MAJOR INTRINSIC PROTEINS OF *LACCARIA BICOLOR*: CHARACTERIZATION, TRANSCRIPT PROFILING AND FUNCTIONS IN ECTOMYCORRHIZAL ASSOCIATIONS WITH *PICEA GLAUCA*

by

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ABSTRACT

In mycorrhizal associations, water transport properties of the fungal hyphae may affect water transport of the host plants. The importance of aquaporins, water-transporting members of the Major Intrinsic Protein (MIP) family, in facilitating water transport has been widely acknowledged and extensively studied in plants. However, the structure, function, and regulation of fungal MIPs are little understood. The rapid increase in the number of sequenced fungal genomes, including *Laccaria bicolor*, has enabled functional and comparative genomic investigations to delineate the role that fungal MIPs play in water transport of mycorrhizal plants.

In this thesis project, phylogenetic analysis of 229 fungal MIPs from 88 species has revealed that MIPs of mycorrhizal fungal species fall into four clusters delineated by functionally characterized fungal MIPs: the orthodox aquaporins, the aquaglyceroporins, the facultative fungal aquaporins, and the X intrinsic proteins (XIPs). This comparative genomics analysis, together with *in silico* structural characterization of predicted MIPs and recently published functional characterization of MIPS from a small number of ectomycorrhizal and arbuscular mycorrhizal species, provide new insight into MIP gene families of mycorrhizal fungi, and possible roles for fungal aquaporins in water relations of mycorrhizal plant-fungus symbioses. To further characterize mycorrhizal MIPs, *in silico* analyses and water transport functional assay were conducted for six MIP genes cloned from ectomycorrhizal (ECM) fungus *L. bicolor* strain UAMH8232 which correspond to five of seven putative MIP genes of the species, and their roles in two important mycorrhizal processes – ECM root water transport and basidiocarp formation, were investigated.

The aquaporin-encoding *JQ585595* from *L. bicolor* was selected for its high water transport capacity and high transcript abundance, and transgenic *L. bicolor*

overexpressing *JQ585595* was generated to test the role of this fungal aquaporin in facilitating water transport in ECM-associated white spruce (*Picea glauca*). The hypothesis was tested that root hydraulic conductivity of mycorrhizal plants would be altered by overexpression of the *L. bicolor* aquaporin, reflecting the increased contribution of water transport through fungal hyphae to water transport of the mycorrhizal root system. In this study, *P. glauca* was inoculated with wild-type (WT), mock transgenic, or *L. bicolor* aquaporin *JQ585595*-overexpressing (OE) strains and exposed to root temperatures ranging from 5°C to 20°C to examine the root water transport properties, physiological responses and the plasma membrane intrinsic protein (PIP) expression in colonized plants. Mycorrhization increased shoot water potential, transpiration, net photosynthetic rates, root hydraulic conductivity, and root cortical cell hydraulic conductivity in seedlings. At 20°C, OE plants had higher root hydraulic conductivity compared with WT plants and the increases were accompanied by higher expression of *P. glauca* PIP *GQ03401_M18.1* in roots. Contrary to WT *L. bicolor*, the effects of OE fungi on root and root cortical cell hydraulic conductivities were abolished at 10°C and 5°C in the absence of major changes in the examined transcript levels of *P. glauca* root PIPs. The results of this study provide evidence for the importance of fungal aquaporins in root water transport of mycorrhizal plants. They also demonstrate links between hyphal water transport, root aquaporin expression and root water transport in ECM plants.

Mycorrhizal fungal aquaporins can transport not only water but also small neutral molecules such as glycerol, ammonia, CO_2 and NO, therefore may contribute to substrate transport and cellular signaling, which is intensively required to drive rapid cell differentiation, division and expansion in certain phases of fungal growth, such as sporocarp initiation and maturation. It was hypothesized that some of these *L. bicolor* MIPs involved in CO_2 signaling or water transport may contribute to the primordium initiation or rapid cell expansion during basidiocarp formation. Therefore, changes in their transcript profiles during the initiation and development of the basidiocarps of *L. bicolor* ECM with *P. glauca* were investigated; and morphological changes that took place in each developmental stage were examined. Based on the previous understanding of the transport capacities of *L. bicolor* MIPs, the involvement of the most significantly upregulated MIPs *JQ585592* and *JQ585595* provided important

clues concerning their possible functions during the different stages of basidiocarp development.

The significant involvement of *JQ585595* in ECM water transport, as well as *JQ585592* and *JQ585595* in basidiocarp formation, highlights that fungal MIPs are of great importance to fundamental processes taking place in ECM fungi and associated plants.

Keywords: Aquaporin, basidiocarp formation, ectomycorrhiza-water relation, fungal major intrinsic protein (MIP) clusters, mycorrhizal fungal aquaporin, plasma membrane intrinsic protein (PIP), root hydraulic conductivity, root water transport pathways, transmembrane facilitate transport

PREFACE

The work presented in this thesis is part of the research project of Dr. Janusz Zwiazek and Dr. Janice Cooke on *Laccaria bicolor* aquaporins and their roles in ectomycorrhizal associations. With the exceptions explained below, all experiments were carried out and the data analyzed by Hao Xu under the supervision of Dr. Janusz Zwiazek and Dr. Janice Cooke.

The thesis work consists of the general introduction and literature review chapter, three data chapters and one chapter describing general conclusions. Chapters 2 and 3 are based on published journal articles, to which the contributions of Hao Xu and each coauthor are stated below in details.

Chapter 2 is based on the published journal article "Xu H, Cooke JEK, Zwiazek JJ. 2013. Phylogenetic analysis of fungal aquaporins provides insight into their possible role in water transport of mycorrhizal associations. *Botany* 91: 495-504". The article was written by Hao Xu, and revised by Dr. Janusz Zwiazek and Dr. Janice Cooke. Dr. Janusz Zwiazek and Dr. Janice Cooke guided literature review and writing of the work. Guided by Dr. Janice Cooke, Hao Xu conducted the sequence searching and the phylogenetic analysis of the work.

Chapter 3 is based on the published journal article "Xu H, Kemppainen M, El Kayal W, Lee SH, Pardo AG, Cooke JEK, Zwiazek JJ. 2015. Overexpression of *Laccaria bicolor* aquaporin *JQ585595* alters root water transport properties in ectomycorrhizal white spruce (*Picea glauca*) seedlings. *New Phytologist* 205: 757-770". The article was written by Hao Xu, and revised mainly by Dr. Janusz Zwiazek and Dr. Janice Cooke. Dr. Janusz Zwiazek and Dr. Janice Cooke guided the work. Dr. Minna Kemppainen, Dr. Alejandro Pardo and Dr. Seonghee Lee also contributed to the revision. In §3.2.5, the important fungal transgenic materials that made this study possible were constructed and provided by Dr. Minna Kemppainen and Dr. Alejandro Pardo. Dr. Minna Kemppainen also provided suggestions to ensure the success of Southern blot assay. The cortical cell hydraulic conductivity data in §3.2.10.2 and §3.3.7 were collectly by Dr. Seonghee Lee with her superb expertise in cell pressure probe technique. Dr. Walid El Kayal generously provided Hao Xu with training on

molecular biology experimental techniques. Some putative aquaporin sequences used in plant MIP phylogenetic analysis in §3.2.11 were provided by Dr. Janice Cooke.

Chpater 4 is based on the article "Xu H, Navarro-Ródenas A, Cooke JEK, Zwiazek JJ. 2015. Transcript profiling of aquaporins during basidiocarp development in *Laccaria bicolor* ectomycorrhizal with *Picea glauca*" submitted to the journal *Mycorrhiza*. The article was written by Hao Xu, and revised by Dr. Janusz Zwiazek and Dr. Janice Cooke. Dr. Alfonso Navarro-Ródenas provided insightful opinions for the experimental design and the writing of Discussion.

For the *Xenopus laevis* oocyte assay presented in §3.2.3 and §3.3.2, Dr. Warren Gallin and Dr. Rheanna Sand provided training and experimental apparatus in Dr. Gallin's laboratory. Approval for using *Xenopus* to obtain oocytes for this study had been granted to Hao Xu upon passing the Animal Research Ethnics Application Trainings provided by the Research Ethics Office, University of Alberta.

For my grandparents

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LIST OF ABBREVIATIONS AND SYMBOLS

AM	Arbuscular mycorrhiza
A	Cell surface area
C_t	Threshold cycle in qPCR
E	Transpiration rate
E	Efficiency of primer amplification in qPCR
E in Clustal sequence alignment analysis	*E*-value in statistics of sequence similarity scores
ECM	Ectomycorrhiza
ER	Endoplasmic reticulum
g_s	Stomatal conductance
h	Hour
K_r	Root hydraulic conductance
L_{pr}	Root hydraulic conductivity
L_{pc}	Hydraulic conductivity of root cortical cell
min	minute
MIP	Major intrinsic protein
mg	Milligram
mL	Millilitre
ng	Nanogram
NIP	Nodulin 26-like intrinsic protein
N	Nitrogen
PIP	Plasma membrane intrinsic protein
P_n	Net photosynthetic rate
P	Significance level in ANOVA test
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
SIP	Small intrinsic protein
s	Second
TIP	Tonoplast intrinsic protein
TMD	Transmembrane domain
WUE	Water use efficiency
XIP	X intrinsic protein
ε	Cell elastic modulus
V	Cell volume
π^i	Osmotic cell pressure
ΔP	Change in cell turgor
Ψ_{midday}	Midday water potential
$\Psi_{osmotic}$	Osmotic water potential
Ψ_{total}	Total water potential
Ψ_{turgor}	Turgor water potential
µg	Microgram
§	Chapter

1 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Major Intrinsic Proteins (MIPs) are a family of integral membrane proteins that universally exist in eukaryotic and prokaryotic organisms (Gomes *et al*. 2009). MIPs form pores in the phospholipid bilayer of cellular membranes, to facilitate the transmembrane transport of water and small neutral (uncharged) molecules. Phylogenetically, MIPs of organisms from different kingdoms are highly related, sharing similar transmembrane secondary structures and well-conserved amino acid motifs. Although MIPs also have kingdom-specific features, they are sometimes broadly classified into two large groups - aquaporins and glyceroporins - according to the specificity of transported substrates and phylogenetic distances (Gomes *et al*. 2009). In this classification, aquaporins are defined as water channels, whereas glyceroporins are considered to be channels for the transport of glycerol and other small neutral molecules. Studies in the last two decades have characterized transport capacities, subcellular localization, mechanisms of transcriptional and post-translational regulations, and diverse physiological functions of MIPs in various species of plants and animals (Chaumont *et al*. 2005; Maurel *et al*. 2008; Chaumont & Tyerman 2014). Because MIPs are significant determinants of cellular and tissue hydraulics, efforts have been made to elucidate the functions of MIPs in controlling plant-water relations, especially root water transport and leaf transpiration (Javot & Maurel 2002; Maurel *et al*. 2008).

The available information concerning MIPs in fungi is relatively limited, but it is fair to assume that they are equally crucial to the life of fungi as to the other forms of life (Pettersson *et al.* 2005; Nehls & Dietz 2014). In yeasts and filamentous fungi, most of the described MIPs exhibit the canonical aquaporin structure, consisting of six transmembrane domains, five loops and signature motifs (Soveral *et al*. 2010). They facilitate transmembrane transport of one or more substrates, including water, glycerol, urea, boric acid, ammonia, and CO_2 (Navarro-Ródenas *et al*. 2012; Li *et al*. 2013a; Nehls & Dietz 2014). In the last decade, increasing attention has been drawn to fungal

MIPs in terms of their classification, functional characterization and contributions to processes such as substrate transport, osmotic regulation and cross-membrane signaling. As new sequencing platforms have been used to generate ever-larger amounts of genomic resources, including whole genome sequences, comparative genomics of fungi has become a powerful tool for fungal gene discovery.

Advances in fungal comparative genomics not only benefits microbiologists and mycologists, but also provides exceptional advantages for plant biologists in the field of plant-fungal interactions. Mycorrhizas are mutualistic plant-fungal associations that develop between plant roots and certain soil-borne fungi (Smith & Read 2008). The highly specialized structures of mycorrhizal roots can allow efficient substrate exchanges between the plant and the fungus, and consequently enhance the growth of both organisms. Due to its universal presence, the mutual benefits it may bring to the associated species, and its remarkable ecological significance, mycorrhizal interactions have been a research hotspot. Since the first mycorrhizal fungal genome was sequenced and assembled in 2008, genomic resources of 33 mycorrhizal fungal species have become available in less than a decade (van der Heijden *et al*. 2015). This has substantially accelerated our understanding about crucial biological processes of mycorrhizal interactions, such as molecular signaling during symbiosis recognition, transport and exchange of nutrients and carbohydrates, stimulation of growth in plants and fungi, etc.

Attributed to the superb power of comparative genomics, the resources for the study of mycorrhizal fungal MIPs have also become plentiful. A remarkable number of putative MIP genes in these species have become available for phylogenetic analysis, functional characterization, and physiological studies (Nehls & Dietz 2014; Verma *et al*. 2014). It can be expected that the studies on MIPs of mycorrhizal fungi will help elucidate the distinct roles of fungal MIPs in resource requisition by the growing hyphae, in the water relation of mycorrhizal association and in the water transport of mycorrhizal roots, as well as in the developmental events involving drastic hyphal fusion, such as fruiting body formation. These studies will enhance our understanding of both fungal MIPs and mycorrhizal interaction.

1.2 Aquaporins and Other Major Intrinsic Proteins

1.2.1 Discovery and definition of aquaporins

Since the water-transporting function of Aquaporin-1 of *Homo sapiens* (Agre *et al*. 1993) and γ-TIP (tonoplast intrinsic protein) of *Arabidopsis thaliana* (Maurel *et al.* 1993) being firstly reported in 1993, our understanding about these transmembrane water channel proteins has been greatly expanded. Aquaporins belong to the Major Intrinsic Protein (MIP) family of integral membrane proteins. In keeping with their role as channels that facilitate diffusion of water and other small molecules such as urea, glycerol, boric acid, ammonia and CO_2 (Uehlein *et al.* 2003; Wu & Beitz 2007; Gomes *et al*. 2009), MIPs are localized in plasma and intracellular membranes (reviewed by Maurel *et al*. 2008). They are also universal in all the other known kingdoms of organisms (Zardoya 2005) and particularly well characterized in plants and mammals. To avoid definition ambiguity, the terms "aquaporin" and "water channel protein" are usually used to refer to functionally water-transporting MIPs, whereas "MIP" is used more generally to refer to the proteins transporting both water and other small neutral molecules.

X-ray crystallography shows highly conserved structural features of MIP homologues across plants, animals and microbes. Typically, these 23-35 kDa proteins consist of six hydrophobic transmembrane helical domains (TMD) connected by five hydrophilic loops (Fig. 1.1). Three loops (A, C, E) are located on the extracytoplasmic side of the membrane, while two loops (B, D) and both the N- and C- termini localize to the intracytoplasmic side (Walz *et al*. 1997). The six transmembrane helices, and Loop B and E, which extend from either side of the membrane and dip into the core of the molecule, form a central aqueous pore through which a single file of water molecules may pass. Loop B and E each possess a highly conserved NPA (Alanine-Proline-Asparagine) motif (Fig. 1.1) that function in pore constriction and water molecule dipole orientation (Murata *et al*. 2000). In addition, an array of aromatic residues on the extracytoplasmic Loop E and in the TMD 2 and 5 face an arginine residue to form the aromatic/Arg site. This aromatic/Arg site is usually the narrowest site in the pore, and is considered important for proton repulsion and selective filtering of molecules (Mitani-Ueno *et al*. 2011). Convenient *in silico* analysis tools such as SOSUI (Hirokawa *et al.* 1998) and TMHMM (Krogh *et al.* 2001) are widely used to rapidly

predict transmembrane protein topology and classify membrane proteins, which largely accelerates the discovery of new MIPs.

1.2.2 MIP phylogenetic classification and functional characterization

1.2.2.1 Phylogenetic classification

Although MIPs are well conserved in all organisms, their nomenclature differs considerably between kingdoms. In animals and bacteria, MIPs are broadly classified into aquaporins and glyceroporins (Gomes *et al*. 2009), as described in §1.1.

The classification of plant MIPs takes into account not only transport capacities and phylogenetic distances, but also protein subcellular localizations; accordingly, plant MIPs are categorized into plasma membrane intrinsic protein (PIP), tonoplast intrinsic protein (TIP), nodulin-26 like intrinsic protein (NIP), small intrinsic proteins (SIP), X intrinsic proteins (XIP), GlpF-like intrinsic protein (GIP) and hybrid intrinsic protein (HIP) subfamilies, with the latter two subfamilies found only in the non-vascular moss *Physcomitrella patens* (Gustavson *et al*. 2005; Danielson & Johanson 2008; Maurel *et al*. 2008; Lopez *et al*. 2012). Phylogenetic analyses use different metrics to compare nucleotide or deduced amino acid similarity between sequences, and can be used to evaluate putative MIPs and categorize them into major clusters according to the calculated inter-sequence distance (*Tamura et al*. 2011). Based on position within a tree, it is possible to infer subcellular localization due to the presence of distinctive nucleotide targeting sequences that contribute to the differentiation between clusters such as PIPs and TIPs. However, such inference is not always reliable, because phylogenetic analysis takes into account the entire sequence for pairwise alignment, whereas subcellular localization is largely determined by certain N-terminal motifs. For more precise prediction, the *in silico* tool Target P uses the N-terminal sequence motifs that are well-known for directing proteins to the secretory pathway, mitochondria and chloroplasts as the sorting signals, to predict MIP subcellular localization (Emanuelsson *et al*. 2007). Such *in silico* predictions can be tested by visualization using techniques such as immunohistological fluorescence staining (Ma *et al*. 2006; Laur & Hacke 2014a) and fluorescence protein fusion (Li *et al*. 2013b).

In fungi, MIP classification was based mainly on analyses conducted with yeast MIPs, and resembled the mammalian MIP nomenclature of two major groups – orthodox

aquaporins and aquaglyceroporins (Pettersson *et al.* 2005; Soveral *et al.* 2010). In the first comprehensive phylogenetic analysis of 19 yeasts and three filamentous fungi (two ascomycete and one basidiomycete), Pettersson *et al*. (2005) classified 55 fungal MIPs into four groups: orthodox aquaporins, Fps-like aquaglyceroporins, Yfl054c-like aquaglyceroporins, and other aquaglyceroporins. Functional characterization of fungal MIP transport capacity for water and small neutral molecules was limited to six yeast species, *i.e*., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe, Zygosaccharomyces rouxii*, *Candida albicans, Kluyveromyces lactis* and *Kluyveromyces marxianus*. Subsequently, Gupta and Sankararamakrishnan (2009) identified nine fungal XIP-like genes from the genomic sequences of eight fungal species, based on sequence similarity with plant XIPs. More recently, Dietz *et al*. (2011) took advantage of the rapidly expanding catalogue of fungal genome sequences to conduct a phylogenetic analysis of 100 fungal MIPs sequences from 29 species representing two phyla. Dietz *et al.* (2011) identified four groups, which they termed classical aquaporins, Fps-like aquaglyceroporins, other aquaglyceroporins and fungal XIPs. Based on about 400 putative fungal MIP sequences, Verma *et al*. (2014) proposed a phylogenetic system consisting of four clusters – orthodox aquaporins, aquaglyceroporins, XIPs and SIP-like fungal MIPs, in which the cluster of aquaglyceroporins can be divided into subgroups of Fps1-like, Yfl054-like, α, β, γ1, γ2 and δ. With the facilitation of above-mentioned *in silico* analysis tools and visualization techniques, it can be expected that the phylogenetic classification of fungal MIPs will be continuously updated as more fungal genomic resources make more MIP sequences available.

1.2.2.2 Functional characterization

Theoretically, a sequence remains a putative MIP until its transport capacity is confirmed by functional assay. *Xenopus* oocytes and yeasts are the most commonly used expression systems for functional assays of MIP transport capacities. In oocyte expression system, the RNA corresponding to a candidate MIP cDNA is microinjected into oocytes. After incubation that allows gene incubation to occur, oocytes are placed in hypotonic solutions in the swelling assay to examine the MIP transport capacity for water (Zhang & Verkman 1991), glycerol (Hansen *et al*. 2002; Dietz *et al*. 2011), urea (Hansen *et al*. 2002; Dietz *et al*. 2011) or boric acid (Mitani *et al.* 2008). Use of

radioactive substrates can be incorporated in oocyte assay to examine the transport capacity of the expressed MIP gene for glycerol (Ma *et al.* 2006), lactic acid (Choi & Roberts 2007) and silicon (Ma *et al*. 2006; Mitani-Ueno *et al*. 2011).

The radioactive labeling techniques can also be incorporated in yeast system to examine the transport of substrates such as arsenite (AsO_3^{-3}), by counting the radioactivity retained on the filter of a scintillation detector (Wysocki *et al.* 2001). With use of advanced spectrometry techniques, such as graphite furnace atomic absorption spectrometer or inductively coupled plasma mass spectrometer, arsenic content in lysates of oocytes and yeast can be detected to determine the transport capacity for arsenate (AsO_4^{-3}) (Bienert *et al.* 2008; Mitani-Ueno *et al*. 2011). Another technique used for detection with yeast expression system is the stopped-flow spectrophotometry. The technique can detect the rapid change in scattered light intensity of yeast protoplast and has been successfully used to examine water permeability of the expressed MIP genes as an alternative for oocyte swelling assay (Fischer & Kaldenhoff 2008). Stopped-flow spectrophotometry can also instantly detect the change in fluorescence emitted during the reaction of carboxufluorescein and carbonic anhydrase, which is triggered by the change in pH due to the influx of CO_2, and therefore it has been used with the yeast coexpression system to detect the MIP transport capacity for CO_2 (Prasad *et al*. 1998). Presumably, similar approaches can be used to examine the MIP transport capacities for other unconventional small neutral molecules. In addition, nutrient or hypersensitivity mutant strains of yeasts can be used to examine the transport capacity for NH_3 in nutrient complementation assay (Dietz *et al*. 2011), for methylamine in toxicity assay (Dietz *et al*. 2011) and for antimonite in hypersensitivity assay (Wysocki *et al.* 2001).

Other less common expression systems have been used in transport assays of unusual substrates. For example, animal cell transfection and fluorescent detection techniques have been incorporated to examine the NO and H_2O_2 transport by MIPs (Herrera *et al.* 2006; Miller *et al*. 2010).

1.2.3 Roles of plant MIPs in plants

MIPs are important for the movement of water and other small neutral molecules across membranes in the cell-to-cell pathway and abundant in various types of plant

cells (reviewed by Kaldenhoff & Fischer 2006; Maurel *et al*. 2008; Chaumont & Tyerman 2014). Plant MIPs are expressed differentially in actively dividing cells and elongating cells in different tissues, and some are exclusively expressed in certain tissues (Obroucheva & Sin'kevich 2010; Almeida-Rodriguez & Hacke 2012). Transport assays, *in silico* simulation, transcript profiling, and physiological experiments with transgenic plants have been integrated to study their transport selectivity and physiological functions (Maural *et al*. 2008; Ludewig & Dynowski 2009). Many such studies on development-dependent or stress-induced expression of MIP genes have been reviewed and compared between large varieties of regulation profiles in different species (Maurel *et al*. 2008; Chaumont & Tyerman 2014).

PIPs are expressed in roots, shoots, leaves and reproductive parts (Kaldenhoff & Fischer 2006). The subcellular localization of PIPs in the plasma membrane renders them the crucial roles in facilitating water transport, determining tissue water permeability (Kaldenhoff *et al*. 2008) and regulating cell hydraulic conductivity and root water uptake (Javot & Maurel 2002; Javot *et al.* 2003; Ehlert *et al*. 2009; Aroca *et al*. 2012; Gambetta *et al*. 2013) (See §1.3.2 & 1.3.3). PIPs regulate leaf hydraulic conductivity, stomatal movement and transpiration (Fraysse *et al*. 2005; Maurel *et al*. 2008; Prado & Maurel 2013). For example, PIPs contribute to the response of leaf hydraulic conductivity to change in light intensity in *Quercus macrocarpa* (Voicu *et al*. 2009) and in the recovery of leaf hydraulic conductivity from water stress in *Populus trichocarpa* (Laur & Hacke 2014a). PIPs are also involved in needle water uptake in *Picea glauca* (Laur & Hacke 2014b). Some PIPs are preferentially expressed in vascular cell types such as xylem parenchyma cells and phloem-associated cells, indicating their roles in sap transport through the whole plant and in the whole-plant rehydration (Maurel *et al*. 2008). For instance, it has been shown that the up-regulation of PIPs could contribute to embolism repair of stem xylem in walnut tree (Sakr *et al*. 2003) and *P. glauca* (Laur & Hacke 2014b). PIPs could also play roles in desiccation and rehydration of reproductive parts, such as during water efflux from the anthers in *Arabidopsis* (Bots *et al*. 2005), and seed imbibition and germination in *Brassica napus* (canola) (Gao *et al*. 1999). In addition, CO_2-transporting PIPs may play important roles in carbon metabolism. It has been found that the expression of the PIP gene *Nt*AQP1 in *Nicotiana tabacum* (tobacco) was positively correlated with mesophyll conductance to

CO_2, maximal stomatal conductance and CO_2 assimilation capacity (Uehlein *et al.* 2003; Flexas *et al*. 2006).

TIPs are expressed in roots, leaves and seeds (Kaldenhoff & Fischer 2006). The main subcellular localization of TIPs is the tonoplast membrane of plant vacuole that is involved in turgor regulation, cell signaling and degradation (Kaldenhoff & Fischer 2006). TIPs usually have multiple transport capacities for water, urea and glycerol. Because of the similar transport capacity for water, the expression patterns of TIPs and PIPs are often linked and they are both important to the processes related to water exchange and cell volume regulation, including cell expansion in various plant tissues, and water efflux from specialized cell types such as stomatal guard cells. Due to the multifunctional capacities, TIPs are not only important to regulation of central vacuoles, but can also contribute to vesicle trafficking, N requisition and carbohydrate compartmentation (Maurel *et al*. 2008).

NIPs are preferentially expressed in roots. The first NIP soybean nodulin 26 was discovered in symbiotic N fixing root nodules (Fortin *et al.* 1987). NIPs locate in peribacteroid membranes (Kaldenhoff & Fischer 2006), plasma membranes and endoplasmic reticulum (ER) membranes (Maurel *et al*. 2008). It is suggested that these multifunctional transporters are involved in bidirectional exchange of water, gylcerol, NH_3 and other small solutes between plant cytoplasm and symbiotic bacteroids (Kaldenhoff & Fischer 2006) or mycorrhizal fungi (Giovannetti *et al*. 2012). In addition, *At*NIP2;1 in *Arabidopsis* is a lactic acid transporter and is induced during hypoxia stress, which suggests the roles of NIPs in cytosolic pH adjustment and plant metabolic adaption to anoxia (Choi & Roberts 2007). Being the efficient transporters for boric acid and silicon, some NIPs also significantly contribute to root uptake of these nutrients in *Arabidopsis* (Takano *et al*. 2006) and rice (Ma *et al*. 2006).

Compared with other subgroups of plant MIPs, the physiological functions of SIPs and XIPs are less understood. So far it has been confirmed that SIPs locate in the ER membranes and are efficient water channels (Maeshima & Ishikawa 2008). Since the first report in *P. patens* (Danielson & Johanson 2008), XIPs have also been found in vascular plants *P. trichocarpa* (Gupta & Sankararamakrishnan 2009), *Vitis vinifera* (Shelden *et al.* 2009), *Gossypium hirsutum* (Park *et al*. 2010) and *Solanaceae* (Bienert *et al.* 2011). Locating on plasma membrane, they are not significant water transporters;

instead, they facilitate transport of glycerol, urea and boric acid (Gupta & Sankararamakrishnan 2009; Bienert *et al*. 2011).

1.2.4 Transcriptional and post-translational regulation of MIPs

Transcription and post-translational modifications of plant MIPs are sensitive to various environmental factors and plant hormones such as abscisic acid and salicylic acid (Javot & Maurel 2002; Beaudette *et al*. 2007; Maurel *et al*. 2008; Ruiz-Lozano *et al*. 2009; Gambetta *et al*. 2013; Prado & Maurel 2013). The abundance of MIPs is largely determined by the transcription of MIP genes, whereas MIP activity and membrane water permeability are regulated by post-translational regulation that includes gating, heterotetramerization and membrane trafficking (Maurel *et al.* 2008). It has been found that cytosolic pH, Ca^{2+}, high solute concentration, pressure pulses as well as the presence of hydroxyl radicals and reactive oxygen species can affect MIP gating. Gating of water flux can be achieved via protonation of a His residue in Loop D or dephosphorylation of two conserved Ser residues situated in the consensus phosphorylation sites of Loop B and the C-terminal region (Fig. 1.1), which results in a closed conformation. Conversely, phosphorylation of Loop B renders an open conformation (Hedfalk *et al*. 2006). These two gating mechanisms have been described for the spinach (*Spinacia oleracea*) aquaporin SoPIP2;1 by protein crystallization and molecular dynamics simulations (Törnroth-Horsefield *et al.* 2006). The protonation of the conserved His residue causes conformational change of Loop D that is relocated close to Loop B and blocks the exit of the pore, and consequently leads to the occlusion of the pore. Upon phosphorylation of Loop B, Loop D is unlocked, permitting the open conformation. Most interestingly, phosphorylation of the C-terminal tail would be able to prevent Loop D of an adjacent monomer from adopting a closed-pore conformation, which partially explains how tetramerization would increase the water transport capacity of each monomer in the aquaporin tetramer (Törnroth-Horsefield *et al.* 2006). Divalent cations such as Ca^{2+} are also proposed to function in gating of the spinach SoPIP2;1 by facilitating ionic interactions and hydrogen bonds that enable the closed conformation (Törnroth-Horsefield *et al.* 2006).

Maurel *et al.* (2008), Li *et al*. (2013b) and Chaumont & Tyerman (2014) have reviewed the studies on heterotetramerization and membrane trafficking of plant MIPs and the advance in relevant techniques. The model of direct interaction between PIP1s

and PIP2s have been supported by the studies using affinity copurification, coimmunopurification and Förster resonance energy transfer / fluorescence lifetime imaging microscopy, suggesting that the heterotetramerizarion enhances the function of water channels and facilitates PIP trafficking (Fetter *et al*. 2004; Zelazny *et al.* 2007). MIP trafficking has been observed as relocalization of TIPs and internalization of PIPs during stress (Li *et al*. 2011; Prak *et al*. 2008).

While the above-mentioned mechanisms have been relatively well investigated in plant and animal MIPs, studies of post-translational regulation for fungal MIPs have so far been limited to yeasts (Hub & Groot 2008). However, the gating mechanisms are likely similar across eukaryotic species (Törnroth-Horsefield *et al*. 2010). Given the degree of sequence similarity and conservation of functional motifs shared between plant, animal and fungal MIPs, similar secondary structure and regulatory mechanisms are likely to be present in fungal MIPs.This assumption is supported by *in silico* prediction of protein secondary structures of fungal MIPs, but it still remains to be tested experimentally. Meanwhile, novel regulatory mechanisms may exist in fungal MIP gating. For instance, a tyrosine residue in N-terminus in a yeast aquaporin was found to form a H-bond with the water molecule in the pore of the channel and therefore caused the closing conformation (Fischer *et al.* 2009). Since the signature motifs for phosphorylation and protonation can be identified in fungal MIP sequences, it will be of interest to determine the extent to which these post-translational mechanisms function in fungal MIP conformational change and gating, thus regulating MIP-mediated water transport in fungi. It would be of particular interest to determine whether post-translational modifications regulate water transport in mycorrhizal associations in response to diverse environmental cues.

1.2.5 Roles of fungal MIPs in fungal biology

Much of the knowledge of MIP structure, function and regulation in fungi was based on studies of the Ascomycota yeasts (Pettersson *et al.* 2005; Soveral *et al.* 2010). Classic aquaporin secondary structures with six TMDs and two NPA motifs, as well as water permeability, have been confirmed for ADC55543, ADC55259 and EGA57700 of the yeast *S. cerevisiae* (Laizé *et al*. 1999; Soveral *et al*. 2010) and XP2492992 of *Komagataella pastoris* (Fischer *et al*. 2009). These orthodox aquaporins mediate rapid water transport across membranes and play important roles in cell osmotic regulation

(Laizé *et al*. 1999; Soveral *et al*. 2010). Fps1 was the first reported osmogated glycerol export channel in *S. cerevisiae*, facilitating transmembrane transport of other small neutral molecules such as urea and charged arsenite (Wysocki *et al*. 2001). These channels function in osmoregulation of intracellular glycerol levels in response to changes in extracellular osmolarity (Bill *et al*. 2001).

Nehls & Dietz (2014) recently reviewed the studies on the roles of fungal MIPs in fungal biology and pointed out their potential functions in water transport, solute transport and cellular adaptations of yeasts, as well as water permeability, nutrient transfer, osmolyte transfer and hyphal fusion of filamentous fungi. The significance of MIPs for many biological processes of fungal growth and interactions between fungi and other organisms is worthy of further investigation (See §1.4.5 for the roles of fungal MIPs in mycorrhizas). For instance, it has been shown that aquaporin Aqy1 in *S. cerevisiae* was involved in sporulation (Sidoux-Walter *et al.* 2004). In addition to relatively well-understood model fungal species, and in the species with application in fermentation and other aspects of the food industry, there are a plethora of other fungi that play key roles in diverse ecological processes. The post-genomic era heralds new possibilities for examining how these fungi function in their environment, and investigating the roles that the fungi play in their diverse ecological niches. For instance, the lichen-forming fungi *Cladonia grayi* and *Xanthoria parietina* are extremely drought resistant, and the marine yeast *Debaryomyces hansenii* inhabits a highly hypertonic environment, indicating high efficiency or unique mechanisms of MIP regulation. Along similar lines, members of the Basidiomycota rapidly absorb water upon fruiting body formation, whereas their spores are usually highly dehydrated. It is tempting to speculate on the significance of MIPs in these fascinating biological processes of fungal growth, which can be understood by studying MIP expression and regulation in different tissues and growth stages.

1.3 Root water transport

1.3.1 Root anatomy and the composite model of root water transport pathways

Root water uptake along the water potential gradient is a fundamental process in plant life. A typical primary root structure consists of epidermis, exodermis or hypodermis

(not always present), cortex, endodermis, pericycle and vascular tissues of the primary phloem and xylem (Taiz & Zeiger 2010). Root epidermal cells outgrow into filamentous root hairs that greatly increase root surface area for water and nutrient uptake. The zones near root tips absorb water most readily, whereas mature regions are usually less permeable to water due to the presence of suberinized outer layers of cells, which may include the epidermis, exodermis and/or hypodermis (Taiz & Zeiger 2010). Therefore root tips play a determining role in root water permeability.

The passive transport process along the water potential gradient does not consume energy. On its way from the rhizosphere via the epidermis, cortex, and endodermis to the xylem, water transport in plant root tips involves apoplastic and cell-to-cell (symplastic plus transmembrane) pathways, as demonstrated by the composite model (Steudle & Peterson 1998) (Fig. 1.2). The apoplast is defined as the continuous system of cell walls, intercellular air spaces and lumens of nonliving cells (Taiz & Zeiger 2010). In the apoplastic pathway, water travels across the root cortex in the intercellular space and through the cell wall matrix without entering the cytoplasmic space (Fig. 1.2). The symplast is defined as the entire network of cell cytoplasm interconnected by plasmodesmata, through which water is transported from cell to cell through the cortex via the symplastic pathway (Taiz & Zeiger 2010). In the transmembrane pathway, water enters and exits a cell, presumably on different faces. In this route, water passes each cell across the plasma membrane twice, through direct diffusion in the phospholipid bilayer and by facilitated transport via transmembrane water channels – the aquaporins (Fig. 1.2). The transmembrane pathway may also involve the tonoplast (Taiz & Zeiger 2010). In the radial cell walls of the endodermis, the Casparian strip of hydrophobic suberin blocks the apoplastic pathway, forcing water to cross endodermis through the plasma membrane (Taiz & Zeiger 2010). The hydrophobic substances of the exodermis cell wall may also form a variable barrier for apoplastic water pathway in certain plants (Hose *et al*. 2001).

1.3.2 Root hydraulic conductivity and roles of plant PIPs in root water transport

Root hydraulic conductance (K_r) is a measure of the ease and ability of the entire plant root as an entity to conduct water. Root hydraulic conductivity (L_{pr}) is a property of the ability of the plant root to conduct water across specified dimensions, such as per unit

of root length, surface area or volume (Martinez-Ballesta *et al*. 2011). Therefore, when K_r is expressed by a specific root dimension, L_{pr} is obtained. L_{pr} is one of the major parameters of root water uptake ability in vascular plants. When expressed on the basis of root surface area, higher L_{pr} indicates stronger water transport capacity per unit of root surface area. L_{pr} can be determined by hydrostatic methods (Rüdinger *et al*. 1994; Miyamoto *et al.* 2001), and techniques such as high pressure flow meter (HPFM; Tyree *et al*. 1995) and root pressure probe (Frensch & Steudle 1989; Steudle 1993).

In the composite model of root water transport, L_{pr} is the inverse of resistance and is a function of apoplastic and cell-to-cell pathways (Steudle & Peterson 1998; Steudle 2000). Water flow follows the least resistance pathway. In apoplastic pathway, Casparian bands and suberin lamellae in endodermis and exodermis form barriers to water flow; the deposition of these hydrophobic substances causes increase in resistance and decrease in L_{pr} (Steudle 2000; Aroca *et al.* 2012). Environmental stresses may cause increased suberin deposition, which decreases water fluidity in these layers of cells, hinders apoplastic water transport and consequently decreases L_{pr}.

The hydraulic resistance and L_{pr} are also determined by the transmembrane pathway that is controlled by the transcriptional and post-translational regulation of MIPs (Törnroth-Horsefield *et al*. 2006; Maurel *et al*. 2008). Because of their subcellular localization in the plasma membrane, expression and regulation of root PIPs play significant roles in determining transmembrane water transport of root cells (Javot & Maurel 2002). Increased PIP abundance and enhanced phosphorylation in the root cortex result in more open water channels, leading to higher root cortical cell hydraulic conductivity L_{pc} (Lee *et al.* 2010). L_{pc} is a crucial component of L_{pr} (Steudle 1993). Therefore, PIP expression and regulation in the cortex have significant impact on L_{pr} (Beaudette *et al*. 2007). The direct measurements of L_{pc} can be conducted by the cell pressure probe technique, to determine the rate of water flow across the plasma membrane of root cortical cells (Steudle 1993; Javot & Maurel 2002). In the endodermis and exodermis, strong expression of PIPs has been observed in the mature zone of maize roots (*Zea mays*) (Hachez *et al*. 2006) and in grapevine roots (Gambetta *et al*. 2013), indicating the enhanced transmembrane pathway in these cell layers where the apoplastic pathway is blocked by suberin.

1.3.3 Role of PIPs in regulation of root hydraulic conductivity

Root water transport and L_{pr} readily respond to various external abiotic factors and environmental stresses, such as drought, flooding, salinity, nutrients, pH, anoxia, low temperature, light intensity and photoperiod (Fennell & Markhart 1998; Martínez-Ballesta *et al*. 2011; Aroca *et al*. 2012). L_{pr} may change synchronously with diurnal changes in environmental factors such as light intensity, whereas adverse external stimuli usually lead to the decline in L_{pr}. Many studies have shown that root PIP regulation contributes to the change in L_{pr} as the responses to environmental cues (reviewed by Javot & Maurel 2002; Maurel *et al*. 2008).

Aerial abiotic and biotic factors can change L_{pr} by regulating the expression and post-translational regulation of root PIPs. For example, the diurnal pattern in L_{pr} and its response to nutrient stress could be correlated with PIP expression in *Lotus japonicus* (Clarkson *et al*. 2000). In maize roots, PIP transcription responds to the diurnal pattern of light intensity, and an increase in the transcript abundance of PIPs precedes the diurnal peak of root water transport around midday (Lopez *et al*. 2003). In *Populus*, root water hydraulics and PIP expression are regulated by transpiration demands (Almeida-Rodriguez *et al*. 2011; Laur & Hacke 2013). A significant reduction in the transcript abundance of root PIPs coincided with a decline in L_{pr} due to defoliation in *Populus tremuloides* (Liu *et al*. 2014) and shoot topping in maize, soybean and grapevine (Vandeleur *et al.* 2014). These results indicate that xylem-mediated shoot-to-root hydraulic signals are involved in root PIP regulation to achieve hydraulic adjustment of the plants.

In soil, low temperature and anaerobic conditions repress root respiration and cause an increase in intracellular pH. It has been found that at high pH, root PIPs switch to a closed conformation, which significantly hinders transmembrane water transport in roots and therefore inhibits root water permeability (Tournaire-Roux *et al*. 2003). During prolonged chilling, however, phosphorylation modifies root PIPs into the open conformation, which increases root water transport (Aroca *et al*. 2005), which explains the recovery of L_{pr} after longer period of chilling. Under salinity stress, PIP internalization is found to be responsible for reduced L_{pr} (Boursiac *et al.* 2005).

The studies describing possible effects of rhizosphere microorganisms on root water transport have been recently reviewed (Groppa *et al*. 2012). In the review, Groppa *et al.* (2012) postulated that plant growth promoting microorganisms such as mycorrhizal fungi, opportunistic symbiotic and saprophytic fungi, as well as growth promoting rhizobacteria, improve L_{pr}, alter plant MIP gene expression and alleviate abiotic stresses. The effects are often complex, varying as a function of the interacting species and environmental stresses. The effects of mycorrhizas on L_{pr} and root MIP expression are separately addressed in details in §1.4.4.1 & 1.4.4.2 due to their importance to this study.

1.4 Mycorrhiza and plant-water relation

1.4.1 Overview of mycorrhizas and their functional types

As its original meaning in Greek indicates, mycorrhiza, the fungi-roots, refers to the symbiotic association between a fungus and the roots of a vascular plant, which is usually mutualistic, but occasionally weakly antagonistic to the plants especially in nutrient-rich soil (Smith & Read 2008; Kivlin *et al.* 2013).

The earliest fossil record shows at least 400-million-year-old history of mycorrhizal associations that might start at the dawn of terrestrial lifestyle of plants (Taylor & Osborn 1996; Brundrett 2002). This observation of the presence of mycorrhizas at the very early stage of vascular land plant evolution has been supported by phylogenetic studies based on DNA sequence data from living fungal taxa (Wang *et al*. 1999; Brundrett 2002). Among the species that have been studied so far, 83% of dicots, 79% of monocots, and all gymnosperms regularly maintain a mutualistic relationship with mycorrhizal fungi (Smith & Read 2008). Mycorrhizas have been found in diverse ecosystems ranging from boreal and temperate forests, to croplands and deserts (Smith & Read 2008). The major fungal phyla involved are Basidiomycota, Ascomycota, and Glomeromycota which have aseptate hyphae.

Based on the profound anatomical changes that arise in colonized root cells, mycorrhizas can be broadly classified into two major groups: endomycorrhizas and ectomycorrhizas (Smith & Read 2008). The most distinct difference between the two groups is that the hyphae of endomycorrhizal fungi penetrate the cell wall and invaginate the cell membrane, while the hyphae of ectomycorrhizal fungi expand only

in the intercellular space without penetrating individual root cells (Bonfante & Genre 2010).

1.4.1.1 Endomycorrhizas

Endomycorrhizas develop intracellularly but do not penetrate the protoplasts. Due to their high diversity, they are further categorized into arbuscular, ericoid, arbutoid, monotropoid, and orchid mycorrhizas. Ericoid, arbutoid and monotropoid mycorrhizas are found in the plant order Ericales, and orchid mycorrhizas are found in the plant family Orchidaceae (Smith & Read 2008). These four groups are of variable morphological characteristics and ecologically significant to specific plant species, but less understood, while arbuscular mycorrhizal (AM) fungi have been relatively well studied due to their universal presence in many plants (Hodge *et al*. 2010).

AM fungi belong to the division Glomeromycota (derived from the Zygomycota) and usually have an asexual life history. However, the genetic diversity can be ensured by a common phenomenon, heterokaryosis, which means that the individuals contain many genetically different nuclei (Parniske 2008). The hyphae of AM fungi enter into the plant root cells and produce structures of arbuscules (dichotomously-branching invaginations of cell membrane) and often, vesicles (balloon-like structure). These structures have been highly conserved since their first appearance in the fossil record 400-460 million years ago (Smith & Read 2008). AM is considered to be the ancestral form of mycorrhizas. AM fungi are associated with about 80% of studied plant species, including various crop plants. Therefore, AM is deemed as the most prevalent symbiotic interaction found in the plant kingdom (Parniske 2008).

1.4.1.2 Ectomycorrhizas

Ectomycorrhizas (ECMs) are typically formed in the roots of more than 10% of plant families, mainly involving fungi of the Basidiomycota and Ascomycota. The association is mostly formed with woody plants (Kottke & Oberwinkler 1986). Hundreds of ECM fungal species have been found in the upper layers of the soil in boreal and temperate forests. They interact with species of trees belonging to the Pinaceae, Fagaceae, Dipterocarpaceae and Caesalpinoidaceae families (Marjanović & Nehls 2008; Smith & Read 2008), and function as a crucial component of forest ecosystems. Fossil records show the evidence for a 180-million-year ECM symbiosis

history in forest ecosystems. In the case of basidiomycete and some ascomycete ECM macrofungi, spores are produced to complete the life cycle by aboveground or underground fruiting bodies, often spotted as mushrooms or truffles grown in forest soil (Yun & Hall 2004).

ECM fungi colonize the plant lateral roots in an extracellular manner. The typical ECM structure consists of a mantle (hyphal sheath) that envelops the root tip, and an intercellular Hartig net of hyphae surrounding the plant epidermal and outer cortical cells (Smith &Read 2008; Taiz & Zeiger 2010) (Fig. 1.3). Outside the root, the free-living fungal mycelia form an extensive network within the soil and leaf litter to absorb nutrients and water (Smith & Read 2008). Free-living fungal hyphae extend in soil by cell elongation and infinite cell division, and can develop into rope-like strands called rhizomorphs. According to their patterns of morphological and anatomical differentiation, and putative functional importance in terms of exploration of soil, ECM mycelial systems can be classified into (1) contact exploration type with a smooth mantle and only a few emanating hyphae, (2) medium-distance exploration type that forms rhizomorphs, (3) long-distance exploration type with rather smooth mycelia and few but highly differentiated rhizomorphs, and (4) “pick-a-back” exploration type that grows within rhizomorphs and/or mantles and can become ectendomycorrhizal (Agerer 2001). It has been suggested that the distance exploration types of mycelia, especially the most distal parts of rhizomorphal hyphae, exclusively perform acquisition and transport of water and nutrients; whereas the mantle forms an outwardly sealed compartment only for storage and exchange between the two ECM partners (Agerer 2001; Smith & Read 2008). Such exploration types are usually species- and strain-specific.

1.4.2 Ecological significance of mycorrhizal associations

1.4.2.1 Overall ecological significance

Mycorrhizal association is considered to be highly beneficial from an ecological perspective, because mineral nutrients, carbohydrates, amino acids and small secreted proteins are frequently exchanged between mycorrhizal partners (Martin & Nehls 2009; Bonfante & Genre 2010). Generally speaking, mycorrhizal fungi absorb resources from the soil through their extensive extraradical network of fine hyphae, transporting

them to the interface with root cells; meanwhile, plants translocate photosynthates to roots and share them with fungal partners (Agerer 2001; Smith & Read 2008).

It has been frequently reported that mycorrhiza can substantially improve plant nutrient conditions and alleviate the impacts of environmental stresses, such as drought, severe pH, high salinity and heavy metal accumulation, on plant growth (Boyle & Hellenbrand 1991; Schützendübel & Polle 2002; Bois *et al*. 2006; Calvo-Polanco *et al*. 2008; Calvo-Polanco *et al*. 2009; Lee *et al*. 2010; Turgeman *et al*. 2011; Navarro-Ródenas *et al*. 2012; Navarro-Ródenas *et al*. 2013). It has been suggested that ECMs can activate stress-related genes and signaling pathways, which leads to priming of pathways enhancing abiotic stress tolerance (Luo *et al*. 2009). However, mycorrhizal effect on plant growth varies a lot with the interacting species, the developmental stages of the species and interaction, as well as environmental stresses imposed on the associated species. Beneficial effects are not always significant, and in some cases, effects can be detrimental (Boyle & Hellenbrand 1991; Smith & Read 2008; Kivlin *et al.* 2013). For instance, it was reported that *Hebeloma longicaudum* improved the growth of *Picea mariana* under drought, but had no effect on *Pinus banksiana* (Boyle & Hellenbrand 1991). ECM fungal community had significant negative effects on productivity of *Pinus sylvestris* in a high fertility substrate but no apparent effects in a low fertility substrate (Jonsson *et al.* 2001).

As an important component in soil community, mycorrhizas can physically and chemically alter the availability of nutrients and water. Consequently, they play a crucial role in maintaining the balance of soil-borne microbial communities, and therefore are important to ecosystem health and carbon sequestration. In practice, mycorrhizas are widely used in agriculture, forestry, horticulture, land reclamation and ecological restoration to improve plant growth and soil quality (Boyle & Hellenbrand 1991; Siemens & Zwiazek 2011).

1.4.2.2 Carbohydrate/mineral exchange

Carbohydrate acquisition from plants is an important resource for fungal growth, and can be essential for some ECM species to complete their full life cycle of sexual reproduction. The mutualistic association provides the mycorrhizal fungal partners with consistent and direct access to carbohydrates (Fajardo-López *et al*. 2008; Martin

& Nehls 2009). In the associated plants, the disaccharides are translocated via phloem from their source (usually leaves as the photosynthesis location) to root tissue, and degraded into monosaccharides in form of glucose or fructose that are uptaken by mycorrhizal fungi. Carbohydrate acquisition by mycorrhizal fungi can enhance carbon assimilation by plant partners. For example, in spruce and aspen, the capacity of sucrose synthesis in source leaves increases upon mycorrhization (Loewe *et al*. 2000). Carbohydrates transferred into mycorrhizal fungi form a crucial component of underground carbon sink in ecosystems. For example, the hyphae of AM fungi produce the glycoprotein glomalin, forming one of the major stores of carbon in the soil (Simard *et al*. 1997).

In exchange, the plants gain significant benefits from the mycelium because of its higher absorptive capacity and larger absorptive area for nutrient (especially nitrogen and phosphorus, and trace metals such as zinc and copper) and water uptake (Hodge *et al*. 2010, Marjanović & Nehls 2008). The structures of the AM arbuscules and ECM Hartig net largely contribute to the increase in the contact surface area between the hyphae and the cell cytoplasm to facilitate the mutual nutrient transfer. It has also been proven that nutrients and water can be horizontally redistributed between different plants in the same community through the fungal network (Egerton-Warburton *et al*. 2007, Mayor *et al*. 2009). Generally, the plant nutritional status can be largely improved by mycorrhizal formation.

Under certain circumstances, such as in nutrient-limited or water-deficit soils, mycorrhizal association can be crucial for plant survival and growth (Smith & Read 2008). For instance, mycorrhizal association enables plants to utilize soil water resources that roots cannot directly access. For example, plant roots alone may be incapable of taking up demineralized phosphate ions in soils with a basic pH value (Smith *et al*. 2003). The mycelium of the mycorrhizal fungus can, however, access these phosphorus sources and make them available to the plant partners. In addition, ECMs have considerable saprotrophic capabilities. For instance, the ECM fungus *Laccaria bicolor* has been found to be able to hydrolyse organic litter, obtain nitrogen from microfauna, and transfer the nutrients to the mycorrhizal partner plants. Furthermore, ECMs promote mineral weathering and enable plants to receive nutrients from not-yet-decomposed and non-mineralized materials via the association (Taylor &

Peterson 2005; Martin *et al*. 2008). Rhizosphere acidification by respiratory CO_2 production and carbonic anhydrase promotes carbonate mineral weathering, which is considered as one of the three key weathering mechanisms of mycorrhizal fungi (Landeweert *et al*. 2001; Thorley *et al.* 2014). This process is enhanced by H^+ extrusion of ECM and AM fungi (Koele *et al*. 2014). Mycorrhizal fungi can also promote weathering of carbonate rocks by physically penetrating or trenching minerals, and secreting organic acids and chelating cations to acidify rhizosphere and complex metal cations (Thorley *et al.* 2014). However, sometimes, the loss of carbohydrates by the plant outweighs the benefits, and some other times, the plant may not benefit at all.

1.4.2.3 Resistance to disease and toxicity

Mycorrhizal plants are often more resistant to soil-borne microbial pathogens and soil toxicity due to heavy metal contamination. For instance, the ECM fungus *L. bicolor* has been discovered to possess expanded multigene families associated with hydrolysis of bacterial and microfauna polysaccharides and proteins, which may result in reduced amount of soil-borne pathogens and enhanced plant resistance to relevant diseases (Martin *et al*. 2008). Mycorrhizas can play a protective role in contaminated or acidic soil, due to both the physical barrier the mycelia construct, and the binding of the metal to the extramatrical mycelium of the fungus, without affecting the exchange of beneficial substances (Smith & Read 2008). Attributed to the above-mentioned ecological contributions of mycorrhizal fungi, the probability of successful survival of plants in barren soils usually increases when plant roots are colonized by mycorrhizal fungi (Smith & Read 2008; Onwuchekwa *et al*. 2014). By contrast, the absence of mycorrhizal fungi may slow plant growth in early succession or in degraded ecosystems.

The following sections review recent breakthroughs in understanding molecular mechanisms involved in symbiosis establishment and nutrient and water exchange.

1.4.3 Molecular biology studies on mycorrhizal symbiosis and nutrient exchange

1.4.3.1 Molecular processes involved in ECM establishment

Since the genomes of two representative ECM fungal species, the basidiomycete *L. bicolor* and the ascomycete *Tuber melanosporum*, were fully sequenced in 2008 and 2010 respectively (Martin *et al*. 2008, Martin *et al*. 2010), the physiological and ecological research of mycorrhizas has entered a new era. The characteristics of their genomes have shed new light upon the understanding of the fundamental molecular events involved in symbiosis recognition and mycorrhizal establishment (Martin *et al*. 2008; Plett & Martin 2012; Hacquard *et al*. 2013).

Symbiotic lifestyle of ECM *L. bicolor* is achieved via the orchestration of elementary processes in both plant and fungal partners through regulation of gene expression at both transcriptional and post-transcriptional levels (Martin *et al*. 2008; Plett *et al.* 2011; Plett & Martin 2012). Firstly, the genome of *L. bicolor* possesses several expanded multigene families, suggesting that the adaptation to symbiosis was preceded by gene duplication largely due to the existence of enormous transposable elements (Martin *et al*. 2008). The expanded multigene families involved in hydrolysis of bacterial and microfauna polysaccharides and proteins not only contribute to enhance plant defence against these negative soil microorganisms, but also, and more importantly, to ensure the high saprotrophic capacity of the mycorrhizal fungus that enables it to grow in soil in the absence of living plant roots.

Secondly, the genome lacks the genes coding enzymes involved in the degradation of plant cell wall components of cellulose, hemicellulose, pectins and pectates, such as fungal hydrolysase. This prevents the symbiotic fungus from degrading plant cells during the root colonization and makes it distinct from the pathogenic fungi. Lack of carbohydrate-hydrolysing enzymes enables mycorrhizal fungi to efficiently avoid triggering plant chitinase gene expression and plant defense reaction, which is the prerequisite for symbiosis establishment.

Thirdly, transcript profiling in different symbiosis developmental stages shows that a dozen of genes are surprisingly upregulated exclusively in symbiotic tissues, with the

extent of 100 folds higher than their regular expression levels (Martin *et al*. 2008). The symbiosis is established upon the communication via extruded molecules from both sides (Veneault-Fourrey *et al*. 2013). These protein products are released into the apoplastic space, and are predicted to function either in the construction of the novel symbiotic apoplastic interface, or in other relevant biological processes such as increased hyphae mitotic division, auxin metabolism, stimulation of lateral root formation, and cell wall synthesis and remodelling. These proteins are therefore considered to be the "symbiosis toolkit". Among these recently found proteins, symbiosis-regulated protein SRAP32 contains an arginine-glycine-aspartic acid cell-adhesion motif. Immunolocalisation experiment showed that when the motif contacts the root surface, hyphae are attached to the electron-dense layer which breakdown can be observed clearly afterwards (Martin *et al*. 2008). Other proteins that are likely involved in the hyphae attachment on the root surface include lectins and hydrophobins. However, further studies are needed to clarify their specific functions. Another important contributor to hyphae attachment is the mycorrhiza-induced small cystein-rich secreted proteins (MiSSPs). More than 300 species-specific *MiSSP* genes in *L. bicolor* have been found to be upregulated in ECM root tips, which suggested the role of MiSSPs in the communication between symbiotic partners. MiSSP7 is found to be necessary for symbiosis development (Plett *et al*. 2011) with its expression induced by compounds secreted from plant roots (Plett *et al*. 2012). MiSSP7 is transported from intrahyphal exosomes, to the symbiotic apoplastic space of the Hartig net and then into the plant cortical cells where it plays a role in repressing jasmonic acid responsive genes (Plett *et al*. 2014). Jasmonic acid is a negative modulator during mycorrhizal symbiosis, therefore MiSSP7 promotes symbiosis recognition by disarming the jasmonic acid signaling. In addition, clitocypin and mycocypin, cysteine protease inhibitors, are highly expressed in symbiotic tissues and are assumed to play a role in the host protease inhibition which prevents plant defence reactions. By binding to plant chitinases, clitocypin may protect chitin in fungal cell walls from degradation (Martin *et al*. 2008).

1.4.3.2 Signaling in AM symbiosis

The discoveries in the last decade indicate that the plant and AM fungus can perceive each other prior to their physical interaction (Harrison 2005; Parniske 2008; Harrison

2012). Although the mechanisms of the diffusible signals require further explanation, the abundant phosphate in root exudates has been suggested to be a signal candidate. It is also found that AM fungi produce diffusible symbiotic signals of lipochitooligosaccharides (Maillet *et al*. 2011). It has been shown recently that AM fungi secrete a mixture of sulphated and non-sulphated lipochitooligosaccharides (referred to as Myc-LCOs) with structures very similar to Nod factors of the nitrogen-fixing symbiosis formed between legumes and rhizobia, and AM symbiosis and rhizobium-legume symbiosis both requires a signaling pathway - the Common symbiosis signaling pathway (CSSP) (Harrison 2012). The hypothesis is that Myc-LCO factors secreted by fungal partner are perceived by the Myc-LCO factor receptor of host plants. Three downstream plant components of the pathway, a receptor kinase, a putative ion channel, and a calcium/calmodulin-dependent protein kinase (CCaMK), have been recently identified, which implicates calcium as a signal in the AM symbiosis (Harrison 2005). Myc factor perception could trigger a rapid transient elevation of cytosolic calcium. In *Lotus japonicus*, another membrane protein, *LjSYMRK,* codes for a receptor-like kinase that has the potential to directly or indirectly perceive AM fungal signals, and transduces the signal to the cytoplasm by phosphorylating the kinase domain of an unidentified substrate (Bonfante & Genre 2010). The input signals to this pathway and the downstream events under its control remain to be explored. The research on *Medicago truncatula* and AM fungus *Glomus versiforme* (Zhang *et al*. 2010) suggests that STR (Stunted Arbuscule) is a representative of a novel clade in the ABCG subfamily (ATP binding cassette transporter). *STR* and *STR2* are coexpressed constitutively in the vascular tissue (Zhang *et al*. 2010). Their expression is induced in arbuscule-containing cortical cells, and their protein products, the transporters, are located in the periarbuscular membrane (the plant-derived membrane, which is continuous with the plant plasma membrane and excludes the fungus from the plant cytoplasm) (Zhang *et al*. 2010). The activity of STR and STR2 is required for normal arbuscule development and consequently a functional AM symbiosis, and their silencing by RNA interference results in impaired arbuscule development and the failure of AM symbiosis (Zhang *et al*. 2010). It is also found that VAMP721 proteins are required for periarbuscular membrane formation, and Vapryin/PAM1 punctate bodies in the plant epidermal cells are a component in cellular programming for accommodation of AM symbionts (Harrison 2012). These finding

indicates that the development of the arbuscule–cortical cell interface is accompanied by AM-specific gene expression in the root cortical cells.

Upon normal arbuscule development, complex gene regulation is triggered to achieve a functional AM symbiosis interaction. For example, development of the AM symbiosis leads to the increase in lateral root formation and a decrease in phosphate-starvation-induced gene expression in a systemic signaling pattern (Harrison 2005).

1.4.3.3 Mineral nutrient transport from fungi to plants

The hyphal network allows mycorrhizal fungi to absorb mineral nutrients from narrower soil pores and more extensive soil profiles. In addition, large glycosyl hydrolase families, secreted proteases, and other secreted enzymes such as chitinases and glucanases have been discovered in the genome of ECM fungus, suggesting a high capacity for decomposing soil organic matter (Martin *et al*. 2008, Mayor *et al*. 2009).

Once the symbiosis surface is constructed, the nutrient exchange will be initiated in the plant-fungus interface in AM arbuscules and ECM Hartig network. *L. bicolor* has expanded groups of GTPases involved in transmembrane signaling transduction and active transportation (Martin *et al*. 2008). Potential influx and efflux transporters for organic and inorganic nitrogen (amino acids, ammonia and ammonium), sulphate and potassium, as well as potential high-affinity transporters for inorganic phosphate, have been found in the membranes of both fungal Hartig net and root cortical cells (Martin *et al*. 2008). In the mantle sheath, expression of a high affinity ammonium influx transporter is upregulated to trap the leaked ammonium, while it is repressed in Hartig net to prevent re-import. Urease transcript level has been observed to increase in Hartig net, followed by the release of ammonium (Martin *et al*. 2008; Lucic *et al*. 2008). Such differential expression suggests highly intense traffic of amino acids, oligopeptides and polyamines via the symbiotic interface.

Transcript profiling in mantle and Hartig net also revealed functional heterogeneity of the ECM compartments in *T. melanosporum* (Hacquard *et al*. 2013). Laser microdissection and microarray analysis shows the genes involved in soil nitrogen and water acquisition, secondary metabolite synthesis and detoxification are upregulated in the fungal mantle, while the expression of carbohydrate and nitrogen-derived transporter genes is enhanced in the Hartig net (Hacquard *et al*. 2013).

1.4.3.4 Carbohydrate transfer from plants to fungi

The other aspect of the 'fair trade' is fungal import of carbohydrates from plants. Though litter and humus layers in forest soil are usually rich in cellulose, lignin and other complex carbon sources, ECM fungi don't seem to have cell wall degrading enzymes to hydrolyse these polysaccharides; therefore most ECM fungi are highly dependent on external monosaccharides, such as glucose and fructose. Therefore, they have to largely rely on their host plants as a direct sugar source, due to the lack of both sucrose influx transporter genes and invertase (Fajardo-López *et al*. 2008).

In the genome of *L. bicolor*, 15 potential hexose transporter proteins are encoded, and the transcripts of 14 of them are detectable. ECM formation resulted in a strongly enhanced expression of six genes, and a function as hexose influx transporter was proven for three of them (Fajardo-López *et al*. 2008). Expression patterns and import kinetics of these genes are different. One group of *L. bicolor* hexose transporters is responsible for uptake of carbohydrates in soil-growing hyphae, to improve carbon acquisition and reduce nutrient uptake competition with other soil microorganisms. The other group is responsible for efficient hexose uptake at the plant–fungus symbiotic interface, where precise regulation plays important role in maintaining the balance between the mycorrhizal partners (Fajardo-López *et al*. 2008). *L. bicolor* has relatively lower exploitation efficiency of sugar source, because fructose can be taken up only when external glucose concentration drops below the K_m value (Michaelis constant in kinetic reactions) of the high affinity hexose influx transporters (Martin *et al*. 2008). This trade-off feature may be crucial for constructing a successful symbiosis relationship.

It has been observed that plants exudate cell wall invertase into apoplastic symbiotic interface in order to help their fungal partners to hydrolyse disaccharides (Martin *et al*. 2008). In such a pattern, continuous host-derived carbohydrate feeding to the mycobiont is maintained. In the Hartig net, for example, an increase in trehalose synthesis was observed following an influx of glucose and fructose (Fajardo-López *et al*. 2008).

1.4.4 Mycorrhizas and plant-water relation

1.4.4.1 The effects of mycorrhizas on root hydraulic conductivity

Being the most water permeable zone in roots, root tips play a determinant role in root water permeability (Steudle & Peterson 1998). Mycorrhiza usually forms at root tips, therefore is assumed to have significant impact on root water flow (Smith & Read 2008; Lehto & Zwiazek 2011). Many studies have illustrated that mycorrhizal fungi affect plant water relations and root hydraulic properties in the colonized host plants (Muhsin & Zwiazek 2002a, b; Augé 2004; Marjanović *et al*. 2005; Uehlein *et al*. 2007; Aroca *et al*. 2009; Lee *et al*. 2010). The contribution of mycorrhiza formation to plant water uptake has been well recognized on ecological and physiological scales (Marjanović & Nehls 2008). Firstly, the extensive hyphal network of mycorrhizas significantly increases absorbing surface and represents greater capability to take up water from lower-water-potential soil and narrower soil pores, which can directly boost plant water support by introducing additional water transport routes from the soil towards the plant root. The contribution of extraradical fungal hyphae to root water transport can be significant, as evidenced by decreased root hydraulic conductance K_r following removal of these hyphae (Muhsin & Zwiazek 2002b). Secondly, in an indirect manner, mycorrhizas modulate plant water requisite by improved nutrition and consequently reduced transpiration demand (Marjanović & Nehls 2008). For these reasons, improved plant water relations have been frequently attributed to ECM (Plamboeck *et al.* 2007; Lehto & Zwiazek 2011) and AM (Uehlein *et al*. 2007; Aroca *et al*. 2009; Bárzana *et al*. 2012). Increased L_{pr} and enhanced stress resistance attributed to mycorrhization with different ECM fungi has been reported in many tree species under various abiotic conditions, such as *P. glauca* (Landhäusser *et al*. 2002; Muhsin & Zwiazek 2002a, b), *Populus tremula* x *tremuloides* (Marjanović *et al*. 2005), *Ulmus americana* (Calvo-Polanco *et al*. 2008, 2009), *Populus balsamifera* (Siemens & Zwiazek 2008) and *P. tremuloides* (Marjanović & Nehls 2008).

Effects of mycorrhiza on root water transport may involve the root apoplastic pathway (Nylund 1987; Muhsin & Zwiazek 2002a; Bárzana *et al*. 2012) and / or MIP-mediated cell-to-cell water transport in roots (Marjanović *et al*. 2005; Porcel *et al*. 2006; Aroca *et al*. 2007; Uehlein *et al*. 2007; Lee *et al.* 2010). On one hand, changes in root anatomy and internal surfaces can substantially alter the properties of apoplastic

pathway. Relative apoplastic flow in mycorrhizal plants could be determined using apoplastic tracer dyes (Siemens & Zwiazek 2003; Bárzana *et al*. 2012) or inhibitors of aquaporin activity (Muhsin & Zwiazek 2002a; Siemens & Zwiazek 2003; Bárzana *et al.* 2012). However, these approaches can be problematic since they also potentially affect hyphal water transport. On the other hand, mycorrhizal associations alter expression of root MIPs in AM and ECM plants (Marjanović *et al*. 2005; Porcel *et al*. 2006; Aroca *et al*. 2007; Uehlein *et al*. 2007; Dietz *et al*. 2011; Giovannetti *et al*. 2012; Navarro-Ródenas *et al*. 2013) and increase L_{pc} (Lee *et al.* 2010), leading to a change in cell-to-cell water transport and L_{pr}. Lack of effect of mycorrhization on host plant root water flow properties has also been reported in some species (Coleman *et al.* 1990; Nardini *et al*. 2000; Calvo-Polanco *et al*. 2008; Siemens & Zwiazek 2008; Yi *et al*. 2008), which might be caused by the inconsistent responses of transmembrane and apoplastic water transport pathways in mycorrhizal roots.

1.4.4.2 The effects of mycorrhiza on plant root PIP expression

Extensive substrate exchanges occur in the interface between mycorrhizal partners, therefore the presence of mycorrhizal fungi can be expected to have a significant impact on membrane transporters of root cells. Numerous studies have demonstrated that the expression levels of plant PIPs and TIPs change upon mycorrhizal formation; and specific interacting species and environmental conditions may lead to different profiling patterns of root MIPs (Marjanović *et al*. 2005; Aroca *et al*. 2007; Uehlein *et al*. 2007; Navarro-Ródenas *et al*. 2013).

AM development increases PIP transcript abundance in *M. truncatula* (Krajinski *et al*. 2000). AM formation with the moderately salt-tolerant *Glomus intraradices* upregulates *LsPIP1* in *Lactuca sativa* under salt stress and alleviates salt stress injury (Jahromi *et al*. 2008). In addition, AM formation also upregulates the expression of two putative plant MIP genes – *LjNIP1* and *LjXIP1* in *L. japonicus* (Giovannetti *et al*. 2012). The water transporter *LjNIP1* is expressed solely in arbuscule-containing root cells and its protein products accumulate on the inner membrane system of arbusculated cells, suggesting its involvement in water transport in these cells.

By contrast, a decrease in the expression of PIPs and TIPs induced by AM formation and heavy metal stress has been also reported in tomato (*Solanum lycopersicum*)

(Ouziad *et al*. 2005). Porcel *et al*. (2006) observed that in *Glycine max* and *L*. *sativa* plants, AM formation affected the responses of PIP expression to drought stress, and proposed that the water loss of mycorrhizal root cells may be minimized by the reduction of the PIP expression under stress. Mycorrhization with *G*. *intraradices* reduced both K_r and PIP2 expression and phosphorylation in *Phaseolus vulgaris*, but prevented K_r and PIP2 expression from further decrease during the drought, cold and salinity stress (Aroca *et al*. 2007). Furthermore, the upregulation of *G. intraradices* putative MIP *GinAQP1* caused downregulation of AM host root PIP (Aroca *et al*. 2009). AM formation in maize also led to lower osmotic root hydraulic conductivity and reduced expression of PIPs, and this effect became more prominent upon application of abscisic acid (Ruiz-Lozano *et al*. 2009).

ECM formation also significantly influences root MIP expression. Inhibitory experiment by $HgCl_2$ suggested that the mercury-sensitive, aquaporin-mediated water transport was largely responsible for increased L_{pc} and L_{pr} of *P. banksiana* seedlings after ECM formation with fungus *Suillus tomentosus* (Lee *et al*. 2010). Differential expression of PIPs was reported in *P. tremula* x *tremuloides* upon ECM formation with *Amanita muscarua* (Marjanović *et al*. 2005). While the expression of *PttPIP2;1*, *PttPIP2;2* and *PttPIP2;*4 is downregulated in mycorrhizal fine roots, the increase in transcript abundance of *PttPIP1;1*, *PttPIP2;*3 and *PttPIP2;*5 was proposed to be responsible for a significant increase in L_{pr}, as PttPIP2;5 demonstrated strong water transport capacity (Marjanović *et al*. 2005). Differential expression of four PIPs and one TIP was also reported in both roots and leaves of *Helianthemum almeriense* upon ECM formation with hypogeous desert truffle *Terfezia claveryi* and drought stress (Navarro-Ródenas *et al*. 2013). *HaPIP1;1* was downregulated by mycorrhizal formation, whereas *HaPIP1;2* was upregulated. Under drought stress, fungal MIP *TcAQP1* (JF491353) was upregulated, tuned with the downregulation in *HaTIP1;1* and *HaPIP*s (including *HaPIP2;1* that encodes a strong water-transporting aquaporin). The authors suggest that such MIP expression pattern helps enhance plant drought resistance, as one of the beneficial effects brought by mycorrhizal symbiosis. Interestingly, similar compensatory expression pattern was also observed in the *G. intraradices* putative MIP *GinAQP1* and the AM host root PIP (Aroca *et al*. 2009).

Based on the studies of AM associations, Ruiz-Lozano & Aroca (2010) suggested that under drought stress, AM formation usually leads to PIP downregulation in mycorrhizal host plants, but tends to cause PIP upregulation in most cases of salinity stress. When stresses and AM have an interacting impact on a plant, the specific PIP responses largely depend on intrinsic osmotic stress and endogenous ABA level in the plant. The frequently observed outcome of PIP regulation by AM formation can be the improved plant-water status and increased plant resistance to stresses (Ruiz-Lozano & Aroca 2010). More species need to be studied in order to examine whether this rule also applies to ECM interactions, as ECM and AM fungi cause distinct changes in root structures that have likely different impacts on root water transport. However, based on the above-mentioned studies, it can be generalized that, although root water flow can be either increased or reduced in plants by mycorrhizal formation, and such interaction can be further complicated by environmental stresses, the changes in L_{pr} usually coincide with the changes in root PIP expression. Systematic studies of environmental factors are necessary to explain the plant MIP expression as response to the synergistic regulation by mycorrhizal symbiosis and environmental stresses (Uehlein *et al*. 2007; Maurel *et al*. 2008).

1.4.4.3 Water transport in mycorrhizas

It has been well established that mycorrhizal formation improves mineral nutrition and water relations in colonized plants via the uptake and transfer of nutrients and water through the fungal hyphae (Egerton-Warburton *et al*. 2003, Egerton-Warburton *et al*. 2007). Water transfer between mycorrhizal fungi and associated trees has been directly traced in several studies (Querejeta *et al*. 2003, 2007; Egerton-Warburton *et al*. 2008). These studies demonstrate the nocturnal water transfer from oak trees to the mycorrhizal fungi during soil drying (Querejeta *et al*. 2003) and the alleviation of the severe soil drying effect on rhizosphere hyphae due to the hydraulic lift of oak trees (Querejeta *et al*. 2003). Furthermore, hydraulically lifted water moves out of the mycorrhizal fungal hyphae during imposed drought, which may improve the surrounding moisture (Egerton-Warburton *et al*. 2008). These studies indicate that the mycorrhizal plant roots and associated fungi are integrated in water transfer.

Given these observations, it is important to understand the mechanisms underlying water transport processes from soil to fungal free-living mycelia, between different

hyphal compartments, as well as at the hyphal-root interface. Abundant fungal hyphae dramatically increase the surface and capacity of water and nutrient uptake from the soil, which usually occur simultaneously (Lehto & Zwiazek 2011, Turgeman *et al*. 2011). Additionally, the small diameter of fungal mycelia allows them to penetrate narrower soil pores where water and nutrients are present but inaccessible for plant roots with wider diameter (Augé 2004). Theoretically, water can move in the extracellular space of hyphal cells in a manner equivalent to the apoplastic pathway in plant roots. In the cell-to-cell pathway, water and nutrients enter hyphal cells via an array of membrane channels. The advantages of a symplastic pathway for hyphal water transport include the possibility of hydraulic regulation by fungal MIPs as water enters and subsequently leaves the hyphae. Both pathways influence the water status of hyphae therefore have impact on water transport of each other. Once in the hyphal protoplasts, these molecules can be transported between adjacent cells either via individual hyphae or via rhizomorphs, which comprise multiple interconnected hyphae (Agerer 2001; Peterson *et al.* 2004). Rhizomorphs show substantial hyphal differentiation that may facilitate water transport. For example, vessel hyphae have enlarged central hyphae with highly modified or absent septae for efficient water movement, while some peripheral hyphae display thickened and pigmented cell walls that may act to reduce water loss (Agerer 2001; Peterson *et al.* 2004).

Delineating the precise pathways for water transport from the fungal partner to the host roots in mycorrhizal associations remains a challenge. Some studies support the view that hydrophobic fungal cell walls in mantle may block the apoplastic water pathway and hinder root water uptake (Duddridge *et al*. 1980; Unestam & Sun 1995), whereas others argue that fungal hyphae are more likely to form a water transport highway for plant roots, which substantially increases water availability to the roots (Khalvati *et al.* 2005; Allen 2007; Egerton-Warburton *et al.* 2007; Lehto & Zwiazek 2011). Since water can be transported in the cell walls of hydrophilic fungi, including *L. bicolor* (Weatherley 1982; Agerer 2001; Lehto & Zwiazek 2011), it could be argued that this route presents the least resistance and thus could be predominant pathway for water transport to the root cortex. In the symplastic pathway, fungal MIPs regulate the entry and exit of water through the hyphae, and influence water permeability of the hphae; therefore they could impact water availability in root extracellular space.

Increased water availability in root extracellular space was postulated to be a significant factor triggering PIP transcriptional and post-translational regulation in root cells (Steudle & Peterson 1998; Javot & Maurel 2002). Since the water transporting capacity of mycorrhizal roots increases with the increasing volume of fungal hyphae (Duddridge *et al*. 1980; Brownlee *et al*. 1983; Plamboeck *et al*. 2007), it is plausible that an increase in root hydration by the fungal hyphae may provide a positive feedback mechanism regulating root MIP expression and/or function. Regulation of aquaporin-mediated water transport involves changes in the abundance of aquaporins in cell membranes and aquaporin gating which is affected by various factors including protein phosphorylation and dephosphorylation (Johansson *et al*. 1998; Kline *et al*. 2010), protonation (Tournaire-Roux *et al*. 2003; Fischer & Kaldenhoff 2008), divalent cations (Gerbeau *et al*. 2002; Verdoucq *et al*. 2008), trafficking (Prak *et al*. 2008; Maurel *et al*. 2009; Zelazny *et al*. 2009), heteromerization (Fetter *et al*. 2004), as well as turgor pressure, solute gradients and temperature (Chaumont *et al*. 2005).

In the soil-fungus-plant pathway of water movement, it can be speculated that fungal MIPs impact the symplastic water transport through hyphae, the water availability in root extracellular space, and ultimately, the water uptake of the mycorrhizal roots. As the mycorrhizal fungal MIPs become better understood, it is time to explore their roles in mycorrhizal water transport.

1.4.5 Mycorrhizal fungal MIPs and their potential roles in mycorrhizal associations

In mycorrhizal fungi, MIPs could play distinct roles in resource requisition by the growing front of substrate mycelia, in the water relation of mycorrhizal association, as well as in the developmental events involving drastic hyphal fusion, such as fruiting body formation (Nehls & Dietz 2014). Nehls & Dietz (2014) suggest the potential functions of fungal MIPs in water permeability and hyphal fusion of the growing mycelial front, and in water permeability and nutrient transfer of ECM. Efficient osmoregulation via fungal MIPs may enable mycorrhizal roots to more effectively cope with soil-associated stresses such as water deficit, high salinity or extreme pH.

Filamentous species in the Basidiomycota, Ascomycota and Glomeromycota that are capable of forming mycorrhizal associations with plants have very different lifestyles

than those of the budding yeasts. The rapidly expanding catalogue of sequenced fungal genomes across the five phyla of Kingdom Mycota (Martin *et al.* 2011; van der Heijden *et al*. 2015), including a growing list of mycorrhizal fungal species, provides an unprecedented opportunity to test the hypothesis that fungal MIPs mediate water movement from soil to the plant partner. To date, ten MIPs from the mycorrhizal fungi *G. intraradices* (AM), *L. bicolor* (ECM) and *T. claveryi* (ECM) have been functionally characterized (Aroca *et al*. 2009; Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012; Li *et al.* 2013a) (Table 1.1). The studies demonstrate strong to moderate capacity for transporting water, urea, glycerol, ammonia and CO_2. The expression of these fungal MIPs can be altered by mycorrhization or abiotic cues such as drought, salt, low temperature, and pH (Aroca *et al*. 2009; Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012; Li *et al*. 2013a; Navarro-Ródenas *et al*. 2013), suggesting their multiple roles in plant-fungal interactions, and their involvement in water transport and nutrient transfer of the mycorrhizal partners (Maurel & Plassard 2011).

In *T. claveryi*, TcAQP1 facilitates water and CO_2 conductivity. Its expression increases in vegetative mycelia grown on nutrient medium under moderate osmotic stress, and also increases in mycorrhizal root tips along with the increase in root colonization rate (Navarro-Ródenas *et al*. 2012). Navarro-Ródenas *et al*. (2012) suggest that *T. claveryi* TcAQP1 may contribute to the adaptation of the mycorrhizal association to water deficit, and may also function as a signal transduction channel during presymbiotic phase of the fungal growth and in carbon metabolism.

In *G. intraradices*, MIP JQ412060 demonstrated water transport capacity; its transcript abundance increased in mycorrhizal association with maize roots and under drought stress (Li *et al*. 2013a). In addition, JQ412060 is predicted to be located in both the plasma and intracellular membranes, supporting possible roles in regulating water flux across plasma membrane and osmotic adjustment among cellular compartments. JQ412059, the only fungal XIP with demonstrated water channel activity so far, was also upregulated in arbuscule-enriched maize root cortical cells and extraradical mycelia under drought stress, which suggests a role for this MIP in transporting water to mycorrhizal plants, thereby increasing their drought tolerance (Li *et al.* 2013a).

Fungal MIPs may be involved in nutrient uptake due to their transport capacity for small neutral molecules (Borgnia *et al*. 1999; Hohmann *et al*. 2000). In *L. bicolor*

monokaryotic strain S238N, the expression of the MIPs responded to change in temperature and mycorrhizal formation. *Lacbi2:443240* was upregulated at low temperature. Mycorrhizal formation with *P. tremuloides* significantly alters the expression of all analyzed *L. bicolor* MIPs (Dietz *et al*. 2011). Lacbi2:317173 and Lacbi2:443240 show high water transport efficiency as well as permeability for NH_3, glycerol or methylamine (Dietz *et al*. 2011). In addition to their potential roles in ECM water transport, the authors also suggest that the two MIPs with strong NH_3 transport capacity may contribute to N exchange between the fungus and the mycorrhizal root.

Another indication for the importance of fungal MIPs in mycorrhizas is their differential expression in different types of hyphal cells. In ECM between the black truffle *T. melanosporum* and the hazel *Corylus avellana*, three putative fungal MIPs (GSTUMT00010663001, GSTUMT00003976001 and GSTUMT00004612001) were upregulated in ECM root tips compared with free-living mycelium (Supplementary Table 27 of Martin *et al*. 2010). GSTUMT00003976001 (a putative orthodox fungal MIP, TmeAQP1) was significantly upregulated in the fungal mantle compared with in the Hartig net, suggesting its differential involvement in transporting substrates in mantle and Hartig net (Hacquard *et al.* 2013). GSTUMT00004612001 (a putative fungal XIP, TmeAQP2) was significantly regulated in the Hartig net, indicating its involvement in ammonia translocation into the apoplastic space as a predominant nitrogen form present at the fungal/plant interface. Differential expression of fungal MIPs was also reported in fruiting bodies, such as the upregulation of GSTUMT00010663001 in the truffles of *T. melanosporum* (Martin *et al*. 2010, Supplementary Table 27), the upregulation of a putative MIP (partial mRNA AA_35507) in *Agrocybe aegerita* fruiting bodies (Table S6 of Wang *et al*. 2013), the exclusive expression of a putative MIP (CC1G_11437T0, *i.e.*, NCBI XM_001837740.2) in primordium stage of *Coprinopsis cinerea* fruiting bodies (Additional File 1 of Cheng *et al*. 2013), and the upregulation of orthodox fungal MIP *Lacbi1:392091* in *L. bicolor* stipe (Nehls & Dietz 2014).

These studies suggest that the regulation of fungal MIPs may be important for mycorrhizal symbiosis establishment and for mycorrhizal responses to environmental stimuli such as altered nitrogen abundance, water availability and temperature. Building upon the current phylogenetic analysis and functional characterization, *in vivo*

studies on mycorrhizal associations under abiotic stresses or environmental variables will be important to deepen the understanding of the physiological and ecological importance of fungal MIPs in the mutualistic interactions.

1.5 Sporocarp formation of mycorrhizal fungi

1.5.1 Developmental stages and environmental factors of sporocarp formation

The sporocarp, often referred as a fungal fruiting body, is a multicellular structure that bears the spore-producing structures, such as basidia in basidiomycete fungi or asci in ascomycete fungi. Sporocarps of basidiomycete and ascomycete fungi are hence known as basidiocarps and ascocarps, respectively (Watkinson 2008). As one of the tissue-like structures (pseudoparenchyma) of sporocarp-bearing filamentous fungi, sporocarp is easily distinguishable from other commonly observed hyphal subnetworks, such as the growing hyphal front, hyphal cords with long-distance transporting capacities, and ECM hyphal structures (Nehls & Dietz 2014). Sporocarp formation and spore production are crucial for many basidiomycete and ascomycete mycorrhizal fungi that rely on sexual reproduction to complete their life cycle, maintain species fitness and expand their ecological distribution (Smith & Read 2008; Watkinson 2008; Fortin & Lamhamedi 2009), and are indicators of forest health (Egli 2011).

Previous research has addressed the processes of sporocarp initiation and development from the perspectives of fungal reproduction, systematics, ecological distribution and biodiversity as well as industrial production techniques and physiological mechanisms in the fungal species with significant economic importance (Yun & Hall 2004; Fortin & Lamhamedi 2009). Recent advances in fungal genomics and transcriptomics have made it possible to study major molecular events during sporocarp initiation and development of mycorrhizal fungi (Gabella *et al*. 2005; Joh *et al*. 2007; Martin *et al*. 2008; Martin & Nehls 2009; Martin *et al*. 2010) and saprophytic macrofungi (Cheng *et al*. 2013; Wang *et al*. 2013).

The sequence of events involved in basidiomycete sporocarp formation was described to include hyphal knot formation, initial aggregation, bipolar fruiting body primordium pinning, primordium differentiation with pileus and stipe, fruiting body maturation with expanded cap and elongated stipe, and spore formation (Kües & Liu 2000) (Fig.

1.4). Initiating from the vegetative stage, the mycelial tissue undergoes a sequential series of cell mitotic divisions, differentiation, elongation and meiosis to reach the final stage of spore production (Massicotte *et al*. 2005). In the model species for sporocarp studies, *Agaricus bisporus*, formation and early outgrowth of primordia are found to be "supra-exponentially" growing and highly substrate-demanding stages (Straatsma *et al*. 2013).

Sporocarp formation is controlled by complex environmental factors, including the relatively well-understood temperature, light, humidity, soil N and carbohydrates, and host genotype, growth and photosynthate supply, and its phenological dynamics (Last *et al*. 1984; Fortin & Lamhamedi 2009; Teramoto *et al*. 2012; Le Tacon *et al*. 2013). In natural forest habitats, it has been reported that the formation of basidiocarps was determined by climatic factors such as temperature, humidity and photoperiod, soil factors such as soil water content and nutrient availability, and growth of the host plants (Kües & Liu 2000). Most sporocarps of ectomycorrhizal fungal species are produced in late summer and autumn (Gévry & Villeneuve 2009). In many tree species, this coincides with the cessation of shoot growth and the second peak of seasonal root growth, which is characterized by the enhanced root respiration (Johnson-Flanagan & Owens 1985b). Under controlled cultivation conditions, the decrease in CO_2 can serve as signal for basidiocarp initiation of saprophytic macrofungi (Stamets 2000). With regard to internal signaling, it has been found that cyclopropane fatty acid synthase gene is essential for sporocarp initiation in *C. cinerea* (Liu *et al*. 2006). Sporocarp development is regulated by chitin deacetylase gene in *Flammulina velutipes* (Yamada *et al*. 2008) and by glyceraldehyde-3-phosphate dehydrogenase genes in *Pleurotus ostreatus* (Tasaki *et al*. 2014).

1.5.2 Potential significance of MIPs in sporocarp formation

Recent genomic and transcriptomic studies have started unveiling profound changes in gene regulation during sporocarp formation of several saprophytic (Nowrousian & Kück 2006; Joh *et al*. 2007; Chum *et al*. 2011; Morin *et al*. 2012; Yu *et al*. 2012; Cheng *et al*. 2013; Traeger *et al*. 2013; Wang *et al*. 2013; Rahmad *et al*. 2014) and ECM fungi such as *Tuber borchii* (Gabella *et al*. 2005), *L. bicolor* (Martin *et al*. 2008) and *T. melanosporum* (Martin *et al*. 2010) (Nowrousian 2014). The regulated genes

included clusters of transcription factor genes, mating-type genes and genes encoding photoreceptors, small secreted proteases, and enzymes of sulfur metabolic pathways.

Strong upregulation was reported for genes encoding major facilitator superfamily transporters (MFS), aquaporin-related MIPs, and amino acid permeases during mycorrhizal establishment and in mycorrhizal structures of mantle and Hartig net (Fajardo-López *et al*. 2008; Lucic *et al*. 2008; Martin *et al*. 2010; Hacquard *et al*. 2013). Sporocarp formation, during which there is considerable hyphal fusion, is a resource-demanding developmental process. Therefore, it can be speculated that these membrane transporters are highly active during the process of sporocarp formation. The upregulation of some MIPs has been reported in sporocarps compared with other hyphal structures (Martin *et al*. 2010; Cheng *et al*. 2013; Wang *et al*. 2013; Nehls & Dietz 2014) (See §1.4.5). Based on the upregulation of water-transporting orthodox fungal MIP Lacbi1:392091 in *L. bicolor* stipe (Nehls & Dietz 2014), the upregulation of a putative MIP in *A. aegerita* fruiting bodies (Wang *et al*. 2013), and the exclusive expression of a putative MIP in *C. cinerea* fruiting bodies (Cheng *et al*. 2013), Nehls & Dietz (2014) suggest that high water influx through fungal MIPs drives cell expansion to support exponential increase in fruiting body biomass. In addition, the multiple transport capacities may confer these fungal MIPs with roles beyond water transport. The impact of gene regulation of MIPs on sporocarp formation process remains a fascinating area to be explored.

1.6 Studied species

To study the roles of fungal MIPs in mycorrhizal associations, a suitable model of a pair of plant and fungal species is required. ECM between *L. bicolor* and *P. glauca* provides such a model, because of the wealth of knowledge concerning the physiology of these species and the availability of their genomic resources. In addition, mycorrhizal associations between the two species have been well characterized, and are easy to induce and maintain. Also, successful mycorrhization of *P. glauca* with *L. bicolor* makes it possible to produce abundant mature basidiocarps in controlled environments (Godbout & Fortin 1990).

1.6.1 Biology and ECM studies of Laccaria bicolor

Laccaria bicolor (Maire) P.D. Orton is a basidiomycete fungus in Order Agaricales (Bastide *et al.* 1994). Being a soil-borne filamentous fungus, its hyphal networks can develop as growing hyphal front, hyphal cords for distance transport, pseudoparenchyma-containing fruiting bodies and ectomycorrhiza (Nehls & Dietz 2014). The fungus forms ectomycorrhizal associations with roots of tree species in a broad range of genera including *Picea*, *Pinus*, *Pseudotsuga*, *Populus* and *Betula*. The ECMs of *L. bicolor* are commonly spotted as small, tan-colored mushrooms with lilac gills in temperate and boreal forests of North America in late summer and autumn (Mueller 1992; Bastide *et al.* 1994). The creamy white to light purple mycelium can be easily cultivated from spores and hyphal plugs on growth medium in a relatively wide range of temperatures, pH and nutrient availabilities and in the presence of osmotic stress (Coleman *et al*. 1989). Its mycorrhizal viability can be well resumed after a long-term storage in laboratory conditions. Although saprophytic capacity of *L. bicolor* makes it possible for the hyphae to grow in soil without living roots, it is deemed as a strictly symbiotic species because it produces basidiocarps only in the presence of the host-derived carbohydrates (Fortin & Lamhamedi 2009). The initiation and development of its basidiocarps follow the typical stages of a basidiomycete macrofungus species, which are driven by the photoperiod and the photosynthate flow from the host tree, and are influenced by the phenology of the host tree as well as climatic and soil factors (Godbout & Fortin 1990; Fortin & Lamhamedi 2009). Under controlled conditions, *L. bicolor* basidiocarp development was stimulated by short photoperiod, and low level of nitrogen and phosphorus fertilization (Godbout & Fortin 1990; Fortin & Lamhamedi 2009). There was clearly a relationship between the species involved in the mycorrhizal symbiosis and the production of basidiocarps, and this relationship may be temperature-dependent: at 24°C/18°C, more basidiocarps were produced in the association of *L. bicolor* with *Pinus strobus* than with *P. glauca* and *Pinus taeda*, whereas at 18°C/12°C, basidiocarps were produced only in the association with *P. glauca* and *P. taeda* (Godbout & Fortin 1990).

The impacts of *L. bicolor* on associated plants vary depending on plant species and abiotic conditions imposed on the ECM. It has been reported that *L. bicolor* promotes seedling growth of *Pinus banksiana* at moderate NaCl concentrations; but at severe levels of salt stress, it causes more photochemical stress and dehydration of *P.*

banksiana, and reduction in seedling biomass of *P. glauca* (Bois *et al*. 2006). Mycorrhization of *U. americana* with *L. bicolor* was found to reduce the impact of soil compaction on seedling growth, g_s and K_r (Calvo-Polanco *et al*. 2008) and to enhance seedling salt tolerance by increasing L_{pr} and alleviating chlorophyll reduction at low and high soil pH (Calvo-Polanco *et al*. 2009).

L. bicolor was the first mycorrhizal fungus with a sequenced genome (Martin *et al*. 2008). The availability of genomic resources has made *L. bicolor* an unprecedented species for the study of the exchange of water, nutrients, carbohydrates and signaling molecules on the interfaces between hyphal and root cells in mantle and Hartig net (Fajardo-López *et al*. 2008; Martin *et al*. 2008; Dietz *et al*. 2011; Plett *et al.* 2011; Plett & Martin 2012). The genome of *L. bicolor* monokaryotic strain S238N is about 65 megabase, containing around 20,000 protein-encoding genes (Martin *et al*. 2008). These include seven MIP genes predicted in the most recently released genome portal of *L. bicolor* v2.0 (JGI portal version 7.14.2, released on February 5 2015, accessed on February 22 2015, http://genome.jgi-psf.org/Lacbi2/Lacbi2.home.html), *i.e.*, Lacbi2:456764, Lacbi2:671860, Lacbi2:568479, Lacbi2:443240, Lacbi2:317173, Lacbi2:482072 and Lacbi2:576801. Mycorrhization with *L. bicolor* confers gene regulations of cell wall modification and defence mechanisms in roots of *Pinus sylvestris* (Heller *et al*. 2008). In ECM roots of *Populus spp*., *L. bicolor* led to differential expression of auxin-related genes, causing auxin accumulation and stimulating lateral root formation (Felten *et al.* 2009). *L. bicolor* is also an excellent ECM model fungus to study the role of MIPs in sporocarp formation, since it readily produces basidiocarps under controlled growth conditions with a variety of host plants and the MIPs of strain S238N have been well characterized (Godbout & Fortin 1990; Dietz *et al*. 2011). The dikaryotic strain UAMH8232, collected from the roots of *P. banksiana* in Burt Lake, Ontario, Canada, was made available by the University of Alberta Microfungus Collection and Herbarium.

1.6.2 Biology and ECM studies of Picea glauca

White spruce *Picea glauca* (Moench) Voss is one of the evergreen coniferous species in Family Pinaceae. It is widely distributed in boreal, mountainous and temperate forest regions throughout northern latitudes of North America (Hosie 1969). It grows in northeastern United States, through the northern Great Lakes and into regions below

Hudson Bay, west all the way into the northeastern region of British Columbia, and northern Montana, and up into east of the coast mountain range in Alaska, and becomes dominant species on a wide range of boreal plains (Grossnickle 2000). This species is of great ecological and economic importance in forest management and protection. Much of the early research effort has been made concentrated on investigating its biology in natural stands and nurseries, and the responses of its water relations, gas exchange, nutrition, dormancy, morphological development and stress tolerances to various environmental cues including light, humidity, air and soil temperature, wind, soil water and mineral nutrition (Goldstein *et al*. 1985; Johnson-Flanagan & Owens 1985a, b; Johnson-Flanagan & Owens 1986; Husted & Lavender 1989; Wang & Zwiazek 1999a, b; Grossnickle 2000).

P. glauca generally grows in well-drained, slightly acidic soils. Tolerance to low soil fertility, freezing and moderate shading enables *P. glauca* to eventually form climax canopy in many cases of boreal forest succession (Grossnickle 2000). In tree nurseries and growth chamber conditions, the seedlings usually undergo a series of distinct growth phases, including germination, early growth, rapid growth, bud initiation and stem finishing (Roberts & Zwiazek 2001).

Like many boreal forest species (Malloch & Malloch 1982), *P. glauca* forms mycorrhizal symbiosis with many basidiomycete fungi such as *Thelephora americana* and *Amphinema byssoides*, and ascomycete fungi, such as *Wilcoxina mikolae* (Kernaghan *et al*. 2003). Mycorrhization can be efficiently induced in controlled conditions with many other fungal species including *L. bicolor*, *Leccinum aurantiacum*, *Hebeloma crustuliniforme* and *S. tomentosus*, making *P. glauca* a popular choice for many ECM studies. Complex impacts of ECM on *P. glauca* largely depend on the interacting fungal species, and the nature and severity of stresses imposed. For instance, it has been reported that *L. aurantiacum* and *H. crustuliniforme* increase K_r under low soil temperature of weeks, but had no effect on shoot water potential, transpiration or dry mass (Landhäusser *et al*. 2002). *S. tomentosus* significantly improves seedling growth under salt stress (Bois *et al.* 2006). Under moderate salt stress, *L. bicolor* and *H. crustuliniforme* lead to positive impacts such as limited sodium uptake to shoots, enhanced K_r and increased seedling dry weight (Muhsin & Zwiazek 2002b; Nguyen *et al*. 2006). In oil sands reclamation areas, the survival rate of *P. glauca* increased by

mycorrhization with *H. crustuliniforme*, *S. tomentosus* and *L. bicolor* (Onwuchekwa *et al*. 2014).

The EST database of *ca.* 200,000 expressed sequence taqs that correspond to *ca.* 27,000 unique sequences of *P. glauca*, and the recent advancement in assembling its whole draft genome of 20.8GB in 4.9 million scaffolds, have provided the researchers with a valuable platform for gene identification (Birol et al. 2013). The improved assembly contiguity allows us to discover more genes of function with higher accuracy and to investigate the molecular processes in a wide range of biological events such as apical bud formation (El Kayal *et al*. 2011) and needle water uptake and xylem refilling (Laur & Hacke 2014b). Taking advantage of the draft genome, researchers can uncover *P. glauca* genes that are orthologs of many important genes in other plants, such as MIPs (Laur & Hacke 2014b). With the previous in-depth studies on the ecophysiology, the genomic and transcriptomic platforms has made *P. glauca* a strong candidate species for the studies of ECM water relations.

1.7 Study objectives and hypotheses

1.7.1 Hypotheses

Based on the previous mychorrhizal studies and current understanding of fungal MIPs, it can be speculated that fungal MIPs play important roles in crucial processes in ECM fungi, associated plants and their interactions. Since one of the primary functions of MIPs is water transport, fungal MIPs are expected to have significant impact on water flow properties in the hyphae of the ECM fungus and of the ECM-associated roots.

The study presented in this thesis is based on the central hypothesis that MIP-mediated transport plays important roles in hyphal water transport of the ECM fungus and, consequently, has significant impact on fungal growth and on water transport in associated roots.

Considering the advantages described in §1.6, *L. bicolor* and *P. glauca* were chosen for this thesis project to test the formulated hypotheses. Based on *in silico* analysis, water permeability assay and transcript abundance profiling of MIPs in *L. bicolor* UAMH8232, the strongest water-transporting aquaporin was selected to study its role in water transport of the mycorrhizal roots. It is hypothesized that higher transcript

abundance of the fungal aquaporin will lead to increased root water transport, reflected as higher root hydraulic conductivity and increased transcript abundance of major PIPs in root tips.

Due to rapid cell division and expansion, water transport and substrate allocation are especially important during the basidiocarp growth, one of the most drastically developing phases in the life cycle of the filamentous fungi. Given the multifunctional transport capacities of many fungal MIPs, it is hypothesized in this thesis that MIPs will be involved and significantly regulated during this developmental event, which can be revealed by transcript profiling in different stages of basidiocarp growth in *L. bicolor*.

1.7.2 Study objectives

The primary goal of this research was to characterize the MIPs of the basidiomycete fungus *L. bicolor* strain UAMH8232, and to investigate their roles in: **(1)** hyphal growth, **(2)** root water transport of the associated tree species *P. glauca* and **(3)** basidiocarp formation. The specific objectives are: **(1)** to predict the putative MIP genes in *L. bicolor* UAMH8232 by conducting phylogenetic analysis and protein secondary structure prediction; **(2)** to clone and functionally characterize MIPs for their water permeability by conducting *Xenopus* oocyte swelling assays and analyzing their transcript abundance in medium-grown mycelia by qRT-PCR quantification; **(3)** to verify the transgenic strains that have been constructed to alter the transcript abundance of the most significant water-transporting endogenous MIP gene, by Southern blot, TAIL-PCR, transcript abundance quantification and morphological observation; **(4)** to investigate the effects of altered *JQ585595* transcript abundance on mycelial growth and water relation; **(5)** to examine the transcript abundance of *L. bicolor* MIPs in induced ECM root tips of *P. glauca* seedlings, and evaluate the effectiveness of transgenic approach in regulating transgene transcription in ECM *in vivo*; **(6)** to examine the effects of mycorrhization and altered fungal MIP transgene expression on seedling growth, root hydraulics and transcript abundance of putative root PIPs; **(7)** to examine transcript profiles of *L. bicolor* MIPs during basidiocarp and compare with that during vegetative growth, in order to provide implications for the involvement of the MIP family in this important developmental event.

In this thesis, the above-mentioned studies were presented as three research chapters, *i.e.*, Chapter 2, 3 and 4, respectively. Chapter 2 of the thesis presented the phylogenetic analysis of fungal aquaporins and the discussion about their possible role in water transport of mycorrhizal associations. Chapter 3 showed the study on the role of *L. bicolor* aquaporin *JQ585595* in altering root water transport properties in ectomycorrhizal *P. glauca* seedlings. Chapter 4 showed the study on the transcript profiling of MIPs during basidiocarp development in *L. bicolor* ectomycorrhizal with *P. glauca*. At last, general conclusions were given in the Chapter 5.

1.8 References

Agerer R. 2001. Exploration types of ectomycorrhizae—a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **11**: 107–114.

Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S. 1993. Aquaporin CHIP: the archetypal molecular water channel. *American Journal of Physiology* **265**: F463-F463.

Allen MF. 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal* **6**: 291–297.

Almeida-Rodriguez AM, Hacke UG, Laur J. 2011. Influence of evaporative demand on aquaporin expression and root hydraulics of hybrid poplar. *Plant, Cell and Environment* **34**: 1318-1331.

Almeida-Rodriguez AM, Hacke UG. 2012. Cellular localization of aquaporin mRNA in hybrid poplar stems. *American Journal of Botany* **99**: 1249-1254.

Aroca R, Amodeo G, Fernández-Illescas S, Herman EM, Chaumont F, Chrispeels MJ. 2005. The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiology* **137**: 341-353.

Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* **173**: 808-816.

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Rulz-Lozano JM. 2009. Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. *Molecular Plant-Microbe Interactions* **22**: 1169-1178.

Aroca R, Porcel R, Ruiz-Lozano JM. 2012. Regulation of root water uptake under abiotic stress conditions. *Journal of Experimental Botany* **63**: 43-57.

