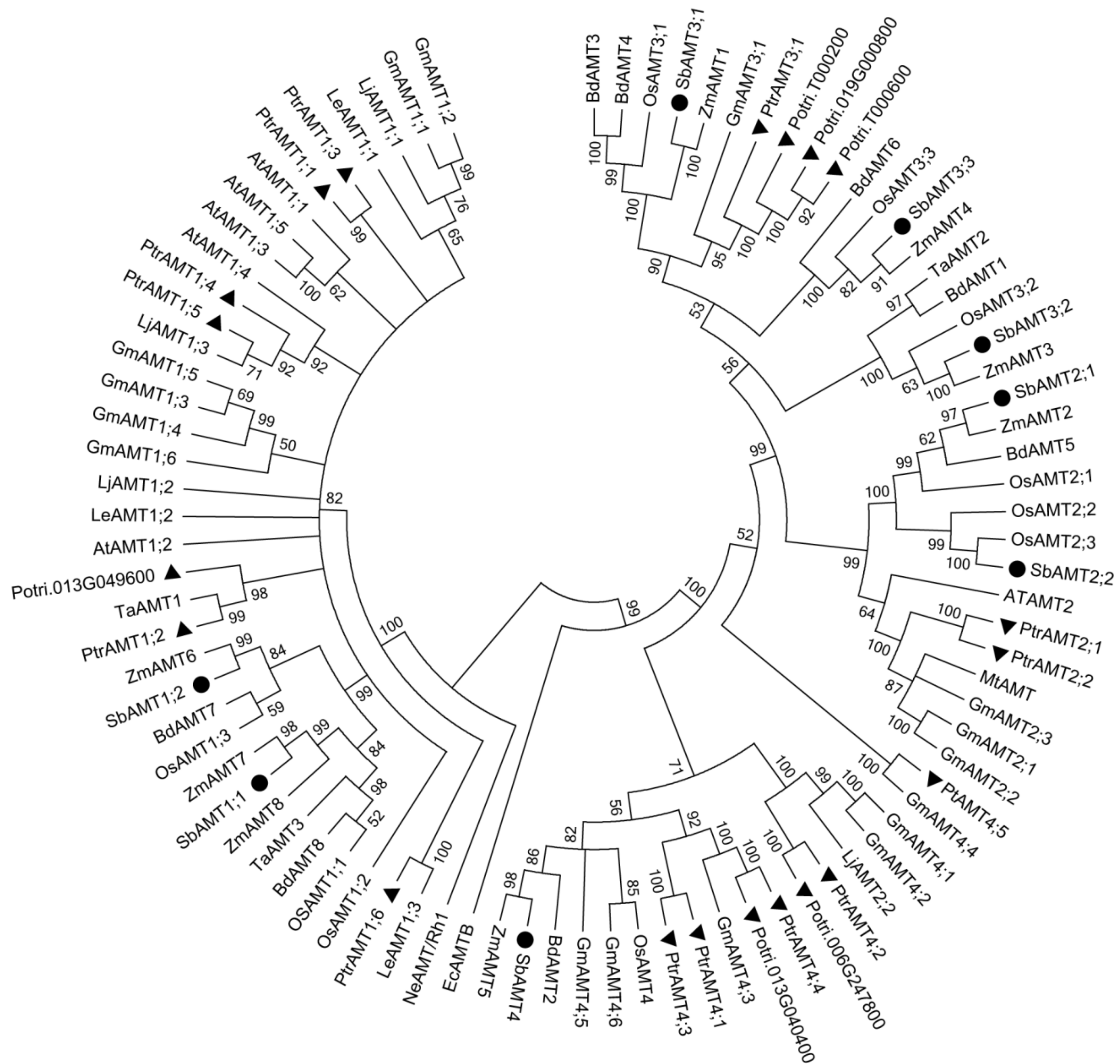

**Supplementary Fig. S5.2. Shoots and roots P content of mycorrhizal (+AM) and non-mycorrhizal (-AM) *P. trichocarpa* and *S. bicolor* plants.** P content was measured in low-P (lP) and high-P (hP) treatments. Values are means of six biological replicates. Differences between treatments were estimated by analysis of variance (ANOVA) per plant species and organ, followed by Tuckey honestly significant test (Tuckey HSD; $p<0.05$). Statistical differences are indicated by lower case letters.



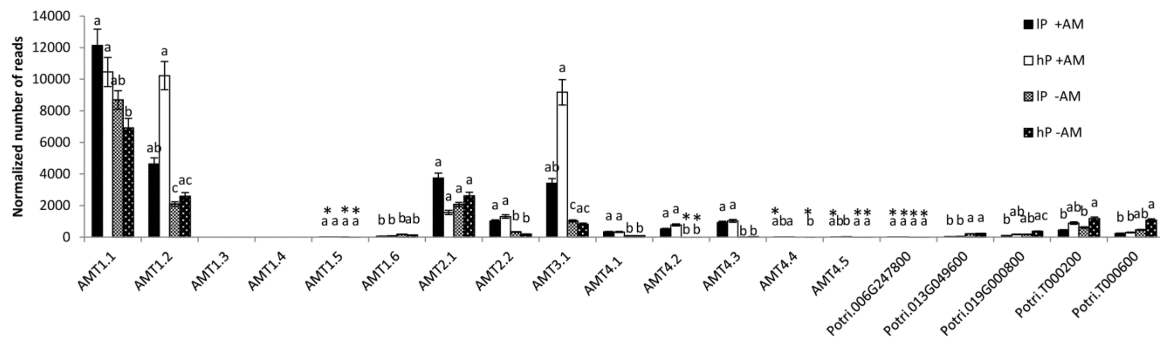

**Supplementary Fig. S5.3. Quantification by mRNA – Sequencing (RNAseq) of the transcript abundances of Pht1 phosphate transporters in the roots of *P. trichocarpa.*** Transcript abundances of the 12 Pht genes in *P. trichocarpa* were quantified in mycorrhized (+AM) and non-mycorrhized (-AM) roots in high-P (hP) and low-P (lP) treatment. PtrPht1.6 and PtrPht1.12 were not expressed (ne). Bars labeled with an asterisk had less than 10 reads and were considered as not expressed. Significant differences were estimated per gene using the Wald test. Lower case letters indicate significant differences in transcript abundances (p-value < 0.05). Numbers of reads in the three biological replicates per condition were normalized per gene using cufflink and cuffquant.



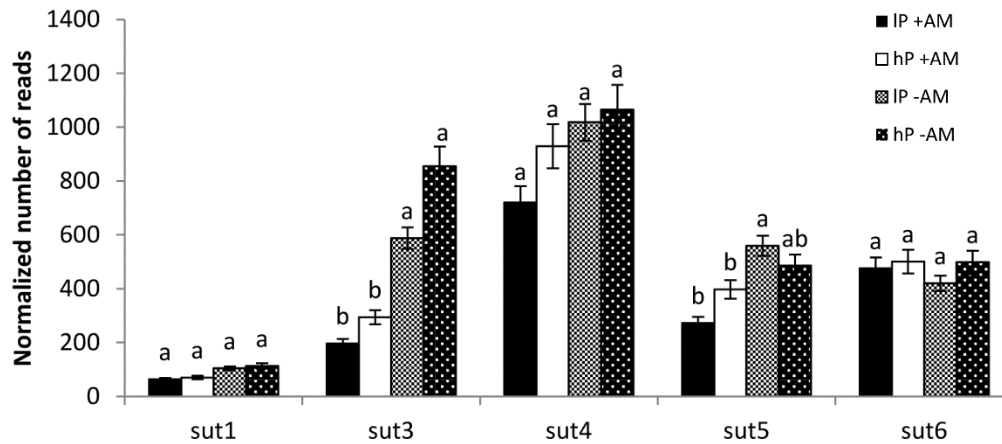

**Supplementary Fig. S5.4. Quantification by qRT-PCR of the transcript abundances of phosphate and monosaccharide transporters in *R. irregularis*.** Quantification of transcript levels of phosphate (A) and monosaccharide (B) transporters in the ERM and in the IRM of the host plant sorghum high-P (hP) and low-P (lP) conditions. Values are means of three biological and three technical replicates. Error bars represent the SE. Translational elongation factor was used as a reference transcript. Statistical analysis was performed by analysis of variance for each gene, followed by Tuckey honest significant difference test (Tuckey HSD; $p<0.05$)



**Supplementary Fig. S5.5.** Neighbor joining tree of plant ammonium transporters (AMTs). Bootstrap values are derived from 1000 replication, only values >50% are displayed. AMTs of poplar are labeled with closed triangle and sorghum AMTs with a closed circle. Phylogenetic tree was constructed using MEGA6.06 package (Tamura *et al.*, 2013).