Augé RM. 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science* **84**: 373–381.

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Rulz-Lozano JM. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Annuals of Botany* **109**: 1009-1017.

Bastide PY, Kropp BR, Piché Y. 1994. Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist* **127(3)**: 547-556.

Beaudette PC, Chlup M, Yee J, Emery RN. 2007. Relationships of root conductivity and aquaporin gene expression in *Pisum sativum*: diurnal patterns and the response to $HgCl_2$ and ABA. *Journal of Experimental Botany* **58**: 1291-1300.

Bienert GP, Thorsen M, Schüssler MD, Nilsson HR, Wagner A, Tamás MJ, Jahn TP. 2008. A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes. *BMC Biology* **6**: 26.

Bienert GP, Bienert MD, Jahn TP, Boutry M, Chaumont F. 2011. Solanaceae XIPs are plasma membrane aquaporins that facilitate the transport of many uncharged substrates. *The Plant Journal* **66**: 306-317.

Bill RM, Hedfalk K, Karlgren S, Mullins JGL, Rydstrom J, Hohmann S. 2001. Analysis of the pore of the unusual Major Intrinsic Protein channel, Yeast Fps1p. *The Journal of Biological Chemistry* **276**: 36543-36549.

Birol I, Raymond A, Jackman SD, Pleasance S, Coope R, Taylor GA, Man Saint Yuen M, Keeling CI, Brand D, Vandervalk BP, Kirk H, Pandoh P, Moore RA,

Zhao Y, Mungall AJ, Jaquish B, Yanchuk A, Ritland C, Boyle B, Bousquet J, Ritland, K, MacKay J, Bohlmann J, Jones SJ. 2013. Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* **29**: 1492-1497.

Bois G, Bigras FJ, Bertrand A, Piché Y, Fung MY, Khasa DP. 2006. Ectomycorrhizal fungi affect the physiological responses *of Picea glauca* and *Pinus banksiana* seedlings exposed to an NaCl gradient. *Tree Physiology* **26**: 1185-1196.

Bonfante P, Genre A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications* **1**: 48.

Borgnia M, Nielsen S, Engel A, Agre P. 1999. Cellular and molecular biology of the aquaporin water channels. *Annual Reviews of Biochemistry* **68**: 425–458.

Bots M, Vergeldt F, Wolters-Arts M, Weterings K, van As H, Mariani C. 2005. Aquaporins of the PIP2 class are required for efficient anther dehiscence in tobacco. *Plant physiology* **137**: 1049-1056.

Boursiac Y, Chen S, Luu DT, Sorieul M, van den Dries N, Maurel C. 2005. Early effects of salinity on water transport in *Arabidopsis* roots - molecular and cellular features of aquaporin expression. *Plant Physiology* **139**: 790-805.

Boyle CD, Hellenbrand KE. 1991. Assessment of the effect of mycorrhizal fungi on drought tolerance of conifer seedlings. *Canadian Journal of Botany* **69**: 1764-1771.

Brownlee C, Duddridge JA, Malibari A, Read DJ. 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant and Soil* **71**: 433-443.

Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New phytologist* **154**: 275-304.

Calvo-Polanco M, Zwiazek JJ, Voicu MC. 2008. Responses of ectomycorrhizal American elm (*Ulmus americana*) seedlings to salinity and soil compaction. *Plant and Soil* **308**: 189–200.

Calvo-Polanco M, Jones MD, Zwiazek JJ. 2009. Effects of pH on NaCl tolerance of american elm (*Ulmus americana*) seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria bicolor*. *Acta Physiologiae Plantarum* **31**: 515-522.

Chaumont F, Moshelion M, Daniels MJ. 2005. Regulation of plant aquaporin activity. *Biology of the Cell* **97**: 749-764.

Chaumont F, Tyerman SD. 2014. Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiology* **164**: 1600-1618.

Cheng CK, Au CH, Wilke SK, Stajich JE, Zolan ME, Pukkila PJ, Kwan HS. 2013. 5'-Serial Analysis of Gene Expression studies reveal a transcriptomic switch during fruiting body development in *Coprinopsis cinerea*. *BMC Genomics* **14**: 195.

Choi WG, Roberts DM. 2007. Arabidopsis NIP2; 1, a major intrinsic protein transporter of lactic acid induced by anoxic stress. *Journal of Biological Chemistry* **282**: 24209-24218.

Chum WW, Kwan HS, Au CH, Kwok IS, Fung YW. 2011. Cataloging and profiling genes expressed in *Lentinula edodes* fruiting body by massive cDNA pyrosequencing and LongSAGE. *Fungal Genetics and Biology* **48**: 359–369.

Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E. 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* **51**: 61-70.

Coleman MD, Bledsoe CS, Lopushinsky W. 1989. Pure culture response of ectomycorrhizal fungi to imposed water stress. *Canadian Journal of Botany* **67**: 29-39.

Coleman MD, Bledsoe CS, Smit B. 1990. Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings. *New Phytologist* **115**: 275–284.

Danielson JAH, Johanson U. 2008. Unexpected complexity of the aquaporin gene family in the moss *Physcomitrella patens*. *BMC Plant Biology* **8**: 45.

Dietz S, von Bülow J, Beitz E, Nehls U. 2011. The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: lessons for symbiotic functions. *New Phytologist* **190**: 927-40.

Duddridge JA, Malibari A, Read DJ. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* **287**: 834–836.

Egerton-Warburton LM, Graham RC, Hubbert KR. 2003. Spatial variability in mycorrhizal hypae and nutrient and water availability in a soil-weathered bedrock profile. *Plant and Soil* **249**: 331–342.

Egerton-Warburton LM, Querejeta JI, Allen MF. 2007. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *Journal of Experimental Botany* **58**: 1473–1483.

Egerton-Warburton LM, Querejeta JI, Allen MF. 2008. Efflux of hydraulically lifted water from mycorrhizal fungal hyphae during imposed drought. *Plant Signal Behav* **3**: 68-71.

Egli S. 2011. Mycorrhizal mushroom diversity and productivity—an indicator of forest health? *Annals of Forest Science* **68**: 81-88.

Ehlert C, Maurel C, Tardieu F, Simonneau T. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology* **150**: 1093-1104.

El Kayal W, Allen CCG, Ju CJT, Adams E, King-Jones S, Zaharia LI, Abrams SR, Cooke JE. 2011. Molecular events of apical bud formation in white spruce, *Picea glauca*. *Plant, Cell and Environment* **34**: 480-500.

Emanuelsson O, Brunak S, Heijne G, Nielsen H. 2007. Locating proteins in the cell using TargetP, SignalP, and related tools. *Nature Protocols* **2**: 953-971.

Fajardo-López M, Dietz S, Grunze N, Bloschies J, Weiß M, Nehls U. 2008. The sugar porter gene family of *Laccaria bicolor*: function in ectomycorrhizal symbiosis and soil-growing hyphae. *New Phytologist* **180**: 365-378.

Felten J, Kohler A, Morin E, Bhalerao RP, Palme K, Martin F, Ditengou FA, Legué V. 2009. The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar and Arabidopsis through auxin transport and signaling. *Plant physiology* **151**:1991-2005.

Fennell A, Markhart AH. 1998. Rapid acclimation of root hydraulic conductivity to low temperature. *Journal of Experimental Botany* **49**: 879-884.

Fetter K, Van Wilder V, Moshelion M, Chaumont F. 2004. Interactions between plasma membrane aquaporins modulate their water channel activity. *The Plant Cell* **16**: 215-228.

Fischer M, Kaldenhoff R. 2008. On the pH regulation of plant aquaporins. *Journal of Biological Chemistry* **283**: 33889-33892.

Fischer G, Kosinska-Eriksson U, Aponte-Santamaría C, Palmgren M, Geijer C, Hedfalk K, Hohmann S, de Groot BL, Neutze R, Lindkvist-Petersson K. 2009. Crystal structure of a yeast aquaporin at 1.15 Å reveals a novel gating mechanism. *PLoS Biology* **7**: e1000130.

Flexas J, Ribas-Carbó M, Hanson DT, Bota J, Otto B, Cifre J, McDowell N, Medrano H, Kaldenhof R. 2006. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. *The Plant Journal* **48**: 427-439.

Fortin JA, Lamhamedi MS. 2009. Ecophysiology of sporocarp development of ectomycorrhizal basidiomycetes associated with boreal forest gymnosperms. In: Khasa D, Piché Y, Coughlan AP (eds) *Advances in mycorrhizal science and technology*. Ottawa, Canada: NRC Research Press, 161-173.

Fortin MG, Morrison NA, Verma DPS. 1987. Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment. *Nucleic Acids Research* **15**: 813-824.

Fraysse LC, Wells B, McCann MC, Kjellbom P. 2005. Specific plasma membrane aquaporins of the PIP1 subfamily are expressed in sieve elements and guard cells. *Biology of the Cell* **97**: 519-534.

Frensch J, Steudle E. 1989. Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). *Plant Physiology* **91**: 719-726.

Gabella S, Abbá S, Duplessis S, Montanini B, Martin F, Bonfante P. 2005. Transcript profiling reveals novel marker genes involved in fruiting body formation in *Tuber borchii*. *Eukaryotic Cell* **4**: 1599–1602.

Gambetta GA, Fei J, Rost TL, Knipfer T, Matthews MA, Shackel KA, Walker MA, McElrone AJ. 2013. Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport. *Plant Physiology* **163**: 1254-1265.

Gao YP, Young L, Bonham-Smith P, Gusta LV. 1999. Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions. *Plant Molecular Biology* **40**: 635-644.

Gerbeau P, Amodeo G, Henzler T, Santoni V, Ripoche P, Maurel C. 2002. The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. *The Plant Journal* **30**: 71-81.

Gévry M, Villeneuve N. 2009. Ecology and management of edible ectomycorrhizal mushrooms in eastern Canada. In: Khasa D, Piché Y, Coughlan AP (eds) *Advances in mycorrhizal science and technology*. Ottawa, Canada: NRC Research Press, 175-191.

Giovannetti M, Balestrini R, Volpe V, Guether M, Straub D, Costa A, Ludewig U, Bonfante P. 2012. Two putative-aquaporin genes are differentially expressed during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *BMC Plant Biology* **12**: 186.

Godbout C, Fortin JA. 1990. Cultural control of basidiome formation in *Laccaria bicolor* with container-grown white pine seedlings. *Mycological Research* **94**: 1051-1058.

Goldstein GH, Brubaker LB, Hinckley TM. 1985. Water relations of white spruce (*Picea glauca* (Moench) Voss) at tree line in north central Alaska. *Canadian Journal of Forest Research* **15**: 1080–1087.

Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F. 2009. Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. *Biochimica et Biophysica Acta* **1788**: 1213–1228.

Groppa MD, Benavides MP, Zawoznik MS. 2012. Root hydraulic conductance, aquaporins and plant growth promoting microorganisms: A revision. *Applied Soil Ecology* **61**: 247-254.

Grossnickle SC. 2000. *Ecophysiology of northern spruce species - The Performance of Planted Seedlings.* Ottawa, Canada: NRC Research Press, 1-5, 115-170.

Gupta AB, Sankararamakrishnan R. 2009. Genome-wide analysis of major intrinsic proteins in the tree plant *Populus trichocarpa*: characterization of XIP subfamily of aquaporins from evolutionary perspective. *BMC Plant Biology* **9**: 134.

Gustavsson S, Lebrun A-S, Norden K, Chaumont F, Johanson U. 2005. A novel plant major intrinsic protein in *Physcomitrella patens* most similar to bacterial glycerol channels. *Plant Physiology* **139**: 287–295.

Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F. 2006. Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. *Plant Molecular Biology* **62**: 305-323.

Hacquard S, Tisserant E, Brun A, Legué V, Martin F, Kohler A. 2013. Laser microdissection and microarray analysis of *Tuber melanosporum* ectomycorrhizas reveal functional heterogeneity between mantle and Hartig net compartments. *Environmental Microbiology* **15**: 1853-1869.

Hansen M, Kun JF, Schultz JE, Beitz E. 2002. A single, bi-functional aquaglyceroporin in blood-stage *Plasmodium falciparum* malaria parasites. *Journal of Biological Chemistry* **277**: 4874–4882.

Harrison MJ. 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Reviews of Microbiology* **59**: 19 -42.

Harrison MJ. 2012. Cellular programs for arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology* **15**: 691-698.

Hedfalk K, Törnroth-Horsefield S, Nyblom M, Johanson U, Kjellbom P, Neutze R. 2006. Aquaporin gating. *Current Opinions in Structural Biology* **16**: 447-456.

Van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406-1423.

Heller G, Adomas A, Li G, Osborne J, van Zyl L, Sederoff R, Finlay RD, Stenlid J, Asiegbu FO. 2008. Transcriptional analysis of *Pinus sylvestris* roots challenged with the ectomycorrhizal fungus *Laccaria bicolor*. *BMC Plant Biology* **8**: 19.

Herrera M, Hong NJ, Garvin JL. 2006. Aquaporin-1 transports NO across cell membranes. *Hypertension* **48**: 157-164.

Hirokawa T, Boon-Chieng S, Mitaku S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* **14**: 378-379.

Hodge A, Helgason T, Fitter AH. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* **3**: 267-273.

Hohmann S, Bill RM, Kayingo G, Prior BA. 2000. Microbial MIP channels. *Trends in Microbiology* **8**: 33–38.

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. 2001. The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany* **52**: 2245-2264.

Hosie RC. 1969. *Native Trees of Canada*. 7th Edition, Canadian Forestry Service, Department of Fisheries and Forestry, Ottawa, Canada: Queen's Printer, 64-65.

Hub JS, De Groot BL. 2008. Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proceedings of the National Academy of Sciences USA* **105**: 1198-1203.

Husted L, Lavender DP. 1989. Effect of soil temperature upon the root growth and mycorrhizal formation of white spruce (*Picea glauca* (Moench) Voss) seedlings grown in controlled environments. *Annals of Forest Science* **46**: 750–753.

Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM. 2008. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecology* **55**: 45-53.

Javot H, Maurel C. 2002. The role of aquaporins in root water uptake. *Annals of Botany* **90**: 301-313.

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Güçlü J, Vinh J, Heyes J, Franck KI, Schäffner AR, Bouchez D, Maurel C. 2003. Role of a single aquaporin isoform in root water uptake. *The Plant Cell* **15**: 509-522.

Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, Kjellbom P. 1998. Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *The Plant Cell* **10**: 451-459.

Johnson-Flanagan AM, Owens JN. 1985a. Development of white spruce (*Picea glauca*) seedling roots. *Canadian Journal of Botany* **63**: 456–462.

Johnson-Flanagan AM, Owens JN. 1985b. Root growth and root growth capacity of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Canadian Journal of Forest Research* **15**: 625-630.

Johnson-Flanagan AM, Owens JN. 1986. Root respiration in white spruce (*Picea glauca* [Moench] Voss) seedlings in relation to morphology and environment. *Plant Physiology* **81**: 21–25.

Joh JH, Lee JS, Kim KH, Jeong SJ, Youn WH, Kim NK, Son ES, Cho YS, Yoo YB, Lee CS, Kim BG. 2007. Isolation of genes expressed during the developmental stages of the oyster mushroom, *Pleurotus ostreatus*, using expressed sequence tags. *FEMS Microbiology Letters* **276**: 19–25.

Jonsson LM, Nilsson MC, Wardle DA, Zackrisson O. 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos* **93**: 353-364.

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N. 2008. Aquaporins and plant water balance. *Plant, Cell and Environment* **31**: 658-666.

Kernaghan G, Sigler L, Khasa D. 2003. Mycorrhizal and root endophytic fungi of containerized *Picea glauca* seedlings assessed by rDNA sequence analysis. *Microbial Ecology* **45**: 128-136.

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U. 2005. Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water

relations, and gas exchange of barley subjected to drought stress. *Plant Biology* **7**: 706–712.

Kivlin SN, Emery SM, Rudgers JA. 2013. Fungal symbionts alter plant responses to global change. *America Journal of Botany* **100**: 1445-1457.

Kline KG, Barrett-Wilt GA, Sussman MR. 2010. In planta changes in protein phosphorylation induced by the plant hormone abscisic acid. *Proceedings of the National Academy of Sciences USA* **107**: 15986-15991.

Koele N, Dickie IA, Blum JD, Gleason JD, de Graaf L. 2014. Ecological significance of mineral weathering in ectomycorrhizal and arbuscular mycorrhizal ecosystems from a field-based comparison. *Soil Biology and Biochemistry* **69**: 63-70.

Kottke I, Oberwinkler F. 1986. Mycorrhiza of forest trees—structure and function. *Trees* **1**: 1-24.

Krajinski F, Biela A, Schubert D, Gianinazzi-Pearson V, Kaldenhoff R, Franken P. 2000. Arbuscular mycorrhiza development regulates the mRNA abundance of Mtaqp1 encoding a mercury-insensitive aquaporin of *Medicago truncatula*. *Planta* **211**: 85-90.

Krogh A, Larsson B, Von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *Journal of Molecular Biology* **305**: 567-580.

Kües U, Liu Y. 2000. Fruiting body production in basidiomycetes. *Applied Microbiology and Biotechnology* **54**: 141-152.

Laizé V, Gobin R, Rousselet G, Badler C, Hohmann S, Ripoche P, Tacnet,F. 1999. Molecular and functional study of *AQY1* from *Saccharomyces cerevisiae*: role of the C-terminal domain. *Biochemical and Biophysical Research Communications* **257**: 139-144.

Landeweert R, Hoffland E, Finlay RD, Kuyper TW, van Breemen N. 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution* **16**: 248-254.

Landhäusser SM, Muhsin TM, Zwiazek JJ. 2002. The effect of ectomycorrhizae on water relations in aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) at low soil temperatures. *Canadian Journal of Botany* **80**: 684-689.

Last FT, Mason PA, Pelham J, Ingleby K. 1984. Fruitbody production by sheathing mycorrhizal fungi: effects of 'host'genotypes and propagating soils. *Forest Ecology and Management* **9**: 221-227.

Laur J, Hacke UG. 2013. Transpirational demand affects aquaporin expression in poplar roots. *Journal of Experimental Botany* **64**: 2283-2293.

Laur J, Hacke UG. 2014a. The role of water channel proteins in facilitating recovery of leaf hydraulic conductance from water stress in *Populus trichocarpa*. *PloS One* **9**: e111751.

Laur J, Hacke UG. 2014b. Exploring *Picea glauca* aquaporins in the context of needle water uptake and xylem refilling. *New Phytologist* **203**: 388-400.

Lee SH, Calvo-Polanco M, Chung GC, Zwiazek JJ. 2010. Role of aquaporins in root water transport of ectomycorrhizal jack pine (*Pinus banksiana*) seedlings exposed to NaCl and fluoride. *Plant, Cell and Environment* **33**: 769–780.

Lehto T, Zwiazek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* **21**: 71–90.

Liu J, Equiza MA, Navarro-Ródenas A, Lee SH, Zwiazek JJ. 2014. Hydraulic adjustments in aspen (*Populus tremuloides*) seedlings following defoliation involve root and leaf aquaporins. *Planta* **240**: 553-564.

Liu Y, Srivilai P, Loos S, Aebi M, Kües U. 2006. An essential gene for fruiting body initiation in the basidiomycete *Coprinopsis cinerea* is homologous to bacterial cyclopropane fatty acid synthase genes. *Genetics* **172**: 873-884.

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B. 2013a. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617-630.

Li X, Wang X, Yang Y, Li R, He Q, Fang X, Luu DT, Maurel C, Lin J. 2011. Single-molecule analysis of PIP2;1 dynamics and partitioning reveals multiple modes of *Arabidopsis* plasma membrane aquaporin regulation. *Plant Cell* **23**: 3780–3797.

Li X, Luu DT, Maurel C, Lin J. 2013b. Probing plasma membrane dynamics at the single-molecule level. *Trends in Plant Science* **18**: 617-624.

Loewe A, Einig W, Shi L, Dizengremel P, HAMPP R. 2000. Mycorrhiza formation and elevated CO_2 both increase the capacity for sucrose synthesis in source leaves of spruce and aspen. *New Phytologist* **145**: 565-574.

Lopez F, Bousser A, Sissoëff I, Gaspar M, Lachaise B, Hoarau J, Mahé A. 2003. Diurnal regulation of water transport and aquaporin gene expression in maize roots: contribution of PIP2 proteins. *Plant and Cell Physiology* **44**: 1384-1395.

Lucic E, Fourrey C, Kohler A, Martin F, Chalot M, Brun-Jacob A. 2008. A gene repertoire for nitrogen transporters in *Laccaria bicolor*. *New Phytologist* **180**: 343-364.

Ludewig U, Dynowski M. 2009. Plant aquaporin selectivity: where transport assays, computer simulations and physiology meet. *Cellular and Molecular Life Sciences* **66**: 3161-3175.

Luo ZB, Janz D, Jiang X, Göbel C, Wildhagen H, Tan Y, Rennenberg H, Feussner I, Polle A. 2009. Upgrading root physiology for stress tolerance by ectomycorrhizas: insights from metabolite and transcriptional profiling into reprogramming for stress anticipation. *Plant Physiology* **151**: 1902-1917.

Maeshima M, Ishikawa F. 2008. ER membrane aquaporins in plants. *Pflügers Archiv-European Journal of Physiology* **456**: 709-716.

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Bécard G, Dénarié J. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469**: 58-63.

Malloch D, Malloch B. 1982. The mycorrhizal status of boreal plants: additional species from northeastern Ontario. *Canadian Journal of Botany* **60**: 1035-1040.

Marjanović Ž, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiss M, Hampp R, Nehls U. 2005. Aquaporins in poplar: what a difference a symbiont makes! *Planta* **222**: 258–268.

Marjanović Ž, Nehls U. 2008. Ectomycorrhiza and water transport. In: Varma A (ed) *Mycorrhiza*. Berlin, Heidelberg, Germany: Springer, 149-159.

Martinez-Ballesta MC, Rodriiguez-Hernández MC, Alcaraz-López C, Mota-Cadenas C, Muries B, Carvajal M. 2011. Plant Hydraulic Conductivity: The Aquaporins Contribution. In: *Hydraulic Conductivity - Issues, Determination and Applications*. (Ed.) Elango L. InTech, doi: 10.5772/18580.

Martin F, Aerts A, Ahrn D, Brun A, Danchin EGJ. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452**: 88-92.

Martin F, Nehls U. 2009. Harnessing ectomycorrhizal genomics for ecological insights. *Current Opinion in Plant Biology* **12**: 508–515.

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, Porcel B, Rubini A, Amicucci A, Amselem J, Anthouard V, Arcioni S, Artiguenave F, Aury J, Ballario P, Bolchi A, Brenna A, Brun A, Buee M, Cantarel B, Chevalier G, Couloux A, Da Silva C, Denoeud F, Duplessis S, Ghignone S, Hilselberger B, Iotti M, Marcais B, Mello A, Miranda M, Pacioni G, Quesneville H, Riccioni C, Ruotolo R, Splivallo R, Stocchi V, Tisserant E, Viscomi AR, Zambonelli A, Zampieri E, Henrissat B, Lebrun M, Paolocci F, Bonfante P, Ottonello S, Wincker P. 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature*. **464**: 1033-1038.

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV. 2011. Sequencing the fungal tree of life. *New Phytologist* **190**: 818-821.

Massicotte HB, Melville LH, Peterson RL. 2005. Building a basidiocarp: a case study of *Laccaria spp*. fruitbodies in the extraradical mycelium of *Pinus* ectomycorrhizas. *Mycologist* **19**: 141-149.

Maurel C, Reizer J, Schroeder JI, Chrispeels MJ. 1993. The vacuolar membrane protein gamma-TIP creates water specific channels in *Xenopus oocytes*. *The EMBO Journal* **12**: 2241.

Maurel C, Verdoucq L, Luu D, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology* **59**: 595-624.

Maurel C, Plassard C. 2011. Aquaporins: for more than water at the plant–fungus interface? *New Phytologist* **190**: 815–817.

Mayor JR, Schuur EAG, Henkel TW. 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecology Letters* **12**: 171-183.

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M. 2006. A silicon transporter in rice. *Nature* **440**: 688-691.

Miller EW, Dickinson BC, Chang CJ. 2010. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. *Proceedings of the National Academy of Sciences USA* **107**: 15681-15686.

Mitani N, Yamaji N, Ma JF. 2008. Characterization of substrate specificity of a rice silicon transporter, Lsi1. *Pflügers Archiv-European Journal of Physiology* **456**: 679-686.

Mitani-Ueno N, Yamaji N, Zhao FJ, Ma JF. 2011. The aromatic/arginine selectivity filter of NIP aquaporins plays a critical role in substrate selectivity for silicon, boron, and arsenic. *Journal of Experimental Botany* **62**: 4391-4398.

Miyamoto N, Steudle E, Hirasawa T, Lafitte R. 2001. Hydraulic conductivity of rice roots. *Journal of Experimental Botany* **52**: 1835-1846.

Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL, Bailey AM, Billette C, Coutinho PM, Deakin G, Doddapaneni H, Floudas D, Grimwood J, Hildén K, Kües U, LaButti KM, Lapidus A, Lindquist EA, Lucas SM, Murat C, Riley RW, Salamov AA, Schmutz J, Subramanian V, Wösten HAB, Xu J, Eastwood DC, Foster GD, Sonnenberg ASM, Cullen D, de Vries RP, Lundell T, Hibbett DS, Henrissat B, Burton KS, Kerrigan RW, Challen MP, Grigoriev IV, Martin F. 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. *Proceedings of the National Academy of Sciences USA* **109**: 17501–17506.

Mueller GM. 1992. *Systematics of Laccaria (Agaricales) in the continental United States and Canada, with discussions on extralimital taxa and descriptions of extant types*. Chicago, USA: Field Museum of Natural History.

Muhsin TM, Zwiazek JJ. 2002a. Ectomycorrhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus americana* seedlings. *New Phytologist* **153**: 153-158.

Muhsin TM, Zwiazek JJ. 2002b. Ectomycorrhizae increase water conductance and protect white spruce (*Picea glauca*) seedlings against salt stress. *Plant and Soil* **238**: 217-225.

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi, Y. 2000. Structural determinants of water permeation through aquaporin-1. *Nature* **407**: 599-605.

Nardini A, Salleo S, Tyree MT, Vertovec M. 2000. Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Annuals of Forest Science* **57**: 305–312.

Navarro-Ródenas A, Ruíz-Lozano JM, Kaldenhoff R, Morte A. 2012. The aquaporin TcAQP1 of the desert truffle *Terfezia claveryi* is a membrane pore for water and CO_2 transport. *Molecular Plant-Microbe Interaction* **25**: 259-266.

Navarro-Ródenas A, Bárzana G, Nicolás E, Carra A, Schubert A, Morte A. 2013. Expression analysis of aquaporins from desert truffle mycorrhizal symbiosis reveals a fine-tuned regulation under drought. *Molecular Plant-Microbe Interactions* **26**: 1068-1078.

Nehls U, Dietz S. 2014. Fungal aquaporins: cellular functions and ecophysiological perspectives. *Applied Microbiology and Biotechnology* **98**: 8835-8851.

Nguyen H, Calvo Polanco M, Zwiazek JJ. 2006. Gas exchange and growth responses of ectomycorrhizal *Picea mariana*, *Picea glauca*, and *Pinus banksiana* seedlings to NaCl and Na_2SO_4. *Plant Biology* **8**: 646-652.

Nowrousian M, Kück U. 2006. Comparative gene expression analysis of fruiting body development in two filamentous fungi. *FEMS Microbiology Letters* **257**: 328–335.

Nowrousian M. 2014. Genomics and transcriptomics to analyze fruiting body development. In: Esser K (ed) *Fungal Genomics XIII*. 2nd Edition, Berlin, Germany: Springer, 149-172.

Nylund J-E. 1987. The ectomycorrhizal infection zone and its relation to acid polysaccharides of cortical cell walls. *New Phytologist* **106**: 505–516.

Obroucheva NV, Sin'kevich IA. 2010. Aquaporins and cell growth. *Russian Journal of Plant Physiology* **57**: 153-165.

Onwuchekwa NE, Zwiazek JJ, Quoreshi A, Khasa DP. 2014. Growth of mycorrhizal jack pine (*Pinus banksiana*) and white spruce (*Picea glauca*) seedlings planted in oil sands reclaimed areas. *Mycorrhiza* **24**: 431-441.

Ouziad F, Hildebrandt U, Schmelzer E, Bothe H. 2005. Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. *Journal of Plant Physiology* **162**: 634-649.

Park W, Scheffler BE, Bauer PJ, Campbell BT. 2010. Identification of the family of aquaporin genes and their expression in upland cotton (*Gossypium hirsutum* L.). *BMC Plant Biology* **10**: 142.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763-775.

Peterson RL, Massicotte HB, Melville LH. 2004. *Mycorrhizas: Anatomy and Cell Biology*. Ottawa, Canada: NRC Research Press, 173.

Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S. 2005. Aquaporins in yeasts and filamentous fungi. *Biology of the Cell* **97**: 487–500.

Plamboeck AH, Dawson TE, Egerton-Warburton LM, North M, Bruns TD, Querejeta JI. 2007. Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. *Mycorrhiza* **17**: 439–447.

Plett JM, Kemppainen M, Kale SD, Kohler A, Legué V, Brun A, Tyler BM, Pardo AG, Martin F. 2011. A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology* **21**: 1197-1203.

Plett JM, Martin F. 2012. Poplar root exudates contain compounds that induce the expression of *MiSSP7* in *Laccaria bicolor*. *Plant Signaling and Behavior* **7**: 12-15.

Plett JM, DaguerreY, Wittulsky S, Vayssières A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C, Martin F. 2014. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences USA* **111**: 8299-8304.

Porcel R, Aroca R, Azcón R, Ruiz-Lozano J. 2006. PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Molecular Biology* **60**: 389-404.

Prado K, Maurel C. 2013. Regulation of leaf hydraulics: from molecular to whole plant levels. *Frontiers in Plant Science.* doi: 10.3389/fpls.2013.00255.

Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. 2008. Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2; 1 in response to salt stress. *Molecular and Cellular Proteomics* **7**: 1019-1030.

Prasad GV, Coury LA, Finn F, Zeidel ML. 1998. Reconstituted aquaporin 1 water channels transport CO_2 across membranes. *Journal of Biological Chemistry* **273**: 33123–33126.

Querejeta J, Egerton-Warburton LM, Allen MF. 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* **134**: 55-64.

Querejeta JI, Egerton-Warburton LM, Allen MF. 2007. Hydraulic lift may buffer rhizosphere hyphae against the negative effects of severe soil drying in a California Oak savanna. *Soil Biology and Biochemistry* **39**: 409-417.

Rahmad N, Al-Obaidi JR, Rashid NM, Zean NB, Yusoff MH, Shaharuddin NS, Jamil NA, Saleh NM. 2014. Comparative proteomic analysis of different developmental stages of the edible mushroom *Termitomyces heimii*. *Biological Research* **47**: 30.

Roberts JJ, Zwiazek JJ. 2001. Growth, morphology, and gas exchange in white spruce (*Picea glauca*) seedlings acclimated to different humidity conditions. *Canadian Journal of Forest Research* **31**: 1038-1045.

Rüdinger M, Hallgren SW, Steudle E, Schulze ED. 1994. Hydraulic and osmotic properties of spruce roots. *Journal of Experimental Botany* **45**: 1413-1425.

Ruiz-Lozano JM, del Mar Alguacil M, Bárzana G, Vernieri P, Aroca R. 2009. Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. *Plant Molecular Biology* **70**: 565-579.

Ruiz-Lozano JM, Aroca R. 2010. Modulation of aquaporin genes by the arbuscular mycorrhizal symbiosis in relation to osmotic stress tolerance. In: Seckbach J, Grube M (eds) *Symbioses and Stress*. Netherlands: Springer, 357-374.

Sakr S, Alves G, Morillon R, Maurel K, Decourteix M, Guilliot A, Fleurat-**Lessard P, Julien JL, Chrispeels MJ. 2003.** Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiology* **133**: 630-641.

Schützendübel A, Polle A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany* **53**: 1351-1365.

Shelden MC, Howitt SM, Kaiser BN, Tyerman SD. 2009. Identification and functional characterization of aquaporins in the grapevine, *Vitis vinifera*. *Functional Plant Biology* **36**: 1065–1078.

Sidoux-Walter F, Pettersson N, Hohmann S. 2004. The *Saccharomyces cerevisiae* aquaporin Aqy1 is involved in sporulation. *Proceedings of the National Academy of Sciences USA* **101**: 17422-17427.

Siemen J, Zwiazek JJ. 2003. Effects of water deficit stress and recovery on the root water relations of trembling aspen (*Populus tremuloides*) seedlings. *Plant Science* **165**: 113-120.

Siemens AJ, Zwiazek JJ. 2008. Root hydraulic properties and growth of balsam poplar (*Populus balsamifera*) mycorrhizal with *Hebeloma crustuliniforme* and *Wilcoxina mikolae* var. *mikolae*. *Mycorrhiza* **18**: 393–401.

Siemens J, Zwiazek J. 2011. *Hebeloma crustuliniforme* modifies root hydraulic responses of trembling aspen (*Populus tremuloides*) seedlings to changes in external pH. *Plant and Soil* **345**: 247–256.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579-582.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16-20.

Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. 3rd Edition, Cambridge, UK: Academic Press.

Soveral G, Prista C, Moura TF, Loureiro-Dias MC. 2010. Yeast water channels: an overview of orthodox aquaporins. *Biology of the Cell* **103**: 35-54.

Stamets P. 2000. *Growing gourmet and medicinal mushrooms*. 3rd Edition, Berkeley, CA: Ten Speed Press, 113, 164, 185.

Steudle E. 1993. Pressure probe techniques: basic principles and application to studies of water and solute relations at the cell, tissue, and organ level. In: Smith JAC, Griffiths H (eds) *Water Deficits: Plant Responses from Cell to Community*. Oxford, UK: Bios Scientific Publishers Ltd, 5–36.

Steudle E, Peterson CA. 1998. How does water get through roots? *Journal of Experimental Botany* **49**: 775–788.

Steudle E. 2000. Water uptake by roots: effects of water deficit. *Journal of Experimental Botany* **51(350)**: 1531-1542.

Straatsma G, Sonnenberg AS, Van Griensven LJ. 2013. Development and growth of fruit bodies and crops of the button mushroom, *Agaricus bisporus*. *Fungal Biology* **117**: 697–707.

Le Tacon F, Zeller B, Plain C, Hossann C, Bréchet C, Robin C. 2013. Carbon transfer from the host to *Tuber melanosporum* mycorrhizas and ascocarps followed using a ^{13}C pulse-labeling technique. *PloS One* **8**: e64626.

Taiz L, Zeiger E. 2010. *Plant physiology*. 5th Edition, Sunderland, MA: Sinauer Associates, 87-89, 125-126.

Takano J, Wada M, Ludewig U, Schaaf G, Von Wirén N, Fujiwara T. 2006. The *Arabidopsis* major intrinsic protein NIP5; 1 is essential for efficient boron uptake and plant development under boron limitation. *The Plant Cell* **18**: 1498-1509.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods**.** *Molecular Biology and Evolution* **28**: 2731-2739.

Tasaki Y, Sato R, Toyama S, Kasahara K, Ona Y, Sugawara M. 2014. Cloning of glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycete mushroom *Pleurotus ostreatus* and analysis of their expression during fruit-body development. *Mycoscience* **55**: 280-288.

Taylor JH, Peterson CA. 2005. Ectomycorrhizal impacts on nutrient uptake pathways in woody roots. *New Forests* **30**: 203-214.

Taylor TN, Osborn JM. 1996. The importance of fungi in shaping the paleoecosystem. *Review of Paleobotany and Palynology* **90**: 249–262.

Teramoto M, Wu B, Hogetsu T. 2012. Transfer of ^{14}C-photosynthate to the sporocarp of an ectomycorrhizal fungus *Laccaria amethystina*. *Mycorrhiza* **22**: 219-225.

Thorley RMS, Taylor LL, Banwart SA, Leake JR, Beerling DJ. 2014. The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling. *Plant, Cell and Environment*. doi: 10.1111/pce.12444.

Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P. 2006. Structural mechanism of plant aquaporin gating. *Nature* **439**: 688–694.

Törnroth-Horsefield S, Hedfalk K, Fischer G, Lindkvist-Petersson K, Neutze R. 2010. Structural insights into eukaryotic aquaporin regulation. *FEBS Letters* **584**: 2580–2588.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**: 393-397.

Traeger S, Altegoer F, Freitag M, Gabaldon T, Kempken F, Kumar F, Marcet-Houben M, Pöggeler S, Stajich, JE, Nowrousian M. **2013.** The genome anddevelopment-dependent transcriptomes of *Pyronema confluens*: a window into fungal evolution. *PLoS Genetics* **9**: e1003820.

Turgeman T, Asher JB, Roth-Bejerano N, Kapulnik Y, Sitrit Y. 2011. Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions. *Mycorrhiza* **21**: 623-630.

Tyree MT, Patiño S, Bennink J, Alexander J. 1995. Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany* **46**: 83-94.

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R. 2003. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. *Nature* **425**: 734-737.

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R. 2007. Arbuscular mycorrhizal symbiosis and plant aquaporin expression. *Phytochemistry* **68**: 122–129.

Unestam T, Sun YP. 1995. Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. *Mycorrhiza* **5**: 301–311.

Vandeleur RK, Sullivan W, Athman A, Jordans C, Gilliham M, Kaiser BN, Tyerman SD. 2014. Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. *Plant, Cell and Environment* **37**: 520-538.

Veneault-Fourrey C, Plett JM, Martin F. 2013. Who is Controlling whom within the Ectomycorrhizal Symbiosis: Insights from Genomic and Functional Analyses. In: de Bruijn FJ (ed) *Molecular Microbial Ecology of the Rhizosphere*. Wiley-Blackwell, Volume 1: 501-512.

Verdoucq L, Grondin A, Maurel C. 2008. Structure-function analysis of plant aquaporin AtPIP2; 1 gating by divalent cations and protons. *Biochemical Journal* **415**: 409-416.

Verma RK, Prabh ND, Sankararamakrishnan R. 2014. New subfamilies of major intrinsic proteins in fungi suggest novel transport properties in fungal channels: implications for the host-fungal interactions. *BMC Evolutionary Biology* **14**: 173.

Voicu MC, Cooke JE, Zwiazek JJ. 2009. Aquaporin gene expression and apoplastic water flow in bur oak (*Quercus macrocarpa*) leaves in relation to the light response of leaf hydraulic conductance. *Journal of Experimental Botany* **60**: 4063-4075.

Walz T, Hirai T, Murata K, Heymann JB, Mitsuoka K, Fujiyoshi Y, Smith BL, Agre P, Engel A. 1997. The three-dimensional structure of aquaporin-1. *Nature* **387**: 624-626.

Wang M, Gu B, Huang J, Jiang S, Chen Y, Yin Y, Pan Y, Yu G, Li Y, Wong BHC, Liang Y, Sun H. 2013. Transcriptome and proteome exploration to provide a resource for the study of *Agrocybe aegerita*. *PloS One* **8**: e56686.

Wang DYC, Kumar S, Hedges SB. 1999. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society of London B* **266**: 163 –171.

Wang Y, Zwiazek J. 1999a. Spring changes in water relations, gas exchange and carbohydrates of white spruce (*Picea glauca*) seedlings. *Canadian Journal of Forest Research* **29**: 332–338.

Wang Y, Zwiazek J. 1999b. Effects of early spring photosynthesis on carbohydrate content, bud flushing, and root and shoot growth of *Picea glauca* bareroot seedlings. *Scandinavian Journal of Forest Research* **14**: 295–302.

Watkinson SC. 2008. Basidiomycota. In: *Encyclopedia of Life Sciences (ELS)*. Chichester, UK: John Wiley & Sons, doi:10.1002/9780470015902.a0000347.pub2.

Weatherley PE. 1982. Water uptake and flow in roots. In: Lange O, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological plant ecology II*. Berlin, Germany: Springer, 79–109.

Wu B, Beitz E. 2007. Aquaporins with selectivity for unconventional permeants. *Cellular and Molecular Life Sciences* **64**: 2413-2421.

Wysocki R, Chéry CC, Wawrzycka D, Van Hulle M, Cornelis R, Thevelein JM, Tamás MJ. 2001. The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*. *Molecular Microbiology* **40**: 1391-1401.

Yamada M, Kurano M, Inatomi S, Taguchi G, Okazaki M, Shimosaka M. 2008. Isolation and characterization of a gene coding for chitin deacetylase specifically expressed during fruiting body development in the basidiomycete *Flammulina velutipes* and its expression in the yeast *Pichia pastoris*. *FEMS Microbiology Letters* **289**: 130-137.

Yi H, Calvo-Polanco M, MacKinnon MD, Zwiazek JJ. 2008. Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. *Environmental and Experimental Botany* **62**: 357–363.

Yun W, Hall IR. 2004. Edible ectomycorrhizal mushrooms: challenges and achievements. *Canadian Journal of Botany* **82**: 1063-1073.

Yu GJ, WangM, Huang J, Yin YL, Chen YJ, Jian S, Jin YX, Lan XQ, Wong BHC, Liang Y, Sun H. 2012. Deep insight into the *Ganoderma lucidum* by comprehensive analysis of its transcriptome. *PLoS One* **7**: e44301.

Zardoya R. 2005. Phylogeny and evolution of the major intrinsic protein family. *Biology of the Cell* **97**: 397-414.

Zelazny E, Borst JW, Muylaert M, Batoko H, Hemminga MA, Chaumont F. 2007. FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. *Proceedings of the National Academy of Sciences USA* **104**: 12359-12364.

Zhang Q, Blaylock LA, Harrison MJ. 2010. Two *Medicago truncatula* half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. *The Plant Cell* **22**: 1483-1497.

Zhang R, Verkman AS. 1991. Water and urea permeability properties of *Xenopus* oocytes: expression of mRNA from toad urinary bladder. *American Journal of Physiology* **260**: C26–34.

Table 1.1 Functionally assayed fungal major intrinsic proteins

Cluster	Canonical MIPs					
	Protein ID	**Fungal species and types**	**# of amino acids and TMDs**	**NPA motifs**		**Functions and subcellular localization (TargetP1.1[6])**
				Loop B	**Loop E**	
Cluster I: orthodox aquaporins	ADC55259[1]	*Saccharomyces cerevisiae*, budding yeast	289aa, 6	NPA	NPA	Water transporter; plasma membrane
	JF491353[2]	*Terfezia claveryi,* ECM desert truffle	307aa, 6	NPA	NPA	Water and CO_2 transporter; plasma membrane
	Lacbi2:456764[3]	*Laccaria bicolor,* ECM	311aa, 6	NPN	NSA	Water transporter; secretary pathway
Cluster II: aquaglyceroporins	CAA38096[1]	*S. cerevisiae*	669aa, 6	NPS	NLA	Glycerol and methylamine facilitators; plasma membrane
	Lacbi2:671860[3]	*L. bicolor*	330aa, 6	NPC	NSA	CH_3NH_2, NH_4^+/NH_3; plasma membrane
	Lacbi2:482072[3]		343aa, 6	NPC	NTA	Glycerol, urea, CH_3NH_2, NH_4^+/NH_3, limited water; mitochondrion
Cluster III: facultative aquaporins	GAA23030[1]	*S. cerevisiae*	646aa, 5	NPA	NPA	Unknown; plasma membrane
	Lacbi2:443240[3]	*L. bicolor*	312aa, 5	NPA	NPA	Glycerol, water, CH_3NH_2, NH_4^+/NH_3; plasma membrane
	Lacbi2:317173[3]		332aa, 6	NPA	NPA	Water, CH_3NH_2, NH_4^+/NH_3; plasma membrane
	Lacbi2:568479[3]		263aa, 6	NPA	NPA	Water, CH_3NH_2; secretary pathway
	JQ412060[4]	*Glomus intraradices* AM	316aa, 6	NPA	NAA	Water; plasma membrane and intracellular membranes (shown in GFP-fusion analysis)
Cluster IV: Fungal XIPs	ACV52007[5]	*G. intraradices,*	253aa, 6	NPA	NPA	No transport capacity detected; plasma membrane
	JQ412059[4]		276aa, 6	NPK	NPA	Water; plasma membrane (shown in GFP-fusion analysis)

Note: Protein ID refers to the accession number for the sequence in the database from which the sequence was retrieved. AM, arbuscular mycorrhizal; ECM, ectomycorrhizal; GFP, green fluorescent protein; MIP, major intrinsic protein; TMD, transmembrane domain; XIP, X intrinsic protein. [1.] Soveral *et al*. 2010; [2.] Navarro-Ródenas *et al*. 2012; [3.] Dietz *et al*. 2011; [4.] Li *et al.* 2013a; [5.] Aroca *et al*. 2009; [6.] Subcellular localization was predicted according to the signal peptide on N-terminus using TargetP1.1, unless noted otherwise.

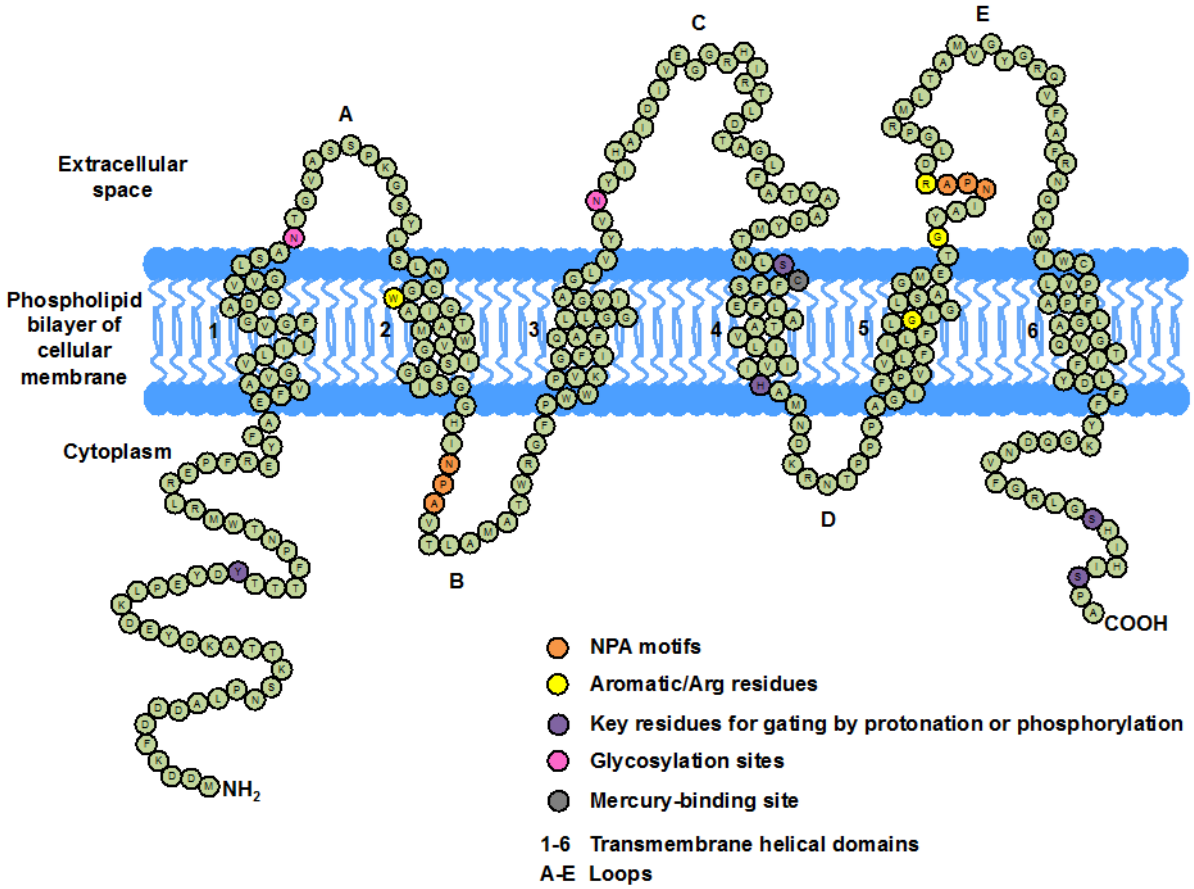


Figure 1.1 Classical transmembrane secondary structure and key residues of an aquaporin
The diagram was drawn based on the protein secondary structure predicted using SOSUI (Hirokawa *et al*. 1998). Key residues for selective filtering and gating were distinguished (Murata *et al*. 2000; Hedfalk *et al*. 2006; Törnroth-Horsefield *et al.* 2006; Mitani-Ueno *et al*. 2011).

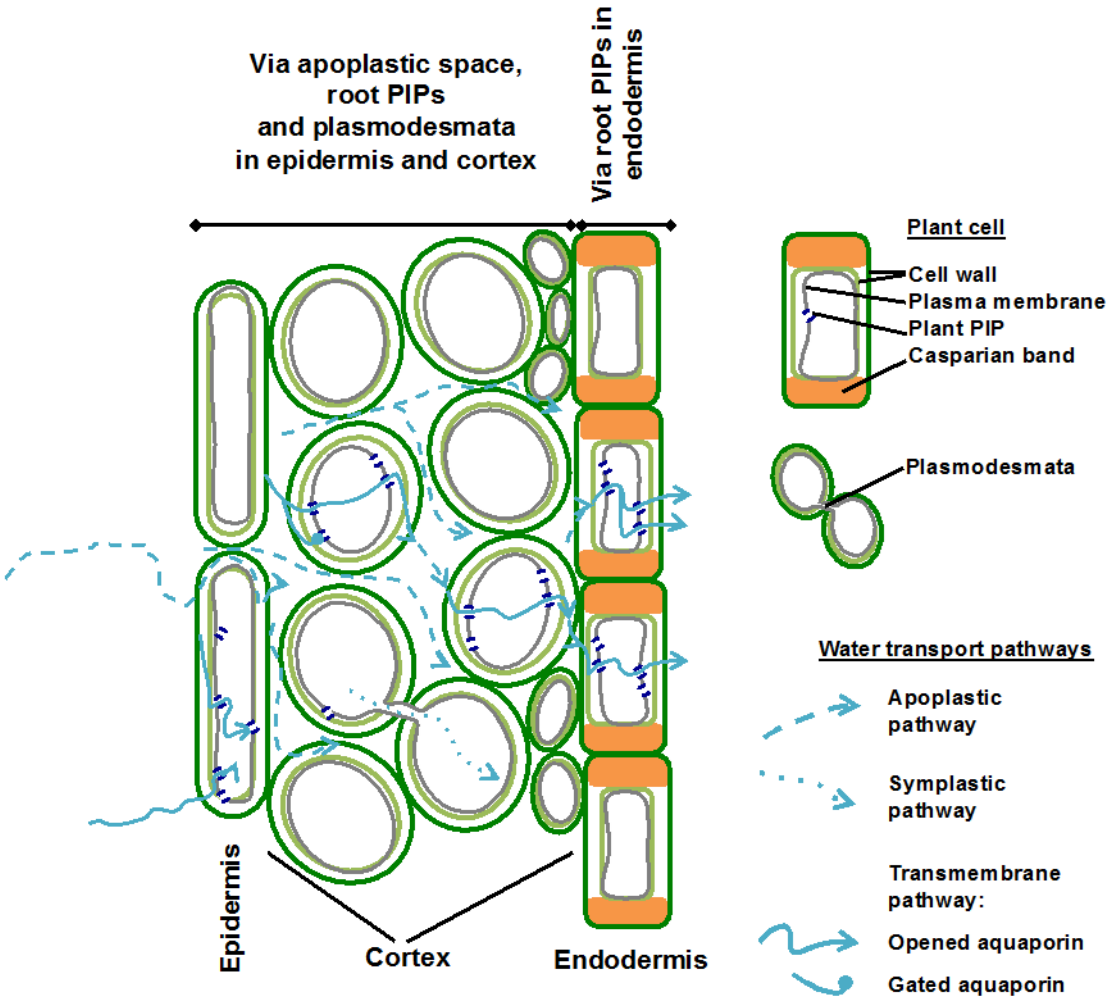


Figure 1.2 The schematic water transport pathways in root epidermis, cortex and endodermis

The schematic diagram was drawn based on the composite model of root water transport (Steudle & Peterson 1998).

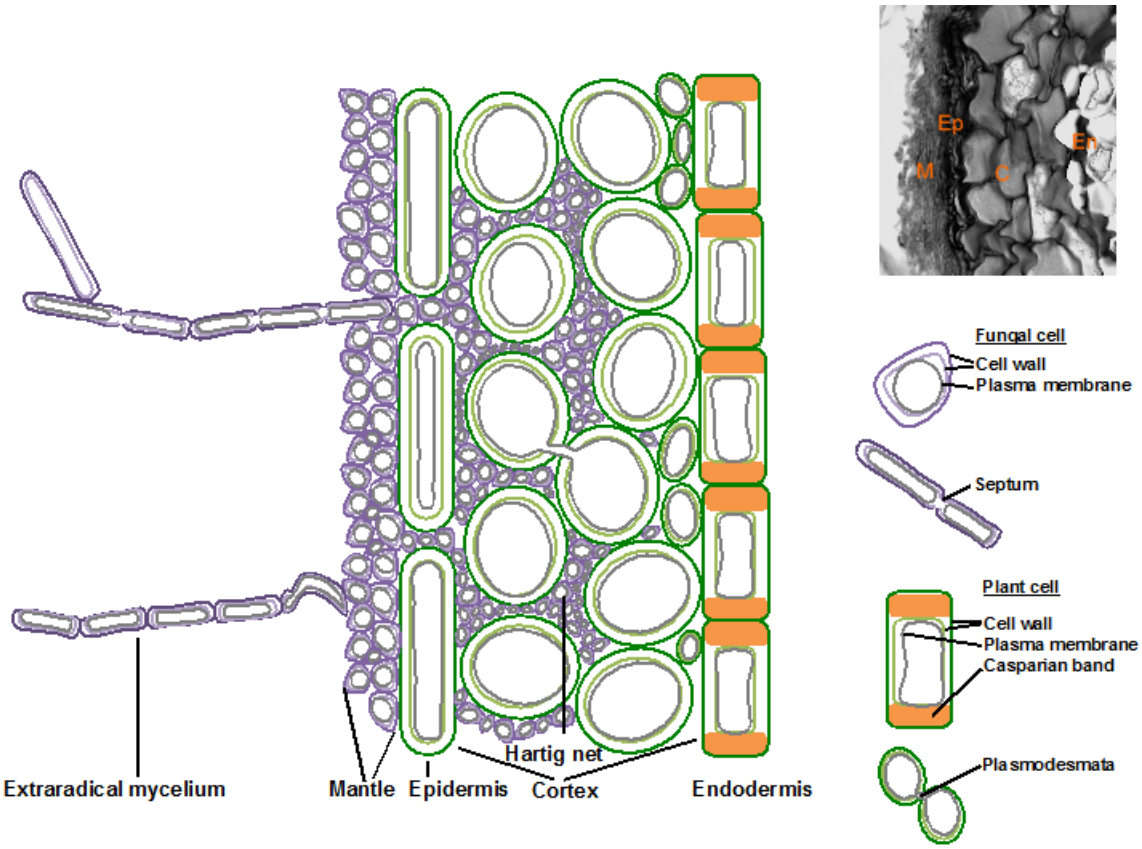


Figure 1.3 The schematic anatomical structures of ectomycorrhizal root
The diagram was drawn based on the cross section of a *Picea glauca* root tip colonized with *Laccaria bicolor* (the image in grey in the upper right corner; M for mantle, Ep for epidermis, C for cortex, and En for endodermis).

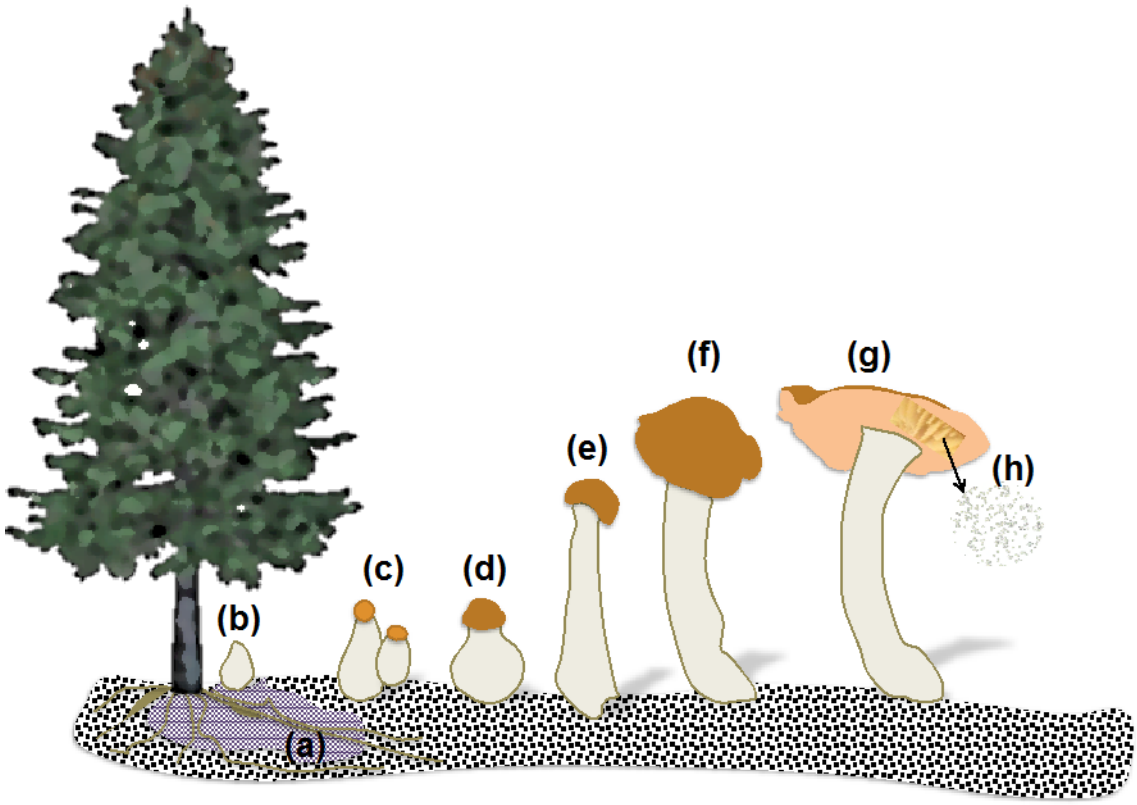


Figure 1.4 The sequence of events involved in sporocarp formation
Sporocarp formation goes through hyphal knot formation from vegetative mycelia (a) associated with tree roots, initial aggregation and bipolar fruiting body primordium pinning (b), primordium differentiation with pileus and stipe (c-d), fruiting body maturation with elongated stipe (e) and expanded cap (f), and full cap expansion (g) with spore production in hymenium beneath the cap (h). The diagram was drawn according to the typical process of basidiocarp formation (Kües & Liu 2000).

2 PHYLOGENETIC ANALYSIS OF FUNGAL AQUAPORINS PROVIDES INSIGHT INTO THEIR POSSIBLE ROLE IN WATER TRANSPORT OF MYCORRHIZAL ASSOCIATIONS

2.1 Introduction and literature review

2.1.1 General introduction

Root water transport in plants involves apoplastic and cell-to-cell (symplastic plus transmembrane) pathways (Steudle & Peterson 1998). Aquaporins, integral membrane proteins with water channel activity belonging to the MIP family of integral membrane proteins, are important for the movement of water across membranes in the cell-to-cell pathway (Maurel *et al*. 2008). It has been recognized that root aquaporins play a key role in plant water flow regulation (Javot & Maurel 2002; Javot *et al.* 2003; Ehlert *et al*. 2009; Lee *et al*. 2012). The processes of water flow have been extensively studied in plant roots, and studies describing possible effects of rhizosphere microorganisms on root water transport have been recently reviewed (Groppa *et al*. 2012). In the review, Groppa *et al.* (2012) postulated that plant growth promoting microorganisms such as arbuscular mycorrhizal and ectomycorrhizal fungi, opportunistic symbiotic and saprophytic *Trichoderma* fungi, as well as growth promoting rhizobacteria, improve root hydraulic conductance, alter plant aquaporin gene expression and alleviate abiotic stresses. The effects are often complex, varying as a function of the interacting species and environmental stresses. Under natural conditions, most plant species form mycorrhizal associations. Many studies have illustrated that ectomycorrhizal fungi affect plant water relations and root hydraulic conductivity in the colonized host plants (Muhsin & Zwiazek 2002; Marjanović *et al*. 2005; Lee *et al*. 2010). On one hand, it has been demonstrated that this effect may involve the root apoplastic pathway (Muhsin & Zwiazek 2002; Bárzana *et al*. 2012). On the other hand, ectomycorrhizal establishment may trigger increases in the aquaporin-mediated cell-to-cell water transport in roots (Lee *et al.* 2010) and produce changes in root hydraulic conductivity through their effects on the transcriptional levels of root plasma membrane intrinsic protein (PIP) aquaporins (Marjanović *et al*. 2005). Similar responses were recorded for

arbuscular mycorrhizal associations with roots of *Lactuca sativa, Medicago truncatula*, *Nicotiana tabacum* and *Phaseolus vulgaris* (Uehlein *et al*. 2007; Aroca *et al*. 2009). Given these observations, it is important to understand the mechanisms underlying water transport processes from soil to fungal free-living mycelia, between different hyphal compartments, as well as at the hyphal-root interface.

At each of these points in the soil-fungus-plant pathway of water movement, fungal aquaporins could contribute to efficient water uptake of the mycorrhizal plant and ultimately, to the success of the mycorrhizal association. Significant advances have been made in understanding MIP structure, function and regulation in a number of plant and animal species. However, the knowledge of MIP structure, function and regulation in fungi is still fairly limited (Pettersson *et al.* 2005; Soveral *et al.* 2010; Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012; Li *et al*. 2013). Much of this knowledge is based on studies of the yeast *Saccharomyces cerevisiae*, a member of the Ascomycota. By contrast, most fungi capable of forming mycorrhizal associations with plants are filamentous species in the Basidiomycota, Ascomycota and Glomeromycota, and have very different lifestyles than those of the budding yeasts. The rapidly-expanding catalogue of sequenced fungal genomes across the five phyla of Kingdom Mycota (Martin *et al.* 2011), including a growing list of mycorrhizal fungal species, provides an unprecedented opportunity to test the hypothesis that fungal aquaporins mediate water movement from soil to the plant partner.

This chapter reviews the current knowledge of water transport processes in mycorrhizal fungal hyphae, water transfer via mycorrhizal fungal hyphae to plant roots, as well as the overall impact of mycorrhizal symbiosis to plant water relations. The current state of knowledge of the classification, structure and transport capacities of fungal MIPs is also summarized based largely on *in silico* structural characterization of mycorrhizal fungal MIPs and phylogenetic analysis of 229 presumed and functionally confirmed fungal MIPs across four major phyla of Mycota. This comparative genomics approach, together with results of recently published studies, is used to infer the fungal MIPs that potentially play roles in water transport and osmotic adjustment of mycorrhizal associations.

2.1.2 Water transport in mycorrhizal associations

It has been well established that mycorrhizal formation improves mineral nutrition and water relations in colonized plants via the uptake and transfer of nutrients and water through the fungal hyphae (Egerton-Warburton *et al*. 2003, Egerton-Warburton *et al*. 2007). Abundant fungal hyphae dramatically increase the surface and capacity of water and nutrient uptake from the soil, which usually occur simultaneously (Lehto & Zwiazek 2011, Turgeman *et al*. 2011). Additionally, the small diameter of fungal mycelia allows them to penetrate narrower soil pores where water and nutrients are present but inaccessible for plant roots with wider diameter (Augé 2004). Theoretically, water can move in the extracellular space of hyphal cells in a manner equivalent to the apoplastic pathway in plant roots. In the cell-to-cell pathway, water and nutrients enter hyphal cells via an array of membrane channels. Once in the hyphal protoplasts, these molecules can be transported between adjacent cells either via individual hyphae or via rope-like strands called rhizomorphs, which comprise multiple interconnected hyphae (Agerer 2001; Peterson *et al.* 2004). Rhizomorphs show substantial hyphal differentiation that may facilitate water transport. For example, vessel hyphae have enlarged central hyphae with highly modified or absent septae for efficient water movement, while some peripheral hyphae display thickened and pigmented cell walls that may act to reduce water loss (Agerer 2001; Peterson *et al.* 2004).

In ectomycorrhizal (ECM) associations, extensive interfaces exist between plant root cortex cells and the Hartig net of the ECM fungus. The ECM mantle is also likely an important component of the plant-fungal interface. In arbuscular mycorrhizal (AM) associations, the interfaces exist between root cortical cell membranes and the specialized fungal structures known as arbuscules (Smith & Read 2008). Ectendomycorrhizas exhibit both the Hartig net and intracellular hyphae that form soon after the development of the Hartig net (Yu *et al.* 2001; Peterson *et al.* 2004); this combination presumably further enhances the interfaces for substance exchange between the symbionts and complicates the dynamics of their interaction. Other classes of mycorrhizal associations, such as the ericoid, arbutoid, monotropoid, and orchid mycorrhizas display further specialized interfaces, often involving branched or coiled hyphal complexes within colonized plant cells (Peterson *et al*. 2004). Plant-fungal interfaces can also be enhanced by modifications of root cells, for example by

differentiation of transfer cells (Peterson *et al.* 2004).

Evidence exists for considerable molecular exchange occurring at these interfaces of mycorrhizal symbionts with their plant hosts. For example, mycorrhizal fungi obtain sugars from plant roots while providing mineral nutrients and water to plants (Govindarajulu *et al*. 2005; Allen 2007; Parniske 2008; Martin & Nehls 2009;). While transfer of mineral nutrients from mycorrhizal fungi to plants has received considerable attention, comparatively little is known about water exchange from mycorrhizal fungi to their plant host. Studies have demonstrated water uptake by mycorrhizal hyphae, and quantified this contribution to plant-water relations using split-root hyphae compartment system and stable isotopes (Khalvati *et al*. 2005, Plamboeck *et al*. 2007). It has been shown that association with the AM fungal species *Glomus intraradices* increases relative apoplastic water flow in roots (Bárzana *et al*. 2012). However, there is still some debate concerning a possible reduction of water movement by the mycorrhizal structure due to increased cell wall hydrophobicity and additional hydraulic resistance when water is transported across the fungal membranes (Lehto & Zwiazek 2011). The contribution of aquaporins is likely to be significant to this process (Maurel & Plassard 2011). Extensive studies have been carried out to investigate the effect of mycorrhizal fungi on plant aquaporins (Marjanovic *et al*. 2005; Uehlein *et al*. 2007). However, so far, the question of the contribution of fungal aquaporins to water transport of the mycorrhizal fungi themselves as well as to that of the host plant-water relations has not been adequately addressed. Recently, it has been shown that expression of specific mycorrhizal fungal aquaporins is enhanced upon mycorrhizal association and under conditions of water limitation (Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012; Li *et al*. 2013), providing further evidence to support the model that fungal aquaporins are important to water movement in mycorrhizal plants. The results arising from the few studies conducted to date suggest that aquaporin-mediated water movement in fungal hyphae could improve efficiency of water transport to meet the plant's hydraulic demand, especially when abiotic factors such as water stress, low temperature, high salinity and hostile pH inhibit root water flow (Muhsin & Zwiazek 2002; Calvo-Polanco *et al*. 2008, Calvo-Polanco *et al*. 2009, Lee *et al*. 2010; Siemens & Zwiazek 2011; Bárzana *et al*. 2012; Navarro-Ródenas *et al*. 2012; Li *et al*. 2013). However, at this point there is no clear understanding of how root hydraulic properties and water transport are modulated by altering the abundance

of mycorrhizal fungal aquaporins.

2.1.3 Aquaporins and water transport

Members of the MIP family, including aquaporins, have been well characterized in many taxa, particularly plants and mammals (Zardoya 2005). In addition to their major function in facilitating transmembrane water transport, MIPs are involved in the transport of other small neutral solutes and gases (Gomes *et al*. 2009). MIPS classified as orthodox aquaporins are generally considered to be water transporters, while other MIPs may be channels that facilitate movement of urea, glycerol, or boric acid across membranes (Maurel *et al*. 2008).

In plant and mammalian cells, MIPs are abundant. In keeping with their role as channels that facilitate diffusion of small molecules, MIPs are membrane-spanning proteins that are localized in plasma and intracellular membranes (Maurel *et al*. 2008). X-ray crystallography shows highly conserved structural features of MIP homologues across plants, animals and microbes. Typically, these 23-35 kDa proteins consist of six hydrophobic transmembrane helical domains connected by five hydrophilic loops. Three loops (A, C, E) are located on the extracytoplasmic side of the membrane, while two loops (B, D) and both the N- and C- termini localize to the intracytoplasmic side. The six transmembrane helices and loop B and E, which extend from either side of the membrane into the core of the molecule, form a central aqueous pore through which a single file of water molecules may pass. Loops B and E each possess a highly conserved NPA (Asn-Pro-Ala) motif that function in pore constriction and water molecule dipole orientation. In addition, an array of aromatic residues on the extracytoplasmic Loop E and in the second and fifth transmembrane domains face an arginine residue to form the aromatic/Arg site. This aromatic/Arg site is usually the narrowest site in the pore, and is considered important for proton repulsion and selective filtering of molecules (Mitani-Ueno *et al*. 2011). Gating of water flux can be achieved via protonation of a His residue in loop D, which results in a closed conformation (Hedfalk *et al*. 2006). Conversely, phosphorylation of loop B renders an open conformation (Hedfalk *et al*. 2006). Given the degree of sequence similarity and conservation of functional motifs shared between plant, animal and fungal MIPs (described in the following sections), similar secondary structure and regulatory

mechanisms may also be found for fungal MIPs, although this remains to be tested experimentally.

2.1.4 Classification of fungal MIPs using comparative genomics

Although MIPs are well conserved throughout the tree of life, their nomenclature differs considerably between major groups. Plant MIPs are classified into subfamilies PIP (plasma membrane intrinsic proteins), TIP (tonoplast intrinsic proteins), NIP (nodulin26-like intrinsic proteins), SIP (small basic intrinsic proteins) and XIP (X intrinsic proteins) based on phylogenetic analyses of amino acid sequence similarities and classical subcellular localization (Gupta & Sankararamakrishnan 2009; Maurel *et al.* 2008). Water channel activity for MIPs belonging to each of these subclasses has been demonstrated (Maurel *et al*. 2009; Lopez *et al*. 2012), and transport of small neutral solutes has been shown for several MIPs (Maurel *et al*. 2009). As such, orthodox aquaporins, *i.e.*, water channel MIPs, cannot be inferred by their phylogenetic relationships. By comparison, mammalian MIPs are classified into two groups based on sequence comparison and permeability studies: the orthodox aquaporins that transport water, and the aquaglyceroporins that are permeable to water as well as glycerol, urea and other small neutral solutes (Hara-Chikuma & Verkman 2006; Krane & Goldstein 2007; Gena *et al.* 2011).

In fungi, MIP classification is based mainly on that of yeast species, and resembles the mammalian MIP nomenclature (Pettersson *et al.* 2005; Soveral *et al.* 2010). In the first comprehensive phylogenetic analysis of 19 yeasts and three filamentous fungi (two Ascomycota and one Basidiomycota), Pettersson *et al*. (2005) classified 55 fungal MIPs into four groups: orthodox aquaporins, Fps-like aquaglyceroporins, Yfl054c-like aquaglyceroporins, and other aquaglyceroporins. At the time of this analysis, functional characterization of fungal MIP transport capacity for water and small neutral molecules was mainly limited to six yeast species, *i.e.*, *S. cerevisiae*, *Schizosaccharomyces pombe, Zygosaccharomyces rouxii*, *Candida albicans, Kluyveromyces lactis* and *K. marxianus*. Subsequently, Gupta and Sankararamakrishnan identified nine fungal XIP-like genes from the genomic sequences of eight fungal species, based on sequence similarity with plant XIPs (Gupta & Sankararamakrishnan 2009).

More recently, Dietz *et al*. (2011) took advantage of the rapidly expanding catalogue of fungal genome sequences to conduct a phylogenetic analysis of 100 fungal MIPs sequences from 29 species representing two phyla. Dietz *et al.* (2011) identified four groups, which they termed classical aquaporins, Fps-like aquaglyceroporins, other aquaglyceroporins and fungal XIPs. Dietz *et al.* (2011) additionally conducted functional analysis for six MIPs of the basidiomycete ECM fungus *Laccaria bicolor* strain S238N. These authors demonstrated water transport capacity for five aquaporins, with three of these exhibiting sufficient water transport capacity to be of physiological relevance.

With sequencing of fungal genomes continuing at an ever-increasing rate and recent publication of functionally characterized MIPs from additional mycorrhizal species (Aroca *et al*. 2009; Dietz *et al.* 2011; Grigoriev *et al*. 2011; Navarro-Ródenas *et al*. 2012), it was timely to conduct an updated phylogenetic analysis of the suite of functionally characterized and putative fungal MIPs available at the time of writing.

2.2 Materials and Methods

A total of 229 MIPs from 88 fungal species were included in this analysis, along with eight well-characterized MIPs from other kingdoms (Appendix 1 provides details on protein IDs, species names, and sequences used in this analysis). The fungal MIPs included the most recently available gene models and annotations for sequences used in previously published phylogenetic analyses (Pettersson *et al*. 2005; Dietz *et al*. 2011), four functionally assayed MIPs from mycorrhizal fungi (Aroca *et al*. 2009; Navarro-Ródenas *et al*. 2012; Li *et al*. 2013), six MIPs cloned from *L. bicolor* strain UAMH8232 (Xu *et al.* unpublished), and 119 additional sequences from fungal species obtained in early 2012 from databases at the Department of Energy's Joint Genome Institute and the National Center for Biotechnology Information (Matheny *et al*. 2006, Martin *et al*. 2008, Martin *et al*. 2010, Grigoriev *et al*. 2012). Fungi from the Basidiomycota, Ascomycota, Glomeromycota, and Zygomycota are represented in this analysis, which includes the following mycorrhizal species: *L. bicolor* (Basidiomycota, ECM), *Terfezia claveryi* (Ascomycota, ECM), *G. intraradices* (*i.e.*, *Rhizophagus intraradices,* Glomeromycota, AM) and *Tuber melanosporum* (Ascomycota, ECM).

Deduced amino acid sequences of 229 fungal MIPs from 88 fungal species representing four phyla (Appendix 1) were aligned using ClustalW, and phylogenetic tree was constructed using neighborjoining (Felsenstein 1985; Saitou & Nei 1987) in MEGA 5.2.1 (Tamura *et al*. 2011). A bootstrap consensus tree consisting of 1000 iterations was obtained. Evolutionary distances were computed using the JTT matrix-based method (Jones *et al*. 1992). Secondary structure for these sequences was determined using SOSUI, a programme for *in silico* analysis of predicted membrane proteins (Hirokawa *et al*. 1998).

2.3 Results

Similar to the analysis of Dietz *et al*. (2011), the dendrogram resulting from this analysis exhibits four distinct clusters (Fig. 2.1). Based on the identity of member sequences, Clusters I to IV correspond to the orthodox fungal water channels, fungal aquaglyceroporins, facultative fungal aquaporins, and fungal XIPs, respectively.

2.3.1 Cluster I: orthodox fungal water channels

This cluster includes aquaporins of species belonging to yeast Ascomycota, filamentous Ascomycota, and Basidiomycota, but interestingly, does not contain aquaporins from species of the Glomeromycota or Zygomycota. Among the 102 fungal aquaporins comprising Cluster I (Fig. 2.2), most deduced amino acid sequences have six predicted transmembrane domains (TMDs) and five loops, although some members of this cluster exhibit five or seven TMDs in *in silico* prediction. NPA signature motifs are well conserved in Loop B and Loop E (or Loop D, depending on the number of transmembrane domains), although occasional variants are observed (Fig. 2.3 a). Asparagine (N) and proline (P) are strictly conserved in the Loop B motif, and alanine (A) may be substituted for threonine (T), valine (V), serine (S), cysteine (C) or N. In the last loop, N and A are strictly conserved with few exceptions such as NPV, while P may alter into phenylalanine (F), V, T, S or tryptophan (W). A few highly conserved domains can be observed; within these, glycine (G) in TMD2, glutamic acid (E) in TMD4, and G and arginine (R, crucial for the aromatic/R selectivity site formation) are conserved across all 101 sequences in Cluster I (Fig. 2.3 a) (Appendix 2 Note A2.1). A residue proximal to the NPA motif of Loop B is conserved as T or S, with infrequent substitution of N or C. This is the presumed phosphorylation site.

2.3.2 Cluster II: fungal aquaglyceroporins

Twenty-three sequences are found in Cluster II (Fig. 2.4). This cluster contains CAA38096 (*S. cerevisiae*), AAS47031 (*K. marxianus*) and AAQ01788 (*K. lactis*). Along with three other yeast MIPs, they form a subgroup of Ascomycota yeast aquaglyceroporins within Cluster II.

The other subgroup of Cluster II contains genes from species belonging to the Basidiomycota and filamentous Ascomycota. This subgroup-clustering pattern hints at an interesting evolutionary relationship between Ascomycota yeasts, Ascomycota filamentous fungi and Basidiomycota. No Glomeromycota or Zygomycota MIPs belong to Cluster II. All the sequences in Cluster II are predicted to have five to seven TMDs, relatively long termini and a total of 11 consensus residues among 53 well-conserved positions (Appendix 2 Note A2.2). The NPA motif in Loop B is sometimes replaced by SPA or NPC; within the last loop, the P in NPA is sometimes substituted by N, L, S, M, T or A, while N and A are well conserved. Some MIPs in this subgroup were characteristic of two fairly long termini. For example, Lacbi2:671860 from *L. bicolor* strain S238N was predicted to have relatively long N-terminus of 39 amino acids and C-terminus of 46 amino acids (Fig. 2.3 b).

2.3.3 Cluster III: facultative fungal aquaporins

There are 82 fungal MIPs that group into Cluster III (Fig. 2.5). The Cluster III form a more disparate group that is clearly not monophyletic; within Cluster III, three subgroups were identified, designated α, β, and γ. *Escherichia coli* GlpF is included in Cluster III but does not fall within the α, β or γ subgroups. *In silico* analysis by SOSUI indicates that most MIPs in Cluster III have six TMDs (Fig. 2.3 c), although some yeast MIPs may have as few as five or as many as seven. NPA motifs may vary as NPA, NPS, NPV or NPT in Loop B, and NLA, NPA, NGA, NFA, NMA or NAA in the last loop. Residues G in TMD1, W in TMD2, R in Loop B, G in TMD3, F in Loop C, as well as T, R, D, and R in Loop E are conversed across all sequences (Appendix 2 Note A2.3).

There are 65 sequences within the α subgroup of Cluster III. Cluster III β consists of 11 Ascomycota MIPs of the species of *Aspergillus* or closely related species. Functional analysis has not been conducted on any of the putative MIPs in subgroup β. Cluster III

γ includes six MIPs: five sequences from the Zygomycota (four in *Phycomyces blakesleeanus* and one in *Mucor circinelloides*), and JQ412060 from *G. intraradices* of the Glomeromycota.

Within Cluster III α, 30 Basidiomycota MIPs, 31 filamentous Ascomycota MIPs and four ascomycete yeast MIPs tend to fall into distinct branches, reflecting the evolutionary divergence of these phyla. The four yeast MIPs include GAA23030 in *S. cerevisiae* and NP592788 in *S. pombe*, which were formerly classifed as Yfl054-like aquaglyceroporins, although their transport capacity had not been experimentally determined. It is worth noting that the four yeast MIPs are predicted to have a relatively long N-terminus containing the conserved sequence PVWSLNQPLPHVLD, while the C-terminus contains the conserved sequence ESPVNYPDNGYIE is conserved. Both motifs are pvostulated to play a role in regulation of protein function.

2.3.4 Cluster IV: fungal XIPs

Cluster IV comprises 22 putative fungal XIPs (Fig. 2.6). Most of these sequences, which have not been functionally characterized, exhibit six TMDs and share eight consensus residues at 37 relatively well-conserved positions (Appendix 2 Note A2.4). Several variants of the NPA motif of Loop B are observed, e.g. NPA/T/L/M, NSM or SPT, whereas the one in Loop D is more conserved, with occasional substitution of S or T for A.

Phylogenetic distances indicate that Cluster IV of fungal MIPs is relatively close to plant MIPs, although the functions of this cluster remain mostly unclear. The main branch of Cluster IV includes the representative plant XIP, TIP and SIP sequences from *Arabidopsis thaliana* included in this analysis (Fig. 2.6). Nineteen XIPs of filamentous Ascomycota localize to this branch. The branch is proximal to the branch that includes the *A. thaliana* PIP and *Mus musculus* water transport channel AQP1, suggesting a more recent evolutionary divergence of fungal XIPs from MIPs of species from other kingdoms than for the other fungal MIPs, particularly the plant XIPs, TIPs and SIPs. Interestingly, Zardoya *et al.* (2002) suggested that plant glycerol transporters might originate from horizontal gene transfer and functional recruitment from other kingdoms.

Previous functional assays of *G. intraradices* MIP ACV52007 did not demonstrate a transport capacity for water, urea or glycerol (Aroca *et al*. 2009). Recently, however, Li *et al.* demonstrated that *G. intraradices* MIP JQ412059 increased water transport across the plasma membrane of transformed yeast cells (Li *et al.* 2013). Given the phylogenetic placement of these *G. intraradices* sequences relative to the fungal XIPs of Cluster IV and the apparent differences in transport capacities between these closely related genes, little can be inferred from this phylogenetic analysis regarding the function of sequences in Cluster IV. As one of the three putative MIPs identified from the ECM fungus *T. melanosporum*, TmeAQP2 (GSTUMT00004612001) (Fig. 2.3 d) is a putative mycorrhizal fungal XIP in Cluster IV, showing five secondary helices and a long N-terminus. The transport capacity of TmeAQP2 has not been determined. However, this gene was significantly regulated in Hartig net in ECM between *T. melanosporum* and *C. avellana*, indicating that it might be required for ammonia translocation into the apoplastic space as a predominant nitrogen form present at the fungal/plant interface. Functional analyses of *G. intraradices* JQ412059 and *T. melanosporum* TmeAQP2 may provide greater insight on the role of Cluster IV MIPs, as well as reveal important understanding of the role for MIPs in transport processes that occur in AM associations.

Sequences in the branches of filamentous Ascomycota, Glomeromycota and Zygomycota might originate from different ancestors; however, due to approximity of their locations on the main phylogenetic tree, they were included into the same cluster. No MIP sequence from yeast in Ascomycota or Basidiomycota available at the time of this analysis was grouped in Cluster IV. In this neighbor-joining analysis, ACV52007 (GintAQP1) and JQ412059 from *G. intraradices* (Glomeromycota), and Mucci2|114802 from *M. circinelloides* (Zygomycota), form a related but distinct branch that was not apparent in previous phylogenetic analyses.

2.4 Discussion

The currently available sequences of both well-characterized and putative fungal MIPs can be classified into four clusters. Although the current understanding of fungal MIP functions is still limited to several closely related model yeasts and a small number of mycorrhizal species, this latest phylogenetic analysis suggests a rich and valuable resource for the study of MIP structure, functions and phylogenetic evolution within

this kingdom and across kingdoms. Understanding genetic, structural and functional diversity of mycorrhizal MIPs will help to resolve the classification of fungal MIPs. Similarly, a more mechanistic model of how mycorrhizal MIPs are regulated at the transcriptional and post-translational level in response to complex environmental cues will enable a better understanding of the fungal contribution to mycorrhizal associations and the many underground ecological processes to which these associations contribute.

To date, 16 MIPs from the mycorrhizal fungi *G. intraradices*, *L. bicolor* and *T. claveryi* have been functionally characterized (Aroca *et al*. 2009, Dietz *et al*. 2011, Navarro-Ródenas *et al*. 2012, Li *et al.* 2013, Xu *et al.* unpublished). These data, together with the phylogenetic and *in silico* structural analyses described above allow us to make inferences about functional roles, regulatory mechanisms and ecological significance of mycorrhizal fungal MIPs that translate into testable hypotheses for experimentation. The release of more fungal genomes and the discovery of new fungal MIPs will enable the refinement of the classification presented here, and shed new light on the evolutionary relationship between fungal phyla as well as between kingdoms. The features of fungal MIPs in Clusters I to IV are discussed, with particular focus on the mycorrhizal aquaporins.

2.4.1 Water transporters in Cluster I, III and IV

Orthodox aquaporins mediate rapid cross-membrane water transport, playing considerable roles in osmoregulation of hyphal cells as well as water uptake of mycorrhizal association at a larger scale.

In Cluster I, functionally tested water-transporting aquaporins include yeast orthodox aquaporins such as ADC55259, and mycorrhizal aquaporins JF491353 in *T. claveryi*, and Lacbi2:456764 in *L. bicolor* (Table 2.1). Given the high degree of sequence similarity and conservation of important motifs, putative fungal aquaporins within Cluster I that have not been functionally characterized may be hypothesized to preferentially transport water as these known aquaporins do. The proven or potential water transport capacity of these Cluster I fungal aquaporins make them attractive candidates for functional analysis of the role that they may play in water uptake by the mycorrhizal plant system. This includes the putative aquaporin TmeAQP1

(GSTUMT00003976001) of 322 amino acids in the ECM truffle *T. melanosporum* (Appendix 1). In ECM between *T. melanosporum* and *Corylus avellana*, this gene was significantly upregulated in the fungal mantle compared with in Hartig net, suggesting its differential involvement in transporting substrates in mantle and Hartig net (Hacquard *et al.* 2013). It should also be noted that water transport capacity of gene products in this cluster might not be as exclusive as it had been thought to be, since JF491353 has been proven to transport CO_2 (Navarro-Ródenas *et al.* 2012), an molecule potentially important for mycorrhizal communication, symbiosis establishment and sexual reproduction (Bahn & Mühlschlegel 2006).

Six aquaporins isolated from *L. bicolor* strain S238N show water transport capacity; among these, Lacbi2:456764 (previously annotated as Lacbi1:392091) exclusively transports water with a considerable water permeability coefficient of 62 μm s^{-1} (Dietz *et al*. 2011). This *L. bicolor* aquaporin clusters with other obligate fungal aquaporins in Cluster I, and possesses the typical secondary structure of the classic major intrinsic protein, as predicted by SOSUI (Fig. 2.3 a). Both this aquaporin and the allele for this locus (JQ585592) in *L. bicolor* strain UAMH8232 are predicted to localize to secretary pathways, based on analysis in TargetP1.1 (Emanuelsson *et al*. 2007). JQ585592 also exhibits moderate water transport capacity (Xu *et al.* unpublished).

Another Cluster I mycorrhizal fungal aquaporin, *T. claveryi* JF491353, facilitates water and CO_2 conductivity (Navarro-Ródenas *et al*. 2012). Navarro-Ródenas *et al*. (2012) suggested that *T. claveryi* JF491353 might contribute to the adaptation of the mycorrhizal association to water deficit, and might also function as a signal transduction channel during presymbiotic phase of the fungal growth and in carbon metabolism. Interestingly, a Cluster I MIP from the ascomycete ECM *T. melanosporum*, TmeAQP1, is closely related to *T. claveryi* JF491353. Considering the predicted subcellular localization of the gene product to the plasma membrane (TargetP1.1) and its hydrophobicity of 0.5 (SOSUI), attention should be given to its potential role in water relation and symbiosis establishment with mycorrhizal tree species in temperate forest ecosystems.

G. intraradices MIP JQ412060 from Cluster IV has demonstrated water transport capacity; transcript abundance corresponding to this gene increased when the mycorrhizal association between *G. intraradices* and maize roots was exposed to

drought stress (Li *et al*. 2013). In addition, JQ412060 is predicted to be located in both the plasma and intracellular membranes (Li *et al*. 2013), supporting possible roles in regulating water flux across plasma membrane and osmotic adjustment among cellular compartments. *L. bicolor* strain S238N Lacbi2:317173 (Fig. 2.3 c) and Lacbi2:443240 from Cluster III of facultative aquaporins (Fig. 2.5) show high water transport efficiency as well as permeability for glycerol or methylamine, suggestive of dual roles in transporting both water and small neutral molecules (Dietz *et al*. 2011). The cDNAs JQ585596 and JQ585597 isolated from *L. bicolor* strain UAMH8232 are closely related to Lacbi2:317173, but exhibit distinct expression patterns and water permeability coefficients. It remains to be determined whether these sequences represent distinct loci. The allelic variation exhibited by *L. bicolor* UAMH8232 compared to the reference sequence of *L. bicolor* S238N is not unexpected given their distinct geographic origins: UAMH8232 was isolated in Ontario (Calvo-Polanco *et al*. 2008), while S238N originated in Oregon (Di Battista *et al*. 1996). Observed differences in transcript abundance for sequences from each strain putatively corresponding to the same loci (Xu *et al*. unpublished) suggest some degree of adaptive variation, possibly reflecting local adaptation to distinct environmental pressures such as drought and cold, or interaction with different host plants. Ecological implications of potential adaptive variation associated with fungal aquaporins may be revealed by further functional and population genetics analyses of aquaporins and their functions of different *L. bicolor* strains.

Although the transport capacity of most of the XIPs in Cluster IV is currently unknown, their roles in mycorrhizal association should not be discounted. For instance, *JQ412059*, thus far the only gene in the fungal XIP group gene with demonstrated water channel activity of its gene product, was up-regulated in arbuscule-enriched maize (*Zea mays*) root cortical cells and extraradical mycelia under drought stress. These findings suggest a role for this MIP in transporting water to mycorrhizal plants, thereby increasing their drought tolerance (Li *et al.* 2013). The putative XIP TmeAQP2 of *T. melanosporum* may play important roles in ECM association with trees, and its characterization may provide insight into the novel functions of fungal XIPs in general.

2.4.2 Osmoregulators in Cluster II and III

Cluster II fungal aquaglyceroporins contains CAA38096 (*S. cerevisiae*), AAS47031 (*K.*

marxianus) and AAQ01788 (*K. lactis*), the first fungal MIPs to be functionally characterized as glycerol efflux channels (Bill *et al*. 2001) and methylamine (CH_3NH_2) transport facilitators important for yeast osmoprotection (Hohmann 2002). Historically, these yeast MIPs have been designated Fps1-like aquaglyceroporins, although their function had nothing in common with farnesyl diphosphate synthase (Bill *et al*. 2001). Close phylogenetic distance between this cluster and *E. coli* AqpZ indicates the possibility of horizontal gene transfer and functional recruitment across kingdoms (Fig. 2.4). It also contains Lacbi2:671860 and Lacbi1:387054, two functionally analyzed aquaglyceroporins from *L. bicolor* strain S238N (Dietz *et al*. 2011). Lacbi2:671860 showed no water permeability but rather a weak capacity for NH_4^+/NH_3 and CH_3NH_2 transport (Dietz *et al*. 2011). Subcellular location prediction suggests that the protein is located on the plasma membrane. The other Cluster II *L. bicolor* gene, Lacbi1:387054 (updated to Lacbi2:482072), exhibited weak water permeability but transported NH_4^+/NH_3, CH_3NH_2, glycerol and urea efficiently (Dietz *et al*. 2011). These substitutions may be related to the limited water permeability predicted for this group, and enhanced capacity for glycerol or CH_3NH_2 transport. Mycorrhizal genes falling into this cluster are less interesting candidates to investigate for their role in water movement within the mycorrhizal-plant symbiosis, but may play crucial roles in osmotic adjustment.

Fps1 was the first reported osmogated glycerol export channel in *S. cerevisiae*, facilitating transmembrane transport of other small neutral molecules such as urea and some charged molecules arsenite (Wysocki *et al*. 2001). These channels function in osmoregulation of intracellular glycerol levels in response to changes in extracellular osmolarity. This osmoregulation mechanism may also apply to filamentous fungi. In the context of mycorrhizal associations, efficient osmoregulation via fungal MIPs may enable plant roots to more effectively cope with soil-associated stresses such as water deficit, high salinity or extreme pH.

In addition, fungal MIPs may be involved in nutrient uptake due to their transport capacity for small neutral molecules (Borgnia *et al*. 1999; Hohmann *et al*. 2000). In the study of Dietz *et al*. (2011), two *L. bicolor* Cluster III MIPs showed differential permeabilities to urea, glycerol, ammonium/ammonia or methylamine using a yeast assay. These data, together with a high transcript abundance corresponding to these

genes suggested their involvement in nitrogen nutrition exchange. Although Lacbi2:671860 from Cluster II didn't show significant transport capacity for either water or other small neutral molecules, transcript abundance corresponding to this gene increased considerably upon mycorrhizal symbiosis establishment, suggesting a possible role in the symbiotic relationship other than transport of water or previously reported small solutes (Dietz *et al*. 2011).

Cluster III includes *E. coli* GlpF, which is a classic glycerol facilitator with limited permeability to water and polyols (Hénin *et al*. 2008). Among the 30 Basidiomycota MIPs, Lacbi2:443240 (previously annotated as Lacbi1:391485), Lacbi2:317173 (Lacbi1:317173) and Lacbi2:568479 (Lacbi1:247946) from *L. bicolor* strain S238N showed varying degrees of water and methylamine transport capacity (Dietz *et al*. 2011). The term "facultative fungal aquaporins" was proposed in place of the former term "Yfl054-like" for this group to more accurately reflect their transport function. Recently, the *G. intraradices* subgroup γ MIP was demonstrated to exhibit water transport capacity using a yeast osmotic shock assay, and translational fusions with GFP showed that this *G. intraradices* MIP localizes to both plasma membrane and intracellular membranes (Li *et al*. 2013). This indicates that at least some Cluster III fungal MIPs have water transport capacity. Taken together, these recent findings suggest that further functional characterization of other fungal MIPs within this subgroup will yield important insight for the role of these genes in plant-fungal relationships.

In Cluster III α, *Sporobolomyces roseus* Sporo1|13459 and *Wallemia sebi* Walse1|59835, the two amino acid sequences deduced from their corresponding putative MIP genes found in Basidiomycota genomes, share 68 residues with four yeast MIPs. The placement of *Wallemia* MIP in this subcluster supports the previous parsimony analysis by Matheny *et al*. (2006), who propose Wallemiomycetes to be an early diverging lineage of Basidiomycota.

2.4.3 Transcriptional and post-translational regulation

Transcript abundance data suggests involvement of ECM and AM fungal partner MIPs in water or nutrient transport of the symbiont association. Several recent studies have identified ECM and AM fungal MIPs that exhibit differential transcript abundance

upon mycorrhizal formation, as well as in response to abiotic stresses such as drought, salt, low temperature, and pH (Aroca *et al*. 2009; Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012). For instance, *Lacbi2:443240* in *L. bicolor* strain S238N was upregulated at low temperature (Dietz *et al*. 2011), whereas *JF491353* in *T. claveryi* was upregulated upon mycorrhizal association (Navarro-Ródenas *et al*. 2012). These studies suggest that transcriptional-level regulation of some MIPs may be important for mycorrhizal responses to altered nitrogen abundance, water availability or temperature, and possibly for mycorrhizal symbiosis establishment. Building upon these phylogenetic and functional characterization investigations, *in situ* studies of mycorrhizal associations exposed to diverse abiotic stresses or environmental gradients will be important to enhance the understanding of the physiological and ecological importance of MIPs in symbiont fungi under natural conditions.

In addition to the transcription of MIP genes, MIP activity is determined by post-translational regulation, such as gating, heterotetramerization and membrane trafficking (Maurel *et al.* 2008). However, while these mechanisms have been relatively well investigated in plant and animal MIPs, studies of post-translational regulation for fungal MIPs have thus far been limited to yeasts (Fischer *et al*. 2009). It has been found that cytosolic pH, Ca^{2+}, high solute concentration, pressure pulses as well as the presence of hydroxyl radicals and reactive oxygen species are all external factors influential in MIP gating. Two gating mechanisms involving post-translational modification have been described for the spinach aquaporin SoPIP2;1(Törnroth-Horsefield *et al.* 2006): protein crystallization and molecular dynamics simulations revealed that channel closure is brought about by protonation of of a conserved His residue in Loop D and dephosphorylation of two conserved Ser situated in the consensus phosphorylation sites of Loop B and the C-terminal region (Törnroth-Horsefield *et al.* 2006). The protonation of this conserved His residue causes conformational change of Loop D which is relocated close to Loop B and blocks the exit of the pore, and consequently leads to the occlusion of the pore. Upon phosphorylation of Loop B, Loop D is unlocked, permitting the open conformation. Most interestingly, phosphorylation of the C-terminal tail would be able to prevent Loop D of an adjacent monomer from adopting a closed-pore conformation, which partially explains how tetramerization would increase the water transport capacity of each monomer in the aquaporin tetramer (Törnroth-Horsefield *et al.* 2006). Divalent

cations such as Ca^{2+} are also proposed to function in gating of the spinach SoPIP2;1 by facilitating ionic interactions and hydrogen bonds that enable the closed conformation (Törnroth-Horsefield *et al.* 2006). Since the signature motifs for phosphorylation and protonation can be identified in fungal aquaporin sequences, it will be of interest to determine the extent to which these post-translational mechanisms function in fungal aquaporin conformational change and gating, thus regulating aquaporin-mediated water transport in fungi. Although challenging, it will be of particular interest to determine whether post-translational modifications function to regulate mycorrhizal water transport in response to diverse environmental cues.

2.4.4 Other ecological perspectives of fungal MIPs

In addition to relatively well-understood model fungal species and those species with application in fermentation and other aspects of the food industry, there are a plethora of other fungal species that play key roles in diverse ecological processes in forestry, agriculture and extreme natural habitats. The post-genomic era heralds new possibilities for examining how these fungi function in their environment, and investigating the roles that they play in their diverse ecological niches. For instance, the lichen-forming fungi *Cladonia grayi* and *Xanthoria parietina* are extremely drought resistant, and the marine yeast *Debaryomyces hansenii* inhabits a highly hypertonic environment, indicating high efficiency or unique mechanisms of MIP regulation. Along similar lines, members of the Basidiomycota rapidly absorb water upon fruiting body formation, whereas their spores are usually highly dehydrated. It is tempting to speculate on roles that the MIPs may play in these fascinating biological processes.

From a practical perspective, understanding the contribution of fungal MIPs to mycorrhizal associations could be used in developing applications for use of mycorrhizal symbiosis to combat various stress conditions in the scenarios of agriculture, forestry, or land reclamation (Boyle & Hellenbrand 1991; Siemens & Zwiazek 2011), as well as in cultivation of economically important species of the Ascomycota and Basidiomycota.

Although the understanding of fungal MIP functions is still limited to several closely related model yeasts and a small number of mycorrhizal species, this latest

phylogenetic analysis suggests a rich and valuable resource for the study of MIP structure, functions and phylogenetic evolution within this kingdom and across kingdoms. Understanding genetic, structural and functional diversity of mycorrhizal MIPs will help to resolve the classification of fungal MIPs. Similarly, a more mechanistic model of how mycorrhizal MIPs are regulationed at the transcriptional and post-translational level in response to complex environmental cues will enable a better understanding of the fungal contribution to mycorrhizal associations and the many underground ecological processes to which these associations contribute.

2.5 References

Agerer R. 2001. Exploration types of ectomycorrhizae—a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **11**: 107–114.

Allen MF. 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal* **6**: 291–297.

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Rulz-Lozano JM. 2009. Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. *Molecular Plant-Microbe Interactions* **22**: 1169-1178.

Augé RM. 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science* **84**: 373–381.

Bahn Y, Mühlschlegel FA. 2006. CO_2 sensing in fungi and beyond. *Current Opinion in Microbiology* **9**: 572-578.

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Rulz-Lozano JM. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Annuals of Botany* **109**: 1009-1017.

Bill RM, Hedfalk K, Karlgren S, Mullins JGL, Rydstrom J, Hohmann S. 2001. Analysis of the pore of the unusual Major Intrinsic Protein channel, Yeast Fps1p. *The Journal of Biological Chemistry* **276**: 36543-36549.

Borgnia M, Nielsen S, Engel A, Agre P. 1999. Cellular and molecular biology of the aquaporin water channels. *Annual Reviews of Biochemistry* **68**: 425–458.

Boyle CD, Hellenbrand KE. 1991. Assessment of the effect of mycorrhizal fungi on drought tolerance of conifer seedlings. *Canadian Journal of Botany* **69**: 1764-1771.

Calvo-Polanco M, Zwiazek JJ, Voicu MC. 2008. Responses of ectomycorrhizal American elm (*Ulmus americana*) seedlings to salinity and soil compaction. *Plant and Soil* **308**: 189–200.

Calvo-Polanco M, Jones MD, Zwiazek JJ. 2009. Effects of pH on NaCl tolerance of american elm (*Ulmus americana*) seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria bicolor*. *Acta Physiologiae Plantarum* **31**: 515-522.

Dietz S, von Bülow J, Beitz E, Nehls U. 2011. The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: lessons for symbiotic functions. *New Phytologist* **190**: 927-40.

Di Battista C, Selosse MA, Bouchard D, Stenström E, Le Tacon F. 1996. Variations in symbiotic efficiency, phenotypic characters and ploidy level among different isolates of the ectomycorrhizal basidiomycete *Laccaria bicolor* strain S238. *Mycological Research* **100**: 1315-1324.

Egerton-Warburton LM, Graham RC, Hubbert KR. 2003. Spatial variability in mycorrhizal hypae and nutrient and water availability in a soil-weathered bedrock profile. *Plant and Soil* **249**: 331–342.

Egerton-Warburton LM, Querejeta JI, Allen MF. 2007. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *Journal of Experimental Botany* **58**: 1473–1483.

Ehlert C, Maurel C, Tardieu F, Simonneau T. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology* **150**: 1093-1104.

Emanuelsson O, Brunak S, Heijne G, Nielsen H. 2007. Locating proteins in the cell using TargetP, SignalP, and related tools. *Nature Protocols* **2**: 953-971.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Fischer G, Kosinska-Eriksson U, Aponte-Santamaría C, Palmgren M, Geijer C, Hedfalk K, Hohmann S, de Groot BL, Neutze R, Lindkvist-Petersson K. 2009. Crystal structure of a yeast aquaporin at 1.15 Å reveals a novel gating mechanism. *PLoS Biology* **7**: e1000130.

Gena P, Pellegrini-Calace M, Biasco A, Svelto M, Calamita G. 2011. Aquaporin membrane channels: biophysics, classification, functions, and possible biotechnological applications. *Food Biophysics* **6**: 241–249.

Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F. 2009. Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. *Biochimica et Biophysica Acta* **1788**: 1213–1228.

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819–823.

Grigoriev IV, Cullen D, Goodwin SB, Hibbett D, Jeffries TW, Kubicek CP, Kuske C, Magnuson JK, Martin F, Spatafora JW, Tsang A, Baker SE. 2011. Fueling the future with fungal genomics. *Mycology* **2**: 192–209.

Grigoriev IV, Nordberg H, Shabalov I, Aerts A, Cantor M, Goodstein D, Kuo A, Minovitsky S, Nikitin R, Ohm RA, Otillar R, Poliakov A, Ratnere I, Riley R, Smirnova T, Rokhsar D, Dubchak I. 2012. The genome portal of the department of energy joint genome institute. *Nucleic Acids Research* **40**: D26-D32.

Groppa MD, Benavides MP, Zawoznik MS. 2012. Root hydraulic conductance, aquaporins and plant growth promoting microorganisms: A revision. *Applied Soil Ecology* **61**: 247-254.

Gupta AB, Sankararamakrishnan R. 2009. Genome-wide analysis of major intrinsic proteins in the tree plant *Populus trichocarpa*: characterization of XIP subfamily of aquaporins from evolutionary perspective. *BMC Plant Biology* **9**: 134.

Hacquard S, Tisserant E, Brun A, Legué V, Martin F, Kohler A. 2013. Laser microdissection and microarray analysis of *Tuber melanosporum* ectomycorrhizas

reveal functional heterogeneity between mantle and Hartig net compartments. *Environmental Microbiology* **15**: 1853-1869.

Hara-Chikuma M, Verkman AS. 2006. Physiological roles of glyceroltransporting aquaporins: the aquaglyceroporins. *Cellular and Molecular Life Sciences* **63**: 1386-1392.

Hedfalk K, Törnroth-Horsefield S, Nyblom M, Johanson U, Kjellbom P, Neutze R. 2006. Aquaporin gating. *Current Opinions in Structural Biology* **16**: 447-456.

Hénin J, Tajkhorshid E, Schulten K, Chipot C. 2008. Diffusion of glycerol through *Escherichia coli* aquaglyceroporin GlpF. *Biophysical Journal* **94**: 832-839.

Hirokawa T, Boon-Chieng S, Mitaku S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* **14**: 378-379.

Hohmann S, Bill RM, Kayingo G, Prior BA. 2000. Microbial MIP channels. *Trends in Microbiology* **8**: 33–38.

Hohmann S. 2002. Osmotic stress signaling and osmoadaptation in yeasts. *Microbiology and Molecular Biology Reviews* **66**: 300-372.

Javot H, Maurel C. 2002. The role of aquaporins in root water uptake. *Annals of Botany* **90**: 301-313.

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Güçlü J, Vinh J, Heyes J, Franck KI, Schäffner AR, Bouchez D, Maurel C. 2003. Role of a single aquaporin isoform in root water uptake. *The Plant Cell* **15**: 509-522.

Jones DT, Taylor WR, Thornton JM. 1992. The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences* **8**: 275-282.

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U. 2005. Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biology* **7**: 706–712.

Krane CM, Goldstein DL. 2007. Comparative functional analysis of aquaporins/glyceroporins in mammals and anurans. *Mammaliam Genome* **18**: 452–462.

Laizé V, Gobin R, Rousselet G, Badler C, Hohmann S, Ripoche P, Tacnet,F. 1999. Molecular and functional study of *AQY1* from *Saccharomyces cerevisiae*: role of the C-terminal domain. *Biochemical and Biophysical Research Communications* **257**: 139-144.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. ClustalW and ClustalX version 2. *Bioinformatics* **23**: 2947-2948.

Lee SH, Calvo-Polanco M, Chung GC, Zwiazek JJ. 2010. Role of aquaporins in root water transport of ectomycorrhizal jack pine (*Pinus banksiana*) seedlings exposed to NaCl and fluoride. *Plant, Cell and Environment* **33**: 769–780.

Lee SH, Chung GC, Jang JY, Ahn SJ, Zwiazek JJ. 2012. Overexpression of PIP2;5 aquaporin alleviates effects of low root temperature on cell hydraulic conductivity and growth in Arabidopsis. *Plant Physiology* **159**: 479-488.

Lehto T, Zwiazek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* **21**: 71–90.

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B. 2013. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617-630.

Lopez D, Bronner G, Brunel N, Auguin D, Bourgerie S, Brignolas F, Carpin S, Tournaire-Roux C, Maurel C, Fumanal B, Martin F, Sakr S, Label P, Julien JL, Gousset-Dupont A, Venisse JS. 2012. Insights into *Populus* XIP aquaporins: evolutionary expansion, protein functionality, and environmental regulation. *Journal of Experimental Botany* **63**: 2217-2230.

Marjanović Ž, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiss M, Hampp R, Nehls U. 2005. Aquaporins in poplar: what a difference a symbiont makes! *Planta* **222**: 258–268.

Martin F, Aerts A, Ahrn D, Brun A, Danchin EGJ. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452**: 88-92.

Martin F, Nehls U. 2009. Harnessing ectomycorrhizal genomics for ecological

insights. *Current Opinion in Plant Biology* **12**: 508–515.

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, Porcel B, Rubini A, Amicucci A, Amselem J, Anthouard V, Arcioni S, Artiguenave F, Aury J, Ballario P, Bolchi A, Brenna A, Brun A, Buee M, Cantarel B, Chevalier G, Couloux A, Da Silva C, Denoeud F, Duplessis S, Ghignone S, Hilselberger B, Iotti M, Marcais B, Mello A, Miranda M, Pacioni G, Quesneville H, Riccioni C, Ruotolo R, Splivallo R, Stocchi V, Tisserant E, Viscomi AR, Zambonelli A, Zampieri E, Henrissat B, Lebrun M, Paolocci F, Bonfante P, Ottonello S, Wincker P. 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature*. **464**: 1033-1038.

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV. 2011. Sequencing the fungal tree of life. *New Phytologist* **190**: 818-821.

Matheny PB, Gossmann JA, Zalar P, Kumar TKA, Hibbett DS. 2006. Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. *Canadian Journal of Botany* **84**: 1794-1805.

Maurel C, Verdoucq L, Luu D, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology* **59**: 595-624.

Maurel C, Plassard C. 2011. Aquaporins: for more than water at the plant–fungus interface? *New Phytologist* **190**: 815–817.

Mitaku S, Hirokawa T. 1999. Physicochemical factors for discriminating between soluble and membrane proteins: hydrophobicity of helical segments and protein length. *Protein Engineering* **12**: 953–957.

Mitani-Ueno N, Yamaji N, Zhao FJ, Ma JF. 2011. The aromatic/arginine selectivity filter of NIP aquaporins plays a critical role in substrate selectivity for silicon, boron, and arsenic. *Journal of Experimental Botany* **62**: 4391-4398.

Muhsin TM, Zwiazek JJ. 2002. Ectomycorrhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus americana* seedlings. *New Phytologist* **153**: 153-158.

Navarro-Ródenas A, Ruíz-Lozano JM, Kaldenhoff R, Morte A. 2012. The aquaporin TcAQP1 of the desert truffle *Terfezia claveryi* is a membrane pore for water and CO_2 transport. *Molecular Plant-Microbe Interaction* **25**: 259-266.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

Peterson RL, Massicotte HB, Melville LH. 2004. *Mycorrhizas: Anatomy and Cell Biology*. Ottawa, Canada: NRC Research Press, 173.

Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S. 2005. Aquaporins in yeasts and filamentous fungi. *Biology of the Cell* **97**: 487–500.

Plamboeck AH, Dawson TE, Egerton-Warburton LM, North M, Bruns TD, Querejeta JI. 2007. Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. *Mycorrhiza* **17**: 439–447.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Siemens J, Zwiazek J. 2011. *Hebeloma crustuliniforme* modifies root hydraulic responses of trembling aspen (*Populus tremuloides*) seedlings to changes in external pH. *Plant and Soil* **345**: 247–256.

Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. 3rd Edition, Cambridge, UK: Academic Press.

Soveral G, Prista C, Moura TF, Loureiro-Dias MC. 2010. Yeast water channels: an overview of orthodox aquaporins. *Biology of the Cell* **103**: 35-54.

Steudle E, Peterson CA. 1998. How does water get through roots? *Journal of Experimental Botany* **49**: 775–788.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods**.** *Molecular Biology and Evolution* **28**: 2731-2739.

Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P. 2006. Structural mechanism of plant aquaporin gating. *Nature* **439**: 688–694.

Turgeman T, Asher JB, Roth-Bejerano N, Kapulnik Y, Sitrit Y. 2011. Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions. *Mycorrhiza* **21**: 623-630.

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R. 2007. Arbuscular mycorrhizal symbiosis and plant aquaporin expression. *Phytochemistry* **68**: 122–129.

Wysocki R, Chéry CC, Wawrzycka D, Van Hulle M, Cornelis R, Thevelein JM, Tamás MJ. 2001. The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*. *Molecular Microbiology* **40**: 1391-1401.

Yu TEJ-C, Egger KN, Peterson RL. 2001. Ectendomycorrhizal associations-characteristics and functions. *Mycorrhiza* **11**: 167–177.

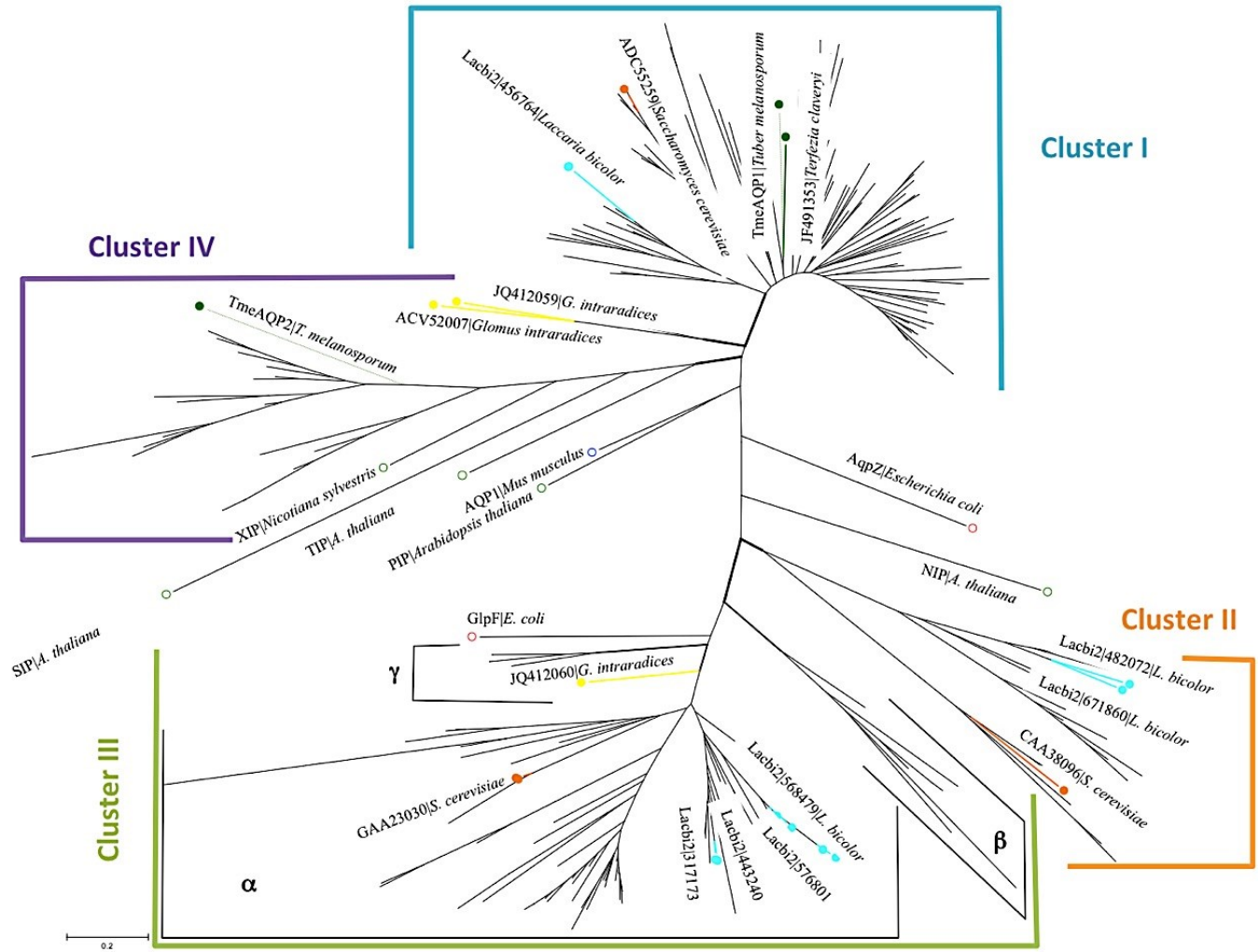
Zardoya R, Ding X, Kitagawa Y, Chrispeels J. 2002. Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment. *Proceedings of the National Academy of Sciences USA* **99**: 14893-14896.

Table 2.1 Functionally assayed fungal MIPs

Cluster	Canonical MIPs					
	Protein ID[1]	**Fungal species and types**	**# of amino acids and TMDs**	**NPA motifs**		**Functions and subcellular localization (TargetP1.1[2])**
				Loop B	**Loop E**	
Cluster I: orthodox aquaporins	ADC55259[3]	*S. cerevisiae*, budding yeast	289aa, 6	NPA	NPA	Water transporter; plasma membrane
	JF491353[4]	*T. claveryi,* ECM desert truffle	307aa, 6	NPA	NPA	Water and CO_2 transporter; plasma membrane
	Lacbi2:456764[5]	*L. bicolor,* ECM	311aa, 6	NPN	NSA	Water transporter; secretary pathway
Cluster II: aquaglyce-roporins	CAA38096[3]	*S. cerevisiae*	669aa, 6	NPS	NLA	Glycerol and methylamine facilitators; plasma membrane
	Lacbi2:671860[5]	*L. bicolor*	330aa, 6	NPC	NSA	CH_3NH_2, NH_4^+/NH_3; plasma membrane
	Lacbi2:482072[5]		343aa, 6	NPC	NTA	Glycerol, urea, CH_3NH_2, NH_4^+/NH_3, limited water; mitochondrion
Cluster III: facultative aquaporins	GAA23030[3]	*S. cerevisiae*	646aa, 5	NPA	NPA	Unknown; plasma membrane
	Lacbi2:443240[5]	*L. bicolor*	312aa, 5	NPA	NPA	Glycerol, water, CH_3NH_2, NH_4^+/NH_3; plasma membrane
	Lacbi2:317173[5]		332aa, 6	NPA	NPA	Water, CH_3NH_2, NH_4^+/NH_3; plasma membrane
	Lacbi2:568479[5]		263aa, 6	NPA	NPA	Water, CH_3NH_2; secretary pathway
	JQ412060[6]	*G. intraradices* AM	316aa, 6	NPA	NAA	Water; plasma membrane and intracellular membranes (shown in GFP-fusion analysis)
Cluster IV: Fungal XIPs	ACV52007[7]	*G. intraradices,*	253aa, 6	NPA	NPA	No transport capacity detected; plasma membrane
	JQ412059[6]		276aa, 6	NPK	NPA	Water; plasma membrane (shown in GFP-fusion analysis)

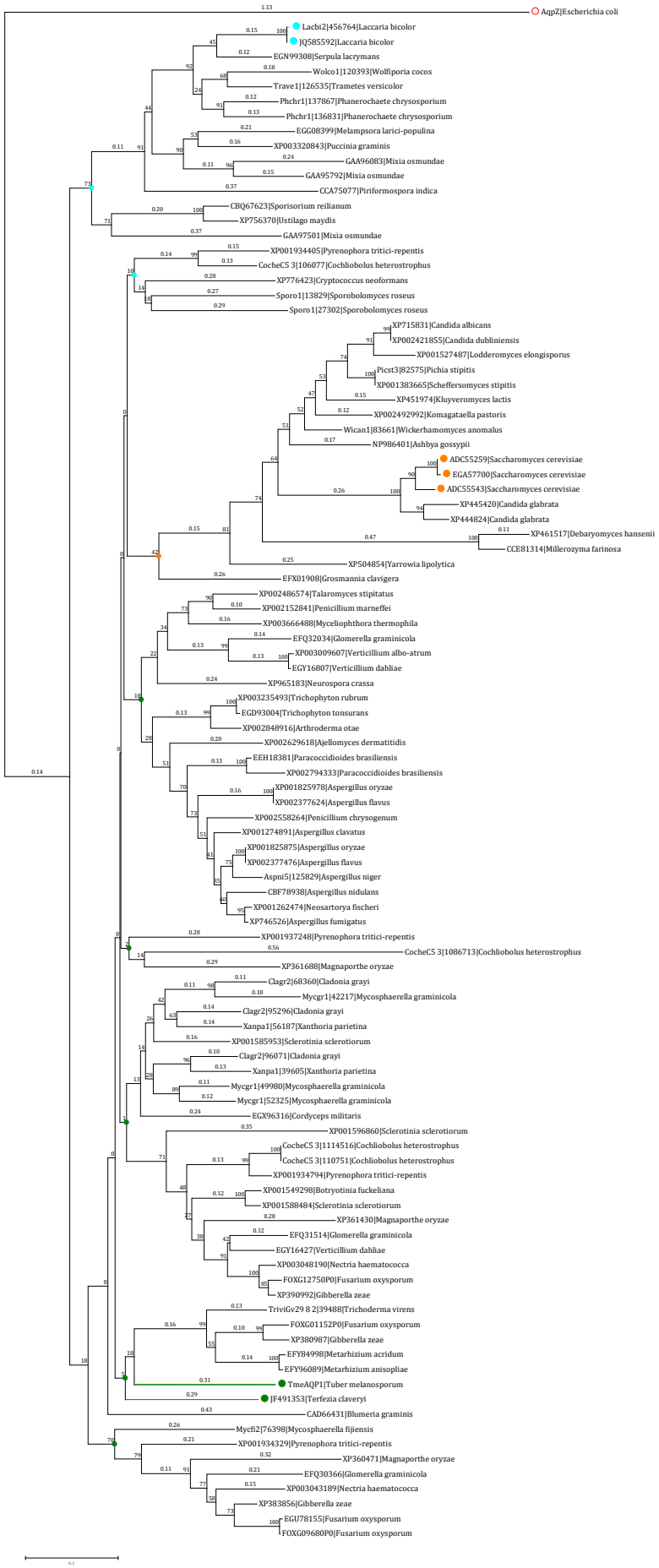
Note: 1. Protein ID refers to the accession number for the sequence in the database from which the sequence was retrieved; 2. Subcellular localization was predicted according to the signal peptide on N- terminus using TargetP1.1, unless noted otherwise; 3. Soveral *et al*. 2010; 4. Navarro-Ródenas *et al*. 2012; 5. Dietz *et al*. 2011; 6. Li *et al.* 2013; 7. Aroca *et al*. 2009.

Figure 2.1 Phylogenetic analysis of 229 fungal MIPs from 88 fungal species representing four phyla shows clustering of these sequences into four distinct groups



Deduced amino acid sequences were aligned using ClustalW followed by analysis using neighbor-joining in MEGA5. Numbers at nodes indicate bootstrap support following 1000 iterations. Cluster I comprises MIPs that include putative water channels, Cluster II comprises MIPs that include putative aquaglyceroporins preferentially transporting small neutral molecules, Cluster III includes MIPs that putatively possess both water and small neutral molecule transport capacities, and Cluster IV includes fungal XIPs. PIP, TIP, NIP and SIP in *Arabidopsis thaliana* (green open circles), XIP in *Nicotina sylvestris* (green open circles), GlpF and AqpZ in *Escherichia coli* (red open circles) and AQP1 in *Mus musculus* (blue open circles) were used as reference MIPs. The branches corresponding to MIPs which transport capacities have been functionally characterized are indicated in bold and color-coded green for filamentous Ascomycota, orange for yeast Ascomycota, cyan for Basidiomycota, and yellow for Glomeromycota.

Figure 2.2 Cluster I of 102 orthodox fungal water channels



Evolutionary distances were computed using the JTT matrix-based method in the units of the number of amino acid substitutions per site (Jones *et al*. 1992). Protein ID, species names, phyla, branch length longer than 0.1 (values above the branch) and Bootstrap frequency (values at the node) are shown. The branches of the MIPs that have been functionally assayed were strengthened and color-coded as green for filamentous Ascomycota, orange for yeast Ascomycota, cyan for Basidiomycota, and yellow for Glomeromycota. AqpZ in *Escherichia coli* (red open circles) was used as the reference outgroup MIP. Node markers were used to show which phylum the species on the corresponding sub-trees affiliate to: green filled circle for filamentous Ascomycota, orange filled circle for yeast Ascomycota, and cyan filled circle for Basidiomycota.

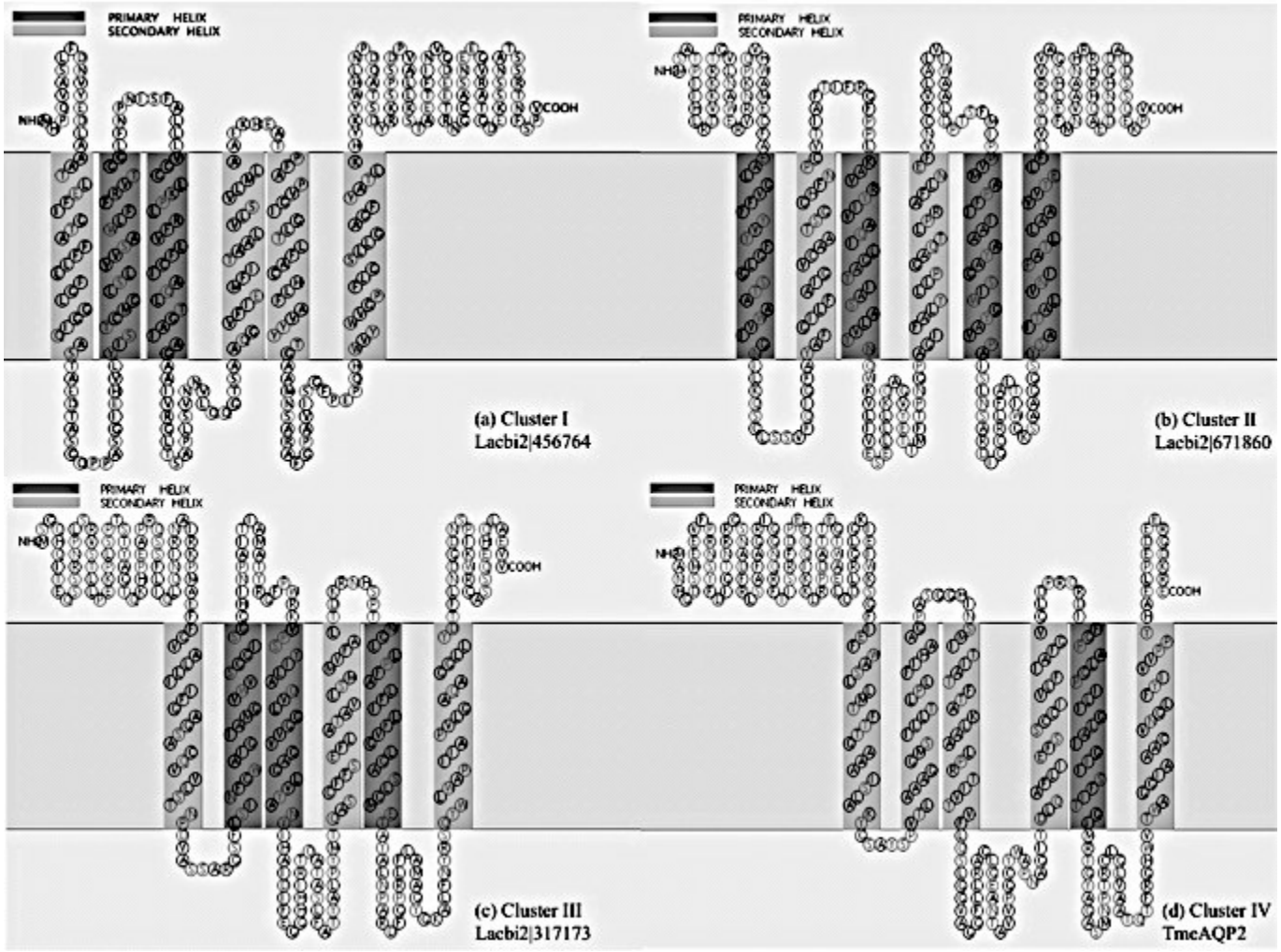


Figure 2.3 Four mycorrhizal fungal MIPs representative of the four different phylogenetic clusters show differences in the number of transmembrane secondary helices, the length of termini, and NPA signature motifs
(a) Orthodox aquaporins (cluster I) represented by *Laccaria bicolor* Lacbi2:456764; **(b)** Aquaglyceroporins (cluster II) represented by *L. bicolor* Lacbi2:671860; **(c)** Facultative aquaporins (cluster III) represented by *L. bicolor* Lacbi2:317173; **(d)** Fungal X intrinsic protein (XIPs) (cluster IV) represented by *Tuber melanosporum* TmeAQP2.

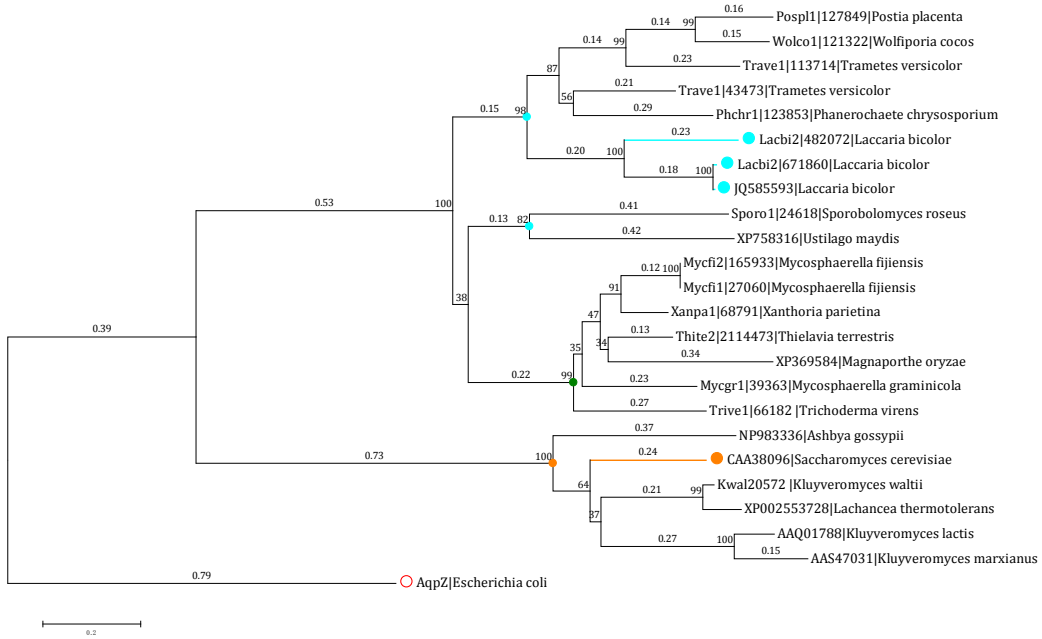


Figure 2.4 Cluster II of 23 fungal aquaglyceroporins
The evolutionary distances were computed using the JTT matrix-based method in the units of the number of amino acid substitutions per site (Jones *et al*. 1992). Protein ID, species names, phyla, branch length longer than 0.1 (values above the branch) and Bootstrap frequency (values at the node) are shown. The branches of the MIPs that have been functionally assayed were strengthened and color-coded as orange for yeast Ascomycota and cyan for Basidiomycota. AqpZ in *Escherichia coli* (red open circles) was used as the reference outgroup MIP. Node markers were used to show which phylum the species on the corresponding sub-trees affiliate to: green filled circle for filamentous Ascomycota, orange filled circle for yeast Ascomycota, and cyan filled circle for Basidiomycota.

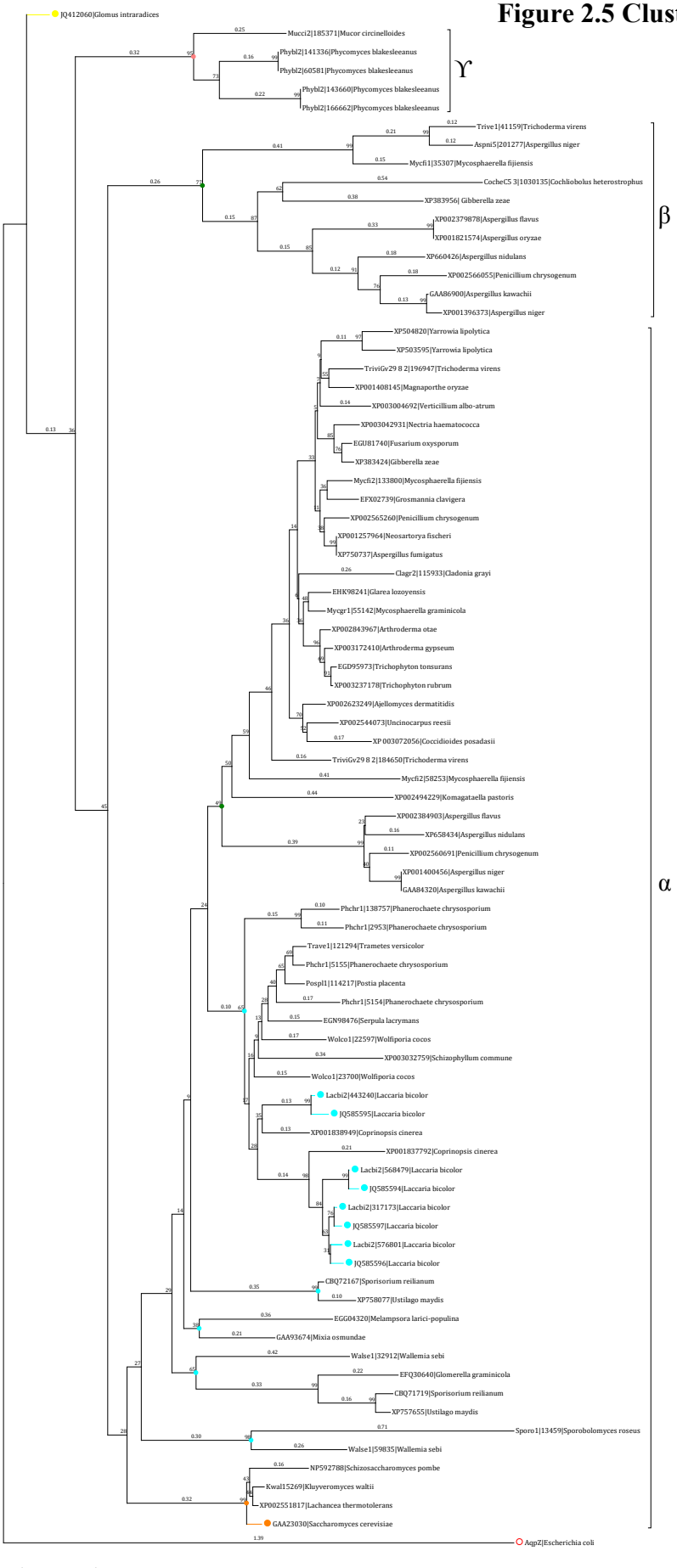


Figure 2.5 Cluster III of 82 fungal facultative aquaporin

The evolutionary distances were computed using the JTT matrix-based method in the units of the number of amino acid substitutions per site (Jones *et al*. 1992). Protein ID, species names, phyla, branch length longer than 0.1 (values above the branch) and Bootstrap frequency (values at the node) are shown. The branches of the MIPs that have been functionally assayed were strengthened and color-coded as orange for yeast Ascomycota and cyan for Basidiomycota. AqpZ in *Escherichia coli* (red open circles) was used as the reference outgroup MIP. Node markers were used to show which phylum the species on the corresponding sub-trees affiliate to: green filled circle for filamentous Ascomycota, orange filled circle for yeast Ascomycota, cyan filled circle for Basidiomycota, yellow filled circle for Glomeromycota, and rose pink filled circle for Zygomycota.

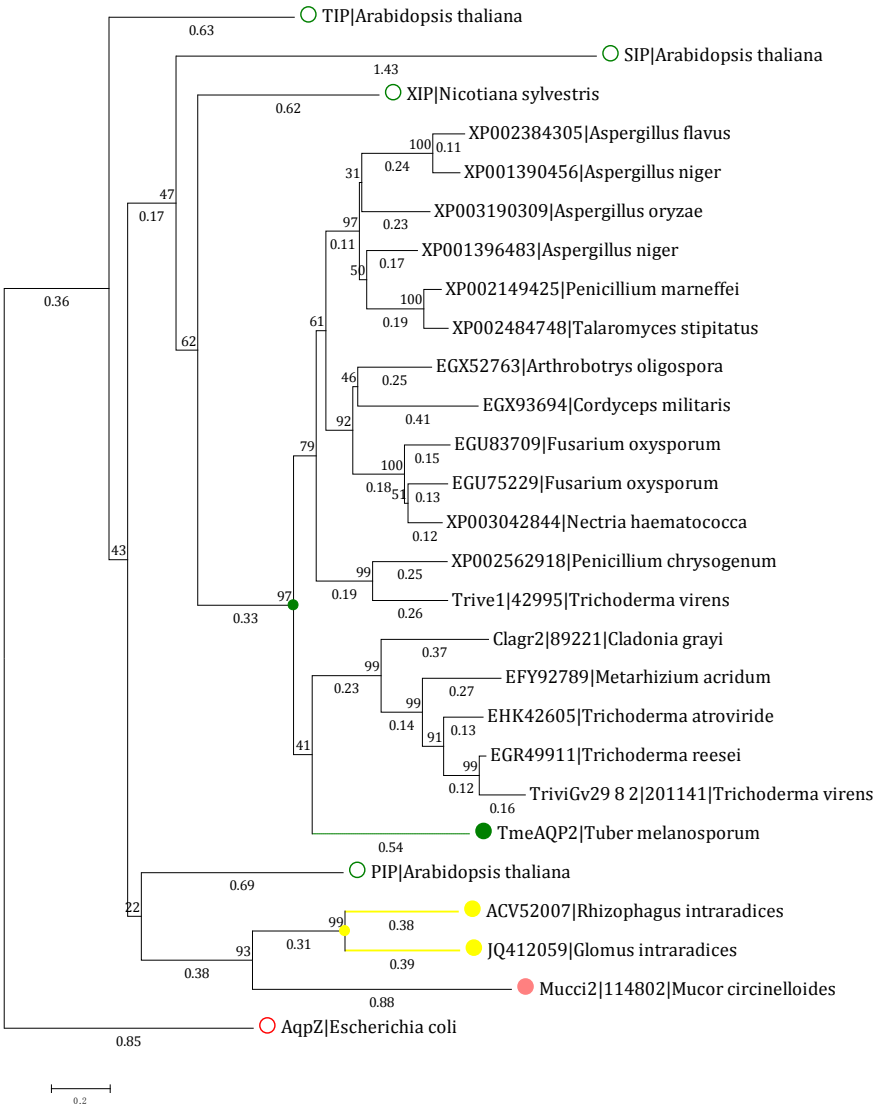


Figure 2.6 Cluster IV of 22 fungal XIPs

Cluster IV MIPs are closely related to plant MIPs (green open circles). Deduced amino acid sequences were aligned with ClustalW and the bootstrap consensus dendrogram was constructed using the neighbor-joining algorhithm of 1000 replicates (Felsenstein 1985, Saitou & Nei 1987) in MEGA5 (Tamura *et al*. 2011). The evolutionary distances were computed using the JTT matrix-based method in the units of the number of amino acid substitutions per site (Jones *et al*. 1992). Protein ID, species names, phyla, branch length longer than 0.1 (values above the branch) and Bootstrap frequency (values at the node) are shown. The branches of the MIPs that have been functionally assayed were strengthened and color-coded as yellow for Glomeromycota. AqpZ in *E. coli* (red open circles) was used as the reference outgroup MIP. Node markers were used to show which phylum the species on the corresponding sub-trees affiliate to: green filled circle for filamentous Ascomycota, yellow filled circle for Glomeromycota, and rose pink filled circle for Zygomycota.

3 OVEREXPRESSION OF *LACCARIA BICOLOR* AQUAPORIN JQ585595 ALTERS ROOT WATER TRANSPORT PROPERTIES IN ECTOMYCORRHIZAL WHITE SPRUCE (*PICEA GLAUCA*) SEEDLINGS

3.1 Introduction

Ectomycorrhizal (ECM) fungi absorb water and nutrients through extensive extraradical hyphal networks, transporting these resources to the mantle and Hartig net where resources are exchanged with the host plant root (Agerer 2001). Processes involved in water uptake by mycorrhizal plants have received less attention than nutrient acquisition. Improved plant water relations have been frequently attributed to ECM (Plamboeck *et al.* 2007; Lehto & Zwiazek 2011) and arbuscular mycorrhizas (AM) (Uehlein *et al.* 2007; Bárzana *et al.* 2012). The effects of mycorrhizal associations often include increased root hydraulic conductivity (Muhsin & Zwiazek 2002a, b; Marjanović *et al*. 2005), which has been attributed to increased apoplastic (Nylund 1987; Muhsin & Zwiazek 2002a; Bárzana *et al*. 2012) and transmembrane water transport (Marjanović *et al*. 2005; Porcel *et al*. 2006; Aroca *et al*. 2007; Uehlein *et al*. 2007; Lee *et al*. 2010). The contribution of extraradical fungal hyphae to root water transport can be significant, as evidenced by decreased root hydraulic conductance following removal of these hyphae (Muhsin & Zwiazek 2002b). Increased relative apoplastic flow in mycorrhizal plants has also been determined by use of apoplastic tracer dye (Bárzana *et al*. 2012) and inhibitors of aquaporin activity (Muhsin & Zwiazek 2002a; Bárzana *et al*. 2012). However, use of apoplastic tracer dyes and aquaporin inhibitors can be problematic because they also potentially affect hyphal water transport.

Mycorrhizal associations have been reported to increase hydraulic conductivity of root cortical cells (Lee *et al.* 2010) and alter the expression of root aquaporins in AM and ECM plants (Marjanović *et al*. 2005; Porcel *et al.* 2006; Aroca *et al*. 2007; Uehlein *et al*. 2007; Dietz *et al*. 2011; Giovannetti *et al*. 2012; Navarro-Ródenas *et al*. 2013). Plant aquaporins are categorized into PIP (plasma membrane intrinsic protein), TIP

(tonoplast intrinsic protein), NIP (nodulin-26 like intrinsic protein), SIP (small intrinsic proteins) and XIP (X intrinsic proteins) subfamilies, based on subcellular localization and transport capacities (Maurel *et al*. 2008). PIPs play a crucial role in facilitating water transport and regulating root (Javot & Maurel 2002; Aroca *et al*. 2012; Gambetta *et al*. 2013) and leaf (Maurel *et al.* 2008; Prado & Maurel 2013) hydraulic conductivity. Their expression and post-translational modifications are sensitive to various environmental factors (Javot & Maurel 2002; Maurel *et al*. 2008; Gambetta *et al.* 2013). The relative contributions of transmembrane and apoplastic water transport pathways in mycorrhizal roots may partly explain the reported lack of effect of mycorrhization on host plant root water flow properties (Coleman *et al*. 1990; Nardini *et al*. 2000; Calvo-Polanco *et al*. 2008; Siemens & Zwiazek 2008; Yi *et al*. 2008).

Delineating the precise pathways for water transport from the fungal partner to the host roots in mycorrhizal associations remains a challenge. Some studies support the view that hydrophobic fungal cell walls in the mantle may block the apoplastic water pathway and hinder root water uptake (Duddridge *et al*. 1980; Unestam & Sun 1995), whereas others argue that fungal hyphae are more likely to form a water transport highway for plant roots, which substantially increases water availability to the roots (Khalvati *et al*. 2005; Allen 2007; Egerton-Warburton *et al*. 2007; Lehto & Zwiazek 2011). Because water can be transported in the cell walls of hydrophilic fungi, including *Laccaria bicolor* (Weatherley 1982; Lehto & Zwiazek 2011), it could be argued that this route offers the least resistance and thus could be the predominant pathway for water transport to the root cortex. However, the advantages of a symplastic pathway for hyphal water transport include the possibility of hydraulic regulation by fungal aquaporins as water enters and subsequently leaves the hyphae.

This study addressed the question of the contribution of aquaporin-mediated transport in mycorrhizal fungal hyphae to water transport of the host plant. Fungal aquaporins have been described from several fungal taxa, and can be classified into four distinct groups: orthodox fungal water channels, fungal aquaglyceroporins, facultative fungal aquaporins and fungal XIPs (Dietz *et al*. 2011; Xu *et al.* 2013). Recent studies have demonstrated the capacity for transport of water and other small molecules by several aquaporins from ECM and AM fungi (Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012; Li *et al*. 2013), which may play multiple roles in plant–fungal interactions (Maurel &

Plassard 2011). In *L. bicolor* strain S238N, five aquaporin genes heterologously expressed in *Xenopus laevis* oocytes showed strong to moderate water transport capacity; some of these were also permeable to urea, glycerol and ammonia (Dietz *et al.* 2011). TcAQP1 from the hypogeous mycorrhizal desert truffle (*Terfezia claveryi*) also showed water and CO_2 transport capacity (Navarro-Ródenas *et al*. 2013), whereas GintAQPF1 and GintAQPF2 from the AM species *Glomus intraradices* showed significant water permeability (Li *et al*. 2013). The expression of these fungal aquaporins could be altered by mycorrhization or abiotic cues (Dietz *et al*. 2011; Li *et al*. 2013; Navarro-Ródenas *et al*. 2013), suggesting their involvement in water transport of the mycorrhizal partners.

One means to assess the relative significance of the different pathways for water movement in mycorrhizal plants is to alter the aquaporin-mediated water transport properties of the mycorrhizal fungus partner. Accordingly, the aquaporin- encoding *JQ585595* (protein ID AFJ15558.1) was selected from *L. bicolor* strain UAMH8232 for its high water transport capacity and high transcript abundance, and generated transgenic L. bicolor overexpressing *JQ585595* to test the role of this fungal aquaporin in facilitating water transport in ectomycorrhizal white spruce (*Picea glauca* [Moench] Voss). *P. glauca* seedlings inoculated with wildtype (WT), *JQ585595*-overexpressing (OE) and mock-transformed strains were examined for the effect of these fungal genotypes on water transport properties of the host plant. The study tested the hypothesis that root hydraulic conductivity of mycorrhizal plants would be enhanced by overexpression of the *L. bicolor* aquaporin, reflecting the increased contribution of water transport through fungal hyphae to water transport of the mycorrhizal root system.

3.2 Materials and Methods

3.2.1 Gene cloning of putative MIPs of Laccaria bicolor *UAMH8232*

Laccaria bicolor (strain UAMH8232, University of Alberta Microfungus Collection and Herbarium) mycelia were grown on solid modified Melin-Norkans (MMN) medium (Marx 1969; Pham *et al.* 2004) at 20°C with cellophane placed on the surface for three weeks before mycelia were harvested and immediately frozen in liquid nitrogen. Mycelia were ground in liquid nitrogen using a mortar and pestle. Total RNA

was extracted using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), and used for first strand cDNA synthesis (Superscript II, Life Technologies, Carlsbad, CA, USA). Six full-length cDNAs - designated according to their NCBI accession numbers as *JQ585592, JQ585593, JQ585594, JQ585595, JQ585596,* and *JQ585597* - corresponding to five of the seven *L. bicolor* S238N MIPs reported by Dietz *et al.* (2011), were amplified using gene-specific primer (Table 3.1), and ligated into pGEM-T Easy (Promega, Madison, WI, USA). Primers for cloning were designed using Primer 3 Plus (Untergasser *et al*. 2007). Sequences were confirmed by Sanger sequencing and were deposited in GenBank (accession numbers *JQ585592* - *JQ585597*).

3.2.2 Secondary structure prediction of putative MIPs and inter-strain deduced amino acid sequence alignment

Protein transmembrane secondary structure of the six putative MIPs was predicted using SOSUI (Hirokawa *et al*. 1998) and TMHMM2.0 (Krogh *et al.* 2001). Protein subcellular localization was predicted using Target P (Emanuelsson *et al*. 2007). Deduced amino acid sequences of the UAMH8232 and S238N strains were aligned to compare the inter-strain amino acid variations, using ClustalW2.1 (Larkin *et al*. 2007).

3.2.3 Oocyte assay for water transport capacity of **Laccaria bicolor** *MIPs*[1]

Full-length cDNAs were sub-cloned into the multiple cloning site of pXT7 containing the *T7* promoter and the 5' and 3' UTR of *Xenopus laevis* ß-globin gene (Dominguez *et al*. 1995), between the restriction sites *Xho*I and *Spe*I (Table 3.1). Correct insertion orientation was confirmed by sequencing, then the expression vector was linearized at the *Nde*I site located downstream of the *Xenopus* ß-globin gene. The linearized vector was used for *in vitro* synthesis of capped RNA (cRNA) using *T7* RNA polymerase (mMESSAGE mMACHINE *T7* kit, Ambion, Austin, TX, USA).

For the *X. laevis* oocyte swelling assay, healthy Stage V-VI oocytes were treated with collagenase and potassium phosphate (Cao *et al*. 1992). Ten ng of cRNA or nuclease-free water (as the negative control) was microinjected into each oocyte using an automatic nanoliter injector (Nanoject II, Drummond Scientific, Broomall, PA, USA).

[1] The pXT7 vector, oocytes and the facilities for oocyte assay were provided by Dr. Warren Gallin, Department of Biological Sciences, University of Alberta.

After incubation in 200 mOsmol (Kg^{-1} H_2O) modified Barth's solution (MBM) in scintillation vials at 18°C for 48 hours, each injected oocyte was transferred into MBM in one well of a four-well Petri dish and viewed under the 4x objective of an Olympus compound microscope. An initial image was taken with an Olympus QCapture digital camera; upon transfer of an oocyte into a well containing D = 0.2 hypotonic MBM (40 mOsmol Kg^{-1} H_2O), serial images were captured at 10 second intervals for three minutes to track changes in oocyte volume due to water influx. The diameter and surface area of oocytes were analyzed using ImageJ (V.1.44o; Schneider *et al.* 2012). The initial transmembrane volume flux and osmotic water permeability coefficiency (P_f) were calculated based on Zhang and Verkman (1991) to represent the water permeability of the oocytes injected with cRNAs of each putative aquaporin.

3.2.4 Quantitative RT-PCR for transcript abundance analysis of water-transporting aquaporins in vegetative mycelia on pure culture

Transcript abundance of water-transporting aquaporins was quantified in *L. bicolor* mycelia grown on solid MMN medium at 20°C for three weeks using the standard curve method of absolute quantification, as described in Appendix 3 (Pfaffl 2004; El Kayal *et al*. 2011). Three biological replicates representing independent mycelial cultures were sampled. Total RNA was prepared as described in §3.2.1. First strand cDNA was synthesized from 1 µg of total RNA using Superscript II (Life Technologies, Carlsbad, CA, USA), and used for SYBR Green™ quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Primers were designed using Primer Express 3.0 (Applied Biosystems, Life Technologies) (Table 3.2). Each 10 µl of the reaction consisted of 2.5 µl of 1.6 µM primer, 2.5 µl of 10 ng/µl cDNA and 5 µl of qPCR Mastermix. The 2X qPCR Mastermix used in this study was a proprietary mix developed by the Molecular Biology Service Unit in Department of Biological Science at University of Alberta (Edmonton, Alberta, Canada), which contained Tris (pH 8.3), KCl, $MgCl_2$, glycerol, Tween 20, DMSO, dNTPs, ROX™ as a normalizing dye, SYBR™ Green (Molecular Probes) as the detection dye, and an antibody inhibited Taq polymerase. The qPCR mastermix and cDNAs were dispensed into each well of the 384-well reaction plate by an automated liquid dispenser (Biomek 3000). Thermal cycling conditions were 95°C for 2 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, and a dissociation step of 95°C for 15 s, 60°C for 15 s and 95°C for 15 s,

carried out in ABI 7900HT qPCR System (Applied Biosystems). The transcript abundance of target aquaporin genes was normalized against the transcript abundance of reference gene *translation elongation factor EF2* (XM_001887160).

3.2.5 Construction of transgenic strains using Agrobacterium-mediated transformation to alter transcript abundance of aquaporin JQ585595

Transgenic *L. bicolor* strains overexpressing *JQ585595* were generated using the pHg/pSILBAγ- plasmid system under hygromycin B selection (Fig. 3.1) (Kemppainen & Pardo 2010)[2]. The aquaporin cDNA was liberated from the pGEM-T Easy vector with *Apa*I/*Pst*I, blunt ends were generated with T4 DNA polymerase and the cDNA fragment cloned into *Sna*BI/*Stu*I-digested pSILBAγ between the constitutive *Agaricus bisporus gpdII* promoter and *Aspergillus nidulans trpC* terminator. The correct cDNA orientation in the expression cassette of pSILBAγ was confirmed by sequencing. The full-length pSILBAγ/*JQ585595* -expression vector was cloned as a *Sac*I linearized fragment into the *Sac*I site in the T-DNA of the pHg binary vector to generate the final pHg/pSILBAγ/*JQ585595* transformation and overexpression construct. The vector was introduced into the *Agrobacterium tumefaciens* strain AGL1 by electroporation. The *L. bicolor* UAMH8232 WT strain was transformed with pHg/pSILBAγ/*JQ585595* via *Agrobacterium* according to Kemppainen *et al*. (2005) with the following modifications: the fungal colonies were pre-grown on cellophane membranes for three days, co-cultivation with *Agrobacterium* lasted for three days and elimination of *Agrobacterium* during transformant selection was carried out with 200 μg/mL of ceftriaxone in the growth medium. To generate the mock transformant strains, *L. bicolor* UAMH8232 WT was *Agro*-transformed with pHg/pSILBAγ. Thirteen and 12 independent transgenic strains were obtained for pHg/pSILBAγ/*JQ585595* and pHg/pSILBAγ transformation, respectively. Transformed strains that showed normal phenotypes of the species were selected for further validation.

Transgenic *L. bicolor* strains aiming to downregulate *JQ585595* were generated by RNA interference (RNAi) approach using the pHg/pSILBAγ- plasmid system under hygromycin B selection (Kemppainen *et al*. 2009). The inverted repeated sequence

[2] Fungal transgenic strains were constructed through collaboration with Dr. Minna Kemppainen and Dr. Alejandro Pardo, Laboratorio de Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Argentina.

arms in the ihpRNA expression cassette corresponded to 329 bp PCR amplicon produced with *JQ585595* cDNA plasmid clone as template. The sequence arms were cloned into *Sna*BI/*Hind*III and *Stu*I/*Bgl*II sites in pSILBAγ. The RNAi expression cassette was further cloned as full-length *Sac*I linearized vector into *Sac*I site in pHg vector. Transformation was conducted as described above. Twelve independent transgenic strains were obtained for pHg/pSILBAγ/*JQ585595*RNAi.

3.2.6 Verification of transgenic Laccaria bicolor *strains by Southern blot, TAIL-PCR and qRT-PCR*

3.2.6.1 Southern blot

Southern blot analysis was used to determine transgene copy number. Ten μg of gDNA extracted using the DNeasy Plant Maxi Kit (Qiagen, Valencia, CA, USA) was digested using *Sac*I or *Bam*HI (FastDigest™, Fermentas). An 870 bp PCR product was amplified using the 1026 bp hygromycin phosphotransferase gene (*hph*) in the binary vector as the template and gene-specific primers (Table 3.1), and used as both positive control and probe. The digested gDNA was purified and denatured at 65°C for 3 min before loading for electrophoresis using 0.8% agarose gel in 0.5x TBE buffer at 80V for four hours. The gel was depurinated in 0.25M HCl for 15 min with gentle shake, rinsed twice with deionized distilled water, and denatured in 0.5M NaOH for 30 min with gentle shake. DNA in the gel was transferred onto H^+-bond membrane (GE Healthcare, Buckinghamshire, UK) using a vacuum blotter (Model 785, BioRad, Richmond, CA, USA), by applying a constant 5 inch Hg vacuuming force for two hours on the gel which was completely immersed in 1.5L of 10x SSC buffer during transferring (Saline Sodium Citrate, pH = 7). Transferred membrane was washed in 2x SSC buffer for 5 min, cross-linked at UV 254nm twice and baked at 80°C for 2 h. Probe hybridization, stringent wash and detection reaction were conducted according to the manufacturer's protocol (Amersham AlkPhos Direct Labeling and Detection System with CDP-*Star*™, GE Healthcare), as previously described (Kemppainen *et al*. 2008). Chemiluminescence generated via an alkaline phosphatase reaction was detected by CCD sensor using a 30 min exposure time (BioRad ChemiDoc)[3].

[3] The BioRad ChemiDoc was provided by Dr. Jonathan Dennis, Department of Biological Sciences, University of Alberta.

3.2.6.2 TAIL-PCR

To reveal the insertion site of the transgenic cassette in the genome of *L. bicolor*, TAIL-PCR (Thermal Asymmetrical Interlacing PCR) was conducted to amplify the part of the T-DNA right border and its flanking sequence from the genome. The gDNAs were the same as used in Southern blot analysis. The templates and primers for each of the sequential reactions were listed in Table 3.3.

The three specific primers, RB1, RB2 and RB3, were designed to anneal to the region close to the right border of T-DNA, allowing amplification towards the 3' end of T-DNA (in forward direction) in the interlacing pattern: the secondary primer (RB2) was designed to anneal to the sequence amplified by the primary primer (RB1), and the tertiary primer (RB3) was designed to anneal to the sequence amplified by RB2. The arbitrary degenerate primer (AD) was used as the reverse primer in TAIL-PCR reactions, aiming to anneal to the flanking sequence out of the T-DNA right border in the reverse direction. The PCR conditions were programmed in the thermal asymmetrical pattern to both ensure specific annealing of RB primers and allow sufficient annealing of AD primer in the first three reactions (Table 3.3, modified according to a study on *Glomus intraradices* [Liu 2012]). After the tertiary PCR reaction, the quaternary PCR reaction was conducted with higher annealing temperature for the primer RB3 and lower concentration of the AD primer to increase amplification specificity (Table 3.3). PCR reaction was run in total volume of 50 µL, including 0.5 µL of 5 U/µL recombinant *Taq* DNA polymerase (Invitrogen), 1.5 µL of 50 mM $MgCl_2$, and 1 µL of 10 mM dNTP mix. Fifteen microliter of each PCR reaction or 0.5µg of 1kb DNA ladder (Fermentas) was loaded in each lane of 1% agarose gel stained by SYBR™ Safe DNA Gel Stain (Life Technologies). The electrophoresis ran in 0.5x TBE buffer at 90 V for 45 min. After gel electrophoresis, the specific band of quaternary PCR products were excised from 1% agarose gel, and DNA was extracted using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) and sequenced by Sanger DNA sequencing using the RB3 primer. By aligning the sequence result with the last 100bp T-DNA at the right border, the flanking sequence was identified and then used for BLASTn in *L. bicolor* S238N genome (JGI V2.0 masked assembly database) to find the highest hit that indicated the T-DNA insertion site.

3.2.6.3 Transcript abundance assay by qRT-PCR

Transcript abundance of *JQ585595* was quantified in transgenic *L. bicolor* mycelia grown on solid MMN medium at 20°C for three weeks using the standard curve method of absolute quantification, as described in §3.2.4 (Appendix 3). Three biological replicates representing independent mycelial cultures were sampled for each strain. The transcript abundance of *JQ585595* was normalized against the geometric mean of that of reference genes *EF2* and *α-tubulin* (XM_001876554), which did not change significantly across all tested samples of mycelia in WT and transgenic strains ($P = 0.76$).

3.2.7 Growth characteristics of transgenic Laccaria bicolor *strains*

A thermocouple psychrometer (Decagon SC10A) with NT-3 Nanovoltmeter thermometer (Luard & Griffin 1981) was used to measure total water potential Ψ_{total} and osmotic potential $\Psi_{osmotic}$ in the mycelia of selected *L. bicolor* strains which grew on solid MMN medium at 5°C and 20°C for one month.

To determine Ψ_{total}, the stripe of mycelium was taken from the surface of cellophane-covered MMN medium and rolled along the inner wall of sample cup. The sample was equilibrated in the sealed chamber for 30 min before collecting the microvolt reading that corresponded to Ψ_{total}. The stripe of mycelia was then taken out, wrapped in a piece of Parafilm™, frozen in liquid N_2 and quickly brought back to 20°C to break the cell walls and plasma membranes in the freeze-thaw process. After the flash freeze-thaw process, the sample was equilibrated in the sealed chamber for another 30 min before collecting the microvolt reading that corresponded to $\Psi_{osmotic}$. Microvolt readings were converted to Ψ data, according to the standard curve made from a serial dilution of KCl solutions. The difference between Ψ_{total} and $\Psi_{osmotic}$ was calculated to represent turgor potential Ψ_{turgor} (Equation 3.1) (two mycelial stripes from each of three plates of culture, $n = 3$). Six replications of individual culture were taken. Dry mass was weighed after 48 h oven drying at 80°C ($n = 6$, six individual cultures). Phenotype was photographed for each strain.

Equation 3.1 Calculation of mycelial turgor water potential

$$\Psi_{turgor} = \Psi_{total} - \Psi_{osmotic}$$

3.2.8 Growth and ectomycorrhiza inoculation of Picea glauca

3.2.8.1 Seedling growth and inoculation

Picea glauca seeds (National Tree Seed Centre, Canadian Forest Service, Fredericton, NB, Canada) were surface sterilized with 1% (v/v) sodium hypochlorite and stratified at 4°C according to Groome *et al*. (1991). Stratified seeds were germinated at 20°C on sterile, moistened cellulose paper (Kimpak; Kimberly-Clark, Mississauga, ON) (Fig. 3.2 a). One week after germination, seedlings were transplanted into autoclaved peat moss: vermiculite (2:1) in sterilized 170 mLSpencer-Lemaire root trainers (Spencer-Lemaire Industries Ltd. Edmonton, AB, Canada) (Carlson 1983) covered with plastic domes (Fig. 3.2 b). Seedlings were grown in a controlled environment growth room with 16 h photoperiod, 22°C/18°C (day/night temperature), 400 µmol m^{-2} s^{-1} photosynthetic photon flux density, and 50-60% relative humidity.

Mycelia of *L. bicolor* strains grown on solid MMN medium (Fig. 3.2 c) were cut into pieces and cultured in liquid MMN medium at 20°C with shaking at 0.8 x *g* for four weeks (Fig. 3.2 d). Cultures prepared from the WT strain, one mock strain, or two transgenic *JQ585595*-OE strains (designated OE1 and OE2) were homogenized in a blender to make liquid inoculum of OD_{600} = 1.5. Immediately after transplanting, seedlings were inoculated by injecting 10 mL of homogenized liquid inoculum from one of the four strains described above into the sterilized potting mix. Autoclaved fungal-free liquid MMN was used to treat non-mycorrhizal control seedlings. After one month, a second inoculation was conducted by applying 10 mL of the respective inoculum to the potting mix (Fig. 3.2 e). Eighteen plants were maintained for each of the five inoculation treatments for another two months before terminal buds set (Fig. 3.2 f, g). Spatial separation of plants minimized the possibility for cross-contamination. Root trainer positions were re-randomized every three days to minimize the impact of any growth chamber heterogeneity.

Two months after the second inoculation, seedlings were ready for mycorrhizal examination and physiological measurements (Fig. 3.2 g, h).

3.2.8.2 Inoculation rate and ectomycorrhizal root tip anatomy

Two months after the second inoculation, mycorrhizal colonization was examined by directly estimating the number of mycorrhizal root tips (Brundrett *et al.* 1996; Peterson *et al*. 2004) and observing extraradical hyphae around the root tips. This was followed by microscopic examination of 30 root tips randomly sampled from five seedlings (six root tips from each sampled seedling) for each type of mycorrhizal treatment (n = 5). The root segments for microscopy were fixed in formalin-acetic acid-alcohol (FAA). Fixed root tips were embedded in paraffin and sectioned using microtome (model RM2125 RTS, Leica; Solms, Germany). Thin sections were stained with toluidine blue. The sectioned samples were observed under ZEISS AXIO compound light microscope (Carl Zeiss; Jena, Germany) with the MacroFire Digital Camera (Optronics; Goleta, CA, USA). Inoculation rate for each examined seedling was calculated as the percentage of the root tips with distinct ECM structure out of the total sectioned root tips.

3.2.8.3 Gas exchange, shoot water potential and dry mass

Net photosynthetic (P_n) and transpiration (E) rates of lateral branches of three-month-old seedlings were measured between 09:00 and 12:00 using a Li-6400 with a 2x3 cm^2 red-blue light chamber (LI-COR, Lincoln, NB, USA). Subsequent to these measurements, needles were collected, scanned and total surface area of needles calculated using ImageJ (V.1.44o; Schneider *et al.* 2012). P_n and E were expressed as a function of needle surface area. Measurements were carried out for six plants from each inoculation treatment (n = 6).

Terminal shoots of 10-15 cm in length were excised at noon and immediately placed into a Scholander pressure chamber (PMS instruments, Corvallis, OR, USA) for mid-day shoot water potential (Ψ_{shoot}) measurements (Scholander *et al*. 1965; Wan *et al.* 1999). The cutting section of the stem was observed under an illuminated magnifier when pressure was increased steadily and slowly into the sample chamber. The reading of the supplied pressure (in Bar) was recorded at the first sight of moisture emerging from the cutting section, and converted to water potential (Ψ) in MPa (n = 6). Dry mass was determined after oven drying at 80°C for 48 h (n = 6).

3.2.9 Transcript profiling of Laccaria bicolor *MIPs in mycorrhizal root tips*

Three months after the first inoculation, root tip segments of about 1 cm in length were collected, stored and ground in liquid N_2 with mortar and pestle, prior to total RNA extraction using the RNeasy Plant Mini extraction method (Qiagen, Valencia, CA, USA), with the addition of 20 mg of polyethylene glycol 8000/mL RLT buffer to facilitate extraction of good quality RNA from the samples. First strand cDNA was synthesized from 1 µg total RNA as described in §3.2.4, and cDNA of 10 ng/µl was used as template for SYBR™ Green qRT-PCR as described in §3.2.4. Primers were listed in Table 3.2. Thermal cycling conditions were 95°C for 2 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, carried out in QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Life Technologies).

Transcript abundance corresponding to the six *L. bicolor* MIPs was quantified in roots mycorrhizal with WT, mock and two OE strains of *L. bicolor* using the standard curve method of absolute quantification (Pfaffl 2004; El Kayal *et al*. 2011) (Appendix 3). *EF2* was used as the reference gene, as it exhibited stable transcript abundance across all tested samples at 20°C and 5°C ($P = 0.81$).

3.2.10 Root hydraulic conductivity and cortical cell hydraulic conductivity

3.2.10.1 Root hydraulic conductivity

A high pressure flow meter (HPFM; Tyree *et al*. 1995) was used to determine whole root hydraulic conductivity (L_{pr}) of three-month-old seedlings immediately after gas exchange measurements. The root with potting mix was removed from the root trainer and kept in a plastic bag submerged in a circulating water bath set to 20°C (Thermo Scientific, Hampton, NH, USA) for 30 min prior to the first measurement of root hydraulic conductance (K_r). Increasing pressure was applied to the root to obtain a smooth linear regression between supplied pressure and flow rate. Slope was calculated as K_r value. The temperature of the circulating water bath was then lowered to 10°C for 30 min before the second measurement and to 5°C for 30 min before the final measurement. To determine root volumes, the potting mix was gently washed off from the roots immediately after K_r measurements. Root volumes were determined using the water displacement method. To calculate L_{pr}, K_r was divided by the root volume (Equation 3.2) (Kamaluddin & Zwiazek 2002).

Equation 3.2 Calculation of L_{pr}

$L_{pr} = K_r$ / root volume

3.2.10.2 Root cortical cell hydraulic conductivity

A cell-pressure probe was used to determine hydraulic conductivity of individual cortical cells (L_{pc}) in the roots of three-month-old mycorrhizal and non-mycorrhizal *P. glauca* (Lee *et al.* 2010)[4]. Roots were collected from six plants per inoculation treatment and kept in a circulating water bath at either 20°C or 10°C for 30 min before measurement (n = 6). A single cortical cell was punctured at a distance of about 20 mm from the root tip with a silicon oil-filled micro-capillary (5 to 6 μm tip diameter). The position of cortical cell was estimated from the depth of the insertion of the micro-capillary tip inside the root. The measurements of half-time of water exchange ($T_{1/2}$) were carried out for up to 20 min as previously described (Lee *et al*. 2005). The hydraulic conductivity of root cortical cells (L_{pc}) was calculated using Equation 3.3 (Steudle 1993).

Equation 3.3 Calculation of L_{pc}

$L_{pc} = V \times \ln(2) / \{A \times T_{1/2} \times (\varepsilon + \pi^i)\}$

Where ε is cell elastic modulus, V is the cell volume, A is the cell surface area, and π^i is osmotic cell pressure.

Cell dimensions for V and A were examined microscopically in the root cortical cells and π^i was estimated from the steady-state cell turgor. Cell elastic modulus (ε) was calculated from changes in cell volumes (ΔV) produced by the cell pressure probe and corresponding changes in cell turgor (ΔP) (Equation 3.4) (Steudle 1993).

Equation 3.4 Calculation of ε

$\varepsilon = V \times \Delta P / \Delta V$

3.2.11 In silico analysis and transcript assay of putative PIPs in Picea glauca

To identify putative *P. glauca* PIPs, phylogenetic analysis and protein secondary structure prediction were conducted. Twenty-six full-length or near full-length *P. glauca* cDNAs corresponding to putative MIP genes were identified from the large-

[4] Root cortical cell hydraulic conductivity data were collected by Dr. Seonghee Lee, Department of Renewable Resources, University of Alberta.

scale white spruce expressed gene resource (Rigault *et al*. 2011) using BLASTx with well-characterized plant MIPs in *Arabidopsis thaliana* (Johanson *et al*. 2001) and *Populus trichocarpa* (Almeida-Rodriguez *et al*. 2010; Lopez *et al*. 2012) as queries (Appendix 1). A neighbor-joining tree (Felsenstein 1985; Saitou & Nei 1987) was generated with MEGA 5.2.1 (Tamura *et al*. 2011) using deduced amino acid sequences. A bootstrap consensus tree of 1000 iterations was obtained. Protein secondary structure of these putative MIPs was predicted on TMHMM2.0 server to confirm transmembrane structure and aquaporin signature motifs.

Nine full-length putative PIPs, most of which were represented in sequenced cDNA libraries of *P. glauca* root tissue (Rigault *et al*. 2011) (Appendix 5), were selected for transcript profiling by qRT-PCR of *P. glauca* roots sampled at 20°C and one hour after the treatment of placing root trainers in the circulating water bath at 5°C. RNA was extracted as described in §3.2.1. The qRT-PCR conditions were as described in §3.2.4. *PgCDC2* (*cell division cycle 2*, GQ0197_L17.1, BT106071) was used as the reference gene (Bedon *et al.* 2009), as its C_t value did not change significantly across all tested samples of mycorrhizal and non-mycorrhizal root tips at 20°C and 5°C ($P = 0.84$). Primers for target PIP genes were designed using Primer Express 3 (Applied Biosystems, Life Technologies) (Table 3.5).

Relative transcript abundance of these PIPs was calculated using the standard curve method of comparative quantification ($\Delta\Delta C_t$ with efficiency correction, Appendix 3) (Livak & Schmittgen 2001; Pfaffl 2004). To evaluate amplification efficiencies for each primer pair, cDNAs of all samples were pooled to generate a 10x dilution series used as the template for each pair of primers. The slope of standard curves for the target and reference genes ranged between -3.01and -3.38, corresponding to the range of the efficiencies between 114.9% and 97.8% used in the efficiency correction of $\Delta\Delta C_t$ values. To assess the impact of mycorrhization on transcript abundance of these *P. glauca* PIPs, the cDNA samples of non-mycorrhizal roots harvested at 20°C were used as calibrator for ratio calculation. To assess the impact of 5°C temperature on transcript abundance of the PIPs, the corresponding samples at 20°C was used as calibrator.

3.2.12 Statistical analysis

Descriptive statistics and ANOVA were conducted using Origin 8.0 (OriginLab, Northampton, MA). Tukey test was used to compare means for statistically significant differences ($P \leq 0.05$).

3.3 Results

3.3.1 Secondary structure prediction and clustering of six MIPs of Laccaria bicolor UAMH8232 in phylogenetic analysis of fungal MIPs

Taking advantage of the genome sequence of the *L. bicolor* model strain S238N, six MIP genes from *L. bicolor* UAMH8232 were obtained. The deduced amino acid sequences of the successfully cloned six putative MIP genes were included in phylogenetic analysis of fungal MIPs. It demonstrated that JQ585592 grouped with orthodox fungal water channels of Cluster I, JQ585593 grouped with fungal aquaglyceroporins of Cluster II, and JQ585594, JQ585595, JQ585596, and JQ585597 all grouped with facultative fungal aquaporins of Cluster III.

3.3.1.1 Transmembrane domains, signature motifs and subcellular localization

The length of the six putative MIPs ranges from 254 to 332 amino acids (Table 3.4). *In silico* analysis using TMHMM2.0 showed that these six MIPs exhibited canonical aquaporin secondary structure: six transmembrane domains (TMD) and five loops (A-E), with each of the two NPA signature motifs or their variations locating at Loop B and E respectively (Fig. 3.3), possible aromatic/Arg sites at TMD2, TMD5 and Loop E to form the selective filter (Table 3.4), and two termini in cytosol. Loops B and E form a seventh half-transmembrane helix and two NPA motifs form a pore for selective transport. The signature motifs NPAs are well conserved in JQ585594-97 (Fig. 3.2 c-f), clustering into Cluster III, while in JQ585592 (Fig. 3.3 a) and JQ585593 (Fig. 3.3 b), NPA motif diverges into NPN or NPC in loop B and NSA in loop E (Table 3.4).

Target P predicted a plasma membrane subcellular localization for JQ585593, and 95-97, whereas a subcellular localization in secretory pathway for JQ585592 and 94 (Table 3.4).

3.3.1.2 Sequence variation between the strains S238N and UAMH2832

The alignment analysis reveals that although none of the MIP gene sequences in UAMH8232 are 100% identical with their counterparts in the strain S238N (Table 3.4), extremely high phylogenetic proximity exists (Appendix 4 Fig. A4.1). Single nucleotide polymorphism causes seven nucleotide differences between *JQ585592* and *Lacbi2:456764* (Fig. A4.2), and 28 nucleotide differences between *JQ585593* and *Lacbi2:671860* (Fig. A4.4). Differences between *JQ585594* and *Lacbi2:568479* were caused by both single nucleotide polymorphism and frame shifting due to alternative splicing (Fig. A4.6). The deduced amino acid sequence of *JQ585595* was 94% identical to that of *Lacbi2:443240*, exhibiting 16 amino acid substitutions over the 312 amino acids of the predicted protein (Fig. A4.7). The deduced amino acid sequences of *JQ585596* and *97* were 90% and 95% identical to *Lacbi2:317173*, exhibiting 20 and five amino acid substitutions over the 332 amino acids, respectively.

3.3.2 Water transport capacity of MIPs of Laccaria bicolor *and transcript abundance of water-transporting aquaporins*

3.3.2.1 Water transport capacity of MIPs of *Laccaria bicolor* by oocyte assay

Four of the six MIPs, JQ585594-97, of *L. bicolor* UAMH8232 showed significant water transport capacity in the oocyte swelling assay (Fig. 3.4). The water permeability coefficient (P_f) of oocytes microinjected with cRNAs corresponding to *JQ585595* was significantly higher than that of oocytes injected with other *L. bicolor* MIPs (Fig. 3.4).

3.3.2.2 Transcript abundance of water-transporting aquaporins in vegetative mycelia grown on pure culture and in mycorrhizal mycelia

Transcript abundance profiling by qRT-PCR was carried out for the three facultative fungal aquaporins that showed the greatest water transport capacity (*JQ585595*, *JQ585596* and *JQ585597*) as well as *JQ585592* that belonged to the cluster of orthodox fungal water channel (Fig. 3.5). The transcript abundance of *JQ585595* was significantly higher than the other three MIPs in both MMN-grown mycelium and mycorrhizal tissue (Fig. 3.5). All the analyzed MIP genes were significantly upregulated in mycorrhizal tissue, compared with mycelium grown on MMN medium, with the most pronounced increase in *JQ585595* (Fig. 3.5). Accordingly, *JQ585595*

was considered to be of the highest physiological relevance and therefore selected for generating transgenic *L. bicolor*, in order to investigate the role of mycorrhizal aquaporins in ECM plant water relations.

3.3.3 Selection of transgenic strains for ectomycorrhiza-water relation study

Abnormal phenotypes may indicate the unfavorable insertion of the transgenic cassette at crucial coding or regulating location in *L. bicolor* genome, therefore only transformed strains that showed normal phenotypes of the species were selected for further validation. The screening outcomes for *JQ585595* OE strains were shown in this section. RNAi strains showed obvious knock-down effect on *JQ585595* transcript abundance, but did not yield significant down-regulation of *JQ585595* expression in mycorrhizal root tips (Appendix 6 Fig. A6.6), therefore could not be considered as a different inoculation treatment from WT or mock strains. So instead of showing the relevant data for RNAi strains in this chapter, they were summarized in Transcript abundance

Seven *L. bicolor* strains transformed with *JQ585595* overexpression construct under control of the constitutive *A. bisporus gpdII* promoter (Fig. 3.1) were tested for transgene expression by qRT-PCR. OE1 and OE2 showed the highest levels of *JQ585595* transcript abundance, with values about 1.5-fold higher compared with WT (Fig. 3.6). Transcript abundance of *JQ585595* in mock strains as well as OE5 and OE7 strains was slightly lower than that of WT (Fig. 3.6). In the three mock strains, the *JQ585595* transcript abundance of Mock 2 was closest to that of WT.

3.3.3.1 Southern blot

Southern blot analysis using a labeled probe targeting the *hph* gene confirmed that the OE1, OE2, and Mock 2 strains each harbored a random single insertion of the transgenic cassette, indicated by a single clear hybridization band (Fig. 3.7 a, b).

3.3.3.2 TAIL-PCR to identify T-DNA insertion site in the genome of transgenic *Laccaria bicolor*

The genomic DNAs of the three transgenic strains, OE1, OE2 and Mock 2 (referred as the mock strain below), were used as the template for the primary reaction of TAIL-PCR. The gel electrophoresis for TAIL-PCR products showed that by using the

corresponding tertiary PCR products as the template (Fig. 3.8 a), the quaternary PCR reaction yielded specific amplification result for these three strains (Fig. 3.8 b). This showed that the T-DNA was integrated into the genome as a single insertion, consistent with the single hybridization signal in the Southern blot analysis (Fig. 3.7).

Sequencing and alignment analysis showed that the flanking sequence in the mock strain was identical with the sequence at the loci 1775745-1776200 on Scaffold LG 9 of *L. bicolor* genome ($E = 0.0$), indicating that T-DNA was inserted between the loci 1775744 and 1775745 on Scaffold LG 9. The flanking sequence in the strain OE1 was highly similar with the sequence at the loci 935396-935508 on Scaffold LG 1 ($E = 8.68\text{x}10^{-48}$), indicating that T-DNA was inserted between the loci 935395 and 935396 on Scaffold LG 1. The flanking sequence in the strain OE2 was highly similar with the sequence at the loci 6869024-6869281 on Scaffold LG 3 ($E = 2.86\text{x}10^{-30}$), indicating that T-DNA was inserted between the loci 6869023 and 6869024 on Scaffold LG 3.

This demonstrated that in OE1, OE2 and mock strains, the transgene cassette was inserted into different scaffold locations of the genome. No ORF (open reading frame) was predicted in these single insertion sites in JGI *L. bicolor* V2.0 database of Lacbi2 Assembly Scaffolds Repeat Masked, indicating that the insertion of T-DNA in the genome of the mock, OE1 and OE2 strains did not disrupt any coding sequence of any putative gene. Based on these analyses, OE1, OE2 and Mock 2 were chosen as the OE strains and mock control, respectively.

3.3.4 The effect of low temperature on growth and mycelial water potential of Laccaria bicolor strains grown on MMN medium

No obvious difference in phenotypes was observed between WT, mock and the two OE strains at either 20°C or 5°C (Fig. 3.9). Hyphae expanded radically along the surface of cellophane disc, and grew into a round mycelial mat of creamy white to light purple color. For all the strains, mycelia radically expanded to cover the entire 9 cm Petri dishes in four weeks at 20°C (Fig. 3.9 a), whereas the mycelia grew thicker but stopped expanding at diameter of about 4-5 cm (Fig. 3.9 b). The dry mass of all the strains was significantly higher at 20°C than at 5°C, but the difference between the strains was not significant at either 20°C or 5°C (Fig. 3.10).

WT and mock strains had similar Ψ_{turgor} at 20°C. The Ψ_{turgor} of OE1 and OE2 was similar and significantly higher than that of WT and mock strains (Fig. 3.11). At 5°C, Ψ_{turgor} did not change significantly in WT, but increased in mock and OE strains. The increase in OE strains was more pronounced than in mock strain, indicating the stronger water influx related with overexpression of *JQ585595*. Although MMN medium and potting mix are totally different growth environments for the hyphae, these data may be used as an indication of turgidity of hyphal cells in ECM grown in potting mix, which may influence the property of apoplastic pathway in the roots.

3.3.5 Mycorrhizal structures and growth of Picea glauca *inoculated with different Laccaria bicolor strains*

3.3.5.1 Inoculation rate and mycorrhizal structures

All seedlings treated with *L. bicolor* were successfully inoculated. Massive mycelia grown in rhizosphere were observed two months after the second inoculation (Fig. 3.12 a). Evident and similar mantle and Hartig net structures were found in about 90% of the 30 sampled root tips from inoculated plants (Fig. 3.12 b-e). There were no significant differences between *L. bicolor* strains in terms of colonization rates: 93.3% ± 4.1% (± SE), 86.7% ± 3.3%, 90% ± 4.1% and 93.3% ± 4.1% for WT (Fig. 3.12 b), mock (Fig. 3.12 c), OE1 (Fig. 3.12 d) and OE2 (Fig. 3.12 e), respectively. There was neither extraradical mycelium in soil nor distinct ECM structures in thin sections of the root tips observed in non-inoculated plants (Fig. 3.12 f).

3.3.5.2 Gas exchange, shoot water potential, dry mass

Seedlings inoculated with OE1 strain had lower dry mass compared with the non-inoculated and mock-inoculated plants, and both OE1 and OE2 plants had also lower dry mass compared with the mock-inoculated plants (Fig. 3.13 a). Mycorrhizal plants had higher shoot water potential Ψ_{midday} (Fig. 3.13 b), net photosynthesis (Fig. 3.13 c) and transpiration rates (Fig. 3.13 d) than non-inoculated plants. However, there was no significant difference in these parameters between the different inoculation treatments (Fig. 3.13).

3.3.6 Transcript abundance of **Laccaria bicolor** *MIPs in mycorrhizal root tips of Picea glauca*

Of the fungal MIPs, *JQ585595* exhibited the highest transcript abundance in mycorrhizal root tips, followed by *JQ585593* and *JQ585594* (Fig. 3.14). The transcript abundance levels of *JQ585592*, *JQ585596* and *JQ585597* were 5- to 10-fold lower than those of *JQ585595*.

The transcript abundance of *JQ585592* in mycorrhizal root tips was low and not significantly different between the different strains at each examined temperature, and increased in all strains with the decrease in temperature from 20ºC to 5ºC (Fig. 3.14 a). The transcript abundance of *JQ585593* was also low at 20ºC and not significantly different between mycorrhizal treatments; however, in all strains, it increased by more than 10-fold with the decrease in temperature to 5ºC (Fig. 3.14 b). Transcript levels of J*Q585594* at 20ºC were similar in all strains with the exception of a small, but statistically significantly higher level in OE1 compared with the mock strain (Fig. 3.14 c). In all strains, *JQ585594* levels were higher at 5ºC compared with 20ºC (Fig. 3.14 c). Transcript levels of *JQ585595* were significantly higher in OE strains than in WT and mock strains at 20°C (Fig. 3.14 d). In all strains, a decrease in temperature from 20ºC to 5ºC induced a significant increase in *JQ585595* transcript abundance (Fig. 3.14 d). All strains maintained low transcript abundance levels of *JQ595596* and *JQ585597* at 20ºC and 5ºC (Fig. 3.14 e, f). Temperature decrease from 20°C to 5ºC had little effect on transcript abundance of these MIPs with the exception of small, but statistically significant decreases observed in OE2 (Fig. 3.14 e, f).

3.3.7 Root hydraulic properties of **Picea glauca** *inoculated with Laccaria bicolor strains of different JQ585595 transcript abundance*

At 20ºC, L_{pr} in mycorrhizal seedlings of the WT and mock strains was about 2-fold higher than L_{pr} in the non-inoculated control seedlings (Fig. 3.15). In the seedlings inoculated with either of the OE strains, L_{pr} was more than 50% higher than in the seedlings inoculated with WT or mock strains, and about 3-fold higher compared with non-inoculated control (Fig. 3.15).

When root temperature was decreased from 20ºC first to 10°C and then 5°C, only small decreases in L_{pr} were measured in non-inoculated plants and in the mock-mycorrhizal plants (Fig. 3.15). There was no effect of the decreased temperatures on L_{pr} in WT plants (Fig. 3.15). However, in both OE lines, the decline in temperature from 20°C to 10°C and 5ºC resulted in a greater than 2-fold decrease in L_{pr} (Fig. 3.15).

At 20ºC, L_{pc} was about 2-fold higher in WT-mycorrhized plants compared with non-inoculated control and more than 3-fold higher in both OE lines (Fig. 3.16). There was no significant effect on L_{pc} in non-inoculated and WT plants when the temperature was decreased to 10ºC (Fig. 3.16). However, in both OE lines the decrease in temperature from 20ºC to 10ºC lowered L_{pc} by more than 3-fold which brought the L_{pc} levels to approximately those that were measured in non-inoculated plants (Fig. 3.16).

3.3.8 The effect of inoculation with Laccaria bicolor *strains of different JQ585595 transcript abundance on the transcript abundance of root PIPs of* Picea glauca

3.3.8.1 Characteristics of putative MIPs of *Picea glauca*

Thirteen full-length or near full-length PIPs, five TIPs, five NIPs, one SIP and no XIP of *P. glauca* were identified in MIP phylogenetic analysis (Fig. 3.17). Two *P. glauca* MIPs did not cluster clearly with any of these groups, although they were well incorporated into the dendrogram, and thus cannot be assigned to a MIP subfamily based on the outcome of the neighbor-joining tree. Four of them, GQ03610_A06.1, GQ03401_M18.1, GQ02828_J14.1, and GQ02902_L14.2 clustered with previously characterized poplar and *Arabidopsis* PIP1s. Eight PIPs, including GQ03010_E09.1, GQ03011 G23.1, GQ03002_G07.1, GQ03001_P18.1, GQ03818 D05.2, GQ03111_E12.1, GQ02901_B20.1, and GQ02905_E13.1, clustered with PIP2s. GQ03703_H07.1 did not group conclusively with the PIP2 cluster, but appeared to be more closely related to PIP2s than PIP1s.

Most of the nine PIPs selected for transcript profiling were represented in sequenced cDNA libraries of *P. glauca* root tissue, taken as evidence of being expressed in roots. *In silico* analysis showed that the deduced amino acid sequences of the nine putative PIPs exhibited the canonical aquaporin transmembrane structure of six TMDs and two NPA signature motifs (Appendix 5). The most highly expressed PIP*s* among these nine

genes in the non-inoculated roots at 20°C were *GQ03401_M18.1*, *GQ03703_H07.1* and *GQ02905_E13.1*, followed by *GQ03610_A06.1*, *GQ03010_E09.1*, *GQ03001_P18.1* and *GQ02901_B20.1*; transcript levels of *GQ03002_G07.1* and *GQ03111_E12.1* were low (Fig. 3.18).

3.3.8.2 Transcript profiling of *Picea glauca* PIPs in inoculated roots

Transcript profiling of the nine *P. glauca* PIPs showed varying responses to mycorrhization with WT, mock, OE1 and OE2 strains. Mycorrhization with WT and mock strains resulted in an approximately 3- to 4- fold increase in *GQ03401_M18.1* transcript abundance, while in both OE lines, *GQ03401_M18.1* transcript abundance increased by 40- to 56- fold (Fig. 3.19). Both OE lines also showed a strong increase in *GQ03703_H07.1* transcript abundance, while the opposite was observed for transcript abundance of *GQ03610_A06.1* (Fig. 3.19). Transcript abundance of *GQ03001_P18.1* was decreased by mycorrhization (Fig. 3.19).

Transcript abundance of most of the *P. glauca* PIPs in roots was significantly downregulated at 5ºC compared with 20ºC in all inoculation treatments (Fig. 3.20). With the exception of a higher *GQ03610_A06.1* transcript abundance 5ºC to 20ºC ratio in both OE lines compared with the other inoculation treatments, there was no clear pattern showing consistent differences in the temperature responses between the inoculation treatments (Fig. 3.20).

3.4 Discussion

3.4.1 MIPs of Laccaria bicolor *UAMH8232*

Taking advantage of the genome sequence of the *L. bicolor* model strain S238N (Martin *et al*. 2008), six aquaporin genes were obtained from *L. bicolor* UAMH8232. Orthodox fungal aquaporins JQ585592 in *L. bicolor* strain UAMH8232 and Lacbi1:392091 in strain S238N both consist of six transmembrane helix domains and five loops and share 99.04% amino acid sequence identity. However unlike Lacbi1:392091-a moderate water transporter (Dietz *et al.* 2011), JQ585592 was found not to be significantly permeable to water (Fig. 3.4). Its aquaporin signature NPA motifs altered into NPN (Alanine-Proline-Alanine) and NSA (Alanine-Serine-Asparagine) in Loop B and E, respectively, which probably led to change in the

conformation of the selective filter and decrease in water permeability of the pore. In addition, its most possible subcellular localization was predicted in secretory pathways with a weak prediction index (Table 3.4), indicating high mobility of trafficking between different subcellular locations. This could also the reason for the low P_f value of JQ585592 in oocyte assay, since the osmotic swelling assay was primarily designed to test the water permeability of microinjected aquaporins that are expressed and localized on oocyte plasma membrane.

In S238N, *Lacbi1:392091* was not highly expressed in mycelium growth in liquld culture at 15.5°C, but upregulated upon *in vitro* mycorrhization with *Populus tremula* x *tremuloides* grown on MS medium (Dietz *et al.* 2011). Similarly, the transcript abundance of *JQ585592* was low in mycelium of UAMH8232 grown on solid culture at 20°C (Fig. 3.5), and its upregulation in mycorrhiza was significant (Fig. 3.5).

JQ585593 in UAMH8232 (Fig. 3.3) and Lacbi1:307192 in S238N (Dietz *et al.* 2011) in Cluster II shared 98% sequence identity and the signature motifs of NPC/NSA. Neither of them demonstrates significant water transport capacity, which again suggests the conservation of NPA motifs may be an important prerequisite for a MIP to maintain its water permeability. JQ585594 and Lacbi1:247946 (Lacbi2:568479) shared 93% sequence identity. Both of them had significant but the lowest water permeability in all the *L. bicolor* MIPs of Cluster III (Dietz *et al.* 2011; Fig. 3.4). The transcript abundance of *Lacbi1:247946* was among the lowest in mycorrhizal tissue (Dietz *et al.* 2011).

Of these six MIPs, JQ585595 demonstrated the highest water transport capacity in the heterologous *X. laevis* oocyte expression system, and the highest transcript abundance in mycelium grown on MMN medium. The 16 amino acid difference between JQ585595 and Lacbi1:391485 (Lacbi2:443240) (Appendix 4 Note A4.7) led to the 6th TMD conformation simulated in JQ585595 but not in Lacbi1:391485 (Fig. 3.3 d). This change in conformation might confer JQ585595 with enhanced water transport capacity (Fig. 3.4). As the highest expressed MIP in mycelium grown on MMN medium, *JQ585595* transcript abundance further increased significantly in mycorrhiza and was significantly higher than that of other MIPs (Fig. 3.5), indicating the significance of JQ585595 to the fungus under two distinct environments. The corresponding MIP gene *Lacbi1:391485* in strain S238N was also upregulated upon

mycorrhization (Dietz *et al*. 2011), despite many differences in the experimental conditions of the two scenarios.

JQ585596 and JQ585597 in strain UAMH8232 are closely related to Lacbi2:317173 – the MIP that showed the highest P_f in strain S238N. It is possible that Lacbi2:317173 in the monokaryotic strain diverged into two isoforms in the dikaryotic strain. This change in amino acid sequence might cause decrease in water permeability of JQ585596 and JQ585597, conferring JQ585595 to be the strongest water transporter in UAMH8232, shown in the heterologous *X. laevis* oocyte expression system (Fig. 3.4). In another study carried out using the same *X. laevis* oocyte assay in the same time frame, JQ585595 demonstrated 40% higher water-transporting capacity compared to the maize ZmPIP2;8 aquaporin and about 160% higher water-transporting capacity than ZmTIP2;2 aquaporin (Lawrence *et al*. 2013). A commonly used positive control, such as AQP1 of *Homo sapiens* or γ-TIP of *Arabidopsis thaliana*, should be included in future oocyte assays to allow comparisons between the data sets of different publications.

In S238N, *Lacbi2:317173* was the highest expressed MIP in liquld culture at 15.5°C, followed by *Lacbi1:391485*. Interestingly, in UAMH8232, although *JQ585595* transcript abundance was significantly higher than either *JQ585596* or *97*, the sum of transcript abundance of *JQ585596* and *97* was similar with that of *JQ585595* in mycelium on pure culture at 20°C, indicating that these five MIPs may have overlapping functions in *L. bicolor*.

It remains to be determined whether these sequences represent distinct loci in monokaryotic and dikaryotic strains. The allelic variation exhibited by *L. bicolor* UAMH8232 compared to the reference sequence of *L. bicolor* S238N is not unexpected given their distinct geographic origins: UAMH8232 was isolated in Ontario (Calvo-Polanco *et al*. 2008), while S238N originated in Oregon (Bastide *et al.* 1994; Di Battista *et al*. 1996). Interstrain differences were observed not only in the number of MIPs and the deduced amino acid sequences of MIPs, but also in MIP gene transcript profiling, for example, as responses to temperature. In strain S238N, the transcript abundance of *Lacbi1:392091* and *Lacbi1:307192* decreased in mycelium grown in liquid medium with temperature decline to 5°C (Dietz *et al.* 2011); in contrary, the corresponding MIPs *JQ585592* and *JQ585593* in strain UAMH8232 were

both upregulated in mycorrhiza when root temperature dropped from 20°C to 5°C (Fig. 3.14). Besides the possible effects of many differences in cultivation conditions and associated plants between Dietz's work (Dietz *et al*. 2011) and this study, observed differences in transcript abundance for sequences from each strain putatively corresponding to the same loci also suggest some degree of adaptive variation, possibly reflecting local adaptation to distinct environmental pressures such as drought and cold, or interaction with different host plants. Ecological implications of potential adaptive variation associated with fungal MIPs may be revealed by further functional and population genetics analyses of MIPs and their functions of different *L. bicolor* strains.

3.4.2 Mycorrhizal effect on seedling growth

Mycorrhizal effect on plant growth varies a lot with the interacting species, the developmental stages of the species and interaction, as well as environmental stresses imposed on the associated species. For instance, it was reported that *Hebeloma longicaudum* improved the growth of *Picea mariana* under drought, but had no effect on *P. banksiana* (Boyle & Hellenbrand 1991). In this study, mycorrhization with WT and mock strains did not affect the total dry mass of *P. glauca* seedlings after three months of growth. Mycorrhization with OE1 reduced seedling dry mass compared with non-inoculated plants. Both OE1- and OE2-inoculated seedlings also showed reduced dry mass compared with mock strain-inoculated seedlings (Fig. 3.13 a). The effects of mycorrhization on plant growth vary, depending on mycorrhization stage and various abiotic and biotic environmental factors (Smith & Read 2008). Growth reductions may occur due to increased carbohydrate demand by the mycorrhizal fungus. Carbon use efficiency theory stated that carbon demands of the fungus might counteract increase in dry weight and growth enhancement in source-limited plants (Tinker *et al*. 1994), which indicated that stronger carbon demands from the fungal symbiont might counteract more dry mass of the seedlings. Considering that in all inoculation treatments, net photosynthetic rates were higher compared with non-mycorrhizal seedlings, the differences in growth responses are likely related to higher carbohydrate demands of the OE strains, likely caused by their higher growth rates or functional demands. OE strains lowered the total seedling dry mass (Fig. 3.13 a) but not seedling net photosynthesis (Fig. 3.13 c), which indicated that part of the photosynthates were consumed or stored elsewhere. It showed that the *JQ585595* overexpression led to significant higher tissue turgor potential of mycelium grown on MMN medium (Fig.

3.11), however did not cause significant change in mycelial dry mass (Fig. 3.10), probably because the glucose supply from the growth medium was depleted. Given sufficient carbohydrate supply, the higher tissue potential would trigger faster growth of fungal hyphae (Lew 2011). So it could be assumed that faster growth of OE strains was initiated by increased tissue turgor potential due to *JQ585595* overexpression, and consequently these strains functioned as a stronger carbohydrate sink which led to reduced seedling dry mass. This suggests that although the positive impacts of mycorrhization on plant-water relations could be enhanced under certain abiotic stresses such as drought, salinity and low soil temperature (Landhäusser *et al*. 2002; Calvo-Polanco *et al*. 2008; Lee *et al*. 2010; Bárzana *et al*. 2012), mycorrhizal association is not always mutual beneficial; given that the resource becomes depleted or abiotic stresses are imposed, the balance of symbiosis may lean to favor one particular side of the symbionts. This complicates the potential application of transgenic mycorrhizal fungi in forestry and land reclamation, and requires more specific studies prior to application in field.

Increased net photosynthetic rates of mycorrhizal plants were paralleled by increased transpiration rates (Fig. 3.13 c, d), suggesting that stomatal factors were likely largely responsible for differences in photosynthetic rates. Similar increases in transpiration and photosynthetic rates of mycorrhizal plants were previously reported for ECM and AM associations (Allen *et al*. 1981; Dosskey *et al*. 1990; Caravaca *et al*. 2003; Birhane *et al*. 2012). In this study, shoot water potentials were higher in mycorrhizal plants despite higher transpiration rates, likely due to higher L_{pr}. Increased shoot water potential and higher rates of gas exchange due to AM mycorrhization were also observed in squash (*Cucurbita* L.), soybean (*Glycine max*) and maize (Subramanian *et al*. 1997; Porcel & Ruiz-Lozano 2004; Augé *et al*. 2008). Leaf water potential was stable despite increased transcription in *Citrus jambhiri* (Levy & Krikun 1980). This indicated that water supply to photosynthetic tissues of mycorrhizal plants enabled sufficient stomatal opening for gas exchange to meet carbon needs of both symbionts. In future studies, needle water content can be determined by measuring fresh and dry mass ratio or specific leaf area, to provide additional parameters to reflect the mycorrhizal impact on plant water status.

3.4.3 Mycorrhizal effect on root hydraulics and PIP transcript abundance

Increase in L_{pr} or root hydraulic conductance attributed to mycorrhization with different ECM fungi has been reported in many tree species under various abiotic conditions, such as *U. americana* (Calvo-Polanco *et al*. 2008, 2009), *P. glauca* (Landhäusser *et al*. 2002; Muhsin & Zwiazek 2002b) and *Populus balsamifera* (Siemens & Zwiazek 2008). In this study, the increase in L_{pr} of *P. glauca* was significant upon mycorrhization with all the *L. bicolor* strains (Fig. 3.15).

In the composite model of root water transport, L_{pr} is a function of apoplastic and cell-to-cell (transmembrane and symplastic) pathways (Steudle & Peterson 1998). Water flow follows the least resistance pathway and this resistance is largely controlled by the transmembrane pathway through the transcriptional and post-translational regulation of PIPs (Törnroth-Horsefield *et al*. 2006; Maurel *et al*. 2008). These changes can be determined by the direct measurements of L_{pc} in root cortical cells (Steudle 1993; Javot & Maurel 2002). In this study, mycorrhization increased L_{pr} and L_{pc} by a similar magnitude in *P. glauca* seedlings, suggesting that the decreased resistance of the transmembrane pathway was likely responsible for the increased root water transport capacity. Similar enhancements of L_{pr} and L_{pc} were previously reported for mycorrhizal plants and may involve both apoplastic and cell-to-cell pathways (Muhsin & Zwiazek 2002a, 2002b; Marjanović & Nehls 2008; Lee *et al.* 2010; Bárzana *et al.* 2012). For instance, it was reported that ECM fungus *Suillus tomentosus* increased L_{pr} and L_{pc} of *P. banksiana*, and enhanced the plant salt resistance by alleviating the impact of NaCl and fluoride on L_{pc} (Lee *et al*. 2010).

GQ03401_M18.1 was annotated as *PIP1;1* in a recent study of the *P. glauca* MIP gene family (Laur & Hacke 2014). The increase in its transcript abundance upon mycorrhization, particularly with OE strains, was accompanied by increased L_{pc} and L_{pr} of mycorrhizal *P. glauca*. *PttPIP1;1* and *PttPIP2;*5 transcript abundance were proposed to be the principal factors responsible for the increase in L_{pr} of ectomycorrhizal *Populus tremula* x *tremuloides* (Marjanović *et al*. 2005). However, the signaling pathways leading to this response are not known. Regulation of aquaporin-mediated water transport involves changes in the abundance of aquaporins in cell membranes and aquaporin gating which is affected by various factors including protein phosphorylation and dephosphorylation (Johansson *et al*. 1998; Kline *et al*. 2010),

protonation (Tournaire-Roux *et al*. 2003; Fischer & Kaldenhoff 2008), divalent cations (Gerbeau *et al*. 2002; Verdoucq *et al*. 2008), trafficking (Prak *et al*. 2008; Maurel *et al*. 2009; Zelazny *et al*. 2009), heteromerization (Fetter *et al*. 2004), as well as turgor pressure, solute gradients and temperature (Chaumont *et al*. 2005). Increased water availability in root extracellular space was postulated to be a significant factor triggering PIP transcriptional and post-translational regulation in root cells (Steudle & Peterson 1998; Javot & Maurel 2002). Since the water transporting capacity of mycorrhizal roots increases with the increasing volume of fungal hyphae (Duddridge *et al*. 1980; Plamboeck *et al*. 2007), it is plausible that an increase in root hydration by the fungal hyphae may provide a positive feedback mechanism regulating root aquaporin expression and/or function.

The high and sensitive transcript abundance of *PIP1;2* in *P. glauca* (Fig. 3.19) suggested its important role in symplastic water transport in root cells, probably not only in cortex but also in other cells which are gateways to water transport, such as endodermis. The increased transcript abundance of *PIP1;2* was relevant with the increase in L_{pc}, and might enhance symplastic water transport of other root cells which were not measured in this study. Therefore, the contribution of ECM *L. biolocr* to root water transport of *P. glauca* can come from the following approaches: (1) Hyphae lead to the formation of an apoplastic water transport highway in the root intercellular space, and L_{pr} is increased due to increased water conductivity in the apoplastic pathway; (2) Hyphae release water into apoplastic space of Hartig net so water availability to root cortical cells is increased, leading to up-regulation of certain PIPs and increased L_{pc}, which further contribute to L_{pr}; (3) Enhanced PIP expression in other root cells, such as endodermis, may be a contributor to increased L_{pr}.

3.4.4 The role of Laccaria bicolor *JQ585595 in root water transport of* Picea glauca *seedlings*

3.4.4.1 The effects of JQ585595 overexpression

JQ585595 was selected for construction of OE transgenic strains because of its highest water transport capacity and transcript abundance in MIPs of strain UAMH8232. Similarly to earlier studies (Kemppainen *et al*. 2005; Kemppainen *et al*. 2008; Kemppainen & Pardo 2010), *Agrobacterium*-meditated transformation was effective in

yielding successful *L. bicolor* transgenic strains. In the transgenic cassette, the constitutive *A. bisporus gpd*II promoter was used to drive expression of *JQ585595* (Fig. 3.1). Previous studies have demonstrated the effectiveness of gene expression induced by this promoter in transgenic basidiomycete fungi (Burns *et al*. 2006; Kilaru *et al*. 2006; Ding *et al*. 2011). Compared with WT, the OE strains did not demonstrate a multiple-fold increase in transcript abundance for *JQ585595* (Fig. 3.6), probably because the transcript abundance for the endogenous aquaporin gene was already high in WT grown on MMN medium at 20^{o}C (Fig. 3.5). Two selected OE strains demonstrated about 1.5-fold greater transcript abundance of *JQ585595* compared with WT (Fig. 3.6). Considering the high transcript abundance of *JQ585595* in WT, the 50%-100% observed increase represents a considerable increase in transcript quantity (Fig. 3.6). Importantly, the elevated level of transcript abundance was sustained in the mycorrhizal root tips (Fig. 3.14 d) and sufficient to produce significant functional effects (Fig. 3.15). In contrast, RNAi construction did not sufficiently suppress gene expression in ECM *in vivo* (Appendix 6). Alternative transgenic systems can be tried to achieve more effective overexpressing or silencing effect in basidiomycete dikaryotic species (Kemppainen *et al*. 2005; Burns *et al*. 2006; Kilaru *et al*. 2006; Kemppainen & Pardo 2010; Ding *et al*. 2011). Meanwhile, the potential impacts of transgenic cassette insertion sites in the genome should be carefully evaluated.

In general, the higher transcript abundance of *JQ585595* in OE strains did not cause significant changes in transcript abundance of other *L. bicolor* aquaporins at 20^{o}C compared with the WT and mock strains, with exception of *JQ585594* in OE1 (Fig. 3.14 c), and *JQ585596* and *JQ585597* in OE2 (Fig. 3.14 e, f). Transcript profiles of the six *L. bicolor* MIPs responded differently to mycorrhizal treatments and temperature decline (Fig. 3.14). At 20^{o}C, transcript profiles were not significantly different between WT and mock strains, indicating the mock strain behaved as an appropriate control. In contrast, at 5^{o}C, five of the six *L. bicolor* MIPs showed significantly higher transcript abundance in the mock strain than WT. TAIL-PCR results demonstrated that no known ORF was disrupted by the insertion in the mock; thus, this effect does not appear to be due to unintended interruption of gene function. It remains unclear whether the insertion sites had impact on gene expression at low temperature via possible mechanisms such as chromatin modification, therefore more mock strains with different insertion sites need to be examined in future studies. Interestingly, the

enhanced expression of these *L. bicolor* MIPs - especially *JQ585595* - in the mock strain at 5°C corresponded to a greater decrease in L_{pr} of mock-inoculated roots at 5°C compared to WT-inoculated roots (Fig. 3.15). This partly explained why the L_{pr} profile of mock-inoculated roots was different than that of WT-inoculated roots, but similar to that of the OE-inoculated roots as a function of temperature. The mechanism by which *JQ585595* might contribute to this process remains to be explored.

Similar to the differences in *L. bicolor* MIP transcript abundance observed in WT- and mock-inoculated seedlings, differences in transcript abundance of some *P. glauca* PIPs were observed between WT- and mock-inoculated seedlings. While transcript profiles of *L. bicolor* MIPs were not significantly different between OE1 and OE2 mycorrhizal strains, greater differences were observed between transcript profiles of some *P. glauca* PIPs in OE1 and OE2 strains. One possible explanation for these observations is that there may have been differences in fungal-plant dynamics between plants inoculated with OE1 versus OE2. Future studies should include longer-term low temperature treatments and examine root tissue distribution of PIPs in response to temperature to explain the reasons why the transcript profiles of some root PIP*s* were not always consistent between the plants inoculated with different strains.