**Supplementary Fig. S5.6. Quantification by mRNA – Sequencing (RNAseq) of the transcript abundances** of ammonia transporters in the roots of *P. trichocarpa*. Transcript abundances of 19 AMT genes in *P. trichocarpa* when mycorrhized (+AM) or not (-AM) by the AM fungus *R. irregularis* in low-P (lP) and high-P (hP) conditions. Bars labeled with an asterisk had less than ten reads and were considered as not expressed. Significant differences were estimated per gene using the Wald test. Lower case letters indicate significant differences in transcript abundances (p-value < 0.05). Number of reads in the three biological replicates per condition were normalized per gene using cufflink and cuffquant.




**Supplementary Fig. S5.7. Quantification by mRNA – Sequencing (RNAseq) of the transcript abundances** of sugar transporters (SUT) in the roots of *P. trichocarpa*. Transcript abundances of six SUT genes in *P. trichocarpa* were quantified in mycorrhized (+AM) and non-mycorrhized (-AM) roots under high P (hP) and low P (lP) availability. Significant differences were estimated per gene using the Wald test. Lower case letters indicate significant differences in transcript abundances (p-value < 0.05). Number of reads in the three biological replicates per condition were normalized per gene using cufflink and cuffquant.

# 6 General discussion

In natural and agro-ecosystems, nutrient availability plays a key role in plant growth, health and propagation. Depending on the soil composition, nutrient availability varies in quantity, quality and chemical complexity. In agriculture, fertilizers are applied on fields in vast amounts to increase crop yields. But, fertilizers are expensive and pollute the environment when applied inappropriately. Moreover, the macro-element P, an essential nutrient for plant growth and yield, limits crop production in most of the agricultural soil. Moreover, P fertilizers are produced from rock phosphate which is in the verge to run out the next 70-200 years (Schroeder *et al.*, 2013). Therefore, it is of importance to find alternative nutrient sources and to improve nutrient use efficiency of agricultural crops. Transporters are here important targets as they contribute not only to nutrient uptake but also for nutrient distribution in the organisms and they are targets for biotic stress tolerances (i.e. salt and aluminum stress) and increased mobilization of micronutrients from the soil to seeds (Schroeder *et al.*, 2013). The AM symbiosis represents a promising approach to improve nutrient supply in agricultural systems and to naturally recycle nutrients. However, a comprehensive understanding of AM symbiosis under changing environmental conditions is requested. In my thesis, I focused on the analysis of nutrient transport during AM symbiosis between *R. irregularis* and a perennial woody plant *P. trichocarpa* and an annual cereal plant *S. bicolor*, respectively, under N and P-deprived conditions. By using the beneficial traits of nutrient uptake in the AM symbiosis for agricultural crops, it might be possible to reduce fertilizer inputs and to reduce pollution of the environment. Therefore, it is a priority to illuminate the importance and mechanisms of nutrient transport in the AM symbiosis.

## 6.1 AM fungi and their role in symbiotic N and P transfer

An important aspect of the AM symbiosis is the uptake of N and P from the substrate and translocation from the mycorrhizal fungus to the host plant. As a major component of amino- and nucleic acids, N is an essential macronutrient for life. The preferred N source for AM fungi and plants is ammonium, which can be directly assimilated to the GS/GOGAT pathway (Johansen *et al.*, 1996; Marzluf, 1996; Villegas *et al.*, 1996; Hawkins *et al.*, 2000; Bago *et al.*, 2001; Breuninger *et al.*, 2004; Toussaint *et al.*, 2004; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005). In the ERM, ammonium is incorporated into arginine and transported to the IRM (Govindarajulu *et al.*, 2005; Cruz *et al.*, 2007). In arbuscules, ammonium is released and translocated to the periarbuscular space where it is freely available to the plant. Key players for ammonium transport are AMTs that accomplish

transport from the arbuscule to the periarbuscular space and thus make ammonium accessible to the plant.

In AM fungi, only five AMTs have been previously identified (López-Pedrosa *et al.*, 2006; Pérez-Tienda *et al.*, 2011; Ellerbeck *et al.*, 2013). In the AM fungus *G. pyriformis,* three more AMTs have been identified, but these transporters have not been characterized so far (Ellerbeck et al., 2013). First experiments have shown that a biphasic transport system exists in *G. pyriformis* (Ellerbeck et al., 2013). In *R. irregularis,* two AMTs were described (López-Pedrosa et al., 2006; Pérez-Tienda et al., 2011). López-Pedrosa *et al.* (2006) characterized the first AMT as an HATS and predicted the existence of at least one other transporter encoding a LATS in the AM fungus *R. irregularis*. During my thesis, I characterized the third functional AMT in *R. irregularis* as a LATS and showed that it is highly induced in the IRM when compared to the ERM. High induction of GintAMT3 in the arbuscule containing cells in comparison to the previously identified AMTs suggests it to be the main export carrier for ammonium from the arbuscules to the periarbuscular space.

Further, I showed that fungal N metabolism-related genes are not affected by N availability indicating that fungal N metabolism is rather altered by changes in protein turnover or enzyme activity, and/or by metabolite reallocation and interconversion. High expression of enzymes coupled to transport and N metabolism under N-deprivation indicate a high metabolic rate and turnover of N in the IRM (Gomez *et al.*, 2009; Guether *et al.*, 2009). However, the amount of transferred nutrients does not correlate necessarily with transporter expression levels (Walder *et al.,* 2015). Nevertheless, data indicate that the plant receives N also upon nutrient shortening, which may be exploited to improve plant growth in nutrient-poor soils or in low-input agro-ecosystems. To verify that metabolic rates are indeed high under nutrient deprivation and nutrient transfer from the AM fungus to the plant is efficient under those conditions, further investigations are needed.

Apart from N, P is also of great importance for symbiotic nutrient exchange. P is taken up by AM fungal PT located in the membrane of the ERM, incorporated into poly-P and transported to the IRM (Ezawa *et al.*, 2002; Javot *et al.*, 2007). Only few AM fungal PTs are known. In *G. versiforme* (Harrison and van Buuren, 1995) and *G. mosseae* (Benedetto et al., 2005), only one PT has been identified (Maldonado-Mendoza *et al.*, 2001). But, in *R. irregularis* the number of described PT has recently increased to seven (Walder *et al.*, 2016), suggesting that also the genome of other AM fungi encode for several PTs. To elucidate the symbiotic P transfer, I investigated PT expression in the ERM and IRM under contrasting P conditions. I showed that PT expression is regulated depending on nutrient availability in both, the ERM and IRM. Moreover, I measured an induction

of PTs under P and N deficiency, indicating that under nutrient limiting conditions the AM fungus transfers more macronutrients to the host plant. Consistent with previous studies, I have shown that under nutrient deficiency the mycorrhizal and non-mycorrhizal plant down-regulates its defense mechanisms, likely to stimulate symbiotic interaction and to overcome nutrient deficiency. Together with the induction of fungal nutrient transporters under nutrient deficiency in the fungal mycelium, the data indicate that nutrient deficient conditions are favorable conditions to promote AM symbiosis. Under nutrient limiting conditions, the AM fungus offers the plant more nutrients and in return the plant provides carbohydrates as it requires the essential N and P.

Differential expression of nutrient transporters in the ERM and IRM shows that the AM fungus is able to regulate local expression of nutrient transporters. Strong induction of nutrient transporters in the IRM indicates that the transporters are located at the arbuscular membrane and actively transport nutrients from the AM fungus to the host plant. However, to determine the exact localization, an immunolocalization of transporters is necessary.

Taken together, my data show that the AM fungus adapts transporter expression according to nutrient availability. Even if the hyphae of AM fungi are not septated, the studied PTs and AMTs were differentially expressed in the ERM and IRM, which suggests a regulatory mechanism that spatially restricts gene regulation. Further it seems that under nutrient limiting conditions the AM fungus transfers essential macro nutrients to the host plants. This beneficial trait may be exploited to improve agriculture in areas with nutrient shortage.

## 6.2 **AM-inducible transporters: a relict from old times**

It is assumed that AM fungi are over 400 million years old and that they enabled colonization of the land by plants as, with their rudimentary root system, the plants were not able to efficiently extract nutrients from the new substrate (Remy *et al.*, 1994; Harrison, 1999; Redecker *et al.*, 2000; Smith and Read, 2008). By forming symbiosis, the AM fungus supplied the plant with essential nutrients while the plant adapted to the new environmental condition. The basis of this nutrient exchanges are specialized transporters, which allow nutrient exchange between symbionts. In root cortical cells, the AM fungus forms arbuscules, which are the site of mineral nutrient transfer from the AM fungus to the host plant and from the plant to the fungus. Fungal transporters located in the arbuscular membrane transport nutrients from the arbuscule to the PAS. The PAM surrounds the dichotomous branches of the arbuscules and the nutrient transporters in the PAM enables nutrient uptake from the PAS. In recent years, more and more plant transporters were found to be specifically induced upon root colonization by AM fungi. In arbusculated root, cells specialized

AMTs and PT were induced and localized in the PAM (Rausch *et al.*, 2001; Harrison *et al.*, 2002; Glassop *et al.*, 2005; Nagy *et al.*, 2005; Couturier *et al.*, 2007; Gomez *et al.*, 2009; Guether *et al.*, 2009; Kobae *et al.*, 2010; Loth-Pereda *et al.*, 2011; Koegel *et al.*, 2013). Consistent with these findings, I demonstrated that root colonization induced specific expression of known and novel AMTs and PTs in poplar and sorghum plants. Specific induction of these AM-inducible transporters in roots suggests that the function of these transporters is nutrient uptake at the plant-fungal interface. Further, I observed specific induction of nutrient transporters in shoots when plant roots are colonized, indicating increased metabolic rates due to the improved nutrition by the AM fungus. By improving plant nutrition, the plant will gain in fitness and increase seed production. On the other hand, the AM fungus will profit by the increased carbohydrate production of the plant.

To gain further insight into nutrient transfer in AM symbiosis, I measured transporter expression changes in mycorrhizal plants. Specific induction of amino acid transporters and $H^+$/oligopeptide symporters in mycorrhizal root tissue indicates that also amino acids are transferred between the symbionts providing an additional N and/or sulfur source for the plant. This hypothesis is consistent with the finding that root colonization led to major changes in the sulfur assimilation pathway. A beneficial effect of sulfur nutrition has been already reported (Casieri *et al.*, 2012; Sieh *et al.*, 2013; Giovannetti *et al.*, 2014; Gerlach *et al.*, 2015).

To support my conclusions further investigations to determine predicted localization of AMT and amino acid transporters are needed. Additional experiments will also clarify which amino acids are transported. Mass spectrometry imaging may be a way to reveal new insights at the plant-fungal interface at the single cell level, as it allows the analysis of metabolites, proteins and peptides of thin sample sections at high spatial resolution (McDonnell and Heeren, 2007; Wiseman *et al.*, 2008).

## 6.3 **Mycorrhizal plants and their dependence on the arbuscular mycorrhizal symbiont**

The majority of land plant species form symbioses with AM fungi, although some plant families that do not form AM symbioses exist. Non-mycorrhizal plants can be found in different, evolutionary rather young clades, strongly indicating that these plant species have lost their ability to form functional AM symbiosis (Brundrett, 2002; Paszkowski, 2006). Here, I compared the effects of nutrient availability on expression of poplar and sorghum nutrient transporters in the presence and absence of mycorrhizal symbiosis with *R. irregularis*. My work revealed that the annual crop sorghum is more dependent to AM symbiosis than the perennial wooden plant poplar. Dependeny to symbiosis was correlated by increased uptake of P via the AM fungus as well

as by the regulation of nutrient transporters. Equal low expression of plant AMT and PT in mycorrhizal roots was comparable to transporter expression in non-mycorrhizal roots, whereas P deficiency induced expression of these transporters in non-mycorrhizal roots. This suggests that the AM fungus sufficiently transferred nutrients to the plant and covered P needs of the crop. Annual plants in comparison to perennial plants need to reach maturity rather quickly and therefore are more willing to trade with the AM symbionts on expense of the carbohydrate.

For poplar, I could show that PT expression was correlated with P concentration in the substrate and P concentration inside the plant, respectively. In mycorrhizal roots, the expression of these transporters was not affected. In perennial plants, a symbiosis-independent nutrition is important as mycorrhizal abundance varies with the seasons (Courty *et al.*, 2008; Dumbrell *et al.*, 2011).

## 6.4 **Carbohydrates: a good day's wages for a good day's work**

The plant's payments for the fungal supply of mineral nutrients are carbohydrates. Several times it was shown that carbohydrates are transferred from the plant to the AM symbiont but until today it is not clear how and by which mechanism the carbohydrates are transferred. In recent years, more and more sugar transporters were identified and their role in plant sugar transport is currently being elucidated (reviewed in (Doidy *et al.*, 2012; Casieri *et al.*, 2013). However, the role of sugar transporters in AM symbiosis needs still to be clarified. In the model species *M. truncatula*, induction of a sugar transporter upon AM colonization was observed, which suggested that sugars are actively transferred from the plant to the AM fungus (Doidy *et al.*, 2012). Several studies concerned with symbiotic carbohydrate exchange reported induction of sucrose-cleaving enzymes, which support the general assumption that in AM colonized root sugars are cleaved to monosaccharides and transferred to the AM fungus (Wright *et al.*, 1998; Ravnskov *et al.*, 2003; García-Rodríguez *et al.*, 2007; Tejeda-Sartorius *et al.*, 2008).

My study enabled the investigation of plant carbohydrate transporters in the model species poplar and sorghum. Astonishingly, we observed the down-regulation of plant carbohydrate transporter in mycorrhizal roots when colonized by the AM fungus. In the AM fungus *R. irregularis*, several MSTs were identified (Helber *et al.*, 2011). The MST RiMST2 was shown to be expressed in the arbuscules and in the hyphae of the IRM. The down-regulation of carbohydrate transporters and the fact that RiMST2 was also found to be expressed in the IRM suggests that the fungus itself takes up carbohydrates from the apoplast. Down-regulated expression of plant carbohydrate transporters upon root colonization results in limited access to those nutrients by the fungus. Hence, the plant may restrict access of the fungus to carbohydrates to minimize the loss of

carbohydrates while still receiving mineral nutrients. On the other hand, the AM ensures that it gets enough carbohydrates from the host plant by taking up the carbohydrates in addition to the carbohydrates given by the plant. However, an active sugar transport from the plant to the fungus cannot be excluded as we also observed induction of one predicted carbohydrate transporter in colonized roots.