Overexpression of *JQ585595* led to significant increase in L_{pr} at 20°C, however, the change in L_{pc} was not statistically significant. On one hand, this suggested that L_{pc} had reached its capacity when WT Hartig net developed in the apoplastic space between cortical cells, therefore would not respond to more hydration brought by *JQ585595*-overexpressing hyphae; on other other hand, this indicated a greater contribution of *JQ585595* overexpression to apoplastic water transport rather than cell-to-cell transport in cortex. An increase in root hydration by the fungal hyphae may also regulate root aquaporin expression in other types of root cells ranther than cortical cells. The expected outcome of *JQ585595* fungal aquaporin expression was an increase in the hyphal water flow likely leading to an increase in hydration at the hyphal-root interphase. Evidence in support of this hypothesis is the increases in PIP *GQ03401_M18.1* root expression in OE-inoculated plants compared with the plants mycorrhized with WT and mock strains. Tissue-specific PIP expression has been reported in roots of grapevine, showing upregulation of certain PIP genes in endodermis (Gambetta *et al*. 2013). The effect of mycorrhiza and *JQ585595*

overexpression on spatially differential expression of PIPs across root section would be worthy of investigation.

3.4.4.2 The effects of low temperature

Interestingly, the stimulating effects of the OE mycorrhizas on L_{pr} and L_{pc} were totally abolished at low temperatures. Although low soil temperature inhibits root water uptake in most plants, including many boreal tree species (Wan *et al*. 1999; Wan *et al*. 2001; Lee *et al*. 2005; Aroca *et al*. 2012; Lee *et al*. 2012), low temperature-tolerant plants, including *P. glauca*, show little responsiveness of root hydraulic properties to low temperature (Landhäusser *et al*. 2002). In this study, when temperature was decreased to 10°C, L_{pr} and L_{pc} were little affected in the non-mycorrhizal and WT-mycorrhizal seedlings. Similar tolerance of L_{pc} to low temperature was reported for chilling-tolerant figleaf gourd, contrary to chilling-sensitive cucumber (Lee *et al*. 2005). The responses of L_{pc} to low temperature have been explained by the aquaporin gene expression and inhibition of aquaporin phosphorylation and/or dephosphorylation (Lee *et al*. 2005; Lee *et al*. 2012). In this study, the L_{pc} in non-mycorrhizal and WT-inoculated plants was not affected despite the reductions in the transcript abundance of the examined PIPs, pointing to possible gating processes in *P. glauca* responsible for low temperature tolerance of transmembrane water transport as in figleaf gourd (Lee *et al*. 2005) and rice (Matsumoto *et al*. 2009). The overexpression of fungal aquaporin *JQ585595* increased the sensitivity of root water transport to low temperature. Since the effect of low root temperature on root hydraulic properties was accompanied by inconsistent differences in root PIP expression compared with the WT and mock lines, it is possible that the overexpression of *JQ585595* could have affected root aquaporin gating processes, as previously reported for chilling-sensitive plants (Aroca *et al*. 2005; Lee *et al*. 2005; Murai-Hatano *et al*. 2008; Lee *et al*. 2012). It is also worth noting that contrary to root *P. glauca* PIPs, most of the *L. bicolor* MIPs exhibited increased transcript abundance when subjected to low temperatures. It can be speculated that the functionality of aquaporin-mediated transport is important to hyphal water transport, and its protection under unfavorable environmental conditions is among the priorities for the fungus.

The increase in *PIP1;2* transcript abundance in WT-mycorrhizal roots was further correlated with the increase in *JQ585595* transcript abundance in WT strain at 5°C.

JQ585595 was up-regulated to the level similar with that of OE lines at 20°C (Fig. 3.14), which might contribute to the increase in water released from hyphal cells through JQ585595 into root intercellular space, offsetting negative impact of chilling and consequently balancing root water transport. High transcript abundance at 5°C suggested the importance of both *Lacbi1:391485* (Dietz *et al.* 2011) and JQ585595 (Fig. 3.14 d) to the corresponding strains at low temperature, despite the difference in growth medium and associated plant species. In addition to relatively high water transport capacity, Dietz *et al.* (2011) also demonstrated that Lacbi1:391485 had capacity to transport glycerol and ammonia. Whether JQ585595 plays roles in transporting similar substrates at mycorrhizal interfaces, is a fascinating topic to be explored. In addition, MIPs *JQ585592*, *93* and *94* were upregulated significantly (Fig. 3.14). Their contribution should be taken into account, although it might not be as distinct as that of *JQ585595* because their absolute transcript abundance was low.

The increase in L_{pr} in OE-mycorrhizal roots at 20°C was reversed at 5°C (Fig. 3.15), accompanied with significant drop in L_{pc} (Fig. 3.16) and down-regulation of most of the PIPs in the profile including *PIP1;2* (Fig. 3.20). This indicated that root hydraulics altered according to the change in surrounding water availability that was influenced by the abundance of JQ585595 in the adjacent hyphae. The transcript level of *JQ585595* further increased at 5°C beyond the level at 20°C (Fig. 3.14 d). This suggested that increased *JQ585595* in OE lines at 5°C might facilitate hyphal cells to recruit water from extracellular space. More water influx and less water efflux led to decreased water availability in the apoplastic space for roots and rendered L_{pr} and L_{pc} to drop back to the level similar with the WT-mycorrhizal roots (Fig. 3.15; Fig. 3.16).

The study has demonstrated the enhancement of L_{pc} and L_{pr} in *P. glauca* roots mycorrhized with *L. bicolor* overexpressing aquaporin *JQ585595*. It can be proposed that the contribution of *L. bicolor* hyphae to root water transport in *P. glauca* involves increased apoplastic water transport in the root intercellular spaces, which may lead to increased hydration at the fungal–root interface and, consequently, impact aquaporin expression and cell-to-cell water transport in mycorrhizal roots. During chilling, PIP post-translational regulation may influence L_{pc} in *P. glauca* roots mycorrhized with *L. bicolor* strains overexpressing *JQ585595*, as increased fungal aquaporin transcription may alter hydration in the root intercellular spaces and, consequently, affect root PIP

regulation and root hydraulic dynamics.

3.5 References

Agerer R. 2001. Exploration types of ectomycorrhizae—a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **11**: 107–114.

Allen MF, Smith WK, Moore TS, Christensen M. 1981. Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* (HBK) Lag. ex Steud. *New Phytologist* **88**: 683-693.

Allen MF. 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal* **6**: 291–297.

Almeida-Rodriguez AM, Cooke JEK, Yeh F, Zwiazek JJ. 2010. Functional characterization of drought-responsive aquaporins in *Populus balsamifera* and *Populus simonii* x *balsamifera* clones with different drought resistance strategies. *Physiologia Plantarum* **140**: 321-333.

Aroca R, Amodeo G, Fernández-Illescas S, Herman EM, Chaumont F, Chrispeels MJ. 2005. The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiology* **137**: 341-353.

Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* **173**: 808-816.

Aroca R, Porcel R, Ruiz-Lozano JM. 2012. Regulation of root water uptake under abiotic stress conditions. *Journal of Experimental Botany* **63**: 43-57.

Augé RM, Toler HD, Sams CE, Nasim G. 2008. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. *Mycorrhiza* **18**: 115-121.

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Rulz-Lozano JM. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Annuals of Botany* **109**: 1009-1017.

Bastide PY, Kropp BR, Piché Y. 1994. Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist* **127**: 547-556.

Bedon F, Levasseur C, Grima-Pettenati J, Séguin A, MacKay J. 2009. Sequence analysis and functional characterization of the promoter of the *Picea glauca* cinnamyl alcohol dehydrogenase gene in transgenic white spruce plants. *Plant Cell Reports* **28**: 787-800.

Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW. 2012. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of Frankincense seedlings under pulsed water availability conditions. *Oecologia* **169**: 895-904.

Boyle CD, Hellenbrand KE. 1991. Assessment of the effect of mycorrhizal fungi on drought tolerance of conifer seedlings. *Canadian Journal of Botany* **69**: 1764-1771.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. *Working with Mycorrhizas in Forestry and Agriculture*. Canberra, Australia: Australian Centre for International Agricultural Research, 196-208.

Burns C, Leach KM, Elliott TJ, Challen MP, Foster GD, Bailey A. 2006. Evaluation of Agrobacterium-mediated transformation of *Agaricus bisporus* using a range of promoters linked to hygromycin resistance. *Molecular Biotechnology* **32**: 129-138.

Calvo-Polanco M, Zwiazek JJ, Voicu MC. 2008. Responses of ectomycorrhizal American elm (*Ulmus americana*) seedlings to salinity and soil compaction. *Plant and Soil* **308**: 189–200.

Calvo-Polanco M, Jones MD, Zwiazek JJ. 2009. Effects of pH on NaCl tolerance of american elm (*Ulmus americana*) seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria bicolor*. *Acta Physiologiae Plantarum* **31**: 515-522.

Cao Y, Anderova M, Crawford NM, Schroeder JI. 1992. Expression of an outward-rectifying potassium channel from maize mRNA and complementary RNA in *Xenopus* oocytes. *Plant Cell* **4**: 961–969.

Caravaca F, Díaz E, Barea J.M, Azcón-Aguilar C, Roldan A. 2003. Photosynthetic and transpiration rates of *Olea europaea* subsp. *sylvestris* and *Rhamnus lycioides* as affected by water deficit and mycorrhiza. *Biologia Plantarum* **46**: 637-639.

Carlson LW. 1983. *Guidelines for rearing containerized conifer seedlings in the prairie provinces* (Vol. 214) Logan PA, Waldron RM (eds). Northern Forest Research Centre, Canada.

Chaumont F, Moshelion M, Daniels MJ. 2005. Regulation of plant aquaporin activity. *Biology of the Cell* **97**: 749-764.

Coleman MD, Bledsoe CS, Smit B. 1990. Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings. *New Phytologist* **115**: 275–284.

Dietz S, von Bülow J, Beitz E, Nehls U. 2011. The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: lessons for symbiotic functions. *New Phytologist* **190**: 927-40.

Ding Y, Liang S, Lei J, Chen L, Kothe E, Ma A. 2011. *Agrobacterium tumefaciens* mediated fused *egfp-hph* gene expression under the control of *gpd* promoter in *Pleurotus ostreatus*. *Microbiological Research* **166**: 314-322.

Di Battista C, Selosse MA, Bouchard D, Stenström E, Le Tacon F. 1996. Variations in symbiotic efficiency, phenotypic characters and ploidy level among different isolates of the ectomycorrhizal basidiomycete *Laccaria bicolor* strain S238. *Mycological Research* **100**: 1315-1324.

Dominguez I, Itoh K, Sokol SY. 1995. Role of glycogen synthase kinase 3b as a negative regulator of dorsoventral axis formation in *Xenopus* embryos. *Proceedings of the National Academy of Sciences USA* **92**: 8498–8502.

Dosskey MG, Linderman RG, Boersma L. 1990. Carbon–sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. *New Phytologist* **115**: 269-274.

Duddridge JA, Malibari A, Read DJ. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* **287**: 834–836.

Egerton-Warburton LM, Querejeta JI, Allen MF. 2007. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *Journal of Experimental Botany* **58**: 1473–1483.

El Kayal W, Allen CCG, Ju CJT, Adams E, King-Jones S, Zaharia LI, Abrams SR, Cooke JE. 2011. Molecular events of apical bud formation in white spruce, *Picea glauca*. *Plant, Cell and Environment* **34**: 480-500.

Emanuelsson O, Brunak S, Heijne G, Nielsen H. 2007. Locating proteins in the cell using TargetP, SignalP, and related tools. *Nature Protocols* **2**: 953-971.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Fetter K, Van Wilder V, Moshelion M, Chaumont F. 2004. Interactions between plasma membrane aquaporins modulate their water channel activity. *The Plant Cell* **16**: 215-228.

Fischer M, Kaldenhoff R. 2008. On the pH regulation of plant aquaporins. *Journal of Biological Chemistry* **283**: 33889-33892.

Gambetta GA, Fei J, Rost TL, Knipfer T, Matthews MA, Shackel KA, Walker MA, McElrone AJ. 2013. Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport. *Plant physiology* **163**: 1254-1265.

Gerbeau P, Amodeo G, Henzler T, Santoni V, Ripoche P, Maurel C. 2002. The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. *The Plant Journal* **30**: 71-81.

Giovannetti M, Balestrini R, Volpe V, Guether M, Straub D, Costa A, Ludewig U, Bonfante P. 2012. Two putative-aquaporin genes are differentially expressed during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *BMC Plant Biology* **12**: 186.

Groome MC, Axler SR, Gifford DJ. 1991. Hydrolysis of lipid and protein reserves in loblolly pine seeds in relation to protein electrophoretic patterns following imbibition. *Physiology Plantarum* **83**: 99-106.

Hirokawa T, Boon-Chieng S, Mitaku S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* **14**: 378-379.

Javot H, Maurel C. 2002. The role of aquaporins in root water uptake. *Annals of Botany* **90**: 301-313.

Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, Kjellbom P. 1998. Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *The Plant Cell* **10**: 451-459.

Johanson U, Karlsson M, Johansson I, Gustavsson S, Sjovall S, Fraysse L, Weig AR, Kjellbom P. 2001. The complete set of genes encoding major intrinsic proteins in *Arabidopsis* provides a framework for a new nomenclature for major intrinsic proteins in plants. *Plant Physiology* **126**: 1358-1369.

Kamaluddin M, Zwiazek JJ. 2002. Ethylene enhances water transport in hypoxic aspen (*Populus tremuloides*). *Plant Physiology* **128**: 962-969.

Kemppainen M, Circosta A, Tagu D, Martin F, Pardo AG. 2005. *Agrobacterium*-mediated transformation of the ectomycorrhizal symbiont *Laccaria bicolor* S238N. *Mycorrhiza* **16**: 19–22.

Kemppainen M, Duplessis S, Martin F, Pardo AG. 2008. T-DNA insertion, plasmid rescue and integration analysis in the model mycorrhizal fungus *Laccaria bicolor*. *Microbial Biotechnology* **1**: 258-269.

Kemppainen M, Duplessis S, Martin F, Pardo AG. 2009. RNA silencing in the model mycorrhizal fungus *Laccaria bicolor*: gene knock‐down of nitrate reductase results in inhibition of symbiosis with *Populus*. *Environmental Microbiology* **11**: 1878-1896.

Kemppainen MJ, Pardo AG. 2010. pHg/pSILBAγ vector system for efficient gene silencing in homobasidiomycetes: optimization of ihpRNA - triggering in the mycorrhizal fungus *Laccaria bicolor*. *Microbial Biotechnology* **3**: 178–200.

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U. 2005. Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biology* **7**: 706–712.

Kilaru S, Hoegger PJ, Majcherczyk A, Burns C, Shishido K, Bailey A, Foster GD, Kües U. 2006. Expression of laccase gene *lcc1* in *Coprinopsis cinerea* under control of various basidiomycetous promoters. *Applied Microbiology and Biotechnology* **71**: 200-210.

Kline KG, Barrett-Wilt GA, Sussman MR. 2010. In planta changes in protein phosphorylation induced by the plant hormone abscisic acid. *Proceedings of the National Academy of Sciences USA* **107**: 15986-15991.

Krogh A, Larsson B, Von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *Journal of Molecular Biology* **305**: 567-580.

Landhäusser SM, Muhsin TM, Zwiazek JJ. 2002. The effect of ectomycorrhizae on water relations in aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) at low soil temperatures. *Canadian Journal of Botany* **80**: 684-689.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. ClustalW and ClustalX version 2. *Bioinformatics* **23**: 2947-2948.

Laur J, Hacke UG. 2014. Exploring *Picea glauca* aquaporins in the context of needle water uptake and xylem refilling. *New Phytologist* **203**: 388-400.

Lawrence SD, Novak NG, Xu H, Cooke JEK. 2013. Herbivory of maize by southern corn rootworm induces expression of the major intrinsic protein. *Plant Signaling and Behavior*. doi: 10.4161/psb.24937.

Lee SH, Chung GC, Steudle E. 2005. Gating of aquaporins by low temperature in

roots of chilling-sensitive cucumber and chilling-tolerant figleaf gourd. *Journal of Experimental Botany* **56**: 985-995.

Lee SH, Calvo-Polanco M, Chung GC, Zwiazek JJ. 2010. Role of aquaporins in root water transport of ectomycorrhizal jack pine (*Pinus banksiana*) seedlings exposed to NaCl and fluoride. *Plant, Cell and Environment* **33**: 769–780.

Lee SH, Chung GC, Jang JY, Ahn SJ, Zwiazek JJ. 2012. Overexpression of PIP2;5 aquaporin alleviates effects of low root temperature on cell hydraulic conductivity and growth in Arabidopsis. *Plant Physiology* **159**: 479-488.

Lehto T, Zwiazek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* **21**: 71–90.

Levy Y, Krikun J. 1980. Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. *New Phytologist* **85**: 25-31.

Lew RR. 2011. How does a hypha grow? The biophysics of pressurized growth in fungi. *Nature Reviews Microbiology* **9**: 509-518.

Liu Y. 2012. *Calcium-related fungal genes implicated in arbuscular mycorrhiza*. Ph.D. Thesis, Huazhong Agricultural University, Wuhan, China, and Burgundy University, Burgundy, France.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**: 402-408.

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B. 2013. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617-630.

Lopez D, Bronner G, Brunel N, Auguin D, Bourgerie S, Brignolas F, Carpin S, Tournaire-Roux C, Maurel C, Fumanal B, Martin F, Sakr S, Label P, Julien JL, Gousset-Dupont A, Venisse JS. 2012. Insights into *Populus* XIP aquaporins: evolutionary expansion, protein functionality, and environmental regulation. *Journal of Experimental Botany* **63**: 2217-2230.

Luard EJ, Griffin DM. 1981. Effect of water potential on fungal growth and turgor.

Transactions of the British Mycological Society **76**: 33-40.

Marjanović Ž, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiss M, Hampp R, Nehls U. 2005. Aquaporins in poplar: what a difference a symbiont makes! *Planta* **222**: 258–268.

Marjanović Ž, Nehls U. 2008. Ectomycorrhiza and water transport. In: Varma A (ed) *Mycorrhiza*. Berlin, Heidelberg, Germany: Springer, 149-159.

Martin F, Aerts A, Ahrn D, Brun A, Danchin EGJ. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452**: 88-92.

Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**: 153-163.

Matsumoto T, Lian HL, Su WA, Tanaka D, Liu CW, Iwasaki I, Kitagawa Y. 2009. Role of the aquaporin PIP1 subfamily in the chilling tolerance of rice. *Plant and Cell Physiology* **50**: 216-229.

Maurel C, Verdoucq L, Luu D, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology* **59**: 595-624.

Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L. 2009. The cellular dynamics of plant aquaporin expression and functions. *Current Opinion in Plant Biology* **12**: 690-698.

Maurel C, Plassard C. 2011. Aquaporins: for more than water at the plant–fungus interface? *New Phytologist* **190**: 815–817.

Muhsin TM, Zwiazek JJ. 2002a. Ectomycorrhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus americana* seedlings. *New Phytologist* **153**: 153-158.

Muhsin TM, Zwiazek JJ. 2002b. Ectomycorrhizae increase water conductance and protect white spruce (*Picea glauca*) seedlings against salt stress. *Plant and Soil* **238**: 217-225.

Murai-Hatano M, Kuwagata T, Sakurai J, Nonami H, Ahamed A, Nagasuga K, Matsunami T, Fukushi K, Maeshima M, Okada M. 2008. Effect of low root temperature on hydraulic conductivity of rice plants and the possible role of aquaporins. *Plant and Cell Physiology* **49**: 1294-1305.

Nardini A, Salleo S, Tyree MT, Vertovec M. 2000. Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Annuals of Forest Science* **57**: 305–312.

Navarro-Ródenas A, Ruíz-Lozano JM, Kaldenhoff R, Morte A. 2012. The aquaporin TcAQP1 of the desert truffle *Terfezia claveryi* is a membrane pore for water and CO_2 transport. *Molecular Plant-Microbe Interaction* **25**: 259-266.

Navarro-Ródenas A, Bárzana G, Nicolás E, Carra A, Schubert A, Morte A. 2013. Expression analysis of aquaporins from desert truffle mycorrhizal symbiosis reveals a fine-tuned regulation under drought. *Molecular Plant-Microbe Interactions* **26**: 1068-1078.

Navarro-Ródenas A, Xu H, Kemppainen M, Pardo A, Zwiazek JJ. 2015. *Laccaria bicolor* Aquaporin LbAQP1 is required for Hartig Net Development in Trembling Aspen (*Populus tremuloides*). *Plant, Cell and Environment*. In Press.

Nylund J-E. 1987. The ectomycorrhizal infection zone and its relation to acid polysaccharides of cortical cell walls. *New Phytologist* **106**: 505–516.

Peterson RL, Massicotte HB, Melville LH. 2004. *Mycorrhizas: Anatomy and Cell Biology*. Ottawa, Canada: NRC Research Press, 173.

Pfaffl MW. 2004. Quantification strategies in real-time PCR. In: Bustin SA (ed) *A-Z of quantitative PCR*. La Jolla, USA: International University Line (IUL), 87-112.

Pham GH, Kumari R, Singh A, Malla R, Prasad R, Sachdev M, Kaldorf M, Buscot F, Oelmüller R, Hampp R, Saxena AK, Rexer K, Kost G, Varma A. 2004. Axenic culture of symbiotic fungus *Piriformospora indica*. In: Varma A, Abbott L, Werner D, Hampp R (eds) *Plant surface microbiology*. Berlin, Heidelberg, Germany: Springer, 593-613.

Plamboeck AH, Dawson TE, Egerton-Warburton LM, North M, Bruns TD,

Querejeta JI. 2007. Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. *Mycorrhiza* **17**: 439–447.

Porcel R, Ruiz-Lozano JM. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *Journal of Experimental Botany* **55**: 1743-1750.

Porcel R, Aroca R, Azcón R, Ruiz-Lozano J. 2006. PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Molecular Biology* **60**: 389-404.

Prado K, Maurel C. 2013. Regulation of leaf hydraulics: from molecular to whole plant levels. *Frontiers in Plant Science.* doi: 10.3389/fpls.2013.00255.

Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. 2008. Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2; 1 in response to salt stress. *Molecular and Cellular Proteomics* **7**: 1019-1030.

Rigault P, Boyle B, Lepage P, Cooke JEK, Bousquet J, MacKay JJ. 2011. A white spruce gene catalogue resource for conifer genome analyses. *Plant Physiology* **157**: 14-28.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671-675.

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. 1965. Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. *Science* **148**: 339-346.

Siemens AJ, Zwiazek JJ. 2008. Root hydraulic properties and growth of balsam poplar (*Populus balsamifera*) mycorrhizal with *Hebeloma crustuliniforme* and *Wilcoxina mikolae* var. *mikolae*. *Mycorrhiza* **18**: 393–401.

Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. 3rd Edition, Cambridge, UK: Academic Press.

Steudle E. 1993. Pressure probe techniques: basic principles and application to studies of water and solute relations at the cell, tissue, and organ level. In: Smith JAC, Griffiths H (eds) *Water Deficits: Plant Responses from Cell to Community*. Oxford, UK: Bios Scientific Publishers Ltd, 5–36.

Steudle E, Peterson CA. 1998. How does water get through roots? *Journal of Experimental Botany* **49**: 775–788.

Subramanian KS, Charest C, Dwyer LM, Hamilton RI. 1997. Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. *Canadian Journal of Botany* **75**: 1582-1591.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods**.** *Molecular Biology and Evolution* **28**: 2731-2739.

Tinker PB, Durall DM, Jones MD. 1994. Carbon use efficiency in mycorrhizas theory and sample calculations. *New Phytologist* **128**:115-122.

Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P. 2006. Structural mechanism of plant aquaporin gating. *Nature* **439**: 688–694.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**: 393-397.

Tyree MT, Patiño S, Bennink J, Alexander J. 1995. Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany* **46**: 83-94.

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R. 2007. Arbuscular mycorrhizal symbiosis and plant aquaporin expression. *Phytochemistry* **68**: 122–129.

Unestam T, Sun YP. 1995. Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. *Mycorrhiza* **5**: 301–311.

Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. 2007. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* **35**: W71-W74.

Verdoucq L, Grondin A, Maurel C. 2008. Structure-function analysis of plant aquaporin AtPIP2; 1 gating by divalent cations and protons. *Biochemical Journal* **415**: 409-416.

Wan X, Landhäusser SM, Zwiazek JJ, Lieffers VJ. 1999. Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. *Tree Physiology* **19**: 879-884.

Wan X, Zwiazek JJ, Lieffers VJ, Landhäusser SM. 2001. Hydraulic conductance in aspen (*Populus tremuloides*) seedlings exposed to low root temperatures. *Tree Physiology* **21**: 691-696.

Weatherley PE. 1982. Water uptake and flow in roots. In: Lange O, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological plant ecology II*. Berlin, Germany: Springer, 79–109.

Xu H, Cooke JEK, Zwiazek JJ. 2013. Phylogenetic analysis of fungal aquaporins provides insight into their possible role in water transport of mycorrhizal associations. *Botany* **91**: 495-504.

Yi H, Calvo-Polanco M, MacKinnon MD, Zwiazek JJ. 2008. Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. *Environmental and Experimental Botany* **62**: 357–363.

Zelazny E, Miecielica U, Borst JW, Hemminga MA, Chaumont F. 2009. An N-terminal diacidic motif is required for the trafficking of maize aquaporins ZmPIP2; 4 and ZmPIP2; 5 to the plasma membrane. *The Plant Journal* **57**: 346-355.

Zhang R, Verkman AS. 1991. Water and urea permeability properties of *Xenopus* oocytes: expression of mRNA from toad urinary bladder. *American Journal of Physiology* **260**: C26–34.

Table 3.1 Polymerase chain reaction primers for gene cloning

Genes	Application	Primers in 5'- 3' orientation
JQ585592	Coding sequence cloning	Forward: TCGATGCATCCACAAGTTGC; Reverse: TGAATAGACAGGCATTAGACC
JQ585593		Forward: ATGTCCGCTACTCCAATCATC; Reverse: TCATACAGGTTTCTCTTGCGATG
JQ585594		Forward: ATGAAGTTAACCATCTCTCACCA; Reverse: CTACCGCCACTTGATTGGAC
JQ585595		Forward: ATGGACGACAAATTCGACGACG; Reverse: TTAAGCTGGGGAGATGTGTATGTG
JQ585596		Forward: ATGTCTGGCCAACATCAGATCAC; Reverse: TCAAACAACCTCAGCGAGC
JQ585597		Forward: ATGTCTGGCCAACATCAGATCAC; Reverse: TCAAACAACCTCAGCGAGC
α-tubulin		Forward: TTGATCTCGAACCGGGATG; Reverse: ATGGCGGTGGTGTTTGAC
EF2		Forward: ATGGAGGATATTGAATCCATTG; Reverse: TCATGATTGGCTCCAAAATAAC
Hygromycin phosphotransferase gene	Sequence amplification for Southern blot	Forward: ATGCAGCTGTCGGAGGGCGA; Reverse: GCGCTTCTGCGGGCGATTTG
JQ585592	Sub-cloning for oocyte assay	Forward: GTCGACGCCACCATGCATCCACAAGTTG; Reverse: GTCGACGCCACCATGCATCCACAAGTTG
JQ585593		Forward: GTCGACGCCACCATGTCCGCTACTCCAA; Reverse: TCTAGATCATACAGGCTTCTCTTGC
JQ585594		Forward: GTCGACGCCACCATGGCCGAATTTGTTG; Reverse: TCTAGACTACCGCCACTTGATTGGA
JQ585595		Forward: GTCGACGCCACCATGGACGACAAATTCG; Reverse: TCTAGATTAAGCTGGGGAGATGTGT
JQ585596		Forward: GTCGACGCCACCATGTCTGGCCAACATC; Reverse: TCTAGATCAAACAACCTCAGCGAGC
JQ585597		Forward: GTCGACGCCACCATGTCTGGCCAACATC; Reverse: TCTAGATCAAACAACCTCAGCGAGC

Table 3.2 Primers for qRT-PCR assay of *Laccaria bicolor* MIPs

Genes	Primer orientation	5'- 3' sequence
JQ585592	Forward	TGGCCCTGCTGAAATCAACT
	Reverse	CCCATTGCGAGTCTCTTCGT
JQ585593	Forward	GTGACATTGGTTGCCGTTTG
	Reverse	ATCCTCCCGCAGCTGACTTT
JQ585594	Forward	TAATGGCGCACTCACAAACG
	Reverse	ATGCCCCAAGACCAATGAAC
JQ585595	Forward	TAACCCCGCTCGTGATCTTG
	Reverse	CCTGTCTTCCATAGCCAACCA
JQ585596	Forward	ACGCTTGTTCCTCGCTATGTC
	Reverse	TGCCCAGAGCCAATATTGACT
JQ585597	Forward	CGCCCTCACTGACAAACGTA
	Reverse	ATAAAGAGCGCAAATGGCAAAA
EF2	Forward	GGCATGGGAGAACTTCAATCA
	Reverse	GCCAGAGACGCAATCAGTGTT
α-tubulin	Forward	CAGACAAGGCAAATCACGAACA
	Reverse	GCCATTTTCGAAGCAAGAGAA

Table 3.3 TAIL-PCR conditions

Reaction	Cycle No.	Thermal Condition
Primary (1st)	1	92°C, 3min
Template: About 100ng gDNA of the	1	94°C, 3min
transgenic strains;	2	94°C, 1min; 60°C 1min; 72°C 3min
Final concentration of primers:		
0.5 µM RB1 & 3 µM of AD	1	94°C, 1min
	1	25°C, 2min
	1	72°C, 3min
	30	94°C, 30s; 60°C 1min; 72°C 3min
	15	94°C, 30s; 43°C 1min; 72°C 3min
	1	72°C, 5min
Secondary (2nd)	1	94°C, 1min
Template: 1 µl out of 50µl primary	24	94°C, 30s; 57°C 1min; 72°C 3min
PCR reaction; Final concentration of	12	94°C, 30s; 45°C 1min; 72°C 3min
primers:	1	72°C, 5min
0.5 µM RB2 & 3 µM of AD		
Tertiary (3rd)	1	94°C, 1min
Template: 1 µl out of 50µl secondary	20	94°C, 30s; 45°C 1min; 72°C 3min
PCR reaction; Final concentration of	1	72°C, 30s
primers:		
0.5 µM RB3 & 3 µM of AD		
Quaternary (4th)	1	94°C, 1min
Template: 1 µl out of 50µl tertiary	24	94°C, 30s; 54°C 1min; 72°C 3min
PCR reaction; Final concentration of	12	94°C, 30s; 45°C 1min; 72°C 3min
primers:	1	72°C, 5min
0.5 µM RB3 & 0.5 µM of AD		
Primers:		
RB1**: 5' GCAGCCTGAATGGCGAATGCTAG 3'**		
RB2**: 5' TGGATCAGATTGTCGTTTCCCG 3'**		
RB3**: 5' ATATTGGCGGGTAAACCTAAG 3'**		
AD**: 5' NTCGA(G/C)T(A/T)T(G/C)G(A/T)GTT 3'**		

Table 3.4 Characteristics of six MIPs in *Laccaria bicolor* UAMH8232

MIP accession number[1]	Protein ID[1]	Length of deduced amino acid sequences	TMHs[2]	NPA motifs	ar/R	Subcellular localization[3]	Phylogenetic cluster	P_f (μm s^{-1}) [4]	CO_2 transport capacity[5]	Corresponding MIPs in strain S238N and their transport capacity[6]	Inter-strain amino acid sequence identity, positive rate and gaps
JQ585592	AFJ15555	311	6	NPN, NSA	FHAR	Secretory RC 5	I: Orthodox fungal aquaporins	25.8 ± 2.1	Yes	Lacbi1:392091 (Lacbi2:456764) H_2O	Id 99%, Pos99%, Gap0%
JQ585593	AFJ15556	330	6	NPC, NSA	FGIR	Plasma membrane RC 5	II: Fungal aquaglyceroporin	46.6 ± 10.2	Yes	Lacbi1:307192 (Lacbi2:671860)	Id 98%, Pos99%, Gap0%
JQ585594	AFJ15557	254	6	NPA, NPA	WGYR	Secretory RC 1	III: Facultative fungal aquaporin	124.0 ± 11	No	Lacbi1:247946 (Lacbi2:568479) H_2O	Id 93%, Pos94%, Gap3%
JQ585595	AFJ15558	312	6	NPA, NPA	WIYR	Plasma membrane RC 1	III: Facultative fungal aquaporin	260.0 ± 8.9	No	Lacbi1:391485 (Lacbi2:443240) H_2O, glycerol, ammonia	Id 94%, Pos96%, Gap0%
JQ585596	AFJ15559	332	6	NPA, NPA	WGYR	Plasma membrane RC 3	III: Facultative fungal aquaporin	166.5 ± 2.7	No	Lacbi1:317173 (Lacbi2:317173)H_2O, ammonia	Id 90%, Pos94%, Gap0%
JQ585597	AFJ15560	332	6	NPA, NPA	WGYR	Plasma membrane RC 3	III: Facultative fungal aquaporin	138.4 ± 1.5	No	Lacbi1:317173 (Lacbi2:317173)H_2O, ammonia	Id 95%, Pos97%, Gap0%

Note: [1.] GenBank accession number in NCBI. [2.] The number of transmembrane helix domains was predicted by TMHMM server 2.0 (Krogh *et al*. 2001). [3.] Subcellular localization was predicted by Target P (Emanuelsson *et al*. 2007); 1 indicated strongest prediction and 5 the weakest. [4.] P_f for negative control was 20.5 ± 0.6 μm s^{-1}. [5.] The assay was conducted in yeast heterologous expression system by Navarro-Ródenas *et al*. (2015). [6.] Functional assays were conducted by Dietz *et al*. (2011); Lacbi2 proteins were searched in JGI *Laccaria bicolor* genome V2.0.

Table 3.5 Primers for qRT-PCR assay of *Picea glauca* PIPs

Genes	Primer orientation	5'- 3' sequence
GQ03610_A06.1	Forward	GATTGT GGGCACATTTGTCCTT
	Reverse	AGTCTCTGGCGCTTCGTTTG
GQ03401_M18.1	Forward	CCACCGATGCCAAAAGAAGT
	Reverse	TGCGAACCCTATGGGAAGTG
GQ03010_E09.1	Forward	CCAACGGAGGAGGATCGAA
	Reverse	CTCCGCAAGAAGGGCACTT
GQ03002_G07.1	Forward	GCTTTCGGCGGAATGATCT
	Reverse	GTTGACGTGACCCCCTGAAAT
GQ03001_P18.1	Forward	GCGGTATTCTTGGTCCATTTG
	Reverse	TGGCAGGATTGATGCTTGTG
GQ03111_E12.1	Forward	ACCTCCTGCTGCTCTCATTGA
	Reverse	GGCAACGAATTCTGCTATCAGA
GQ03703_H07.1	Forward	TCTTGGCTCCATTGCCTATTG
	Reverse	GTTGATGCCAGTCCCAGTGA
GQ02901_B20.1	Forward	CATTTGTGGGACTGGATTGGT
	Reverse	GCTCCACCTCCATTTTGATCA
GQ02905_E13.1	Forward	GCTGGGATTTCAGGTGGACAT
	Reverse	CCTTGCCAAAAACAGTCCAAA
PgCDC2	Forward	GTGCAGAGAAAAAGTCGAAC
	Reverse	CCACACCATATGTTCCTTCT

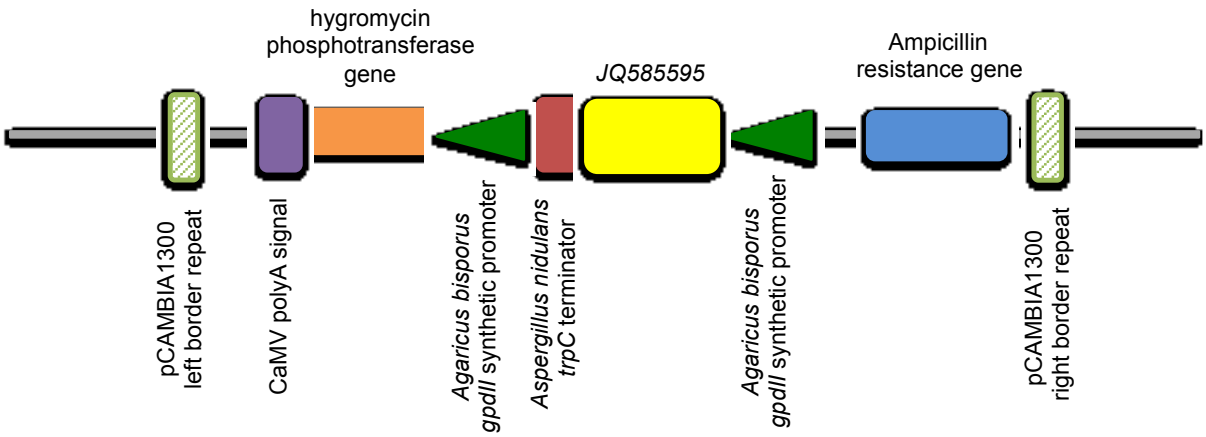


Figure 3.1 Construction of binary vectors for *Agrobacterium*-mediated transformation to overexpress *JQ585595*
This transgenic cassette rendering *JQ585595* overexpression and hygromycin resistance randomly inserted into the genome of *L. bicolor* UAMH8232 via *Agrobacterium*-mediated transformation.

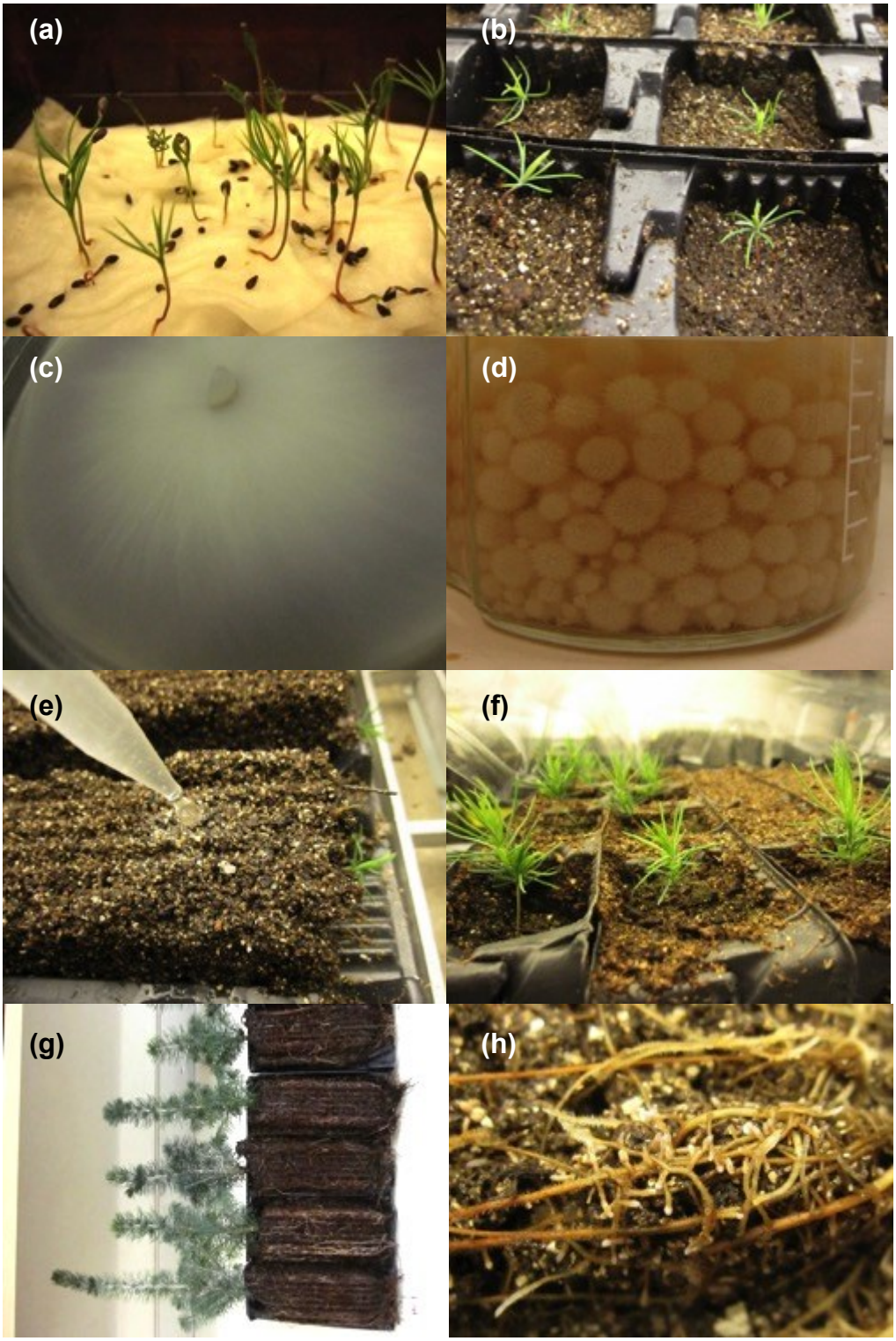


Figure 3.2 *Picea glauca* seedlings preparation and inoculation
(a) Seed germination on sterile, moistened cellulose paper; **(b)** Germinants transplanted into potting mix in root trainers; **(c)** *Laccaria bicolor* mycelium grown on solid MMN medium at 20°C for two weeks; **(d)** *L. bicolor* cultivated in liquid MMN medium at 20°C with shaking at 0.8 x *g* for four weeks; **(e)** Seedling root inoculated with 10 mL homogenized liquid inoculum after transplanting; **(f)** Seedlings, four weeks after transplanting; **(g)** Seedlings and **(h)** inoculated roots, three months after transplanting.

Figure 3.3 Canonical aquaporin transmembrane-domain structure and NPA signature motifs of *Laccaria bicolor* MIPs
Deduced amino acid sequence of *L. bicolor* JQ585592 **(a)**, JQ585593 **(b)**, JQ585594 **(c)**, JQ585595 **(d)**, JQ585596 **(e)** and JQ585597 **(f)** were predicted as a canonical aquaporin with six-transmembrane-domain structure and NPA signature motifs in the *in silico* assay. Transmembrane secondary structure of the protein was predicted using TMHMM. Deduced amino acid sequence fragments in termini, transmembrane domains TMD 1-6 and Loop A-E were highlighted in pink, yellow and turquoise, respectively. NPA motifs or their variations were in bold and underlined in Loop B & E.

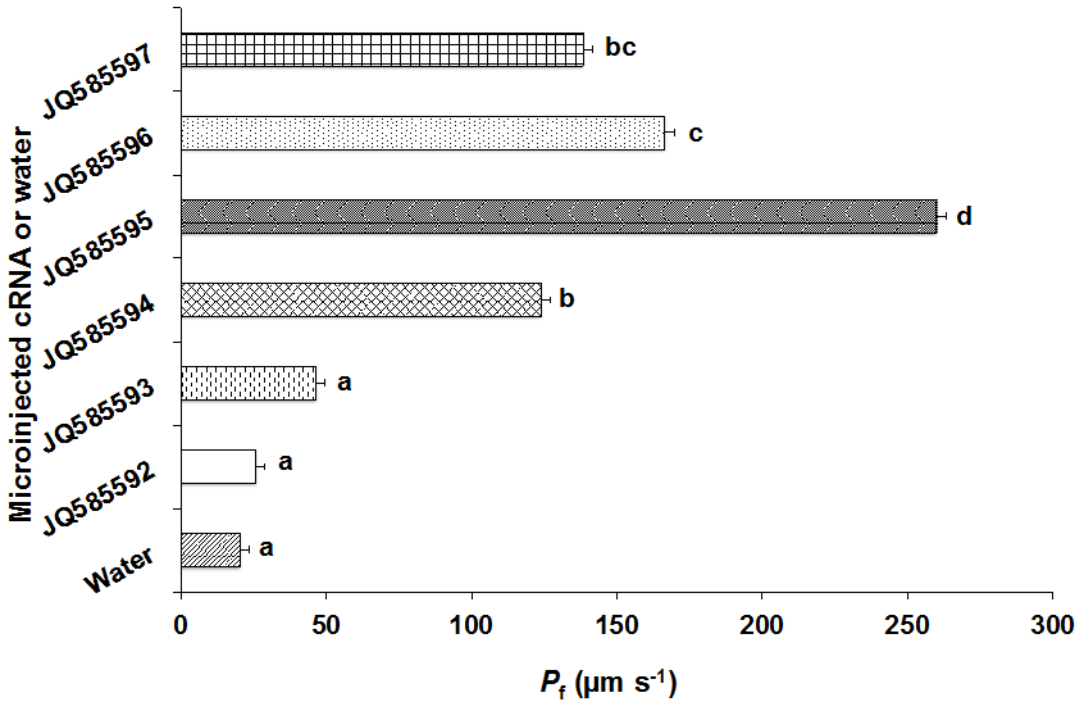


Figure 3.4 Water permeability of oocytes microinjected with the cRNAs of *MIP* genes of *Laccaria bicolor* UAMH8232

P_f is the osmotic permeability coefficient value of *Xenopus laevis* oocytes in which the corresponding *L. bicolor MIP* genes were heterologously expressed; either cRNAs of each *MIP* gene or water (as negative control) was microinjected into the oocytes. Means (n = 10) ± SE are shown. Values with different letters are significantly different at $P \leq 0.05$ (ANOVA, Tukey's test).

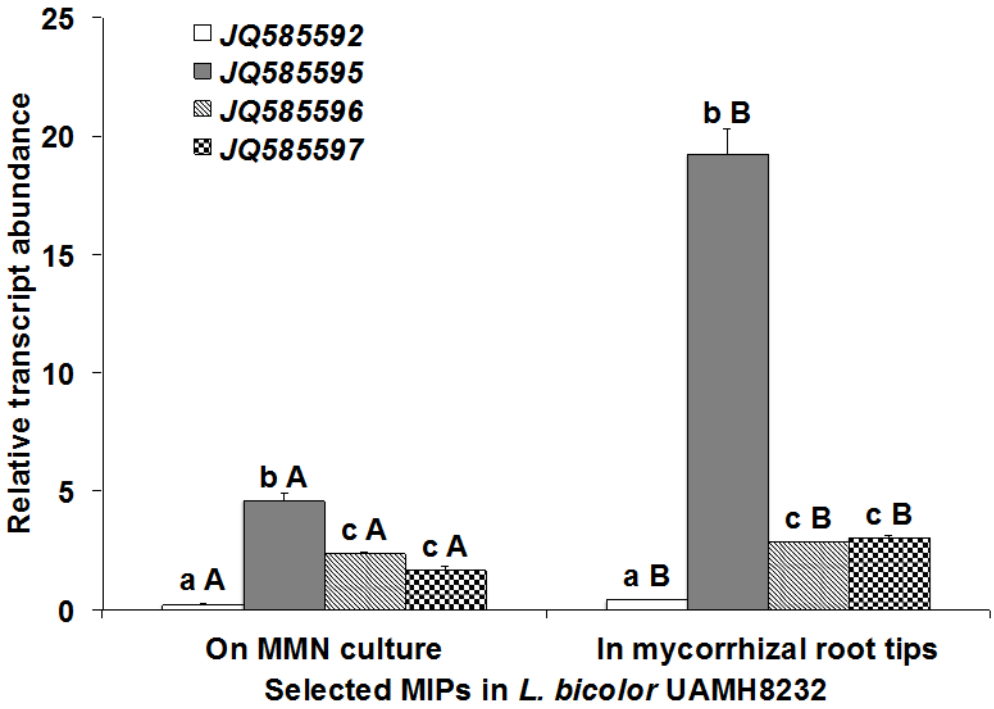


Figure 3.5 Transcript abundance of water-transporting aquaporins and orthodox aquaporin of *Laccaria bicolor* UAMH8232 in vegetative mycelial tissues grown on MMN medium and in mycorrhizal root tips of *Picea glauca*
Relative transcript abundance of selected MIPs in *L. bicolor* wild-type mycelia measured by standard curve method of absolute quantification in qRT-PCR assay; the transcript abundance of the aquaporin genes was normalized against that of the reference gene, *EF2*. Means (n = 3) ± SE are shown. The different letters in lowercase mean significantly difference between analyzed genes in the same type of tissue at $P \leq 0.05$ (ANOVA, Tukey's test). The different letters in UPPERCASE mean significantly difference of the same gene between the two types of tissues at $P \leq 0.05$ (ANOVA, Tukey's test).

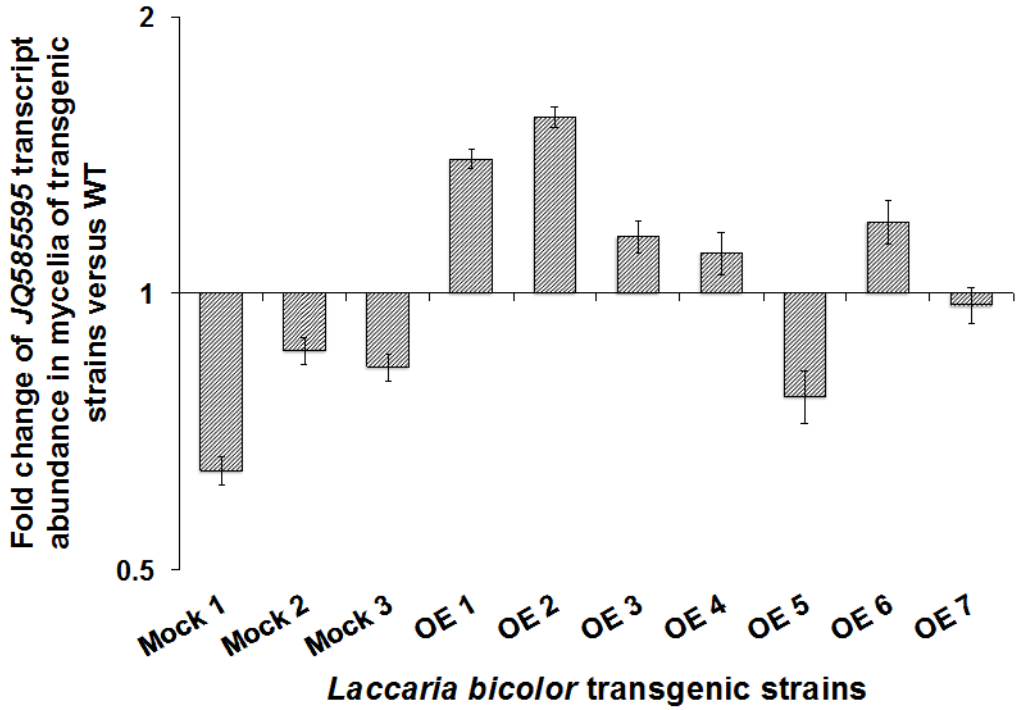


Figure 3.6 Transcript abundance of *JQ585595* in mycelia of *Laccaria bicolor* transgenic overexpression (OE) and mock strains
Transcript abundance in transgenic strains was compared with that in wild type (WT)(n = 3± SE).

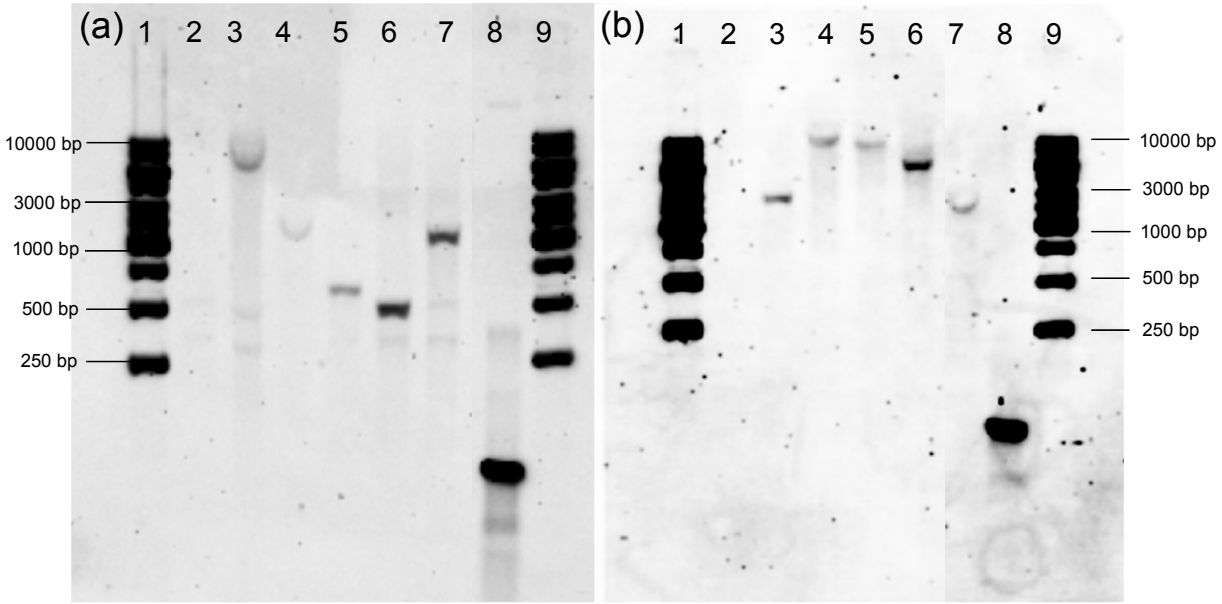


Figure 3.7 Southern blot analysis of *Laccaria bicolor* genomic DNA digested by restriction enzymes *Sac*I (a) or *Bam*HI (b)
Southern blot showing the transgenic strains for single copy insertion events in the genomic DNA digested by *Sac*I **(a)** and *Bam*HI **(b)**; one clear band of hybridization indicating single copy insertion; DNA ladder and digested genomic DNA were loaded in the following order: Lane 1 and 9 for 1Kb DNA ladder (GeneRuler™; Fermentas); Lane 2 for WT; Lane 3 for Mock 1; Lane 4 for Mock 2; Lane 5 for Mock 3; Lane 6 for OE1; Lane 7 for OE2; Lane 8 for 0.2 ng of 870 bp PCR amplicon of hygromycin phosphotransferase gene as positive control.

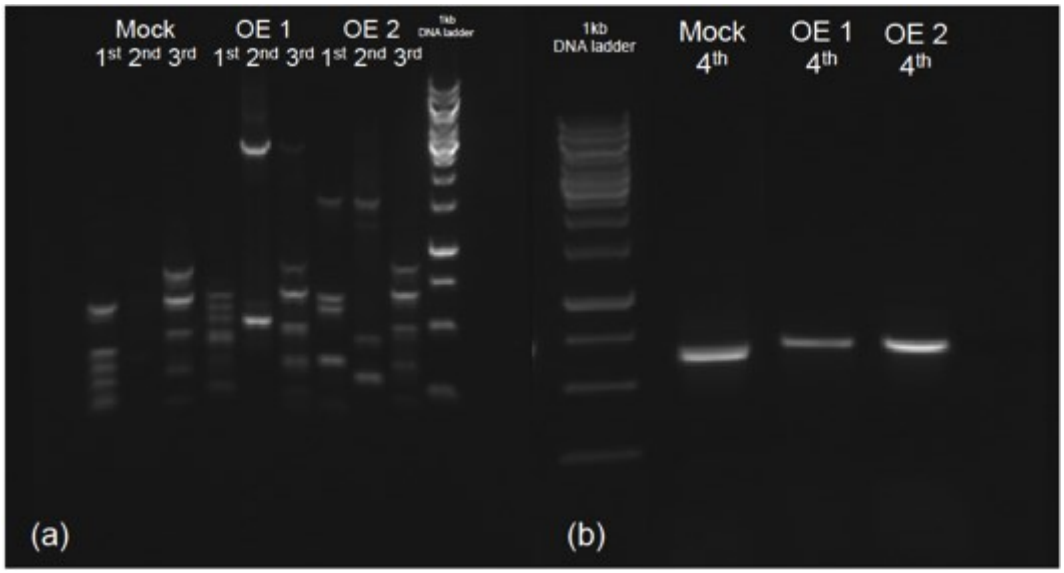


Figure 3.8 Gel electrophoresis of TAIL-PCR products to amplify the part of the T-DNA right border and its flanking sequence from the genome of *Laccaria bicolor* transgenic strains

(a) Products of the primary, secondary and tertiary PCRs; **(b)** Products of the quaternary PCRs. Fifteen microliter of each PCR reaction or 0.5µg of 1kb DNA ladder was loaded in each lane of 1% agarose gel stained by SYBR™ Safe DNA Gel Stain (Life Technologies). The electrophoresis ran in 0.5x TBE buffer at 90 V for 45 min.

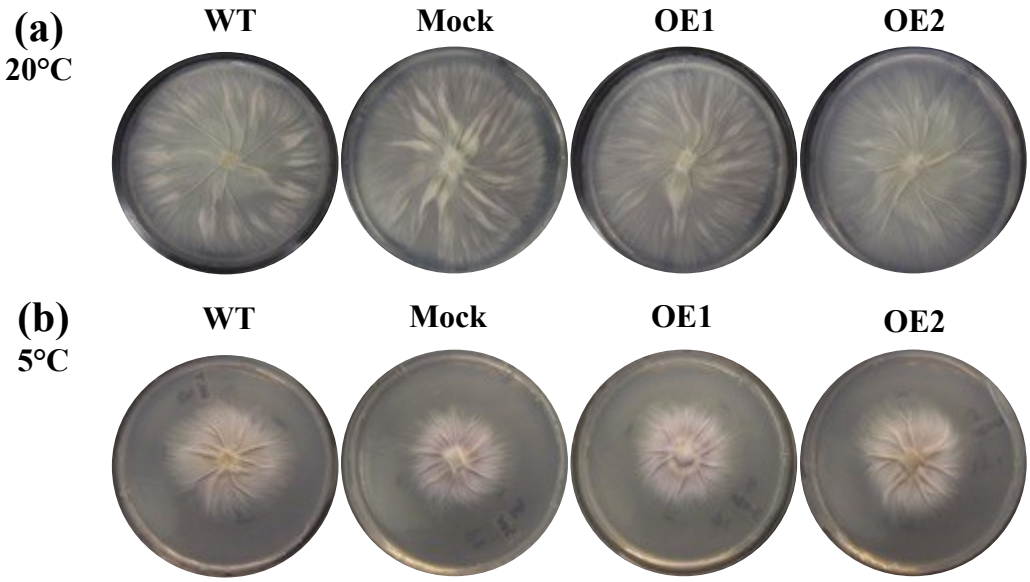


Figure 3.9 Phenotype of vegetative mycelia of *Laccaria bicolor* strains grown on MMN medium at 20°C (a) and 5°C (b)

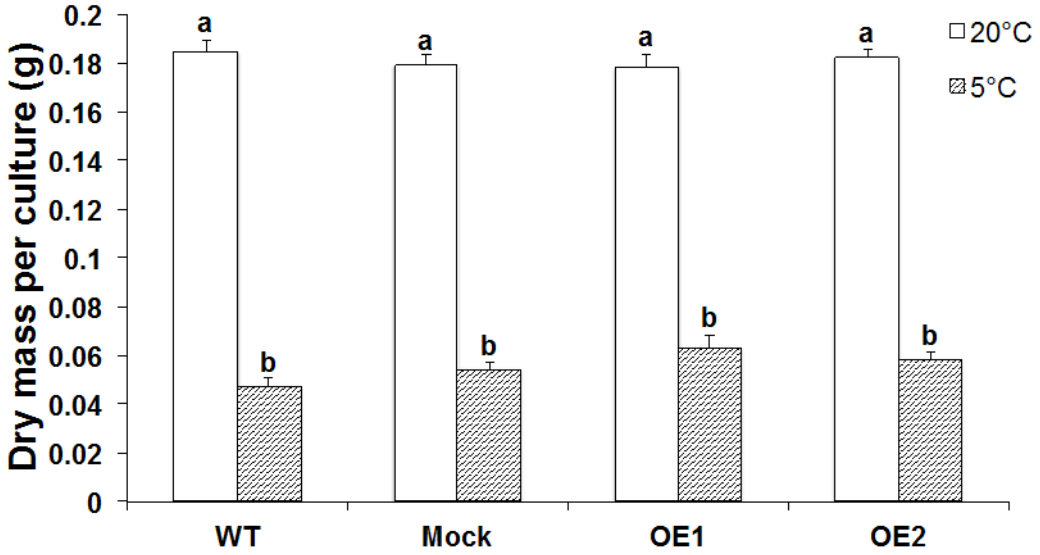


Figure 3.10 Dry mass of mycelia of WT, mock and OE strains grown on MMN medium at 20°C and 5°C for three weeks

Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 6 \pm$ SE).

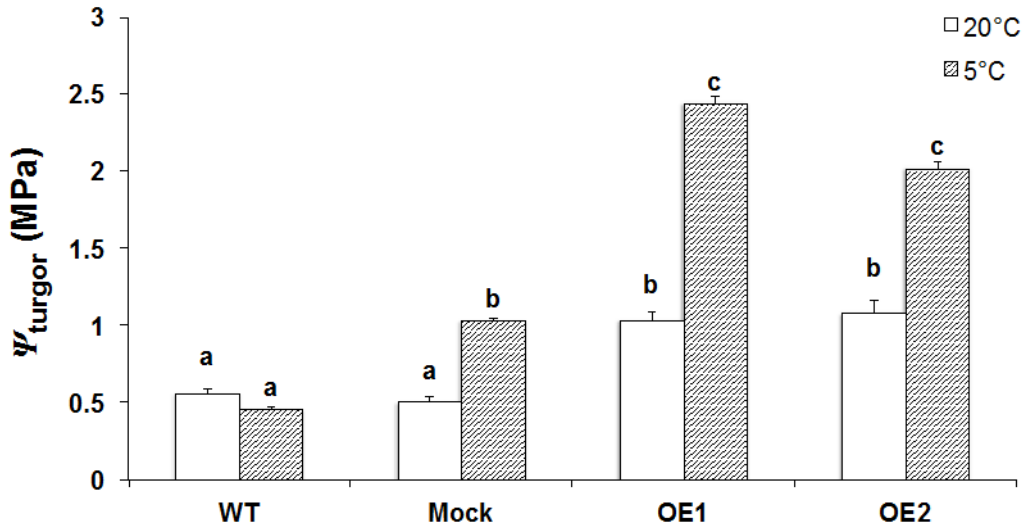


Figure 3.11 Turgor water potential Ψ_{turgor} of mycelia of WT, mock and OE strains grown on MMN medium at 20°C and 5°C for three weeks
Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 3 \pm$ SE).

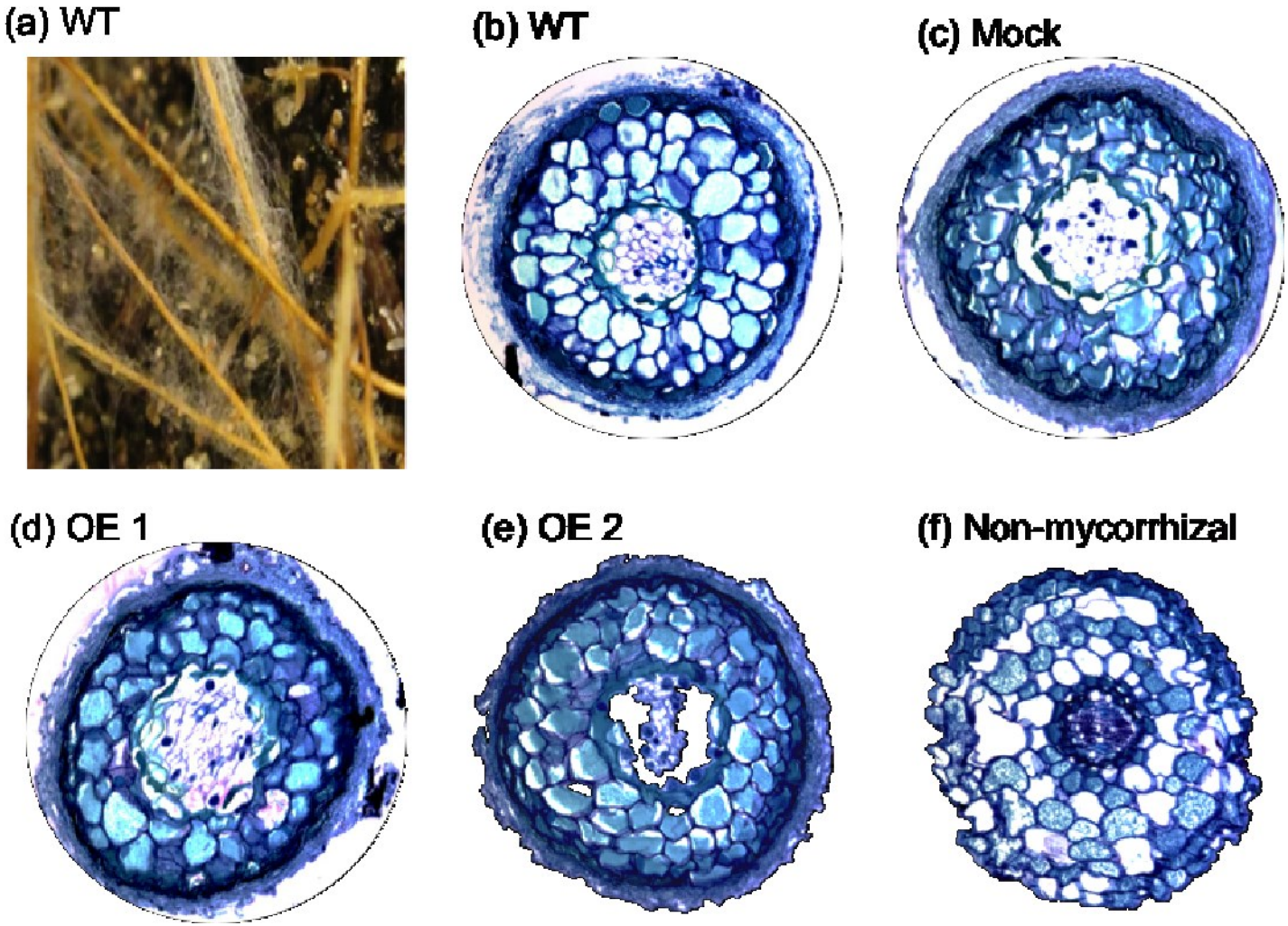


Figure 3.12 Morphologic characteristics of *Picea glauca* roots ectomycorrhizal with *Laccaria bicolor*
(a) Massive mycelia grown in rhizosphere were observed two months after the second inoculation, with mantle and Hartig net structure well developed in root tips **(b-e)**; **(f)** No such structures were observed in the roots of *P. glauca* seedlings that had not been inoculated.

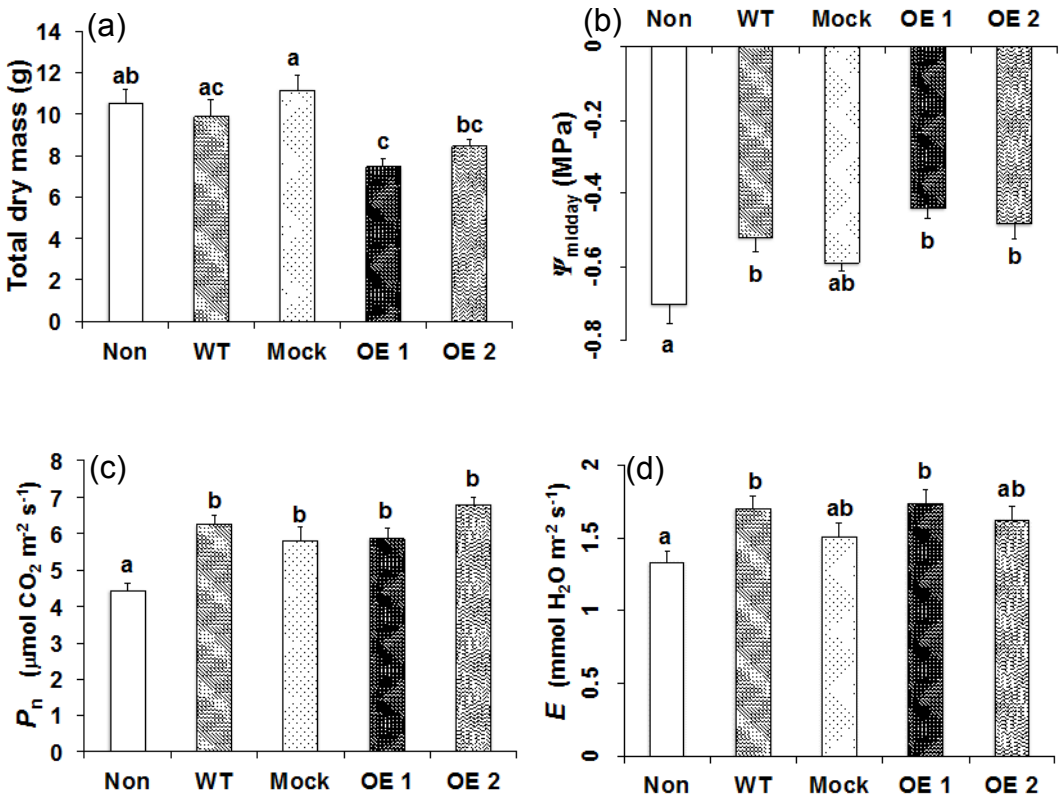


Figure 3.13 The effects of mycorrhization with *Laccaria bicolor* on (a) total dry mass, (b) midday shoot water potential Ψ_{midday}, (c) net photosynthetic rate P_n and (d) transpiration rate E of *Picea glauca* seedlings

The treatments were non-inoculated (Non), and mycorrhized with wild-type *L. bicolor* (WT), mock (Mock) and two *JQ585595*-overexpression strains (OE1 and OE2). Means (n = 6) ± SE are shown. Different letters indicate significant difference at $P \leq 0.05$ (ANOVA, Tukey's test).

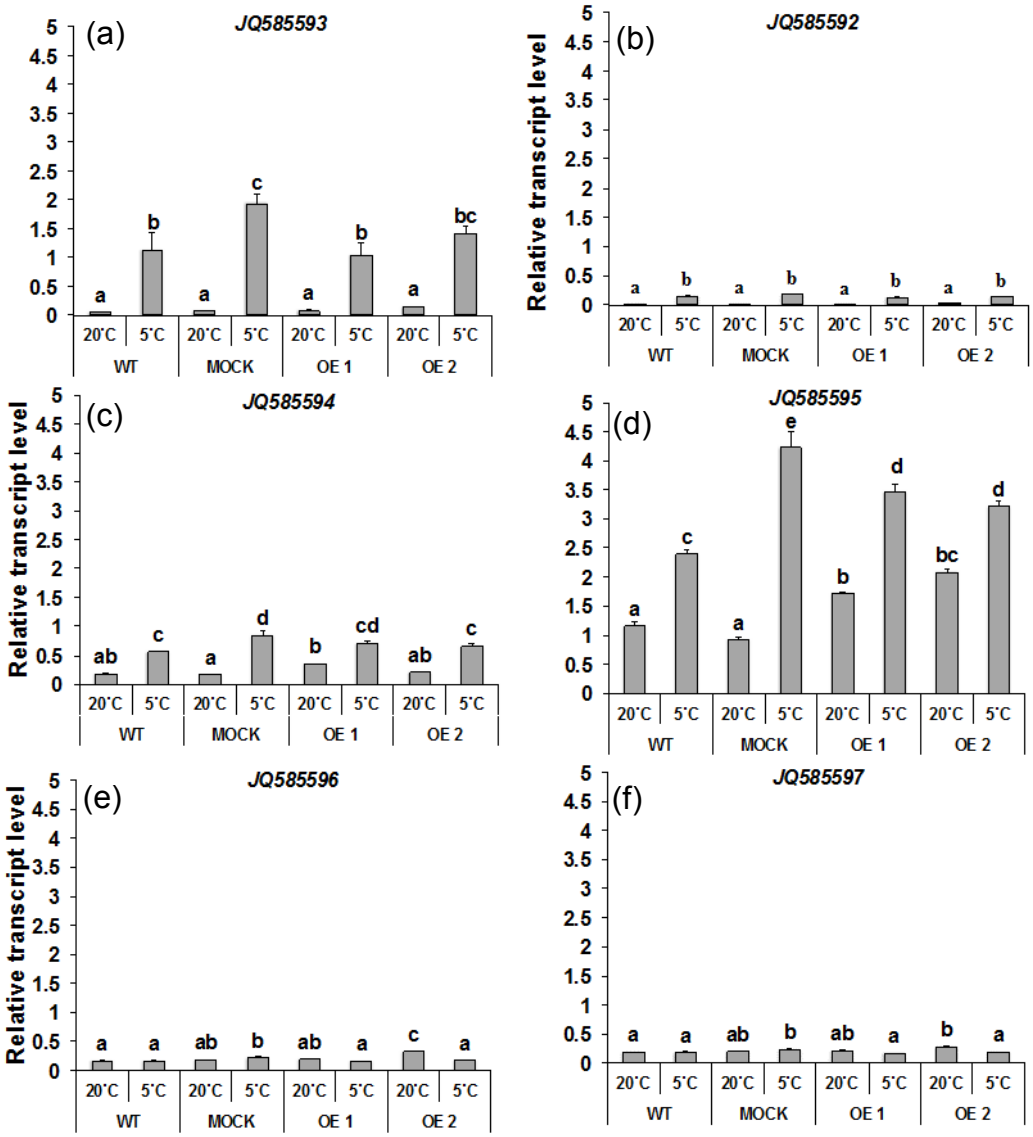


Figure 3.14 The relative transcript level of *Laccaria bicolor* MIPs in roots of *Picea glauca* mycorrhized with the wild-type (WT), mock (Mock), and two overexpression (OE1 and OE2) strains of *L. bicolor* and exposed to root temperature of 20°C and 5 °C

The transcript abundance of target MIPs was normalized to that of the reference gene *EF2*. Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 3 \pm$ SE).

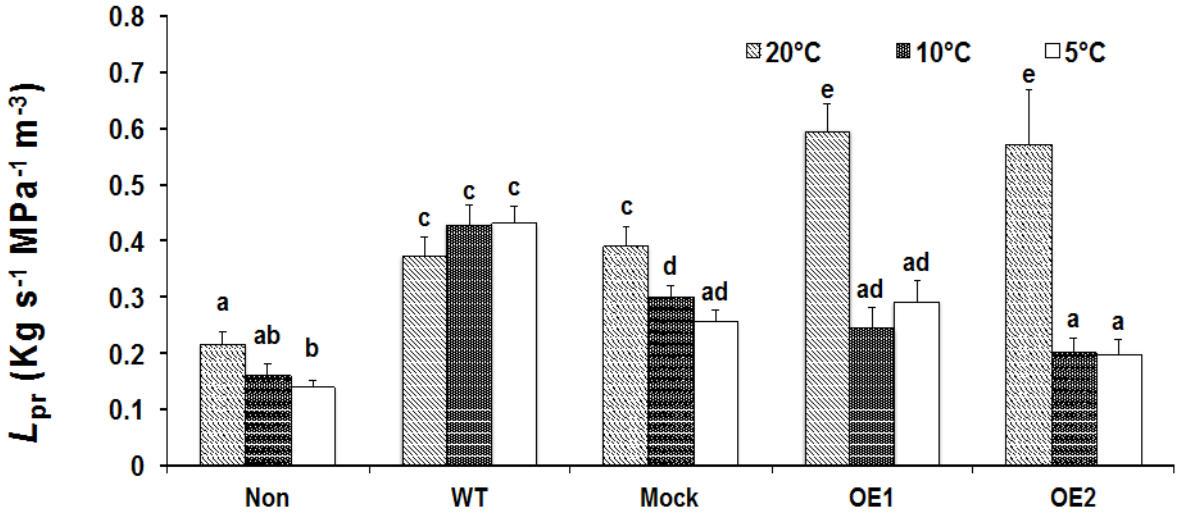


Figure 3.15 Root hydraulic conductivity (L_{pr}) in non-inoculated (Non) *Picea glauca* seedlings and in seedlings inoculated with the wild-type (WT), mock (Mock), and two overexpression (OE1 and OE2) strains of *Laccaria bicolor*
Means (n = 6) ± SE are shown. Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey's test).

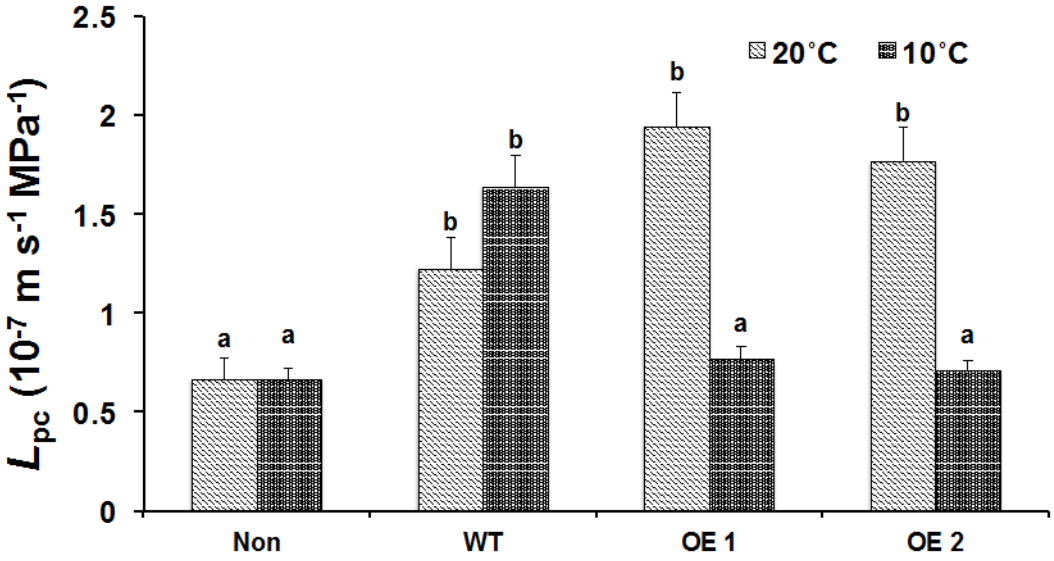


Figure 3.16 Cell hydraulic conductivity of root cortical cells (L_{pc}) in non-inoculated (Non) *Picea glauca* seedlings and in seedlings inoculated with the wild-type (WT), mock (Mock), and two overexpression (OE1 and OE2) strains of *Laccaria bicolor*

Means (n = 6) ± SE are shown. Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey's test).

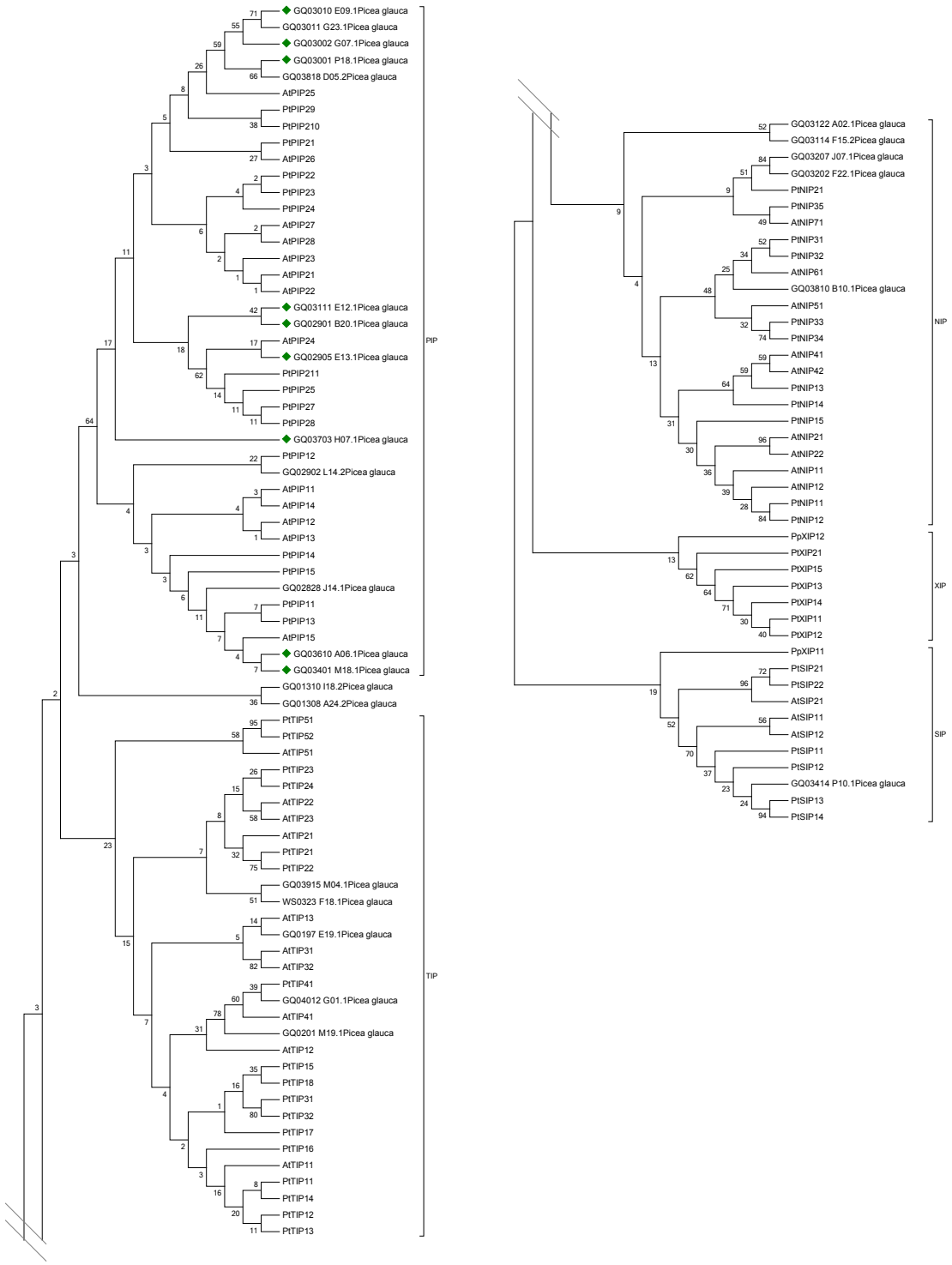


Figure 3.17 Phylogenetic analysis of putative *Picea glauca* MIPs using 36 MIPs of *Arabidopsis thaliana* and 57 MIPs of *Populus* as reference proteins
Deduced amino acid sequences were aligned using ClustalW, followed by analysis using neighborjoining in MEGA5. Green diamonds indicate the PIPs investigated in this study.

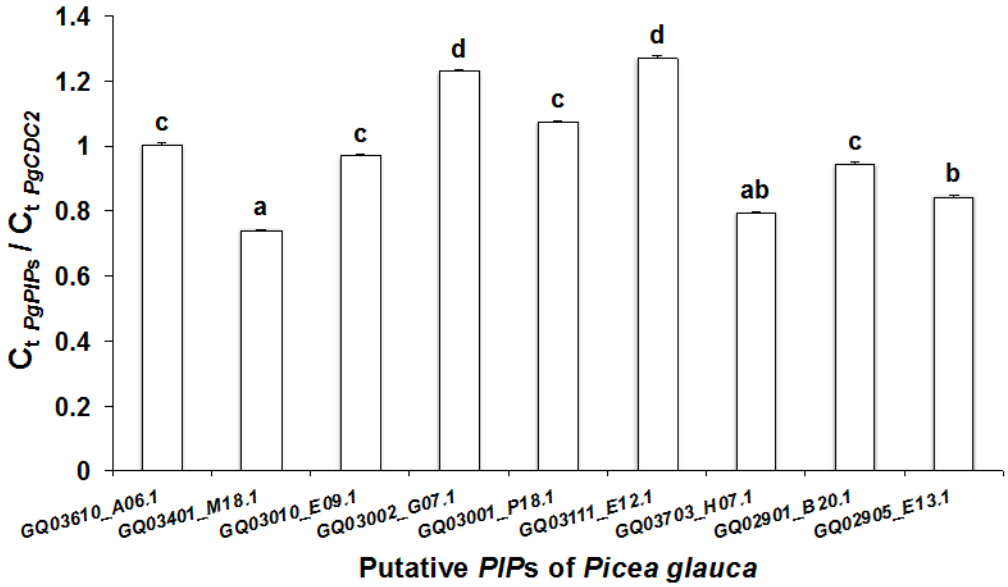


Figure 3.18 Relative transcript abundance of nine *Picea glauca* PIPs in non-inoculated root tips at 20°C

Comparative quantification of standard curve method was used to examine the relative transcript abundance of PIPs normalized to that of the reference gene *PgCDC2* for three biological replicates in SYBR™ Green qPCR assay. Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 3 \pm$ SE).

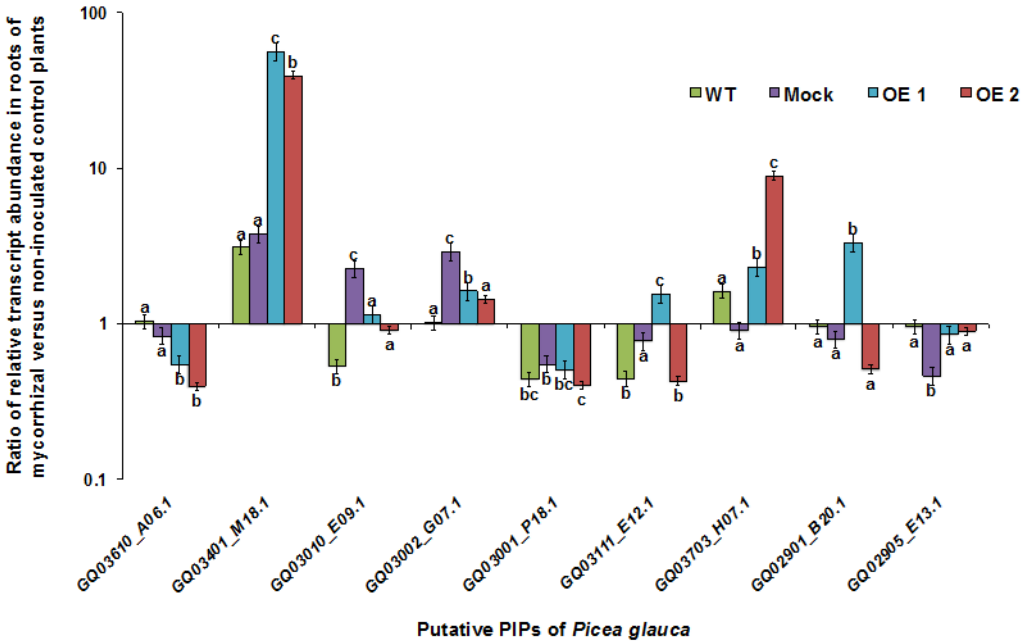


Figure 3.19 Changes in transcript abundance of nine putative PIP genes in *Picea glauca* root tips due to mycorrhizal inoculation with the *Laccaria bicolor* wild-type (WT), mock (Mock), and two overexpression strains (OE1 and OE2) Relative transcript abundance was measured in qPCR assay using standard curve method of comparative quantification with *PgCDC2* as the reference gene. Fold change is displayed on the log scale. Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 3 \pm$ SE). Letter "a" indicates no significant difference from non-inoculated control.

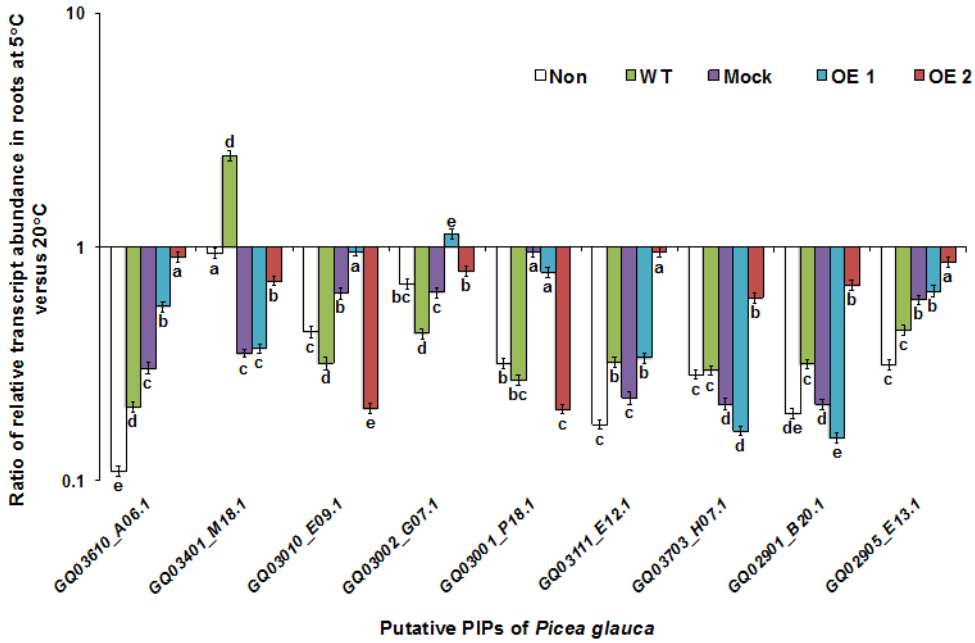


Figure 3.20 Changes in transcript abundance of nine putative PIP genes in *Picea glauca* non-inoculated (Non) root tips and in root tips mycorrhized with the *Laccaria bicolor* wild-type (WT), mock (Mock), and two overexpression strains (OE1 and OE2) due to temperature decrease from 20°C to 5°C
Relative transcript abundance was measured in qPCR assay using standard curve method of comparative quantification with *PgCDC2* as the reference gene. Fold change is displayed on the log scale. Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 3 \pm$ SE). Letter "a" indicates no significant difference from 20°C control.

4 TRANSCRIPT PROFILING OF AQUAPORINS DURING BASIDIOCARP DEVELOPMENT IN *LACCARIA BICOLOR* ECTOMYCORRHIZAL WITH *PICEA GLAUCA*

4.1 Introduction

The sporocarp is a tissue-like structure (pseudoparenchyma) produced by filamentous fungi that is easily distinguishable from other commonly observed hyphal subnetworks and ectomycorrhizal (ECM) hyphal structures (Nehls & Dietz 2014). Sexual reproductive processes involving sporocarp formation and spore production help maintain species fitness and expand ecological distribution of many basidiomycete and ascomycete mycorrhizal fungi (Smith & Read 2008; Watkinson 2008; Fortin & Lamhamedi 2009). Sporocarps are also considered to be good indicators of forest health (Egli 2011). Due to the ecological and commercial importance of fungal fruiting bodies, the processes of sporocarp formation have been often investigated in economically important species from the perspectives of fungal reproduction, systematics, ecological distribution and biodiversity as well as industrial production techniques (Yun & Hall 2004; Fortin & Lamhamedi 2009).

The sequence of events involved in sporocarp formation comprises hyphal knot formation, initial aggregation, bipolar fruiting body primordium pinning, primordium differentiation with pileus (young cap) and stipe, fruiting body maturation with expanded cap and elongated stipe, and spore formation (Kües & Liu 2000). The vegetative mycelial tissue undergoes a sequential series of cell mitotic divisions, differentiation, elongation and meiosis to reach the final stage of spore production (Massicotte *et al.* 2005). Sporocarp formation is controlled by complex environmental factors that include temperature, light, humidity, soil nitrogen and carbohydrates, and growth, photosynthate supply and their phenological dynamics of host plants (Last *et al.* 1984; Fortin & Lamhamedi 2009; Teramoto *et al.* 2012; Le Tacon *et al.* 2013). Molecular approaches are providing new insight into processes that are important for sporocarp development. For example, it was found that the gene encoding cyclopropane fatty acid synthase was essential for sporocarp initiation in *Coprinopsis*

cinerea (Liu *et al.* 2006), while sporocarp development was regulated by a gene encoding chitin deacetylase in *Flammulina velutipes* (Yamada *et al.* 2008) and glyceraldehyde-3-phosphate dehydrogenase genes in *Pleurotus ostreatus* (Tasaki *et al.* 2014).

Recent genomic and transcriptomic studies have unveiled profound changes in gene regulation during sporocarp formation of several saprophytic (Nowrousian & Kück 2006; Joh *et al.* 2007; Chum *et al.* 2011; Morin *et al*. 2012; Yu *et al*. 2012; Cheng *et al*. 2013; Traeger *et al.* 2013; Rahmad *et al*. 2014) and ECM fungi including *Tuber borchii* (Gabella *et al.* 2005), *Tuber melanosporum* (Martin *et al.* 2010), and *Laccaria bicolor* (Martin *et al*. 2008) (Nowrousian 2014). These studies identified key players such as mating-type genes and genes encoding transcription factors, photoreceptors, small secreted proteases, and enzymes of sulfur metabolic pathways. Genomic investigations have also revealed that genes encoding major facilitator superfamily transporters (MFS), aquaporin-related major intrinsic proteins (MIP), and amino acid permeases were highly upregulated during mycorrhizal establishment and in the mycorrhizal structures of the mantle and Hartig net (Fajardo-López *et al.* 2008; Lucic *et al.* 2008; Martin *et al.* 2010; Hacquard *et al.* 2013). Higher expression of some MIPs also has been reported recently for sporocarp stipes relative to other hyphal structures (Nehls & Dietz 2014). In mycorrhizal fungi, MIPs may play important roles in resource acquisition by the growing front of substrate mycelia, in regulating water relations of the mycorrhizal association (Xu *et al*. 2015), as well as in the resource-demanding developmental events involving substantial hyphal fusion, such as sporocarp formation (Nehls & Dietz 2014). The impacts of transcriptional regulation of these transporters on sporocarp formation processes remain a fascinating area to be explored.

Given the high demand of substrate translocation due to rapid cell division and expansion, aquaporin-mediated transport is likely to play a major role in sporocarp formation of mycorrhizal fungi. Fungal MIPs comprise four distinct groups: orthodox fungal water channels, fungal aquaglyceroporins, facultative fungal aquaporins and fungal XIPs (Xu *et al.* 2013). Aquaporins of several mycorrhizal fungi demonstrated versatile transport capacities, including the capacity to transport water, glycerol, urea, ammonia, CO_2, NO and H_2O_2 (Dietz *et al*. 2011; Navarro-Ródenas *et al*. 2012; Li *et al*.

2013; Xu *et al*. 2015; Navarro-Ródenas *et al*. 2015). Relative transcript abundances corresponding to these aquaporins were impacted by mycorrhizal formation (Dietz *et al.* 2011; Navarro-Ródenas *et al.* 2012; Li *et al.* 2013; Hacquard *et al.* 2013), and their functions in plant-fungal interactions included signaling during symbiosis establishment (Navarro-Ródenas *et al*. 2015) and water transport in mycorrhizal roots (Xu *et al*. 2015).

L. *bicolor* is an excellent ECM model fungus to study the role of aquaporins in sporocarp formation since it readily produces basidiocarps under controlled growth conditions with a variety of host plants (Godbout & Fortin 1990) and its aquaporins have been well characterized (Dietz *et al*. 2011; Xu *et al*. 2015). ECM associations between *L. bicolor* and trees of *Picea*, *Pinus*, *Pseudotsuga*, *Populus* and *Betula* species are widely distributed in North American temperate forests (Mueller 1992). Although *L. bicolor* has saprophytic capacity, it is considered to be a strictly symbiotic species because it produces basidiocarps only from the host-derived carbohydrates (Lamhamedi *et al.* 1994; Fortin & Lamhamedi 2009). The initiation and development of its basidiocarps proceeds through the stages typical of a basidiomycete macrofungus species, which can be driven by short photoperiod and photosynthate flow from the host, and are influenced by phenology of the host tree as well as climatic and soil factors such as low level of nitrogen and phosphorus fertilization (Godbout & Fortin 1990; Fortin & Lamhamedi 2009). Different stages of sporocarp formation involve different cellular and molecular processes that require transcription of different suites of genes. Since some of these stages are characterized by rapid growth, they likely involve increased transport of water and other small molecules. In the present study, we hypothesized that the aquaporins that are involved in transport of water and small neutral signaling molecules such as CO_2 and NO will be upregulated during the stages of basidiocarp development involving rapid cell expansion. Therefore, we characterized the different stages of basidiocarp formation in *L. bicolor* mycorrhizal with *Picea glauca* and examined the responses of gas exchange in *P. glauca* seedlings in response to mycorrhization. For each basidiocarp developmental stage, we investigated changes in the transcript abundance of the genes encoding the aquaporin family in *L. bicolor*. The transcript profiles corresponding to the aquaporins with demonstrated transport properties enabled us to infer the relative degree of

involvement and potential roles of these aquaporins at the different developmental stage of basidiocarp formation.

4.2 Materials and Methods

4.2.1 Fungal and plant culture

Picea glauca (Moench) Voss seeds (National Tree Seed Centre, Canadian Forest Service, Fredericton, NB, Canada) were surface-sterilized using diluted Tween-20 solution (*ca.* 1:1000 [v/v]) and 20% (v/v) commercial bleach (1% [v/v] sodium hypochlorite) according to Groome *et al*. (1991). Seeds were stratified at 4ºC for two weeks and germinated at 20ºC on sterile, moistened crepe cellulose paper (Kimpak; Kimberly-Clark, Mississauga, ON, Canada). One week after germination, the seedlings were transplanted into autoclaved peat moss: vermiculite (2:1) in sterilized 170 ml Spencer-Lemaire root trainers (Spencer-Lemaire Industries Ltd., Edmonton, AB, Canada) covered with plastic domes. The seedlings were grown in a controlled environment growth room with 16 h photoperiod, 22 /18ºC (day/night) temperature, 400 μmol m^{-2} s^{-1} photosynthetic photon flux density, and 50-60% relative humidity.

Laccaria bicolor (Maire) P.D. Orton strain UAMH8232 was made available by the University of Alberta Microfungus Collection and Herbarium, and cultured in liquid modified Melin-Norkans (MMN) medium (Marx 1969; Pham *et al*. 2004) at 20°C with shaking at 0.8 x *g* for four weeks. The culture was homogenized in a blender to make the liquid inoculum of OD_{600} = 1.5. Immediately after transplanting, seedlings were inoculated by injecting 10 mL of homogenized liquid inoculum into the sterilized potting mix. Autoclaved fungal-free liquid MMN was used to treat the non-inoculated control seedlings. After one month, a second inoculation was conducted by applying 10 mL of the respective inoculum onto the roots that had developed along the inner wall of the root container. Eighteen plants each were maintained for the inoculated and non-inoculated treatments. The seedlings were spatially separated to minimize the possibility of cross-contamination. The plants were grown in a completely randomized design, with root containers randomly rearranged every three days to minimize the impact of growth chamber heterogeneity on plant growth.

Two months after the second inoculation, mycorrhizal colonization was examined by observing extraradical hyphae around the root tips and directly estimating the number

of mycorrhizal root tips (Brundrett *et al.* 1996). This was followed by microscopic examination of six root tips randomly sampled from each of the five inoculated and five non-inoculated seedlings, respectively (n = 5). In total, 30 root tips were sampled for each treatment. The root segments for microscopy were fixed in formalin-acetic acid-alcohol (FAA), embedded in paraffin and sectioned with a microtome. The 5-µm-thin sections were stained with toluidine blue. The sectioned samples were observed under a Zeiss AXIO compound light microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany) with a MacroFire Digital Camera (Optronics, Goleta, CA, USA). Colonization rate for each examined seedling was calculated as the percentage of mycorrhizal root tips out of the total root tips randomly selected for sectioning.

4.2.2 Basidiocarp development

About two months after the second inoculation, basidiocarps started to continuously emerge in the containers with inoculated seedlings. Basidiocarps with normal phenotype were randomly selected to track the growth. The duration of each developmental stage and the height of the entire aboveground basidiocarp were recorded (n = 10). Fresh mass and dry mass (oven-dried at 80°C for 72 hours) of basidiocarps were measured at each stage to determine the tissue water content (n = 6). Since a distinct structural separation of the cap and the stipe occurred in Stage 5 and 6, caps and stipes were sampled separately for water content determinations at these two stages.

Spore prints were collected by attaching a piece of gill from the mature basidiocarp onto a glass microscope slide, covering with a slide cover and placing it in a sealed plastic bag for 48 h. The images of spores were captured under a Zeiss AXIO compound light microscope. Spore size and density were determined using ImageJ computer software (V.1.44o; Schneider *et al.* 2012).

4.2.3 Measurements of gas exchange

Net photosynthetic rate (P_n), transpiration rate (E) and stomatal conductance (g_s) of lateral branches of *P. glauca* seedlings were measured at approximately 09:00-12:00 using a Licor-6400 portable photosynthesis system with a 2x3 cm^2 red-blue light chamber (LI-COR, Lincoln, NB, USA). Subsequent to these measurements, needles were collected, scanned and the total surface area of the needles calculated using

ImageJ. The P_n, E and g_s were expressed as a function of needle surface area. The P_n was divided by E to calculate transient water use efficiency (*WUE*) (Equation 4.1). The measurements were carried out for six plants of non-inoculated control (Non-inoculated), six associated with *L. bicolor* at ECM vegetative stage (ECM with vegetative mycelia), and six associated with basidiocarp-bearing *L. bicolor* (ECM bearing basidiocarps of Stage 3-5), respectively ($n = 6$).

Equation 4.1 Calculation of transient *WUE*

$$WUE = P_n / E$$

4.2.4 Quantification of transcript abundance of Laccaria bicolor *MIPs during basidiocarp development*

Basidiocarps collected for quantitative RT-PCR (qRT-PCR) were flash-frozen in liquid nitrogen and ground to a fine powder prior for total RNA extraction using the RNeasy Plant Mini extraction method according to the manufacturer's instructions (Qiagen, Valencia, CA, USA), with RLC buffer. The samples were collected on days 1, 6, 9, 13, 15 and 22, representing Stages 1-6, respectively ($n = 4$). At Stages 5 and 6, caps and stipes were collected separately; hymenium tissue in caps was collected at Stage 6. First strand cDNA was synthesized from 0.5 µg of total RNA using Superscript II (Life Technologies, Carlsbad, CA, USA). Primers were designed using Primer Express 3.0 (Applied Biosystems, Life Technologies) (Table 4.1). Each 10 µL reaction consisted of 2.5 µL of 1.6 µM primer, 2.5 µL of 5 ng/µL cDNA and 5 µL of SYBR Green qPCR Mastermix as outlined in Xu *et al*. (2015). Final concentration for primers and cDNA was 0.4 µM and 1.25 ng/µL, respectively. Thermal cycling conditions were 95°C for 2 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, carried out in QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Life Technologies).

Transcript abundance corresponding to the six *L. bicolor* aquaporins – *JQ585592, JQ585593, JQ585594, JQ585595, JQ585596,* and *JQ585597* – was quantified in basidiocarps at Stages 0 to 6 using the standard curve method (Pfaffl 2004), with *Translation Elongation Factor 2* (*EF2*) as the reference gene. *EF2* was selected as an appropriate reference gene as it showed statistically invariant transcript abundance over all samples that were compared ($P = 0.91$). The standard curve template was prepared as a series of dilutions of the mixture of the PCR amplicons of each analysed

genes, with the template concentration ranging from $1.6x10^2$ to $1.6x10^8$ molecules mL^{-1} for each gene.