## 6.5 General conclusion and outlook

My research helped to deepen our understanding of basic mechanism of symbiotic nutrient exchange. Nutrient exchange is a tightly linked interplay of fungal and plant transporters. The picture emerges that the plants try to get as many mineral nutrients from the AM symbionts but are willing to pay only the minimum. Such unequal terms of trade were already observed in a microcosm study in which the AM fungi delivered mineral nutrients to the host plants flax and sorghum but did not receive equal amounts of carbohydrates in exchange (Walder *et al.* 2012). A subsequent transporter expression study could not explain the differences of nutrient uptake in the two plants (Walder *et al.*, 2015). One factor complicating the understanding of plant mycorrhizal nutrient transfer is the fact that transporter expression alone is not sufficient to estimate the amounts of transferred nutrients from AM fungus to the plant or vice versa. Affinities of single transporters play also a significant role in the transport rates of mineral nutrients. To gain further insight into plant-mycorrhizal nutrient exchange a combination of several biochemical and cell biological methods is needed. It is necessary to determine the localization of transporters and their physiological properties. By using immune localization or fluorescently tagged transporters it is possible to determine protein localization. Patch clamp and heterologous expression experiments could give us more information about physiological properties of the transporters. Here are of special interest AM-inducible transporters, which may differ from transporters responsible for nutrient uptake from the substrate or for inter cellular distribution. To identify the importance of the transporters for symbiotic nutrient exchange and in order to see how important single transport components are for the maintenance of the symbiotic structures it will be necessary to analyze knockout mutants or to knockdown the genes of interest. If knockdown of single transporters or of a combination of transporters would lead to degeneration symbiotic structures than this would mean that these transporters are essential for the symbiosis.

Further, it would be interesting to know whether also amino acids are transferred from the AM fungi to the plant. Amino acids could be an additional source for the plant to receive N and sulfur. Using matrix-assisted laser desorption ionization imaging mass spectrometry would allow to

determine metabolites in colonized root cells as well as spatial distribution of proteins within the samples.

With comprehensive knowledge of mycorrhizal symbiosis under different environmental conditions, it may, in future, be possible to reduce application of mineral fertilizers so that the plant needs can be covered via the mycorrhizal symbiont (Zhang *et al.*, 2014). It may also be possible to optimize fertilizer composition as the AM fungi are able to take up nutrients from more complex molecules. An optimization of fertilizer to AMs capacity to take up nutrients from complex matter might reduce the diffusion rate and leakage of e.g. nitrate into the ground water. My work presented in this thesis contributed an important piece to the puzzle of symbiotic nutrient exchange and helped to get a step closer to reaching these goals.

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# 8 Appendix

**Table A1 Relative abundances of metabolites detected in the ERM of *R. irregularis*.** Data was log transformed and tested by Wilcoxon rank sum test ($p<0.05$, n=3) in MeV v4.9 (http://www.tm4.org/mev.html). Significant p-values are highlighted in bold.

| Class | Name | Average abundance (x1000) | | Standard deviation | | log2 ratio lP vs hP | p-value |
|---|---|---|---|---|---|---|---|
| | | lP ERM | hp ERM | lP ERM | hp ERM | | |
| Acids | Aconitic acid, cis- | 0.4 | 0.6 | 0.1 | 0.2 | -0.53 | 0.275 |
| Acids | Benzoic acid | 7.7 | 7.6 | 0.9 | 0.5 | 0.02 | 0.827 |
| Acids | Benzoic acid, 4-hydroxy- | 1.0 | 1.0 | 0.2 | 0.2 | 0.04 | 0.827 |
| Acids | Citric acid | 1.3 | 1.1 | 0.4 | 0.2 | 0.25 | 0.827 |
| Acids | Fumaric acid | 3.7 | 2.9 | 1.8 | 0.6 | 0.36 | 0.513 |
| Acids | Isocitric acid | 0.9 | 0.8 | 0.3 | 0.2 | 0.32 | 0.513 |
| Acids | Lactic acid | 9.0 | 11.4 | 3.6 | 2.7 | -0.34 | 0.275 |
| Acids | Malic acid | 0.3 | 0.2 | 0.1 | 0.1 | 0.60 | 0.275 |
| Acids | Pyruvic acid | 19.5 | 18.0 | 1.2 | 1.7 | 0.11 | 0.275 |
| Acids | Quinic acid | 0.1 | 0.4 | >0.1 | 0.4 | -1.88 | 0.513 |
| Acids | Succinic acid | 1.7 | 1.4 | 0.4 | 0.2 | 0.26 | 0.827 |
| Alcohols | Benzylalcohol | 2.8 | 3.1 | 0.7 | 0.4 | -0.18 | 0.513 |
| Amino Acids | Aspartic acid | 1.0 | 1.2 | 0.5 | 0.2 | -0.24 | 0.513 |
| Amino Acids | Glutamic acid | 4.7 | 6.3 | 2.4 | 1.2 | -0.44 | 0.513 |
| Amino Acids | Glycine | 13.7 | 7.8 | 5.4 | 2.9 | 0.80 | 0.275 |
| Amino Acids | Isoleucine | 3.9 | 2.5 | 1.6 | 0.2 | 0.63 | 0.513 |
| Amino Acids | Leucine | 7.4 | 6.5 | 0.1 | 0.6 | 0.19 | **0.050** |
| Amino Acids | Lysine | 2.7 | 2.5 | 0.2 | 1.3 | 0.11 | 0.564 |
| Amino Acids | Ornithine | 3.5 | 8.6 | 0.5 | 6.0 | -1.28 | 0.127 |
| Amino Acids | Phenylalanine | 1.0 | 1.1 | 0.4 | 0.3 | -0.12 | 0.827 |
| Amino Acids | Pyroglutamic acid | 9.2 | 9.6 | 3.1 | 0.7 | -0.06 | 0.513 |
| Amino Acids | Serine | 7.9 | 6.9 | 1.9 | 0.5 | 0.19 | 0.827 |
| Amino Acids | Valine | 4.5 | 2.9 | 2.1 | 0.7 | 0.62 | 0.513 |
| N- Compounds | Ethanolamine | 16.3 | 14.8 | 0.8 | 1.7 | 0.15 | 0.275 |
| N- Compounds | Putrescine | 21.6 | 19.6 | 0.7 | 2.8 | 0.14 | 0.513 |
| N- Compounds | Pyridine, 2-hydroxy- | 21.4 | 21.0 | 2.1 | 4.1 | 0.02 | 0.827 |
| Phenylpropanoids | Caffeic acid, trans- | 1.3 | 1.3 | 0.2 | 0.2 | -0.03 | 1.000 |
| Phosphates | Phosphoric acid | 0.5 | 0.4 | 0.1 | 0.0 | 0.25 | 0.513 |
| Phosphates | Phosphoric acid monomethyl ester | 5.9 | 6.2 | 3.2 | 2.1 | -0.06 | 0.827 |
| Polyols | Arabitol | 0.4 | 0.4 | 0.1 | 0.1 | -0.07 | 0.827 |
| Polyols | Glycerol | 9.5 | 11.2 | 2.4 | 0.7 | -0.24 | 0.513 |
| Polyols | Inositol, myo- | 0.6 | 0.6 | 0.2 | 0.1 | 0.05 | 0.827 |
| Polyols | Mannitol | 3.7 | 6.9 | 4.0 | 5.1 | -0.92 | 0.513 |
| Sugar Conjugates | Galactinol | 0.7 | 3.6 | 1.0 | 3.6 | -2.29 | 0.248 |
| Sugar Conjugates | Salicin | 0.2 | 4.7 | 0.2 | 4.7 | -4.25 | 1.000 |
| Sugars | Glucose | >0.1 | >0.1 | >0.1 | >0.1 | 0.41 | 0.127 |
| Sugars | Glucose, 1,6-anhydro-, beta- | 7.4 | 6.9 | 0.7 | 1.3 | 0.11 | 0.513 |
| Sugars | Rhamnose | 2.1 | 1.9 | 0.5 | 0.3 | 0.16 | 0.513 |
| Sugars | Ribose | 0.8 | 1.0 | 0.2 | 0.1 | -0.32 | 0.564 |
| Sugars | Sucrose | 0.3 | 0.3 | 0.1 | 0.2 | -0.06 | 0.827 |
| Sugars | Trehalose, alpha,alpha'- | 26.6 | 20.3 | 4.2 | 3.4 | 0.39 | 0.127 |

**Table A2 Relative abundances of metabolites detected in poplar roots**. Abundances were measured in the mycorrhized (+AM) and non-mycorrhized (-AM) poplar roots under high (hP) and low (lP) P availability. Data was log transformed and tested by y 2-way analysis of variance (ANOVA) ($p<0.05$, n=3) in MeV v4.9 (http://www.tm4.org/mev.html). Significant p-values are highlighted in bold.

| Class | Name | Average abundance (x1000) | | | | | | Standard deviation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | +AM | -AM | lP +AM | hP +AM | lP- AM | hP - AM | +AM | -AM | lP +AM | hP +AM | lP - AM | hP - AM |
| Acids | Aconitic acid, cis- | 1.1 | 3.4 | 1.1 | 1.1 | 3.2 | 3.6 | 0.4 | 1.5 | 0.2 | 0.5 | 0.8 | 2.0 |
| Acids | Benzoic acid | 9.7 | 15.0 | 9.1 | 10.3 | 18.7 | 11.3 | 1.4 | 5.2 | 1.4 | 0.9 | 4.7 | 2.1 |
| Acids | Benzoic acid, 3,4-dihydroxy- | 17.1 | 19.1 | 16.6 | 17.7 | 19.9 | 18.2 | 5.8 | 7.3 | 6.5 | 5.0 | 4.2 | 9.3 |
| Acids | Benzoic acid, 4-hydroxy- | 18.0 | 16.9 | 17.4 | 18.7 | 21.6 | 12.1 | 6.0 | 6.3 | 7.9 | 3.1 | 3.2 | 4.9 |
| Acids | Citric acid | 25.4 | 18.5 | 25.9 | 24.8 | 22.6 | 14.4 | 3.0 | 6.6 | 3.5 | 2.3 | 1.8 | 7.1 |
| Acids | Fumaric acid | 10.1 | 18.4 | 10.9 | 9.2 | 20.6 | 16.2 | 3.6 | 6.0 | 3.7 | 3.3 | 2.8 | 7.4 |
| Acids | Glutaric acid, 2-hydroxy- | 5.9 | 17.7 | 5.3 | 6.4 | 21.2 | 14.2 | 3.1 | 5.2 | 1.7 | 3.9 | 3.7 | 3.9 |
| Acids | Glutaric acid, 2-oxo- | 4.0 | 12.9 | 4.2 | 3.7 | 19.6 | 6.3 | 2.0 | 7.6 | 2.1 | 2.0 | 4.9 | 1.3 |
| Acids | Glutaric acid, 3-hydroxy-3-methyl- | 9.4 | 17.2 | 8.2 | 10.6 | 21.8 | 12.5 | 4.4 | 6.3 | 3.7 | 4.8 | 3.2 | 5.1 |
| Acids | Isocitric acid | 22.9 | 15.4 | 24.3 | 21.4 | 19.8 | 10.9 | 5.3 | 6.2 | 5.8 | 4.3 | 1.9 | 5.8 |
| Acids | Lactic acid | 7.0 | 11.8 | 5.4 | 8.7 | 16.9 | 6.6 | 2.7 | 6.8 | 2.1 | 2.1 | 6.0 | 1.7 |
| Acids | Malic acid | 17.4 | 16.6 | 16.2 | 18.7 | 20.7 | 12.6 | 6.5 | 4.3 | 5.6 | 7.0 | 1.5 | 1.4 |
| Acids | Malic acid, 2-isopropyl- | ND | 13.6 | ND | ND | 18.3 | 8.9 | ND | 5.9 | ND | ND | 5.0 | 0.9 |
| Acids | Malic acid, 2-methyl- | ND | 16.5 | ND | ND | 21.2 | 11.8 | ND | 5.6 | ND | ND | 3.8 | 2.1 |
| Acids | Pyruvic acid | 20.9 | 16.9 | 21.3 | 20.6 | 18.6 | 15.2 | 3.6 | 3.6 | 4.6 | 2.1 | 1.8 | 4.1 |
| Acids | Quinic acid | 3.7 | 4.1 | 2.7 | 4.7 | 5.4 | 2.8 | 1.5 | 2.3 | 1.0 | 1.3 | 1.9 | 1.8 |
| Acids | Shikimic acid | 2.6 | 12.8 | 1.9 | 3.2 | 16.3 | 9.3 | 0.9 | 3.8 | 0.1 | 1.0 | 1.0 | 1.8 |
| Acids | Succinic acid | 8.2 | 14.8 | 8.3 | 8.1 | 18.4 | 11.1 | 4.0 | 4.3 | 2.4 | 5.0 | 2.7 | 1.8 |
| Acids | Vanillic acid | 14.5 | 17.7 | 15.4 | 13.6 | 20.9 | 14.6 | 4.9 | 4.8 | 4.8 | 4.9 | 3.2 | 4.0 |
| Alcohols | Benzylalcohol | 9.4 | 12.2 | 9.1 | 9.7 | 16.0 | 8.3 | 1.3 | 6.4 | 1.5 | 1.0 | 6.7 | 2.8 |
| Amino Acids | Aspartic acid | 0.7 | 15.5 | 0.9 | 0.5 | 18.6 | 12.4 | 0.3 | 6.0 | 0.4 | 0.2 | 6.1 | 4.1 |
| Amino Acids | Butanoic acid, 4-amino- | 0.8 | 6.1 | 0.9 | 0.6 | 7.2 | 5.1 | 0.2 | 2.0 | 0.1 | 0.1 | 2.3 | 0.5 |
| Amino Acids | Glutamic acid | 1.8 | 12.8 | 2.5 | 1.0 | 9.2 | 16.5 | 1.2 | 4.9 | 1.3 | 0.6 | 1.6 | 4.2 |
| Amino Acids | Glycine | 4.8 | 9.0 | 5.5 | 4.1 | 11.0 | 7.1 | 1.5 | 3.2 | 1.5 | 1.3 | 2.7 | 2.3 |
| Amino Acids | Isoleucine | 4.4 | 14.9 | 5.2 | 3.6 | 18.2 | 11.5 | 1.4 | 4.8 | 1.3 | 1.1 | 4.9 | 0.9 |
| Amino Acids | Leucine | 7.1 | 16.8 | 6.9 | 7.3 | 18.8 | 14.9 | 1.1 | 4.9 | 1.3 | 0.7 | 3.0 | 5.7 |
| Amino Acids | Phenylalanine | 1.4 | 15.5 | 1.7 | 1.0 | 18.9 | 12.0 | 0.5 | 5.1 | 0.5 | >0.1 | 4.5 | 2.6 |
| Amino Acids | Pyroglutamic acid | 5.4 | 14.7 | 5.8 | 4.9 | 14.4 | 14.9 | 1.0 | 5.3 | 0.7 | 1.1 | 2.1 | 7.2 |
| Amino Acids | Serine | 6.8 | 14.5 | 7.9 | 5.8 | 17.4 | 11.6 | 1.5 | 4.1 | 1.3 | 0.7 | 4.2 | 0.6 |
| Amino Acids | Valine | 2.9 | 14.6 | 2.8 | 3.0 | 18.4 | 10.7 | 0.7 | 5.5 | 0.7 | 0.6 | 5.6 | 0.5 |
| Aromatic | Catechol | 2.7 | 6.7 | 3.9 | 1.5 | 10.6 | 2.9 | 3.2 | 8.4 | 4.2 | 0.5 | 10.5 | 1.8 |
| N- Compounds | Ethanolamine | 16.6 | 17.2 | 15.7 | 17.6 | 17.1 | 17.3 | 1.5 | 4.2 | 1.4 | 0.8 | 2.5 | 5.3 |
| N- Compounds | Phenol, 2-amino- | 5.1 | 12.4 | 5.5 | 4.7 | 16.0 | 8.8 | 1.9 | 5.3 | 2.6 | 0.7 | 4.2 | 3.6 |
| N- Compounds | Putrescine | 23.0 | 20.2 | 23.2 | 22.8 | 21.6 | 18.9 | 3.9 | 4.1 | 3.3 | 4.4 | 1.2 | 5.3 |
| N- Compounds | Pyridine, 2-hydroxy- | 19.1 | 20.3 | 17.0 | 21.3 | 22.6 | 17.9 | 4.6 | 5.0 | 4.0 | 4.0 | 1.1 | 6.2 |
| Phenylpropanoids | Caffeic acid, cis- | 16.3 | 14.7 | 16.5 | 16.2 | 19.1 | 10.2 | 2.5 | 5.5 | 3.1 | 1.6 | 1.8 | 4.1 |
| Phenylpropanoids | Caffeic acid, trans- | 14.4 | 14.9 | 14.7 | 14.1 | 20.5 | 9.3 | 2.6 | 6.6 | 3.0 | 2.1 | 2.3 | 4.3 |
| Phenylpropanoids | Cinnamic acid, 4-hydroxy-, trans- | 5.6 | 9.8 | ND | ND | 11.8 | 7.8 | 0.0 | 2.9 | ND | ND | 0.5 | 2.9 |
| Phenylpropanoids | Epicatechin | 15.1 | 16.7 | 14.3 | 16.0 | 20.2 | 13.3 | 3.4 | 5.0 | 2.9 | 3.7 | 2.7 | 4.4 |
| Phenylpropanoids | Ferulic acid, trans- | 14.7 | 17.1 | 15.3 | 14.3 | 21.1 | 11.0 | 4.0 | 5.0 | 4.0 | 4.0 | 0.4 | 1.2 |
| Phenylpropanoids | Quinic acid, 3-caffeoyl-, cis- | 16.7 | 8.3 | 17.6 | 15.8 | 8.5 | 8.1 | 6.9 | 5.4 | 9.4 | 2.4 | 2.7 | 7.1 |
| Phenylpropanoids | Quinic acid, 3-caffeoyl-, trans- | 15.3 | 8.8 | 16.0 | 14.6 | 10.0 | 7.5 | 6.6 | 5.2 | 9.1 | 2.3 | 1.4 | 7.0 |
| Phosphates | Fructose-6-phosphate | 4.8 | 17.3 | 3.9 | 5.6 | 16.0 | 18.6 | 1.6 | 5.7 | 0.5 | 1.9 | 5.1 | 6.0 |
| Phosphates | Glucose-6-phosphate | 3.4 | 15.7 | 2.1 | 4.6 | 13.4 | 18.0 | 1.8 | 5.3 | 0.6 | 1.7 | 4.8 | 4.6 |
| Phosphates | myo-Inositol-phosphate | 0.6 | 2.0 | ND | ND | 1.2 | 2.7 | 0.0 | 1.5 | ND | ND | 0.1 | 1.9 |
| Phosphates | Phosphoric acid | 0.6 | 6.3 | 0.6 | 0.5 | 1.5 | 11.0 | 0.3 | 11.0 | 0.4 | 0.0 | 0.1 | 14.0 |
| Phosphates | Phosphoric acid monomethyl ester | 3.3 | 16.8 | 2.9 | 3.7 | 16.5 | 17.1 | 1.2 | 8.2 | 1.0 | 1.3 | 7.0 | 9.2 |