4.2.5 Statistical analysis

Descriptive statistics and ANOVA were conducted using Origin 8.0 (OriginLab, Northampton, MA, USA). A Tukey test was used to compare means for statistically significant differences ($P \leq 0.05$).

4.3 Results

4.3.1 Development of Laccaria bicolor *basidiocarps*

Two months after the second inoculation, all of the inoculated *P. glauca* seedlings had become mycorrhizal with *L. bicolor*. Abundant mycelia grew in the rhizosphere, and the mantle and Hartig net structures were well developed in the root tips of inoculated seedlings (Fig. 4.1 a). The root tip colonization rate was 93.3 ± 4.1% (mean ± SE, n = 5). In contrast, no ECM structures were observed in the roots of non-inoculated *P. glauca* seedlings.

Basidiocarps started to emerge in the containers with inoculated seedlings two months after the second inoculation. Abundant mycelia were present in the upper soil layers around the roots (Fig. 4.1 a).

The first visible structure was the initial aggregate of bipolar primordia (Fig. 4.1 b) at the mean height of 2.9 mm (Fig. 4.2 a). On an average, it took five days for the initial aggregate bipolar primordium (defined as Stage 1 in this study; Fig. 4.1 b, Fig. 4.2 a) to develop into the primordium with distinct cap and stipe (Stage 2, Fig. 4.1 c) that was as twice as tall as the initial bipolar primordium in Stage 1 (Fig. 4.2 a). After the pinning primordium in Stage 1 (Fig. 4.1 b), bipolar growth led to the formation of distinct pileus and stipe structures in Stage 2 (Fig. 4.1 c). Caps were orange-brown in color and turned darker as they grew, whereas stipes were creamy white. It took another three days to enter the next stages of maturation, distinguished by swollen stipe base that stored a considerable amount of liquid in Stage 3 (Fig. 4.1 d), followed by rapid stipe elongation in Stage 4 four days later (Fig. 4.1 e), and cap expansion and opening in Stage 5 two days after Stage 4 (Fig. 4.1 f). The stipe consisted of elongated polarized

tubular-structured cells (Fig. 4.1 e). The next day, the development reached Stage 6 – the final stage – of basidiospore formation in the hymenium of a fully opened cap, where the hymenium developed underneath with distantly spaced, smoothly margined gills (Fig. 4.1 g). The stipe base became less swollen, but was distinctly bulbous. This stage continued for more than 10 days until the basidiocarps dehydrated and wilted. No veil was formed. The height of basidiocarps increased by 10-fold between Stage 2 (day 6) and the beginning of Stage 6 (day 16), and reached a maximum of *ca.* 92 mm on day 22 (Fig. 4.2 a). The diameter of caps expanded from about 3-5 mm at Stage 3 (Fig. 4.1 d) to 35-40 mm at Stage 6 after full expansion (Fig. 4.1 g). The majority of the basidiocarps developed into maturity and produced spherical, light-colored spores (Fig. 4.1 g). The ornamented spore wall appeared smooth when examined under the light microscope at 630 x magnification. The thickness of the protective walls was 0.08 - 0.15 µm, with two distinct layers (Fig. 4.1 g). The average diameter of the spores was 0.544 ± 0.013 µm (mean ± SE, n = 30). The color of spore print was creamy white to light purple. Spore density was higher than 5.5×10^5 mm^{-2}. Several basidiocarps stopped growing at Stages 2 and 3, in cases where more than one basidiocarp was present per container.

Tissue water content was about 85 - 86% at Stages 0-2, followed by an increase in Stages 3-5, and a decrease at Stage 6 (Fig. 4.2 b). In the two latter stages, the stipes contained less water than the caps, however the difference was not significant.

4.3.2 Transcript profiling of MIPs during the development of Laccaria bicolor *basidiocarps*

The transcript profiles of *L. bicolor* aquaporins *JQ585592*, *JQ585593*, *JQ585594*, *JQ585595*, *JQ585596* and *JQ585597* showed differential transcript abundance at different developmental stages (Fig. 4.3). Of the aquaporins that were examined, *JQ585595* was the most highly expressed in vegetative mycelia (Stage 0, Fig. 4.1 a), followed by *JQ585594*, *JQ585596* and *JQ589957*; the expression levels of *JQ585592* and *JQ585593* were low (Fig. 4.3). All aquaporin genes were upregulated during Stage 1 of basidiocarp formation, with the most significant fold increase in *JQ585592*, *JQ585596* and *JQ585597* (Fig. 4.3 a, c). *JQ585592*, the orthodox fungal aquaporin of Cluster I, was upregulated 78-fold in Stage 1, 670-fold in Stage 2 and more than 1200-fold in Stage 3 relative to Stage 0 (Fig. 4.3 a). The expression of *JQ585592* was

maintained at a high level in Stage 4 and in cap tissues of Stage 5 and 6, but significantly declined in the stipe tissues during the latter two stages. *JQ585593*, the fungal aquaglyceroporin of Cluster II, was upregulated to a lower extent compared with *JQ585592*, and the expression of this aquaporin was higher in the cap than in the stipe at Stages 5 and 6 (Fig. 4.3 b). The three aquaporins of Cluster III (facultative fungal aquaporins) – *JQ585595, JQ585596* and *JQ585597* – showed similar expression patterns through the basidiocarp development, with *JQ595595* being consistently higher than the other two aquaporins (Fig. 4.3 c). The expression levels of these three aquaporins declined in Stage 2 and increased again in Stage 3. The expression of *JQ585595* and *JQ585596* was significantly higher in caps than in stipes. The expression levels of *JQ585596* and *JQ585597* did not significantly change from Stage 4 to the cap tissues of Stage 5 and 6, whereas *JQ585595* was upregulated in the cap tissues of Stages 5 and 6, with the highest expression level in the hymenium tissue of Stage 6 (Fig. 4.3 c). The expression of *JQ585594* did not change to as great an extent as that of the other three facultative fungal aquaporins (Fig. 4.3 c). *JQ585592* and *JQ585595* were the two most highly expressed aquaporins in the later stages of the basidiocarp development.

4.3.3 Seedling morphology and gas exchange

Basidiocarps emerged when the inoculated seedlings were three-month old and about 15.6 ± 0.78 cm high (mean ± SE, n = 12), and the terminal buds had set in most of the inoculated seedlings. The non-inoculated seedlings started to set terminal buds about two weeks later and reached an average height of 16.7 ± 0.90 cm (mean ± SE, n = 12), which was not significantly different from the inoculated seedlings (P = 0.33). No clear correlation was observed between the timing of bud set and the timing or amount of basidiocarp formation.

Mycorrhization significantly increased P_n and a further increase occurred after the emergence of basidiocarps (Fig. 4.4 a). Compared with non-inoculated seedlings, g_s was higher in the seedlings that contained only vegetative mycelia (Fig. 4.4 b), whereas *WUE* of seedlings with basidiocarp-bearing *L. bicolor* was significantly higher compared with non-inoculated seedlings and the inoculated seedlings that contained only vegetative mycelia (Fig. 4.4 c).

4.4 Discussion

Basidiocarp formation is influenced by carbon flow from the host plant to the mycorrhizal fungus and, therefore, must be strongly linked to the growth of the associated host plants (Fortin & Lamhamedi 2009; Egli 2011). *P. glauca* seedlings inoculated with *L. bicolor* had higher P_n compared with non-inoculated seedlings, suggesting higher carbon demand due to mycorrhization. The formation of basidiocarps further enhanced P_n, indicating the role of basidiocarps as a carbon sink. In addition, the formation of basidiocarps also enhanced *WUE* (Fig. 4.4 c), supporting the notion that more efficient plant photosynthesis could be an indicator for strong carbohydrate demand of colonized roots (Nehls *et al*. 2010). Cessation of shoot elongation in seedlings and setting of terminal buds likely resulted in increased carbohydrate supply to *L. bicolor* to support greater fungal growth by shifting source-sink dynamics in the plant to favor translocation of photosynthates to roots and onwards to the fungal hyphae. The dependence of basidiocarp formation on photosynthesis was also clearly demonstrated in *L. bicolor* mycorrhizal with *P. strobus* (Lamhamedi *et al*. 1994). A more recent study using ^{14}C-labeling showed that recently-assimilated photosynthates were derived from the host plant, and then transferred and translocated to the basidiocarp of ECM fungus *Laccaria amethystina* (Teramoto *et al*. 2012). Photosynthate supply from the host plant would allow sufficient hyphal growth for the formation of hyphal knot and initial aggregation, which are two prerequisites for initiation of basidiocarps and subsequently lead to primordium pinning and differentiation. Future studies should evaluate the impacts of mycorrhization and basidiocarp formation on plant growth, by examining dry mass accumulation and allocation, total organic carbon reserve in different tissues, tissue density, as well as carbon fixation efficiency. The exact amount of carbohydrates transferred to mycorrhizal fungi and soil should also be investigated to better understand the roles of mycorrhiza in ecosystem carbon sequestration.

In this study, the complete life cycle and basidiocarp morphology of *L. bicolor* UAMH8232 was consistent with those of the previously published *L. bicolor* strain CRBF 0101 (Godbout & Fortin 1990). The change in morphological characteristics in Stages 1 and 2 suggests that the basidiocarps underwent considerable cell division and cell differentiation, which is known as the switch from mycelial extension to the

successive development of basidiocarp primordia (Kües & Liu 2000). Rapid cell division likely contributed to the bipolar growth prior to cell differentiation in Stage 2, during which cell differentiation led to the formation of distinct pileus and stipe (Fig. 4.1 c). Such cytological events have been reported during the fruiting of basidiomycete model species for mushroom development such as *Coprinus cinereus* (Kües 2000), and also of *Laccaria spp*. (Massicotte *et al*. 2005). While cell expansion is thought to slow or cease during bipolar primordium development, cells usually rapidly elongate in all directions in the subsequent steps of fruiting. This likely leads to the increased volume; in contrast, cell division probably becomes quiescent during this time (Kües 2000; Kües & Liu 2000; Massicotte *et al*. 2005). Both the cap and stipe expanded further during Stage 3, and the swelling at the base of the stipe coincided with a significant increase in water content, which is commonly associated with turgor pressure buildup that can act as a driving force for cell expansion. Under the controlled environment conditions of our study, basidiocarp development was completed within 22 days. Given the rapid growth that occurs at certain times during *L. bicolor* basidiocarp formation, we hypothesized that aquaporin-mediated movement of water and other small solutes may play important roles in these growth processes. Accordingly, we conducted detailed transcript profiling of the *L. bicolor* aquaporin gene family over the course of basidiocarp formation, in order to better understand possible roles for each of these genes in the sequential steps of basidiocarp development.

In basidiomycetes, the switch from vegetative to reproductive growth and subsequent fast growth of the basidiocarps requires a major shift in metabolism as well as an efficient transporting system and therefore, presumably involves massive reprogramming of gene expression (Martin *et al*. 2008). In *L. bicolor* S238N, six aquaporins have been described, some of which demonstrated transport capacity for water, urea, glycerol or ammonia (Dietz *et al*. 2011, Table 4.1). Recently, it was reported that *Lacb1:392091*, *Lacbi1:247946 and Lacbi1:391485* were upregulated in a single mature sporocarp compared with the entire ECM mycelium (Nehls & Dietz 2014). In the dikaryotic strain *L. bicolor* UAMH8232, we also reported six aquaporins, JQ585592-97, which correspond to five of the six genes in *L. bicolor* S238N (Xu *et al*. 2013, Table 4.1). We demonstrated that most of these *L. bicolor* aquaporin genes were upregulated at different points during basidiocarp development, suggesting their involvement in water influx, nutrient uptake and potentially signaling during

basidiocarp formation (Fig. 4.3). Transcript abundances corresponding to Cluster I, II and III aquaporins (*sensu* Xu *et al*. 2013) differed between clusters, while the four aquaporins within Cluster III showed considerable coregulation. These findings suggest that Cluster I, II and III aquaporins play non-redundant roles in basidiocarp development. This is consistent with their substrate transport capacities: whereas all four Cluster III aquaporins demonstrate significant water permeability (Xu *et al.* 2015), the Cluster I JQ585592 demonstrates the most significant CO_2 transport capacity and is also a strong channel for NO and a moderate channel for H_2O_2; it was shown to be essential to the development of the mycorrhizal symbiosis (Navarro-Ródenas *et al.* 2015). The Cluster II JQ585593 does not show significant water permeability, but rather moderate CO_2 transport capacity.

JQ585592 reached the peak of transcript abundance in Stage 3 and maintained similarly high expression in caps during Stages 5 and 6 (Fig. 4.3 a). The corresponding aquaporin in strain S238N, *i.e.*, *Labci1:392091*, was also found to be the most highly expressed aquaporin in the sporocarp (Nehls & Dietz 2014). The high expression level of the CO_2 transporting JQ585592 could be related to the cell differentiation that takes place in caps to produce the gilled spore-bearing hymenium. Many fungal species have sensing mechanisms to determine the concentration of surrounding CO_2 and respond effectively to this environmental cue (Cummins *et al*. 2014). CO_2 has been reported to stimulate growth of AM fungi (Bécard & Piché 1989), increase hyphal extension rate of mushroom mycelia (Wiegant *et al*. 1992) and regulate the timing of saprotrophic mushroom formation (Stamets 2000). In natural conditions, most sporocarps of ECM fungal species are produced in late summer and autumn (Gévry & Villeneuve 2009), which may correspond with altered CO_2 levels in the ecosystem. In many tree species, late summer and autumn coincides with the cessation of shoot growth as well as a second peak of seasonal root growth, which is characterized by enhanced root respiration (Johnson-Flanagan & Owens 1985). In spruce-dominated conifer forests, it has been reported that declining air temperatures and litter fall also contribute to increased ratio of soil respiration versus ecosystem respiration in the autumn (Davidson *et al*. 2006). Under controlled cultivation conditions, the decrease in CO_2 can serve as signal for basidiocarp initiation of saprophytic macrofungi (Stamets 2000), which is used in practice to induce the growth of basidiocarps outside of the substrate and to ensure the efficient spore dissemination. This suggests a possibility that the

change in intercellular CO_2 concentration may serve as a signal for basidiocarp development. The upregulation of *JQ585592* could contribute to the enhancement of CO_2 sensitivity as a signaling cue in carbonic anhydrase and fungal adenylyl pathways during basidiocarp formation and maturation. CO_2 signaling is known to play crucial roles in several pathways for fungal cell division and differentiation (Bahn & Mühlschlegel 2006; Hall *et al*. 2010; Cummins *et al*. 2014). CO_2 and H_2O, both of which are likely to be transported through aquaporins, are converted into HCO_3^- and H^+ in the presence of Zn^{2+} and carbonic anhydrase. Adenylyl cyclase is activated by HCO_3^- and further catalyzes ATP to cAMP, which has been shown to promote hyphal formation in *Candida albicans*, and capsule formation and sexual reproduction in *Cryptococcus neoformans* (Bahn & Mühlschlegel 2006). The resulting H^+ lowers pH of the compartment in which it is formed, which favored the late stage of mating (Bahn & Mühlschlegel 2006). In addition, lower pH was an external acidifying stimulus for modification and extension of the cell wall, which is essential for cellular growth and differentiation, and also has key roles in intercellular communication and carbohydrate storage (Latgé 2007; Ruiz-Herrera 2012). Furthermore, rhizosphere acidification by respiratory CO_2 production and carbonic anhydrase promotes carbonate mineral weathering, which is considered to be among the three key weathering mechanisms of mycorrhizal fungi (Landeweert *et al.* 2001; Thorley *et al*. 2014). This process is enhanced by H^+ extrusion by ECM and arbuscular (AM) fungi (Koele *et al.* 2014). Increased abundance of JQ585592 could lead to more CO_2 transported, inducing the production of carbonic acid and H^+. One possible consequence would be promoted mineralization and increased mineral availability for both symbiont partners during the resource-demanding process of basidiocarp growth. JQ585592 is also capable of transporting H_2O_2 and NO, the two molecules that are considered to be important regulators in mycorrhizal symbiosis (Puppo *et al*. 2013; Navarro-Ródenas *et al*. 2015). The upregulation of *JQ585592* indicates that the signaling of these molecules could also play important roles in sporocarp formation and fungal sexual reproduction, which mechanisms remain to be explored. In addition, the subcellular localization of JQ585592 indicated the regulation could occur on not only plasma membrane but also on the membrane of reticulum endoplasm or vesicles (Table 4.1; predicted using TargetP, Emanuelsson *et al*. 2007). This suggests a possible involvement of JQ585592 in the continuous flow of secretion vesicles from the hyphal cell body to the growing hyphal tip in vigorously growing tissues, which is regulated by rearrangement of

cytoskeleton and considered essential for cell wall and membrane extension (Fischer *et al.* 2008).

JQ585595 was another significantly upregulated aquaporin, particularly in Stage 4 entire basidiocarps, Stage 5 caps and Stage 6 hymenium (Fig. 4.3 c). The aquaporin JQ585595 exhibits the highest water transport capacity of the *L. bicolor* aquaporins, and has been demonstrated to affect water transport in mycorrhizal roots of *P. glauca* (Xu *et al.* 2015). *JQ585595* was the most abundantly expressed aquaporin gene in the vegetative mycelium prior to basidiocarp formation. Its differential expression in Stage 1 and 2 indicated that water transport via JQ585595 might be less engaged during cellular events in Stage 2. The swelling of stipe base in Stage 3, the elongation of stipes in Stage 3-6, and the expansion of caps in Stage 5-6 were accompanied with higher tissue water content (Fig. 4.2 b) and upregulation of *JQ585595* (Fig. 4.3 c), suggesting a correlation between cell expansion and water uptake via this aquaporin. The increase in *JQ585595* expression could contribute to rapid water uptake, providing sufficient intracellular hydrostatic pressures to drive stipe elongation, cap expansion and opening during Stages 3-6. Moore-Landecker (2002) described that in the later stages, basidiospores were actively discharged from sterigmata-producing basidia when the drop of water had accumulated and reached its full size at the point where the basidiospore and sterigma joined. The increased expression of water-transporting JQ585595 in hymenium might contribute to this process of gill expansion and basidiospore release. In addition, the amino acid sequence of this aquaporin is *ca.* 94% identical with Lacbi1:391485, an aquaporin permeable to water, glycerol and ammonia in strain S238N (Dietz *et al.* 2011). *Lacbi1:391485* was also upregulated in the sporocarps (Nehls & Dietz 2014). This suggests that these aquaporins could play key roles in facilitating translocation of substrates, such as glycerol and ammonia, required for turgor pressure regulation, cell expansion and division.

JQ585594, and the pair of isoforms JQ585596 and 97 grouped into the same cluster of facultative fungal aquaporins with JQ585595 (Xu *et al*. 2013). All four Cluster III aquaporins showed coregulation, indicating the possibility of hetero-tetramerization. Interestingly, transcription of these water-transporting aquaporins was upregulated in Stage 1 but downregulated in Stage 2, in contrast to the CO_2, NO and H_2O_2 -transporting *JQ585592*, which was strongly upregulated at Stage 2. This suggests the

importance of transmembrane water transport to the initiation of primordium development in Stage 1, and the importance of the movement of CO_2 and other small neutral molecules to the events of cap and stipe differentiation in Stage 2. Although *JQ585593* was significantly upregulated in basidiocarp tissues in comparison to Stage 0, its transcript abundance was consistently low (Fig. 4.3 b). This aquaporin belongs to Cluster II aquaglyceroporins (Xu *et al*. 2013). It shows high sequence similarity to aquaporin Lacbi1:387054 from strain S238N, which demonstrated capacity to transport ammonium/ammonia, methylamine, glycerol, and/or urea (Dietz *et al*. 2011). It remains to be explored whether these aquaporins contribute to signaling or osmosis regulation as a transporter for glycerol or ammonia, or whether they play role in equilibration and subcellular routing and compartmentation of both aquaporin-transported substrates and carbohydrates to cell expansion and division as Maurel *et al*. suggested for plant cells (2009).

The differential aquaporin expression in caps and stipes indicated transmembrane transporting was more active in caps in Stage 5-6, and low expression of aquaporins indicated that transport pathways other than aquaporin-mediated transmembrane transport might dominate transport of water and other substrates in stipes (Fig. 4.3). In contrast, the transcript level of aquaporins in strain S238N were higher in stipe than in pileus of a single mature fruiting body (Nehls & Dietz 2014). To elucidate this difference between the strains of *L. bicolor*, more research with a larger sample size should be carried out to further refine and characterize developmental stages.

In conclusion, the significantly regulated transcript abundance of the *L. bicolor* aquaporin gene family, particularly *JQ585592* and *JQ585595*, implied their contributions in fungal cellular processes during the basidiocarp formation of *L. bicolor* mycorrhizal with *P. glauca* seedlings. Increased transcript abundance of *JQ585592* suggests the importance of possible signaling molecules such as CO_2 during basidiocarp development. Enhanced water transport attributed to increased *JQ585595* transcript abundance may contribute to cell expansion during the later stages of basidiocarp development. In-depth understanding of these fungal aquaporins can be improved by future studies of their subcellular localization, post-translational regulation as well as their precise roles in signaling and transporting processes in the context of fungal and mycorrhizal development.

4.5 References

Bahn Y, Mühlschlegel FA. 2006. CO_2 sensing in fungi and beyond. *Current Opinion in Microbiology* **9**: 572-578.

Bécard G, Piché Y. 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. *Applied and Environmental Microbioloy* **55**: 2320-2325.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. *Working with Mycorrhizas in Forestry and Agriculture*. Canberra, Australia: Australian Centre for International Agricultural Research, 196-208.

Cheng CK, Au CH, Wilke SK, Stajich JE, Zolan ME, Pukkila PJ, Kwan HS. 2013. 5'-Serial Analysis of Gene Expression studies reveal a transcriptomic switch during fruiting body development in *Coprinopsis cinerea*. *BMC Genomics* **14**: 195.

Chum WW, Kwan HS, Au CH, Kwok IS, Fung YW. 2011. Cataloging and profiling genes expressed in *Lentinula edodes* fruiting body by massive cDNA pyrosequencing and LongSAGE. *Fungal Genetics and Biology* **48**: 359–369.

Cummins EP, Selfridge AC, Sporn PH, Sznajder JI, Taylor CT. 2014. Carbon dioxide-sensing in organisms and its implications for human disease. *Cellular and Molecular Life Sciences* **71**: 831-45.

Davidson EA, Richardson AD, Savage KE, Hollinger DY. 2006. A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. *Global Change Biology* **12**: 230-239.

Dietz S, von Bülow J, Beitz E, Nehls U. 2011. The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: lessons for symbiotic functions. *New Phytologist* **190**: 927-40.

Egli S. 2011. Mycorrhizal mushroom diversity and productivity—an indicator of forest health? *Annals of Forest Science* **68**: 81-88.

Emanuelsson O, Brunak S, Heijne G, Nielsen H. 2007. Locating proteins in the cell using TargetP, SignalP, and related tools. *Nature Protocols* **2**: 953-971.

Fajardo-López M, Dietz S, Grunze N, Bloschies J, Weiß M, Nehls U. 2008. The sugar porter gene family of *Laccaria bicolor*: function in ectomycorrhizal symbiosis and soil-growing hyphae. *New Phytologist* **180**: 365-378.

Fischer R, Zekert N, Takeshita N. 2008. Polarized growth in fungi–interplay between the cytoskeleton, positional markers and membrane domains. *Molecular Microbioloy* **68**: 813-826.

Fortin JA, Lamhamedi MS. 2009. Ecophysiology of sporocarp development of ectomycorrhizal basidiomycetes associated with boreal forest gymnosperms. In: Khasa D, Piché Y, Coughlan AP (eds) *Advances in mycorrhizal science and technology*. Ottawa, Canada: NRC Research Press, 161-173.

Gabella S, Abbá S, Duplessis S, Montanini B, Martin F, Bonfante P. 2005. Transcript profiling reveals novel marker genes involved in fruiting body formation in *Tuber borchii*. *Eukaryotic Cell* **4**: 1599–1602.

Gévry M, Villeneuve N. 2009. Ecology and management of edible ectomycorrhizal mushrooms in eastern Canada. In: Khasa D, Piché Y, Coughlan AP (eds) *Advances in mycorrhizal science and technology*. Ottawa, Canada: NRC Research Press, 175-191.

Godbout C, Fortin JA. 1990. Cultural control of basidiome formation in *Laccaria bicolor* with container-grown white pine seedlings. *Mycological Research* **94**: 1051-1058.

Groome MC, Axler SR, Gifford DJ. 1991. Hydrolysis of lipid and protein reserves in loblolly pine seeds in relation to protein electrophoretic patterns following imbibition. *Physiologia Plantarum* **83**: 99-106.

Hacquard S, Tisserant E, Brun A, Legué V, Martin F, Kohler A. 2013. Laser microdissection and microarray analysis of *Tuber melanosporum* ectomycorrhizas reveal functional heterogeneity between mantle and Hartig net compartments. *Environmental Microbiology* **15**: 1853-1869.

Hall RA, De Sordi L, MacCallum DM, Topal H, Eaton R, Bloor JW, Robinson GK, Levin LR, Buck J, Wang Y. 2010. CO_2 acts as a signalling molecule in populations of the fungal pathogen *Candida albicans*. *PLoS Pathogens* **6**: e1001193.

Johnson-Flanagan AM, Owens JN. 1985. Root growth and root growth capacity of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Canadian Journal of Forest Research* **15**: 625-630.

Joh JH, Lee JS, Kim KH, Jeong SJ, Youn WH, Kim NK, Son ES, Cho YS, Yoo YB, Lee CS, Kim BG. 2007. Isolation of genes expressed during the developmental stages of the oyster mushroom, *Pleurotus ostreatus*, using expressed sequence tags. *FEMS Microbiology Letters* **276**: 19–25.

Koele N, Dickie IA, Blum JD, Gleason JD, de Graaf L. 2014. Ecological significance of mineral weathering in ectomycorrhizal and arbuscular mycorrhizal ecosystems from a field-based comparison. *Soil Biology and Biochemistry* **69**: 63-70.

Kües U, Liu Y. 2000. Fruiting body production in basidiomycetes. *Applied Microbiology and Biotechnology* **54**: 141-152.

Kües U. 2000. Life history and developmental processes in the basidiomycete *Coprinus cinereus*. *Microbiology and Molecular Biology Reviews* **64**: 316-353.

Lamhamedi MS, Godbout C, Fortin JA. 1994. Dependence of *Laccaria bicolor* basidiome development on current photosynthesis of *Pinus strobus* seedlings. *Canadian Journal of Forest Research* **24**: 1797-1804.

Landeweert R, Hoffland E, Finlay RD, Kuyper TW, van Breemen N. 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution* **16**: 248-254.

Last FT, Mason PA, Pelham J, Ingleby K. 1984. Fruitbody production by sheathing mycorrhizal fungi: effects of 'host'genotypes and propagating soils. *Forest Ecology and Management* **9**: 221-227.

Latgé JP. 2007. The cell wall: a carbohydrate armour for the fungal cell. *Molecular Microbiology* **66**: 279-290.

Liu Y, Srivilai P, Loos S, Aebi M, Kües U. 2006. An essential gene for fruiting body initiation in the basidiomycete *Coprinopsis cinerea* is homologous to bacterial cyclopropane fatty acid synthase genes. *Genetics* **172**: 873-884.

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B. 2013. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617-630.

Lucic E, Fourrey C, Kohler A, Martin F, Chalot M, Brun-Jacob A. 2008. A gene repertoire for nitrogen transporters in *Laccaria bicolor*. *New Phytologist* **180**: 343-364.

Martin F, Aerts A, Ahrn D, Brun A, Danchin EGJ. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452**: 88-92.

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, Porcel B, Rubini A, Amicucci A, Amselem J, Anthouard V, Arcioni S, Artiguenave F, Aury J, Ballario P, Bolchi A, Brenna A, Brun A, Buee M, Cantarel B, Chevalier G, Couloux A, Da Silva C, Denoeud F, Duplessis S, Ghignone S, Hilselberger B, Iotti M, Marcais B, Mello A, Miranda M, Pacioni G, Quesneville H, Riccioni C, Ruotolo R, Splivallo R, Stocchi V, Tisserant E, Viscomi AR, Zambonelli A, Zampieri E, Henrissat B, Lebrun M, Paolocci F, Bonfante P, Ottonello S, Wincker P. 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* **464**: 1033-1038.

Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**: 153-163.

Massicotte HB, Melville LH, Peterson RL. 2005. Building a basidiocarp: a case study of *Laccaria spp*. fruitbodies in the extraradical mycelium of *Pinus* ectomycorrhizas. *Mycologist* **19**: 141-149.

Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L. 2009. The cellular dynamics of plant aquaporin expression and functions. *Current Opinion in Plant Biology* **12**: 690-698.

Moore-Landecker E. 2002. Fungal spores. In: *Encyclopedia of Life Sciences (ELS)*. Chichester: John Wiley & Sons Ltd, doi: 10.1038/npg.els.0000378.

Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL, Bailey AM, Billette C, Coutinho PM, Deakin G, Doddapaneni H, Floudas D, Grimwood J, Hildén K, Kües U, LaButti

KM, Lapidus A, Lindquist EA, Lucas SM, Murat C, Riley RW, Salamov AA, Schmutz J, Subramanian V, Wösten HAB, Xu J, Eastwood DC, Foster GD, Sonnenberg ASM, Cullen D, de Vries RP, Lundell T, Hibbett DS, Henrissat B, Burton KS, Kerrigan RW, Challen MP, Grigoriev IV, Martin F. 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. *Proceedings of the National Academy of Sciences USA* **109**: 17501–17506.

Mueller GM. 1992. *Systematics of Laccaria (Agaricales) in the continental United States and Canada, with discussions on extralimital taxa and descriptions of extant types*. Chicago, USA: Field Museum of Natural History.

Navarro-Ródenas A, Ruíz-Lozano JM, Kaldenhoff R, Morte A. 2012. The aquaporin TcAQP1 of the desert truffle *Terfezia claveryi* is a membrane pore for water and CO_2 transport. *Molecular Plant-Microbe Interaction* **25**: 259-266.

Navarro-Ródenas A, Xu H, Kemppainen M, Pardo A, Zwiazek JJ. 2015. *Laccaria bicolor* Aquaporin LbAQP1 is required for Hartig Net Development in Trembling Aspen (*Populus tremuloides*). *Plant, Cell and Environment*. In Press.

Nehls U, Göhringer F, Wittulsky S, Dietz S. 2010. Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. *Plant Biology* **12**: 292-301.

Nehls U, Dietz S. 2014. Fungal aquaporins: cellular functions and ecophysiological perspectives. *Applied Microbiology and Biotechnology* **98**: 8835-8851.

Nowrousian M, Kück U. 2006. Comparative gene expression analysis of fruiting body development in two filamentous fungi. *FEMS Microbiology Letters* **257**: 328–335.

Nowrousian M. 2014. Genomics and transcriptomics to analyze fruiting body development. In: Esser K (ed) *Fungal Genomics XIII*. 2nd Edition, Berlin, Germany: Springer, 149-172.

Pfaffl MW. 2004. Quantification strategies in real-time PCR. In: Bustin SA (ed) *A-Z of quantitative PCR*. La Jolla, USA: International University Line (IUL), 87-112.

Pham GH, Kumari R, Singh A, Malla R, Prasad R, Sachdev M, Kaldorf M, Buscot F, Oelmüller R, Hampp R, Saxena AK, Rexer K, Kost G, Varma A. 2004. Axenic culture of symbiotic fungus *Piriformospora indica*. In: Varma A, Abbott L, Werner D, Hampp R (eds) *Plant surface microbiology*. Berlin, Heidelberg, Germany: Springer, 593-613.

Puppo A, Pauly N, Boscari A, Mandon K, Brouquisse R. 2013. Hydrogen peroxide and nitric oxide: key regulators of the legume—Rhizobium and mycorrhizal symbioses. *Antioxidants and Redox Signaling* **18**: 2202-2219.

Rahmad N, Al-Obaidi JR, Rashid NM, Zean NB, Yusoff MH, Shaharuddin NS, Jamil NA, Saleh NM. 2014. Comparative proteomic analysis of different developmental stages of the edible mushroom *Termitomyces heimii*. *Biological Research* **47**: 30.

Ruiz-Herrera J. 2012. *Fungal Cell Wall - Structure, synthesis and assambly*. 2nd Edition, Boca Raton, USA: CRC Press, 149-165.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671-675.

Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. 3rd Edition, Cambridge, UK: Academic Press.

Stamets P. 2000. *Growing gourmet and medicinal mushrooms*. 3rd Edition, Berkeley, CA: Ten Speed Press, 113, 164, 185.

Le Tacon F, Zeller B, Plain C, Hossann C, Bréchet C, Robin C. 2013. Carbon transfer from the host to *Tuber melanosporum* mycorrhizas and ascocarps followed using a ^{13}C pulse-labeling technique. *PloS One* **8**: e64626.

Tasaki Y, Sato R, Toyama S, Kasahara K, Ona Y, Sugawara M. 2014. Cloning of glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycete mushroom *Pleurotus ostreatus* and analysis of their expression during fruit-body development. *Mycoscience* **55**: 280-288.

Teramoto M, Wu B, Hogetsu T. 2012. Transfer of ^{14}C-photosynthate to the sporocarp of an ectomycorrhizal fungus *Laccaria amethystina*. *Mycorrhiza* **22**: 219-225.

Thorley RMS, Taylor LL, Banwart SA, Leake JR, Beerling DJ. 2014. The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling. *Plant, Cell and Environment*. doi: 10.1111/pce.12444.

Traeger S, Altegoer F, Freitag M, Gabaldon T, Kempken F, Kumar F, Marcet-Houben M, Pöggeler S, Stajich, JE, Nowrousian M. **2013.** The genome anddevelopment-dependent transcriptomes of *Pyronema confluens*: a window into fungal evolution. *PLoS Genetics* **9**: e1003820.

Watkinson SC. 2008. Basidiomycota. In: *Encyclopedia of Life Sciences (ELS)*. Chichester, UK: John Wiley & Sons, doi: 10.1002/9780470015902.a0000347.pub2.

Wiegant WM, Wery J, Buitenhuis ET, de Bont JA. 1992. Growth-promoting effect of thermophilic fungi on the mycelium of the edible mushroom *Agaricus bisporus*. *Applied and Environmental Microbiology* **58**: 2654-2659.

Xu H, Cooke JEK, Zwiazek JJ. 2013. Phylogenetic analysis of fungal aquaporins provides insight into their possible role in water transport of mycorrhizal associations. *Botany* **91**: 495-504.

Xu H, Kemppainen M, El Kayal W, Lee SH, Pardo AG, Cooke JEK, Zwiazek JJ. 2015. Overexpression of *Laccaria bicolor* aquaporin *JQ585595* alters root water transport properties in ectomycorrhizal white spruce (*Picea glauca*) seedlings. *New Phytologist* **205**: 757-770.

Yamada M, Kurano M, Inatomi S, Taguchi G, Okazaki M, Shimosaka M. 2008. Isolation and characterization of a gene coding for chitin deacetylase specifically expressed during fruiting body development in the basidiomycete *Flammulina velutipes* and its expression in the yeast *Pichia pastoris*. *FEMS Microbiology Letters* **289**: 130-137.

Yun W, Hall IR. 2004. Edible ectomycorrhizal mushrooms: challenges and achievements. *Canadian Journal of Botany* **82**: 1063-1073.

Yu GJ, WangM, Huang J, Yin YL, Chen YJ, Jian S, Jin YX, Lan XQ, Wong BHC, Liang Y, Sun H. 2012. Deep insight into the *Ganoderma lucidum* by comprehensive analysis of its transcriptome. *PLoSOne* **7**: e44301.

Table 4.1 Characteristics of the six aquaporins of Laccaria bicolor UAMH8232

Aquaporin[1]	Protein ID[1]	Length of deduced amino acid sequences	TMHs[2]	NPA motifs	Possible ar/R residues	Subcellular localization[3]	Phylogenetic cluster[4]	P_f (μm s^{-1}) [5]	CO_2 transport capacity[6]	Primers for qRT-PCR[7]	Corresponding aquaporins in strain S238N and their transport capacity[8]
JQ585592	AFJ15555	311	6	NPN, NSA	F H A R	Secretory RC 5	I: Orthodox fungal aquaporins	25.8 ± 2.1	Yes	F: TGGCCCTGCTGAAATCAACT R: CCCATTGCGAGTCTCTTCGT	Lacbi1:392091 (Lacbi2:456764) H_2O
JQ585593	AFJ15556	330	6	NPC, NSA	F G I R	Plasma membrane RC 5	II: Fungal aquaglyceroporin	46.6 ± 10.2	Yes	F: GTGACATTGGTTGCCGTTTG R: ATCCTCCCGCAGCTGACTTT	Lacbi1:307192 (Lacbi2:671860)
JQ585594	AFJ15557	254	6	NPA, NPA	W G G R	Secretory RC 1	III: Facultative fungal aquaporin	124.0 ± 11.0	No	F: TAATGGCGCACTCACAAACG R: ATGCCCCAAGACCAATGAAC	Lacbi1:247946 (Lacbi2:568479) H_2O
JQ585595	AFJ15558	312	6	NPA, NPA	W G G R	Plasma membrane RC 1	III: Facultative fungal aquaporin	260.0 ± 8.9	No	F: TAACCCCGCTCGTGATCTTG R: CCTGTCTTCCATAGCCAACCA	Lacbi1:391485 (Lacbi2:443240) H_2O, glycerol, ammonia
JQ585596	AFJ15559	332	6	NPA, NPA	W G A R	Plasma membrane RC 3	III: Facultative fungal aquaporin	166.5 ± 2.7	No	F: ACGCTTGTTCCTCGCTATGTC R: TGCCCAGAGCCAATATTGACT	Lacbi1:317173 (Lacbi2:317173) H_2O, ammonia
JQ585597	AFJ15560	332	6	NPA, NPA	W G A R	Plasma membrane RC 3	III: Facultative fungal aquaporin	138.4 ± 1.5	No	F: CGCCCTCACTGACAAACGTA R: ATAAAGAGCGCAAATGGCAAAA	Lacbi1:317173 (Lacbi2:317173) H_2O, ammonia

Note:

1. GenBank accession number in NCBI.

2. The number of transmembrane helix domains was predicted by TMHMM server 2.0 (Xu *et al*. 2015).

3. Subcellular localization was predicted by Target P (Emanuelsson *et al*. 2000); 1 indicated strongest prediction and 5 the weakest.

4. Phylogenetic analysis was refereed to Xu *et al*. 2013.

5. P_f was water permeability determined in *Xenopus laevis* oocyte assay (Xu *et al.* 2015); P_f for negative control was 20.5 ± 0.6 μm s^{-1}.

6. The assay was conducted in yeast heterologous expression system by Navarro-Ródenas *et al.* (2015).

7. F and R referred to forward and reverse primers, respectively.

8. Functional assays were conducted by Dietz *et al.* (2011); Lacbi2 proteins were searched in JGI *Laccaria bicolor* genome V2.0.

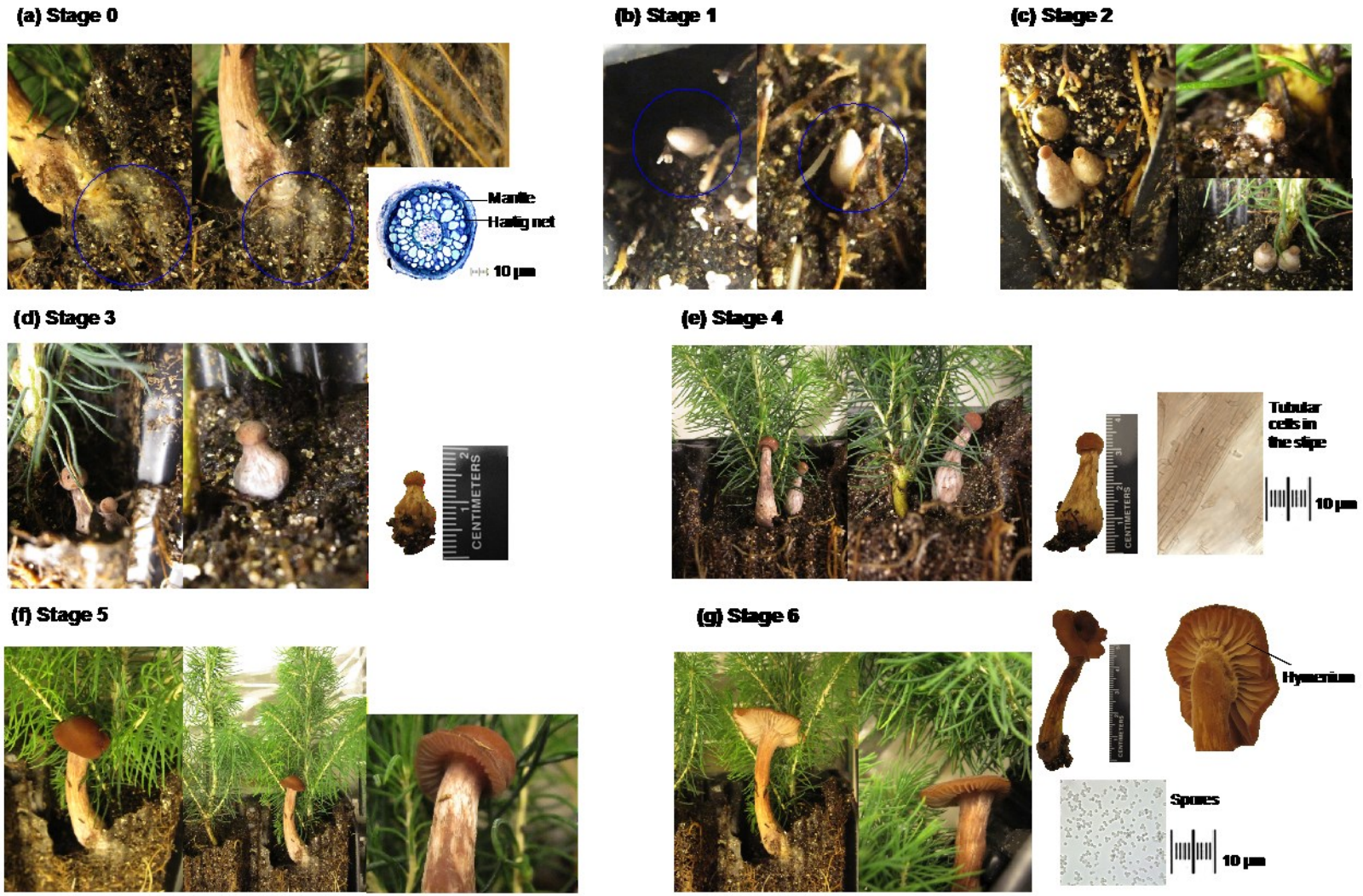


Figure 4.1 Developmental stages of basidiocarp formation in *Laccaria bicolor* UAMH 8232 ectomycorrhizal with *Picea glauca* seedlings
(a) Stage 0: localized intense hyphal branching around the ectomycorrhizal roots (circled); massive extraradical forage hyphae growing around the roots; mantle and Hartig net structures present; **(b)** Stage 1: formation of an initial aggregate bipolar primordium, *i.e.*, pinning (circled); **(c)** Stage 2: differentiation of primordium with cap and stipe; **(d-f)** Fruiting body maturation: **(d)** Stage 3-swollen stipe base, **(e)** Stage 4-rapid stipe elongation and tubular-shaped hyphal cells, **(f)** Stage5-cap expanding; **(g)** Stage 6: basidiospore formation in hymenium of fully developed basidiocarps; numerous spores were produced.

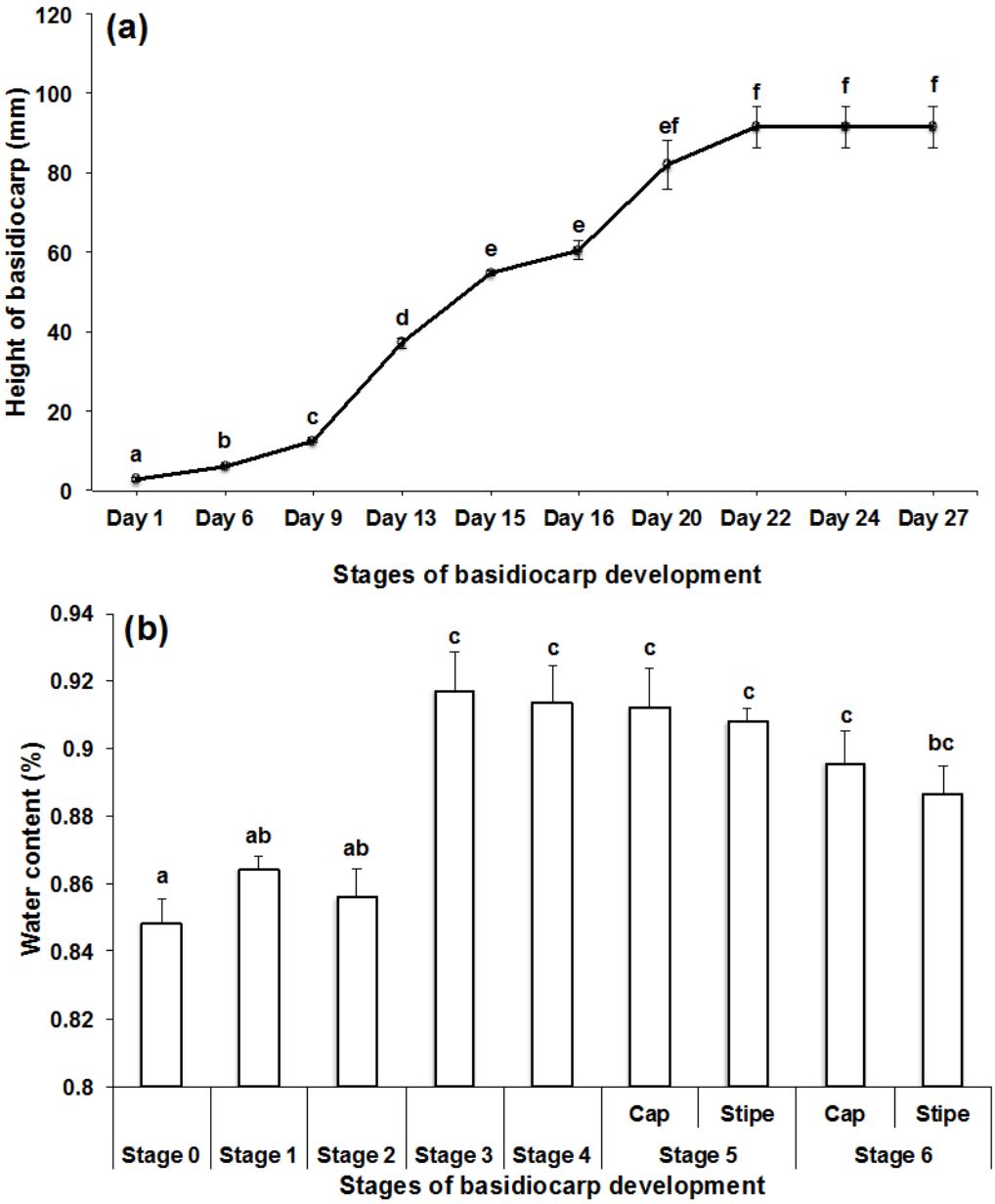
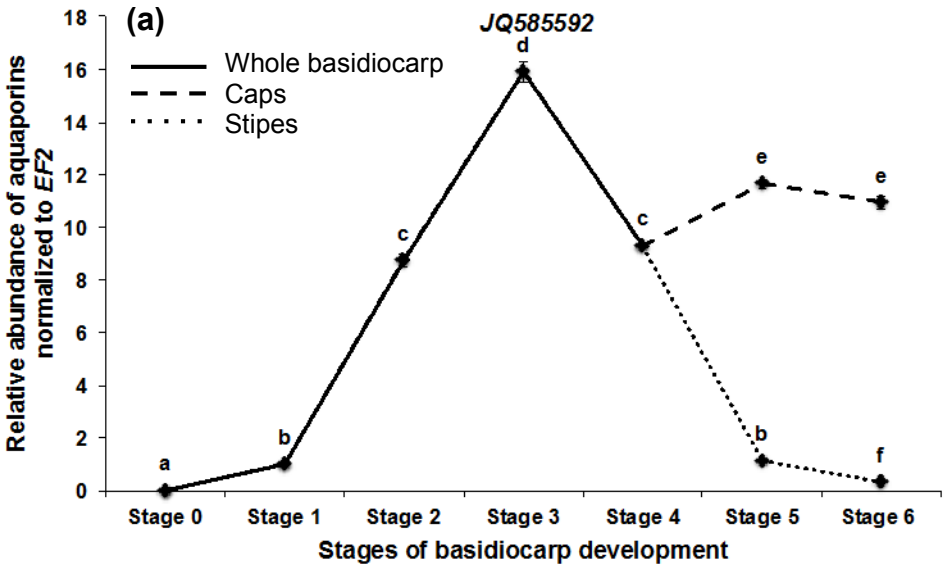
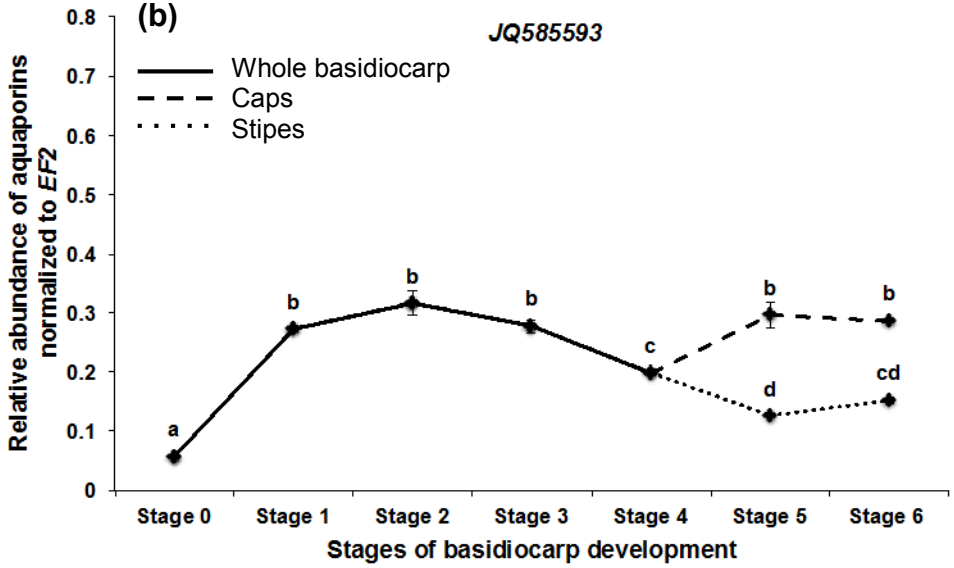


Figure 4.2 Heights (a) and tissue water contents (b) of basidiocarps in *Laccaria bicolor* UAMH 8232 at different developmental stages

Water content data in (b) for Stages 1-5 were collected on the same days as for heights in (a), and the water content in Stage 6 was measured on day 22. The means (n = 10) ± SE are shown in (a) and n = 6 + SE in (b). Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test.





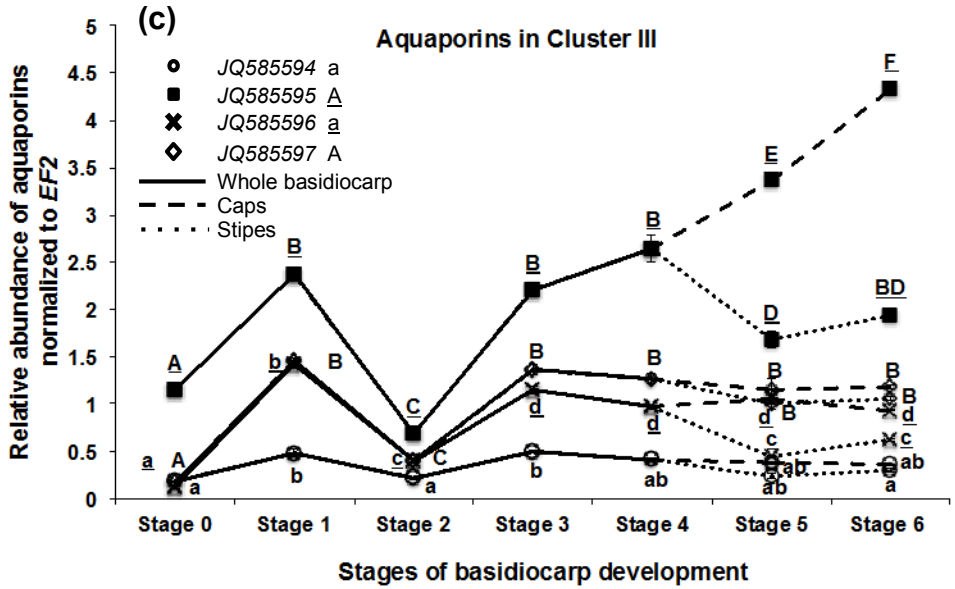
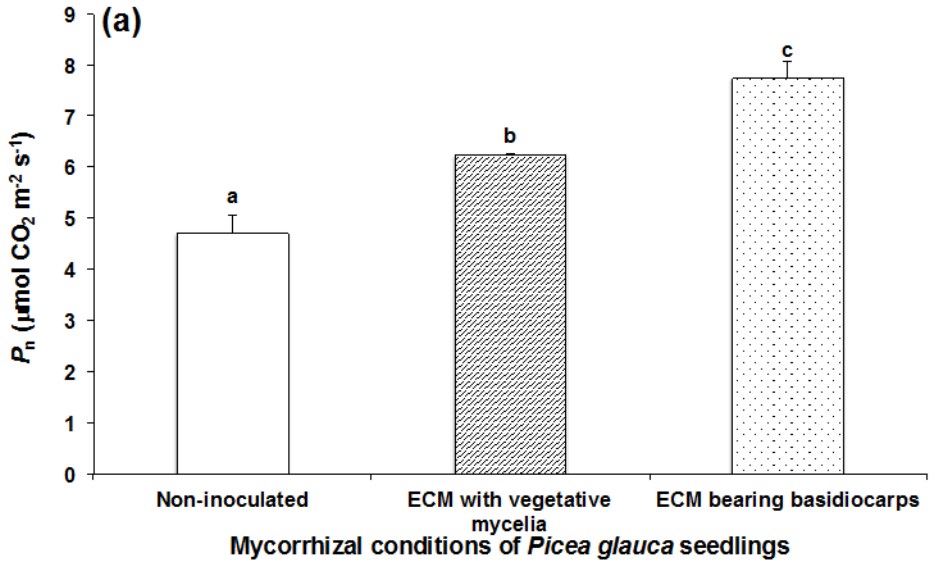
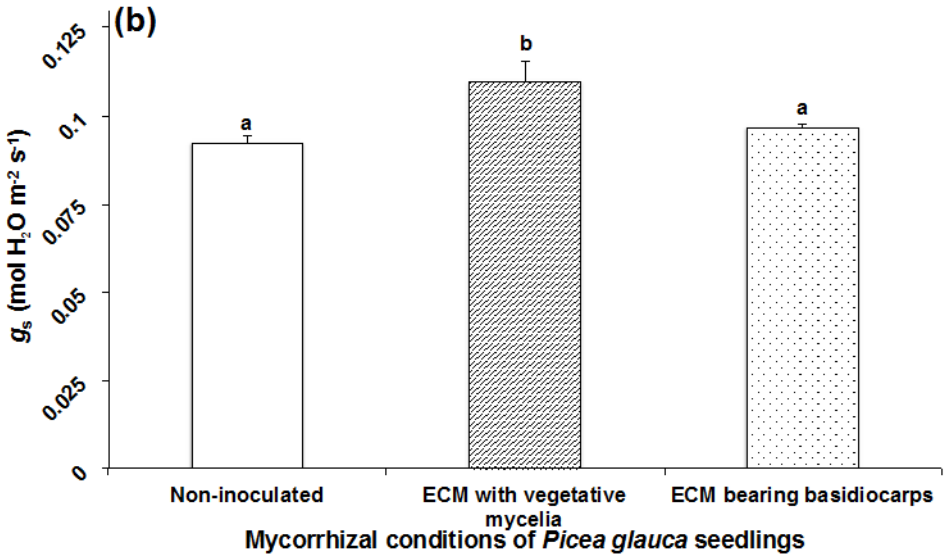


Figure 4.3 Transcript abundance of aquaporin genes in *Laccaria bicolor* basidiocarps at different developmental stages

Relative abundance of the Cluster I fungal aquaporin *JQ585592* **(a)**, Cluster II fungal aquaglyceroporin *JQ585593* **(b)** and Cluster III facultative fungal aquaporins *JQ585594*, *JQ585595*, *JQ585596* and *JQ585597* **(c)** in basidiocarps at the different development stages. The qRT-PCR assay was conducted using the standard curve method, and the transcript abundance of each aquaporin gene was normalized to that of the reference gene *EF2*. Solid lines represent the expression in the whole basidiocarp. Dashed lines represent the expression in the caps in Stages 5 and 6. Hymenium tissue was collected in Stage 6; Dotted lines represent the expression in the stipes in Stages 5 and 6. The means ($n = 4$) ± SE are shown. Different letters indicate significantly different time points of the transcript abundance for each gene at $P \leq 0.05$ determined with ANOVA, Tukey's test. Letters used for *JQ585594*, *JQ585595*, *JQ585596* and *JQ585597* are lowercase, underlined uppercase, underlined lowercase, and uppercase, respectively.





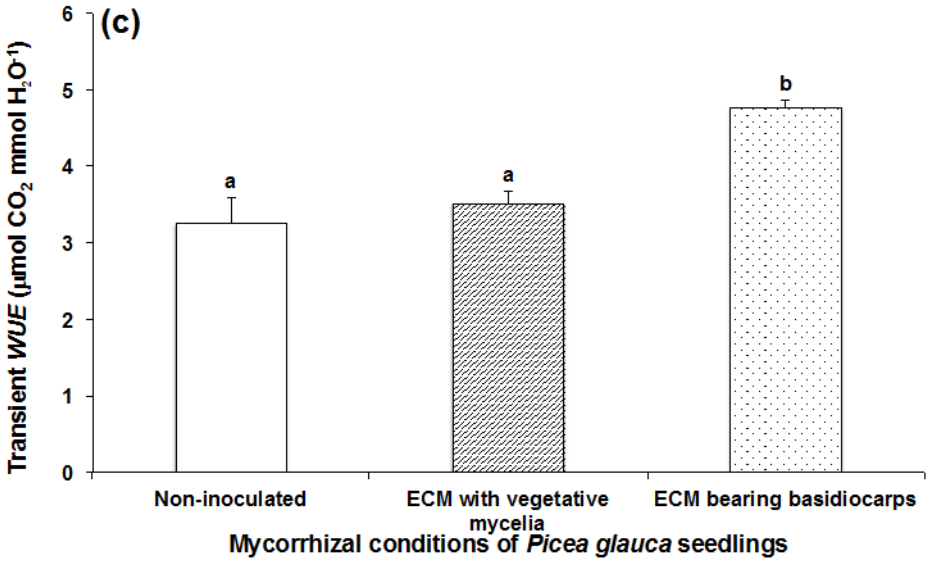


Figure 4.4 Net photosynthetic rate P_n (a), stomatal conductance g_s (b) and transient water use efficiency (*WUE*) (c) in non-inoculated -*Picea glauca* seedlings and in seedlings associated with vegetative mycelium and basidiocarp-bearing mycelium (Stages 3-5) of ectomycorrhizal *Laccaria bicolor* UAMH8232

Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 6$ + SE).

5 GENERAL DISCUSSION AND CONCLUSIONS

5.1 Outcomes of the studies

The studies in Chapter 2, 3 and 4 addressed the hypotheses and the seven objectives introduced in §1.7. The verification of the hypotheses and the fulfillment of the objectives deepen the understanding of the classification and transport capacities of mycorrhizal fungal MIPs, and suggest their multiple roles in water transport of mycorrhizal association and in signaling processes during basidiocarp growth. The major outcomes of the studies are listed below.

- Phylogenetic classification of fungal MIPs: Fungal MIPs can be grouped into four major phylogenetic clusters, *i.e.*, Cluster I the orthodox aquaporins, Cluster II the aquaglyceroporins, Cluster III the facultative fungal aquaporins, and Cluster IV the fungal XIPs. The relationship between putative MIPs of mycorrhizal fungal species and functionally characterized fungal MIPs provide insight into MIP gene families of mycorrhizal fungi and their possible roles in plant-fungus symbioses.

- Water permeability of *Laccaria bicolor* MIPs: In the six MIP genes cloned from ECM fungus *L. bicolor* UAMH8232 (Fig. 5.1), *JQ585592* and *JQ585593* encode an orthodox aquaporin and an aquaglyceroporin, respectively, which do not demonstrate significant water transport capacity in the swelling assay of oocyte heterologous expression. JQ585594, JQ585595, and the pair of isoforms JQ585596 and JQ585597, are water-transporting facultative fungal aquaporins, in which JQ585595 demonstrates the highest water transport capacity. In addition, JQ585592 demonstrates permeability for CO_2, NO and H_2O_2, and JQ585595 demonstrates permeability for NO in yeast expression systems (Navarro-Ródenas *et al.* 2015). Transport capacity for more than one substrate suggests that fungal MIPs may play multiple physiological roles.

- Transcript profiles of *L. bicolor* MIPs: The six MIP genes show different transcript abundance in mycelia grown on pure culture, and demonstrate significantly different transcript profiles in vegetative mycelia, in ECM root tips at root temperature of 20°C and 5°C, and in each of the six developmental

stages of basidiocarp formation. In general, the transcript abundance of these MIPs is higher in ECM root tips than in vegetative mycelia, and higher at root temperature 5°C than 20°C, with *JQ585595* being the highest expressed under all these conditions. During development of the basidiocarp, *JQ585592* and *JQ585595* show the greatest degree of upregulation. This indicates the differential involvement of these MIPs in different tissues and phases of fungal growth.

- The contribution of *L. bicolor* MIPs to water transport in ECM water transport: ECM fungus and fungal aquaporins can have significant impact on root water transport pathways (Fig. 5.2; Fig. 5.3 a, b). As the conceptual model suggests (Fig. 5.2), water can be transported through fungal hyphae by apoplastic pathway and transmembrane pathway; in the latter pathway, the regulation of fungal aquaporins plays a determining role. The presence of fungal hyphae as mantle and Hartig net may directly alter the apoplastic pathway of root water transport; by changing the hydration in the apoplastic space, it may have impact on PIP regulation in different types of root cells in the cell-to-cell root water transport pathways (Fig. 5.2). The study shows the increase in L_{pr} and L_{pc} of *Picea glauca* upon mycorrhization with *L. bicolor* and the enhancement of L_{pr} in *P. glauca* roots mycorrhizal with *L. bicolor* overexpressing the aquaporin *JQ585595*. This indicates that the contribution of *L. bicolor* hyphae to root water transport in *P. glauca* involves increased apoplastic water transport in the root intercellular spaces, which may be attributed to water released from the hyphae and may lead to increased hydration at the fungal-root interface, and consequently impact PIP expression and fully load the transmembrane pathway in mycorrhizal roots (Fig. 5.3 b). Moderate increase in *JQ585595* expression may lead to a further increase in hydration in the root intercellular space; therefore, apoplastic water transport in roots is further enhanced (Fig. 5.3 c). However, at low root temperature, upregulation of fungal MIPs to a furthest extent may lead to the increase in water influx into hyphal cells and the decrease in hydration in the root intercellular space (Fig. 5.3 d), which consequently affects PIP regulation and reduces L_{pc} and L_{pr} in *P. glauca* roots mycorrhizal with *L. bicolor* strains overexpressing *JQ585595*. This suggests that the role of fungal MIPs in ECM root water transport is highly dynamic.

- The possible roles of *L. bicolor* MIPs during basidiocarp formation: The significantly upregulated transcription of the studied MIPs, particularly *JQ585592* and *JQ585595*, suggests their contributions in fungal cellular processes during the basidiocarp formation of *L. bicolor* ectomycorrhizal with *P. glauca* seedlings. As a functional channel for CO_2, NO and H_2O_2 (Navarro-Ródenas *et al.* 2015), upregulated *JQ585592* may serve as a channel for a possible signaling molecule such as CO_2 during the basidiocarp initiation and maturation. Enhanced water transport attributed to upregulated *JQ585595*, the functional water channel, may contribute to cell expansion during the stipe elongation and cap development.

- The involvement of *L. bicolor* MIPs in ECM water transport and basidiocarp formation gives examples of the importance of fungal MIPs in fundamental processes in the growth of ECM fungi and in their interactions with associated plants, which invites future studies on their roles in important processes of mycorrhizal and fungal biology.

5.2 Perspectives for future studies

To date, the genomes of *ca.* 400 fungal species, including 33 mycorrhizal fungi, have been sequenced, assembled and released by the Joint Genome Institute (van der Heijden *et al*. 2015). These expanding genomic and transcriptomic resources will allow researchers to improve the current fungal MIP classification, as the number of known fungal MIPs increases (Verma *et al*. 2014). These resources also provide a platform for the research on subcellular localization and post-translational regulation of fungal MIPs, as well as their refined roles in signaling and transporting in the context of fungal developmental biology and mycorrhizas - an essential process in carbon and nutrient cycling of temperate and boreal forests.

This study characterized six genes in the MIP family of *L. bicolor* dikaryotic strain UAMH8232 by phylogenetic analysis and water transport assay, and examined their transcript profiles in three distinct phases of fungal growth, *i.e.*, medium-cultured vegetative mycelia, ECM root tips and basidiocarps. This study also investigated the role of a particular MIP *JQ585595* in ECM-associated root water transport using transgenic strains. The contribution of the strongly upregulated *JQ585592* and

JQ585595 to basidiocarp formation was discussed, and should be further investigated by examining the effects of altered gene expression on basidiocarp growth. The contributions of other characterized MIPs to ECM root water transport and basidiocarp formation have been briefly touched in this study, and may be examined in depth in future study in similar approaches.

Certain inter-strain differences shown in sequence analysis and water transport assay were found between the MIPs of the strain UAMH8232 and their homologues of the monokaryotic strain S238N. In addition, previous gene cloning in the strain UAMH8232 did not yield a homologue gene for Lacbi2:482072 of the strain S238N (61.82% identical with JQ585593, grouped in Cluster II, containing a mitochondrial targeting peptide) (Fig. 5.1). Recently released genome portal of *L. bicolor* v2.0 (JGI portal version 7.14.2, released on February 5 2015, accessed on February 22 2015, http://genome.jgi-psf.org/Lacbi2/Lacbi2.home.html) reveals a new putative MIP in the strain S238N, *i.e.*, Lacbi2:576801 (92.4% identical with JQ585596, grouped in Cluster III, containing a secretory pathway targeting peptide). The knowledge of the MIP family of the species may keep expanding along with the update of the genome platform. Therefore, research efforts should be made to clone and characterize the homologues of the new putative MIP genes in the strain UAMH8232, and examine their roles in important processes such as ECM water transport and basidiocarp formation.

The contributions of mycorrhizal fungal MIPs to root-water relations can be further investigated by using techniques such as qRT-PCR based on laser capture microdissection RNA extraction and mRNA *in situ* hybridization, to examine the transcript abundance of both fungal and plant MIPs in each important type of cells in root water transport pathways (Almeida-Rodriguez & Hacke 2012; Giovannetti *et al*. 2012; Gambetta *et al*. 2013; Hacquard *et al*. 2013), including fungal MIPs in extraradical mycelium, mantle and Hartig net of the fungus, and plant PIPs in epidermis, cortex and endodermis (Fig. 5.2). In addition, using antibody and immunofluorescence techniques to examine protein phosphorylation and to verify the *in silico* prediction of subcellular localization (Fig. 5.1), the MIP post-translational modifications can be elucidated to link gating and trafficking of fungal MIPs and major root PIPs to changes in root hydraulic conductivity (Javot & Maurel 2002; Maurel *et al.*

2008; Chaumont & Tyerman 2014). Meanwhile, methods should be improved to accurately track and quantify water transported in root apoplastic pathway (Muhsin & Zwiazek 2002; Bárzana *et al*. 2012). The application of stable isotopes and new fluorescent dyes may shed light on this area. Efficient methods, such as a cell pressure probe specialized for fungal cells, also need to be designed to directly measure hydraulic status of fungal mycelia and individual hyphal cells. These studies will help to examine the status of each pathway, in order to verify the proposed processes of water transport at the interface of hyphal cells of and root cortical cells (Fig. 5.3). In addition, the currently proposed model of ECM root water transport mainly addresses the importance of hydraulic signal itself in regulating MIP-mediated processes. Many other signals, such as plant hormones and fungal secreted metabolites, may play roles in signaling at the plant-fungal interface and regulating both plant and fungal MIPs. Therefore relevant consideration should be incorporated into future experimental designs.

Other transport capacities and related physiological functions of fungal MIP family would be very interesting areas to explore, as they may play multiple crucial roles in fungal growth and reproduction as well as interaction with mycorrhizal plants (Nehls & Dietz 2014; Verma *et al*. 2014). For instance, in addition to their direct contribution to water transport, *L. bicolor* MIPs may be involved in important signaling pathways in the processes of mutualism recognition and substrate exchanges, which consequently shape the extracellular environment of root cells. Furthermore, it would be important to examine the precise mechanisms by which MIPs regulate the transport of signaling molecules such as CO_2 to trigger the switch of fungal growth stages. These signaling pathways remain unclear and should be paid attention to in future studies that involve more mycorrhizal fungal and plant species. MIP regulations as responses to different environmental cues should be considered as well, because the nature of mycorrhizal interaction is often complicated by factors such as chilling, drought, and salinity. The techniques proposed in the paragraph above can also be used to examine the spatially differential expression and post-translational regulation of *L. bicolor* MIPs in these contexts.

From a practical perspective, the knowledge of the roles of fungal MIPs in mycorrhizal associations can help to guide the applications of mycorrhizal symbiosis in agriculture,

forestry and land reclamation, as well as in sporocarp cultivation of economically important macrofungi of Ascomycota and Basidiomycota. The general understanding of fungal MIPs may provide insight for the research on fungal diversity, and for the practice in plant protection against fungal pathogens, as well as in biocontrol and biorefinery using beneficial fungal species.

5.3 References

Almeida-Rodriguez AM, Hacke UG. 2012. Cellular localization of aquaporin mRNA in hybrid poplar stems. *American Journal of Botany* **99**: 1249-1254.

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Rulz-Lozano JM. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Annuals of Botany* **109**: 1009-1017.

Chaumont F, Tyerman SD. 2014. Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiology* **164**: 1600-1618.

Emanuelsson O, Brunak S, Heijne G, Nielsen H. 2007. Locating proteins in the cell using TargetP, SignalP, and related tools. *Nature Protocols* **2**: 953-971.

Fischer R, Zekert N, Takeshita N. 2008. Polarized growth in fungi–interplay between the cytoskeleton, positional markers and membrane domains. *Molecular Microbioloy* **68**: 813-826.

Gambetta GA, Fei J, Rost TL, Knipfer T, Matthews MA, Shackel KA, Walker MA, McElrone AJ. 2013. Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport. *Plant Physiology* **163**: 1254-1265.

Giovannetti M, Balestrini R, Volpe V, Guether M, Straub D, Costa A, Ludewig U, Bonfante P. 2012. Two putative-aquaporin genes are differentially expressed during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *BMC Plant Biology* **12**: 186.

Hacquard S, Tisserant E, Brun A, Legué V, Martin F, Kohler A. 2013. Laser microdissection and microarray analysis of *Tuber melanosporum* ectomycorrhizas

reveal functional heterogeneity between mantle and Hartig net compartments. *Environmental Microbiology* **15**: 1853-1869.

Van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406-1423.

Javot H, Maurel C. 2002. The role of aquaporins in root water uptake. *Annals of Botany* **90**: 301-313.

Lehto T, Zwiazek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* **21**: 71–90.

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV. 2011. Sequencing the fungal tree of life. *New Phytologist* **190**: 818-821.

Maurel C, Verdoucq L, Luu D, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology* **59**: 595-624.

Muhsin TM, Zwiazek JJ. 2002. Ectomycorrhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus americana* seedlings. *New Phytologist* **153**: 153-158.

Navarro-Ródenas A, Xu H, Kemppainen M, Pardo A, Zwiazek JJ. 2015. *Laccaria bicolor* Aquaporin LbAQP1 is required for Hartig Net Development in Trembling Aspen (*Populus tremuloides*). *Plant, Cell and Environment*. In Press.

Nehls U, Dietz S. 2014. Fungal aquaporins: cellular functions and ecophysiological perspectives. *Applied Microbiology and Biotechnology* **98**: 8835-8851.

Steudle E, Peterson CA. 1998. How does water get through roots? *Journal of Experimental Botany* **49**: 775–788.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods**.** *Molecular Biology and Evolution* **28**: 2731-2739.

Verma RK, Prabh ND, Sankararamakrishnan R. 2014. New subfamilies of major intrinsic proteins in fungi suggest novel transport properties in fungal channels: implications for the host-fungal interactions. *BMC Evolutionary Biology* **14**: 173.

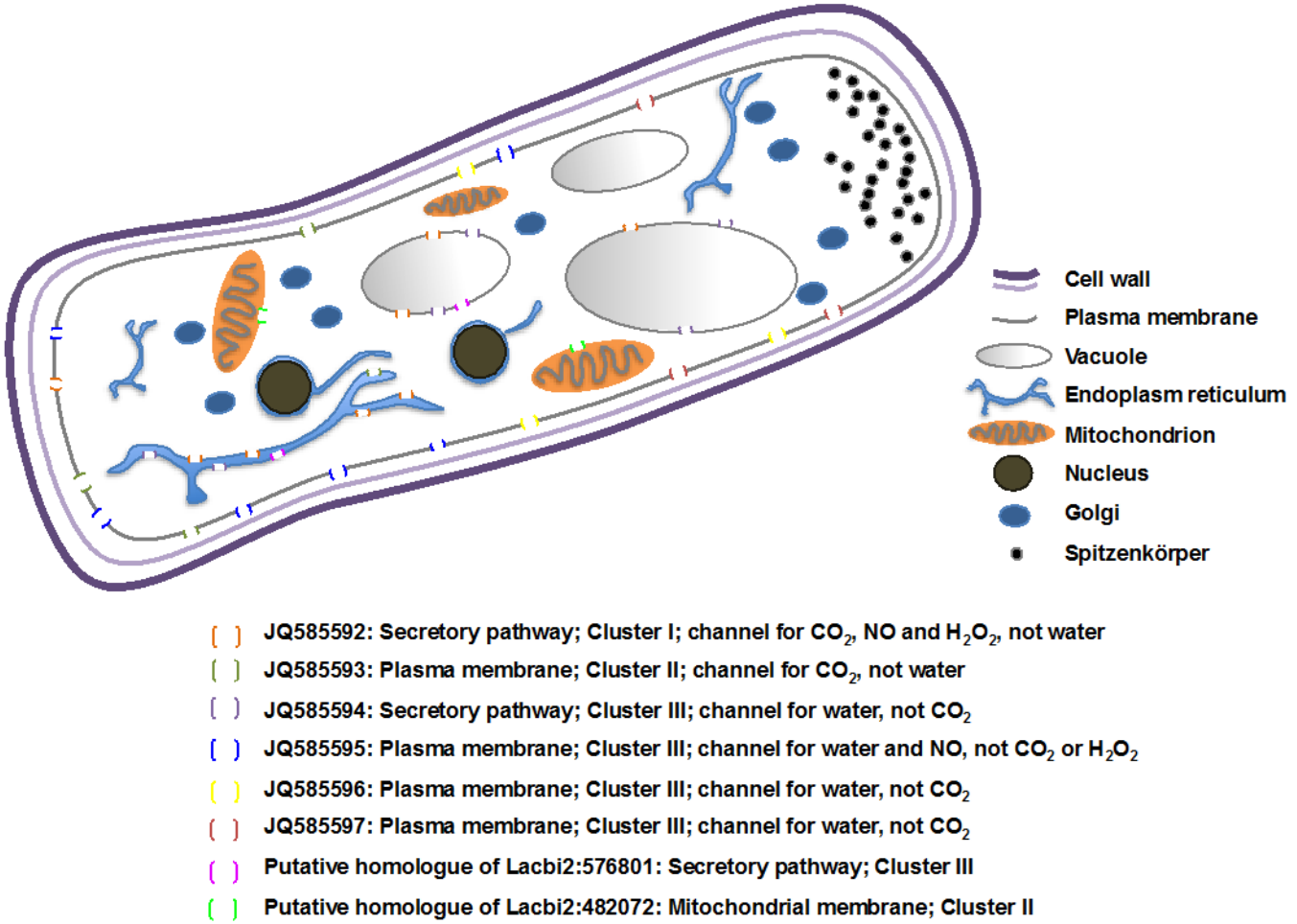


Figure 5.1 Known MIP proteins of Laccaria bicolor UAMH8232 and their putative subcellular localization
Subcellular localization of the MIPs was predicted using TargetP (Emanuelsson *et al.* 2007). Phylogenetic analysis of MIP clustering was conducted using MEGA 5 (Tamura *et al.* 2011), as shown in Chpater 2. Water transport capacity of the MIPs was examined in *Xenopus* oocyte swelling assay, as shown in Chapter 3. Transport capacity for CO_2, NO and H_2O_2 was examined in yeast expression system (Navarro-Ródenas *et al.* 2015). The diagram of the fungal hyphal cell was drawn with reference to Fischer *et al*. (2008).

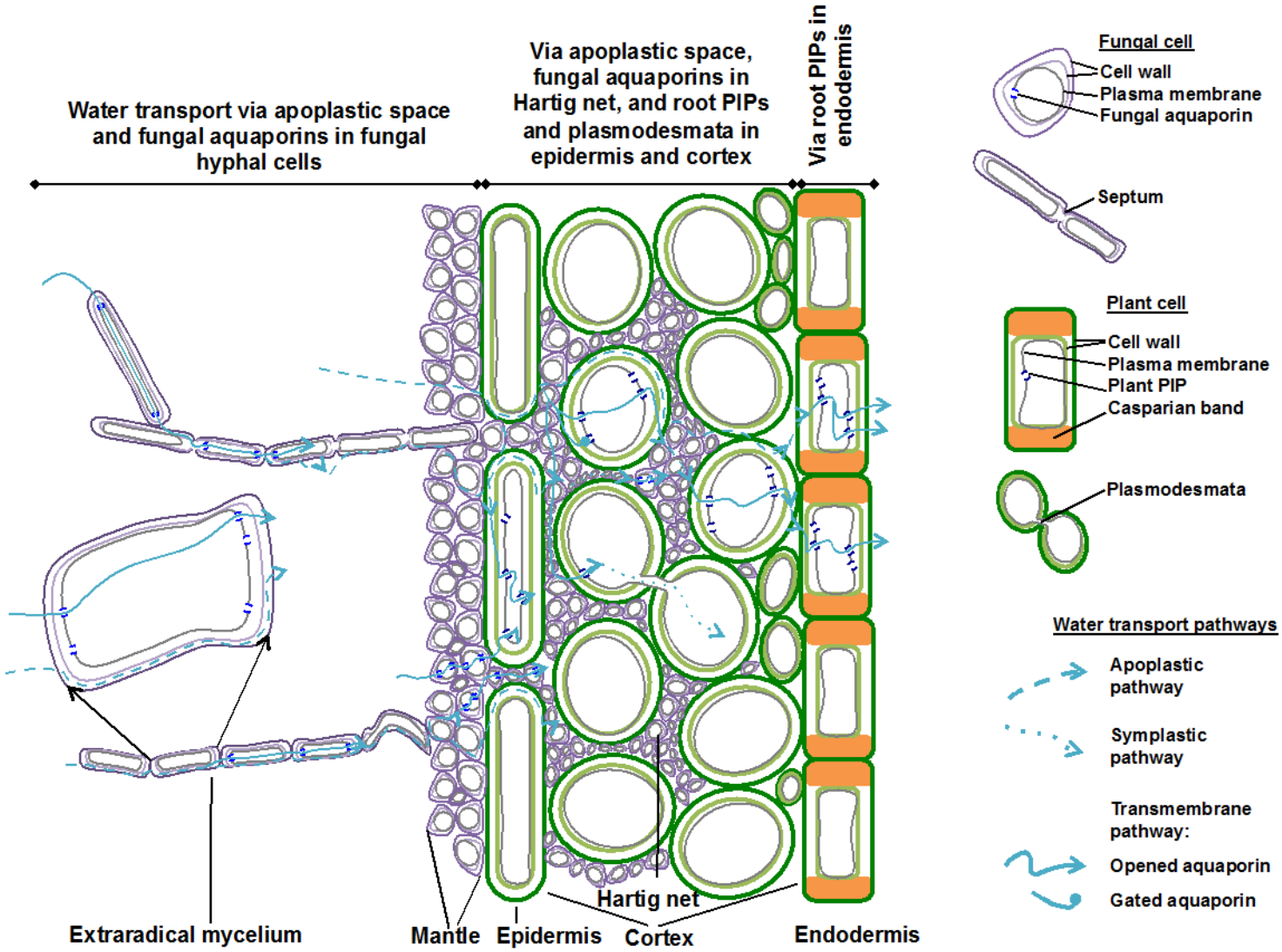


Figure 5.2 A conceptual model of water pathways through ectomycorrhizal fungus-root association
The conceptual model was developed based on the composite model of root water transport (Steudle & Peterson 1998). The diagram was drawn with reference to that of the ECM root water transport suggested by Lehto & Zwiazek (2011).

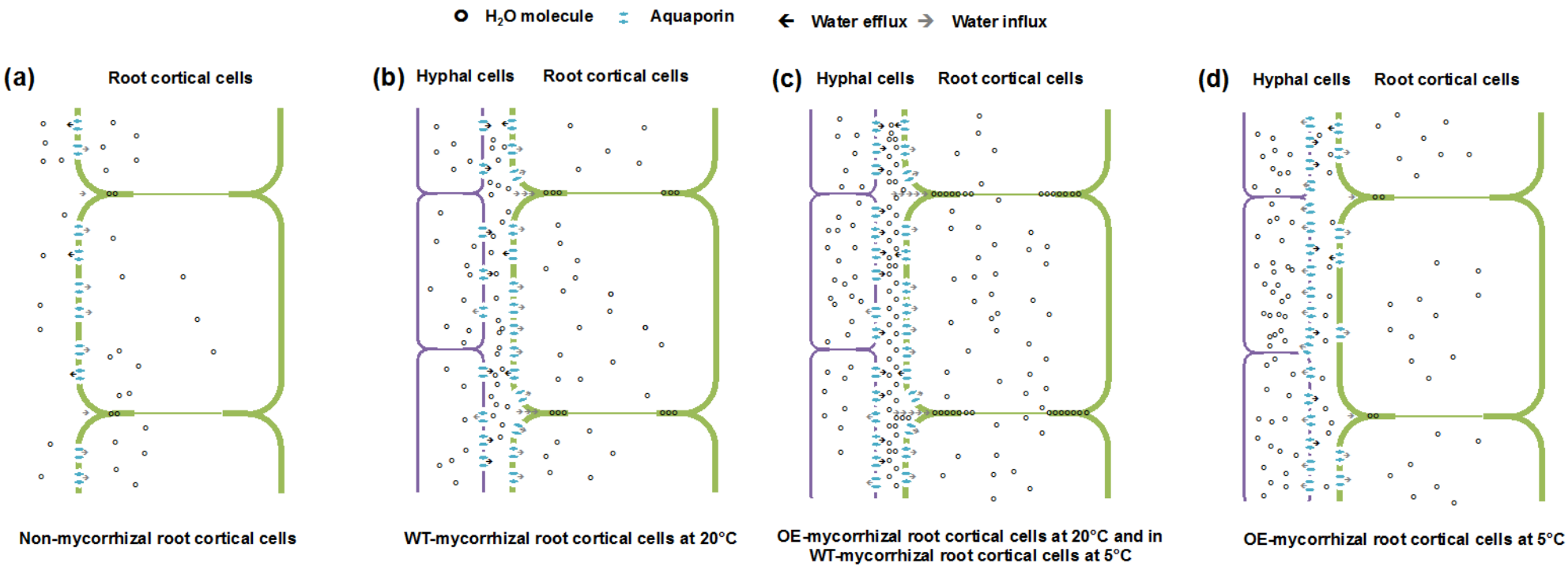


Figure 5.3 Proposed processes of water transport at the ECM interface of hyphal cells of *Laccaria bicolor* and root cortical cells of *Picea glauca* with emphasis on the roles of fungal and root aquaporins

(a) In non-mycorrhizal root tips, water is transported in apoplastic and cell-to-cell pathways in cortical cells; **(b)** In WT-mycorrhizal root tips, root water transport in apoplastic and cell-to-cell pathways is enhanced by the presence of mycorrhizal hyphae, as water released from mycorrhizal hyphae increases the hydration in the intercellular space of cortical cells, and fully loads the root aquaporins for transmembrane water transport; **(c)** Transcript abundance of the fungal aquaporins is up-regulated in OE-mycorrhizal root tips at 20°C and in WT-mycorrhizal root tips at root temperature 5°C, and moderate increase in fungal aquaporins contributes to the increase in water efflux from hyphal cells and in hydration of root intercellular space, which leads to further enhancement of root apoplastic water transport; **(d)** In OE-mycorrhizal root tips at 5°C, transcript abundance of fungal aquaporins is further up-regulated, which causes more water influx into hyphal cells and less water in intercellular space available for root transport. The simplified model was developed based on the study of root hydraulic dynamics of *Picea glauca* mycorrhizal with ECM fungus *Laccaria bicolor*. WT stands for the *L. bicolor* strain UAMH8232. OE stands for the strains overexpressing the endogenous fungal aquaporin *JQ585595*.

REFERENCES

Agerer R. 2001. Exploration types of ectomycorrhizae—a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **11**: 107–114.

Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S. 1993. Aquaporin CHIP: the archetypal molecular water channel. *American Journal of Physiology* **265**: F463-F463.

Allen MF, Smith WK, Moore TS, Christensen M. 1981. Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* (HBK) Lag. ex Steud. *New Phytologist* **88**: 683-693.

Allen MF. 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal* **6**: 291–297.

Almeida-Rodriguez AM, Cooke JEK, Yeh F, Zwiazek JJ. 2010. Functional characterization of drought-responsive aquaporins in *Populus balsamifera* and *Populus simonii* x *balsamifera* clones with different drought resistance strategies. *Physiologia Plantarum* **140**: 321-333.

Almeida-Rodriguez AM, Hacke UG, Laur J. 2011. Influence of evaporative demand on aquaporin expression and root hydraulics of hybrid poplar. *Plant, Cell and Environment* **34**: 1318-1331.

Almeida-Rodriguez AM, Hacke UG. 2012. Cellular localization of aquaporin mRNA in hybrid poplar stems. *American Journal of Botany* **99**: 1249-1254.

Aroca R, Amodeo G, Fernández-Illescas S, Herman EM, Chaumont F, Chrispeels MJ. 2005. The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiology* **137**: 341-353.

Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* **173**: 808-

816.

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Rulz-Lozano JM. 2009. Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. *Molecular Plant-Microbe Interactions* **22**: 1169-1178.

Aroca R, Porcel R, Ruiz-Lozano JM. 2012. Regulation of root water uptake under abiotic stress conditions. *Journal of Experimental Botany* **63**: 43-57.

Augé RM. 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science* **84**: 373–381.

Augé RM, Toler HD, Sams CE, Nasim G. 2008. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. *Mycorrhiza* **18**: 115-121.

Bahn Y, Mühlschlegel FA. 2006. CO_2 sensing in fungi and beyond. *Current Opinion in Microbiology* **9**: 572-578.

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Rulz-Lozano JM. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Annuals of Botany* **109**: 1009-1017.

Bastide PY, Kropp BR, Piché Y. 1994. Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist* **127**: 547-556.

Beaudette PC, Chlup M, Yee J, Emery RN. 2007. Relationships of root conductivity and aquaporin gene expression in *Pisum sativum*: diurnal patterns and the response to $HgCl_2$ and ABA. *Journal of Experimental Botany* **58**: 1291-1300.

Bécard G, Piché Y. 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. *Applied and Environmental Microbioloy* **55**: 2320-2325.

Bedon F, Levasseur C, Grima-Pettenati J, Séguin A, MacKay J. 2009. Sequence analysis and functional characterization of the promoter of the *Picea glauca* cinnamyl

alcohol dehydrogenase gene in transgenic white spruce plants. *Plant Cell Reports* **28**: 787-800.

Bienert GP, Thorsen M, Schüssler MD, Nilsson HR, Wagner A, Tamás MJ, Jahn TP. 2008. A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes. *BMC Biology* **6**: 26.

Bienert GP, Bienert MD, Jahn TP, Boutry M, Chaumont F. 2011. Solanaceae XIPs are plasma membrane aquaporins that facilitate the transport of many uncharged substrates. *The Plant Journal* **66**: 306-317.

Bill RM, Hedfalk K, Karlgren S, Mullins JGL, Rydstrom J, Hohmann S. 2001. Analysis of the pore of the unusual Major Intrinsic Protein channel, Yeast Fps1p. *The Journal of Biological Chemistry* **276**: 36543-36549.

Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW. 2012. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of Frankincense seedlings under pulsed water availability conditions. *Oecologia* **169**: 895-904.

Birol I, Raymond A, Jackman SD, Pleasance S, Coope R, Taylor GA, Man Saint Yuen M, Keeling CI, Brand D, Vandervalk BP, Kirk H, Pandoh P, Moore RA, Zhao Y, Mungall AJ, Jaquish B, Yanchuk A, Ritland C, Boyle B, Bousquet J, Ritland, K, MacKay J, Bohlmann J, Jones SJ. 2013. Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* **29**: 1492-1497.

Bois G, Bigras FJ, Bertrand A, Piché Y, Fung MY, Khasa DP. 2006. Ectomycorrhizal fungi affect the physiological responses *of Picea glauca* and *Pinus banksiana* seedlings exposed to an NaCl gradient. *Tree Physiology* **26**: 1185-1196.

Bonfante P, Genre A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications* **1**: 48.

Borgnia M, Nielsen S, Engel A, Agre P. 1999. Cellular and molecular biology of the aquaporin water channels. *Annual Reviews of Biochemistry* **68**: 425–458.

Bots M, Vergeldt F, Wolters-Arts M, Weterings K, van As H, Mariani C. 2005.

Aquaporins of the PIP2 class are required for efficient anther dehiscence in tobacco. *Plant Physiology* **137**: 1049-1056.

Boursiac Y, Chen S, Luu DT, Sorieul M, van den Dries N, Maurel C. 2005. Early effects of salinity on water transport in *Arabidopsis* roots - molecular and cellular features of aquaporin expression. *Plant Physiology* **139**: 790-805.

Boyle CD, Hellenbrand KE. 1991. Assessment of the effect of mycorrhizal fungi on drought tolerance of conifer seedlings. *Canadian Journal of Botany* **69**: 1764-1771.

Brownlee C, Duddridge JA, Malibari A, Read DJ. 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant and Soil* **71**: 433-443.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. *Working with Mycorrhizas in Forestry and Agriculture*. Canberra, Australia: Australian Centre for International Agricultural Research, 196-208.

Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New phytologist* **154**: 275-304.

Burns C, Leach KM, Elliott TJ, Challen MP, Foster GD, Bailey A. 2006. Evaluation of Agrobacterium-mediated transformation of *Agaricus bisporus* using a range of promoters linked to hygromycin resistance. *Molecular Biotechnology* **32**: 129-138.

Calvo-Polanco M, Zwiazek JJ, Voicu MC. 2008. Responses of ectomycorrhizal American elm (*Ulmus americana*) seedlings to salinity and soil compaction. *Plant and Soil* **308**: 189–200.

Calvo-Polanco M, Jones MD, Zwiazek JJ. 2009. Effects of pH on NaCl tolerance of american elm (*Ulmus americana*) seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria bicolor*. *Acta Physiologiae Plantarum* **31**: 515-522.

Cao Y, Anderova M, Crawford NM, Schroeder JI. 1992. Expression of an outward-rectifying potassium channel from maize mRNA and complementary RNA in *Xenopus* oocytes. *Plant Cell* **4**: 961–969.

Caravaca F, Díaz E, Barea J.M, Azcón-Aguilar C, Roldan A. 2003. Photosynthetic and transpiration rates of *Olea europaea* subsp. *sylvestris* and *Rhamnus lycioides* as affected by water deficit and mycorrhiza. *Biologia Plantarum* **46**: 637-639.

Carlson LW. 1983. *Guidelines for rearing containerized conifer seedlings in the prairie provinces* (Vol. 214) Logan PA, Waldron RM (eds). Northern Forest Research Centre, Canada.

Chaumont F, Moshelion M, Daniels MJ. 2005. Regulation of plant aquaporin activity. *Biology of the Cell* **97**: 749-764.

Chaumont F, Tyerman SD. 2014. Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiology* **164**: 1600-1618.

Cheng CK, Au CH, Wilke SK, Stajich JE, Zolan ME, Pukkila PJ, Kwan HS. 2013. 5'-Serial Analysis of Gene Expression studies reveal a transcriptomic switch during fruiting body development in *Coprinopsis cinerea*. *BMC Genomics* **14**: 195.

Choi WG, Roberts DM. 2007. Arabidopsis NIP2; 1, a major intrinsic protein transporter of lactic acid induced by anoxic stress. *Journal of Biological Chemistry* **282**: 24209-24218.

Chum WW, Kwan HS, Au CH, Kwok IS, Fung YW. 2011. Cataloging and profiling genes expressed in *Lentinula edodes* fruiting body by massive cDNA pyrosequencing and LongSAGE. *Fungal Genetics and Biology* **48**: 359–369.

Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E. 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* **51**: 61-70.

Coleman MD, Bledsoe CS, Lopushinsky W. 1989. Pure culture response of ectomycorrhizal fungi to imposed water stress. *Canadian Journal of Botany* **67**: 29-39.

Coleman MD, Bledsoe CS, Smit B. 1990. Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings. *New Phytologist* **115**: 275–284.

Cummins EP, Selfridge AC, Sporn PH, Sznajder JI, Taylor CT. 2014. Carbon dioxide-sensing in organisms and its implications for human disease. *Cellular and*

Molecular Life Sciences **71**: 831-45.

Danielson JAH, Johanson U. 2008. Unexpected complexity of the aquaporin gene family in the moss *Physcomitrella patens*. *BMC Plant Biology* **8**: 45.

Davidson EA, Richardson AD, Savage KE, Hollinger DY. 2006. A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. *Global Change Biology* **12**: 230-239.

Dietz S, von Bülow J, Beitz E, Nehls U. 2011. The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: lessons for symbiotic functions. *New Phytologist* **190**: 927-40.

Ding Y, Liang S, Lei J, Chen L, Kothe E, Ma A. 2011. *Agrobacterium tumefaciens* mediated fused *egfp-hph* gene expression under the control of *gpd* promoter in *Pleurotus ostreatus*. *Microbiological Research* **166**: 314-322.

Di Battista C, Selosse MA, Bouchard D, Stenström E, Le Tacon F. 1996. Variations in symbiotic efficiency, phenotypic characters and ploidy level among different isolates of the ectomycorrhizal basidiomycete *Laccaria bicolor* strain S238. *Mycological Research* **100**: 1315-1324.

Dominguez I, Itoh K, Sokol SY. 1995. Role of glycogen synthase kinase 3b as a negative regulator of dorsoventral axis formation in *Xenopus* embryos. *Proceedings of the National Academy of Sciences USA* **92**: 8498–8502.

Dosskey MG, Linderman RG, Boersma L. 1990. Carbon–sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. *New Phytologist* **115**: 269-274.

Duddridge JA, Malibari A, Read DJ. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* **287**: 834–836.

Egerton-Warburton LM, Graham RC, Hubbert KR. 2003. Spatial variability in mycorrhizal hypae and nutrient and water availability in a soil-weathered bedrock profile. *Plant and Soil* **249**: 331–342.

Egerton-Warburton LM, Querejeta JI, Allen MF. 2007. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *Journal of Experimental Botany* **58**: 1473–1483.

Egerton-Warburton LM, Querejeta JI, Allen MF. 2008. Efflux of hydraulically lifted water from mycorrhizal fungal hyphae during imposed drought. *Plant Signal Behav* **3**: 68-71.

Egli S. 2011. Mycorrhizal mushroom diversity and productivity—an indicator of forest health? *Annals of Forest Science* **68**: 81-88.

Ehlert C, Maurel C, Tardieu F, Simonneau T. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology* **150**: 1093-1104.

El Kayal W, Allen CCG, Ju CJT, Adams E, King-Jones S, Zaharia LI, Abrams SR, Cooke JE. 2011. Molecular events of apical bud formation in white spruce, *Picea glauca*. *Plant, Cell and Environment* **34**: 480-500.

Emanuelsson O, Brunak S, Heijne G, Nielsen H. 2007. Locating proteins in the cell using TargetP, SignalP, and related tools. *Nature Protocols* **2**: 953-971.

Fajardo-López M, Dietz S, Grunze N, Bloschies J, Weiß M, Nehls U. 2008. The sugar porter gene family of *Laccaria bicolor*: function in ectomycorrhizal symbiosis and soil-growing hyphae. *New Phytologist* **180**: 365-378.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783–791.

Felten J, Kohler A, Morin E, Bhalerao RP, Palme K, Martin F, Ditengou FA, Legué V. 2009. The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar and Arabidopsis through auxin transport and signaling. *Plant physiology* **151**: 1991-2005.

Fennell A, Markhart AH. 1998. Rapid acclimation of root hydraulic conductivity to low temperature. *Journal of Experimental Botany* **49**: 879-884.

Fetter K, Van Wilder V, Moshelion M, Chaumont F. 2004. Interactions between plasma membrane aquaporins modulate their water channel activity. *The Plant Cell* **16**: 215-228.

Fischer G, Kosinska-Eriksson U, Aponte-Santamaría C, Palmgren M, Geijer C, Hedfalk K, Hohmann S, de Groot BL, Neutze R, Lindkvist-Petersson K. 2009. Crystal structure of a yeast aquaporin at 1.15 Å reveals a novel gating mechanism. *PLoS Biology* **7**: e1000130.

Fischer M, Kaldenhoff R. 2008. On the pH regulation of plant aquaporins. *Journal of Biological Chemistry* **283**: 33889-33892.

Fischer R, Zekert N, Takeshita N. 2008. Polarized growth in fungi–interplay between the cytoskeleton, positional markers and membrane domains. *Molecular Microbioloy* **68**: 813-826.

Flexas J, Ribas-Carbó M, Hanson DT, Bota J, Otto B, Cifre J, McDowell N, Medrano H, Kaldenhof R. 2006. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. *The Plant Journal* **48**: 427-439.

Fortin JA, Lamhamedi MS. 2009. Ecophysiology of sporocarp development of ectomycorrhizal basidiomycetes associated with boreal forest gymnosperms. In: Khasa D, Piché Y, Coughlan AP (eds) *Advances in mycorrhizal science and technology*. Ottawa, Canada: NRC Research Press, 161-173.

Fortin MG, Morrison NA, Verma DPS. 1987. Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment. *Nucleic acids research* **15**: 813-824.

Fraysse LC, Wells B, McCann MC, Kjellbom P. 2005. Specific plasma membrane aquaporins of the PIP1 subfamily are expressed in sieve elements and guard cells. *Biology of the Cell* **97**: 519-534.

Frensch J, Steudle E. 1989. Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). *Plant Physiology* **91**: 719-726.

Gabella S, Abbá S, Duplessis S, Montanini B, Martin F, Bonfante P. 2005. Transcript profiling reveals novel marker genes involved in fruiting body formation in

Tuber borchii. *Eukaryotic Cell* **4**: 1599–1602.

Gambetta GA, Fei J, Rost TL, Knipfer T, Matthews MA, Shackel KA, Walker MA, McElrone AJ. 2013. Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport. *Plant Physiology* **163**: 1254-1265.

Gao YP, Young L, Bonham-Smith P, Gusta LV. 1999. Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions. *Plant Molecular Biology* **40**: 635-644.

Gena P, Pellegrini-Calace M, Biasco A, Svelto M, Calamita G. 2011. Aquaporin membrane channels: biophysics, classification, functions, and possible biotechnological applications. *Food Biophysics* **6**: 241–249.

Gerbeau P, Amodeo G, Henzler T, Santoni V, Ripoche P, Maurel C. 2002. The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. *The Plant Journal* **30**: 71-81.

Gévry M, Villeneuve N. 2009. Ecology and management of edible ectomycorrhizal mushrooms in eastern Canada. In: Khasa D, Piché Y, Coughlan AP (eds) *Advances in mycorrhizal science and technology*. Ottawa, Canada: NRC Research Press, 175-191.

Giovannetti M, Balestrini R, Volpe V, Guether M, Straub D, Costa A, Ludewig U, Bonfante P. 2012. Two putative-aquaporin genes are differentially expressed during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *BMC Plant Biology* **12**: 186.

Godbout C, Fortin JA. 1990. Cultural control of basidiome formation in *Laccaria bicolor* with container-grown white pine seedlings. *Mycological Research* **94**: 1051-1058.**

Goldstein GH, Brubaker LB, Hinckley TM. 1985. Water relations of white spruce (*Picea glauca* (Moench) Voss) at tree line in north central Alaska. *Canadian Journal of Forest Research* **15**: 1080–1087.

Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F. 2009. Aquaporins are multifunctional water and solute transporters highly divergent in living

organisms. *Biochimica et Biophysica Acta* **1788**: 1213–1228.

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819–823.

Grigoriev IV, Cullen D, Goodwin SB, Hibbett D, Jeffries TW, Kubicek CP, Kuske C, Magnuson JK, Martin F, Spatafora JW, Tsang A, Baker SE. 2011. Fueling the future with fungal genomics. *Mycology* **2**: 192–209.

Grigoriev IV, Nordberg H, Shabalov I, Aerts A, Cantor M, Goodstein D, Kuo A, Minovitsky S, Nikitin R, Ohm RA, Otillar R, Poliakov A, Ratnere I, Riley R, Smirnova T, Rokhsar D, Dubchak I. 2012. The genome portal of the department of energy joint genome institute. *Nucleic Acids Research* **40**: D26-D32.

Groome MC, Axler SR, Gifford DJ. 1991. Hydrolysis of lipid and protein reserves in loblolly pine seeds in relation to protein electrophoretic patterns following imbibition. *Physiology Plantarum* **83**: 99-106.

Groppa MD, Benavides MP, Zawoznik MS. 2012. Root hydraulic conductance, aquaporins and plant growth promoting microorganisms: A revision. *Applied Soil Ecology* **61**: 247-254.

Grossnickle SC. 2000. *Ecophysiology of northern spruce species - The Performance of Planted Seedlings.* Ottawa, Canada: NRC Research Press, 1-5, 115-170.

Gupta AB, Sankararamakrishnan R. 2009. Genome-wide analysis of major intrinsic proteins in the tree plant *Populus trichocarpa*: characterization of XIP subfamily of aquaporins from evolutionary perspective. *BMC Plant Biology* **9**: 134.

Gustavsson S, Lebrun A-S, Norden K, Chaumont F, Johanson U. 2005. A novel plant major intrinsic protein in *Physcomitrella patens* most similar to bacterial glycerol channels. *Plant Physiology* **139**: 287–295.

Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F. 2006. Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. *Plant Molecular Biology* **62**: 305-323.

Hacquard S, Tisserant E, Brun A, Legué V, Martin F, Kohler A. 2013. Laser microdissection and microarray analysis of *Tuber melanosporum* ectomycorrhizas reveal functional heterogeneity between mantle and Hartig net compartments. *Environmental Microbiology* **15**: 1853-1869.

Hall RA, De Sordi L, MacCallum DM, Topal H, Eaton R, Bloor JW, Robinson GK, Levin LR, Buck J, Wang Y. 2010. CO_2 acts as a signalling molecule in populations of the fungal pathogen *Candida albicans*. *PLoS Pathogens* **6**: e1001193.

Hansen M, Kun JF, Schultz JE, Beitz E. 2002. A single, bi-functional aquaglyceroporin in blood-stage *Plasmodium falciparum* malaria parasites. *Journal of Biological Chemistry* **277**: 4874–4882.

Hara-Chikuma M, Verkman AS. 2006. Physiological roles of glyceroltransporting aquaporins: the aquaglyceroporins. *Cellular and Molecular Life Sciences* **63**: 1386-1392.

Harrison MJ. 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Reviews of Microbiology* **59**: 19 -42.

Harrison MJ. 2012. Cellular programs for arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology* **15**: 691-698.

Hedfalk K, Törnroth-Horsefield S, Nyblom M, Johanson U, Kjellbom P, Neutze R. 2006. Aquaporin gating. *Current Opinions in Structural Biology* **16**: 447-456.

Van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406-1423.

Heller G, Adomas A, Li G, Osborne J, van Zyl L, Sederoff R, Finlay RD, Stenlid J, Asiegbu FO. 2008. Transcriptional analysis of *Pinus sylvestris* roots challenged with the ectomycorrhizal fungus *Laccaria bicolor*. *BMC Plant Biology* **8**: 19.

Hénin J, Tajkhorshid E, Schulten K, Chipot C. 2008. Diffusion of glycerol through *Escherichia coli* aquaglyceroporin GlpF. *Biophysical Journal* **94**: 832-839.

Herrera M, Hong NJ, Garvin JL. 2006. Aquaporin-1 transports NO across cell membranes. *Hypertension* **48**: 157-164.

Hirokawa T, Boon-Chieng S, Mitaku S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* **14**: 378-379.

Hodge A, Helgason T, Fitter AH. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* **3**: 267-273.

Hohmann S, Bill RM, Kayingo G, Prior BA. 2000. Microbial MIP channels. *Trends in Microbiology* **8**: 33–38.

Hohmann S. 2002. Osmotic stress signaling and osmoadaptation in yeasts. *Microbiology and Molecular Biology Reviews* **66**: 300-372.

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. 2001. The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany* **52**: 2245-2264.

Hosie RC. 1969. *Native Trees of Canada*. 7th Edition, Canadian Forestry Service, Department of Fisheries and Forestry, Ottawa, Canada: Queen's Printer, 64-65.

Hub JS, De Groot BL. 2008. Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proceedings of the National Academy of Sciences USA* **105**: 1198-1203.

Husted L, Lavender DP. 1989. Effect of soil temperature upon the root growth and mycorrhizal formation of white spruce (*Picea glauca* (Moench) Voss) seedlings grown in controlled environments. *Annals of Forest Science* **46**: 750–753.

Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM. 2008. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecology* **55**: 45-53.

Javot H, Maurel C. 2002. The role of aquaporins in root water uptake. *Annals of Botany* **90**: 301-313.

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Güçlü J, Vinh J, Heyes J, Franck KI, Schäffner AR, Bouchez D, Maurel C. 2003. Role of a single aquaporin isoform in root water uptake. *The Plant Cell* **15**: 509-522.

Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, Kjellbom P. 1998. Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *The Plant Cell* **10**: 451-459.

Johanson U, Karlsson M, Johansson I, Gustavsson S, Sjovall S, Fraysse L, Weig AR, Kjellbom P. 2001. The complete set of genes encoding major intrinsic proteins in *Arabidopsis* provides a framework for a new nomenclature for major intrinsic proteins in plants. *Plant Physiology* **126**: 1358-1369.

Johnson-Flanagan AM, Owens JN. 1985. Development of white spruce (*Picea glauca*) seedling roots. *Canadian Journal of Botany* **63**: 456–462.

Johnson-Flanagan AM, Owens JN. 1985. Root growth and root growth capacity of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Canadian Journal of Forest Research* **15**: 625-630.

Johnson-Flanagan AM, Owens JN. 1986. Root respiration in white spruce (*Picea glauca* [Moench] Voss) seedlings in relation to morphology and environment. *Plant Physiology* **81**: 21–25.

Joh JH, Lee JS, Kim KH, Jeong SJ, Youn WH, Kim NK, Son ES, Cho YS, Yoo YB, Lee CS, Kim BG. 2007. Isolation of genes expressed during the developmental stages of the oyster mushroom, *Pleurotus ostreatus*, using expressed sequence tags. *FEMS Microbiology Letters* **276**: 19–25.

Jones DT, Taylor WR, Thornton JM. 1992. The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences* **8**: 275-282.

Jonsson LM, Nilsson MC, Wardle DA, Zackrisson O. 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos* **93**: 353-364.

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N. 2008. Aquaporins and plant water balance. *Plant, Cell and Environment* **31**: 658-666.

Kamaluddin M, Zwiazek JJ. 2002. Ethylene enhances water transport in hypoxic aspen (*Populus tremuloides*). *Plant Physiology* **128**: 962-969.

Kemppainen M, Circosta A, Tagu D, Martin F, Pardo AG. 2005. *Agrobacterium*-mediated transformation of the ectomycorrhizal symbiont *Laccaria bicolor* S238N. *Mycorrhiza* **16**: 19–22.

Kemppainen M, Duplessis S, Martin F, Pardo AG. 2008. T-DNA insertion, plasmid rescue and integration analysis in the model mycorrhizal fungus *Laccaria bicolor*. *Microbial Biotechnology* **1**: 258-269.

Kemppainen M, Duplessis S, Martin F, Pardo AG. 2009. RNA silencing in the model mycorrhizal fungus *Laccaria bicolor*: gene knock‐down of nitrate reductase results in inhibition of symbiosis with *Populus*. *Environmental Microbiology* **11**: 1878-1896.

Kemppainen MJ, Pardo AG. 2010. pHg/pSILBAγ vector system for efficient gene silencing in homobasidiomycetes: optimization of ihpRNA - triggering in the mycorrhizal fungus *Laccaria bicolor*. *Microbial Biotechnology* **3**: 178–200.

Kernaghan G, Sigler L, Khasa D. 2003. Mycorrhizal and root endophytic fungi of containerized *Picea glauca* seedlings assessed by rDNA sequence analysis. *Microbial Ecology* **45**: 128-136.

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U. 2005. Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biology* **7**: 706–712.

Kilaru S, Hoegger PJ, Majcherczyk A, Burns C, Shishido K, Bailey A, Foster GD, Kües U. 2006. Expression of laccase gene *lcc1* in *Coprinopsis cinerea* under control of various basidiomycetous promoters. *Applied Microbiology and Biotechnology* **71**: 200-210.

Kivlin SN, Emery SM, Rudgers JA. 2013. Fungal symbionts alter plant responses to global change. *America Journal of Botany* **100**: 1445-1457.

Kline KG, Barrett-Wilt GA, Sussman MR. 2010. In planta changes in protein phosphorylation induced by the plant hormone abscisic acid. *Proceedings of the National Academy of Sciences USA* **107**: 15986-15991.

Koele N, Dickie IA, Blum JD, Gleason JD, de Graaf L. 2014. Ecological significance of mineral weathering in ectomycorrhizal and arbuscular mycorrhizal ecosystems from a field-based comparison. *Soil Biology and Biochemistry* **69**: 63-70.

Kottke I, Oberwinkler F. 1986. Mycorrhiza of forest trees—structure and function. *Trees* **1**: 1-24.

Krajinski F, Biela A, Schubert D, Gianinazzi-Pearson V, Kaldenhoff R, Franken P. 2000. Arbuscular mycorrhiza development regulates the mRNA abundance of Mtaqp1 encoding a mercury-insensitive aquaporin of *Medicago truncatula*. *Planta* **211**: 85-90.

Krane CM, Goldstein DL. 2007. Comparative functional analysis of aquaporins/glyceroporins in mammals and anurans. *Mammaliam Genome* **18**: 452–462.

Krogh A, Larsson B, Von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *Journal of Molecular Biology* **305**: 567-580.

Kües U, Liu Y. 2000. Fruiting body production in basidiomycetes. *Applied Microbiology and Biotechnology* **54**: 141-152.

Kües U. 2000. Life history and developmental processes in the basidiomycete *Coprinus cinereus*. *Microbiology and Molecular Biology Reviews* **64**: 316-353.

Laizé V, Gobin R, Rousselet G, Badler C, Hohmann S, Ripoche P, Tacnet,F. 1999. Molecular and functional study of *AQY1* from *Saccharomyces cerevisiae*: role of the C-terminal domain. *Biochemical and Biophysical Research Communications* **257**: 139-144.

Lamhamedi MS, Godbout C, Fortin JA. 1994. Dependence of *Laccaria bicolor* basidiome development on current photosynthesis of *Pinus strobus* seedlings. *Canadian Journal of Forest Research* **24**: 1797-1804.

Landeweert R, Hoffland E, Finlay RD, Kuyper TW, van Breemen N. 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology & Evolution* **16**: 248-254.

Landhäusser SM, Muhsin TM, Zwiazek JJ. 2002. The effect of ectomycorrhizae on water relations in aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) at low soil temperatures. *Canadian Journal of Botany* **80**: 684-689.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. ClustalW and ClustalX version 2. *Bioinformatics* **23**: 2947-2948.

Last FT, Mason PA, Pelham J, Ingleby K. 1984. Fruitbody production by sheathing mycorrhizal fungi: effects of 'host'genotypes and propagating soils. *Forest Ecology and Management* **9**: 221-227.

Latgé JP. 2007. The cell wall: a carbohydrate armour for the fungal cell. *Molecular Microbiology* **66**: 279-290.

Laur J, Hacke UG. 2013. Transpirational demand affects aquaporin expression in poplar roots. *Journal of Experimental Botany* **64**: 2283-2293.

Laur J, Hacke UG. 2014. Exploring *Picea glauca* aquaporins in the context of needle water uptake and xylem refilling. *New Phytologist* **203**: 388-400.

Laur J, Hacke UG. 2014. The role of water channel proteins in facilitating recovery of leaf hydraulic conductance from water stress in *Populus trichocarpa*. *PloS One* **9**: e111751.

Lawrence SD, Novak NG, Xu H, Cooke JEK. 2013. Herbivory of maize by southern corn rootworm induces expression of the major intrinsic protein. *Plant Signaling and Behavior*. doi: 10.4161/psb.24937.

Lee SH, Chung GC, Steudle E. 2005. Gating of aquaporins by low temperature in roots of chilling-sensitive cucumber and chilling-tolerant figleaf gourd. *Journal of Experimental Botany* **56**: 985-995.

Lee SH, Calvo-Polanco M, Chung GC, Zwiazek JJ. 2010. Role of aquaporins in root water transport of ectomycorrhizal jack pine (*Pinus banksiana*) seedlings exposed to NaCl and fluoride. *Plant, Cell and Environment* **33**: 769–780.

Lee SH, Chung GC, Jang JY, Ahn SJ, Zwiazek JJ. 2012. Overexpression of PIP2;5 aquaporin alleviates effects of low root temperature on cell hydraulic conductivity and growth in Arabidopsis. *Plant Physiology* **159**: 479-488.

Lehto T, Zwiazek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* **21**: 71–90.

Levy Y, Krikun J. 1980. Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. *New Phytologist* **85**: 25-31.

Lew RR. 2011. How does a hypha grow? The biophysics of pressurized growth in fungi. *Nature Reviews Microbiology* **9**: 509-518.

Liu J, Equiza MA, Navarro-Ródenas A, Lee SH, Zwiazek JJ. 2014. Hydraulic adjustments in aspen (*Populus tremuloides*) seedlings following defoliation involve root and leaf aquaporins. *Planta* **240**: 553-564.

Liu Y, Srivilai P, Loos S, Aebi M, Kües U. 2006. An essential gene for fruiting body initiation in the basidiomycete *Coprinopsis cinerea* is homologous to bacterial cyclopropane fatty acid synthase genes. *Genetics* **172**: 873-884.

Liu Y. 2012. *Calcium-related fungal genes implicated in arbuscular mycorrhiza*. Ph.D. Thesis, Huazhong Agricultural University, Wuhan, China, and Burgundy University, Burgundy, France.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**: 402-408.

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B. 2013. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617-630.

Li X, Wang X, Yang Y, Li R, He Q, Fang X, Luu DT, Maurel C, Lin J. 2011. Single-molecule analysis of PIP2;1 dynamics and partitioning reveals multiple modes of *Arabidopsis* plasma membrane aquaporin regulation. *Plant Cell* **23**: 3780–3797.

Li X, Luu DT, Maurel C, Lin J. 2013. Probing plasma membrane dynamics at the single-molecule level. *Trends in Plant Science* **18**: 617-624.

Loewe A, Einig W, Shi L, Dizengremel P, HAMPP R. 2000. Mycorrhiza formation and elevated CO_2 both increase the capacity for sucrose synthesis in source leaves of spruce and aspen. *New Phytologist* **145**: 565-574.

Lopez F, Bousser A, Sissoëff I, Gaspar M, Lachaise B, Hoarau J, Mahé A. 2003. Diurnal regulation of water transport and aquaporin gene expression in maize roots:

contribution of PIP2 proteins. *Plant and Cell Physiology* **44**: 1384-1395.

Lopez D, Bronner G, Brunel N, Auguin D, Bourgerie S, Brignolas F, Carpin S, Tournaire-Roux C, Maurel C, Fumanal B, Martin F, Sakr S, Label P, Julien JL, Gousset-Dupont A, Venisse JS. 2012. Insights into *Populus* XIP aquaporins: evolutionary expansion, protein functionality, and environmental regulation. *Journal of Experimental Botany* **63**: 2217-2230.

Luard EJ, Griffin DM. 1981. Effect of water potential on fungal growth and turgor. *Transactions of the British Mycological Society* **76(1)**: 33-40.

Lucic E, Fourrey C, Kohler A, Martin F, Chalot M, Brun-Jacob A. 2008. A gene repertoire for nitrogen transporters in *Laccaria bicolor*. *New Phytologist* **180**: 343-364.

Ludewig U, Dynowski M. 2009. Plant aquaporin selectivity: where transport assays, computer simulations and physiology meet. *Cellular and Molecular Life Sciences* **66**: 3161-3175.

Luo ZB, Janz D, Jiang X, Göbel C, Wildhagen H, Tan Y, Rennenberg H, Feussner I, Polle A. 2009. Upgrading root physiology for stress tolerance by ectomycorrhizas: insights from metabolite and transcriptional profiling into reprogramming for stress anticipation. *Plant Physiology* **151**: 1902-1917.

Maeshima M, Ishikawa F. 2008. ER membrane aquaporins in plants. *Pflügers Archiv-European Journal of Physiology* **456**: 709-716.

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Bécard G, Dénarié J. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469**: 58-63.

Malloch D, Malloch B. 1982. The mycorrhizal status of boreal plants: additional species from northeastern Ontario. *Canadian Journal of Botany* **60**: 1035-1040.

Marjanović Ž, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiss M, Hampp R, Nehls U. 2005. Aquaporins in poplar: what a difference a symbiont makes! *Planta* **222**: 258–268.

Marjanović Ž, Nehls U. 2008. Ectomycorrhiza and water transport. In: Varma A (ed)

Mycorrhiza. Berlin, Heidelberg, Germany: Springer, 149-159.

Martinez-Ballesta MC, Rodriiguez-Hernández MC, Alcaraz-López C, Mota-Cadenas C, Muries B, Carvajal M. 2011. Plant Hydraulic Conductivity: The Aquaporins Contribution. In: *Hydraulic Conductivity - Issues, Determination and Applications*. (Ed.) Elango L. InTech, doi: 10.5772/18580.

Martin F, Aerts A, Ahrn D, Brun A, Danchin EGJ. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452**: 88-92.

Martin F, Nehls U. 2009. Harnessing ectomycorrhizal genomics for ecological insights. *Current Opinion in Plant Biology* **12**: 508–515.

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, Porcel B, Rubini A, Amicucci A, Amselem J, Anthouard V, Arcioni S, Artiguenave F, Aury J, Ballario P, Bolchi A, Brenna A, Brun A, Buee M, Cantarel B, Chevalier G, Couloux A, Da Silva C, Denoeud F, Duplessis S, Ghignone S, Hilselberger B, Iotti M, Marcais B, Mello A, Miranda M, Pacioni G, Quesneville H, Riccioni C, Ruotolo R, Splivallo R, Stocchi V, Tisserant E, Viscomi AR, Zambonelli A, Zampieri E, Henrissat B, Lebrun M, Paolocci F, Bonfante P, Ottonello S, Wincker P. 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* **464**: 1033-1038.

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV. 2011. Sequencing the fungal tree of life. *New Phytologist* **190**: 818-821.

Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**: 153-163.

Massicotte HB, Melville LH, Peterson RL. 2005. Building a basidiocarp: a case study of *Laccaria spp*. fruitbodies in the extraradical mycelium of *Pinus* ectomycorrhizas. *Mycologist* **19**: 141-149.

Matheny PB, Gossmann JA, Zalar P, Kumar TKA, Hibbett DS. 2006. Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. *Canadian Journal of Botany* **84**: 1794-1805.

Matsumoto T, Lian HL, Su WA, Tanaka D, Liu CW, Iwasaki I, Kitagawa Y. 2009. Role of the aquaporin PIP1 subfamily in the chilling tolerance of rice. *Plant and Cell Physiology* **50**: 216-229.

Maurel C, Reizer J, Schroeder JI, Chrispeels MJ. 1993. The vacuolar membrane protein gamma-TIP creates water specific channels in *Xenopus oocytes*. *The EMBO Journal* **12**: 2241.

Maurel C, Verdoucq L, Luu D, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology* **59**: 595-624.

Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L. 2009. The cellular dynamics of plant aquaporin expression and functions. *Current Opinion in Plant Biology* **12**: 690-698.

Maurel C, Plassard C. 2011. Aquaporins: for more than water at the plant–fungus interface? *New Phytologist* **190**: 815–817.

Mayor JR, Schuur EAG, Henkel TW. 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecology Letters* **12**: 171-183.

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M. 2006. A silicon transporter in rice. *Nature* **440**: 688-691.

Miller EW, Dickinson BC, Chang CJ. 2010. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. *Proceedings of the National Academy of Sciences USA* **107**: 15681-15686.

Mitaku S, Hirokawa T. 1999. Physicochemical factors for discriminating between soluble and membrane proteins: hydrophobicity of helical segments and protein length. *Protein Engineering* **12**: 953–957.

Mitani N, Yamaji N, Ma JF. 2008. Characterization of substrate specificity of a rice silicon transporter, Lsi1. *Pflügers Archiv-European Journal of Physiology* **456**: 679-686.

Mitani-Ueno N, Yamaji N, Zhao FJ, Ma JF. 2011. The aromatic/arginine selectivity filter of NIP aquaporins plays a critical role in substrate selectivity for silicon, boron,

and arsenic. *Journal of Experimental Botany* **62**: 4391-4398.

Miyamoto N, Steudle E, Hirasawa T, Lafitte R. 2001. Hydraulic conductivity of rice roots. *Journal of Experimental Botany* **52**: 1835-1846.

Moore-Landecker E. 2002. Fungal spores. In: *Encyclopedia of Life Sciences (ELS)*. Chichester: John Wiley & Sons Ltd, doi: 10.1038/npg.els.0000378.

Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL, Bailey AM, Billette C, Coutinho PM, Deakin G, Doddapaneni H, Floudas D, Grimwood J, Hildén K, Kües U, LaButti KM, Lapidus A, Lindquist EA, Lucas SM, Murat C, Riley RW, Salamov AA, Schmutz J, Subramanian V, Wösten HAB, Xu J, Eastwood DC, Foster GD, Sonnenberg ASM, Cullen D, de Vries RP, Lundell T, Hibbett DS, Henrissat B, Burton KS, Kerrigan RW, Challen MP, Grigoriev IV, Martin F. 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. *Proceedings of the National Academy of Sciences USA* **109**: 17501–17506.

Mueller GM. 1992. *Systematics of Laccaria (Agaricales) in the continental United States and Canada, with discussions on extralimital taxa and descriptions of extant types*. Chicago, USA: Field Museum of Natural History.

Muhsin TM, Zwiazek JJ. 2002. Ectomycorrhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus americana* seedlings. *New Phytologist* **153**: 153-158.

Muhsin TM, Zwiazek JJ. 2002. Ectomycorrhizae increase water conductance and protect white spruce (*Picea glauca*) seedlings against salt stress. *Plant and Soil* **238**: 217-225.

Murai-Hatano M, Kuwagata T, Sakurai J, Nonami H, Ahamed A, Nagasuga K, Matsunami T, Fukushi K, Maeshima M, Okada M. 2008. Effect of low root temperature on hydraulic conductivity of rice plants and the possible role of aquaporins. *Plant and Cell Physiology* **49**: 1294-1305.

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi, Y. 2000. Structural determinants of water permeation through aquaporin-1.

Nature **407**: 599-605.

Nardini A, Salleo S, Tyree MT, Vertovec M. 2000. Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Annuals of Forest Science* **57**: 305–312.

Navarro-Ródenas A, Ruíz-Lozano JM, Kaldenhoff R, Morte A. 2012. The aquaporin TcAQP1 of the desert truffle *Terfezia claveryi* is a membrane pore for water and CO_2 transport. *Molecular Plant-Microbe Interaction* **25**: 259-266.

Navarro-Ródenas A, Bárzana G, Nicolás E, Carra A, Schubert A, Morte A. 2013. Expression analysis of aquaporins from desert truffle mycorrhizal symbiosis reveals a fine-tuned regulation under drought. *Molecular Plant-Microbe Interactions* **26**: 1068-1078.

Navarro-Ródenas A, Xu H, Kemppainen M, Pardo A, Zwiazek JJ. 2015. *Laccaria bicolor* Aquaporin LbAQP1 is required for Hartig Net Development in Trembling Aspen (*Populus tremuloides*). *Plant, Cell and Environment*. In Press.

Nehls U, Göhringer F, Wittulsky S, Dietz S. 2010. Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. *Plant Biology* **12**: 292-301.

Nehls U, Dietz S. 2014. Fungal aquaporins: cellular functions and ecophysiological perspectives. *Applied Microbiology and Biotechnology* **98**: 8835-8851.

Nguyen H, Calvo Polanco M, Zwiazek JJ. 2006. Gas exchange and growth responses of ectomycorrhizal *Picea mariana*, *Picea glauca*, and *Pinus banksiana* seedlings to NaCl and Na_2SO_4. *Plant Biology* **8**: 646-652.

Nowrousian M, Kück U. 2006. Comparative gene expression analysis of fruiting body development in two filamentous fungi. *FEMS Microbiology Letters* **257**: 328–335.

Nowrousian M. 2014. Genomics and transcriptomics to analyze fruiting body development. In: Esser K (ed) *Fungal Genomics XIII*. 2nd Edition, Berlin, Germany: Springer, 149-172.

Nylund J-E. 1987. The ectomycorrhizal infection zone and its relation to acid polysaccharides of cortical cell walls. *New Phytologist* **106**: 505–516.

Obroucheva NV, Sin'kevich IA. 2010. Aquaporins and cell growth. *Russian Journal of Plant Physiology* **57**: 153-165.

Onwuchekwa NE, Zwiazek JJ, Quoreshi A, Khasa DP. 2014. Growth of mycorrhizal jack pine (*Pinus banksiana*) and white spruce (*Picea glauca*) seedlings planted in oil sands reclaimed areas. *Mycorrhiza* **24**: 431-441.

Ouziad F, Hildebrandt U, Schmelzer E, Bothe H. 2005. Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. *Journal of Plant Physiology* **162**: 634-649.

Park W, Scheffler BE, Bauer PJ, Campbell BT. 2010. Identification of the family of aquaporin genes and their expression in upland cotton (*Gossypium hirsutum* L.). *BMC Plant Biology* **10**: 142.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

Peterson RL, Massicotte HB, Melville LH. 2004. *Mycorrhizas: Anatomy and Cell Biology*. Ottawa, Canada: NRC Research Press, 173.

Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S. 2005. Aquaporins in yeasts and filamentous fungi. *Biology of the Cell* **97**: 487–500.

Pfaffl MW. 2004. Quantification strategies in real-time PCR. In: Bustin SA (ed) *A-Z of quantitative PCR*. La Jolla, USA: International University Line (IUL), 87-112.

Pham GH, Kumari R, Singh A, Malla R, Prasad R, Sachdev M, Kaldorf M, Buscot F, Oelmüller R, Hampp R, Saxena AK, Rexer K, Kost G, Varma A. 2004. Axenic culture of symbiotic fungus *Piriformospora indica*. In: Varma A, Abbott L, Werner D, Hampp (eds) *Plant surface microbiology*. Berlin, Heidelberg, Germany: Springer, 593-613.

Plamboeck AH, Dawson TE, Egerton-Warburton LM, North M, Bruns TD, Querejeta JI. 2007. Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. *Mycorrhiza* **17**: 439–447.

Plett JM, Kemppainen M, Kale SD, Kohler A, Legué V, Brun A, Tyler BM, Pardo AG, Martin F. 2011. A secreted effector protein of *Laccaria bicolor* is required for

symbiosis development. *Current Biology* **21**: 1197-1203.

Plett JM, Martin F. 2012. Poplar root exudates contain compounds that induce the expression of *MiSSP7* in *Laccaria bicolor*. *Plant Signaling and Behavior* **7**: 12-15.

Plett JM, DaguerreY, Wittulsky S, Vayssières A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C, Martin F. 2014. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences USA* **111**: 8299-8304.

Porcel R, Ruiz-Lozano JM. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *Journal of Experimental Botany* **55**: 1743-1750.

Porcel R, Aroca R, Azcón R, Ruiz-Lozano J. 2006. PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Molecular Biology* **60**: 389-404.

Prado K, Maurel C. 2013. Regulation of leaf hydraulics: from molecular to whole plant levels. *Frontiers in Plant Science.* doi: 10.3389/fpls.2013.00255.

Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. 2008. Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2; 1 in response to salt stress. *Molecular and Cellular Proteomics* **7**: 1019-1030.

Prasad GV, Coury LA, Finn F, Zeidel ML. 1998. Reconstituted aquaporin 1 water channels transport CO_2 across membranes. *Journal of Biological Chemistry* **273**: 33123–33126.

Puppo A, Pauly N, Boscari A, Mandon K, Brouquisse R. 2013. Hydrogen peroxide and nitric oxide: key regulators of the legume—Rhizobium and mycorrhizal symbioses. *Antioxidants and Redox Signaling* **18**: 2202-2219.

Querejeta J, Egerton-Warburton LM, Allen MF. 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* **134**: 55-64.

Querejeta JI, Egerton-Warburton LM, Allen MF. 2007. Hydraulic lift may buffer rhizosphere hyphae against the negative effects of severe soil drying in a California Oak savanna. *Soil Biology and Biochemistry* **39**: 409-417.

Rahmad N, Al-Obaidi JR, Rashid NM, Zean NB, Yusoff MH, Shaharuddin NS, Jamil NA, Saleh NM. 2014. Comparative proteomic analysis of different developmental stages of the edible mushroom *Termitomyces heimii*. *Biological Research* **47**: 30.

Rigault P, Boyle B, Lepage P, Cooke JEK, Bousquet J, MacKay JJ. 2011. A white spruce gene catalogue resource for conifer genome analyses. *Plant Physiology* **157**: 14-28.

Roberts JJ, Zwiazek JJ. 2001. Growth, morphology, and gas exchange in white spruce (*Picea glauca*) seedlings acclimated to different humidity conditions. *Canadian Journal of Forest Research* **31**: 1038-1045.

Rüdinger M, Hallgren SW, Steudle E, Schulze ED. 1994. Hydraulic and osmotic properties of spruce roots. *Journal of Experimental Botany* **45**: 1413-1425.

Ruiz-Herrera J. 2012. *Fungal Cell Wall - Structure, synthesis and assambly*. 2nd Edition, Boca Raton, USA: CRC Press, 149-165.

Ruiz-Lozano JM, del Mar Alguacil M, Bárzana G, Vernieri P, Aroca R. 2009. Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. *Plant Molecular Biology* **70**: 565-579.

Ruiz-Lozano JM, Aroca R. 2010. Modulation of aquaporin genes by the arbuscular mycorrhizal symbiosis in relation to osmotic stress tolerance. In: Seckbach J, Grube M (eds) *Symbioses and Stress*. Netherlands: Springer, 357-374.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Sakr S, Alves G, Morillon R, Maurel K, Decourteix M, Guilliot A, Fleurat-**Lessard P, Julien JL, Chrispeels MJ. 2003.** Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiology* **133**: 630-641.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671-675.

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. 1965. Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. *Science* **148**: 339-346.

Schützendübel A, Polle A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany* **53**: 1351-1365.

Shelden MC, Howitt SM, Kaiser BN, Tyerman SD. 2009. Identification and functional characterization of aquaporins in the grapevine, *Vitis vinifera*. *Functional Plant Biology* **36**: 1065–1078.

Sidoux-Walter F, Pettersson N, Hohmann S. 2004. The *Saccharomyces cerevisiae* aquaporin Aqy1 is involved in sporulation. *Proceedings of the National Academy of Sciences USA* **101**: 17422-17427.

Siemen J, Zwiazek JJ. 2003. Effects of water deficit stress and recovery on the root water relations of trembling aspen (*Populus tremuloides*) seedlings. *Plant Science* **165**: 113-120.

Siemens JA, Zwiazek JJ. 2008. Root hydraulic properties and growth of balsam poplar (*Populus balsamifera*) mycorrhizal with *Hebeloma crustuliniforme* and *Wilcoxina mikolae* var. *mikolae*. *Mycorrhiza* **18**: 393–401.

Siemens JA, Zwiazek J. 2011. *Hebeloma crustuliniforme* modifies root hydraulic responses of trembling aspen (*Populus tremuloides*) seedlings to changes in external pH. *Plant and Soil* **345**: 247–256.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579-582.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16-20.

Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. 3rd Edition, Cambridge, UK: Academic Press.

Soveral G, Prista C, Moura TF, Loureiro-Dias MC. 2010. Yeast water channels: an overview of orthodox aquaporins. *Biology of the Cell* **103**: 35-54.

Stamets P. 2000. *Growing gourmet and medicinal mushrooms*. 3rd Edition, Berkeley, CA: Ten Speed Press, 113, 164, 185.

Steudle E. 1993. Pressure probe techniques: basic principles and application to studies of water and solute relations at the cell, tissue, and organ level. In: Smith JAC, Griffiths H (eds) *Water Deficits: Plant Responses from Cell to Community*. Oxford, UK: Bios Scientific Publishers Ltd, 5–36.

Steudle E, Peterson CA. 1998. How does water get through roots? *Journal of Experimental Botany* **49**: 775–788.

Steudle E. 2000. Water uptake by roots: effects of water deficit. *Journal of Experimental Botany* **51**: 1531-1542.

Straatsma G, Sonnenberg AS, Van Griensven LJ. 2013. Development and growth of fruit bodies and crops of the button mushroom, *Agaricus bisporus*. *Fungal Biology* **117**: 697–707.

Subramanian KS, Charest C, Dwyer LM, Hamilton RI. 1997. Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. *Canadian Journal of Botany* **75**: 1582-1591.

Le Tacon F, Zeller B, Plain C, Hossann C, Bréchet C, Robin C. 2013. Carbon transfer from the host to *Tuber melanosporum* mycorrhizas and ascocarps followed using a ^{13}C pulse-labeling technique. *PloS One* **8**: e64626.

Taiz L, Zeiger E. 2010. *Plant physiology*. 5th Edition, Sunderland, MA: Sinauer Associates, 87-89.

Takano J, Wada M, Ludewig U, Schaaf G, Von Wirén N, Fujiwara T. 2006. The *Arabidopsis* major intrinsic protein NIP5; 1 is essential for efficient boron uptake and plant development under boron limitation. *The Plant Cell* **18**: 1498-1509.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods**.** *Molecular Biology and Evolution* **28**: 2731-2739.

Tasaki Y, Sato R, Toyama S, Kasahara K, Ona Y, Sugawara M. 2014. Cloning of glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycete mushroom *Pleurotus ostreatus* and analysis of their expression during fruit-body development. *Mycoscience* **55**: 280-288.

Taylor JH, Peterson CA. 2005. Ectomycorrhizal impacts on nutrient uptake pathways in woody roots. *New Forests* **30**: 203-214.

Taylor TN, Osborn JM. 1996. The importance of fungi in shaping the paleoecosystem. *Review of Paleobotany and Palynology* **90**: 249–262.

Teramoto M, Wu B, Hogetsu T. 2012. Transfer of ^{14}C-photosynthate to the sporocarp of an ectomycorrhizal fungus *Laccaria amethystina*. *Mycorrhiza* **22**: 219-225.

Thorley RMS, Taylor LL, Banwart SA, Leake JR, Beerling DJ. 2014. The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling. *Plant, Cell and Environment*. doi: 10.1111/pce.12444.

Tinker PB, Durall DM, Jones MD. 1994. Carbon use efficiency in mycorrhizas theory and sample calculations. *New Phytologist* **128**:115-122.

Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P. 2006. Structural mechanism of plant aquaporin gating. *Nature* **439**: 688–694.

Törnroth-Horsefield S, Hedfalk K, Fischer G, Lindkvist-Petersson K, Neutze R. 2010. Structural insights into eukaryotic aquaporin regulation. *FEBS Letters* **584**: 2580–2588.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**: 393-397.

Traeger S, Altegoer F, Freitag M, Gabaldon T, Kempken F, Kumar F, Marcet-Houben M, Pöggeler S, Stajich, JE, Nowrousian M. **2013.** The genome anddevelopment-dependent transcriptomes of *Pyronema confluens*: a window into fungal evolution. *PLoS Genetics* **9**: e1003820.

Turgeman T, Asher JB, Roth-Bejerano N, Kapulnik Y, Sitrit Y. 2011. Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions. *Mycorrhiza* **21**: 623-630.

Tyree MT, Patiño S, Bennink J, Alexander J. 1995. Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany* **46**: 83-94.

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R. 2003. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. *Nature* **425**: 734-737.

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R. 2007. Arbuscular mycorrhizal symbiosis and plant aquaporin expression. *Phytochemistry* **68**: 122–129.

Unestam T, Sun YP. 1995. Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. *Mycorrhiza* **5**: 301–311.

Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. 2007. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* **35**: W71-W74.

Vandeleur RK, Sullivan W, Athman A, Jordans C, Gilliham M, Kaiser BN, Tyerman SD. 2014. Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. *Plant, Cell and Environment* **37**: 520-538.

Veneault-Fourrey C, Plett JM, Martin F. 2013. Who is Controlling whom within the Ectomycorrhizal Symbiosis: Insights from Genomic and Functional Analyses. In: de Bruijn FJ (ed) *Molecular Microbial Ecology of the Rhizosphere*. Wiley-Blackwell, Volume 1: 501-512.

Verdoucq L, Grondin A, Maurel C. 2008. Structure-function analysis of plant

aquaporin AtPIP2; 1 gating by divalent cations and protons. *Biochemical Journal* **415**: 409-416.

Verma RK, Prabh ND, Sankararamakrishnan R. 2014. New subfamilies of major intrinsic proteins in fungi suggest novel transport properties in fungal channels: implications for the host-fungal interactions. *BMC Evolutionary Biology* **14**: 173.

Voicu MC, Cooke JE, Zwiazek JJ. 2009. Aquaporin gene expression and apoplastic water flow in bur oak (*Quercus macrocarpa*) leaves in relation to the light response of leaf hydraulic conductance. *Journal of Experimental Botany* **60**: 4063-4075.

Walz T, Hirai T, Murata K, Heymann JB, Mitsuoka K, Fujiyoshi Y, Smith BL, Agre P, Engel A. 1997. The three-dimensional structure of aquaporin-1. *Nature* **387**: 624-626.

Wang DYC, Kumar S, Hedges SB. 1999. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society of London B* **266**: 163 –171.

Wang M, Gu B, Huang J, Jiang S, Chen Y, Yin Y, Pan Y, Yu G, Li Y, Wong BHC, Liang Y, Sun H. 2013. Transcriptome and proteome exploration to provide a resource for the study of *Agrocybe aegerita*. *PloS One* **8**: e56686.

Wang Y, Zwiazek J. 1999. Spring changes in water relations, gas exchange and carbohydrates of white spruce (*Picea glauca*) seedlings. *Canadian Journal of Forest Research* **29**: 332–338.

Wang Y, Zwiazek J. 1999. Effects of early spring photosynthesis on carbohydrate content, bud flushing, and root and shoot growth of *Picea glauca* bareroot seedlings. *Scandinavian Journal of Forest Research* **14**: 295–302.

Wan X, Landhäusser SM, Zwiazek JJ, Lieffers VJ. 1999. Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. *Tree Physiology* **19**: 879-884.

Wan X, Zwiazek JJ, Lieffers VJ, Landhäusser SM. 2001. Hydraulic conductance in aspen (*Populus tremuloides*) seedlings exposed to low root temperatures. *Tree Physiology* **21**: 691-696.

Watkinson SC. 2008. Basidiomycota. In: *Encyclopedia of Life Sciences (ELS)*. Chichester, UK: John Wiley & Sons, doi: 10.1002/9780470015902.a0000347.pub2.

Weatherley PE. 1982. Water uptake and flow in roots. In: Lange O, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological plant ecology II*. Berlin, Germany: Springer, 79–109.

Wiegant WM, Wery J, Buitenhuis ET, de Bont JA. 1992. Growth-promoting effect of thermophilic fungi on the mycelium of the edible mushroom *Agaricus bisporus*. *Applied and Environmental Microbiology* **58**: 2654-2659.

Wu B, Beitz E. 2007. Aquaporins with selectivity for unconventional permeants. *Cellular and Molecular Life Sciences* **64**: 2413-2421.

Wysocki R, Chéry CC, Wawrzycka D, Van Hulle M, Cornelis R, Thevelein JM, Tamás MJ. 2001. The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*. *Molecular Microbiology* **40**: 1391-1401.

Xu H, Cooke JEK, Zwiazek JJ. 2013. Phylogenetic analysis of fungal aquaporins provides insight into their possible role in water transport of mycorrhizal associations. *Botany* **91**: 495-504.

Xu H, Kemppainen M, El Kayal W, Lee SH, Pardo AG, Cooke JEK, Zwiazek JJ. 2015. Overexpression of *Laccaria bicolor* aquaporin *JQ585595* alters root water transport properties in ectomycorrhizal white spruce (*Picea glauca*) seedlings. *New Phytologist* **205**: 757-770.

Yamada M, Kurano M, Inatomi S, Taguchi G, Okazaki M, Shimosaka M. 2008. Isolation and characterization of a gene coding for chitin deacetylase specifically expressed during fruiting body development in the basidiomycete *Flammulina velutipes* and its expression in the yeast *Pichia pastoris*. *FEMS Microbiology Letters* **289**: 130-137.

Yi H, Calvo-Polanco M, MacKinnon MD, Zwiazek JJ. 2008. Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. *Environmental and Experimental Botany* **62**: 357–363.

Yun W, Hall IR. 2004. Edible ectomycorrhizal mushrooms: challenges and

achievements. *Canadian Journal of Botany* **82**: 1063-1073.

Yu GJ, WangM, Huang J, Yin YL, Chen YJ, Jian S, Jin YX, Lan XQ, Wong BHC, Liang Y, Sun H. 2012. Deep insight into the *Ganoderma lucidum* by comprehensive analysis of its transcriptome. *PLoS One* **7**: e44301.

Yu TEJ-C, Egger KN, Peterson RL. 2001. Ectendomycorrhizal associations-characteristics and functions. *Mycorrhiza* **11**: 167–177.

Zardoya R, Ding X, Kitagawa Y, Chrispeels J. 2002. Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment. *Proceedings of the National Academy of Sciences USA* **99**: 14893-14896.

Zardoya R. 2005. Phylogeny and evolution of the major intrinsic protein family. *Biology of the Cell* **97**: 397-414.

Zelazny E, Borst JW, Muylaert M, Batoko H, Hemminga MA, Chaumont F. 2007. FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. *Proceedings of the National Academy of Sciences USA* **104**: 12359-12364.

Zelazny E, Miecielica U, Borst JW, Hemminga MA, Chaumont F. 2009. An N-terminal diacidic motif is required for the trafficking of maize aquaporins ZmPIP2; 4 and ZmPIP2; 5 to the plasma membrane. *The Plant Journal* **57**: 346-355.

Zhang Q, Blaylock LA, Harrison MJ. 2010. Two *Medicago truncatula* half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. *The Plant Cell* **22**: 1483-1497.

Zhang R, Verkman AS. 1991. Water and urea permeability properties of *Xenopus* oocytes: expression of mRNA from toad urinary bladder. *American Journal of Physiology* **260**: C26–34.

APPENDICES

APPENDIX 1 Deduced amino acid sequences used in phylogenetic analysis on plant MIPs and fungal MIPs

Protein name and accession number in NCBI or JGI genome databases was listed for each sequence, followed by the species name.

Plant MIPs

>PtPIP1;1 [POPTR_0010s19930][*Populus trichocarpa*]
MEGKEEDVRLGANKFNERQPLGTAAQSQDDKDYKEPPPAPLFEPSELTSWSFY
RAGIAEFMATFLFLYITVLTVMGVFKDTTKCTTVGIQGIAWAFGGMIFALVYC
TAGISGGHINPAVTFGLFLARKLSLTRAVFYMLMQCLGAICGAGVVKGFYGK
KNYELLNGGANMVSPGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHV
PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIFNKDKAWDDHWIFWVGP
FIGAALAALYHQVVIRAIPFKK*

>PtPIP1;2 [POPTR_0008s06580][*Populus trichocarpa*]
MEGKEEDVRLGANRFNERQPIGTAAQSLDDKDYKEPPPAPLFEPGELTSWSFY
RAGIAEFMATFLFLYITVLTVMGVVKDQTKCTTVGIQGIAWAFGGMIFALVYC
TAGISGGHINPAVTFGLFLARKLSLTRAVFYMVMQCLGAICGAGVVKGFYGK
TNYELHNGGANMVAHGYTKGDGLGAEIVGTFILVYTVFSATDAKRSARDSHV
PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIFNKDSAWDDHWIFWVGPF
IGAALAALYHQVVIRAIPFKK*

>PtPIP1;3 [POPTR_0003s12870][*Populus trichocarpa*]
MEGKEEDVKLGANKFSERQPIGTSAQTDKDYKEAPPAPLFEPGELKSWSFYRA
GIAEFIATFLFLYITVLTVMGVTKPGTSKCSTVGIQGIAWAFGGMIFALVYCTA
GISGGHINPAVTFGLFLARKLSLTRAVFYIIMQCLGAICGAGVVKGLQGSHNYE
LQGGGANVVNHGYTKGDGLGAEIVGTFVLVYTVFSATDAKRNARDSHVPILA
PLPIGFAVFLVHLATIPITGTGINPARSLGAAIIFNKDHAWDDHWIFWVGPFIGA
ALAAVYHQIVIRAIPFKSRA*

>PtPIP1;4 [POPTR_0006s09920][*Populus trichocarpa*]
MEEGEEDVKVGANRYGEGQPIGTAAQTQHGKDYTEPPPAPLYQPGEWLSWSF
YRAGIAEFVATFLFLYITVLTVMGVARSSTKCSTVGIQGIAWAFGGMIFVLVYC
TAGISGGHINPAVTFGLLLARKLTLTRAVFYMIMQCLGAICGAGVVKGFQKSP
YEILGGGANTVSTGYSKGSGLGVEILGTFVLVYTVFSATDAKRSARDSHVPVL
APLPIGFAVFLVHLATIPITGTGINPARSLGAALIYNKDKAWDDHWIFWVGPFI
GAALASLYHQIVIRAIPFKSK*

>PtPIP1;5 [POPTR_0016s12070][*Populus trichocarpa*]
MEGREEDVRVGANKYGERQPIGTAAQAQDVKDYTDPPPAPLFEPGELSSWSF
YRAGIAEFVATFLFLYITVLTVMGVAKSPTKCSTVGIQGIAWAFGGMIFALVYC
TAGISGAVFYMLMQCLGAICGAAVVKAFQKSQYEMLGGGANTVSTGYAKGS
GLGAEIVGTFVLVYTVFSATDAKRNARDSHVPILAPLPIGFAVFLVHLATIPITG
TGINPARSLGAALIYNKDQAWDDHWIFWVGPFIGAALASLYHQIVIRAIPFKSK

>PtPIP2;1 [POPTR_0009s13890][*Populus trichocarpa*]
MSKDVIEEGQTHTKDYVDPPPAPLFDVGELKLWSFFRALIAEFIATLLFLYVTV
ATVIGHKKNQDACGGVGLLGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLL
LARKVSLIRAVGYMVAQCLGAVCGVGLVKAFMKPYYNSLGGGANMVAPGY
STGTAVGAEIIGTFVLVYTVFSATDPKRSARDSHIPVLAPLPIGFAVFMVHLATI
PITGTGINPARSFGAAVIINDKKAWDDHWIFWVGPFVGALAAAAYHQYILRAG
AIKALGSFRSHPTN*

>PtPIP2;2 [POPTR_0004s18240][*Populus trichocarpa*]
MSKEVSEVGQTHGKDYVDPPPAPLLDLGELKLWSFYRALIAEFIATLLFLYVT
VATVIGHKSNKDPCDGVGLLGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLF
LARKVSLIRAVAYMVAQCLGAICGVGLVKAFMKKNYNSLGGGANTVAMGY
NTGTALGAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLAT
IPITGTGINPARSFGAAVIFNNEKAWDDHWIFWVGPFVGALAAAAYHQYILRA
AAIKALGSFRSNPAN*

>PtPIP2;3 [POPTR_0010s22950][*Populus trichocarpa*]
MAKDMEVAEAGSFSAKDYHDPPPAPLFDAKELTKWSFYRALIAEFIATLLFLYI
TVLTVIGYKSQIDGSADSCGGVGILGIAWAFGGMIFVLVYCTAGISGGHINPAV
TFGLFLARKVSLIRAVMYMVAQCLGAICGVGLVKAFQKSYYKKYGGGANTL
ADGFSTGTGLGAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMV
HLATIPITGTGINPARSLGAAVIYNQDKAWDGHWIFWVGPFAGAAIAAFYHQFI
LRAGAVKALGSFRSAQRF*

>PtPIP2;4 [POPTR_0008s03950][Populustrichocarpa]
MAKDTEVAEAGSFSAKDYQDPPPAPLIDAEELTKWSFYRALIAEFIATMLFLYI
TVLTVIGYKSQIDGNADPCGGVGILGIAWAFGGMIFVLVYCTAGISGGHINPAV
TFGLFLARKVSLIRAVMYMVAQCAGAICGVGLVKAFQKSYYTKYNGGANVL
ADGYSTGTGLGAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMV
HLATIPITGTGINPARSFGAAVIYNNKKAWHDQWIFWAGPFIGAAIAAFYHQFI
LRAGAIKALGSFRSNPNV*

>PtPIP2;5 [POPTR_0006s12980][*Populus trichocarpa*]
MGKDIEVGGEFSAKDYHDPPPAPLIDAEELTQWSLYRAIIAEFIATLLFLYITVL
TVIGYKSQTDTTKNSDACGGVGILGIAWAFGGMIFVLVYCTAGISGGHINPAV
TFGLFLARKVSLVRAVLYMVAQCLGAICGCGLVKAFQKSYYTKYGGGVNEL
ATGFSKGTGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMV
HLATIPITGTGINPARSFGAAVIYNEDKAWDDHWIFWVGPFIGAAIAALYHQY
VLRAAAVKALGSFRSSSNI*

>PtPIP2;7 [POPTR_0016s09090][*Populus trichocarpa*]
MGKDIEVGGEFSAKDYHDPPPAPLIDAEEITQWSFYRAIIAEFVATLLFLYITVL
TVIGYKSQTDVNKNGDECGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVT
FGLFLARKVSLVRAILYMVAQCLGAICGCGLVKAFQKSYYTNYGGGANGLAN
GYSKGTGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHL
ATIPITGTGINPARSFGAAVIFNKEKAWDDHWIFWVGPFIGAAIAALYHQFILRA
AAVKSLGSFRSSPNI*

>PtPIP2;8 [POPTR_0009s01940][*Populus trichocarpa*]

MAKDIEVAEHGETVKDYQDPPPAPLIDAEELGQWSFYRALIAEFIATLLFLYVT
VLTVIGYKSQTDPDKGLDACGGVGILGIAWAFGGMIFVLVYCTAGISGGHINP
AVTFGLFLARKVSLIRAVLYMVAQCLGAICGCGLVKAFQKSYYNHYGGGANE
LQEGYNKGTGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFM
VHLATIPITGTGINPARSFGAAVIFNQSKAWDDHWLFWVGPFIGAAIAAFYHQF
ILRAAAIKALGSFRSNA*

>PtPIP2;9 [POPTR_0005s11110][*Populus trichocarpa*]
MSTGGKDYRDPPPAPLLDMEELKQWSFYRALIAEFVATFLFLYIGVGTVVGYK
GVHNNLCDGAGYLGVAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFVARKV
SLIRAVAYMMAQCLGAMLGVWMVMILTGIHYDQAGGAVNVVAPGYSKGTA
LGAEIIGTFVLVYTVLAATDPKRMARDSHVPVLAPLPIGFAVFVVHLALIPITGT
GINPARSLGAAVVKNAKEIWDDHWIFWVGPFVGALAAAVYHQYILGSGAAK
ALASFRSNPTS*

>PtPIP2;10 [POPTR_0005s11100][*Populus trichocarpa*]
MSSEERNIERQHGRDYHDPPPAPLLDMGELKQWSFYRAAIAEFIATFLFLFFSV
STVVNYKEPNYTDQCSRVGHLGIAWANGGMIFVLVYCTSGISGGHLNPAVTF
GMLVARKMSLIRAAAYMLAQCLGAILGHLFVFLFMYADEQQSSVGVVNVVS
RNYSKGAGLGAEFIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFVV
HLATIPITGTGINPARSLATNLLHRSTAEAMDDLWIFWVGPFLGALAAAVYHK
YVLRAGAVKTLKSFRALGSFGSQPPV*

> PtPIP2;11 [POPTR_0006s09910][*Populus trichocarpa*]
MGKDVEVRGEFIAKDYHDPPPAPLIDAEELTQWSLYRAIIAEFIATLLFLYITVL
TVIGYKSQTDTTKNSDACGGVGILGIAWAFGGMIFVLVYCTAGISGGHINPAV
TFGLFLARKVSLVRAVLYMVAQCLGAICGCGLVKAFQKSYYTKYGGGANEL
ATGFSKGTGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMV
HLATIPITGTGINPARSFGAAVIYNKDKAWDDHWIFWVGPFIGAAIAALYHQY
VLRAAAVKALGSFRSSSNI

>PtTIP1;1 [POPTR_0001s24200][*Populus trichocarpa*]
MPITSIAFGSPAEAGQPDALRAALAEFISMLIFVFAGEGSGMAFNKLTDNGSSTP
AGLVAASLAHAFALFVAVSVGANISGGHVNPAVTFGAFIGGHITFIRSLLYWV
AQCLGSVVACLLLKLATGGQETSAFALSSGVGAWNAVVFEIVMTFGLVYTVY
ATAVDPKKGDIGIIAPIAIGFIVGANILAGGAFDGASMNPAVSFGPAVVSWTWD
SHWVYWLGPFVGSAIAAIVYEVIFINPSTHEQLPSTDF

>PtTIP1;2 [POPTR_0009s03230][*Populus trichocarpa*]
MAITSIAFGSPAEVGQSDALKAALAEFISMLIFVFAGEGSGMAFNKLTDDGSST
PAGLVAASLAHAFALFVAVSVGANISGGHVNPAVTFGAFLGGHITFIRSILYWV
AQCLGSVVACLLLKLATGGLETSAFSLSSGVGVWNAVVFEIVMTFGLVYTVY
ATAVDPKRGDIGIIAPIAIGFIVGANILAGGAFDGASMNPAVSFGPAVVSWTWD
NHWVYWLGPFVGSAIAAIVYEVCFISPTTHEQLTSSDF

>PtTIP1;3 [POPTR_0010s21700][*Populus trichocarpa*]
MPINRIAFGTPREASHPDALRAALAEFISMLIFVFAGSGSGMAFNKLTDNASTT
PSGLVAAALAHAFALFVAVSVGANISGGHVNPAVTFGALIGGNITLLRSILYWI
AQLLGSVVACLLLKFATGGLETPAFGLSSGVGAWNALVFEIVMTFGLVYTVY

ATAVDPKKGNLGIIAPIAIGFIVGANILAGGAFDGASMNPAVSFGPAVVSWTW
TNHWVYWLGPFIGAAIAALVYDNIFIGSGGHEPLPTNDF

>PtTIP1;4 [POPTR_0008s05050][*Populus trichocarpa*]
MPINRIAVGTPGEASHPDSLRAALAEFISTLIFVFAGSGSGMAFNKLTDSASTTP
AGLVAAALAHAFALFVAVSVGANISGGHVNPAVTFGALIGGNITLLRSILYWI
AQLLGSVVACLLLKFSTGGLETPAFGLSSGVGAWNAVVFEIVMTFGLVYTVY
ATAVDPKKGNLGIIAPIAIGFIVGANILAGGAFDGASMNPAVSFGPAVVSWTW
TNHWVYWLGPFIGAGIAALVYDNIFIGSGGHEPLPTNDF

>PtTIP1;5 [POPTR_0016s10780][*Populus trichocarpa*]
MPIRNIAVGHYRETTQPDALKAALAEFISTLIFVFAGEGSGMAFSKLTDGASNT
PAGLIAAAIAHAFALFVAVSVGANISGGHVNPAVTFGAFIGGNITLFRGILYWIA
QLLGSTVACLLLKFVTGGLETSAFALSTGVGVWNAFVLEIVMTFGLVYTVYA
TAIDPKKGNLGIIAPIAIGFIVGANILVGGAFDGASMNPAVSFGPALVSWSWTN
HWVYWAGPLVGGGLAGLIYELFFIGFGTHEQLPTTDY

>PtTIP1;6 [POPTR_0006s12350][*Populus trichocarpa*]
MPIRNIAVGHYHEATQPDALRAALAEFISTLIFVFAGEGSGMAFAKLTDGAAN
TPAGLIAAAIAHAFALFVAVSVGANISGGHVNPAVTFGAFIGGNITLLRGILYWI
AQLLGSTVACLLLKFTTGGLETSAFALSSGVGVWNAFVLEIVMTFGLVYTVYA
TAVDPKKGNLGIIAPIAIGFIVGANILAGGAFDGASMNPAVSFGPALVSWTWTN
HWVYWAGPLIGGGLAGLIYEFFFIGFGNHEQLPTADY

>PtTIP1;7 [POPTR_0009s01070][*Populus trichocarpa*]
MPNLIVIDRIAIGTVAADFHPNAFKAALAEFISTLIFVFAGQGSTMAYNKLTSNA
PTSPAGLIAVALAHAFGLFVAVATSANISGGHCNPAVTFGAFLGGNITLLRGIL
YWIAQLLGSTVACLLLKFATHYMTVSVFTLSSGVSVWNAFVFEIVMTFALVYT
VYATAIDAKKGDVGVIAPLAIGFVLGANILAGGAFEGAALNPAVPFGPALVSW
NWHHHWVYWAGPLIGGGLAGVVYELIFISHTHEPLAVVEY

>PtTIP1;8 [POPTR_0004s22600][*Populus trichocarpa*]
MRNFIIIERITIGRVEDDFHSNAFKAALAEFISTLIFVFAGQGSTMAYNKLTSNAP
TSPAGLIAVALAHAFGLFVGVAVSANISGGHVNPAVTFGAFIGGNISLLRGILY
WIAQLLGSTVACLLLKYTTHHMTVSVFTLSPGVTVWNAFVFEIVMTFALVYT
VYATAIDPKKGDVGVIAPLAIGFVLGANILVGGAFEGAALNPAVPFGPALVSW
NWYHHWVYWAGPLIGGGLAGIVYELIFMSHSTHEPLPGGEF

>PtTIP2;1 [POPTR_0001s18730][*Populus trichocarpa*]
MAGIAFGRFDDSFSLGSFKAYLAEFISTLLFVFAGVGSAMAYNKLTGDAALDP
AGLVAIAVCHGFALFVAVSVGANISGGHVNPAVTFGLALGGQITILTGIFYWIA
QLLGSIVACYLLKVATGGLAVPIHSVAAGVGAIEGVVMEIIITFALVYTVYATA
ADPKKGSLGTIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVASGDFHDNW
IYWAGPLVGGGIAGLIYGNVFITDHTPLSGDF

>PtTIP2;2 [POPTR_0003s04930][*Populus trichocarpa*]
MARIAFGRFNDSFSLGSLKAYLAEFISTLLFVFAGVGSAMAYNKLTGDAALDP
AGLVAIAVCHGFALFVAVAVGANISGGHVNPAVTLGLALGGQMTILTGIFYWI
AQLLGSIVACYLLKVVTGGLAVPIHSVAAGVGAIEGVVMEIIITFALVYTVYAT

AADPKKGSLGTIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVASGDFHDN
WIYWVGPLIGGGLAGLIYGNLYITDHSPSSYEF

>PtTIP2;3 [POPTR_0003s07550][*Populus trichocarpa*]
MAKIAFGSLGDSFSLASIKAYLSEFIATLLFVFAGVGSAIAYSKLTTDAALDPPG
LVAVAVAHAFALFVGVSIAANISGGHLNPAVTFGLAIGGNITFLTGLLYWIAQC
LGSIVACLLLKVVTSAEGIPTHGVASGMSAIEGVVMEIVITFALVYTVYATAAD
PKKGSLGIIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVVSGDFSQNWIYW
LGPLVGGGLAGLVYGGIFIGSYAPAPVSEDYA

>PtTIP2;4 [POPTR_0001s15700][*Populus trichocarpa*]
MVKIAFGSLGDSFSVGSLKAYLSEFIATLLFVFAGVGSAIAYSKLTTDAALDPP
GLVAVAVAHAFALFVGVSIAANISGGHLNPAVTFGLAIGGNITILTGLLYWIAQ
CLGSIAACLLLKFATSAESIPTHGVASGMSAVEGVVMEIVITFALVYTVYATAA
DPKKGSIGIIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVVSGDFSQNWIY
WLGPLIGGGLAGLVYGDIFIGSYTAAPVSEDYA

>PtTIP3;1 [POPTR_0018s14910][*Populus trichocarpa*]
MPRRYAFGKADEATRPDAMRAALAELVSTFIFVFAGEGSILALDKLYKGTGPP
ASGLLVVALAHALALFSAVASSINISGGHVNPAVTFGSLVGGRISVIRAVSYWV
AQLLGSIFAALLLRLVTNGMIPAGFHVQSEVGEVHGLLLEMALTFGLVYTVYA
TAIDPKRGSLGIIAPLAIGFVVGANILVGGPFDGASMNPARAFGPALVGWRWR
NHWIYWVGPFLGGGLAALIYEYIVISAEPVAHHTHQHQPLAPEDY

>PtTIP3;2 [POPTR_0017s03540][*Populus trichocarpa*]
MRAALAEFVSTFVFVFAGEGSVLALDKLYKETGPLASGLVVVALAHALALFS
AVASSINISGGHVNPAVTFGSLVGGRISVIRAVYYWVAQLLGSIVAALLLRLVT
NGMRPVGFHVQSGVGEVHGLLMEMALTFGVVYTVYATALDPKRGSLGIIAPL
AIGFIVGANILVGGPFDGASMNPARAFGPALIGWRWRNHWIYWVGPFLGGGL
AALIYEYIVIPTEPVPRHAHQHQPLAPEDY

>PtTIP4;1 [POPTR_0006s25620][*Populus trichocarpa*]
MTKIALGSRHEAAQPDCLKALVVEFVTTFLFVFAGVGSAMAADKLTGDALLG
LFVVAVAHAFVVAVMISAGHISGGHLNPAVTIGLLFGGHITVVRSILYWIDQLL
ASTAACFLLKYLTGGLATPVHTLASGMDYLQGVVWEIVLTFSLLFTVYATIVD
PKKGSIDGLGPMLTGFVVGANILAGGAFSGASMNPARSFGPALVSWDWTDH
WVYWVGPLIGGGLAGFIYENFFITRSHRPLPSEEEPF

>PtTIP5;1 [POPTR_0003s10800][*Populus trichocarpa*]
MASTSLTARFKQSVTPASLRAYLAEFISTFFYVFAVVGSAMASRKLLPDAAAV
PSSLVIVAIANAFALSSAVYIAANASGGHVNPAVTFGMAVGGRINVPTALFYWI
SQMLASVMACIFLKVATVGQHVPTNTIAEEMTGFGASLLEGVMAFGLVYTVY
AAGDPRRGSLGAIGPLAVGLTAGANVLAAGPFSGGSMNPACAFGSAVIAGRL
KNQAVYWVGPLIGAAVAGLLYDNVVFPTEAPDSLRGVSDDVGV

>PtTIP5;2 [POPTR_0001s00690][*Populus trichocarpa*]
MAPTSLTARFQQSVTPASLRAYLAEFISTFFYVFAVVGSAMASRKLLPDAAAD
PSSLVIVAIANAFALSSAVYIAANASGGHVNPAVTFGMAVGGHINVPTALFYW
ISQLLASVMASIFLKVTTVGQHVPTYTIAEEMTGFGASLLEGVMTFGLVYTVY

AAGDPRRSSLGAIGPLAVGLMAGANVLAAGPFSGGSMNPACAFGSAVIAGKF
KNQAVYWVGPLIGASVAGLLYDNVVFPTQAPDSVRRGVSEGVGV

>PtNIP1;1 [POPTR_0004s06160] [*Populus trichocarpa*]
MAEIDGTNGNGNHGGVVLDIKDNYPSSSSIKEVSVLNFYVPFMQKLVAEIAGT
YFLIFAGCSSVAVNLNFDKVVTLPGISITWGLAVMVLVYSVGHISGAHFNPAV
TLAFATCKRFPWKQVPAYVACQVIGATLAAGTIRLLFQGDQDHFTGTMPAGS
NLQSFVVEFIITFYLMFIISGVATDNRAIGELAGLAVGSTVLLNVMFAGPISGAS
MNPARSLGPAIVSHQYKGLWIYIVSPILGAQAGAWVYNLIRYTDKPLREITKSA
SFLNGKESS

>PtNIP1;2 [POPTR_0011s06770] [*Populus trichocarpa*]
MAEIDGTNGNGNHGGVVLDIKDNYPSSSSIKEVSVLNFYVPFMQKLVAEIAGT
YFLIFAGCSSVAVNLNFDKVVTLPGISITWGLAVMVLVYSVGHISGAHFNPAV
TLAFATCKRFPWKQVPAYVACQVIGATLAAGTIRLLFQGDQDHFTGTMPAGS
NLQSFVVEFIITFYLMFIISGVATDNRAIGELAGLAVGSTVLLNVMFAGPISGAS
MNPARSLGPAIVSHQYKGLWIYIVSPILGAQAGAWVYNLIRYTDKPLREITKSA
SFLNGKESS

>PtNIP1;3 [POPTR_0010s12330][*Populus trichocarpa*]
MPWNNEFGDDTEGGKKTESSDEDSPPETTVQIIQKIIAEMIGTFFLIFMGCGSVV
VNQMYGSVTFPGVCVVWGLIVMVMVYSVGHISGAHFNPAVTVTFAIFRHFPY
KQVPLYIAAQLLGSLLASGTLSLLFSVTDEAYFGTIPVGPDIRSFVTEIIISFLLMF
VISGVATDNRAIGELAGIAVGMTIMLNVFVAGPVSGASMNPARSLGPAIVMRQ
FKGIWVYIVGPPIGTILGALCYNIIRFTDKPLREITKTASFLKSKN

>PtNIP1;4 [POPTR_0017s03060][*Populus trichocarpa*]
MARKSDGIESQEITSMEEGLATPTDPKENGKFDCCTSPAAVTITQKLIAEVIGTY
FVIFAGCGSVAVNNIYGSVTFPGVCVTWGLIVMVMIYSLGHISGAHFNPAVTIA
FAIFRRFPSWQVPLYIIAQLMGSILASGTLALALDVTPEAFFGTVPVGSDGQSLV
LEIIISFLLMFVISGVSTDDRAVGDLAGIAVGMTILLNVFVAGPVSGASMNPARS
IGPAVVKHQFKGLWVYIVGPIIGAIAGAFACNLIRWTDKPLGELTKVGSFIKSG
SKNYAS

>PtNIP1;5 [POPTR_0002s09740][*Populus trichocarpa*]
MSSSNSITEPSPKFQLPTRRSIMAEAKAASPAPEWLSTRNAALSNFQKIVAELM
GTYILVFVGCGAALTDKVQRLNMLGIAIVWGAVLMAAIYALGHVSGAHFNPA
VSIALAVVRKFSWKEVPMYILAQVLGSTLASLTLRMLFHEQGNIQPIVNQYSDP
TSDLEAIVWEFIITFILMFTICGVATDPRASKDLSGVAIGGAVMFNAMIAGPITG
ASMNPARSLGPALVSGVYKNLWVYIVSPILGAMAAAAVYSVLRVPEPAKPED
TNKSTYNNLNLHADP

>PtNIP2;1 [POPTR_0017s11960][*Populus trichocarpa*]
MATVDQEMNISVESSRFHFVKLFREHYPSGFLRKVVAEVIATYLLVFVTCGAA
AISASDEHKVSKLGASVAGGLIVTVMIYAVGHISGAHMNPAVTTAFAAVLNFP
WKQVPFYAAAQLTGAISASFTLKVLLHPIRNVGTTSPSGTAVQALIMEIVVTFS
MMFITSAVATDTKAVGELAGIAVGSAVCITSILAGPVSGGSMNPARTLGPAIAS
RYFKGVWVYLLGPVTGTLLGAWSYNLIRVTDKPVQAIPRRFSFGSRRTRAIDE
QSPSMGPLDAF

>PtNIP3;1 [POPTR_0003s17930][*Populus trichocarpa*]
MDNAEVPSVPSTPATPGTPGAPLFGGFKGERGVHGRKSLLRSCKCFSVEEWA
MEEGRLPPVSCSLPPPPVSLARKVGAEFIGTLILIFAGTATAIVNQKTQGSETLV
GLAASSGLAVMIVILATGHISGAHLNPSITIAFAALKHFPWKHVPVYIGAQVLA
SLCAAFALKGIFHPVMGGGVTVPSGGYGQAFALEFITSFILMFVVTAVATDTR
AVGELAGIAVGATVMLNIFIAGETTGASMNPVRTLGPAIAVNNYKAIWIYLTA
PILGALCGAGTYSAVKLPEEDGDSNEKTSAARSFRR

>PtNIP3;2 [POPTR_0001s14850][*Populus trichocarpa*]
MDTEEVPSAPSTPATPGTPGAPLFGGFKGERGVHGRKSLLRSCKCFGVEEWA
MEEGRLPPVSCSLPPPPVSLARKLGAEFMGTLILIFAGTATAIVNQKTQGSEALI
GLAASTGLAAMIVILSTGHISGAHLNPSITIAFAALKHFPWKHVPVYIGAQVLA
SLCAAFALKVIFHPMMGGGVTVPSGGHGQAFALEFIISFILMFVVTAVATDTRA
VGELAGIAVGATVMLNILIAGETTGASMNPVRTLGPAIAANNYKAIWVYLTAP
ILGALCGAGTYSAVKLPEEDGDTNEKTSATRSFRR*

>PtNIP3;3 [POPTR_0001s45920][*Populus trichocarpa*]
MPESEAGTPAVSAPNTPGTPGGPLFTGLRVDSLSYSDRKIMPKCKCLPVTAPT
WGQPHTCFLDFPAPDVSLTRKLGAEFVGTFILIFAATAGPIVNQKYNNAETLIG
NAACAGLAVMIIILSTGHISGAHLNPSLTIAFAALRHFPWVQVPAYIAAQVSASI
CASFALKGVFHPFMSGGVTVPSVSTGQAFALEFLITFNLLFVVTAVATDTRAV
GELAGIAVGATVMLNILVAGPSSGGSMNPVRSLGPAVAAGTYKDIWIYLVAPT
LGALVGAATYTAVKLREEEADPPRQVRSFRR

>PtNIP3;4 [POPTR_0011s14990][*Populus trichocarpa*]
MPGPEEAGTPTVTAPNTPGTPGGPLFTGLRVDSLSYSDRKIMPKCKCLPVTAPN
WGQPHTCFLDIPSPDVSLTRKLGAEFVGTFILIFMATAGPIVNQKYDHAETLIG
NAACAGLAVMIIILSTGHISGAHLNPSLTIAFAALRHFPWVQVPAYIAAQVSASI
CASFALKGVFHPFMSGGVTVPSVSTGQAFALEFFITFNLLFVVTAVATDTRAV
GELAGIAVGATVMLNILVAGPSTGGSMNPVRTLGPAIAAGNYKKIWIYLVAPT
LGAVVGAGAYTLVKLRDDETDPPRPVRSFRR

>PtNIP3;5 [POPTR_0008s20750][*Populus trichocarpa*]
MKHLLEEITSAHVPKTAVLPPASSSSSSTDDQEMGSNSMPMKRHIFIKKSSFCSF
LHGMDLNPARMVLAEMVGTFLLLFCVCGIVACTQILRGEVGLMEYASVAGLT
IIVVIFSIGSISGAHVNPAVTIAFATFGHFPWSKVPLYILAQTVGSVSATYVGSSV
YGVKTELMTTRPAIGCSSAFWVEFMATFMLMFLAASLTSQSRSIGPLSGFLYGI
AIGLAVLITGPVSGGSLNPARSLGPAIVSWDFKDIWVYITAPTIGAVAGALMFH
LLRIRPQACSANSSPDDDLLVHSIAFTES

>PtSIP1;1 [POPTR_0013s05030][*Populus trichocarpa*]
MGAVKAAIGDAVFTFMWVFVSSMFGLFTNVIVTALGLQTLVWAPVLANASLI
FAFVFLFNFLGEFLGGATFNPTGTASFYAAGVGGDSLFSMALRFPAQAAGSVG
GSLAILEVMPVQYKHMLGGPTLQVDLQTGGLAEGVLTFLMTFAVLVIILKGPR
SSLVQAWFLATVTVTLVSAGSTYTGPSMNPAFAFGWAYVNKWHNTWEQLYV
YWICPFIGAILAAWVFRVVFPPPAPKQKKT

>PtSIP1;2 [POPTR_0019s04640][*Populus trichocarpa*]
MGAIKAASGDAVLTFMWVFVSSMFGLFTNLIVTALGLQTLVWAPLVITTFIVF
TFVFLFNLIGEALGGASFNPTGTASFYAAGVGGDTLFSMALRFPAQAAGAVGG

ALAIMEVMPVQYKHMLGGPTLQVDLHTGGLAEGVLTFLMSFAVLVIILKGPR
NPLVQTLFLAIATITLVVAGSTYTGPSMNPANAFGWAYVRKWHNTWEQLYV
YWICPFIGAILASWVFRAVFPPPAPKQKKA

>PtSIP1;3 [POPTR_0002s21410][*Populus trichocarpa*]
MGAIKGAIVDGILTAMWVFSVPLLGVFSSIIATYVGVEAMSIAGLFISINVAALF
MLTFSLIGAAFGGASFNPATTITLYIAGLKPDASLLSMALRFPVQAAGGVGGA
MAIRGVMPKHYRHVLKGGPSLRVDLHTGAIAEGVLTFLICLTLHFLLLKGPKN
VVLKVWLLAVATVGLVMAGGKYTGPSMNPANAYGWAYLGNRHTTWDFFY
VYWICPFIGAILAAFVSKFLFKAAPIKEKKA

>PtSIP1;4 [POPTR_0014s15250][*Populus trichocarpa*]
MGAIKGAIVDGILTCMWVFSVPLLGVFSSIIATYVGVEAMSIAGLFITINVAALF
MLTFSLIGAACGGASFNPATTITLYTAGLKPDASLMSMALRFPVQAAGGVAGA
MAITEVMPKQYRYVLRGGPSLKGVLTFLICLALHFVLLKGPKNFVLKVWLLA
VATVGLVMAGGKYTGPSMNPANAYGWAYLSNRHTTWDFFYVYWICPFIGAT
LAALISKFLFKAPPIKDKKA

>PtSIP2;1 [POPTR_0016s02560][*Populus trichocarpa*]
MVSKTRLILSDFVVSLMWVWSGSLIKIFVFKVLGMGHDSRGEFLKNSLSIMNM
FLFAFLGKVTKGGAYNPLTILSSAISGDFSQFLFTIGARIPAQVIGSITGVRLFIDT
FPEIGLGPRLTVDIHKGALTEGLLTFAIVTISLGLARKIPGSFFMKTWISSVSKLS
LHILGSDLTGGCMNPASVMGWAYARGDHITKEHILVYWLAPIEGTLLAVWTF
KLLFRPQKQDEKEKLKGKTE

>PtSIP2;2 [POPTR_0006s02800][*Populus trichocarpa*]
MVSKTRLIVSDFIVSIIWVWNGALIKMFVFKVLQMGHDSRGEFMRQSLTVVSL
FFFAFLAKVTKGASFNPLAVLSSAISGDFSHFLFTIGTRIPAQVIGSITAVRLLIDT
FPEIGRGPRLNVDIHKGALTEGLLAFGVVTISLGLARKIPGSFFMKTWISSISKLS
LHILGSDLTGGCMNPASVMGWAYARGDHITKEHILVYWLAPIQGALLAAYTF
KLLFRPQKQDEKEKLKGKTD

>PtXIP1;1 [POPTR_0009s13100][*Populus trichocarpa*]
MAEALKNEGGKTKQITWREILGLEDLLSLTVWRASVAELLGTAVLVFALDTIV
ISTIQTGTNMPNLILSTLVAIIITILLLATFPISGGHINPIITFAAFLTGLISLSKTFIYI
LAQCVGATFGALALKAVVNSEIENTYSLGGCTLTIVAPGPHGPTVIGLETNQAL
WLEIICGFVFLFASVWMAFDHRQAQGIGRVGVFIIGGIVLGLLVFVSTTVTTTK
GYAGAGLNPARCLGPAIVRGGHLWNGHWVFWVGPAVACVAFAVYTKIIPRQ
LAHTIE

>PtXIP1;2 [POPTR_0009s13080][*Populus trichocarpa*]
MPNLILSTLVAIIITILLLATFPISGGHINPIITFAAFLTGLISLSKTFIYILAQCVGAI
FGALALKAVVNSEIEKTYSLGGCTLTIVAPGPHGPTVIGLETNQALWLEIICGFV
FLFASVWMAFDHRQAQGIGRVRVLIIVGIVLGLLVFVSTTVTATKGYAGAGLN
PARCLGPAIVRGGHLWNGHWVFWVGPAIACVAFAVYTKIIPRQLAHTIE

>PtXIP1;3 [POPTR_0004s17430][*Populus trichocarpa*]
MAGYPGSTVEDEESLYSGKKPQPSATTPMAKVVQNEGGIQKKKSPTLREILGL
EDLFSLTTWRASVAELLGTAVLVFALDTIVISTIQTQTKTPNLILSTLVAIIVTILL
LATYPISGGHINPIVTFAALLTGLISISKAFIYILAQCVGGIVGALALKAVVNSEIE

RTFSLGGCTLTVVAPGPEGPTVVGLETGQALWLEIICGFVFLFASVWMAFDHR
QAKGLGRVNVLIIVGIVLGLLVYVSTTVTATKGYAGAGLNPARCLGPAIVRGG
HLWNGHWVFWVGPAIACVAFAIYTKVIPSQLSHTIE

>PtXIP1;4 [POPTR_0009s13070][*Populus trichocarpa*]
MAGYTEGDEENLFRANKIQRFATTPTAEVVKNEKRMKKQKSTKLSEILGLEDL
VSLTVWRASVAELIGTAVLVFTLDTIVISTIRIETKIPNLILSILAAIIITILILATFPI
SGGHINPLVTFAALLTGLVSLSKAIIYILAQCVGGIFGALALKAVVNREIQQTFS
LGGCTLTVVAPGPDGQTVIGLETSQALWLEIICGFVFLFASVWMAFDQRQAKA
LGRVNVFIIIGIVVGLLVYISTTVTATKGYAGAGLNPARCLGPAIVRGGHLWDG
HWVFWVGPGIACVLFALYTKLIPPQLSHTIE

>PtXIP1;5 [POPTR_0009s13110][*Populus trichocarpa*]
MAGNAGVVQDEEIGYGGNKVQPFASTPRPSKTERGKRDSSALSRILGLDELVS
LNVWRASLAEVFGTAVLVFAMDTIVISSYETQTKTPNLVMATLIAITIAILLLAT
FPISGGHINPAITLSAMFTGLITVSRAAIYILAQCIGAILGALALKAVVNSTIEQTF
SLGGCTLKIVAPGPSGPVAIGLETGQALWLEIICTFVFLFSSIYIAFDRRQAIALG
RVVFCSIIGLVVGLLVFISTTVTATKGYAGVGMNPARCLGPALVRGGHLWKG
HWVFWVGPIVASLTFSLYTKIIPREHLLGAESK

>PtXIP2;1 [POPTR_0009s13090][*Populus trichocarpa*]
MWRATLTELVATACLLFTLTTSIISCLESTTAEPKFLIPFAIFVIAFFFLLTTVPLS
GGHMSPVFTFIAALEGVITPVRALFYMSAQCVGSIVAYLVIKSVMDKNAEEKY
SLGGCMIDGNGEGISPTNAFILEFSCTFIVLFVGVTVAFDKRRCKELGLQMVCG
ILAGAMTLAFFVSISVTGRAGYAGAGLNPARCLGPSLLKGGRLWYGHWVFW
VGPFVACIVYYGFTLTLPTGTS

>AtPIP1;1 [At3g61430][*Arabidopsis thaliana*]
>PIP|[*Arabidopsis thaliana*] in fungal MIP phylogenetic analysis
MEGKEEDVRVGANKFPERQPIGTSAQSDKDYKEPPPAPFFEPGELSSWSFWRA
GIAEFIATFLFLYITVLTVMGVKRSPNMCASVGIQGIAWAFGGMIFALVYCTAG
ISGGHINPAVTFGLFLARKLSLTRALYYIVMQCLGAICGAGVVKGFQPKQYQA
LGGGANTVAHGYTKGSGLGAEIIGTFVLVYTVFSATDAKRNARDSHVPILAPL
PIGFAVFLVHLATIPITGTGINPARSLGAAIIYNKDHSWDDHWVFWVGPFIGAA
LAALYHVVVIRAIPFKSRS

>AtPIP1;2 [At2g45960][*Arabidopsis thaliana*]
MEGKEEDVRVGANKFPERQPIGTSAQSDKDYKEPPPAPLFEPGELASWSFWRA
GIAEFIATFLFLYITVLTVMGVKRSPNMCASVGIQGIAWAFGGMIFALVYCTAG
ISGGHINPAVTFGLFLARKLSLTRAVYYIVMQCLGAICGAGVVKGFQPKQYQA
LGGGANTIAHGYTKGSGLGAEIIGTFVLVYTVFSATDAKRNARDSHVPILAPLP
IGFAVFLVHLATIPITGTGINPARSLGAAIIFNKDNAWDDHWVFWVGPFIGAAL
AALYHVIVIRAIPFKSRS

>AtPIP1;3 [At1g01620][*Arabidopsis thaliana*]
MEGKEEDVRVGANKFPERQPIGTSAQTDKDYKEPPPAPFFEPGELSSWSFYRA
GIAEFIATFLFLYITVLTVMGVKRAPNMCASVGIQGIAWAFGGMIFALVYCTA
GISGGHINPAVTFGLFLARKLSLTRAVFYIVMQCLGAICGAGVVKGFQPNPYQT
LGGGANTVAHGYTKGSGLGAEIIGTFVLVYTVFSATDAKRSARDSHVPILAPLP

IGFAVFLVHLATIPITGTGINPARSLGAAIIYNKDHAWDDHWIFWVGPFIGAAL
AALYHQLVIRAIPFKSRS

>AtPIP1;4 [At4g00430][*Arabidopsis thaliana*]
MEGKEEDVRVGANKFPERQPIGTSAQSTDKDYKEPPPAPLFEPGELSSWSFYR
AGIAEFIATFLFLYITVLTVMGVKRAPNMCASVGIQGIAWAFGGMIFALVYCT
AGISGGHINPAVTFGLFLARKLSLTRAVFYMIMQCLGAICGAGVVKGFQPTPY
QTLGGGANTVAHGYTKGSGLGAEIIGTFVLVYTVFSATDAKRSARDSHVPVW
TPLLVPILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIYNKDHSWDDHWIF
WVGPFIGAALAALYHQIVIRAIPFKSKS

>AtPIP1;5 [At4g23400][*Arabidopsis thaliana*]
MEGKEEDVNVGANKFPERQPIGTAAQTESKDYKEPPPAPFFEPGELKSWSFYR
AGIAEFIATFLFLYVTVLTVMGVKRAPNMCASVGIQGIAWAFGGMIFALVYCT
AGISGGHINPAVTFGLFLARKLSLTRALFYIVMQCLGAICGAGVVKGFQPGLY
QTNGGGANVVAHGYTKGSGLGAEIVGTFVLVYTVFSATDAKRSARDSHVPIL
APLPIGFAVFLVHLATIPITGTGINPARSLGAAIIYNKDHAWDDHWIFWVGPFIG
AALAALYHQIVIRAIPFKSKT

>AtPIP2;1 [At3g53420][*Arabidopsis thaliana*]
MAKDVEAVPGEGFQTRDYQDPPPAPFIDGAELKKWSFYRAVIAEFVATLLFLY
ITVLTVIGYKIQSDTDAGGVDCGGVGILGIAWAFGGMIFILVYCTAGISGGHINP
AVTFGLFLARKVSLPRALLYIIAQCLGAICGVGFVKAFQSSYYTRYGGGANSL
ADGYSTGTGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMV
HLATIPITGTGINPARSFGAAVIYNKSKPWDDHWIFWVGPFIGAAIAAFYHQFV
LRASGSKSLGSFRSAANV

>AtPIP2;2 [At2g37170][*Arabidopsis thaliana*]
MAKDVEGPEGFQTRDYEDPPPTPFFDADELTKWSLYRAVIAEFVATLLFLYITV
LTVIGYKIQSDTKAGGVDCGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAV
TFGLFLARKVSLIRAVLYMVAQCLGAICGVGFVKAFQSSYYDRYGGGANSLA
DGYNTGTGLAAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMV
HLATIPITGTGINPARSFGAAVIYNKSKPWDDHWIFWVGPFIGAAIAAFYHQFV
LRASGSKSLGSFRSAANV

>AtPIP2;3 [At2g37180][*Arabidopsis thaliana*]
MAKDVEGPDGFQTRDYEDPPPTPFFDAEELTKWSLYRAVIAEFVATLLFLYVT
VLTVIGYKIQSDTKAGGVDCGGVGILGIAWAFGGMIFILVYCTAGISGGHINPA
VTFGLFLARKVSLIRAVLYMVAQCLGAICGVGFVKAFQSSHYVNYGGGANFL
ADGYNTGTGLAAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFM
VHLATIPITGTGINPARSFGAAVIFNKSKPWDDHWIFWVGPFIGATIAAFYHQF
VLRASGSKSLGSFRSAANV

>AtPIP2;4 [At5g60660][*Arabidopsis thaliana*]
MAKDLDVNESGPPAARDYKDPPPAPFFDMEELRKWPLYRAVIAEFVATLLFL
YVSILTVIGYKAQTDATAGGVDCGGVGILGIAWAFGGMIFVLVYCTAGISGGH
INPAVTVGLFLARKVSLVRTVLYIVAQCLGAICGCGFVKAFQSSYYTRYGGGA
NELADGYNKGTGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAV
FMVHLATIPITGTGINPARSFGAAVIYNNEKAWDDQWIFWVGPMIGAAAAAFY
HQFILRAAAIKALGSFGSFGSFRSFA

>AtPIP2;5 [At3g54820][*Arabidopsis thaliana*]
MTKEVVGDKRSFSGKDYQDPPPEPLFDATELGKWSFYRALIAEFIATLLFLYVT
IMTVIGYKSQTDPALNPDQCTGVGVLGIAWAFGGMIFILVYCTAGISGGHINPA
VTFGLLLARKVTLVRAVMYMVAQCLGAICGVALVKAFQSAYFTRYGGGANG
LSDGYSIGTGVAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFIV
HLATIPITGTGINPARSLGAAIIYNKDKAWDHHWIFWVGPFAGAAIAAFYHQFV
LRAGAIKALGSFRSQPHV

>AtPIP2;6 [At2g39010][*Arabidopsis thaliana*]
MTKDELTEEESLSGKDYLDPPPVKTFEVRELKKWSFYRAVIAEFIATLLFLYVT
VLTVIGFKSQTDINAGGGACASVGLLGISWAFGGMIFILVYCTAGISGGHINPA
VTFGLFLASKVSLVRAVSYMVAQCLGATCGVGLVKVFQSTYYNRYGGGANM
LSDGYNVGVGVGAEIIGTFVLVYTVFSATDPKRNARDSHIPVLAPLPIGFSVFM
VHLATIPITGTGINPARSFGAAVIYNNQKAWDDQWIFWVGPFVGAAIAAFYHQ
FVLRAGAMKAYGSVRSQLHELHA

>AtPIP2;7 [At4g35100][*Arabidopsis thaliana*]
SKEVSEEGKTHHGKDYVDPPPAPLLDMGELKSWSFYRALIAEFIATLLFLYVT
VATVIGHKKQTGPCDGVGLLGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGL
FLARKVSLVRALGYMIAQCLGAICGVGFVKAFMKTPYNTLGGGANTVADGYS
KGTALGAEIIGTFVLVYTVFSATDPKRSARDSHIPVLAPLPIGFAVFMVHLATIPI
TGTGINPARSFGAAVIYNNEKAWDDQGIFWVGPFLGALAAAAYHQYILRASAI
KALGSFRSNATN

>AtPIP2;8 [At2g16850][*Arabidopsis thaliana*]
MSKEVSEEGRHGKDYVDPPPAPLLDMAELKLWSFYRAIIAEFIATLLFLYVTV
ATVIGHKNQTGPCGGVGLLGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLF
LARKVSLPRAVAYMVAQCLGAICGVGLVKAFMMTPYKRLGGGANTVADGY
STGTALGAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATI
PITGTGINPARSFGAAVIYNNEKAWDDHWIFWVGPFVGALAAAAYHQYILRA
AAIKALASFRSNPTN

>AtTIP1;1 [At2g36830][*Arabidopsis thaliana*]
MPIRNIAIGRPDEATRPDALKAALAEFISTLIFVVAGSGSGMAFNKLTENGATTP
SGLVAAAVAHAFGLFVAVSVGANISGGHVNPAVTFGAFIGGNITLLRGILYWI
AQLLGSVVACLILKFATGGLAVPAFGLSAGVGVLNAFVFEIVMTFGLVYTVYA
TAIDPKNGSLGTIAPIAIGFIVGANILAGGAFSGASMNPAVAFGPAVVSWTWTN
HWVYWAGPLVGGGIAGLIYEVFFINTTHEQLPTTDY

>AtTIP1;2 [At3g26520][*Arabidopsis thaliana*]
>TIP|[*Arabidopsis thaliana*] in fungal MIP phylogenetic analysis
MPTRNIAIGGVQEEVYHPNALRAALAEFISTLIFVFAGSGSGIAFNKITDNGATT
PSGLVAAALAHAFGLFVAVSVGANISGGHVNPAVTFGVLLGGNITLLRGILYW
IAQLLGSVAACFLLSFATGGEPIPAFGLSAGVGSLNALVFEIVMTFGLVYTVYA
TAVDPKNGSLGTIAPIAIGFIVGANILAGGAFSGASMNPAVAFGPAVVSWTWT
NHWVYWAGPLIGGGLAGIIYDFVFIDENAHEQLPTTDY

>AtTIP1;3 [At4g01470][*Arabidopsis thaliana*]

MPINRIAIGTPGEASRPDAIRAAFAEFFSMVIFVFAGQGSGMAYGKLTGDGPAT
PAGLVAASLSHAFALFVAVSVGANVSGGHVNPAVTFGAFIGGNITLLRAILYW
IAQLLGAVVACLLLKVSTGGMETAAFSLSYGVTPWNAVVFEIVMTFGLVYTV
YATAVDPKKGDIGIIAPLAIGLIVGANILVGGAFDGASMNPAVSFGPAVVSWIW
TNHWVYWVGPFIGAAIAAIVYDTIFIGSNGHEPLPSNDF

>AtTIP2;1 [At3g16240][*Arabidopsis thaliana*]
MAGVAFGSFDDSFSLASLRAYLAEFISTLLFVFAGVGSAIAYAKLTSDAALDTP
GLVAIAVCHGFALFVAVAIGANISGGHVNPAVTFGLAVGGQITVITGVFYWIA
QLLGSTAACFLLKYVTGGLAVPTHSVAAGLGSIEGVVMEIIITFALVYTVYATA
ADPKKGSLGTIAPLAIGLIVGANILAAGPFSGGSMNPARSFGPAVAAGDFSGH
WVYWVGPLIGGGLAGLIYGNVFMGSSEHVPLASADF

>AtTIP2;2 [At4g17340][*Arabidopsis thaliana*]
MVKIEIGSVGDSFSVASLKAYLSEFIATLLFVFAGVGSALAFAKLTSDAALDPA
GLVAVAVAHAFALFVGVSIAANISGGHLNPAVTLGLAVGGNITVITGFFYWIA
QCLGSIVACLLLVFVTNGESVPTHGVAAGLGAIEGVVMEIVVTFALVYTVYAT
AADPKKGSLGTIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVVSGDFSQIW
IYWVGPLVGGALAGLIYGDVFIGSYAPAPTTESYP

>AtTIP2;3 [At5g47450][*Arabidopsis thaliana*]
MVKIEVGSVGDSFSVSSLKAYLSEFIATLLFVFAGVGSAVAFAKLTSDGALDPA
GLVAIAIAHAFALFVGVSIAANISGGHLNPAVTLGLAIGGNITLITGFFYWIAQC
LGSIVACLLLVFVTNGKSVPTHGVSAGLGAVEGVVMEIVVTFALVYTVYATA
ADPKKGSLGTIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVVSGDLSQIWI
YWVGPLVGGALAGLIYGDVFIGSYEAVETREIRV

>AtTIP3;1[At1g73190][*Arabidopsis thaliana*]
MATSARRAYGFGRADEATHPDSIRATLAEFLSTFVFVFAAEGSILSLDKLYWE
HAAHAGTNTPGGLILVALAHAFALFAAVSAAINVSGGHVNPAVTFGALVGGR
VTAIRAIYYWIAQLLGAILACLLLRLTTNGMRPVGFRLASGVGAVNGLVLEIIL
TFGLVYVVYSTLIDPKRGSLGIIAPLAIGLIVGANILVGGPFSGASMNPARAFGP
ALVGWRWHDHWIYWVGPFIGSALAALIYEYMVIPTEPPTHHAHGVHQPLAPE
DY

>AtTIP3;2 [At1g17810][*Arabidopsis thaliana*]
MATSARRAYGFGRADEATHPDSIRATLAEFLSTFVFVFAGEGSILALDKLYWD
TAAHTGTNTPGGLVLVALAHALALFAAVSAAINVSGGHVNPAVTFAALIGGRI
SVIRAIYYWVAQLIGAILACLLLRLATNGLRPVGFHVASGVSELHGLLMEIILTF
ALVYVVYSTAIDPKRGSIGIIAPLAIGLIVGANILVGGPFDGASMNPARAFGPAL
VGWRWSNHWIYWVGPFIGGALAALIYEYMIIPSVNEPPHHSTHQPLAPEDY

>AtTIP4;1 [At2g25810][*Arabidopsis thaliana*]
MKKIELGHHSEAAKPDCIKALIVEFITTFLFVFAGVGSAMATDSLVGNTLVGLF
AVAVAHAFVVAVMISAGHISGGHLNPAVTLGLLLGGHISVFRAFLYWIDQLLA
SSAACFLLSYLTGGMGTPVHTLASGVSYTQGIIWEIILTFSLLFTVYATIVDPKK
GSLDGFGPLLTGFVVGANILAGGAFSGASMNPARSFGPALVSGNWTDHWVY
WVGPLIGGGLAGFIYENVLIDRPHVPVADDEQPLLN

>AtTIP5;1 [At3g47440][*Arabidopsis thaliana*]

MRRMIPTSFSSKFQGVLSMNALRCYVSEFISTFFFVLAAVGSVMSSRKLMAGD
VSGPFGVLIPAIANALALSSSVYISWNVSGGHVNPAVTFAMAVAGRISVPTAM
FYWTSQMIASVMACLVLKVTVMEQHVPIYKIAGEMTGFGASVLEGVLAFVLV
YTVFTASDPRRGLPLAVGPIFIGFVAGANVLAAGPFSGGSMNPACAFGSAMVY
GSFKNQAVYWVGPLLGGATAALVYDNVVVPVEDDRGSSTGDAIGV

>AtNIP1;1 [At4g19030][*Arabidopsis thaliana*]
MADISGNGYGNAREEVVMVNLKDEVEHQQEMEDIHNPRPLKKQDSLLSVSVP
FLQKLIAEFLGTYFLVFTGCASVVVNMQNDNVVTLPGIAIVWGLTIMVLIYSLG
HISGAHINPAVTIAFASCGRFPLKQVPAYVISQVIGSTLAAATLRLLFGLDHDVC
SGKHDVFIGSSPVGSDLQAFTMEFIVTFYLMFIISGVATDNRAKLNIGTKCCNIQ
IGELAGLAIGSTVLLNVLIAAPVSSASMNPGRSLGPALVYGCYKGIWIYLVAPT
LGAIAGAWVYNTVRYTDKPLREITKSGSFLKTVRIGST

>AtNIP1;2 [At4g18910][*Arabidopsis thaliana*]
MAEISGNGGDARDGAVVVNLKEEDEQQQQQQAIHKPLKKQDSLLSISVPFLQ
KLMAEVLGTYFLIFAGCAAVAVNTQHDKAVTLPGIAIVWGLTVMVLVYSLGH
ISGAHFNPAVTIAFASCGRFPLKQVPAYVISQVIGSTLAAATLRLLFGLDQDVCS
GKHDVFVGTLPSGSNLQSFVIEFIITFYLMFVISGVATDNRAIGELAGLAVGSTV
LLNVIIAGPVSGASMNPGRSLGPAMVYSCYRGLWIYIVSPIVGAVSGAWVYNM
VRYTDKPLREITKSGSFLKTVRNGSSR

>AtNIP2;1 [At2g34390][*Arabidopsis thaliana*]
>NIP|[*Arabidopsis thaliana*] in fungal MIP phylogenetic analysis
MDDISVSKSNHGNVVVLNIKASSLADTSLPSNKHESSSPPLLSVHFLQKLLAEL
VGTYYLIFAGCAAIAVNAQHNHVVTLVGIAVVWGIVIMVLVYCLGHLSAHFN
PAVTLALASSQRFPLNQVPAYITVQVIGSTLASATLRLLFDLNNDVCSKKHDVF
LGSSPSGSDLQAFVMEFIITGFLMLVVCAVTTTKRTTEELEGLIIGATVTLNVIF
AGEVSGASMNPARSIGPALVWGCYKGIWIYLLAPTLGAVSGALIHKMLPSIQN
AEPEFSKTGSSHKRVTDLPL

>AtNIP2;2 [At2g29870][*Arabidopsis thaliana*]
MMCAARNTMSSSGSSPSGSDLQAFVMEFIITGFLMLVVCAVTTTKRTTEELEG
LIIGATVTLNVIFVGEVSGASMNPARSIGPALVWGCYKGIWIYLLAPTLGAVSR
ALIHKMLPSIPNAEPKFSKTGSSHKRVSDLPL*

>AtNIP4;1 [At5g37810][*Arabidopsis thaliana*]
MSSHSDEIEEEQISRIEKGKGKDCQGGIETVICTSPSIVCLTQKLIAEMIGTYFIVF
SGCGVVVVNVLYGGTITFPGICVTWGLIVMVMIYSTGHISGAHFNPAVTVTFAI
FRRFPWHQVPLYIGAQFAGSLLASLTLRLMFKVTPEAFFGTTPADSPARALVAE
IIISFLLMFVISGVATDNRAVGELAGIAVGMTIMVNVFVAGPISGASMNPARSL
GPALVMGVYKHIWVYIVGPVLGVISGGFVYNLIRFTDKPLRELTKSASFLRAVS
PSHKGSSSKT

>AtNIP4;2 [At5g37820][*Arabidopsis thaliana*]
MTSHGEEIEDEQISRIEKGNCKDSQGGMETAICSSPSIVCLTQKLIAEMIGTYFIIF
SGCGVVVVNVLYGGTITFPGICVTWGLIVMVMIYSTGHISGAHFNPAVTVTFA
VFRRFPWYQVPLYIGAQLTGSLLASLTLRLMFNVTPKAFFGTTPTDSSGQALV
AEIIISFLLMFVISGVATDSRATGELAGIAVGMTIILNVFVAGPISGASMNPARSL

GPAIVMGRYKGIWVYIVGPFVGIFAGGFVYNFMRFTDKPLRELTKSASFLRSV
AQKDNASKSDG

>AtNIP5;1 [At4g10380][*Arabidopsis thaliana*]
MAPPEAEVGAVMVMAPPTPGTPGTPGGPLITGMRVDSMSFDHRKPTPRCKCL
PVMGSTWGQHDTCFTDFPSPDVSLTRKLGAEFVGTFILIFTATAGPIVNQKYDG
AETLIGNAACAGLAVMIIILSTGHISGAHLNPSLTIAFAALRHFPWAHVPAYIAA
QVSASICASFALKGVFHPFMSGGVTIPSVSLGQAFALEFIITFILLFVVTAVATDT
RAVGELAGIAVGATVMLNILVAGPSTGGSMNPVRTLGPAVASGNYRSLWVYL
VAPTLGAISGAAVYTGVKLNDSVTDPPRPVRSFRR

>AtNIP6;1 [At1g80760][*Arabidopsis thaliana*]
MDHEEIPSTPSTPATTPGTPGAPLFGGFEGKRNGHNGRYTPKSLLKSCKCFSVD
NEWALEDGRLPPVTCSLPPPNVSLYRKLGAEFVGTLILIFAGTATAIVNQKTDG
AETLIGCAASAGLAVMIVILSTGHISGAHLNPAVTIAFAALKHFPWKHVPVYIG
AQVMASVSAAFALKAVFEPTMSGGVTVPTVGLSQAFALEFIISFNLMFVVTAV
ATDTRAVGELAGIAVGATVMLNILIAGPATSASMNPVRTLGPAIAANNYRAIW
VYLTAPILGALIGAGTYTIVKLPEEDEAPKERRSFRR

>AtNIP7;1 [At3g06100][*Arabidopsis thaliana*]
MNGEARSRVVDQEAGSTPSTLRDEDHPSRQRLFGCLPYDIDLNPLRIVMAELV
GTFILMFSVCGVISSTQLSGGHVGLLEYAVTAGLSVVVVVYSIGHISGAHLNPSI
TIAFAVFGGFPWSQVPLYITAQTLGATAATLVGVSVYGVNADIMATKPALSCV
SAFFVELIATSIVVFLASALHCDFVQLGNLTGFVIGTVISLGVLITGPISGGSMNP
ARSLGPAVVAWDFEDLWIYMTAPVIGAIIGVLTYRSISLKTRPCPSPVSPSVSSL
LR

>AtSIP1;1 [At3g04090][*Arabidopsis thaliana*]
>SIP|[*Arabidopsis thaliana*] in fungal MIP phylogenetic analysis
MMGVLKSAIGDMLMTFSWVVLSATFGIQTAAIISAGDFQAITWAPLVILTSLIF
VYVSIFTVIFGSASFNPTGSAAFYVAGVPGDTLFSLAIRLPAQAIGAAGGALAIM
EFIPEKYKHMIGGPSLQVDVHTGAIAETILSFGITFAVLLIILRGPRRLLAKTFLL
ALATISFVVAGSKYTGPAMNPAIAFGWAYMYSSHNTWDHIYVYWISSFVGAL
SAALLFRSIFPPPRPQKKKQKKA

>AtSIP1;2 [At5g18290][*Arabidopsis thaliana*]
MSAVKSALGDMVITFLWVILSATFGIQTAAIVSAVGFHGITWAPLVISTLVVFV
SISIFTVIGNVLGGASFNPCGNAAFYTAGVSSDSLFSLAIRSPAQAIGAAGGAITI
MEMIPEKYKTRIGGKPSLQFGAHNGAISEVVLSFSVTFLVLLIILRGPRKLLAKT
FLLALATVSVFVVGSKFTRPFMNPAIAFGWAYIYKSHNTWDHFYVYWISSYTG
AILSAMLFRIIFPAPPLVQKKQKKA

>AtSIP2;1 [At3g56950][*Arabidopsis thaliana*]
MGRIGLVVTDLVLSFMWIWAGVLVNILVHGVLGFSRTDPSGEIVRYLFSIISMFI
FAYLQQATKGGLYNPLTALAAGVSGGFSSFIFSVFVRIPVEVIGSILAVKHIIHVF
PEIGKGPKLNVAIHHGALTEGILTFFIVLLSMGLTRKIPGSFFMKTWIGSLAKLT
LHILGSDLTGGCMNPAAVMGWAYARGEHITKEHLLVYWLGPVKATLLAVWF
FKVVFKPLTEEQEKPKAKSE

>PpXIP1;1 [ProteinID:71087][*Physcomitrella patens*]
MGHSQAADVVVYQHTASAPGTPTLDEDRGTCKIPEPISAISASKFSSEVLHRAV
LRDLQNPEVWRAGVFECVASFAATFVGILCTISTLEAEFSHPVAVIACLQGLVL
SLCIFAAAPATGGHVNPCITWTEMLTGHISPVRGVLYIIGQILGSIVGSFMAKIV
VGNALATQYNLGGCYLQSRVSATSGMMGLGTGRALVLEIVLAFFVLFISYSVA
LDPPRLPRTGYTLAPFMIGGIVGLCIFAGAGLFSGYGGAGINPGRCIGPAVVLG
GSMWTGHWVFWVGPGLSGALMAALYRNIPPTHIQVYKLRKEARKGLVGGRK
NKPFFAKVNNLRLACGNEKDGERIRSDSSEDQSSSHHRGKPLTYGRDHAV