**Table A2** continued

| Name | log2 ratios | | | | | 2-way ANOVA | | |
|---|---|---|---|---|---|---|---|---|
| | +AM vs -AM | lP+AM vs hP+AM | lP-AM vs hP -AM | lP-AM vs hP - AM | hP +AM vs hP- AM | Effect of mycorrhization | Effect of p-availability | Effect of interaction |
| Aconitic acid, cis- | -1.62 | -0.01 | -0.17 | -1.54 | -1.69 | **0.006** | 0.863 | 0.934 |
| Benzoic acid | -0.63 | -0.18 | 0.72 | -1.03 | -0.13 | **0.013** | 0.182 | **0.034** |
| Benzoic acid, 3,4-dihydroxy- | -0.16 | -0.09 | 0.13 | -0.27 | -0.05 | 0.750 | 0.839 | 0.602 |
| Benzoic acid, 4-hydroxy- | 0.09 | -0.10 | 0.83 | -0.32 | 0.62 | 0.767 | 0.426 | 0.162 |
| Citric acid | 0.46 | 0.06 | 0.65 | 0.20 | 0.78 | 0.054 | 0.125 | 0.172 |
| Fumaric acid | -0.87 | 0.25 | 0.34 | -0.92 | -0.82 | **0.038** | 0.325 | 0.814 |
| Glutaric acid, 2-hydroxy- | -1.59 | -0.28 | 0.58 | -2.00 | -1.14 | **0.003** | 0.546 | 0.420 |
| Glutaric acid, 2-oxo- | -1.70 | 0.18 | 1.64 | -2.21 | -0.75 | **0.009** | 0.111 | 0.180 |
| Glutaric acid, 3-hydroxy-3-methyl- | -0.87 | -0.37 | 0.80 | -1.41 | -0.24 | 0.065 | 0.549 | 0.199 |
| Isocitric acid | 0.57 | 0.18 | 0.86 | 0.29 | 0.97 | 0.054 | 0.084 | 0.190 |
| Lactic acid | -0.74 | -0.68 | 1.36 | -1.65 | 0.39 | 0.083 | 0.389 | **0.013** |
| Malic acid | 0.07 | -0.21 | 0.72 | -0.35 | 0.57 | 0.958 | 0.412 | 0.135 |
| Malic acid, 2-isopropyl- | ND | ND | 1.04 | ND | ND | ND | ND | ND |
| Malic acid, 2-methyl- | ND | ND | 0.85 | ND | ND | ND | ND | ND |
| Pyruvic acid | 0.31 | 0.05 | 0.29 | 0.20 | 0.44 | 0.147 | 0.400 | 0.454 |
| Quinic acid | -0.15 | -0.79 | 0.92 | -0.98 | 0.72 | 0.895 | 0.723 | 0.092 |
| Shikimic acid | -2.32 | -0.73 | 0.82 | -3.08 | -1.53 | **0.000** | 0.658 | **0.008** |
| Succinic acid | -0.85 | 0.04 | 0.72 | -1.14 | -0.46 | **0.032** | 0.212 | 0.552 |
| Vanillic acid | -0.29 | 0.18 | 0.51 | -0.44 | -0.10 | 0.353 | 0.258 | 0.631 |
| Benzylalcohol | -0.37 | -0.09 | 0.95 | -0.81 | 0.23 | 0.479 | 0.180 | 0.104 |
| Aspartic acid | -4.52 | 0.82 | 0.59 | -4.43 | -4.66 | **0.000** | 0.134 | 0.845 |
| Butanoic acid, 4-amino- | -3.03 | 0.68 | 0.51 | -2.96 | -3.13 | **0.000** | **0.032** | 0.572 |
| Glutamic acid | -2.87 | 1.27 | -0.85 | -1.88 | -4.01 | **0.001** | 0.574 | 0.090 |
| Glycine | -0.92 | 0.44 | 0.63 | -0.99 | -0.80 | **0.023** | 0.112 | 0.736 |
| Isoleucine | -1.75 | 0.51 | 0.66 | -1.81 | -1.67 | **0.000** | **0.041** | 0.873 |
| Leucine | -1.25 | -0.06 | 0.34 | -1.44 | -1.04 | **0.001** | 0.483 | 0.306 |
| Phenylalanine | -3.55 | 0.76 | 0.65 | -3.51 | -3.61 | **0.000** | **0.048** | 0.964 |
| Pyroglutamic acid | -1.45 | 0.24 | -0.05 | -1.31 | -1.60 | **0.001** | 0.532 | 0.762 |
| Serine | -1.09 | 0.45 | 0.58 | -1.14 | -1.02 | **0.000** | **0.014** | 0.765 |
| Valine | -2.32 | -0.09 | 0.78 | -2.71 | -1.83 | **0.000** | 0.224 | 0.118 |
| Catechol | -1.33 | 1.43 | 1.85 | -1.43 | -1.00 | 0.245 | 0.276 | 0.598 |
| Ethanolamine | -0.05 | -0.16 | -0.02 | -0.13 | 0.02 | 0.927 | 0.722 | 0.594 |
| Phenol, 2-amino- | -1.28 | 0.26 | 0.87 | -1.53 | -0.91 | **0.019** | 0.242 | 0.306 |
| Putrescine | 0.19 | 0.02 | 0.19 | 0.11 | 0.27 | 0.362 | 0.499 | 0.616 |
| Pyridine, 2-hydroxy- | -0.08 | -0.33 | 0.34 | -0.41 | 0.25 | 0.803 | 0.872 | 0.161 |
| Caffeic acid, cis- | 0.15 | 0.03 | 0.91 | -0.21 | 0.66 | 0.298 | 0.070 | 0.075 |
| Caffeic acid, trans- | -0.05 | 0.06 | 1.14 | -0.48 | 0.60 | 0.666 | 0.056 | 0.070 |
| Cinnamic acid, 4-hydroxy-, trans- | ND | ND | 0.60 | ND | -0.47 | ND | ND | ND |
| Epicatechin | -0.14 | -0.16 | 0.60 | -0.50 | 0.26 | 0.668 | 0.323 | 0.138 |
| Ferulic acid, trans- | -0.12 | 0.10 | 0.94 | -0.47 | 0.37 | 0.703 | 0.066 | 0.121 |
| Quinic acid, 3-caffeoyl-, cis- | 1.01 | 0.16 | 0.07 | 1.05 | 0.96 | 0.102 | 0.745 | 0.572 |
| Quinic acid, 3-caffeoyl-, trans- | 0.80 | 0.13 | 0.42 | 0.68 | 0.96 | 0.155 | 0.487 | 0.347 |
| Fructose-6-phosphate | -1.85 | -0.51 | -0.22 | -2.01 | -1.73 | **0.003** | 0.420 | 0.806 |
| Glucose-6-phosphate | -2.22 | -1.10 | -0.43 | -2.64 | -1.97 | **0.000** | 0.065 | 0.453 |
| myo-Inositol-phosphate | ND | ND | -1.11 | -1.17 | ND | ND | ND | ND |
| Phosphoric acid | -3.48 | 0.45 | -2.86 | -1.22 | -4.54 | **0.042** | 0.634 | 0.468 |
| Phosphoric acid monomethyl ester | -2.35 | -0.39 | -0.05 | -2.53 | -2.20 | **0.001** | 0.648 | 0.676 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Polyhydroxy Acids | Arabinonic acid-1,4-lactone | 11.2 | 13.9 | 13.1 | 9.2 | 18.9 | 6.4 | 2.5 | 7.1 | 0.9 | 1.9 | 4.6 | 1.2 |
| Polyhydroxy Acids | Galactaric acid | 6.9 | 15.5 | 6.3 | 7.4 | 19.8 | 11.3 | 2.8 | 5.8 | 2.3 | 3.1 | 5.1 | 2.5 |
| Polyhydroxy Acids | Galactonic acid | 5.0 | 13.7 | 3.9 | 6.1 | 16.7 | 10.7 | 2.4 | 3.4 | 1.7 | 2.6 | 1.1 | 2.0 |
| Polyhydroxy Acids | Gluconic acid | 2.2 | 4.9 | 2.1 | 2.4 | 6.8 | 2.9 | 1.2 | 2.1 | 0.8 | 1.5 | 1.1 | 0.4 |
| Polyhydroxy Acids | Glyceric acid | 6.0 | 18.9 | 5.5 | 6.8 | 20.3 | 17.6 | 1.1 | 3.9 | 1.0 | 0.7 | 2.1 | 4.8 |
| Polyhydroxy Acids | Gulonic acid | 13.4 | 17.1 | 13.5 | 13.2 | 20.7 | 13.5 | 1.7 | 5.8 | 1.5 | 1.9 | 3.3 | 5.6 |
| Polyhydroxy Acids | Lyxonic acid-1,4-lactone | 13.2 | 14.2 | 14.5 | 11.8 | 18.5 | 9.8 | 2.2 | 7.0 | 0.8 | 2.2 | 4.8 | 6.0 |
| Polyhydroxy Acids | Ribonic acid | 7.6 | 16.9 | 8.7 | 6.5 | 19.8 | 13.9 | 1.7 | 4.7 | 1.9 | 0.1 | 3.1 | 4.1 |
| Polyhydroxy Acids | Saccharic acid | 4.2 | 14.5 | 4.2 | 4.2 | 20.9 | 8.1 | 1.3 | 7.1 | 1.6 | 0.8 | 4.0 | 1.7 |
| Polyhydroxy Acids | Threonic acid | 3.4 | 14.1 | 4.0 | 2.9 | 19.1 | 9.2 | 0.9 | 5.9 | 0.6 | 0.7 | 2.7 | 3.5 |
| Polyols | Arabitol | 0.9 | 2.0 | 0.8 | 0.9 | 2.2 | 1.8 | 0.3 | 0.5 | 0.2 | 0.4 | 0.2 | 0.7 |
| Polyols | Galactitol | 13.9 | 15.6 | 16.5 | 11.3 | 13.1 | 18.0 | 4.8 | 4.6 | 5.1 | 2.5 | 1.2 | 5.4 |
| Polyols | Glycerol | 8.5 | 7.6 | 6.1 | 11.0 | 7.3 | 7.9 | 5.1 | 1.9 | 0.3 | 6.3 | 1.8 | 1.9 |
| Polyols | Inositol, myo- | 2.4 | 17.1 | 3.2 | 1.6 | 18.6 | 15.6 | 1.3 | 5.7 | 1.4 | 0.5 | 5.0 | 6.0 |
| Polyols | Mannitol | 0.9 | 1.0 | 1.0 | 0.8 | 0.7 | 1.4 | 0.4 | 0.7 | 0.3 | 0.4 | 0.2 | 0.9 |
| Sugar Conjugates | Galactinol | 2.3 | 11.7 | 2.9 | 1.8 | 7.8 | 15.6 | 0.9 | 9.3 | 0.9 | 0.4 | 2.1 | 11.7 |
| Sugar Conjugates | Salicin | 5.0 | 9.6 | 6.2 | 3.8 | 14.4 | 4.8 | 3.9 | 7.4 | 5.2 | 0.8 | 7.6 | 2.3 |
| Sugar Conjugates | Salicylic acid-glucopyranoside | 1.6 | 8.9 | 1.6 | 1.6 | 5.6 | 12.2 | 0.2 | 9.9 | 0.3 | 0.1 | 0.5 | 13.2 |
| Sugars | Arabinose | 6.6 | 15.2 | 6.2 | 7.0 | 17.2 | 13.2 | 3.0 | 4.5 | 2.1 | 3.6 | 5.3 | 2.2 |
| Sugars | Fructose | 15.9 | 10.1 | 17.6 | 14.2 | 10.1 | 10.1 | 8.3 | 6.0 | 8.8 | 7.5 | 5.5 | 6.4 |
| Sugars | Galactose | 13.1 | 11.3 | 11.4 | 14.8 | 13.7 | 8.8 | 9.4 | 5.3 | 5.5 | 11.8 | 6.5 | 1.5 |
| Sugars | Glucose | 8.2 | 7.3 | 8.9 | 7.6 | 6.7 | 7.8 | 5.0 | 4.1 | 4.6 | 5.3 | 3.2 | 4.8 |
| Sugars | Glucose, 1,6-anhydro-, beta- | 9.4 | 15.8 | 9.0 | 9.8 | 19.6 | 12.0 | 1.1 | 4.8 | 0.7 | 1.3 | 4.2 | 0.7 |
| Sugars | Maltose | 3.9 | 9.9 | 4.3 | 3.4 | 12.7 | 7.0 | 0.9 | 5.8 | 0.8 | 0.8 | 6.8 | 1.9 |