>PpXIP1;2 [ProteinID:71489][*Physcomitrella patens*]
MHSLREPSENSTKGVLGNWVFTSGGLDSPNRPVNLAPIIGAPCFKRAFHDLIGV
TDEFASASERVELLVVVWFNVELSRIVSVEVLEVVNLVFKPEIVLQSCNRILCSF
SLSVFCGLGGIRSTAVPVEHLRTIESGIQSDWENFPQIQPSRVVRNDSVKGHYD
GSVVNVPLTRKVKVWIGLHDSRKADVWRAAAVEFVATAGLTFLSIGAYQQG
KSISVAAHVFIQALIYSLVILAATPISGAHLNPSITFTTFLTGQATLVRTILYVVA
QLLGGILGALGMWALTTHEMRREYSLGGCLLQKLPVEGTDLGLSTLSNKQGL
VAETVFTIIMLFVVYGIGFDSRNVVVTFLISSPFIIGGIFGILIFISQGVGYTTAMN
PARCFGPAILHHNKLWGPLYIFIFGPLIAAGIVAIFQHIMHQKHAAEVEPVLPLN
FFHIVTPDHQRPGFGPRCPTMFYPSIDQEVDHSQQLVSGNIPTMMSSELLQQPS
NQLNSAANKINLVLQVKAFMQPRTGETRQASKTNIMDGARIDQNLLLQQYSN
SLGSSDGIAIHHSGDFEKDR

>XIP|[*Nicotiana sylvestris*]
MASNASHVLGDEESQLSGGSNRVQPFSSTPKKNIDDEGKKHTSLTVAQRLGIS
DFFSLDVWRASMGELLGSAVLVFMLDTIVISTFESDVKMPNLIMSILIAIVITILL
LAVVPVSGGHINPVISFSAALVGIISMSRAIIYMVAQCVGAILGALALKAVVSST
IAQTFSLGGCTITVIAPGPNGPITVGLETAQALWLEIFCSFVFLFASIWMAYDHR
QAKALGLVTVLSIVGIVLGLLVFISTTVTAKKGYAGAGMNPARCFGAAVVRG
GHLWDGHWIFWVGPTIACVAFYVYTKIIPPQHFHADGYKYDFIGVVKASFGLH
E

>GQ03610_A06.1[*Picea glauca*]
MEGKEEDVRLGANKYSERQPLGTAAQTREKDYKDSGPAPLFEPGELASWSFW
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TAGISGGHINPAVTFGLFLARKLSLPRAVFYMICQCLGAICGAGVVKGFMESEY
EMDGGGANSVAHGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHVPM
LAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIYNKSHAWDDHWIFWVGPFL
GAGLAAFYHQMIIRAIPFKSRS

>GQ03401_M18.1[*Picea glauca*]
MEGKEEDVKLGADKYSERQPLGTAAQTMEKDYKEPGPAPLFEPGEFRSWSFW
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MESEYQMDGGGANVVAPGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARD
SHVPLLAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIYNRDHAWDDMWIF
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>GQ03010_E09.1[*Picea glauca*]

MEMEGGDYEEHPPAPLLDSLELKLWSFYRAVIAEFVATLLFLYITMTTVVENK
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PRAVLYVVAQCLGAVCGTALVKGIQGSFYASNGGGSNSVSPGYSKGSALLAEI
IGTFVLVYTVFSATDPKRKARDSHVPVLAPLPIGFAVFSIYLATNSITGTGINPA
RSFGPAVIYGHKKSRDDLWIFWIGPLIGAAVATAYHRYLLRAGAFGSKNLGSL
RSQPASAI

>GQ03002_G07.1[*Picea glauca*]
MEMEGGEEQTRDYEEHPPAPLLDSLELKLWSFYRAVIAEFVATLLFLYITMTT
VVENKQSKGTCGGVGLLGEAWAFGGMIFVLVYCISGISGGHVNPAVTFALFL
ARKVSLPRAVLYVVAQCLGAVCGTALVKGIQGSFYASNGGGSNSVSPGYSKG
TALLAEIIGTFVLVYTVFSATDPKRKARDSHVPVLAPLPIGFAVFLVHLATIPITG
TGINPARSFGPAVIYGHEKSWDDLWIFWVGPLIGAAVAAAHHQYVLKASGFG
LKNLGSLRSHPASAT

>GQ03001_P18.1[*Picea glauca*]
MEAKEAEGIEQAKDYRDPPPAPLLDSLELKRWSFYRAAIAEFVATLLFLYITLT
TVVENNRNKVNCSGVGLLGEAWAFGGMIFVLVYCISGISGGHVNPAVTFALFL
ARKVSLPRAVLYIVAQCLGALCGTALVRGIQGSFYASTGGGSNSVSAGYSKGS
ALLAEIIGTFVLVYTVFSATDPKRNARDSHIPVLAPLPIGFAVFLVHLATIPITGT
SINPARSFGPAVIYGHKKSWDDLWIFWVGPLVGAAIAAAYHQYVLRAGGLGL
KSLRSFRSQPTSLAI

>GQ03111_E12.1[*Picea glauca*]
MTKEERRESEQQGFAPKDYTDPPPAALIETSEFKLWSFYRALIAEFVATLLFLYI
TIATVIGHSRTSTNCGSVGVLGIAWSFGGMIFVLVYCTAGISGGHINPAVTFGLF
LARKVSLPRAILYMIAQCLGAICGTGLVKAFQKSFYDRYGGGANYVHHGYTK
GVGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPI
TGTGINPARSFGAAVIYGHKQSWDDHWIFWVGPFAGAALAAAYHQYILRAAA
IKALGSFRSNANV

>GQ03703_H07.1[*Picea glauca*]
MTKEEGKELEQQGFAPKDYTDPPPAALIDANEFKLWSLYRALIAEFIATLLFLY
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FLARKVSLPRAILYMIAQCLGAICGAGLVKAFQKSFYDRYGGGANFVHPGYTK
GVGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPI
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VKALGSYRSNVDV

>>GQ02901_B20.1[*Picea glauca*]
MTKEEGKEMEQQGFAPKDYTDPPPASFIDSGEFRLWSFYRALIAEFIATLLFLYI
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LARKVSLPRAILYMIAQCLGAICGTGLVKAFQKSFYDQNGGGANFVHPGYTK
GVGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPI
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KALGSFRSNPHV

>GQ02905_E13.1[*Picea glauca*]
MAKEGGKEVEQQGFAAKDYKDPPPAALFDVSEFKLWAFYRAIIAEFIATLLFL
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GLFLARKVSLPRAVLYMVAQCLGAICGCGLVKAFQKSYYDQYGGGANSVAH
GYTKGVGLSAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHL
ATVPITGTGINPARSFGAAVIYGHQKIWDEHWIFWVGPFLGAAGAAAYHQYIL
RAGAIKALGSFRSNPHV

>GQ03011_G23.1[*Picea glauca*]
MEMEGEDYEDHPPAPLLDSLELKLWSFYRAVIAEFVATLLFLYITMTTVVENK
QSKGTCGGVGLLGEAWAFGGMIFVLVYCISGISGGHVNPAVTFGMFLARKVS
LPRAVLYVVAQCLGAVCGTALVRGIQGSFYASNGGGSNSVSPGYSKGSALLA
EIIGTFVLVYTVFSATDPKRKARDSHVPVLAPLPIGFAVFLVYLATNSITGTGIN
PARSFGPAVIYGHKKPRDDLWIFWVGPLIGAAVATVYHRYLLRAGAFGSKNL
GSLRSHPASAI

>GQ03818_D05.2[*Picea glauca*]
MEMESGEEQTRDYEEHPPAPLLDSLELKLWSFYRAVIAEFVATLLFLYITMTTV
VENKQIKGTCGGVGLLGEAWSFGGMIFVLVYCISGISGGHVNPAVTFALFLAR
KVSLPRAVLYIVAQCLGALCGTALVKGIQGSFYASNGGGSNSVSPGYSKGSAL
LAEIIGTFVLVYTVFSATDPKRKARDSHVPVLAPLPIGFAVFLVHLATIPITGTGI
NPARSFGPAVIYGHEKSWDDLWIFWVGPLIGAAVAAAYHQYVLRAGGFGLKS
LGSLRSHPTSAT

>GQ02828_J14.1[*Picea glauca*]
MEDVSVGASKYSERQSLGISAQTQRESKDYNEPGPAPLFEPEELRSWSFWRAG
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SGGHINPAVTFGLFLARKVSLPRTVFYMICQCLGAMCGAGVVKGMQKGMYE
VEGGGANLVAHGYSKGDGLGAEIVGTFVLVYTVFSATDAKRSARDPHVPVLA
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ALAAIYHQLIIRAIPFKSRS

>GQ03915_M04.1[*Picea glauca*]
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>CocheC5_3|106077|[*Cochliobolus heterostrophus*]
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VPREEL

>XP001596860|[*Sclerotinia sclerotiorum*]
MSMRSPTITQHPQPLRASPVSNEDDEVQIRLDDSRYHIVAVIGELIGTTLFLFFG
YAGIEVTRLQGREPPDLEVLFYISATFGASLMVTAWIFFRISGGLFNPAVTLALA
LLKAVSPVRAVLLVITQLGASCLAAILVKEIFPNQLGVGTSLGSGTSTAQGFVIE
AITTAALIFTIIMLAVEKHKATFVAPIGIGLALFVAHMVAVPFTGASLNPARSFG
PSAIVWNFPREHWIYWVGPLLGAGLAVLFFRLIKLLEYEMANPGQDGDPENDP
TQNPEHDVAQHANEREEEVLGLSNGKSWYRDESTSGSMRRKGSVNSFIGNRR
SMDRMGEMWGRLDDVEAQWRRQQYGNFI

>Clagr2|68360|[*Cladonia grayi*]
MNTESPRSTTDTIRGLLRRLPTTVRGHVVAVLGEFVGTISFLFFAFAGTQVANIS
SNTNTGTMVVTTVQQKNPAELLYISLSFGFSLAVNAWVFFRISGGLFNPAVTV
GMVLIGAITWVRGVLLFIIQIVGGIVAAELVQALFQGDLAVSTTLGGGTTIAQG
VLIEFILTAQLVFTIFMLAAEKHPGNFIAPVGIGLSLFIAELVGVFWTGGSLNPA
RSFGPAVAIHKFHSTQWIYWVGPLCGSLLAVLIYALVKSLEYESANPDPELGPT
APAIPAAKDLEHQKSLQDGNGRA

>Mycgr1|42217|[*Mycosphaerella graminicola*]
MKKIPATARGHIVAVVGEAVGTFCFLFFAFTGTQSANVASNPNTGDTVITTTPQ
KNPAQLLYISLAFGFSLAVNAWVYFRISGGLFNPAVTLGMMLIQAITPVRAALL
FIAQMVGAIVAAYVVQALFTGGLNVSTTLSDTTTVAQGVIIEMLLTMQLVFTIF
MLAAEKHTGNFIAPVGIGLSLFIAELTGVFWTGGSLNPARSFAPDVALASFSSD
QWVYWVGPFAGSILAVLLFKLVKSLEYDTANPDPEAALPPAGGPVNDGPRES
MNPGRSSDSDATAMKH

>Clagr2|95296|[*Cladonia grayi*]
MCGEFAGTFLFLFFAFAGTQVANNQIQAPVDVNQGSNPAQLLYISLAFGFSLA
ANAWVFFRVSGGLFNPAVTFGLCLVGALDYVRGALIAVTQILGGITAAAVVSA
LFPGPLSVRTTLSARTSVSRGLFIEMFLTAQLVFTIFMLAAEKHKATFIAPVGIG
LSLFIAELSGVYYTGGSLNPARSLAPDVVLHKFDHYYWIYWLGPILGAVLASG
FYWLMKKLEYETANPGQDFNQEEKEHFDPKAHKSAPAVSFGPSEVLSHEKHE
GSDDMTERTVDTEYYDPVGVKASPGSHTAGLGSHTAAQETVKGPKKNGGRL
LAQEVSKTTNEASSNAYVTGPIEESGYHM

>Xanpa1|56187|[*Xanthoria parietina*]
MTTNEPEGSMILNLPGLRSKKHHAIFLGRFRPASTGSHRSPFMGFLPDSFRNHFI
AMLGESAGTFLFLFFAFAGTQVANTKEPDAPIASAPDPASLLYIALCFGFSLAV
NAWVFFRISGGLFNPAGLIISKDSGEMSLDTYCVSLGMCLVGALSWIRGALLIL
SQILGGIISAAIVSALFPGPLNVRTSLAPTTTIAQGLFIEMFLTAQLVFTIFMLAAE
KHKGTFIAPVGIGLSLFIAELAGVYYTGGSLNPARSFGPSVVLHSFYNYDWIYW
LGPVLGSLLASGLYKFIKILEYETANPGQDFDEREQDFFDPSRGTNRPIVNLAPN
GAAFMEEENGRDGNAANRDGQSHDIKRDQTQKVVKLPQGNLGRCERTSR

>Clagr2|96071|[*Cladonia grayi*]

MQNPLRRPTGNASHNAPHRSRDLERNGPDKNDDGYLPKDYRGHLVAMSGEF
VGTFMFLFFALAATQIANTITPPDQPNLNQLLFISLAFGFSLAVTAWVFYRISGG
LFNPAVTFGMVLTGTLPALRGVLLFPAQILGGMVAAAVVSCLFPGTLAVETTL
TNGTSIAQGLFIEMFLTAELVFTVLMLAAEKTKATFIAPVGIGLSLFVAELAGV
YFTGGSLNPARSFGPCVANRNFQRYHWIYWVGPFLGALISGGYYKFVKFNNY
EEANPGQDDSHHPQDVAEKRR

>Xanpa1|39605|[*Xanthoria parietina*]
MRGHFVAMTGEFVGTVMFLFFAFGGTQIANNVIPPDQASLDRLLFISLAFGFSL
ATTAWVFYRVSGGLFNPAVTLGLVLAGGLPPIRGALLFIPQMLGGMVAAALV
KCMFPGSLSVQTKLTGGTSIAQGLFIEMFLTAELLLTVLFLAAEKSKATFIAPVG
IGLALFVAELTGVYYTGGSLNPARSFGPDVANRQFDGYHYIYWIGPFLGAAIA
AGYHRFAKYLRYEEANPGQDAISEHEAEDAKSQ

>Mycgr1|49980|[*Mycosphaerella graminicola*]
MLSKQAMCGEFIGTILFLFFALGGTQVVNNLPVNPGADGKIQQLLYISLCFGFS
LAVNVWVFFRISGGLFNPAVTLGLCLIGAVPWVRGVLLGISQVLGGITAAALIS
CMLPGPLAAETTLGGGTSVAQGVFLEMILTAELVFTIIMLAAEKHKSTFIAPIGI
GLSLFIAELTGVFFTGGSLNPARSFGPAVVNHNFAKYHWIYWIGPLMGSLLAS
GFYKFIKVLEYE

>Mycgr1|52325|[*Mycosphaerella graminicola*]
MRIPFARRLPNNLRNHLVAMSGEYVGTVLFLFFALGGTQVANNIPSSAGRTVA
EAGSNPQQLQYIALCFGFSLAVNAWVFFRISGGLFNPAVTLGMCLIGALPWFR
GVLLFLVQILGGMTAAALIAAMLPGELSVQTTLGGGTSVAQGLFLEMFLTAEL
VFTIFMLAAEKHKSTFIAPVGIGLSLFVAELVGVYFTGGSLNPARSFGPALVNR
NFHGYHWIYWLGPMLGACLAAGFYKFIKALEYETANPDQDGDGR

>ACV52007|[*Rhizophagus intraradices*]
MGLKDDFVTSLAEFIGTTYFIFIGLGGSDAIAAFSGKSLGDIKLFATAFSFGWSL
MINVWLWSDISGGVLNPAITIALMFTDDQELRIRRGIFYIIAQFAGAILGSLLVK
LFLPAPIAALTTLSDGTTILQGLVIEIITTSLLTLTVYTLAVNERGGFMKSFGMGT
SVLISVLVAGPYTGASLNPARTLGPAIVSGKISGDIWIYFIGPIIGSLLAASFHTYF
KKNFGALHLDRDRDDLDRDLDLDRENLGKD

>Mucci2|114802|[*Mucor circinelloides*]
MRPINDIFGCNKGSPPNLRADLKASLGEFFGMAIFIFLALAGVQGALEAPTFQS
VGAATGPTATQIQSIAFSFGVGITVALFICGAVSGGILNPAVLLSLMITGNINWL
RGIFFFIAEIVGAIIGAYFANFVTAHELQGVNLLNPGFNYAQGFFAECLLTCVLC
LTVLFIIVDKHLLADFAPFVVGSAVFICHMIGAPVDGTSINPARSFAASIVTGKW
ANHWIFWFGPLIGGIFAVMIYLAVKVITEESQEKALLQNAHNQAMIDADKNKE
QNQQNPAQYTSVNV

>XP776423|[*Cryptococcus neoformans*]
MPDLVVTTQELRPSSPVYGHARTIIKNNFVAMAGEYVGTTLFMLCCLGGTHV
ALLPEKSVTGDLSQPLNTSSLFYISLSIGLSLTVNVWIFFRVSGGLFNPAVSLGM
VLAGCLPPMKGALLTVAQVLGGITGAAIINVLLPGELNAGTTLGGGASIAQGL
FIEAILTALLMLTVFFTAAEKNEATFLSPLAIGMALFIAEMVGVPYSGGALNPV
RSLGPAVVTHNFPGYHWIYWVGPALGSVLATCFYSLLKYLEYESVPGPGEAPH

VPPFWRLPARGYLSTLSHPFAKRASTGTYSHEDDPEKGLRSGKDSQANTDNPT
RVNRPGSGDAEIQSPVEDTTMANARLDRIEMLLTQLMQVRTSEGTQKSPAV

>Sporo1|13829|[*Sporobolomyces roseus*]
MTDFVAMVGEFVGSESLYPSTAIAGGTTRSDTADGADAVAAIVNTSNLLYIAL
SFSVSLATTVWIFFRVSGGLFNPAVTLAMWLIGAIKPLRAILLVFSQIAGGIAGS
ALVYGLLPGSFNVQTTLSANTSIVRGIFIEMFLTAMLLMAILMLAAEKNRSTFI
APLGIGMSLFIAEMLGVAFTGGAVNPARAFGPAVVTGSFVSYHYIYWVGPALG
AIIASGFYRFIK

>XP001937248|[*Pyrenophora tritici-repentis*]
MKQKYTEDPQHPAEPRQPGCGWLPNRARHHLISFLGEYVGTFLFLFFAFAATQ
VANNLRGTRPMDVGTLLYISLAFGCSLAITVWVFFRISGGLFNPAVTFAMGLV
GAIGWVKVLLLILAQTLGAITAAAIVSGLFPGPLSVNTSLSKGTSVTRGLFIEMF
LTFMLLLTIFMLAAEKHRATFIAPLGIGLALFIAELAGVYFTGGSLNPARSFGPA
LITGKWPGHHWIYWLGPLLGAAMAAGFYRLLKTLGYETANPGADGDGREEY
STRASDRTPTRYQTGTMPLSTTDGTEEHDFVIQLQSGQIQPAKIHRQAMPALDG
TTSTITDSIEKPMTPTPVHMHNLDGHRSDTINHPEDRPRAHHKRGSSQHEMAS
DSSYRHGPNAESGSSES

>Sporo1|27302|[*Sporobolomyces roseus*]
MSELPTHVPAADTLQTNVHVPHRHRGGTYLRKHLGVGAHKGDRHAQRLLKS
HAIAFIGEFVGTTLFMFFAFSGTTVASLPATSVTNSGATSTQGAVQPSPNTSSLL
YVALSFGFSLSVNIQIFAGVSGGVFNPAISLGLALIGAHTPVRAIILTIAQILGGIA
GAAITSGLLPGPLNVRTTLVAGTNIARGLFIEMFLTTILMLTVLVLTVEESAIGA
KSAGASAAPISIGLALFLAELVGVFYTGGSLNPARSIGPDIVIGTFDNYHWIYWL
GPAMGAALAVVFWKGIQYVHFSVGPRAAGTPDASKMLLENPDEKSAGASGP
AGDQGKNGAALENSTSHYGDAGLARDDNDNQSMVSVIAPEPADRMTVLEQS
NHRIEAMLSQLMHGGGGSPLSTIGSSAGNSLSQRKISTEQTLHENELHGTDHLH
SVGSRPSQYSSR

>CocheC5_3|1086713|[*Cochliobolus heterostrophus*]
MSLTSKDQPPSLPLHEPPTPTPTTPSSQPPRAHHTLRTEAAAFLGEFIGTFMFLSL
AFTGTQIALNATGSKEPSSSDLPLPDLTKLLYISLAFGASLAINVAIFADISGGKF
NPAVTTALYLTRKIHWHRAAQTIFSQLVASIAASAFISGLVPGPLTIGTTLSADM
SVTRGMFLEALVTSQLVLTILMLEGGAAKPMYIGGALFVGHVCSVYYTGASL
NPARSFGAAVVVGFTGYHWIYWVGPLLGAGVASAVYGGVGWLRRERVVVV

>EGX96316|[*Cordyceps militaris*]
MQIIQPEYPRQLVSAIPNKIRNHFVAAIGEFVGTFLFLFFAFSATQVANTALKGK
NAAQQQSQAADSQHAIADIPDTPTLLYIALAFGFSLAVNAWVFFRISGGLFNPA
VTLGLVLIGGLRPVRAVLIFLAQILGATSASAVVDALFPGELQIATALGGGTSV
AQGVWIEAFLTAELVFTIIMLAAEKHKGTFLAPIGIGLSLFIAEMTGVYFTGGSL
NPARSVGPCIVLHSFPSYHWIYWVGPGIGSVVAAGFYHLINILEYETANSGQDF
NEKEMDRFVFDEEAAHTGAHIVRPDATDVLDPIVSPVASPAASGDVHRGPCPG
GLASPAAPSLIPNGSLKKD

>XP001585953|[*Sclerotinia sclerotiorum*]
MNINEPRRGGGFVLPFFNDSKKSREPHAGRDSLQSNGNRFTGWIPGTVGYTTT
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IVGTPSDPAQLLYIALSFGFSLAVNVWVFFRTNGGLFNPAVTVGMCLVGALPY
LRGLFLFIAQIVGGITAAALVSALFPGPITFRTTLGGGTSIVRGLFIEMFLTAQLA
LTIFMLAAEKHKGTFIAPIGIGLALFIAELTGLYFTGGSVNPARSFGPSVVSGQFP
GYHWIYWLGPFLGAILASAFYKFIKVLEYETANPGQDAGRVGESYEPQAHTTN
KVSFAEEGMVGRELDESGASHVHGTDKNLGTPKEYGTHRRPFSGSPAPSHPND
QFAGLNGGGMNGDEFAAGGNAGVGGVKRDKRDSEGTLVDNGKKGTMRGG
AGVMESRAGGSGGASG

>XP001825875|[*Aspergillus oryzae*]
MKFMNHGARADRAEHSGSTPVYSSTQKQLPMLHLKDTARNNVIAVIGEFVGT
FLFLFFSFAGTQVSNTPKPVDGAPPNTANLLYSALSFGFSLMVNVWAFYRVTG
GLFNPAVTLALCLVGGLSPIRGVLVFAAQIVAGIASAGVVSALFPGDLNVGTRL
GGGASISQGLFIEMFLTAQLVFVIIMLAVVKHKSTFLAPVGIGLVFFVTEMIGDY
YTGGSLNPARSLGPDVINRSFPGYHWIY

>XP002377476|[*Aspergillus flavus*]
MKFMNHGARADRAEHSGSTPVYSSTQKQLPMLHLKDTARNNVIAVIGEFVGT
FLFLFFSFAGTQVSNTPKPVDGAPPNTANLLYSALSFGFSLMVNVWAFYRVTG
GLFNPAVTLALCLVGGLSPIRGVLVFAAQIVAGIASAGVVSALFPGDLNVGTRL
GGGASISQGLFIEMFLTAQLVFVIIMLAVVKHKSTFLAPVGIGLVFFVTEMIGDY
YTGGSLNPARSLGPDVINRSFPGYHWIYWVGPLLGSLLACGFFGLLKMMEYTT
ANPGQDYNEWEAKNGPGSYDVSGNRPSVQLSDTSTLNRAHSPTNGHGVQPQ
HVNGAEQV

>Aspni5|125829|[*Aspergillus niger*]
MGPQQWFRKRNPDSQTAPMYRSSNNQLPMLHLADTTRNNFIAAVGEFVGTFL
FLFFSFAGTQVSNTPKPAPGSPPNTSNLLYSSLCFGFSLMVNVWAFYRVTGGLF
NPAVTLALCLVGGLSPIRGVILFGVQLIAGIASAGVVGALFPGDLNVGTRLGGG
ASISQGLFIEVFLTAQLVFIIIMLAVVKHKSTFLAPVGIGLVFFVTEMVGDYYTG
GSLNPARSLGPDVINRSFPGYHWIYWVGPLLGSLLACGFFTFLKMFQYTTVNP
GQDYNEWEAKMGSGGPGSWDMSMHRPSYNSEATTVEQPQSPSAGRRNHTPT
NSQPQNNPNIPNIPHGAEQV

>XP001262474|[*Neosartorya fischeri*]
MLFGKRNGDERRVVRRSQSQLPMLGLADSARNNLIAVVGEFVGTFLFLFFSFA
GTQVSNTPKPVAGAPPNTSNLLYSALSFGFSLTVNIWAFYRVTGGLFNPVVSL
ALCLVGGMPPLRGVFVFAAQLVAGIAAAGVVSALFPGDLNVGTRLGGGASIS
QGLFIEMFLTAQLVFVIIMLAVVKHKSTFLAPVGIGLTFFVTEMIGDYYTGGSL
NPARSLGPDVINRSFPGYHWIYWVGPLLGSLLACGFYTFLRMFKYESVNPGQD
YDEWEAKRGPGSFDENGRDSTAFSDSTAGVRSHSPRGVNRPSGPVSGAEQV

>XP746526|[*Aspergillus fumigatus*]
MAFGKRNGEERRVLHRTQSQLPMLGLADSARNNLIAVVGEFVGTFLFLFFSFA
GTQVSNTPKPVAGAPPNTSNLLYSALSFGFSLTVNIWAFYRVTGGLFNPVVSL
ALCLVGGMPPLRGVLVFMAQLVGGIAAAGVVSALFPGDLNVGTRLGGGASIS
QGLFIEMFLTAQLVFVIIMLAVVKHKSTFLAPVGIGLTFFVTEMIGDYYTGGSL
NPARSLGPDVINRSFPGYHWIYWVGPLLGSLLACGFYAFLRMFKYESVNPGQD
YDEWEAKRNHGSFDGNGRESTAFSDSTAGAQSQSPRGVNRPVSGAEQV

>XP001825978|[*Aspergillus oryzae*]

MPSWIHQRRGDMVNDPASRNVSTTSSTPIMQALAARIRSQPGGNTAPIVRSEK
GQLPMLHVPYTFQNTFVAVVGEFVGTFMFLFFAFAGGQVSNTPKPAEGAAPN
TSNLLYLSLSFGFSLLVNVWTFYRVTGGLFNPVVTLALCLCGGMHPVRGVLVF
ASQIIAGIASAGVVSCLFPGPLSVGTRLGGGTSISQGLFIEMFLTAHLVFVVIML
AVVKQKSTFLAPVGIGLVLFVNQLVGTYYTGCALNPARALGPDVINRSFPGYH
WIYWVGPLLGSLLASGFYGFLSIFHYETVNPGQDFNQWEAAAGPGPWHEEIN
KHSGAPNHSHLSGDQPTLHQDNV

>XP002377624|[*Aspergillus flavus*]
MPSWIHQRRGDMVNDPASRNVSTTSSTPIMQALAARIRSQPGGNTAPIVRSEK
GQLPMLHVPHTFQNTFVAVVGEFVGTFMFLFFAFAGGQVSNTPKPAEGAAPN
TSNLLYLSLSFGFSLLVNVWTFYRVTGGLFNPVVTLALCLCGGMHPVRGVLVF
ASQIIAGIASAGVVSCLFPGPLSVGTRLGGGTSISQGLFIEMFLTAHLVFVVIML
AVVKQKSTFLAPVGIGLVLFVNQLVGTYYTGCALNPARALGPDVINRSFPGYH
WIYWVGPLLGSLLASGFYGFLSIFHYETVNPGQDFNQWEAAAGPGPWHEEIN
KHSGAPNHSHLSGDQPTLHQDNV

>XP002558264[*Penicillium chrysogenum*]
MLFKKRHSSDTSTLEGTQTSGGNGQLPMLRLPDRMRYNLLCLMGEFVGTFLF
LFFSFAGTQVSNTPMPPPGSPPNTSNLLYSALSFGSALTVNVWAFFRVTGGLFN
PAVTLALCLTGGMPPLRGLFVFPAQLVAGIAAAGVVSALFPGPLNCATRLGGG
ASIVQGLFIEMFLTAQLVFVIIMLAVVKHKSTYLAPVGIGLAFFVAELIGDYYT
GGSLNPARSLGPDVINRSFPGYHWIYWVGPLLGSLLASAFYRLLCFVRWERINP
GQDYNEEEVARKESLLGSEHTITANVANPRRDIAPDEHV

>XP001274891|[*Aspergillus clavatus*]
MLHLAPSLRNNMIAMVGEFVGTFLFLFFSYAGTQVSNTPKPAPGSPPNLEALL
YSSLCFGFSLTVNVWAFYRVTGGLFNPSVTLALCLVGGMPPIRGVLVFAAQIV
GGIAAAGVVSALFPGPLNVTTRLGGGASISQGLFIEMFLTAQLVFVIIMLAVVK
QKSTFLAPVAIGLAFFVAEMIGDYYTGGSLNPARSLGPDVINRSFPGYHWIYW
VGPLLGSLLACGFYTFLRLFQYETVNPGQDNNEWEAEAKTRGESFDESNGRTS
TNFSESTFGGRNPSLTPAPTSGAPANFAPHGGIPNGAEQV

>CBF78938|[*Aspergillus nidulans*]
MRSRIPNILKPWGRQETQGDTPVVRRNRNQLPMLHLADTTRNNLIAMTGEFV
GTFLFLFFSFAGTQVANTPKPVEGAPPNTDALLYSALAFGFSLMVNIWAFYRV
TGLLFNPAVTLALCLVGGMPAYRGLFVFAAQIVGGIAAAGVVSALFPGDLNVS
TRLGGGASISQGLFIEMFLTAQLVFVIIMLAVVKHKGTFLAPVAIGIAFFVTEMI
GDYYTGGSLNPARSLGPDVINRSFPGYHWIYWVGPLLGSLLACGFYYFLTFFS
YESVNPGQDFNEWEAKWGPGPTSWDSSMRQHSHSDTTTLNRGMSPRDSRAA
RNGNGDWNGAHGAHVPPPPGEEQV

>EEH18381|[*Paracoccidioides brasiliensis*]
MSAPTPPITTNNDHGGSRPSAFRRFSTLSTRLPGGNIPDTFRNNFIATLGEFVGTF
LFLFFSFAGTQISNEPPPTPGAKPNHIALLYSSLAFGVSLAVNVWVFYRVTGGL
FNPSVTLALFLIGSLSPIRAVLVFAAQIVAGIAAAGVVSCLFPGPLVVYTRLGSG
TSITQGLFIEMFLTAQLVITIIMLAAVKHKATYLAPLGIGLAFFLTELCGDPFTG
GSLNPARSLGPDVINRQFPGYHWIYWVGPGLGSLLAVGMFYILKALHYQTCN
AHQDDDHEKLVETAAPPEQLGEHKAKEGHKGESGRTPLGAHG

>XP002794333|[*Paracoccidioides brasiliensis*]
MSAPNPPITSNNNHGGSPPSAFRRFSTLSTRFPGGNLPDTFRNNFIATLGEFVGT
FLFLFFSYAGTQVSNDPPPNSGAKPNHIVLLYSSLAFGTSLAVNVWVFYRVTG
GLFNPSVTLALFLIGSLSPIRAVLVFAAQIVAGIAAAGVVSCLFPGPLVVFTRLG
SGTSIPQGLFIEMFLTAHCSREAQGDILGAIGIGLAFFLTELCGDPFTGGSLNPAR
SLGPDVINRQFPGYHWIYWVGPGLGSLLAVGMFYILKALHYQTCNPHQDDDH
EKLVETATPPGGHA

>XP002486574|[*Talaromyces stipitatus*]
MRILSSYHRRSGQTEPNLSRLSTNRQAAQLPMLNLADNNRNNLIAVIGEFVGTF
MFLFWSFAGTQISNTPMPPAGSYPNTSNLLYASLAFGFSLTVNVWAFYRVTGG
LFNPSVTLALFLVGGIPAMRSVLIVIAQILGGICAAAVVSALFPGPLNVATTLGG
GANTAQGLFIEMFLTAQLVFVIIMLAVVKHKSTFLAPVGIGLTFFLTELCGIYYT
GGSLNFARSLGPAIVNHSFPHYFWIYFLGPLLGSGLASGFYYLLNKMRYETCNP
GQDADSMEAPTKESTGTSSFNQSIPSAYDRGAGNGVNGNVNGTGGAAGYGH
QRNISEATAVSPGYATTSEKNEPALSPTGHGEYPSTTSAGQYANTPANQPTGTY
TNQPSGNQPGSLNPVRERGESGQINQF

>XP002152841|[*Penicillium marneffei*]
MGVLSTAYRRSDQGQADTELSHVSTNRQSTQLPMLSIADNLRNNMIAAIGEFV
GTFMFLFFGFVGAQVANTPAPEPGSGPDTSKLLYISLVFGFSLTVNVWAFYRV
TGGMFNPAVTLALFLVGGLPAIRGVFVVISQIVAGICAAAVVSALFPGPLNVEN
TLGGGTNVAQGLFIEMFLTSQLVFVIIMLAVVKHKSTFLAPVGIGLTLFLCHLT
GIYFTGASLNFARSLGPAVVNHSFPHYFWIYFLGPMLGSCLASSFYFFLNKMRY
ETCNPGQDAESLEDPAAAKERQLSSSTTGGDLGVSNGVSNGVSNGVNGDVNG
SDRTINEHQTNISETAAINSGYADKNKNEASGGDYQYSSNSASGHPYSNQSNA
N

>XP003009607|[*Verticillium albo-atrum*]
MSAPESPDQRHFFSHLSSRQTSAAPGSPTAAAPPGAPPTRTTTNRLPILKAQSGA
RNNVTAVVGEFVGTFLFLFFSFAGTQLANTPASTSTEPNHTALIFIALSFGVSLT
ANVWVFYRITGGMFNPVVTLALVICGGLPITRALLIMPTQILAGMSAAGVISAL
LPGPLAVTNSLGGGANTAQGLFIEMFLTAQLVFTILMLAVEKHRSTFLAPVGIG
ASFFLAELVGCYWTGGALNPARAFGPAVATRTFPNYHWIYWLGPIMGSLLAS
GFYLLLKAMQYQECNPGQDASGADLERYEDRHAQHHTTTEEPKV

>EGY16807|[*Verticillium dahliae*]
MSAPESPDQRHFFSHLSSRQTSAAPGSPTAAVPAGAAPTRTTTNRLPILKAQSG
ARNNVTAVVGEFVGTFLFLFFSFAGTQLANTPASTSTEPNHTVLIFIALSFGVSL
TANVWVFYRITGGMFNPVVTLALVICGGLPITRALLIMPTQILAGMSAAGVISA
LLPGPLAVTNSLGGGASTAQGLFIEMFLTAQLVFTILMLAVEKHRSTFLAPVGI
GASFFLAELVGCYWTGGALNPARAFGPAVATRTFPNYHWIYWLGPVMGSLL
ASGFYLLLKAMQYQECNPGQDASGADLERYEDRHAQHHTTTEEPKV

>EFQ32034|[*Glomerella graminicola*]
MTIGRKPFGNSDVNQGSLPMLRVANTTRNNIVAVLGEFVGTFLFLLFSFAGTQ
VANTPPGAPDSDPNLPCIIFIALAFGVSLTANVWAFYRITGGMFNPVVTLALVV
CGGFSPLRALLIMPTQIIAGLCAAGVASALFPGPLAVTTTLGGGANVAQGFFIE
TFLTTQLVFVILMVAVEKHRSTFLAPVAIGLSFFLAELTGVYFTGGSLNPARSL

GPAVVARQFTEYHWIYWLGPIFGSMMACGFYLLLHNLRYHECNPGQEADGQ
ETTSKTPLTSTA

>XP002629618|[*Ajellomyces dermatitidis*]
MESSPAQYADINPGPDQDHAPLDNYPSYSTTIVSSNSNPTGSPKYDTSRPRPRA
SFISDRINRIPDPLRNGIVATTGEFVGTFLFLFYPFASGIIANEVVLEPGEQADPTA
LLYTALGFGVSLTVNVWLFFRVTGGMLNPAVTLALYLLGLVPPVRAVFVIVA
QLIGGIAAAGLASAMFPNPLTVGTRLGNGVSIVQGLFIEVFLTALLVLAIIMLAV
VKHKATFIAPLGIGLAFFLAELVGIPFTGGSLNPARSLGPDVINRSFPGYHWIYW
LGPVLGALLAVGWYLFLRALRFETCSPGQDAEFESGAVFEGRRGGMAAATAT
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>XP003666488|[*Myceliophthora thermophila*]
MNSSRRLRLFSFASDTANQNESSGRLPMLTRPLTARNNIVAALGEFVGTFLFLF
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LFNPVVTEEEEEENKTFTLWLKQGVVKVALALFLVGGLSGIRVLVVVIAQFIG
GIAAAAVVSALFPGPMMVATTLGGGASISQGLFIEMFLTAQLVFVIIMLAAEKH
KSTFLAPIGIGIAFFLAELIGVYFTGGSLNPARSLGPAVVNHSFPGYFWIYWVGP
LLGSLLACAFYVLLKYLRWKECNPSQDWNEIEKQESERQLRNKASKNITERPS
TSPTDATHAADVQPQVQPQSNLD

>XP003235493|[*Trichophyton rubrum*]
MGPLPSLFSSESNDSWKKSSVATFGEFVGTFMFLFLSYTGCQIANMSVDPKDSS
PEPNPTVLLYISLSFATALAINVWVFYRVTGGMFNPAVSLALALVGAISPIRAV
MVSIAQVVAGIAAAGVVSALFPGPLVVQTKLGGGTTTTQGLFIEMFLTAELIITI
LMLAIVKHRATFLAPLGIGLALFIAQLSGVYFTGGSLNPARSFGPDVIVGGFPG
YHWIYWVGPLLGSLLATGFYQALNFLRYQNVNPGQDFDGIGSELMGNPERSE

>EGD93004|[*Trichophyton tonsurans*]
MGPLPSLFSSESNDSWKKSSVATFGEFVGTFMFLFLSYTGCQIANMSVDPKDS
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>XP_002848916.1|[*Arthroderma otae*]
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>XP461517|[*Debaryomyces hansenii*]
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>CCE81314|[*Millerozyma farinosa*]
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>EGA57700|[*Saccharomyces cerevisiae*]
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>ADC55259|[*Saccharomyces cerevisiae*]
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>ADC55543|[*Saccharomyces cerevisiae*]
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>XP445420|[*Candida glabrata*]
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>XP444824|[*Candida glabrata*]
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>Picst3|82575|[*Pichia stipitis*]

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>XP451974|[*Kluyveromyces lactis*]
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>XP380987|[*Gibberella zeae*]
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>TriviGv29_8_2|39488|[*Trichoderma virens*]
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>CAD66431|[*Blumeria graminis*]
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>XP756370|[*Ustilago maydis*]
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>Lacbi2|456764|[*Laccaria bicolor*]
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>GAA96083|[*Mixia osmundae*]
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>GAA95792|[*Mixia osmundae*]
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V

>CAA38096|[*Saccharomyces cerevisiae*]
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>XP658434|[*Aspergillus nidulans*]
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>XP002843967|[*Arthroderma otae*]
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>GAA93674|[*Mixia osmundae*]
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>XP002560691|[*Penicillium chrysogenum*]
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>Walse1|32912|[*Wallemia sebi*]
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>EFQ30640|[*Glomerella graminicola*]
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EVL

>CBQ71719|[*Sporisorium reilianum*]
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>XP757655|[*Ustilago maydis*]
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>Pospl1|114217|[*Postia placenta*]
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>Trave1|121294|[*Trametes versicolor*]
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>EGN98476|[*Serpula lacrymans*]
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>Phchr1|5155|[*Phanerochaete chrysosporium*]
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>Phchr1|5154|[*Phanerochaete chrysosporium*]
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>Wolco1|23700|[*Wolfiporia cocos*]
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>XP001837792|[*Coprinopsis cinerea*]
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>XP001838949|[*Coprinopsis cinerea*]
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>Lacbi2|443240|[*Laccaria bicolor*]
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EGGRHVRTLDTAGLFATYAADHMTNVSCFFSEFLATAVLIVVIHAMNDKRNA
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>Lacbi2|317173|[*Laccaria bicolor*]
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>Lacbi2|568479|[*Laccaria bicolor*]
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>Lacbi2|576801|[*Laccaria bicolor*]
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>XP001257964|[*Neosartorya fischeri*]
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>XP750737|[*Aspergillus fumigatus*]
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>XP003004692|[*Verticillium albo-atrum*]
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>XP504820|[*Yarrowia lipolytica*]
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>XP503595|[*Yarrowia lipolytica*]
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>TriviGv29_8_2|196947|[*Trichoderma virens*]
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>XP001408145|[*Magnaporthe oryzae*]
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>XP002565260|[*Penicillium chrysogenum*]

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>TriviGv29_8_2|184650|[*Trichoderma virens*]
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>Mycgr1|55142|[*Mycosphaerella graminicola*]
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>EFX02739|[*Grosmannia clavigera*]
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NTAVDKV

>Mycfi2|133800|[*Mycosphaerella fijiensis*]
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>EGU81740|[*Fusarium oxysporum*]
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>XP383424|[*Gibberella zeae*]
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>Clagr2|115933|[*Cladonia grayi*]
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>Mycfi2|58253|[*Mycosphaerella fijiensis*]
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>Phchr1|138757|[*Phanerochaete chrysosporium*]
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>Kwal20572 |[*Kluyveromyces waltii*]
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>NP983336|[*Ashbya gossypii*]
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>Sporo1|24618|[*Sporobolomyces roseus*]
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>Mycfi2|165933|[*Mycosphaerella fijiensis*]
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>Mycfi1|27060|[*Mycosphaerella fijiensis*]

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>XP758316|[*Ustilago maydis*]
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>XP369584|[*Magnaporthe oryzae*]
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>Thite2|2114473|[*Thielavia terrestris*]
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>Xanpa1|68791|[*Xanthoria parietina*]
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>Mycgr1|39363|[*Mycosphaerella graminicola*]
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>Trive1| 66182 |[*Trichoderma virens*]
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>Lacbi2|482072|[*Laccaria bicolor*]
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>Lacbi2|671860|[*Laccaria bicolor*]
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>Phchr1|123853|[*Phanerochaete chrysosporium*]
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>Pospl1|127849|[*Postia placenta*]
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>Wolco1|121322|[*Wolfiporia cocos*]
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AESVESETFKQAP

>Trave1|43473|[*Trametes versicolor*]
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>Trave1|113714|[*Trametes versicolor*]
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>Phybl2|141336|[*Phycomyces blakesleeanus*]
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VDQ

>Phybl2|60581|[*Phycomyces blakesleeanus*]
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>Mucci2|185371|[*Mucor circinelloides*]
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>XP002379878|[*Aspergillus flavus*]

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>XP001821574|[*Aspergillus oryzae*]
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>Phybl2|143660|[*Phycomyces blakesleeanus*]
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>Phybl2|166662|[*Phycomyces blakesleeanus*]
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>XP002566055|[*Penicillium chrysogenum*]
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>XP660426|[*Aspergillus nidulans*]
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>GAA86900|[*Aspergillus kawachii*]
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>XP001396373|[*Aspergillus niger*]
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>CocheC5_3|1030135|[*Cochliobolus heterostrophus*]
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QVA

>XP383956| [*Gibberella zeae*]
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>Mycfi1|35307|[*Mycosphaerella fijiensis*]
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>Trive1|41159|[*Trichoderma virens*]
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>Aspni5|201277|[*Aspergillus niger*]
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>XP002551817|[*Lachancea thermotolerans*]
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>GAA23030|[*Saccharomyces cerevisiae*]
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>NP592788|[*Schizosaccharomyces pombe*]
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>Kwal15269 |[*Kluyveromyces waltii*]
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>Sporo1|13459|[*Sporobolomyces roseus*]
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>Walse1|59835|[*Wallemia sebi*]
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>XP002384305|[*Aspergillus flavus*]
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>XP001390456|[*Aspergillus niger*]
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AE

>XP003190309|[*Aspergillus oryzae*]
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PPWGRSM

>XP001396483|[*Aspergillus niger*]
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KA

>XP002149425|[*Penicillium marneffei*]
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>XP002484748|[*Talaromyces stipitatus*]
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>XP002562918|[*Penicillium chrysogenum*]
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>Trive1|42995|[*Trichoderma virens*]
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>EGX52763|[*Arthrobotrys oligospora*]
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>EGX93694|[*Cordyceps militaris*]
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>EGU75229|[*Fusarium oxysporum*]
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>XP003042844|[*Nectria haematococca*]
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PWTKEN

>EGU83709|[*Fusarium oxysporum*]

MRLESPAHQMSSYDGDVESRRPVELASSPERIIPRYRSSSHPFAGRVGANQAFT
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>Clagr2|89221|[*Cladonia grayi*]
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>EFY92789|[*Metarhizium acridum*]
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>EGR49911|[*Trichoderma reesei*]
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>TriviGv29_8_2|201141|[*Trichoderma virens*]
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>EHK42605|[*Trichoderma atroviride*]

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>TmeAQP2|GSTUMT00004612001|[*Tuber melanosporum*]
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>JQ412059|[*Glomus intraradices*]
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>JQ412060|[*Glomus intraradices*]
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>JQ585592|[*Laccaria bicolor*]
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>JQ585593|[*Laccaria bicolor*]
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>JQ585594|[*Laccaria bicolor*]
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>JQ585595|[*Laccaria bicolor*]
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>JQ585596|[*Laccaria bicolor*]
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SSQECQLAEVV

>JQ585597|[*Laccaria bicolor*]
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ASSQEQQLAEVV

Bacterial MIPs
>AqpZ|[*Escherichia coli*]
MFRKLAAECFGTFWLVFGGCGSAVLAAGFPELGIGFAGVALAFGLTVLTMAF
AVGHISGGHFNPAVTIGLWAGGRFPAKEVVGYVIAQVVGGIVAAALLYLIASG
KTGFDAAASGFASNGYGEHSPGGYSMLSALVVELVLSAGFLLVIHGATDKFAP
AGFAPIAIGLALTLIHLISIPVTNTSVNPARSTAVAIFQGGWALEQLWFFWVVPI
VGGIIGGLIYRTLLEKRD

>GlpF|[*Escherichia coli*]
MSQTSTLKGQCIAEFLGTGLLIFFGVGCVAALKVAGASFGQWEISVIWGLGVA
MAIYLTAGVSGAHLNPAVTIALWLFACFDKRKVIPFIVSQVAGAFCAAALVYG
LYYNLFFDFEQTHHIVRGSVESVDLAGTFSTYPNPHINFVQAFAVEMVITAILM
GLILALTDDGNGVPRGPLAPLLIGLLIAVIGASMGPLTGFAMNPARDFGPKVFA
WLAGWGNVAFTGGRDIPYFLVPLFGPIVGAIVGAFAYRKLIGRHLPCDICVVEE
KETTTPSEQKASL

Animal MIP

>AQP1|[*Mus musculus*]
MASEIKKKLFWRAVVAEFLAMTLFVFISIGSALGFNYPLERNQTLVQDNVKVS
LAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISILRAVMYIIAQCVGAIVA
TAILSGITSSLVDNSLGRNDLAHGVNSGQGLGIEIIGTLQLVLCVLATTDRRRRD
LGGSAPLAIGLSVALGHLLAIDYTGCGINPARSFGSAVLTRNFSNHWIFWVGPF
IGGALAVLIYDFILAPRSSDFTDRMKVWTSGQVEEYDLDADDINSRVEMKPK

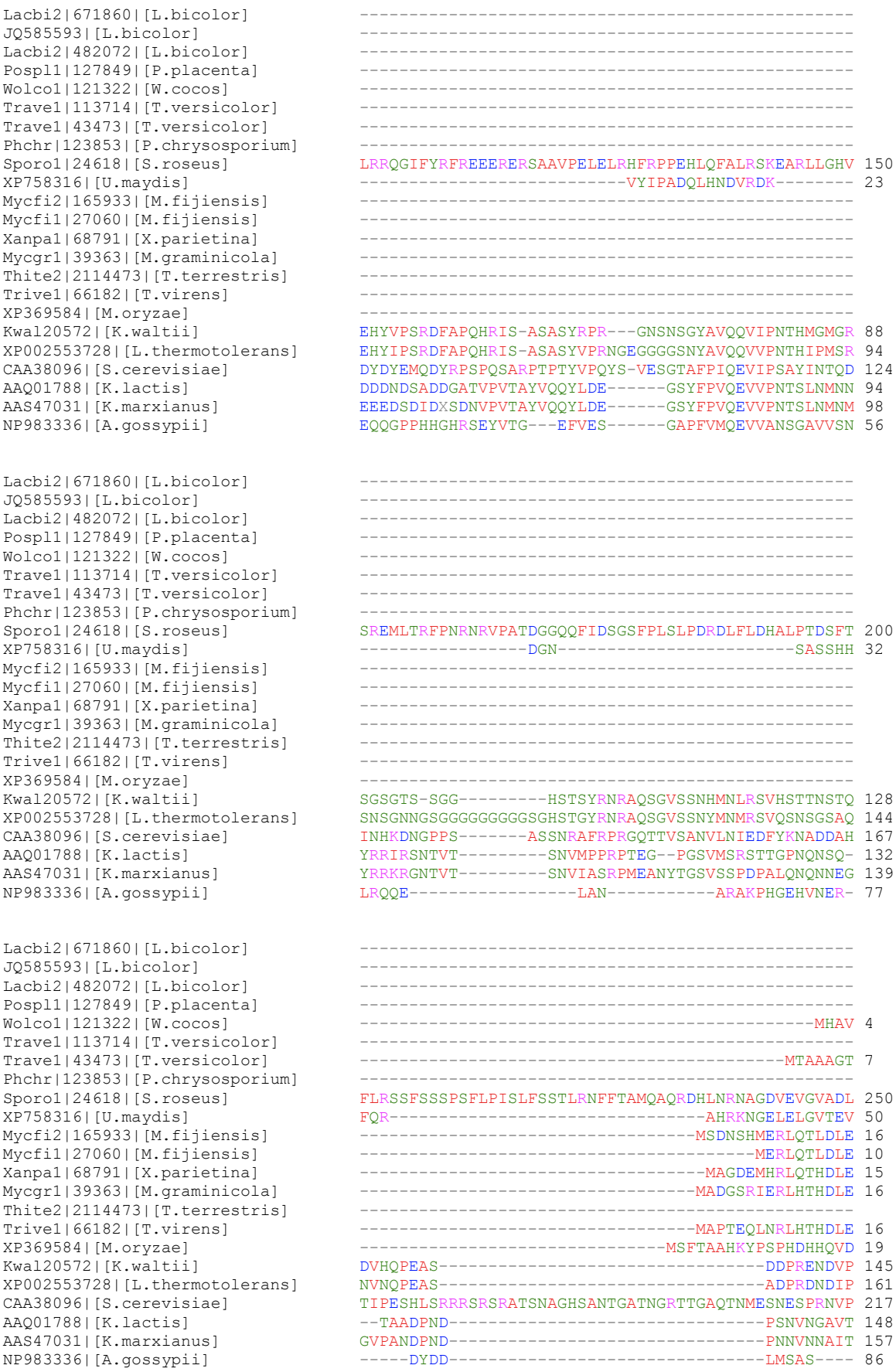
APPENDIX 2 CLUSTAL W 2.1 multiple sequence alignment shows conservation and variation of aligned amino acid residues between five mycorrhizal fungal MIPs in Cluster I, II, III and IV

Note A2.1. CLUSTAL W 2.1 multiple sequence alignment shows conservation and variation of aligned amino acid residues between the mycorrhizal fungal MIPs in Cluster I of the orthodox fungal water channels. The similarity score between putative TmeAQP1 of Ascomycota ECM *Tuber melanosporum* and functionally characterized JF491353 of Ascomycota ECM *Terfezia claveryi* is 0.45, higher than the one between TmeAQP1 and Lacbi2:456764 which is 0.36, and 80 residues are completely conserved among these 3 aquaporins in Cluster I. The consensus symbol asterisk "*" indicates positions which have a single, fully conserved residue among the aligned sequences. The symbol colon ":" indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, and the period "." indicates conservation between groups of weakly similar properties - scoring ≤ 0.5. Colors indicate physicochemical properties of the residues. AVFPMILW are coded RED for small + hydrophobic (including aromatic -Y). DE are coded BLUE for acidic. RK are coded MAGENTA for basic-H. STYHCNGQ are coded GREEN for Hydroxyl + sulfhydryl + amine + G. Others are coded Grey for Unusual amino/imino acids, etc (Larkin *et al*. 2007).

```
JF491353|[T.claveryi]          ----------MSSAFGTKESDNEAVTARG---WRQSVGKNYAIAAVGEFL 37
TmeAQP1|[T.melanosporum]       MAGHKSGTTSRTAPLEGADSENASLNVGSRVPLKQNIPRDYALAVFGEFL 50
Lacbi2|456764|[L.bicolor]      ----------MHPQVAS---------------LFDNVYEDLAAATL-EFI 24
                                          . .                  :.: .: * *.. **:

JF491353|[T.claveryi]          GTFLFLFFAFAGTQCAKLAFDPLKKEQGLAEDAIPTVGFLLYVALAFGFS 87
TmeAQP1|[T.melanosporum]       GTFLFLFFAFGGTQAVKINHSGSGTLPKDPTSAIPSPDLLLYVSICFGFA 100
Lacbi2|456764|[L.bicolor]      GTAFFLLFGLGGIQASTAEDTASGQPP------ASGIEHVLYISTCMGLS 68
                               ** :**:*.:.* *. .                .    :**:: .:*::

JF491353|[T.claveryi]          LAVNVWIFYRISGGIFNPAVTLGVVLLGAMPPLKGVFLVAAQFTGGIFAA 137
TmeAQP1|[T.melanosporum]       LTVNVWVFYRVTGALFNPAITFGCVLVGGVPPLKGALIGIAQLVGGIAAS 150
Lacbi2|456764|[L.bicolor]      LVVSAWLFFRVTGGLFNPNISFALLLVGGLKPLRFVLFCIAQLTGAIAGA 118
                               *.*..*:*:*::*.:*** :::. :*:*.: **: .::  **:.*.* .:

JF491353|[T.claveryi]          GMVELLLPGNLSVNTGLGGGINTAQGLFLEAFLTFELTLTIYMLAVEKHR 187
TmeAQP1|[T.melanosporum]       GLVEGLTPGQLAVGTALAKDVSIVQGLFIEVFLTAQLMITIFLVG-NLHR 199
Lacbi2|456764|[L.bicolor]      AIVRGLTSAPLSVNNVLQQGTSAAQGVFIEMFITAALVLSVLMLAAEKHE 168
                               .:*. * .. *:*.. *  . . .**:*:* *:*  * ::: ::. : *.

JF491353|[T.claveryi]          ATFMAPIGIGLALFIAHLCGIFFTGSSLNPARSFGPAVITRDFPTYHWIY 237
TmeAQP1|[T.melanosporum]       ATFIAPLGIGFSFFITQLFGVYFTGGSLNPARSLGPAVVTGDFPGYHWIY 249
Lacbi2|456764|[L.bicolor]      ATPFAPVGIGLTLFACHLFAVYYTGAAMNSARAFGPAVISGFPEPQHWVY 218
                               ** :**:***:::*  :* .:::**.::*.**::****::      **:*

JF491353|[T.claveryi]          WVGPCLGSVAATGFYKLLLLLEYKTANPGQDF--------DGLHHTGTGF 279
TmeAQP1|[T.melanosporum]       WVGPGLGAALGAAVYKFLLLVNYKTLNPGQDD--------DGLAVVRCEV 291
Lacbi2|456764|[L.bicolor]      WVGPFLGSLLGAGFYATLKHYKYWHLNPDQATSDYRKSPSDPVALLKSTA 268
                               **** **:  .:..*  *   :*   **.*          * :

JF491353|[T.claveryi]          EHSHASNSVSG--GTMSRDEIQAIKGS-ESV------------ 307
TmeAQP1|[T.melanosporum]       GRGGRSGETYDESQVVSGPSGIVPKGSDENV------------ 322
Lacbi2|456764|[L.bicolor]      ETFINVGDEETRNGCASNEEGVRATGDEKSSNATSSRTNFSPV 311
                                     ..        *  .    .*. :.
```

Note A2.2. CLUSTAL W 2.1 multiple sequence alignment shows conservation and variation of aligned amino acid residues among 23 fungal aquaglyceroporins in Cluster II. Fifty-three positions are well conserved among the aligned sequences, including 11 completely aligned consensus residues. Lacbi2:671860 in *L. bicolor* strain S238N and JQ585593 in strain UAMH8232 are 98% identical, with only 5 residue differentiation at underlined positions. The consensus symbol asterisk "*" indicates positions which have a single, fully conserved residue among the aligned sequences. The symbol colon ":" indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, and the period "." indicates conservation between groups of weakly similar properties - scoring ≤ 0.5. Colors indicate physicochemical properties of the residues. AVFPMILW are coded RED for small+ hydrophobic (including aromatic -Y). DE are coded BLUE for acidic. RK are coded MAGENTA for basic-H. STYHCNGQ are coded GREEN for Hydroxyl + sulfhydryl + amine + G. Others are coded Grey for Unusual amino/imino acids, etc (Larkin *et al*. 2007).

```
Lacbi2|671860|[L.bicolor]          ------------------------------------------------
JQ585593|[L.bicolor]               ------------------------------------------------
Lacbi2|482072|[L.bicolor]          ------------------------------------------------
Pospl1|127849|[P.placenta]         ------------------------------------------------
Wolco1|121322|[W.cocos]           ------------------------------------------------
Trave1|113714|[T.versicolor]       ------------------------------------------------
Trave1|43473|[T.versicolor]        ------------------------------------------------
Phchr|123853|[P.chrysosporium]    ------------------------------------------------
Sporo1|24618|[S.roseus]           MSELARHVASTHSATTARNRRGLLLSKVAHNCAVPQLGLVLALAAFTKAG 50
XP758316|[U.maydis]                ------------------------------------------------
Mycfi2|165933|[M.fijiensis]       ------------------------------------------------
Mycfi1|27060|[M.fijiensis]        ------------------------------------------------
Xanpa1|68791|[X.parietina]        ------------------------------------------------
Mycgr1|39363|[M.graminicola]      ------------------------------------------------
Thite2|2114473|[T.terrestris]     ------------------------------------------------
Trive1|66182|[T.virens]           ------------------------------------------------
XP369584|[M.oryzae]               ------------------------------------------------
Kwal20572|[K.waltii]              -------------------------MS--KKSLTD--------SQDGSQR 15
XP002553728|[L.thermotolerans]    -------------------------MS--KKSLPD--------SQDGNQR 15
CAA38096|[S.cerevisiae]           -------------------------MSNPQKALNDFLSSESVHTHDSSRK 25
AAQ01788|[K.lactis]               -------------------------MSQTAQYEPVKDAG-----ISNGDW 20
AAS47031|[K.marxianus]            -------------------------MSENTQYDNTRDSGGQSPVNNNNAW 25
NP983336|[A.gossypii]             ----------------------------------------------MSGKHK 6

Lacbi2|671860|[L.bicolor]          ------------------------------------------------
JQ585593|[L.bicolor]               ------------------------------------------------
Lacbi2|482072|[L.bicolor]          ------------------------------------------------
Pospl1|127849|[P.placenta]         ------------------------------------------------
Wolco1|121322|[W.cocos]           ------------------------------------------------
Trave1|113714|[T.versicolor]       ------------------------------------------------
Trave1|43473|[T.versicolor]        ------------------------------------------------
Phchr|123853|[P.chrysosporium]    ------------------------------------------------
Sporo1|24618|[S.roseus]           SLLFSGLVPADISSQKFSPPTSTPRAQFWYKKTIGTDSEPWSELGSSQFA 100
XP758316|[U.maydis]                ----------------MSQAATTP------------------------- 8
Mycfi2|165933|[M.fijiensis]       ------------------------------------------------
Mycfi1|27060|[M.fijiensis]        ------------------------------------------------
Xanpa1|68791|[X.parietina]        ------------------------------------------------
Mycgr1|39363|[M.graminicola]      ------------------------------------------------
Thite2|2114473|[T.terrestris]     ------------------------------------------------
Trive1|66182|[T.virens]           ------------------------------------------------
XP369584|[M.oryzae]               ------------------------------------------------
Kwal20572|[K.waltii]              KPQE--------------------YMNDYLAEAENTSNVSQDQQ---DSE 42
XP002553728|[L.thermotolerans]    KPQE--------------------YMNDFLSDAESGSNVSHDQQNGGDGG 45
CAA38096|[S.cerevisiae]           QSNKQSSDEGRSSSQPSHHHSGGTNNNNNNNNNNNNNSNNNNGNDGGNDD 75
AAQ01788|[K.lactis]               QNDD--------------------FANVNHRYPTGSVDGNESRISGEGGY 50
AAS47031|[K.marxianus]            QESG--------------------FAHTRPRRYTTRSSVSE-RQSGLSGL 54
NP983336|[A.gossypii]             TEQG--------------------FAQD--------------------L 15
```


Lacbi2|671860|[L.bicolor]
JQ585593|[L.bicolor]
Lacbi2|482072|[L.bicolor]
Pospl1|127849|[P.placenta]
Wolco1|121322|[W.cocos]
Trave1|113714|[T.versicolor]
Trave1|43473|[T.versicolor]
Phchr|123853|[P.chrysosporium]
Sporo1|24618|[S.roseus] LRRQGIFYRFREEERERSAAVPELELRHFRPPEHLQFALRSKEARLLGHV 150
XP758316|[U.maydis] VYIPADQLHNDVRDK 23
Mycfi2|165933|[M.fijiensis]
Mycfi1|27060|[M.fijiensis]
Xanpa1|68791|[X.parietina]
Mycgr1|39363|[M.graminicola]
Thite2|2114473|[T.terrestris]
Trive1|66182|[T.virens]
XP369584|[M.oryzae]
Kwal20572|[K.waltii] EHYVPSRDFAPQHRIS-ASASYRPR---GNSNSGYAVQQVIPNTHMGMGR 88
XP002553728|[L.thermotolerans] EHYIPSRDFAPQHRIS-ASASYVPRNGEGGGGSNYAVQQVVPNTHIPMSR 94
CAA38096|[S.cerevisiae] DYDYEMQDYRPSPQSARPTPTYVPQYS-VESGTAFPIQEVIPSAYINTQD 124
AAQ01788|[K.lactis] DDDNDSADDGATVPVTAYVQQYLDE------GSYFPVQEVVPNTSLNMNN 94
AAS47031|[K.marxianus] EEEDSDIDXSDNVPVTAYVQQYLDE------GSYFPVQEVVPNTSLNMNM 98
NP983336|[A.gossypii] EQQGPPHHGHRSEYVTG---EFVES------GAPFVMQEVVANSGAVVSN 56
Lacbi2|671860|[L.bicolor]
JQ585593|[L.bicolor]
Lacbi2|482072|[L.bicolor]
Pospl1|127849|[P.placenta]
Wolco1|121322|[W.cocos]
Trave1|113714|[T.versicolor]
Trave1|43473|[T.versicolor]
Phchr|123853|[P.chrysosporium]
Sporo1|24618|[S.roseus] SREMLTRFPNRNRVPATDGGQQFIDSGSFPLSLPDRDLFLDHALPTDSFT 200
XP758316|[U.maydis] DGN SASSHH 32
Mycfi2|165933|[M.fijiensis]
Mycfi1|27060|[M.fijiensis]
Xanpa1|68791|[X.parietina]
Mycgr1|39363|[M.graminicola]
Thite2|2114473|[T.terrestris]
Trive1|66182|[T.virens]
XP369584|[M.oryzae]
Kwal20572|[K.waltii] SGSGTS-SGG---------HSTSYRNRAQSGVSSNHMNLRSVHSTTNSTQ 128
XP002553728|[L.thermotolerans] SNSGNNGSGGGGGGGGGSGHSTGYRNRAQSGVSSNYMNMRSVQSNSGSAQ 144
CAA38096|[S.cerevisiae] INHKDNGPPS-------ASSNRAFRPRGQTTVSANVLNIEDFYKNADDAH 167
AAQ01788|[K.lactis] YRRIRSNTVT---------SNVMPPRPTEG--PGSVMSRSTTGPNQNSQ- 132
AAS47031|[K.marxianus] YRRKRGNTVT---------SNVIASRPMEANYTGSVSSPDPALQNQNNEG 139
NP983336|[A.gossypii] LRQQE----------LAN----------ARAKPHGEHVNER- 77
Lacbi2|671860|[L.bicolor]
JQ585593|[L.bicolor]
Lacbi2|482072|[L.bicolor]
Pospl1|127849|[P.placenta]
Wolco1|121322|[W.cocos] MHAV 4
Trave1|113714|[T.versicolor]
Trave1|43473|[T.versicolor] MTAAAGT 7
Phchr|123853|[P.chrysosporium]
Sporo1|24618|[S.roseus] FLRSSFSSSPSFLPISLFSSTLRNFFTAMQAQRDHLNRNAGDVEVGVADL 250
XP758316|[U.maydis] FQR AHRKNGELELGVTEV 50
Mycfi2|165933|[M.fijiensis] MSDNSHMERLQTLDLE 16
Mycfi1|27060|[M.fijiensis] MERLQTLDLE 10
Xanpa1|68791|[X.parietina] MAGDEMHRLQTHDLE 15
Mycgr1|39363|[M.graminicola] MADGSRIERLHTHDLE 16
Thite2|2114473|[T.terrestris]
Trive1|66182|[T.virens] MAPTEQLNRLHTHDLE 16
XP369584|[M.oryzae] MSFTAAHKYPSPHDHHQVD 19
Kwal20572|[K.waltii] DVHQPEAS DDPRENDVP 145
XP002553728|[L.thermotolerans] NVNQPEAS ADPRDNDIP 161
CAA38096|[S.cerevisiae] TIPESHLSRRRSRSRATSNAGHSANTGATNGRTTGAQTNMESNESPRNVP 217
AAQ01788|[K.lactis] --TAADPND PSNVNGAVT 148
AAS47031|[K.marxianus] GVPANDPND PNNVNNAIT 157
NP983336|[A.gossypii] -----DYDD LMSAS---- 86

```
Lacbi2|671860|[L.bicolor]          --MSATPIIHLRDVKKRTGVLNAWERVRNKPQVHWAMECFAEALGVFFYV 48
JQ585593|[L.bicolor]               --MSATPIIHLRDVKKRTGVLNAWERVRNKPQVHWAMECFAEALGVFFYV 48
Lacbi2|482072|[L.bicolor]          --MSNAPLVHLSDLQKRLRVFAVWEKVRNDGKVHWAIECFAEMFGVFLYV 48
Pospl1|127849|[P.placenta]         ----------------------MANWERRR-HGHARWFVEFTAEAMGTFLYT 29
Wolco1|121322|[W.cocos]            SVSSKSAVVHLSDIKRQPQIYARLERYR-RGNARYLIEFIAEATATFFYT 53
Trave1|113714|[T.versicolor]       -----MQHTAVYEVVPRPAVVAHWERRRNRRQVHWVVECIAEATGTFLYT 45
Trave1|43473|[T.versicolor]        HHLRQREFVHLADIKPRSSGHIAWERRR-HRQAHWLVECLAEFMGVFFYV 56
Phchr|123853|[P.chrysosporium]     --------------------------------------------------
Sporo1|24618|[S.roseus]           LRSNQVAEGTHQAAHLPAPPKWL--TTWERRRPPLLVECFAEFLGVGIYV 298
XP758316|[U.maydis]                LQG-RTDVPSAQVAQSAGLSNWSGRVRLEQSMPKLVPALIAEFMGTMFYC 99
Mycfi2|165933|[M.fijiensis]        LGATEAVVQHYARHVVATKPVSQRKLNFERSRPRWLRECMAEATGVFFYV 66
Mycfi1|27060|[M.fijiensis]         LGATEAVVQHYARHVVATKPVSQRKLNFERSRPRWLRECMAEATGVFFYV 60
Xanpa1|68791|[X.parietina]         IGATEAVQRHYSRHVVATKPVSQRKLDFEHSRPRWLRECMAEATGVFYYV 65
Mycgr1|39363|[M.graminicola]       LGATEVLQKHVSRHVVAEKRVSQRKLDFERSRPRWLRECIAEATGVFFYV 66
Thite2|2114473|[T.terrestris]      ----------------------------------MAEATGVFFYV 11
Trive1|66182|[T.virens]            LGTTDAVQKHITRNVVPPRRVSQRRLDFEHRRPRWVRECIAEATGVFMYV 66
XP369584|[M.oryzae]                QVVHSILDHHVENQLPPYPTISTRRLAFERHRPLWLRECIAEATGVFFFV 69
Kwal20572|[K.waltii]               MMVKPKTLYQNPQTPTVLPSTYHPINTWSTLKQTYLKEFLAEFMGTLVMM 195
XP002553728|[L.thermotolerans]     LMVKPKTLYQNPQTPTVLPSTYHPINTWSTLKQTYLKEFLAEFMGTMVMI 211
CAA38096|[S.cerevisiae]            IMVKPKTLYQNPQTPTVLPSTYHPINKWSSVKNTYLKEFLAEFMGTMVMI 267
AAQ01788|[K.lactis]                MMVKPKTLYQNPQTPTVLPSTYYPINKWSSFKYQHMKEFFGEFLGTMIMM 198
AAS47031|[K.marxianus]             MMVKPKTLYQNPQTPTVLPSTYYPINKWSSFKYQHMKEFFGEFLGTMIMM 207
NP983336|[A.gossypii]              MVVKPKPLHQNPQTPTVLPSNYQPINAWSQFKATYLREFFAEFLGTMVLV 136

Lacbi2|671860|[L.bicolor]          YFGLGSTAAWVIG--------------------------NILKQSG---- 68
JQ585593|[L.bicolor]               YFGLGSTAAWVIG--------------------------NILKQSG---- 68
Lacbi2|482072|[L.bicolor]          YFGLGSTAGWVIG--------------------------NIIKETN---- 68
Pospl1|127849|[P.placenta]         FAGVGSTAGWVLG--------------------------NILGLPS---- 49
Wolco1|121322|[W.cocos]            FAGGGSTASYVFG--------------------------NLLGLPN---- 73
Trave1|113714|[T.versicolor]       FAGAGATAAYVLG--------------------------NILELPG---- 65
Trave1|43473|[T.versicolor]        YAGVGSTASYLLA--------------------------NTAQLNG---- 76
Phchr|123853|[P.chrysosporium]     -----------MA--------------------------NQPD------- 6
Sporo1|24618|[S.roseus]           FAGVGASATLLIT--------------------------TAAKESG---- 318
XP758316|[U.maydis]                LAGEMATAGVLVT--------------------------TYAG-SP---- 118
Mycfi2|165933|[M.fijiensis]        FPGIAAVASMVLN--------------------------KANPA------ 84
Mycfi1|27060|[M.fijiensis]         FPGIAAVASMVLN--------------------------KANPA------ 78
Xanpa1|68791|[X.parietina]         FPGIAAIASFTLN--------------------------LENEAGVAA-- 87
Mycgr1|39363|[M.graminicola]       FPGIAAITAFTIN--------------------------KEDAA------ 84
Thite2|2114473|[T.terrestris]      LPGIASVANFTLS--------------------------SASPTLVPLGV 35
Trive1|66182|[T.virens]            LPGIGAIASFTVN--------------------------ATNPVG----S 86
XP369584|[M.oryzae]                FAGLASIATFTLH--------------------------HATDKNS---V 90
Kwal20572|[K.waltii]               FFGCTVVCQVRSG---QQQQRVTFLKQLAGS-TEVPDENKIAMLQYLMPV 241
XP002553728|[L.thermotolerans]     FFGCTVVCQVRSG---QQQQRVTFLKELSAS-TQVPAENKIELLQYLMPV 257
CAA38096|[S.cerevisiae]            IFGSAVVCQVNVAGKIQQDNFNVALDNLNVTGSSAETIDAMKSLTSLVSS 317
AAQ01788|[K.lactis]                MFGTAVNCQRKLS---QQNQINKFNQIIQLN---NMESDQIAMLQYLATP 242
AAS47031|[K.marxianus]             MFGTAVVCQSKLS---EQDKINQFNQILAMN---HKSNDDISMLQYIATP 251
NP983336|[A.gossypii]              FFGDSVVVQTRMS---STARVTAFLGQLESN---GLSGSPVEYMRHLVTP 180

Lacbi2|671860|[L.bicolor]          --LSSVFQIGFAYAFGILFAIGVCA--ATSGGHFNPCVTIAFTIFR---G 111
JQ585593|[L.bicolor]               --LSSVFQIGFAYAFGILFAIGVCA--ATSGGHFNPCVTIAFTIFR---G 111
Lacbi2|482072|[L.bicolor]          --LSSILQIGLAYAFGIWFAIGLCS--SSSGGHFNPCVTLSFVVFK---G 111
Pospl1|127849|[P.placenta]         --ISSLFQIGVAYAIGIVLALTICL--PASKGHVNPAFTVYALVRG---H 92
Wolco1|121322|[W.cocos]            --LGSLFQVGVAYAIGIVMALAVCL--PVSNGHANPAFTIYAVIHG---H 116
Trave1|113714|[T.versicolor]       --LGSLLQIGIAYAVGIALALAVCL--PTSYGQFNPAITIHAAVFH---K 108
Trave1|43473|[T.versicolor]        --LGSLFQIGTAYAFGILFALIVCA--PTSGGHFNPAMTIAFTLMG---R 119
Phchr|123853|[P.chrysosporium]     ---AAVLQIGFAYAFGILFALITCG--STSGGHFNPAVTICQVLYRGFPV 51
Sporo1|24618|[S.roseus]           --FGSLFTVGFAYALGIAFAIVCTA--GTSGGHLSPAFTIAFCLFK---G 361
XP758316|[U.maydis]                --QGNLTMIGFAYAFGITFAIIVCA--TTSGGQFHPAFTIAQVVFK---G 161
Mycfi2|165933|[M.fijiensis]        --YGSFFEVGWAFALGIAFAIITCA--PTSGGHFNPAITICFAVWQ---G 127
Mycfi1|27060|[M.fijiensis]         --YGSFFEVGWAFALGIAFAIITCA--PTSGGHFNPAITICFAVWQ---G 121
Xanpa1|68791|[X.parietina]         --FGSLFQIGWAFAIGIAFAIITCA--PTSGGHFNPAITICFAIWQ---G 130
Mycgr1|39363|[M.graminicola]       --FGSLFSVGMAFAFGIAFAIITCG--PTSGGHFNPAVTISLAIYQ---G 127
Thite2|2114473|[T.terrestris]      ATFSSLFQIGWAFALGIAFAIITCA--PTSGGHFNPAITLSLAIWQ---G 80
Trive1|66182|[T.virens]            TAFGSLFSIGFAFALGIAFAIITCA--PTSGGHFSPAVTICLCIWQ---G 131
XP369584|[M.oryzae]                AGIGSIFQVGWGMGMGVALAIITCA--PTSGGHFNPAITICLAIWQ---H 135
Kwal20572|[K.waltii]               DITGTFDDIALIWGGAVVMGYFAAGGSALSGGHLNPALTLSNCVFR---G 288
XP002553728|[L.thermotolerans]     DQFGTYDDVALIWGGAVVMGYFAAGGSALSGGHMNPALTLSNCVFR---G 304
CAA38096|[S.cerevisiae]            VAGGTFDDVALGWAAAVVMGYFCAGGSAISGAHLNPSITLANLVYR---G 364
AAQ01788|[K.lactis]                DVAGNFATVAFGWAAAVVMGYFAAGGSAISGAHLNPAITVSNFVYR---G 289
AAS47031|[K.marxianus]             NVAGNFVSIAFGWAGAVVMGYFAAGGSAISGAHLNPAITVSNFIYR---G 298
NP983336|[A.gossypii]              DVAGSSISVNLCWASGVVMGYYAAGGPAITGAHMNPAVTLANYCFR---G 227
```

```
                                                .  :   . .: :.        : .:  *..*:

Lacbi2|671860|[L.bicolor]          FPPLKAVRYIVAQILGAYIASALVYNQWKVLIVESELLLKQAGVYETTMF 161
JQ585593|[L.bicolor]               FPPLKAVRYIVAQILGAYIASALVYNQWKVLIVESELLLKQAGVYETTMF 161
Lacbi2|482072|[L.bicolor]          FPKLKACRYIIAQILGAYIASALVYSQWNVLIEECTLGLIKAKAYDTTMF 161
Pospl1|127849|[P.placenta]         CTPQRALMLIVAQILGAYIACLLIYAQYHTIIQEATEALMAKGVYDEIMF 142
Wolco1|121322|[W.cocos]            CTSAKGLRLIVAQIFGAYIACLLIYAQYHNLFKEAEAALVAKGVYNELMF 166
Trave1|113714|[T.versicolor]       LPPLKAVRYIIAQIFGSYIACLLIYVQYKQLIHTAVSALQAKGVYDTVMF 158
Trave1|43473|[T.versicolor]        CTWKKALRYFVAQILGAYVACLLIYVQWHDLIEEASEILAAAGKLDAVMF 169
Phchr|123853|[P.chrysosporium]     YIDASRPRYIVAQILGAFLAALFVYVQWRVNIRATEAALLAEGKWDSVMF 101
Sporo1|24618|[S.roseus]            FPWRKAPYYIVSQILGAMVGALIVAGIFHQQLTEVTAGFRALGLGDTGIF 411
XP758316|[U.maydis]                FPIKLAPLYICAQVVGAMVASLIVVGSWHDELMKITHLLKATGKTAT-IF 210
Mycfi2|165933|[M.fijiensis]        FPWKKVPSYIFSQIFGAFIAGLFLMGLYHQQLSAFQAELRAA---GESSV 174
Mycfi1|27060|[M.fijiensis]         FPWKKVPSYIFSQIFGAFIAGLFLMGLYHQQLSAFQAELRAA---GESSV 168
Xanpa1|68791|[X.parietina]         FPWKKVPHYIFSQIFGAFVAGLLLVGMYWPEIQAFKAESIAA---GKGLV 177
Mycgr1|39363|[M.graminicola]       FPWKKVPYYIFSQVFGSFMAGLLLMGMYHEQIQSYTAGLLAD---GGREV 174
Thite2|2114473|[T.terrestris]      FPWRKVPYYIFSQIFGAFVAGLVLMGMYWTQISEMKAAFIEA---GKPLV 127
Trive1|66182|[T.virens]            FPLKKVPHYILSQLFGGFIAALVLMGIYHQQLEEMKEVLLAA---GKPLV 178
XP369584|[M.oryzae]                FPWRKVPRYIISQIFGAFLAALTIMVLYWEQIQDFAATRRAA---GLPLV 182
Kwal20572|[K.waltii]               FPWRKVPVYWAGQLLGAFCGALIVFIYYKPVIVNVYPDWNGN-------- 330
XP002553728|[L.thermotolerans]     FPWRKVPVYWAGQLLGAYCGALIVFAYYMPVIKYVYPDWNGN-------- 346
CAA38096|[S.cerevisiae]            FPLKKVPYYFAGQLIGAFTGALILFIWYKRVLQEAYSDWWMN-------- 406
AAQ01788|[K.lactis]                FPWRKLGVYFMGQYLGSYIGTLLILWYYREVIEHVYPNWHLE-------- 331
AAS47031|[K.marxianus]             FPFRKLGFYFMGQYVGSYLGSLLMIWYYHKVIAHVYPNWPQE-------- 340
NP983336|[A.gossypii]              LPAVKVLIYWAAQMLGGYMGGLTVFWYYARVIKTTFPDWKTN-------- 269
                                             .* .*.  .   :   :   :

Lacbi2|671860|[L.bicolor]          TPNGPAGIFALYLLPGAQTLPRAFLNEFVNCFVLALVIWAALDPTSFMIP 211
JQ585593|[L.bicolor]               TPNGPAGIFALYLLPGAQTLPRAFLNEFVNCFVLALVIWAALDPTSFMIP 211
Lacbi2|482072|[L.bicolor]          TPNGPAGIFALYLVPGAQSVPRALLNEFVNSTLIGMIIWAALDPTNMMVP 211
Pospl1|127849|[P.placenta]         TSQGPGGIFGLYATPGAS-LGNIFVNEFVCDFILAVCVFGAIEPTNPFSP 191
Wolco1|121322|[W.cocos]            TTQGPAGVIGLYATPGAN-LGFIFLNEFICDFVLALAIFAAIEPTNAFMP 215
Trave1|113714|[T.versicolor]       TSAGPGGIFGLYVNPGSD-LGYVLLNEFVCDFILGIVIFSCTDPGNPIST 207
Trave1|43473|[T.versicolor]        TANGPAGVFALYVPPGTN-LGRVFLNEFVCDFIIGLVIWSCMDPTNFGAS 218
Phchr|123853|[P.chrysosporium]     TPQGLAGIFALYAPAGSN-LGQVLLNEFVCDFLIGLVIWSCMDPTNFLVP 150
Sporo1|24618|[S.roseus]            SAQGPAGLFGLMPAAGQE-LKWAFFNEFVANIFLAILVFSVLDACNFFVS 460
XP758316|[U.maydis]                TAEGPAGAIALFPTPGRS-MGSIFVNEFFANILVGMIVWANLDAANPFTG 259
Mycfi2|165933|[M.fijiensis]        PT--MSSILCAYPLPNQTNLGYLFLIEFFVDSYIGIIIWACLDPANPFIT 222
Mycfi1|27060|[M.fijiensis]         PT--MSSILCAYPLPNQTNLGYLFLIEFFVDSYIGIIIWACLDPANPFIT 216
Xanpa1|68791|[X.parietina]         YNGGPASILCTFPNPNQTNLGYLFLTEFFVDSFIGLIIWANLDPANPFVS 227
Mycgr1|39363|[M.graminicola]       MNGGPASFLVSFPTEDQQNLGYLFLIEFFVCSYLGIVIWAVLDPANPFVS 224
Thite2|2114473|[T.terrestris]      ANGAPASILCSFPNPDQTNLGYVFLIEFFVDAFLAVVIWACLDPANPFVS 177
Trive1|66182|[T.virens]            ANGAPASVLCSFPNPGQS-MGYVFMTEFFCDCFVGLIIWACLDPANPFVS 227
XP369584|[M.oryzae]                GSHTPASIFCSYPHPDQN-LGFVLTIEFVADAFIALVVWAALDPSNPFIH 231
Kwal20572|[K.waltii]               --ETVLSMFCTYPQEYLS-SSRQFVSELICSAVLQIGIFALTDPYTCLRS 377
XP002553728|[L.thermotolerans]     --ETVLGMFCTSPLQYLD-SSRQFISELICSAVLQVGIFALTDPYTCLRS 393
CAA38096|[S.cerevisiae]            --ESVAGMFCVFPKPYLS-SGRQFFSEFLCGAMLQAGTFALTDPYTCLSS 453
AAQ01788|[K.lactis]                --ESVLAMFSVVPLDYLS-TSRQIIAEFLIGAMLQCGIFSLTDPYTCLST 378
AAS47031|[K.marxianus]             --ESVVAMFSVVPLDYLS-TPRQIIAEFVIGAMLQCGIFSLTDPYTCLST 387
NP983336|[A.gossypii]              --ESVIGCFSTVPLPYLD-SNRQFISEFVIGALLIGLIFALTDPYTCLTT 316
                                         . :             :  *:.    :    :.  :. .

Lacbi2|671860|[L.bicolor]          PVMAPFIIAAAYAGSIWGYAVPAISLNSARDIGCRLFALTIWGKSAAGGS 261
JQ585593|[L.bicolor]               PVMAPFIIAAAYAGSIWGYAVPAISLNSARDIGCRLFALTIWGKSAAGGS 261
Lacbi2|482072|[L.bicolor]          PAMGPLFISLAYAAVIWGFATPAVALNTARDLGARLFAMSIWGTKAAGSG 261
Pospl1|127849|[P.placenta]         PTMAPWIIAFTYAIVIWGYAPVGLAANSARDVGGRLAALTLWGLPASGGR 241
Wolco1|121322|[W.cocos]            PAAAPWFIGFIYAVVIWGYAPVGLAANSARDVGGRLAALTLWGARASGGR 265
Trave1|113714|[T.versicolor]       PSTMPWLIALAYGVVVWGYSPIGLAANSARDLGGRFAALTLFGKAASGGN 257
Trave1|43473|[T.versicolor]        PVSAPWIVAFAYAVVVWGYSPVGVATNAARDVGSRLMAMTIWGMPASGGS 268
Phchr|123853|[P.chrysosporium]     PAAGPWIVSFSYSMCIWGYSPVGLSTNTARDLGTRLAVMAIWGTPAAGGS 200
Sporo1|24618|[S.roseus]            LATAPFVIGMGYAMIIWAFSINSVALNNARDMGGRMACGIIYGSKCWTQH 510
XP758316|[U.maydis]                PQAAPYTIGLGFAVVVWCFSSSNVVANSARDIGARLVCSMFWGSECFPSR 309
Mycfi2|165933|[M.fijiensis]        GASAPFVIGLAYAAMVWGFAPITISTNLARDLGTRIVAAIFYGGEAFSYH 272
Mycfi1|27060|[M.fijiensis]         GASAPFVIGLAYAAMVWGFAPITISTNLARDLGTRIVAAIFYGGEAFSYH 266
Xanpa1|68791|[X.parietina]         PTSAPFAIGLAYATMVWGFADIAISTNLARDLGTRIVAAIFFGGESFTYL 277
Mycgr1|39363|[M.graminicola]       PAGAPFVIGLAYATMVWAFADITIGTNMARDLGARLVALIFYGREAFTYR 274
Thite2|2114473|[T.terrestris]      PAGAPFAIGLAYGVMVWGFANVTISTNLARDLGTRIVAAIFFGREAFSYM 227
Trive1|66182|[T.virens]            PSLAPLLIGLAYGAMAWAFGANTLTMNMARDFGPRVVAAIFYGREAFSYM 277
XP369584|[M.oryzae]                PAAIPFIIGIAYADMIWGFGGITLSTNLARDLGARIVAGILFGGEAFTYH 281
Kwal20572|[K.waltii]               DLFPLMLFVLIFCLIGATSLQTGAGLNPARDLGPRLALASIGFDSRALWK 427
XP002553728|[L.thermotolerans]     DLFPLMLFVLIFCLIGATALQTGAGLNPARDLGPRLALATVGFESDTLWR 443
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CAA38096|[S.cerevisiae]          DVFPLMMFILIFIINASMAYQTGTAMNLARDLGPRLALYAVGFDHKMLWV 503
AAQ01788|[K.lactis]              DLFPMMLFILMFILNAAGAYQTGAVLNPARDMGPRLALLTIGMDKDVIFN 428
AAS47031|[K.marxianus]           DLFPVMLFILIFSLNASGAYQTGAVLNPARDMGPRLALWTIGMDKDVILI 437
NP983336|[A.gossypii]            DFFPIMLFLLIFSLLACGSYQTGAILNPARDIGPRLAMWTVGFSRKALWE 366
                                        .   :              * ***.* *.    .

Lacbi2|671860|[L.bicolor]        ----YSAITALVNIPATLLAAVVYELFLVDSDR----------------- 290
JQ585593|[L.bicolor]             ----YSAIAALVNIPATLLAAVVYELFLVDSDR----------------- 290
Lacbi2|482072|[L.bicolor]        ----YSAIACLINIPATLLGVFLYEVFFTDSDR----------------- 290
Pospl1|127849|[P.placenta]      ----YAAIAALTNIPATLLAGVFYEFVLNDSNR----------------- 270
Wolco1|121322|[W.cocos]         ----YAAIAALTNIPATLLAGVFYELVFNDPDR----------------- 294
Trave1|113714|[T.versicolor]    ----YAALAALTNIPATLLAGVFYELVFSDSTRSAY-------------- 289
Trave1|43473|[T.versicolor]     ----YAAIAALTNIPATLLAAVFYEVVLADSSR----------------- 297
Phchr|123853|[P.chrysosporium]  ----YAAIAALTNIPATLLAATFHEFFLADSSRGAF------------- 232
Sporo1|24618|[S.roseus]         SG--YTAIAALTTFPGTIVGAAIQTLLLSDSAR----------------- 541
XP758316|[U.maydis]              ----YSALAALTNIPATLLGVGLYTFFLSDTRR----------------- 338
Mycfi2|165933|[M.fijiensis]     E---YSWIAILVNVPATLFATGYYEFLMRDSLA----------------- 302
Mycfi1|27060|[M.fijiensis]      E---YSWIAILVNVPATLFATGYYEFLMRDSLA----------------- 296
Xanpa1|68791|[X.parietina]      N---YSWIAILVNIPATIFATGYYEFLMRDSLG----------------- 307
Mycgr1|39363|[M.graminicola]    S---YSWIPILVNIPATVLAASFYELIMRDSLQ----------------- 304
Thite2|2114473|[T.terrestris]   T---YSPISILVNIPATLFATAYYEIVLKDSFM----------------- 257
Trive1|66182|[T.virens]         N---YAAIGIFTSIPATLISSAFYEFVMRDSLS----------------- 307
XP369584|[M.oryzae]             G---YAPIGMLVNIPATLFGTAIYEFAFRDSLM----------------- 311
Kwal20572|[K.waltii]            AHHHYFWVPIVAPFVGTLLGGTIYDICIYQGHESPLNWPYTLLKGKFKRA 477
XP002553728|[L.thermotolerans]  AHHHYFWVPIVAPFVGTLLGGTVYDICIYQGHESPLNWPYSLVKHKARRA 493
CAA38096|[S.cerevisiae]         HHHHFFWVPMVGPFIGALMGGLVYDVCIYQGHESPVNWSLPVYKEMIMRA 553
AAQ01788|[K.lactis]             THHHFFWVPMVVPFVGSFTGGLVYDFCIYQGHESPLNLPLSAYTDWFRRH 478
AAS47031|[K.marxianus]          PIITSFGSQWLFHSLAVSAGGLVYDFCIYQGHESPLNLPLSAYTDWFRRQ 487
NP983336|[A.gossypii]           DHHHYFWVALVGPCVGGLVGALIYDLLIFQGHESPVNQPAAHVLKRLKTR 416
                                          .    .    .     . : :

Lacbi2|671860|[L.bicolor]        ----------------------------------------VVAGSHLEFMN 301
JQ585593|[L.bicolor]             ----------------------------------------VVAGSHLEFMN 301
Lacbi2|482072|[L.bicolor]        ----------------------------------------VVSPAALTIMN 301
Pospl1|127849|[P.placenta]      ----------------------------------------TLTPAYLEVAA 281
Wolco1|121322|[W.cocos]         ----------------------------------------TVTSAHLELTN 305
Trave1|113714|[T.versicolor]    ----------------------------------------PSSASATAHTLT 301
Trave1|43473|[T.versicolor]     ----------------------------------------VVTPAHVDYLS 308
Phchr|123853|[P.chrysosporium]  ----------------------------------------PPLGRRSCSFAD 244
Sporo1|24618|[S.roseus]         ----------------------------------------MIVNAPPSHSA 552
XP758316|[U.maydis]              ----------------------------------------PPATVALNHLH 349
Mycfi2|165933|[M.fijiensis]     ----------------------------------------KIGKGAARHEH 313
Mycfi1|27060|[M.fijiensis]      ----------------------------------------KIGKGAARHEH 307
Xanpa1|68791|[X.parietina]      ----------------------------------------KIAKGHAVHEH 318
Mycgr1|39363|[M.graminicola]    ----------------------------------------VIGKGAALHED 315
Thite2|2114473|[T.terrestris]   ----------------------------------------IIAKGHAQHRD 268
Trive1|66182|[T.virens]         ----------------------------------------VIGTGHAVHAD 318
XP369584|[M.oryzae]             ----------------------------------------IVGKGHANAEG 322
Kwal20572|[K.waltii]            WRQRPRFY-RKRAGSDAAVSDWEYDNESNG-ASARDSANDDTPKTGFFQD 525
XP002553728|[L.thermotolerans]  WSNRPRFY-RKRAGSDSAVSDWEYDNESNN-ASARDTDNEDTPKTGFFPE 541
CAA38096|[S.cerevisiae]         WFRRPGWKKRNRARRTSDLSDFSYNNDDDEEFGERMALQKTKTKSSISDN 603
AAQ01788|[K.lactis]             WEL-------LKVKTSSGFVGSDLETIGTN----------NTTSNVESHR 511
AAS47031|[K.marxianus]          WDT-------IKLKTSSGLKGTDLETIDSG----------HTLSHIESHR 520
NP983336|[A.gossypii]           FTS-------FGRKTAS--TG---EYTFSD----------KELTDVSSNN 444

Lacbi2|671860|[L.bicolor]        V----AANHRRHRHQ-----------AEDDNHGDADDSSQEKPV----- 330
JQ585593|[L.bicolor]             V----AANHRRHRQQ-----------AEDDNLVEADDSSQEKPV----- 330
Lacbi2|482072|[L.bicolor]        A----HANHRRLHHG-----------HGEADKRDSTEKPTITTYEHAGG 335
Pospl1|127849|[P.placenta]      A----EKAHEERVQGV----------VPSDASLSSGDSKARVLPQ----- 312
Wolco1|121322|[W.cocos]         G----ARAYEARCRG-----------GDSAESVESETFKQAP------ 332
Trave1|113714|[T.versicolor]    G----SRVQRFTPSGSGSSTR----GMPSSSAKSTGPRRRRSMSLTPP-- 341
Trave1|43473|[T.versicolor]     G----HLAHEEHSQG----------IVRGGSVSPGLDEKSREQTIEHV-- 342
Phchr|123853|[P.chrysosporium]  SRICSDHAAACRRDGRPPCACGAQRHRRTRAVVHCRASPARPARLAVAYI 294
Sporo1|24618|[S.roseus]         EVDMINESRGFQIP--------SRAVTRESVYNHPHPEKSGSSV------ 588
XP758316|[U.maydis]              EEHVRSIERAETLHAEILDEKIAQTLSRGGDATALQKQRTNLGVKNF--- 396
Mycfi2|165933|[M.fijiensis]     GEEGLGLHLTKSGISR----------VRTEGNFKPGSSSTSEHEKV---- 349
Mycfi1|27060|[M.fijiensis]      GEEGLGLHLTKSGISR----------VRTEGNFKPGSSSTSEHEKV---- 343
Xanpa1|68791|[X.parietina]      GEEGLRRHITNTGHIE----------TGAANALRSNQSGSDEYNLGKQA- 357
Mycgr1|39363|[M.graminicola]    GEEGLTRHLTSTGMIR----------ATGDVYFSNESDDSKRKN------ 349
Thite2|2114473|[T.terrestris]   GDTGLVRHLSKVGMIEN-------DEPGTDGGAAQTSAMSSDGVPVKR-- 309
Trive1|66182|[T.virens]         GDEALVRHITRTTTVE---------------GMEERSGEYKS-------- 345
XP369584|[M.oryzae]             GDAALYAYFKKAKLID-----------------EEQGIKA--------- 345
Kwal20572|[K.waltii]            SDPQIQKQVQFKSVSKNFNGKRNPVSGIPTIFEEGDDDEEEENGADEGEN 575
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XP002553728|[L.thermotolerans]      SDPQIQKHVQFKSMSKNFNGKRNPVSGIPTIFEEGDDDDVDD--ADEAAS 589
CAA38096|[S.cerevisiae]             ENEAGEKKVQFKSVQR---GKR-TFGGIPTILEEEDSIETRSLGATTTDS 649
AAQ01788|[K.lactis]                 SQTSENKQVHFKSVLRNS-KTRNPSTGIPTIFESEETTYSRPNFIQKHSD 560
AAS47031|[K.marxianus]              SQLSENKQVHFKSVLRNS-KLRNPSTGVPTIFESEETTYSRPNFEQKTSN 569
NP983336|[A.gossypii]               ---ASGKNINFRSVTRG-----ESTNGVPTIY-------SQSNDPKK--- 476


Lacbi2|671860|[L.bicolor]           -------------------------
JQ585593|[L.bicolor]                -------------------------
Lacbi2|482072|[L.bicolor]          NGVEVSHV----------------- 343
Pospl1|127849|[P.placenta]          -------------------------
Wolco1|121322|[W.cocos]             -------------------------
Trave1|113714|[T.versicolor]        -------------------------
Trave1|43473|[T.versicolor]         -------------------------
Phchr|123853|[P.chrysosporium]     QER---------------------- 297
Sporo1|24618|[S.roseus]            -------------------------
XP758316|[U.maydis]                 -------------------------
Mycfi2|165933|[M.fijiensis]        -------------------------
Mycfi1|27060|[M.fijiensis]         -------------------------
Xanpa1|68791|[X.parietina]         -------------------------
Mycgr1|39363|[M.graminicola]       -------------------------
Thite2|2114473|[T.terrestris]      -------------------------
Trive1|66182|[T.virens]            -------------------------
XP369584|[M.oryzae]                -------------------------
Kwal20572|[K.waltii]               DNVTEKRPPLASKKTKSSDKKNKRY 600
XP002553728|[L.thermotolerans]     GNETEKRPPLKPKSSKSFDKKGKKQ 614
CAA38096|[S.cerevisiae]            IGLSDTS----SEDSHYGNAKKVT- 669
AAQ01788|[K.lactis]                RSAS--------------------- 564
AAS47031|[K.marxianus]             GSI---------------------- 572
NP983336|[A.gossypii]              -------------------------
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Note A2.3. CLUSTAL W 2.1 multiple sequence alignment shows conservation and variation of aligned amino acid residues between five mycorrhizal fungal MIPs in Cluster III. The similarity score between Lacbi2:576801 of Basidiomycota ECM *Laccaria bicolor* and JQ412060 of Glomeromycota AM *Glomus intraradices* is 42. The lowest score is 35, between Lacbi2:443240 and JQ412060; whereas the highest is 89, between Lacbi2:317173 and Lacbi2:576801. The consensus symbol asterisk "*" indicates positions which have a single, fully conserved residue among the aligned sequences. The symbol colon ":" indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, and the period "." indicates conservation between groups of weakly similar properties - scoring ≤ 0.5. Colors indicate physicochemical properties of the residues. AVFPMILW are coded RED for small + hydrophobic (including aromatic -Y). DE are coded BLUE for acidic. RK are coded MAGENTA for basic-H. STYHCNGQ are coded GREEN for Hydroxyl + sulfhydryl + amine + G. Others are coded Grey for Unusual amino/imino acids, etc (Larkin *et al*. 2007).

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Lacbi2|317173|[L.bicolor]      MSGQHQITEQSSRNPLSRVSTLLPEKPLSPTSTYAGTQKHPEAPRQSSFL 50
Lacbi2|576801|[L.bicolor]      ---------------------------------------------------M 1
Lacbi2|568479|[L.bicolor]      ----------------------------------------------------MK 2
Lacbi2|443240|[L.bicolor]      ------MDDKFDDDALP----------NSKTTPEDYGDKLAEYDYTNTFP 34
JQ412060|[G.intraradices]      ------MADERGPINKSGPSSTYGATENNGESGGTRG-APATEDVIVIQD 43

Lacbi2|317173|[L.bicolor]      IQLQNIRNAIRKPMAEFFGVALLIIFGAGSACQVVLSTNPDVASSARGSF 100
Lacbi2|576801|[L.bicolor]      FTLAHHRHAIRKPMAEFFGVALLVIFGAGAACQVVLSTNP-------NSF 44
Lacbi2|568479|[L.bicolor]      LTISHHKCAIRKVMAEFVGVALLVIFGAGTACQVVLSTNP-------SSF 45
Lacbi2|443240|[L.bicolor]      NTWMRLREPFREYIAEFVGVAVLIIFGVGADCQVVLSANTGVAPSPKGDY 84
JQ412060|[G.intraradices]      SGWYYIKFRFKEPFAEFLGTFILVAFGVGAIAQTVLSKGAT------GNW 87
                                     :  ::: :***.*. :*: **.*: .*.*** ..       ..:

Lacbi2|317173|[L.bicolor]      LSINFGWAIGIAMGVWVSGGISGGHINPAITIAMATYRGFPWRKVPSYIL 150
Lacbi2|576801|[L.bicolor]      LSINFGWAIGIAMGAWISGSISGGHINPAITIAMATYRGFPWREVPSYIL 94
Lacbi2|568479|[L.bicolor]      LSINFGWAIGIATGAWVSAGISGGHINPAITIAMATYRGFPWREVPGYIF 95
Lacbi2|443240|[L.bicolor]      LSLNCGWAIGTAMGVWISGGISGGHINPAVTLALATWRGFPWRKVPGFLF 134
JQ412060|[G.intraradices]      ITIALGFGLGLALGIAVSGHYSGGHLNPAVTITLAIYRKFPWVKVPVYIT 137
                               :::  *:.:* * *  :*.  ****:***:*:::* :* *** :** ::

Lacbi2|317173|[L.bicolor]      AQVLGGVVGAGLVYANYIHAIDIFEGGH--HIRTQATASLFATYALPYMT 198
Lacbi2|576801|[L.bicolor]      AQVLGGVVGAALVYANYIHAIDVFEGGR--HIRTQATASLFATYALPYMT 142
Lacbi2|568479|[L.bicolor]      AQALGGFVGAALVYANYFHAIDIFEGG---HIRTQATASLFATFALPYMT 142
Lacbi2|443240|[L.bicolor]      AQLLGGIVGAGLVYVNYIHAIDIVEGGR--HVRTLDTAGLFATYAADHMT 182
JQ412060|[G.intraradices]      AQVLGAFVAAAVIYLNYLPAIYNFAGDKRDVIGANATAGIFATYPQPFMS 187
                               ** **..*.*.::* **: **  . *.    : :  **.:***:.  .*:

Lacbi2|317173|[L.bicolor]      QASCFFSEFLATAVLSMMVFALTDKRNHSPTNGLLPFALFILFVGLGASL 248
Lacbi2|576801|[L.bicolor]      QVSCFFSEFLATAVLAMMVLALTDNRNGAPTNGLSPFALFVLFIGLGASL 192
Lacbi2|568479|[L.bicolor]      QASCFFSEFLATAVLFIVFLALNDKHNGALTNGLLPFALFILFIGLGASL 192
Lacbi2|443240|[L.bicolor]      NVSCFFSEFLATAVLIVVIHAMNDKRNAPPPAGLAPLVLFFLILGIGASL 232
JQ412060|[G.intraradices]      IGGAFFSEALGTFFLLFVILAMTDERNVPTTRIVAPITIGLTLTAIAISL 237
                                 ..**** *.* .* .:. *:.*::* . .  : *:.: . : .:. **

Lacbi2|317173|[L.bicolor]      GMETAYALNPARDFGPRLFLAMAGYGKALFNYRSQYWLWAPIIAPVLGAQ 298
Lacbi2|576801|[L.bicolor]      GMETAYALNPARDFGPRLFLAMAGYGKALFNYRSQYWLWAPIIAPVLGAQ 242
Lacbi2|568479|[L.bicolor]      GMQTGYAVNPARDFGPRLFLAMAGYGKAVFNYRRQYWIWAPIIAPILGAQ 242
Lacbi2|443240|[L.bicolor]      GMETGYAINPARDLGPRMLTAMVGYGRQVFAFRNQYWIWCPVIAPFLGAQ 282
JQ412060|[G.intraradices]      GFETGFSLNAARDFGPRLFTFFIGYGVEVFTAY-KFYFWIPLVAPIVGGL 286
                               *::*.:::*.***:***::  : ***  :*    ::::* *::**.:*.
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Lacbi2|317173|[L.bicolor]      AGGLLYDTFLNDGDNSPIKWRCASSQEHQLAEVV 332
Lacbi2|576801|[L.bicolor]      AGGLLYDTFLYDGDDSPIKWR------------- 263
Lacbi2|568479|[L.bicolor]      AGGLLYDTSIYNGDDSPIKWR------------- 263
Lacbi2|443240|[L.bicolor]      VGTIFYDLFFYKGQDN-VFGRLGSHIHISPA--- 312
JQ412060|[G.intraradices]      VAGFVYDSLLYWGEKS-FLNKNVHHEHRAVA--- 316
                               .. :.**  :  *:.. .  :
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Note A2.4. CLUSTAL W 2.1 multiple sequence alignment shows conservation and variation of aligned amino acid residues among 22 fungal XIPs in Cluster IV. Thirty-seven positions are well conserved among the aligned sequences, including 8 completely aligned consensus residues. The similarity score between Ascomycota ECM *Tuber melanosporum* putative MIP TmeAQP2 and functionally characterized JQ412059 of Glomeromycota AM *Glomus intraradices* is 18.8. The similarity score between JQ412059 and ACV52007 is 41.5. The consensus symbol asterisk "*" indicates positions which have a single, fully conserved residue among the aligned sequences. The symbol colon ":" indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, and the period "." indicates conservation between groups of weakly similar properties - scoring ≤ 0.5. Colors indicate physicochemical properties of the residues. AVFPMILW are coded RED for small+ hydrophobic (including aromatic -Y). DE are coded BLUE for acidic. RK are coded MAGENTA for basic-H. STYHCNGQ are coded GREEN for Hydroxyl + sulfhydryl + amine + G. Others are coded Grey for Unusual amino/imino acids, etc (Larkin *et al*. 2007).

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EGX93694|[C.militaris]              ----------------------------------------------------
EGX52763|[A.oligospora]             ----------------------------------------------------
XP002384305|[A.flavus]              ----------------------------------------------------
XP001390456|[A.niger]               ----------------------------------------------------
XP003190309|[A.oryzae]              ----------------------------------------------------
XP001396483|[A.niger]               ----------------------------------------------------
XP002149425|[P.marneffei]           ----------------------------------------------------
XP002484748|[T.stipitatus]          ----------------------------------------------------
XP002562918|[P.chrysogenum]         ----------------------------------------------------
Trive1|42995|[T.virens]             ----------------------------------------------------
TmeAQP2|[T.melanosporum]            ----------------------------------------------------
EGR49911|[T.reesei]                 ----------------------------------------------------
TriviGv29_8_2|201141|[T.virens]     ----------------------------------------------------
EHK42605|[T.atroviride]             ----------------------------------------------------
EFY92789|[M.acridum]                ----------------------------------------------------
Clagr2|89221|[C.grayi]              ----------------------------------------------------
ACV52007|[R.intraradices]           ----------------------------------------------------
JQ412059|[G.intraradices]           ----------------------------------------------------
Mucci2|114802|[M.circinelloide]     ----------------------------------------------------


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EGX52763|[A.oligospora]             ----------------------------------------------------
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XP001396483|[A.niger]               ----------------------------------------------------
XP002149425|[P.marneffei]           ----------------------------------------------------
XP002484748|[T.stipitatus]          ----------------------------------------------------
XP002562918|[P.chrysogenum]         ----------------------------------------------------
Trive1|42995|[T.virens]             ----------------------------------------------------
TmeAQP2|[T.melanosporum]            ----------------------------------------------------
EGR49911|[T.reesei]                 ----------------------------------------------------
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EHK42605|[T.atroviride]             ----------------------------------------------------
EFY92789|[M.acridum]                ----------------------------------------------------
Clagr2|89221|[C.grayi]              ----------------------------------------------------
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XP002149425|[P.marneffei]           ----------------------------------------------------
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XP002562918|[P.chrysogenum]         ----------------------------------------------------
Trive1|42995|[T.virens]             ----------------------------------------------------
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TriviGv29_8_2|201141|[T.virens]     ----------------------------------------------------
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EFY92789|[M.acridum]                ----------------------------------------------------
Clagr2|89221|[C.grayi]              ----------------------------------------------------
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Mucci2|114802|[M.circinelloide]     ----------------------------------------------------

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XP002149425|[P.marneffei]           ----------------------------------------------------
XP002484748|[T.stipitatus]          ----------------------------------------------------
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JQ412059|[G.intraradices]           ----------------------------------------------------
Mucci2|114802|[M.circinelloide]     ----------------------------------------------------
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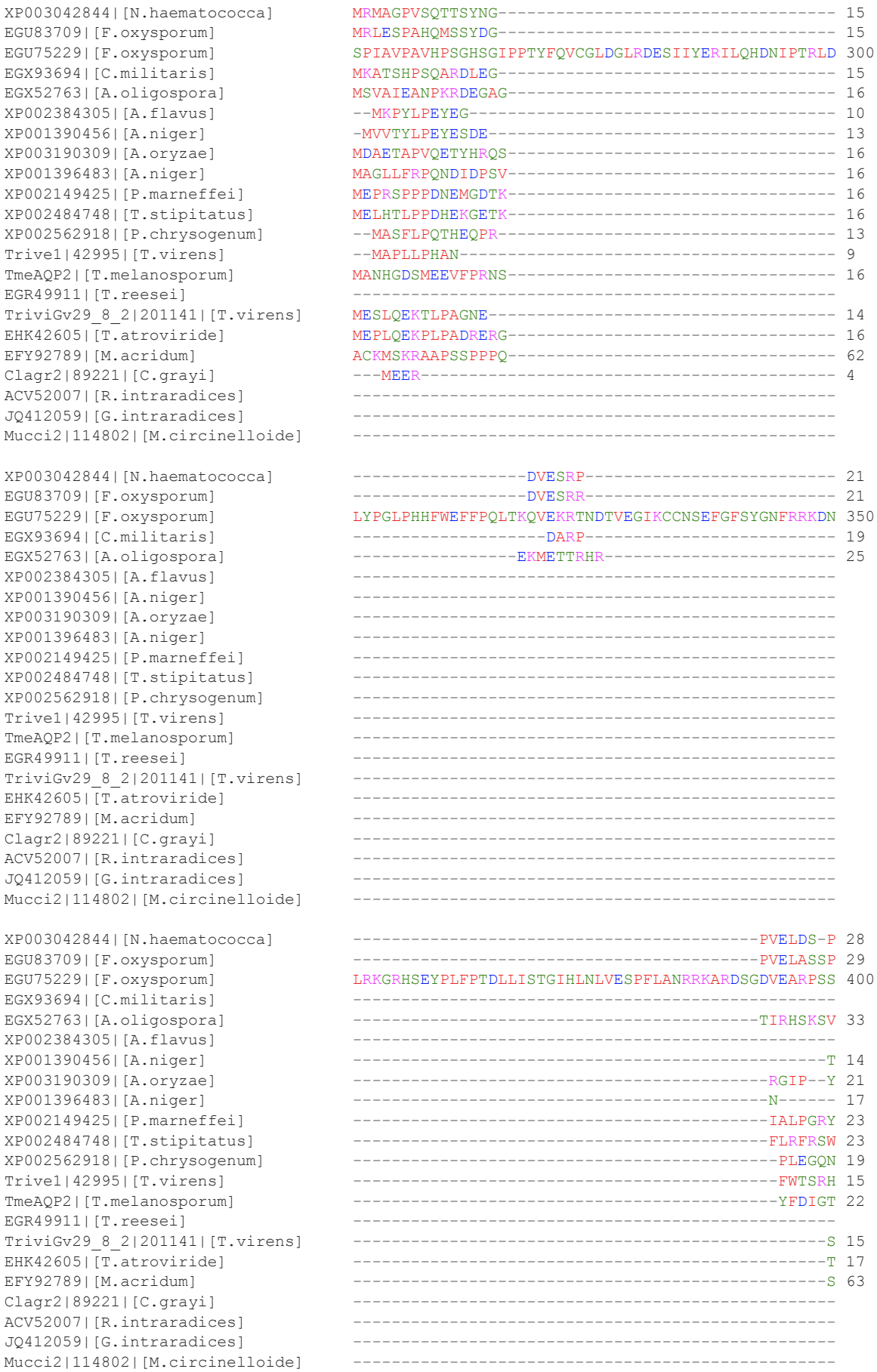
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EGX52763|[A.oligospora] MSVAIEANPKRDEGAG 16
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XP003190309|[A.oryzae] MDAETAPVQETYHRQS 16
XP001396483|[A.niger] MAGLLFRPQNDIDPSV 16
XP002149425|[P.marneffei] MEPRSPPPDNEMGDTK 16
XP002484748|[T.stipitatus] MELHTLPPDHEKGETK 16
XP002562918|[P.chrysogenum] --MASFLPQTHEQPR 13
Trive1|42995|[T.virens] --MAPLLPHAN 9
TmeAQP2|[T.melanosporum] MANHGDSMEEVFPRNS 16
EGR49911|[T.reesei]
TriviGv29_8_2|201141|[T.virens] MESLQEKTLPAGNE 14
EHK42605|[T.atroviride] MEPLQEKPLPADRERG 16
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EGU75229|[F.oxysporum] LRKGRHSEYPLFPTDLLISTGIHLNLVESPFLANRRKARDSGDVEARPSS 400
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XP003190309|[A.oryzae] RGIP--Y 21
XP001396483|[A.niger] N------ 17
XP002149425|[P.marneffei] IALPGRY 23
XP002484748|[T.stipitatus] FLRFRSW 23
XP002562918|[P.chrysogenum] PLEGQN 19
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TmeAQP2|[T.melanosporum] YFDIGT 22
EGR49911|[T.reesei]
TriviGv29_8_2|201141|[T.virens] S 15
EHK42605|[T.atroviride] T 17
EFY92789|[M.acridum] S 63
Clagr2|89221|[C.grayi]
ACV52007|[R.intraradices]
JQ412059|[G.intraradices]
Mucci2|114802|[M.circinelloide]

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EGU83709|[F.oxysporum]              ERIIPRYRSSSHPFAGRVGANQAFTVEGRTSEDGKLLEREPDATPHMPFR 79
EGU75229|[F.oxysporum]              EPSGPHYQSYSQPFAGRLGANQAYVVEGGTSEDDHLLHHAPDATPHMSFW 450
EGX93694|[C.militaris]              ----GATQVDISPFIGRLGGSGTGCLS-RDTANEELLKALPDAAPLMSLS 64
EGX52763|[A.oligospora]             PEPLRAAHIHTTPFAARLGGNQEFVLDRADPKNAAVLEDIPDAATHMTVK 83
XP002384305|[A.flavus]              HEPQSGLTLAVAPFAGRLGGNQDFVVDRSDPRNEKVLEKVPDAAPWMSLS 60
XP001390456|[A.niger]               HPQHHGLEPAIPPFAGRMGGNQDFVVDRTDPKNSKVLERVPDAAPCMTLK 64
XP003190309|[A.oryzae]              GQNDMPLRPVIYPFAGRIGGNQGLVLDRDDPANAELLKKVPDAAPLMSIS 71
XP001396483|[A.niger]               -VQQLSCKPHVQPFVGRIGGNQGIVLDRADPDNAEYLRKVPDAAPLMSAR 66
XP002149425|[P.marneffei]           ESPITGALPAVQPFAGRIGGNQSLVLDRNDPKNSDYLKAVPDAAPFMRIS 73
XP002484748|[T.stipitatus]          STPLTGVHPAVQPFAGRIGGNQALVLDRNDPKNTEYLKAVPDAAPFMRVS 73
XP002562918|[P.chrysogenum]         GGIRRRFQVESTPFAGRIGANQEFSVSP--LEEAELLRKTPDAAPLVPWS 67
Trive1|42995|[T.virens]             DAEALPSPTNVIPFAGRIGANQEFSLEKNNCTQIELLQKFPDAAPWIPLR 65
TmeAQP2|[T.melanosporum]            NRRGSNADFRLSAFAGRIGGNQRFTISRFDPEFQQIKKDRPDATTEGTWA 72
EGR49911|[T.reesei]                 ----------------------MDLAAFDGSFAPAVRPREVRLAPWYRS 27
TriviGv29_8_2|201141|[T.virens]     TGARDQSAASTPR-----TATAKMDLAAFDGSFAPGVRPHAVRLTPWYRS 60
EHK42605|[T.atroviride]             TQARAASSAVSLSNSSTPAATHKMDLAAFDGSFAPLVRPQAVRLTPWYRR 67
EFY92789|[M.acridum]                VMPSAPQTPDRVSEESKPTLERGMSVAAFDGSFAPLVRPTVRREKPWYKD 113
Clagr2|89221|[C.grayi]              ------LSPGQVANFAGSLVVNNLDVAPK----AIVTRGNRGKAAVWAV- 43
ACV52007|[R.intraradices]           ------------------------------------------------MGLK 4
JQ412059|[G.intraradices]           ----------------------------------------------MLDAEQK 7
Mucci2|114802|[M.circinelloide]     ----------------------------MRPINDIFGCNKGSPPNLRAD 21

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EGU75229|[F.oxysporum]              ELMDMRPIKNLDLWKAALIEGIGTLLFVYITIWVNIS--PDIAPAAP--T 496
EGX93694|[C.militaris]              DQFALRPFLTLGLWKAAVIEGVGSLMLVWVTVFANGS--PLVLPSAP--T 110
EGX52763|[A.oligospora]             QALDLRGFRQAILWKAAAVEGVGTMLLVYATDWSTLS--PAAYPPPPDPA 131
XP002384305|[A.flavus]              EIFDLRGFLSLDLWKFACLECIASMMNVFISAWVTLH----EPAAVEAPK 106
XP001390456|[A.niger]               EIFDLRGFLSVDLWKFAVLECIASMMNVFITCWVTTH----PLSATTSPK 110
XP003190309|[A.oryzae]              EGFDPRGFLSIDHWKFGFIECIGTMLNVFVTAWISIR-HSSASQDAQAPS 120
XP001396483|[A.niger]               DAFNVRGFTDLDLWRFAVVECVGTMMLAFITAWAAA---TPANVAPPTPS 113
XP002149425|[P.marneffei]           EALDLRGFLDLNLWKFAIVEGVASFLLIFITGWIAIQPKPTSSSSASTAA 123
XP002484748|[T.stipitatus]          EALDLRGFLDWNLWKFAIVEGVASFLLIFITGWIAIRPRPASSTSSSTEP 123
XP002562918|[P.chrysogenum]         QMICPNQFIQLNIWKAAFVEGVVTCLLVYFTCFLAVGLG----------- 106
Trive1|42995|[T.virens]             DSLSLRPLLEAVLWKAAVVEAIGPIYILLRFAFDSELN----------- 103
TmeAQP2|[T.melanosporum]            ELCDLRGLGKIEIWKSGFIEFWASLLMTFTTGAAAIS------------ 109
EGR49911|[T.reesei]                 RDYFVGQWLDVSVWKSAVVEMVATSCLVFLSGQITAT------------ 64
TriviGv29_8_2|201141|[T.virens]     RDYYIGQWLDLSVWKSAVVEFIATCCMVFLSGQITAT------------ 97
EHK42605|[T.atroviride]             RDYFVGQWFEPALWRSAVVELIATCCQVFVSGQIVAT------------ 104
EFY92789|[M.acridum]                RDYFLAGWLSPAIWRAAVVEAIATSCLAYASLLAAST------------ 150
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ACV52007|[R.intraradices]           DDFVT-SLAEFIGTTYFIFIGLGGSDAIAAFSGKSLG------------ 40
JQ412059|[G.intraradices]           KNYVAGAFGEFVGTAYFLFMGVGG--AVNFLNNAAGS------------ 42
Mucci2|114802|[M.circinelloide]     LKASLGEFFGMAIFIFLALAGVQGALEAPTFQSVGAA------------ 58

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EGU75229|[F.oxysporum]              QRFGSFDNAAFLGPLIGGMTNLIFITLFITSFGAISGAHFNPLITFATFC 546
EGX93694|[C.militaris]              ERWGVFNNATFVGPLVGGVLNFFYISLFIFCFGGVTGAHLNPAITIATLF 160
EGX52763|[A.oligospora]             SESGVFSTASFLGPLVGGVTSAIFIAMYIFCFGAVTGGHLNPLITIATFF 181
XP002384305|[A.flavus]              TEVGIYHTVTFFSPLFGGLTNLLLTPLLIYTFAPSSGGHISPTITLATLF 156
XP001390456|[A.niger]               GQAGVYGTVTFFSPTFGGLTNLLLTPLLIYTFSPSSGGHISPTITLATFF 160
XP003190309|[A.oryzae]              SASGVYSTATFLGPLFGGISNWLFLTLFIFSFSNVSGSHLNPTITMATFF 170
XP001396483|[A.niger]               TPAGIFATTAFLGPLVGAVTNWLLLTLFIFSFSSVSGSHLNPTITLATFF 163
XP002149425|[P.marneffei]           SSAGVFGTASFLGPLVGGITNWFFLTLFIYCFAPVSGGHINPTITLATFF 173
XP002484748|[T.stipitatus]          TAAGVFGTSTFLGPLIGGITNWLFLTLFIYSFAPISGGHINPTITLATFF 173
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Trive1|42995|[T.virens]             -ISSVFASGALVPSLLGGLTAILILPLFIFATGPVSGAHLNPAITFATFF 152
TmeAQP2|[T.melanosporum]            LAKYGSAYSPVTLAAAGGMSILLTLPLFIHAAGPASGGHINSMITIATFT 159
EGR49911|[T.reesei]                 ----IEGYGTPQVGGYIGISNIILLSTFIYATAPASGGHLNPMITFSAIL 110
TriviGv29_8_2|201141|[T.virens]     ----LESYGTPQVGGYIGISNIILLSTFIYATAPASGGHLNPMISFSAIL 143
EHK42605|[T.atroviride]             ----ISTYGTPQLGAYIGISNLVLIATFIYAVAPASGGHMNPTITFAAVL 150
EFY92789|[M.acridum]                ----LVSYNTPRIGGYIGIFNTILLAILIYATSPASGGHMNPMITFSAIL 196
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JQ412059|[G.intraradices]           ------PLPGFAIPFCFGFS----LFVNVFIWAPISGGVFNPSITIALMA 82
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EGX93694|[C.militaris]              AR--LCSLPRAVLYVGFQTGGAAVGGLLARVARGSR---EFKTGGCWLFS 205
EGX52763|[A.oligospora]             TR--LTSLPRAILYVSFQIIGASLAGLLIRASYDSR---EFKVGGCWLNP 226
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XP001390456|[A.niger]               AR--IITFPRMILYLAGQTLGGALAGFAMHSAYGTR---EFTVGGCHIDT 205
XP003190309|[A.oryzae]              AR--LISLPRLVIYLASQTLGGALAGFMLRAAYGSR---DYTVGGCYMNP 215
XP001396483|[A.niger]               AR--LISLPRMVLYLCGQILGGALAGWILQSAFGSG---QYSVGGCVVDT 208
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XP002484748|[T.stipitatus]          AR--LISLPRMTLYLMGQTGGGALAGLVLHNLFGSS---NFVVGGCFIET 218
XP002562918|[P.chrysogenum]         AR--LSTFPRSILYISFQLLGATVAGYLLRGSFDTR---SFVIPGCVIDT 201
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TmeAQP2|[T.melanosporum]            AK--LAALPRTIVYVFLQSLGGVAGGFLLRAGLGEAN--TAVVPGCYWAP 205
EGR49911|[T.reesei]                 TG--LCSVPRGILYMSAQTLGGALAGGLLLGVWGHERATSLQGGGCWYDP 158
TriviGv29_8_2|201141|[T.virens]     TG--LCSVPRGILYMCGQTLGGALAGGILLGVWGPERATSLKGGGCWYDP 191
EHK42605|[T.atroviride]             TG--LCSVPRGLLYLVGQTAGGALGGGILLGIWGKERAIAVRGGGCWYDP 198
EFY92789|[M.acridum]                AG--ICPVPRGVLYLCAQLLGASLAGGILTGVWGEEKAAGIHGGGCFFTP 244
Clagr2|89221|[C.grayi]              TG--LTGFSRGILYMTGQTIGAALAGGTLRGSFGAERATQWQGGGCFREP 172
ACV52007|[R.intraradices]           TDDQELRIRRGIFYIIAQFAGAILGSLLVKLFLPAP----IAALTTLSDG 126
JQ412059|[G.intraradices]           TNPKDFPWYRGILYIVSQFLGALFGSWLIDLIQPEA----PNAATLLADG 128
Mucci2|114802|[M.circinelloide]     TG--NINWLRGIFFFIAEIVGAIIGAYFANFVTAHE-----LQGVNLLNP 145
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EGX93694|[C.militaris]              -DIVPVQDAFAIEFMACLIMLFFAFGTGLDPRQRETVGPTLGPFLVGLSV 254
EGX52763|[A.oligospora]             -AEISVSSAFTNEFSASLVVLFMAFGVGLDPRQRQIFGPSLGPIFVGLAV 275
XP002384305|[A.flavus]              -TLVPVNAALIIEFFACLVLIFLAFGVALDPRQAKIFGHAAGPWLVGVVL 250
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XP003190309|[A.oryzae]              -QLVPVNEGFLLEFVFTLLLIFLSFGVGLDPRQGRIYGAALSPFLVGLAL 264
XP001396483|[A.niger]               -ALVPVREAFVLEFICSLTLIFLSFGVGLDPRQVRVYGAALSPWLVGMVL 257
XP002149425|[P.marneffei]           -NLVEVRQALVLEFMCTLILIFLAFGVALNPRQERIYGPALAPWLVGLAL 267
XP002484748|[T.stipitatus]          -NLVEVRQALLLEFMCTLTLVFIAFGVALNPRQERIFGPALAPWLVGLTL 267
XP002562918|[P.chrysogenum]         -SVVSVGSAFTIEVTTDFMLIFLSFSVGLDPRQREVFGPALGPVFVGIIL 250
Trive1|42995|[T.virens]             -TIVSTGSAFAIEFITDFALIFLSFGVGLDPRQRSVFGPALGPIFVGLVL 246
TmeAQP2|[T.melanosporum]            NAGITDLQAFLIEFSSCCIVLFIAFGVGLDPRQRDIFGPALGPILIGLAI 255
EGR49911|[T.reesei]                 -SQASPGQVYLNEVFSSFVLLFLSFGVGLDPRQAALFGPRMGPLLVGASL 207
TriviGv29_8_2|201141|[T.virens]     -SQANPGQIYLNEVFASFVLLFLSFGVGLDPRQAALFGPRMGPLLVGASL 240
EHK42605|[T.atroviride]             -SQANPGQIYLNETFASFVLLFLAFGVGLDPRQAALFGPRMGPALVGASL 247
EFY92789|[M.acridum]                -SVTSAGQVFLNEAAASFVVLYLAYGVGLDPRQAILFGPRLGPLLVGASL 293
Clagr2|89221|[C.grayi]              -GTVTAGQALLIETMCCFVLLVLAFGVALDPRQQMLMGPVFGPIAVGSSL 221
ACV52007|[R.intraradices]           ---TTILQGLVIEIITTSLLTLTVYTLAVNER---------GGFMKSFGM 164
JQ412059|[G.intraradices]           ---VSVAQGLFMEMFATSVLTMAVLILAGERY---------GKYLAPFGI 166
Mucci2|114802|[M.circinelloide]     --GFNYAQGFFAECLLTCVLCLTVLFIIVDKH--------LLADFAPFVV 185
                                              *      :         :

XP003042844|[N.haematococca]        GTLAFSTGFTRYGYGGAGLNPARCMG-AFVGTRFPTWHWIHWVG------ 311
EGU83709|[F.oxysporum]              GTLAFATSYTRYGYGGPSFNPARCMG-VFVGSRFPSWHWIHWAA------ 312
EGU75229|[F.oxysporum]              GTMSFASAFARYGYGGAGLNPARCMG-AFVGSRFPSWHWIHWVA------ 683
EGX93694|[C.militaris]              AGLTFGTGFARYGYGGAGLNPARCFG-AYVGSHFPGFHWIHWYT------ 297
EGX52763|[A.oligospora]             GTTAFSMAFTRPGYGGAAMNPARCFG-AYVGSSFPTWHWIHWVA------ 318
XP002384305|[A.flavus]              GVVCWATAFTRPGYIGASLNPARCFG-VYVASEFPGYHWVHWVA------ 293
XP001390456|[A.niger]               GIVTWGTAFTREGYIGASVNPARCFG-AYVASDFPTYHWIHWVG------ 297
XP003190309|[A.oryzae]              GLVSWGSAFSRAGYAGASLNPARCFG-VYVATSFPGYHWIHWVA------ 307
XP001396483|[A.niger]               GAVSLGSAYTREGYGGASLNPARCFG-VYVGSSFPGYHWIHWVA------ 300
XP002149425|[P.marneffei]           GLLSWGSGYEKPGYAGASMNPARCFG-VYVGSGFPGYHWIHWVG------ 310
XP002484748|[T.stipitatus]          GLLSWSSSYEKPGYAGASMNPARCFG-VYVGSGFPGYHWIHWVG------ 310
XP002562918|[P.chrysogenum]         GITSFGTGYSQVGYTGFGGNPARCFG-AMVGSHFTSYHWIHWLG------ 293
Trive1|42995|[T.virens]             GMCTFVTGFSRVGYTGFSGNPARCFG-AMVGSHFAPYHWIYWVA------ 289
TmeAQP2|[T.melanosporum]            GIISFITGIMRTGYAGASMNPTRCLG-LVAATQTFRGHWVTWAG------ 298
EGR49911|[T.reesei]                 GLVTFSSSGIIPGYAGAQMNPSRCLA-FGIARRNMSYQWVWWFG------ 250
TriviGv29_8_2|201141|[T.virens]     GLVSFSTSGIIPGYAGAQMNPSRCLA-FGIARQNMALDLVVWAGGGRSHD 289
EHK42605|[T.atroviride]             GLVSFSTSGIIPGYAGAQMNPAKCFG-NGIARMDLSYQWIYWFG------ 290
EFY92789|[M.acridum]                GLVSFASSGIAEGYGGAQANPARCFA-AAIARKDMSYQWIWWFG------ 336
Clagr2|89221|[C.grayi]              GLVVFASGGLVPGYSGAGANPARCFA-FAVARRNFKDQWIWWLG------ 264
ACV52007|[R.intraradices]           GTSVLISVLVAGPYTGASLNPARTLGPAIVSGKISGDIWIYFIG------ 208
JQ412059|[G.intraradices]           GMSLFISALCAGPYTGASLNPARTLGPAIVANQYGRAHWIYYVG------ 210
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Mucci2|114802|[M.circinelloide]     GSAVFICHMIGAPVDGTSINPARSFAASIVTGKWAN-HWIFWFG------ 228
                                    .             *   **:: :.            : :

XP003042844|[N.haematococca]        --------DGIACIIHG--------------LVYYFVPP---------- 328
EGU83709|[F.oxysporum]              --------DGIACIIHG--------------ICYYFVPP---------- 329
EGU75229|[F.oxysporum]              --------DGIACIIHG--------------VCYYFIPP---------- 700
EGX93694|[C.militaris]              --------PPMMASAYQRW------------LTYTGWPTSQLARFTLAF 326
EGX52763|[A.oligospora]             --------TIAASVFHG--------------IVYYMVPPWGG------- 338
XP002384305|[A.flavus]              --------PLAAAVAHG--------------LVYLIDPL---------- 310
XP001390456|[A.niger]               --------PLAAAVAHG--------------LVYFVDPL---------- 314
XP003190309|[A.oryzae]              --------PAIASVGHG--------------IAYFIVPP---------- 324
XP001396483|[A.niger]               --------PIAAAIGHG--------------LVYYLVPP---------- 317
XP002149425|[P.marneffei]           --------VICATLGHG--------------VFYQLLPP---------- 327
XP002484748|[T.stipitatus]          --------VFCATLGHG--------------LFYQLLPP---------- 327
XP002562918|[P.chrysogenum]         --------PIVASILHG--------------IMYYFIPPYQ-------- 312
Trive1|42995|[T.virens]             --------PLSASAIHG--------------MVYYLVPPYSRTR----- 311
TmeAQP2|[T.melanosporum]            --------GIAAACVNG--------------LFYIVVPP---------- 315
EGR49911|[T.reesei]                 -------PAVG-GLIEAL--------------LYNLIPP---------- 267
TriviGv29_8_2|201141|[T.virens]     GRPIQSDPASSYGAVEAKEQRVPFKLDSWAHRDTYGLRGR---------- 329
EHK42605|[T.atroviride]             -------PAVA-GIMMGI--------------LYNLIPP---------- 307
EFY92789|[M.acridum]                -------PAAA-AVLLAV--------------FYNAIPP---------- 353
Clagr2|89221|[C.grayi]              -------PFCG-SWLLAIS-------------YHIAPP---------- 281
ACV52007|[R.intraradices]           --------PIIGSLLAAS----------FHTYFKKNFGALHLD------ 233
JQ412059|[G.intraradices]           --------PTLGSLLAAGY---------WHILRILNIDVVDLKNVLNKC 242
Mucci2|114802|[M.circinelloide]     --------PLIGGIFAV--------------MIYLAVKVITEESQEKAL 255

XP003042844|[N.haematococca]        --WTKEN----------------------------------------- 333
EGU83709|[F.oxysporum]              --WVKRTD---------------------------------------- 335
EGU75229|[F.oxysporum]              --WTEVRQ---------------------------------------- 706
EGX93694|[C.militaris]              TIWRRRGKVGCSSPTVFLVLPILK------------------------ 350
EGX52763|[A.oligospora]             --FAGKHERQLVSDEEK------------------------------- 353
XP002384305|[A.flavus]              --WSDPRLE--------------------------------------- 317
XP001390456|[A.niger]               --WKDPRAE--------------------------------------- 321
XP003190309|[A.oryzae]              --WGRSM----------------------------------------- 329
XP001396483|[A.niger]               --WKA------------------------------------------- 320
XP002149425|[P.marneffei]           --WISEKAK--------------------------------------- 334
XP002484748|[T.stipitatus]          --WTTDKK---------------------------------------- 333
XP002562918|[P.chrysogenum]         --YCV------------------------------------------- 315
Trive1|42995|[T.virens]             -ASCVSGGT--------------------------------------- 319
TmeAQP2|[T.melanosporum]            --YHAELPEDEERGDKRE------------------------------ 331
EGR49911|[T.reesei]                 --HHTELVKKQGIGNDP-DTMVGHTDIPTV------------------ 294
TriviGv29_8_2|201141|[T.virens]     --VHIRQSEREGRDTESNERALGMASIATVAGKRKKDGLMVNEMRQARQP 377
EHK42605|[T.atroviride]             --HHAELCKRKSREMSREMTSDSMAERAEASVIASA------------ 341
EFY92789|[M.acridum]                --HHTDNQQPKERQHQSN------------------------------ 369
Clagr2|89221|[C.grayi]              --YHSSSPP--------------------------------------- 288
ACV52007|[R.intraradices]           --RDRDDLDRDLDLDRENLGKD-------------------------- 253
JQ412059|[G.intraradices]           KKCGKEDPRISLKHCEECLKDDPKPEKYDIESQN-------------- 276
Mucci2|114802|[M.circinelloide]     LQNAHNQAMIDADKNKEQNQQNPAQYTSVNV----------------- 286

XP003042844|[N.haematococca]        ------------------------------------------------
EGU83709|[F.oxysporum]              ------------------------------------------------
EGU75229|[F.oxysporum]              ------------------------------------------------
EGX93694|[C.militaris]              ------------------------------------------------
EGX52763|[A.oligospora]             ------------------------------------------------
XP002384305|[A.flavus]              ------------------------------------------------
XP001390456|[A.niger]               ------------------------------------------------
XP003190309|[A.oryzae]              ------------------------------------------------
XP001396483|[A.niger]               ------------------------------------------------
XP002149425|[P.marneffei]           ------------------------------------------------
XP002484748|[T.stipitatus]          ------------------------------------------------
XP002562918|[P.chrysogenum]         ------------------------------------------------
Trive1|42995|[T.virens]             ------------------------------------------------
TmeAQP2|[T.melanosporum]            ------------------------------------------------
EGR49911|[T.reesei]                 ------------------------------------------------
TriviGv29_8_2|201141|[T.virens]     IETDCPAHRSRDSTHYLLRDARVQLGCYGAITPSEANETSSSRGDCIGTA 427
EHK42605|[T.atroviride]             ------------------------------------------------
EFY92789|[M.acridum]                ------------------------------------------------
Clagr2|89221|[C.grayi]              ------------------------------------------------
ACV52007|[R.intraradices]           ------------------------------------------------
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JQ412059|[G.intraradices]          ------------------------------------------------
Mucci2|114802|[M.circinelloide]    ------------------------------------------------

XP003042844|[N.haematococca]       ------------------------------------------------
EGU83709|[F.oxysporum]              ------------------------------------------------
EGU75229|[F.oxysporum]              ------------------------------------------------
EGX93694|[C.militaris]              ------------------------------------------------
EGX52763|[A.oligospora]             ------------------------------------------------
XP002384305|[A.flavus]              ------------------------------------------------
XP001390456|[A.niger]               ------------------------------------------------
XP003190309|[A.oryzae]              ------------------------------------------------
XP001396483|[A.niger]               ------------------------------------------------
XP002149425|[P.marneffei]           ------------------------------------------------
XP002484748|[T.stipitatus]          ------------------------------------------------
XP002562918|[P.chrysogenum]         ------------------------------------------------
Trive1|42995|[T.virens]             ------------------------------------------------
TmeAQP2|[T.melanosporum]            ------------------------------------------------
EGR49911|[T.reesei]                 ------------------------------------------------
TriviGv29_8_2|201141|[T.virens]     VGRAMEDTQRGERAKLPMPDQTLIQPRIQDIQVARAAARRKAALVFVHLS 477
EHK42605|[T.atroviride]             ------------------------------------------------
EFY92789|[M.acridum]                ------------------------------------------------
Clagr2|89221|[C.grayi]              ------------------------------------------------
ACV52007|[R.intraradices]           ------------------------------------------------
JQ412059|[G.intraradices]           ------------------------------------------------
Mucci2|114802|[M.circinelloide]     ------------------------------------------------

XP003042844|[N.haematococca]       ------------------------------------------------
EGU83709|[F.oxysporum]              ------------------------------------------------
EGU75229|[F.oxysporum]              ------------------------------------------------
EGX93694|[C.militaris]              ------------------------------------------------
EGX52763|[A.oligospora]             ------------------------------------------------
XP002384305|[A.flavus]              ------------------------------------------------
XP001390456|[A.niger]               ------------------------------------------------
XP003190309|[A.oryzae]              ------------------------------------------------
XP001396483|[A.niger]               ------------------------------------------------
XP002149425|[P.marneffei]           ------------------------------------------------
XP002484748|[T.stipitatus]          ------------------------------------------------
XP002562918|[P.chrysogenum]         ------------------------------------------------
Trive1|42995|[T.virens]             ------------------------------------------------
TmeAQP2|[T.melanosporum]            ------------------------------------------------
EGR49911|[T.reesei]                 ------------------------------------------------
TriviGv29_8_2|201141|[T.virens]     TTLLAITALFIAGTLAVPASSYPPPPPEYGDGHKGDVGYGGDHGGDHGKD 527
EHK42605|[T.atroviride]             ------------------------------------------------
EFY92789|[M.acridum]                ------------------------------------------------
Clagr2|89221|[C.grayi]              ------------------------------------------------
ACV52007|[R.intraradices]           ------------------------------------------------
JQ412059|[G.intraradices]           ------------------------------------------------
Mucci2|114802|[M.circinelloide]     ------------------------------------------------

XP003042844|[N.haematococca]       ------------------------------------------------
EGU83709|[F.oxysporum]              ------------------------------------------------
EGU75229|[F.oxysporum]              ------------------------------------------------
EGX93694|[C.militaris]              ------------------------------------------------
EGX52763|[A.oligospora]             ------------------------------------------------
XP002384305|[A.flavus]              ------------------------------------------------
XP001390456|[A.niger]               ------------------------------------------------
XP003190309|[A.oryzae]              ------------------------------------------------
XP001396483|[A.niger]               ------------------------------------------------
XP002149425|[P.marneffei]           ------------------------------------------------
XP002484748|[T.stipitatus]          ------------------------------------------------
XP002562918|[P.chrysogenum]         ------------------------------------------------
Trive1|42995|[T.virens]             ------------------------------------------------
TmeAQP2|[T.melanosporum]            ------------------------------------------------
EGR49911|[T.reesei]                 ------------------------------------------------
TriviGv29_8_2|201141|[T.virens]     HGKDHGGHYPGDPNYPPPPPPGYHNGGSDKPPTDGDGYPSDGGDDGDDSD 577
EHK42605|[T.atroviride]             ------------------------------------------------
EFY92789|[M.acridum]                ------------------------------------------------
Clagr2|89221|[C.grayi]              ------------------------------------------------
ACV52007|[R.intraradices]           ------------------------------------------------
```