| Sugars | Mannose | 10.1 | 9.8 | 7.7 | 12.4 | 9.6 | 9.9 | 8.3 | 4.3 | 3.0 | 10.9 | 4.5 | 4.0 |
| Sugars | Raffinose | 1.9 | 5.7 | 2.6 | 1.1 | 0.6 | 10.8 | 0.9 | 11.3 | 0.7 | 0.3 | 0.1 | 14.2 |
| Sugars | Rhamnose | 7.1 | 14.1 | 7.0 | 7.2 | 16.5 | 11.8 | 2.3 | 3.5 | 2.9 | 1.5 | 3.7 | 0.4 |
| Sugars | Ribose | 3.4 | 13.3 | 3.6 | 3.3 | 17.2 | 9.3 | 1.2 | 5.9 | 1.5 | 0.7 | 5.7 | 2.4 |
| Sugars | Sucrose | 23.5 | 21.9 | 23.4 | 23.7 | 22.8 | 21.1 | 2.9 | 5.2 | 1.9 | 3.7 | 2.1 | 7.0 |
| Sugars | Trehalose, alpha,alpha'- | 2.2 | 1.0 | 1.8 | 2.6 | 0.9 | 1.0 | 1.0 | 0.4 | 0.2 | 1.4 | 0.1 | 0.6 |
| Sugars | Xylose | 7.4 | 10.4 | 7.1 | 7.7 | 13.7 | 7.1 | 3.5 | 6.6 | 3.4 | 3.6 | 7.0 | 4.0 |
| Sugars | Xylulose | 1.1 | 4.1 | 1.1 | ND | 5.7 | 2.6 | 0.0 | 2.2 | ND | ND | 2.0 | 1.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Arabinonic acid-1,4-lactone | -0.18 | 0.51 | 1.57 | -0.52 | 0.53 | 0.914 | **0.001** | **0.041** |
| Galactaric acid | -1.18 | -0.25 | 0.81 | -1.66 | -0.60 | **0.011** | 0.455 | 0.209 |
| Galactonic acid | -1.45 | -0.63 | 0.64 | -2.08 | -0.81 | **0.002** | 0.963 | 0.100 |
| Gluconic acid | -1.14 | -0.22 | 1.22 | -1.73 | -0.29 | **0.018** | 0.217 | 0.139 |
| Glyceric acid | -1.62 | -0.28 | 0.20 | -1.87 | -1.38 | **0.000** | 0.900 | 0.238 |
| Gulonic acid | -0.36 | 0.04 | 0.62 | -0.61 | -0.03 | 0.425 | 0.257 | 0.311 |
| Lyxonic acid-1,4-lactone | -0.11 | 0.30 | 0.91 | -0.35 | 0.26 | 0.772 | 0.080 | 0.292 |
| Ribonic acid | -1.15 | 0.42 | 0.51 | -1.19 | -1.10 | **0.001** | **0.048** | 0.686 |
| Saccharic acid | -1.77 | 0.01 | 1.37 | -2.30 | -0.94 | **0.001** | 0.065 | **0.039** |
| Threonic acid | -2.04 | 0.50 | 1.05 | -2.24 | -1.69 | **0.000** | **0.019** | 0.292 |
| Arabitol | -1.19 | -0.20 | 0.26 | -1.42 | -0.97 | **0.004** | 0.722 | 0.471 |
| Galactitol | -0.16 | 0.54 | -0.46 | 0.33 | -0.67 | 0.507 | 0.853 | 0.143 |
| Glycerol | 0.17 | -0.86 | -0.12 | -0.27 | 0.48 | 0.927 | 0.307 | 0.475 |
| Inositol, myo- | -2.84 | 0.98 | 0.26 | -2.56 | -3.28 | **0.000** | 0.123 | 0.422 |
| Mannitol | -0.23 | 0.26 | -0.86 | 0.39 | -0.74 | 0.896 | 0.895 | 0.468 |
| Galactinol | -2.34 | 0.70 | -0.99 | -1.45 | -3.14 | **0.010** | 0.829 | 0.391 |
| Salicin | -0.94 | 0.72 | 1.58 | -1.21 | -0.35 | 0.193 | 0.137 | 0.275 |
| Salicylic acid-glucopyranoside | -2.48 | -0.06 | -1.13 | -1.84 | -2.90 | **0.038** | 0.913 | 0.998 |
| Arabinose | -1.21 | -0.18 | 0.38 | -1.48 | -0.92 | **0.008** | 0.776 | 0.575 |
| Fructose | 0.65 | 0.30 | >0.01 | 0.79 | 0.49 | 0.300 | 0.769 | 0.986 |
| Galactose | 0.22 | -0.38 | 0.64 | -0.26 | 0.75 | 0.984 | 0.813 | 0.615 |
| Glucose | 0.18 | 0.23 | -0.23 | 0.41 | -0.05 | 0.728 | 0.791 | 0.941 |
| Glucose, 1,6-anhydro-, beta- | -0.75 | -0.13 | 0.70 | -1.13 | -0.29 | **0.001** | 0.076 | **0.018** |
| Maltose | -1.35 | 0.36 | 0.85 | -1.55 | -1.05 | **0.006** | 0.139 | 0.622 |
| Mannose | 0.05 | -0.68 | -0.03 | -0.32 | 0.33 | 0.800 | 0.791 | 0.866 |
| Raffinose | -1.59 | 1.23 | -4.14 | 2.12 | -3.25 | 0.602 | 0.766 | 0.173 |
| Rhamnose | -1.00 | -0.04 | 0.48 | -1.24 | -0.72 | **0.009** | 0.674 | 0.351 |
| Ribose | -1.96 | 0.13 | 0.89 | -2.28 | -1.52 | **0.001** | 0.258 | 0.258 |
| Sucrose | 0.10 | -0.02 | 0.11 | 0.04 | 0.17 | 0.534 | 0.669 | 0.658 |
| Trehalose, alpha,alpha'- | 1.20 | -0.49 | -0.18 | 1.03 | 1.33 | **0.046** | 0.883 | 0.841 |
| Xylose | -0.50 | -0.12 | 0.94 | -0.95 | 0.10 | 0.639 | 0.526 | 0.363 |
| Xylulose | ND | ND | 1.14 | -2.36 | ND | ND | ND | ND |