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JQ412059|[G.intraradices]          ------------------------------------------------
Mucci2|114802|[M.circinelloide]    ------------------------------------------------

XP003042844|[N.haematococca]       ------------------------------------------------
EGU83709|[F.oxysporum]              ------------------------------------------------
EGU75229|[F.oxysporum]              ------------------------------------------------
EGX93694|[C.militaris]              ------------------------------------------------
EGX52763|[A.oligospora]             ------------------------------------------------
XP002384305|[A.flavus]              ------------------------------------------------
XP001390456|[A.niger]               ------------------------------------------------
XP003190309|[A.oryzae]              ------------------------------------------------
XP001396483|[A.niger]               ------------------------------------------------
XP002149425|[P.marneffei]           ------------------------------------------------
XP002484748|[T.stipitatus]          ------------------------------------------------
XP002562918|[P.chrysogenum]         ------------------------------------------------
Trive1|42995|[T.virens]             ------------------------------------------------
TmeAQP2|[T.melanosporum]            ------------------------------------------------
EGR49911|[T.reesei]                 ------------------------------------------------
TriviGv29_8_2|201141|[T.virens]     GGGHGGDRENDPSDLCPTLLYSNPQCCSASVLNIADLDCEPPRKRPSRKH 627
EHK42605|[T.atroviride]             ------------------------------------------------
EFY92789|[M.acridum]                ------------------------------------------------
Clagr2|89221|[C.grayi]              ------------------------------------------------
ACV52007|[R.intraradices]           ------------------------------------------------
JQ412059|[G.intraradices]           ------------------------------------------------
Mucci2|114802|[M.circinelloide]     ------------------------------------------------

XP003042844|[N.haematococca]       --------------------------------
EGU83709|[F.oxysporum]              --------------------------------
EGU75229|[F.oxysporum]              --------------------------------
EGX93694|[C.militaris]              --------------------------------
EGX52763|[A.oligospora]             --------------------------------
XP002384305|[A.flavus]              --------------------------------
XP001390456|[A.niger]               --------------------------------
XP003190309|[A.oryzae]              --------------------------------
XP001396483|[A.niger]               --------------------------------
XP002149425|[P.marneffei]           --------------------------------
XP002484748|[T.stipitatus]          --------------------------------
XP002562918|[P.chrysogenum]         --------------------------------
Trive1|42995|[T.virens]             --------------------------------
TmeAQP2|[T.melanosporum]            --------------------------------
EGR49911|[T.reesei]                 --------------------------------
TriviGv29_8_2|201141|[T.virens]     DFKQICAAQGSDAKCCVLPLLGLGILCTDAIV 659
EHK42605|[T.atroviride]             --------------------------------
EFY92789|[M.acridum]                --------------------------------
Clagr2|89221|[C.grayi]              --------------------------------
ACV52007|[R.intraradices]           --------------------------------
JQ412059|[G.intraradices]           --------------------------------
Mucci2|114802|[M.circinelloide]     --------------------------------
```

APPENDIX 3 Quantification methods in qPCR assay in this study

Absolute quantification of standard curve was used in qPCR assay of *Laccaria bicolor* major intrinsic proteins, as previously described (Pfaffl 2004; El Kayal *et al*. 2011). Target genes were PCR amplified from GOI-containing pGEM-T Easy vectors using M13 primers. The purified PCR amplicons of each target gene were pooled and made into a series of dilutions as templates for standard curve ($1.6E^8$ - $1.6E^2$ molecules per mL for each gene). The number of molecules was calculated from the mass (ng) and the molecular weight (g/mol) of the PCR amplicons of each gene, given that the fragment size (base pairs) of PCR amplicons was known, and the average molecular weight of each base pair is 660 g/mol and there is 6.02×10^{23} of molecules/mol. Based on Ct values of the assayed genes and the corresponding standard curves, the absolute transcript quantity of each gene was calculated. The transcript abundance of target genes was normalized against the transcript abundance of reference gene *translation elongation factor EF2* (XM_001887160) for mycelia grown on pure culture, and against *EF2* for mycorrhizal root tips.

$$\text{Number of molecules} = \text{Mass} \times 6.02 \times 10^{14} / (\text{Number of base pairs} \times 660) \quad (1)$$

The ΔΔCt comparative quantification of standard curve method was used in qPCR assay of *Picea glauca* major intrinsic proteins. The cDNAs of all the samples were pooled and made into a series of dilution as the templates for standard curves to evaluate the amplification efficiency of each pair of primers. The relative transcript abundance of target PIP genes was normalized to that of the reference gene *PgCDC2*. For the gene expression change of the PIPs due to mycorrhizal treatments, non-mycorrhizal root tips were used as the control. For the gene expression change of the PIPs due to temperature decline, root tips at 20°C were used as the control. Gene expression fold change was calculated using $\Delta\Delta C_t$ method with efficiency corrected in Equation (3) (Livak & Schmittgen 2001; Pfaffl 2004).

$$\text{Fold difference} = (E_{\text{target}})^{\Delta Ct\ \text{target}} / (E_{\text{normalizer}})^{\Delta Ct\ \text{normalizer}} \quad (2)$$

Where E is efficiency from standard curve.

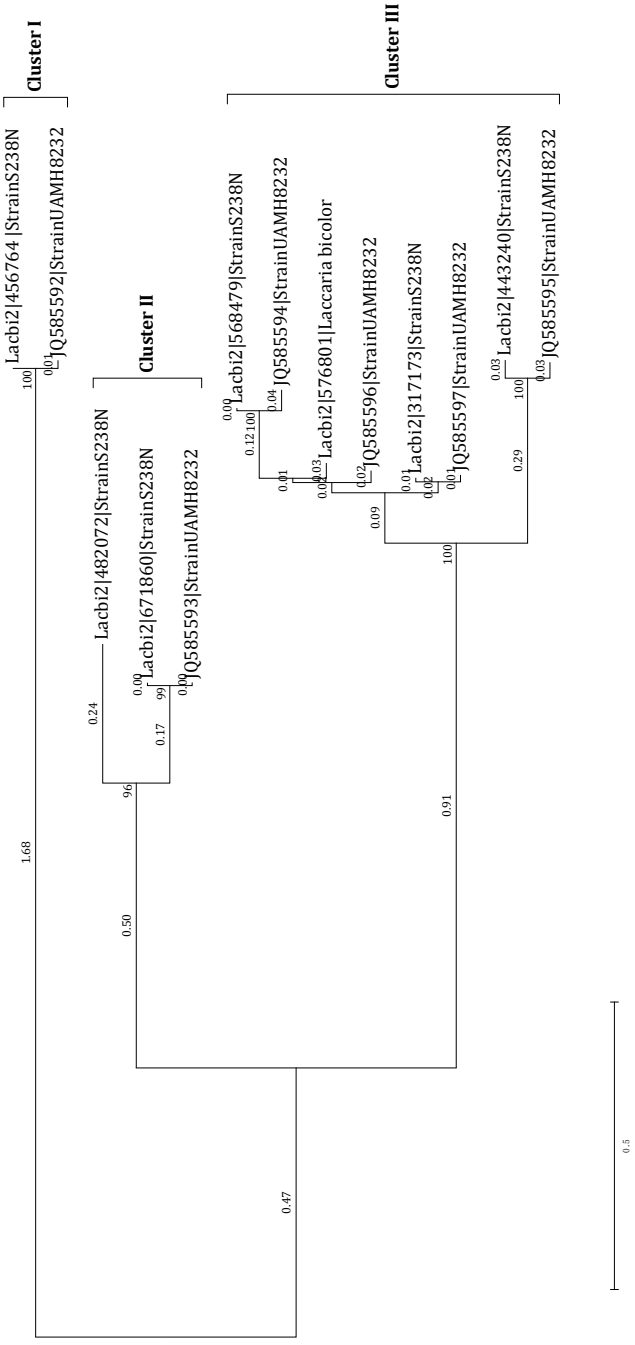
$$E = 10^{(-1/\text{slope})} - 1 \quad (3)$$

$$\Delta C_{t\ \text{target}} = C_{t\ \text{target}}^{\text{control}} - C_{t\ \text{target}}^{\text{Sample}} \quad (4)$$

$$\Delta C_{t\ \text{normalizer}} = C_{t\ \text{normalizer}}^{\text{control}} - C_{t\ \text{normalizer}}^{\text{Sample}} \quad (5)$$

APPENDIX 4 Phylogenetic analysis and homologue sequence alignment of 13 MIPs in the two strains of ECM fungus *Laccaria bicolor*, S238N and UAMH8232

Figure A4. Phylogenetic relation of 13 MIPs in the two strains of ECM fungus *Laccaria bicolor*, S238N and UAMH8232. Deduced amino acid sequences were aligned with ClustalW and the bootstrap consensus dendrogram was constructed using the neighbor-joining algorhithm of 1000 replicates (Felsenstein 1985, Saitou & Nei 1987) in MEGA5 (Tamura *et al*. 2011). The evolutionary distances were computed using the JTT matrix-based method in the units of the number of amino acid substitutions per site (Jones *et al*. 1992). Bootstrap frequency values shown at the nodes indicate the similarity of the pairwise amino acid sequences, and branch length values above the branches indicate the distance to the closest common ancestor. These MIPs fall into Cluster I, II and III, respectively.



Note A4. CLUSTAL W 2.1 multiple sequence alignment shows conserved amino acid residues and conserved nucleotides of the homologues in each cluster. In amino acid sequence alignment, the consensus symbol asterisk "*" indicates positions which have a single, fully conserved residue among the aligned sequences. The symbol colon ":" indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, and the period "." indicates conservation between groups of weakly similar properties - scoring ≤ 0.5. Colors indicate physicochemical properties of the residues. AVFPMILW are coded RED for small+ hydrophobic (including aromatic -Y). DE are coded BLUE for acidic. RK are coded MAGENTA for basic-H. STYHCNGQ are coded GREEN for Hydroxyl + sulfhydryl + amine + G. Others are coded Grey for Unusual amino/imino acids, etc (Larkin *et al*. 2007). In nucleotide sequence alignment, the symbol asterisk "*" indicates consensus nucleotides between aligned sequences. NPA motifs are in bold and underlined.

Note A4.1 Amino acid sequence alignment between Lacbi2:456764 of strain S238N and JQ585592 of strain UAMH8232 in Cluster I.
Identity Score 99.04

```
Lacbi2|456764|[StrainS238N]      MHPQVASLFDNVYEDLAAATLEFIGTAFFLLFGLGGIQASTAEDTASGQP 50
JQ585592|[StrainUAMH8232]        MHPQVASLFDNVYEDLAAATLEFIGTAFFLLFGLGGIQASTAEDTASSQP 50
                                 **************************************************.** 

Lacbi2|456764|[StrainS238N]      PASGIEHVLYISTCMGLSLVVSAWLFFRVTGGLFNPNISFALLLVGGLKP 100
JQ585592|[StrainUAMH8232]        PASGIEHVLYISTCMGFSLVVSAWLFFRVTGGLFNPNISFALLLVGGLKP 100
                                 ****************:*************************************

Lacbi2|456764|[StrainS238N]      LRFVLFCIAQLTGAIAGAAIVRGLTSAPLSVNNVLQQGTSAAQGVFIEMF 150
JQ585592|[StrainUAMH8232]        LRFVLFCIAQLTGAIAGAAIVRGLTSAPLSVNNVLQQGTSAAQGVFIEMF 150
                                 **************************************************

Lacbi2|456764|[StrainS238N]      ITAALVLSVLMLAAEKHEATPFAPVGIGLTLFACHLFAVYYTGAAMNSAR 200
JQ585592|[StrainUAMH8232]        ITAALVLSVLMLAAEKHEATPFAPVGIGLTLFACHLFAVYYTGAAMNSAR 200
                                 **************************************************

Lacbi2|456764|[StrainS238N]      AFGPAVISGFPEPQHWVYWVGPFLGSLLGAGFYATLKHYKYWHLNPDQAT 250
JQ585592|[StrainUAMH8232]        AFGPAVISGFPEPQHWVYWVGPFLGSLLGAGFYATLKHYKYWRLNPDQAT 250
                                 ******************************************:*******

Lacbi2|456764|[StrainS238N]      SDYRKSPSDPVALLKSTAETFINVGDEETRNGCASNEEGVRATGDEKSSN 300
JQ585592|[StrainUAMH8232]        SDYRKSPSDPVALLKSTAETFINVGDEETRNGCASNEEGVRATGDEKSSN 300
                                 **************************************************

Lacbi2|456764|[StrainS238N]      ATSSRTNFSPV 311
JQ585592|[StrainUAMH8232]        ATSSRTNFSPV 311
                                 ***********
```

Note A4.2 Nucleotide sequence alignment between Lacbi2:456764 of strain S238N and JQ585592 of strain UAMH8232 in Cluster I.

```
Lacbi2|456764                 ATGCATCCACAAGTTGCTTCACTCTTCGACAACGTCTACGAGGATCTGGC 50
JQ585592|[StrainUAMH8232]     ATGCATCCACAAGTTGCTTCACTCTTCGACAACGTCTACGAGGATCTGGC 50
                              **************************************************

Lacbi2|456764                 CGCAGCTACCCTAGAGTTCATTGGCACGGCGTTTTTTCTTTTGTTCGGTC 100
JQ585592|[StrainUAMH8232]     CGCAGCTACCCTAGAGTTCATTGGCACGGCGTTTTTCCTTTTGTTCGGTC 100
                              ********************************** ************

Lacbi2|456764                 TGGGGGGTATTCAGGCTAGCACCGCGGAGGACACGGCGAGCGGTCAGCCA 150
JQ585592|[StrainUAMH8232]     TGGGGGGTATTCAGGCTAGCACCGCGGAGGACACGGCGAGCAGTCAGCCA 150
                              ****************************************** ********

Lacbi2|456764                 CCAGCCTCGGGCATCGAACATGTTCTCTATATCTCAACCTGCATGGGGTT 200
JQ585592|[StrainUAMH8232]     CCAGCCTCGGGCATCGAACATGTTCTCTATATCTCAACCTGCATGGGGTT 200
                              **************************************************

Lacbi2|456764                 GTCTCTCGTTGTATCCGCCTGGCTCTTCTTCCGCGTCACTGGTGGACTCT 250
JQ585592|[StrainUAMH8232]     CTCTCTCGTTGTATCCGCCTGGCTCTTCTTCCGCGTCACTGGTGGACTCT 250
                               *************************************************

Lacbi2|456764                 TCAATCCAAATATATCTTTTGCGTTGCTTCTAGTCGGGGGTCTCAAGCCA 300
JQ585592|[StrainUAMH8232]     TCAATCCAAATATATCTTTTGCGTTGCTTCTAGTCGGGGGTCTCAAGCCA 300
                              **************************************************

Lacbi2|456764                 CTTCGCTTCGTGCTGTTCTGCATTGCTCAATTGACTGGTGCGATCGCAGG 350
JQ585592|[StrainUAMH8232]     CTTCGCTTCGTGCTGTTCTGCATTGCTCAATTGACTGGTGCGATCGCAGG 350
                              **************************************************

Lacbi2|456764                 AGCTGCCATCGTTCGCGGTCTGACGTCGGCGCCCCTCTCTGTCAACAACG 400
JQ585592|[StrainUAMH8232]     AGCTGCCATCGTTCGCGGTCTGACGTCGGCGCCCCTCTCTGTCAACAACG 400
                              **************************************************

Lacbi2|456764                 TTCTTCAGCAAGGGACGAGTGCCGCACAAGGCGTTTTTATTGAGATGTTT 450
JQ585592|[StrainUAMH8232]     TTCTTCAGCAAGGGACGAGTGCCGCACAAGGCGTTTTCATTGAGATGTTT 450
                              ********************************** ************

Lacbi2|456764                 ATCACCGCGGCGCTTGTGCTTTCCGTTTTGATGTTAGCGGCAGAGAAACA 500
JQ585592|[StrainUAMH8232]     ATCACCGCGGCGCTTGTGCTTTCCGTTTTGATGTTAGCGGCAGAGAAACA 500
                              **************************************************

Lacbi2|456764                 TGAGGCCACTCCTTTCGCTCCCGTCGGGATTGGTCTTACGCTCTTTGCTT 550
JQ585592|[StrainUAMH8232]     TGAGGCCACTCCTTTCGCTCCCGTCGGGATTGGTCTTACGCTCTTTGCTT 550
                              **************************************************

Lacbi2|456764                 GTCATCTTTTTGCGGTTTACTACACTGGCGCGGCTATGAATTCAGCAAGG 600
JQ585592|[StrainUAMH8232]     GTCATCTTTTTGCGGTTTACTACACTGGCGCTGCTATGAATTCAGCAAGG 600
                              ******************************* ******************

Lacbi2|456764                 GCGTTTGGACCAGCTGTAATCTCTGGATTCCCAGAGCCCCAACACTGGGT 650
JQ585592|[StrainUAMH8232]     GCGTTTGGACCAGCTGTAATCTCCGGATTCCCAGAGCCCCAACACTGGGT 650
                              ********************** *************************

Lacbi2|456764                 GTATTGGGTTGGGCCGTTCTTGGGATCACTCCTCGGTGCAGGCTTCTACG 700
JQ585592|[StrainUAMH8232]     GTATTGGGTTGGGCCGTTCTTGGGATCACTCCTCGGTGCAGGCTTCTACG 700
                              **************************************************

Lacbi2|456764                 CTACCTTGAAGCACTACAAGTATTGGCATCTCAATCCCGATCAAGCTACC 750
JQ585592|[StrainUAMH8232]     CTACCTTGAAGCACTACAAGTATTGGCGTCTCAATCCCGATCAAGCTACC 750
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                           ************************** **********************

Lacbi2|456764              AGTGATTACAGGAAATCGCCTTCAGATCCAGTGGCCCTGCTGAAATCAAC 800
JQ585592|[StrainUAMH8232]  AGTGATTACAGGAAATCGCCTTCAGATCCAGTGGCCCTGCTGAAATCAAC 800
                           **************************************************

Lacbi2|456764              TGCGGAAACCTTCATCAATGTCGGAGACGAAGAGACTCGCAATGGGTGTG 850
JQ585592|[StrainUAMH8232]  TGCGGAAACCTTCATCAATGTCGGAGACGAAGAGACTCGCAATGGGTGTG 850
                           **************************************************

Lacbi2|456764              CGTCAAATGAGGAAGGGGTCAGGGCGACGGGCGATGAAAAGTCGAGCAAC 900
JQ585592|[StrainUAMH8232]  CGTCAAATGAGGAAGGGGTCAGGGCGACGGGCGATGAAAAGTCGAGCAAC 900
                           **************************************************

Lacbi2|456764              GCTACCTCGTCACGCACGAATTTCAGCCCGGTCTAA 936
JQ585592|[StrainUAMH8232]  GCGACCTCGTCACGCACGAATTTCAGCCCGGTCTAA 936
                           ** *********************************
```

Note A4.3 Amino acid sequence alignment between Lacbi2:671860 of strain S238N and JQ585593 of strain UAMH8232 in Cluster II.
Identity Score 98.48

```
Lacbi2|671860|[StrainS238N]      MSATPIIHLRDVKKRTGVLNAWERVRNKPQVHWAMECFAEALGVFFYVYF 50
JQ585593|[StrainUAMH8232]        MSATPIIHLRDVKKRTGVLNAWERVRNKPQVHWAMECFAEALGVFFYVYF 50
                                 **************************************************

Lacbi2|671860|[StrainS238N]      GLGSTAAWVIGNILKQSGLSSVFQIGFAYAFGILFAIGVCAATSGGHFNP 100
JQ585593|[StrainUAMH8232]        GLGSTAAWVIGNILKQSGLSSVFQIGFAYAFGILFAIGVCAATSGGHFNP 100
                                 **************************************************

Lacbi2|671860|[StrainS238N]      CVTIAFTIFRGFPPLKAVRYIVAQILGAYIASALVYNQWKVLIVESELLL 150
JQ585593|[StrainUAMH8232]        CVTIAFTIFRGFPPLKAVRYIVAQILGAYIASALVYNQWKVLIVESELLL 150
                                 **************************************************

Lacbi2|671860|[StrainS238N]      KQAGVYETTMFTPNGPAGIFALYLLPGAQTLPRAFLNEFVNCFVLALVIW 200
JQ585593|[StrainUAMH8232]        KQAGVYETTMFTPNGPAGIFALYLLPGAQTLPRAFLNEFVNCFVLALVIW 200
                                 **************************************************

Lacbi2|671860|[StrainS238N]      AALDPTSFMIPPVMAPFIIAAAYAGSIWGYAVPAISLNSARDIGCRLFAL 250
JQ585593|[StrainUAMH8232]        AALDPTSFMIPPVMAPFIIAAAYAGSIWGYAVPAISLNSARDIGCRLFAL 250
                                 **************************************************

Lacbi2|671860|[StrainS238N]      TIWGKSAAGGSYSAITALVNIPATLLAAVVYELFLVDSDRVVAGSHLEFM 300
JQ585593|[StrainUAMH8232]        TIWGKSAAGGSYSAIAALVNIPATLLAAVVYELFLVDSDRVVAGSHLEFM 300
                                 ***************:********************************

Lacbi2|671860|[StrainS238N]      NVAANHRRHRHQAEDDNHGDADDSSQEKPV 330
JQ585593|[StrainUAMH8232]        NVAANHRRHRQQAEDDNLVEADDSSQEKPV 330
                                 **********:******  :**********
```

Note A4.4 Nucleotide sequence alignment between Lacbi2:671860 of strain S238N and JQ585593 of strain UAMH8232 in Cluster II.

```
Lacbi2|671860|[StrainS238N]      ATGTCCGCTACTCCAATCATCCACCTGCGCGACGTGAAAAAGCGCACTGG 50
JQ585593|[StrainUAMH8232]        ATGTCCGCTACTCCAATCATCCACCTGCGCGACGTGAAAAAGCGTACTGG 50
                                 ********************************************* *****

Lacbi2|671860|[StrainS238N]      AGTCTTGAACGCATGGGAGAGGGTACGGAACAAGCCCCAGGTGCACTGGG 100
JQ585593|[StrainUAMH8232]        AGTCTTGAACGCATGGGAGAGGGTACGGAACAAGCCCCAGGTGCACTGGG 100
                                 **************************************************

Lacbi2|671860|[StrainS238N]      CGATGGAGTGTTTCGCTGAAGCTTTGGGCGTCTTTTTCTACGTGTACTTT 150
JQ585593|[StrainUAMH8232]        CGATGGAGTGTTTCGCTGAGGCTTTGGGCGTCTTTTTCTACGTATACTTT 150
                                 ****************** ********************** ******

Lacbi2|671860|[StrainS238N]      GGACTCGGATCTACCGCAGCTTGGGTGATTGGGAACATCTTGAAACAGTC 200
JQ585593|[StrainUAMH8232]        GGACTCGGATCTACCGCAGCTTGGGTGATTGGGAACATCTTGAAACAGTC 200
                                 **************************************************

Lacbi2|671860|[StrainS238N]      TGGGCTCTCCTCTGTCTTCCAGATCGGTTTTGCCTACGCATTTGGCATTT 250
JQ585593|[StrainUAMH8232]        TGGGCTCTCCTCTGTCTTCCAGATCGGTTTCGCCTACGCATTTGGCATTT 250
                                 ****************************** ******************

Lacbi2|671860|[StrainS238N]      TGTTTGCCATCGGTGTATGTGCAGCGACTTCTGGTGGACACTTCAACCCC 300
JQ585593|[StrainUAMH8232]        TGTTTGCCATCGGTGTCTGTGCAGCTACTTCTGGTGGACACTTCAACCCT 300
                                 ***************** ******** ***********************

Lacbi2|671860|[StrainS238N]      TGTGTTACCATCGCATTCACGATATTCAGAGGCTTTCCACCCCTGAAGGC 350
JQ585593|[StrainUAMH8232]        TGCGTTACCATCGCATTCACGATATTCAGAGGTTTTCCACCCCTGAAGGC 350
                                 ** ****************************** *****************

Lacbi2|671860|[StrainS238N]      TGTCAGATATATCGTTGCGCAAATTCTTGGAGCTTACATTGCGTCCGCCC 400
JQ585593|[StrainUAMH8232]        TGTCAGATATATAGTTGCGCAAATTCTTGGAGCTTACATTGCGTCCGCCC 400
                                 ************ ************************************

Lacbi2|671860|[StrainS238N]      TTGTATACAATCAATGGAAGGTCCTTATCGTGGAGTCGGAACTTCTCTTG 450
JQ585593|[StrainUAMH8232]        TTGTATACAATCAATGGAAGGTCCTTATCGTGGAGTCGGAACTTCTCTTG 450
                                 **************************************************

Lacbi2|671860|[StrainS238N]      AAACAAGCTGGCGTCTACGAAACGACGATGTTCACGCCCAATGGTCCGGC 500
JQ585593|[StrainUAMH8232]        AAACAAGCTGGCGTCTACGAAACGACGATGTTCACGCCCAATGGTCCGGC 500
                                 **************************************************

Lacbi2|671860|[StrainS238N]      CGGAATCTTCGCTCTTTATCTTCTTCCTGGAGCGCAAACTTTGCCTCGCG 550
JQ585593|[StrainUAMH8232]        AGGAATCTTCGCTCTTTATCTTCTTCCTGGAGCGCAAACTTTGCCTCGCG 550
                                  **************************************************

Lacbi2|671860|[StrainS238N]      CTTTTCTTAATGAATTCGTTAATTGTTTTGTGCTCGCCTTGGTTATCTGG 600
JQ585593|[StrainUAMH8232]        CTTTCCTTAATGAATTCGTTAATTGTTTTGTGCTCGCCTTGGTTATCTGG 600
                                 **** ******************************************

Lacbi2|671860|[StrainS238N]      GCTGCTCTTGACCCTACTAGTTTCATGATTCCACCCGTTATGGCTCCTTT 650
JQ585593|[StrainUAMH8232]        GCTGCTCTTGACCCTACTAGTTTCATGATTCCACCCGTTATGGCTCCTTT 650
                                 **************************************************

Lacbi2|671860|[StrainS238N]      CATCATCGCTGCGGCATACGCTGGCTCTATCTGGGGTTATGCGGTACCCG 700
JQ585593|[StrainUAMH8232]        CATCATCGCTGCGGCATACGCTGGCTCTATCTGGGGTTATGCGGTTCCCG 700
                                 ********************************************* ****

Lacbi2|671860|[StrainS238N]      CGATTTCTTTGAATTCGGCCCGTGACATTGGTTGCCGTTTGTTCGCATTG 750
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JQ585593|[StrainUAMH8232]       CGATTTCTTTGAATTCGGCCCGTGACATTGGTTGCCGTTTGTTCGCACTG 750
                                ********************************************** **

Lacbi2|671860|[StrainS238N]    ACCATCTGGGGAAAGTCAGCTGCGGGAGGATCCTACTCGGCAATAACGGC 800
JQ585593|[StrainUAMH8232]       ACCATCTGGGGAAAGTCAGCTGCGGGAGGATCCTACTCGGCAATAGCGGC 800
                                ********************************************** ****

Lacbi2|671860|[StrainS238N]    ACTTGTGAACATTCCAGCCACTTTGCTCGCTGCGGTCGTCTATGAACTGT 850
JQ585593|[StrainUAMH8232]       ACTTGTAAATATTCCAGCCACTTTGCTCGCTGCGGTCGTCTATGAGCTGT 850
                                ****** ** ********************************** ****

Lacbi2|671860|[StrainS238N]    TCCTCGTGGATTCTGATCGAGTCGTAGCTGGCTCACATCTTGAGTTCATG 900
JQ585593|[StrainUAMH8232]       TCCTCGTGGATTCTGATCGAGTTGTAGCTGGCTCACATCTTGAGTTCATG 900
                                ********************** ***************************

Lacbi2|671860|[StrainS238N]    AATGTCGCAGCAAACCACCGAAGGCACCGTCATCAGGCCGAGGATGACAA 950
JQ585593|[StrainUAMH8232]       AACGTTGCAGCAAATCACCGAAGGCACCGTCAGCAGGCCGAGGATGACAA 950
                                ** ** ******** ***************** *****************

Lacbi2|671860|[StrainS238N]    TCATGGTGATGCTGATGACTCATCGCAAGAGAAACCTGTATGA 993
JQ585593|[StrainUAMH8232]       CCTTGTCGAAGCTGATGACTCATCGCAAGAGAAGCCTGTATGA 993
                                 * **  ** ********************** *********
```

Note A4.5 Amino acid sequence alignment between Lacbi2:568479 of strain S238N and JQ585594 of strain UAMH8232 in Cluster IV.
Identity Score 93.31

```
Lacbi2|568479|[StrainS238N]      MKLTISHHKCAIRKVMAEFVGVALLVIFGAGTACQVVLSTNP-------S 43
JQ585594|[StrainUAMH8232]        ---------------MAEFVGVALLVIFGAGAACQVILSTNPGVSPSERG 35
                                                ****************:****:*****       .

Lacbi2|568479|[StrainS238N]      SFLSINFGWAIGIATGAWVSAGISGGHINPAITIAMATYRGFPWREVPGY 93
JQ585594|[StrainUAMH8232]        SFLSINFGWAIGIATGAWVSTGMSEGHINPAITIGMATYRGFPWREVPGY 85
                                 ********************:*:* *********.***************

Lacbi2|568479|[StrainS238N]      IFAQALGGFVGAALVYANYFHAIDIFEGGHIRTQATASLFATFALPYMTQ 143
JQ585594|[StrainUAMH8232]        IFGQVLGGFVGAALVYANYFHAIDIFEGGH-RTQATASLFATFALPYMTQ 134
                                 **.*.************************ ******************

Lacbi2|568479|[StrainS238N]      ASCFFSEFLATAVLFIVFLALNDKHNGALTNGLLPFALFILFIGLGASLG 193
JQ585594|[StrainUAMH8232]        ASCFFSEFLATAVLFIVFLALNDKHNGALTNGLLPFALFILFIGLGASLG 184
                                 **************************************************

Lacbi2|568479|[StrainS238N]      MQTGYAVNPARDFGPRLFLAMAGYGKAVFNYRRQYWIWAPIIAPILGAQA 243
JQ585594|[StrainUAMH8232]        MQTGYAVNPARDFGPRLFLAMAGYGKAVFNYRRQYWIWAPIIAPILGAQA 234
                                 **************************************************

Lacbi2|568479|[StrainS238N]      GGLLYDTSIYNGDDSPIKWR 263
JQ585594|[StrainUAMH8232]        GGLLYDTFIYNGDDSPIKWR 254
                                 ******* ************
```

Note A4.6 Nucleotide sequence alignment between Lacbi2:568479 of strain S238N and JQ585594 of strain UAMH8232 in Cluster IV.

```
Lacbi2|568479|[StrainS238N]      ATGAAGTTAACCATCTCTCACCACAAATGTGCAATCCGCAAAGTCATGGC 50
JQ585594|[StrainUAMH8232]        ----------------------------------------------ATGGC 5
                                                                               *****

Lacbi2|568479|[StrainS238N]      CGAATTTGTTGGTGTGGCACTCTTGGTTATCTTTGGCGCGGGGACTGCTT 100
JQ585594|[StrainUAMH8232]        CGAATTTGTTGGTGTGGCACTCTTGGTTATCTTTGGCGCGGGGGCTGCTT 55
                                 ****************************************** ******

Lacbi2|568479|[StrainS238N]      GCCAGGTTGTCCTCTCGACAAATCCAAGC---------------------- 129
JQ585594|[StrainUAMH8232]        GCCAGGTTATCCTCTCGACAAATCCAGGCGTCTCACCCTCCGAACGAGGT 105
                                 ******** ***************** **

Lacbi2|568479|[StrainS238N]      TCGTTTCTTTCGATCAATTTCGGATGGGCAATCGGTATCGCTACGGGTGC 179
JQ585594|[StrainUAMH8232]        TCGTTTCTTTCGATCAATTTCGGATGGGCAATCGGTATCGCTACGGGTGC 155
                                 **************************************************

Lacbi2|568479|[StrainS238N]      CTGGGTCAGCGCTGGCATATCTGGAGGACACATAAACCCCGCAATTACAA 229
JQ585594|[StrainUAMH8232]        CTGGGTCAGCACTGGCATGTCTGAAGGACACATAAACCCTGCAATTACAA 205
                                 ********** ******* **** ************** **********

Lacbi2|568479|[StrainS238N]      TTGCAATGGCGACGTACCGCGGGTTTCCCTGGCGTGAAGTACCCGGCTAT 279
JQ585594|[StrainUAMH8232]        TTGGGATGGCGACGTACCGCGGGTTTCCCTGGCGTGAAGTACCCGGCTAT 255
                                 ***  ******************************************

Lacbi2|568479|[StrainS238N]      ATTTTCGCCCAGGCGTTGGGTGGGTTTGTTGGTGCAGCGCTGGTGTACGC 329
JQ585594|[StrainUAMH8232]        ATCTTCGGCCAGGTGTTGGGTGGGTTTGTTGGTGCAGCACTGGTGTACGC 305
                                 ** **** ***** ************************ ***********

Lacbi2|568479|[StrainS238N]      AAATTATTTCCATGCAATTGATATTTTCGAGGGAGGGCACATCCGCACGC 379
JQ585594|[StrainUAMH8232]        AAATTATTTCCATGCAATTGATATCTTCGAAGGAGGGCA---CCGCACGC 352
                                 ************************ ***** ********   ********

Lacbi2|568479|[StrainS238N]      AAGCCACTGCTTCTCTCTTTGCGACATTTGCTCTGCCGTACATGACACAA 429
JQ585594|[StrainUAMH8232]        AAGCCACTGCTTCTCTCTTTGCGACATTTGCTCTGCCGTACATGACACAA 402
                                 **************************************************

Lacbi2|568479|[StrainS238N]      GCATCATGTTTCTTTTCAGAGTTTTTAGCCACCGCCGTCCTTTTCATCGT 479
JQ585594|[StrainUAMH8232]        GCATCATGTTTCTTTTCAGAGTTTTTAGCCACCGCCGTCCTTTTCATCGT 452
                                 **************************************************

Lacbi2|568479|[StrainS238N]      GTTCTTGGCTCTCAACGATAAGCATAATGGCGCACTCACAAATGGGCTCC 529
JQ585594|[StrainUAMH8232]        GTTCTTGGCTCTCAACGATAAGCATAATGGCGCACTCACAAACGGGCTCC 502
                                 ****************************************** *******

Lacbi2|568479|[StrainS238N]      TACCATTTGCCCTGTTTATTTTGTTCATTGGTCTTGGGGCATCGCTCGGC 579
JQ585594|[StrainUAMH8232]        TACCATTTGCCCTGTTTATTTTGTTCATTGGTCTTGGGGCATCGCTCGGC 552
                                 **************************************************

Lacbi2|568479|[StrainS238N]      ATGCAAACAGGTTATGCCGTCAACCCAGCGAGAGACTTTGGACCGCGCTT 629
JQ585594|[StrainUAMH8232]        ATGCAAACAGGTTATGCCGTCAACCCAGCGAGAGACTTTGGACCGCGCTT 602
                                 **************************************************

Lacbi2|568479|[StrainS238N]      GTTCCTTGCTATGGCAGGCTACGGAAAGGCCGTTTTCAACTATCGCAGAC 679
JQ585594|[StrainUAMH8232]        GTTCCTTGCTATGGCAGGCTACGGAAAGGCCGTTTTCAACTATCGCAGAC 652
                                 **************************************************

Lacbi2|568479|[StrainS238N]      AATATTGGATTTGGGCACCCATAATTGCTCCAATCCTTGGCGCTCAAGCT 729
```

```
JQ585594|[StrainUAMH8232]        AATATTGGATTTGGGCACCCATAATTGCTCCAATCCTTGGCGCTCAAGCC 702
                                 ************************************************

Lacbi2|568479|[StrainS238N]      GGAGGCCTGCTCTATGATACCTCTATATATAATGGAGATGACAGTCCAAT 779
JQ585594|[StrainUAMH8232]        GGAGGCCTGCTCTATGATACCTTTATATATAATGGAGATGACAGTCCAAT 752
                                 ********************** **************************

Lacbi2|568479|[StrainS238N]      CAAGTGGCGGTAG 792
JQ585594|[StrainUAMH8232]        CAAGTGGCGGTAG 765
                                 *************
```

Note A4.7 Amino acid sequence alignment between Lacbi2:443240 of strain S238N and JQ585595 of strain UAMH8232 in Cluster IV.
Identity Score 93.59

```
Lacbi2|443240|[StrainS238N]      MDDKFDDDALPNSKTTPEDYGDKLAEYDYTNTFPNTWMRLREPFREYIAE 50
JQ585595|[StrainUAMH8232]        MDDKFDDDALPNSKTTAKDYEDKLPEYDYTTTFPNTWMRLREPFREYFAE 50
                                 ****************.:** ***.*****.****************:**

Lacbi2|443240|[StrainS238N]      FVGVAVLIIFGVGADCQVVLSANTGVAPSPKGDYLSLNCGWAIGTAMGVW 100
JQ585595|[StrainUAMH8232]        FVGVAVLIIFGVGADCQVVLSANTGVASSPKGSYLSLNCGWAIGTAMGVW 100
                                 ****************************.****.****************

Lacbi2|443240|[StrainS238N]      ISGGISGGHINPAVTLALATWRGFPWRKVPGFLFAQLLGGIVGAGLVYVN 150
JQ585595|[StrainUAMH8232]        ISGGISGGHINPAVTLAMATWRGFPWWKVPGFIFAQLLGGIVGAGLVYVN 150
                                 *****************:******** *****:****************

Lacbi2|443240|[StrainS238N]      YIHAIDIVEGGRHVRTLDTAGLFATYAADHMTNVSCFFSEFLATAVLIVV 200
JQ585595|[StrainUAMH8232]        YIHAIDIVEGGRHIRTLDTAGLFATYAADYMTNLSCFFSEFLATAVLIIV 200
                                 *************:***************:***:**************:*

Lacbi2|443240|[StrainS238N]      IHAMNDKRNAPPPAGLAPLVLFFLILGIGASLGMETGYAINPARDLGPRM 250
JQ585595|[StrainUAMH8232]        IHAMNDKRNTPPPAGIVPFVLFFLILGIGASLGMETGYAINPARDLGPRM 250
                                 *********:*****:.*:********************************

Lacbi2|443240|[StrainS238N]      LTAMVGYGRQVFAFRNQYWIWCPVIAPFLGAQVGTIFYDLFFYKGQDNVF 300
JQ585595|[StrainUAMH8232]        LTAMVGYGRQVFAFRNQYWIWCPVLAPFLGAQVGTIFYDLFFYKGQDNVF 300
                                 ************************:************************

Lacbi2|443240|[StrainS238N]      GRLGSHIHISPA 312
JQ585595|[StrainUAMH8232]        GRLGSHIHISPA 312
                                 ************
```

Note A4.8 Nucleotide sequence alignment between Lacbi2:443240 of strain S238N and JQ585595 of strain UAMH8232 in Cluster IV.

```
Lacbi2|443240|[StrainS238N]      ATGGACGACAAATTCGACGACGACGCCCTCCCCAACTCGAAGACTACGCC 50
JQ585595|[StrainUAMH8232]        ATGGACGACAAATTCGACGACGACGCTCTCCCCAACTCAAAGACTACGGC 50
                                 ************************** ********** ********* *

Lacbi2|443240|[StrainS238N]      TGAGGACTACGGGGACAAGCTCGCAGAATATGATTATACCAACACATTCC 100
JQ585595|[StrainUAMH8232]        TAAGGACTACGAGGACAAGCTCCCAGAATATGATTATACCACCACATTCC 100
                                 * ********* ********** ****************** ********

Lacbi2|443240|[StrainS238N]      CCAATACGTGGATGAGACTACGTGAACCCTTTCGTGAATATATCGCAGAG 150
JQ585595|[StrainUAMH8232]        CCAATACGTGGATGAGACTACGTGAACCCTTTCGTGAATATTTCGCAGAG 150
                                 ************************************** ********

Lacbi2|443240|[StrainS238N]      TTCGTTGGCGTTGCGGTCCTTATCATCTTTGGTGTCGGTGCCGACTGTCA 200
JQ585595|[StrainUAMH8232]        TTCGTTGGTGTTGCGGTCCTTATCATCTTTGGTGTCGGTGCCGACTGTCA 200
                                 ******** ****************************************

Lacbi2|443240|[StrainS238N]      AGTCGTCTTGTCTGCAAACACTGGCGTTGCACCATCTCCGAAAGGTGACT 250
JQ585595|[StrainUAMH8232]        AGTCGTCTTGTCTGCAAACACTGGCGTTGCATCATCTCCGAAAGGTAGCT 250
                                 ******************************** **************  **

Lacbi2|443240|[StrainS238N]      ATCTATCACTGAATTGCGGTTGGGCCATTGGCACGGCTATGGGCGTTTGG 300
JQ585595|[StrainUAMH8232]        ATCTATCACTGAATTGCGGTTGGGCCATTGGCACAGCTATGGGCGTTTGG 300
                                 ********************************** ***************

Lacbi2|443240|[StrainS238N]      ATCTCGGGCGGAATTTCAGGTGGTCACATTAATCCTGCCGTAACGTTGGC 350
JQ585595|[StrainUAMH8232]        ATCTCGGGCGGAATTTCAGGCGGTCATATTAACCCTGCTGTAACACTGGC 350
                                 ******************** ***** ***** ***** *****  ****

Lacbi2|443240|[StrainS238N]      GTTGGCGACATGGCGCGGCTTCCCATGGAGGAAAGTTCCCGGTTTCCTCT 400
JQ585595|[StrainUAMH8232]        GATGGCGACATGGCGCGGCTTCCCATGGTGGAAAGTTCCCGGTTTCATTT 400
                                 * ************************** ***************** * *

Lacbi2|443240|[StrainS238N]      TCGCTCAGCTGCTAGGTGGTATCGTCGGAGCTGGACTGGTCTATGTGAAT 450
JQ585595|[StrainUAMH8232]        TCGCTCAGCTCCTAGGCGGAATAGTCGGAGCTGGACTGGTCTATGTGAAT 450
                                 ********** ***** ** ** ****************************

Lacbi2|443240|[StrainS238N]      TACATTCACGCCATTGATATCGTAGAAGGCGGCCGCCATGTTCGAACACT 500
JQ585595|[StrainUAMH8232]        TACATTCACGCCATTGATATCGTAGAAGGCGGCCGCCATATCCGAACCCT 500
                                 ************************************ * ***** **

Lacbi2|443240|[StrainS238N]      CGATACCGCTGGATTGTTCGCAACGTATGCGGCTGATCACATGACCAACG 550
JQ585595|[StrainUAMH8232]        CGATACCGCTGGATTGTTCGCAACGTATGCAGCTGATTACATGACGAACT 550
                                 ******************************** ****** ******* ***

Lacbi2|443240|[StrainS238N]      TGTCCTGCTTTTTCTCGGAGTTCCTCGCTACTGCCGTGCTTATCGTCGTC 600
JQ585595|[StrainUAMH8232]        TGTCTTGCTTTTTCTCAGAGTTCCTCGCTACTGCCGTGCTTATCATCGTC 600
                                 **** ********** ***************************** *****

Lacbi2|443240|[StrainS238N]      ATCCACGCCATGAACGACAAGAGAAATGCCCCTCCTCCAGCCGGCCTCGC 650
JQ585595|[StrainUAMH8232]        ATCCACGCGATGAACGACAAGAGAAACACCCCTCCTCCAGCTGGCATCGT 650
                                 ******** *****************  ************* *** ***

Lacbi2|443240|[StrainS238N]      GCCATTGGTTCTCTTCTTCCTTATCCTTGGTATCGGTGCATCCCTTGGAA 700
JQ585595|[StrainUAMH8232]        ACCATTTGTTCTCTTCTTCCTTATCCTTGGTATCGGTGCATCCCTTGGAA 700
                                  ***** ****************************************

Lacbi2|443240|[StrainS238N]      TGGAAACAGGTTATGCTATCAACCCCGCTCGTGATCTTGGCCCCCGCATG 750
```

```
JQ585595|[StrainUAMH8232]        TGGAAACAGGTTACGCCATTAACCCCGCTCGTGATCTTGGCCCCCGCATG 750
                                 ************* ** ** ****************************

Lacbi2|443240|[StrainS238N]     CTCACTGCTATGGTTGGCTATGGAAGACAGGTATTCGCTTTCCGGAATCA 800
JQ585595|[StrainUAMH8232]        CTCACTGCTATGGTTGGCTATGGAAGACAGGTTTTCGCTTTCCGGAATCA 800
                                 ******************************** *****************

Lacbi2|443240|[StrainS238N]     ATATTGGATCTGGTGTCCAGTCATTGCCCCGTTCCTGGGTGCTCAAGTTG 850
JQ585595|[StrainUAMH8232]        ATATTGGATCTGGTGTCCAGTTCTTGCCCCATTCCTGGGCGCTCAGGTTG 850
                                 **********************  ******* ******** ***** ****

Lacbi2|443240|[StrainS238N]     GAACGATTTTCTATGACCTGTTCTTCTACAAAGGACAAGATAATGTTTTC 900
JQ585595|[StrainUAMH8232]        GAACGATCTTCTATGACCTGTTCTTCTACAAAGGACAAGATAATGTTTTC 900
                                 ******* ****************************************

Lacbi2|443240|[StrainS238N]     GGGCGATTAGGGTCACACATACACATCTCCCCAGCTTAA 939
JQ585595|[StrainUAMH8232]        GGGCGATTAGGGTCACACATACACATCTCCCCAGCTTAA 939
                                 ************************************
```

Note A4.9 Amino acid sequence alignment of Lacbi2:317173 and Lacbi2:576801 in strain S238N, and JQ585596 and JQ585597 in strain UAMH8232 in Cluster IV.
317173 VS. JQ585596 Identity Score 90.06
317173 VS. JQ585597 Identity Score 94.88
JQ585596 VS. JQ585597 Identity Score 91.27

```
JQ585597|[StrainUAMH8232]       MSGQHQITEQPSGNPLSRTSTLIQEKPLTPTSSHAGTQKQPEAPRQPTFL 50
Lacbi2|317173|[StrainS238N]     MSGQHQITEQSSRNPLSRVSTLLPEKPLSPTSTYAGTQKHPEAPRQSSFL 50
JQ585596|[StrainUAMH8232]       MSGQHQITEQPSGNPLSRTSTLIQEKPLTPTSSHAETQKHLEAPRQSSFL 50
Lacbi2|576801|[Laccaria         ------------------------------------------------M 1
                                                                                :

JQ585597|[StrainUAMH8232]       IQLQNIRHAIRKPMAEFFGVALLIIFGAGSACQVVLSTNPDVASSARGSF 100
Lacbi2|317173|[StrainS238N]     IQLQNIRNAIRKPMAEFFGVALLIIFGAGSACQVVLSTNPDVASSARGSF 100
JQ585596|[StrainUAMH8232]       IQLQDIRHAIRMPMAEFFGVALLIIFGAGSACQVVLSTNPNVASSDRGSF 100
Lacbi2|576801|[Laccaria         FTLAHHRHAIRKPMAEFFGVALLVIFGAGAACQVVLSTNPN-------SF 44
                                : * . *:*** **********:*****:**********:       **

JQ585597|[StrainUAMH8232]       LSINFGWAIGIAMGVWVSGGISGGHINPAITIAMATYRGFPWCKVPSYIL 150
Lacbi2|317173|[StrainS238N]     LSINFGWAIGIAMGVWVSGGISGGHINPAITIAMATYRGFPWRKVPSYIL 150
JQ585596|[StrainUAMH8232]       LSINLGWAIGIAMGAWVSGGISGGHINPAITIAMATYRGFPWRRVPSYIF 150
Lacbi2|576801|[Laccaria         LSINFGWAIGIAMGAWISGSISGGHINPAITIAMATYRGFPWREVPSYIL 94
                                ****:*********.*:**.********************** .*****:

JQ585597|[StrainUAMH8232]       AQVLGGVVGAALVYANYIHAIDVFEGGHHIRTEATASLFATYALPYMTQA 200
Lacbi2|317173|[StrainS238N]     AQVLGGVVGAGLVYANYIHAIDIFEGGHHIRTQATASLFATYALPYMTQA 200
JQ585596|[StrainUAMH8232]       AQVLGGVVGAALVYANYIHAIDIFEGGRHVRTQATASLFATYALPYMTQV 200
Lacbi2|576801|[Laccaria         AQVLGGVVGAALVYANYIHAIDVFEGGRHIRTQATASLFATYALPYMTQV 144
                                **********.**********:****:*:**:****************.

JQ585597|[StrainUAMH8232]       SCFFSEFLATAVLSMMVFALTDKRNHSPTNGLLPFALFILFVGLGASLGM 250
Lacbi2|317173|[StrainS238N]     SCFFSEFLATAVLSMMVFALTDKRNHSPTNGLLPFALFILFVGLGASLGM 250
JQ585596|[StrainUAMH8232]       SCFFSEFLATAVLSMMVLALTDNRNGAPTNGLLPFALFVLFIGLGASLGM 250
Lacbi2|576801|[Laccaria         SCFFSEFLATAVLAMMVLALTDNRNGAPTNGLSPFALFVLFIGLGASLGM 194
                                *************:***:****:** :***** *****:**:********

JQ585597|[StrainUAMH8232]       ETAYALNPARDFGPRLFLAMAGYGKALFNYRSQYWLWAPIIAPVLGAQAG 300
Lacbi2|317173|[StrainS238N]     ETAYALNPARDFGPRLFLAMAGYGKALFNYRSQYWLWAPIIAPVLGAQAG 300
JQ585596|[StrainUAMH8232]       ETAYALNPARDFGPRLFLAMSGYGKALFNYRSQYWLWAPIIAPVLGAQAG 300
Lacbi2|576801|[Laccaria         ETAYALNPARDFGPRLFLAMAGYGKALFNYRSQYWLWAPIIAPVLGAQAG 244
                                ********************:******************************

JQ585597|[StrainUAMH8232]       GLLYDTFLNDGDNSPIKWRCASSQEQQLAEVV 332
Lacbi2|317173|[StrainS238N]     GLLYDTFLNDGDNSPIKWRCASSQEHQLAEVV 332
JQ585596|[StrainUAMH8232]       GLLYDTFLYDGDNSPIKWRRASSQECQLAEVV 332
Lacbi2|576801|[Laccaria         GLLYDTFLYDGDDSPIKWR------------- 263
                                ******** ***:******
```

Note A4.10 Nucleotide sequence alignment of Lacbi2:317173 and Lacbi2:576801 in strain S238N, and JQ585596 and JQ585597 in strain UAMH8232 in Cluster IV.

```
JQ585596|[StrainUAMH8232]       ATGTCTGGCCAACATCAGATCACTGAGCAACCGTCTGGAAACCCACTCTC 50
Lacbi2|576801|[StrainS238N]     ATGT---------------TCACT----------TTGG-------CTCAC 18
Lacbi2|317173|[StrainS238N]     ATGTCTGGCCAACATCAGATCACCGAGCAATCGTCTCGAAACCCACTCTC 50
JQ585597|[StrainUAMH8232]       ATGTCTGGCCAACATCAGATCACTGAGCAACCGTCTGGAAACCCACTCTC 50
                                ****               ****           * *       *** *

JQ585596|[StrainUAMH8232]       CAGAACTTCTACACTTATTCAAGAGAAACCGCTGACTCCCACATCGTCTC 100
Lacbi2|576801|[StrainS238N]     CA------------------------------------------------ 20
Lacbi2|317173|[StrainS238N]     CAGGGTTTCTACACTCCTCCCCGAGAAACCGCTGAGCCCCACATCGACCT 100
JQ585597|[StrainUAMH8232]       CAGAACTTCTACACTTATTCAAGAGAAACCGCTGACTCCCACATCGTCTC 100
                                **

JQ585596|[StrainUAMH8232]       ACGCTGAGACTCAAAAACATCTCGAGGCCCCTCGACAGTCTTCTTTTCTT 150
Lacbi2|576801|[StrainS238N]     ------------------------------------------------
Lacbi2|317173|[StrainS238N]     ACGCTGGGACACAAAAACATCCAGAGGCCCCTCGACAGTCTTCTTTTCTT 150
JQ585597|[StrainUAMH8232]       ACGCTGGGACTCAAAAACAGCCCGAGGCCCCTCGACAACCTACTTTTCTT 150


JQ585596|[StrainUAMH8232]       ATCCAACTGCAAGATATTAGGCATGCAATCCGCATGCCCATGGCCGAATT 200
Lacbi2|576801|[StrainS238N]     -----------------CAGGCATGCAATCCGCAAGCCCATGGCTGAATT 53
Lacbi2|317173|[StrainS238N]     ATCCAACTGCAAAATATTAGGAATGCGATCCGCAAGCCCATGGCCGAATT 200
JQ585597|[StrainUAMH8232]       ATCCAACTGCAAAATATTAGGCATGCAATCCGCAAGCCAATGGCCGAATT 200
                                          *** **** ******* *** ***** *****

JQ585596|[StrainUAMH8232]       TTTCGGTGTGGCGCTCTTGATCATTTTCGGTGCAGGGTCTGCCTGCCAGG 250
Lacbi2|576801|[StrainS238N]     TTTCGGTGTGGCGCTCTTGGTCATTTTTGGTGCAGGGGCTGCCTGCCAGG 103
Lacbi2|317173|[StrainS238N]     TTTCGGTGTGGCGCTCTTGATCATTTTTGGTGCAGGGTCTGCCTGCCAGG 250
JQ585597|[StrainUAMH8232]       TTTTGGTGTGGCGCTCTTGATCATTTTCGGTGCAGGGTCTGCCTGCCAGG 250
                                *** *************** ******* ********* ************

JQ585596|[StrainUAMH8232]       TTGTACTCTCGACAAATCCAAACGTCGCATCATCTGATCGAGGTTCATTT 300
Lacbi2|576801|[StrainS238N]     TTGTACTCTCGACAAATCCAAAC--------------------TCGTTT 132
Lacbi2|317173|[StrainS238N]     TTGTACTCTCGACAAATCCGGACGTTGCGTCATCTGCTCGAGGTTCGTTT 300
JQ585597|[StrainUAMH8232]       TTGTGCTCTCTACAAATCCGGACGTCGCGTCATCTGCTCGAGGTTCGTTT 300
                                **** ***** ********  **                    ** ***

JQ585596|[StrainUAMH8232]       CTCTCCATAAACCTCGGATGGGCCATCGGTATTGCCATGGGTGCCTGGGT 350
Lacbi2|576801|[StrainS238N]     CTCTCTATAAATTTCGGATGGGCCATCGGTATTGCGATGGGTGCCTGGAT 182
Lacbi2|317173|[StrainS238N]     CTCTCCATAAATTTCGGATGGGCCATCGGTATTGCGATGGGTGTTTGGGT 350
JQ585597|[StrainUAMH8232]       CTCTCCATAAATTTCGGATGGGCCATCGGTATTGCTATGGGTGTCTGGGT 350
                                ***** *****  ********************** *******  *** *

JQ585596|[StrainUAMH8232]       CAGCGGCGGCATCTCTGGAGGACACATTAACCCTGCGATAACAATCGCAA 400
Lacbi2|576801|[StrainS238N]     CAGCGGCAGCATCTCTGGAGGACACATTAACCCTGCGATAACCATCGCAA 232
Lacbi2|317173|[StrainS238N]     CAGCGGCGGCATCTCCGGAGGACACATTAACCCTGCAATTACCATCGCAA 400
JQ585597|[StrainUAMH8232]       CAGCGGCGGCATCTCTGGAGGACACATTAACCCTGCAATTACCATCGCAA 400
                                ******* ******* ******************** ** ** *******

JQ585596|[StrainUAMH8232]       TGGCGACTTATCGCGGCTTTCCTTGGCGTAGAGTGCCCAGCTACATCTTC 450
Lacbi2|576801|[StrainS238N]     TGGCGACTTACCGCGGCTTTCCTTGGCGTGAAGTGCCCAGCTACATCCTC 282
Lacbi2|317173|[StrainS238N]     TGGCGACCTACCGCGGCTTTCCTTGGCGTAAAGTGCCCAGCTACATTCTT 450
JQ585597|[StrainUAMH8232]       TGGCGACCTACCGCGGCTTTCCTTGGTGTAAAGTGCCCAGCTACATCCTC 450
                                ******* ** *************** **  ***************  *
JQ585596|[StrainUAMH8232]       GCCCAGGTGTTAGGTGGGGTCGTTGGTGCCGCGCTGGTATACGCGAATTA 500
Lacbi2|576801|[StrainS238N]     GCCCAGGTGTTAGGTGGGGTCGTCGGTGCCGCACTGGTATACGCGAATTA 332
Lacbi2|317173|[StrainS238N]     GCCCAAGTGTTGGGCGGGGTCGTCGGTGCCGGGCTGGTATACGCGAATTA 500
JQ585597|[StrainUAMH8232]       GCCCAAGTGTTGGGTGGGGTCGTCGGTGCCGCACTGGTATACGCGAATTA 500
                                ***** ***** ** ******** *******  ******************
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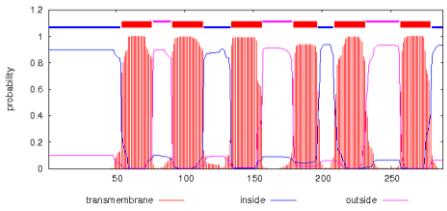
```
JQ585596|[StrainUAMH8232]       TATCCATGCAATCGATATCTTTGAAGGCGGACGTCACGTCCGCACCCAAG 550
Lacbi2|576801|[StrainS238N]     TATCCACGCAATCGATGTCTTCGAAGGCGGACGTCACATCCGCACCCAAG 382
Lacbi2|317173|[StrainS238N]     TATCCATGCAATCGACATCTTCGAAGGCGGGCATCACATCCGCACCCAAG 550
JQ585597|[StrainUAMH8232]       TATCCATGCAATCGACGTCTTCGAAGGCGGGCACCACATCCGCACAGAAG 550
                                ****** ********  **** ******** *  *** *******  ***

JQ585596|[StrainUAMH8232]       CTACTGCTTCTCTCTTCGCAACGTACGCTCTGCCATACATGACACAAGTA 600
Lacbi2|576801|[StrainS238N]     CTACCGCTTCCCTCTTCGCAACGTACGCTCTGCCGTACATGACGCAAGTA 432
Lacbi2|317173|[StrainS238N]     CTACCGCTTCCCTCTTCGCGACGTATGCTCTGCCATACATGACGCAAGCA 600
JQ585597|[StrainUAMH8232]       CTACCGCTTCCCTTTTCGCGACGTATGCTCTGCCGTACATGACGCAAGCA 600
                                **** ***** ** ***** ***** ******** ******** **** *

JQ585596|[StrainUAMH8232]       TCATGTTTCTTTTCGGAATTCTTGGCCACCGCCGTTCTTTCTATGATGGT 650
Lacbi2|576801|[StrainS238N]     TCTTGTTTCTTTTCGGAATTCCTGGCCACCGCCGTTCTGGCTATGATGGT 482
Lacbi2|317173|[StrainS238N]     TCATGTTTCTTTTCGGAATTCTTGGCCACCGCCGTTCTTTCTATGATGGT 650
JQ585597|[StrainUAMH8232]       TCATGTTTCTTTTCGGAATTCTTAGCCACCGCCGTTCTTTCTATGATGGT 650
                                ** ****************** * **************  **********

JQ585596|[StrainUAMH8232]       TTTGGCCCTCACCGATAACCGCAATGGCGCTCCGACAAATGGGCTTTTAC 700
Lacbi2|576801|[StrainS238N]     TTTGGCCCTCACCGATAACCGTAATGGCGCTCCGACAAATGGGCTTTCAC 532
Lacbi2|317173|[StrainS238N]     TTTCGCCCTCACTGACAAACGTAATCACTCTCCGACAAATGGGCTTTTGC 700
JQ585597|[StrainUAMH8232]       TTTCGCCCTCACTGACAAACGTAATCACTCTCCGACAAATGGGCTTTTGC 700
                                *** ******** ** ** ** ***  * ******************  *

JQ585596|[StrainUAMH8232]       CATTTGCACTATTTGTTTTGTTCATCGGCCTTGGGGCGTCGCTCGGCATG 750
Lacbi2|576801|[StrainS238N]     CATTTGCACTATTTGTTTTGTTCATTGGCCTTGGGGCGTCGCTCGGCATG 582
Lacbi2|317173|[StrainS238N]     CATTTGCGCTCTTTATTTTGTTCGTCGGCCTTGGGGCGTCGCTCGGCATG 750
JQ585597|[StrainUAMH8232]       CATTTGCGCTCTTTATTTTGTTCGTCGGCCTTGGGGCATCACTCGGCATG 750
                                ******* ** *** ******** * ********** ** *********

JQ585596|[StrainUAMH8232]       GAAACAGCGTACGCCCTCAATCCTGCGCGAGACTTTGGACCACGCTTGTT 800
Lacbi2|576801|[StrainS238N]     GAAACAGCGTACGCCCTCAATCCTGCGCGAGACTTTGGACCACGCTTGTT 632
Lacbi2|317173|[StrainS238N]     GAAACAGCGTACGCCCTCAACCCCGCGCGAGACTTTGGACCACGCTTGTT 800
JQ585597|[StrainUAMH8232]       GAAACAGCGTACGCCCTCAACCCCGCGCGAGACTTTGGACCACGCTTGTT 800
                                ******************** ** *************************

JQ585596|[StrainUAMH8232]       CCTCGCTATGTCAGGTTACGGAAAGGCTCTCTTCAACTATCGCAGTCAAT 850
Lacbi2|576801|[StrainS238N]     CCTCGCTATGGCAGGTTACGGAAAAGCTCTCTTCAACTATCGCAGTCAAT 682
Lacbi2|317173|[StrainS238N]     CCTTGCTATGGCAGGTTACGGAAAAGCTCTCTTCAACTATCGCAGTCAAT 850
JQ585597|[StrainUAMH8232]       CCTTGCTATGGCAGGTTACGGAAAAGCTCTCTTCAACTATCGCAGTCAAT 850
                                *** ****** ************* *************************

JQ585596|[StrainUAMH8232]       ATTGGCTCTGGGCACCCATTATTGCTCCGGTCCTTGGCGCTCAGGCTGGA 900
Lacbi2|576801|[StrainS238N]     ATTGGCTTTGGGCACCCATTATTGCTCCGGTCCTTGGCGCTCAGGCTGGA 732
Lacbi2|317173|[StrainS238N]     ATTGGCTTTGGGCACCTATTATTGCTCCGGTCCTTGGCGCTCAGGCTGGA 900
JQ585597|[StrainUAMH8232]       ATTGGCTTTGGGCACCCATCATTGCTCCAGTTCTTGGCGCTCAGGCTGGA 900
                                ******* ******** ** ******** ** ******************

JQ585596|[StrainUAMH8232]       GGCTTACTTTATGACACCTTTTTATACGATGGAGATAACAGCCCCATCAA 950
Lacbi2|576801|[StrainS238N]     GGCTTGCTCTACGATACCTTTTTATACGATGGAGATGACAGCCCCATCAA 782
Lacbi2|317173|[StrainS238N]     GGCTTACTCTATGATACCTTTTTAAACGATGGAGATAACAGCCCCATCAA 950
JQ585597|[StrainUAMH8232]       GGCTTACTCTATGATACCTTTTTGAACGATGGAGATAACAGCCCCATCAA 950
                                ***** ** ** ** ********  *********** *************

JQ585596|[StrainUAMH8232]       ATGGCGCCGCGCTTCCTCGCAAGAATGCCAGCTCGCTGAGGTTGTTTGA 999
Lacbi2|576801|[StrainS238N]     ATGGCG------------GTGA-------------------------- 792
Lacbi2|317173|[StrainS238N]     ATGGCGCTGTGCTTCTTCGCAAGAGCACCAGCTCGCTGAGGTTGTTTGA 999
JQ585597|[StrainUAMH8232]       ATGGCGCTGTGCTTCTTCGCAAGAGCAACAGCTCGCTGAGGTTGTTTGA 999
                                ******            *  *
```

APPENDIX 5 *In silico* protein secondary structure prediction on deduced amino acids of putative PIPs of *Picea glauca* analyzed in this study

The NPA motifs were underlined.

>GQ03610_A06.1
MEGKEEDVRLGANKYSERQPLGTAAQTREKDYKDSGPAPLFEPGELASWSFW
RAGIAEFMATFLFLYITILTVMGVKRSDDVCTGSVGIQGIAWAFGGMIFCLVYC
TAGISGGHINPAVTFGLFLARKLSLPRAVFYMICQCLGAICGAGVVKGFMESEY
EMDGGGANSVAHGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHVPM
LAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIYNKSHAWDDHWIFWVGPFL
GAGLAAFYHQMIIRAIPFKSRS*
Length: 288; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 128.51922; Exp number, first 60 AAs: 6.66533; Total prob of N-in: 0.89913; Inside 1-53; TMhelix 54-76; Outside 77-90; TMhelix 91-113; Inside 114-133; TMhelix 134-156; Outside 157-178; TMhelix 179-196; Inside 197-208; TMhelix 209-231; Outside 232-256; TMhelix 257-279; Inside 280-288



>GQ03401_M18.1
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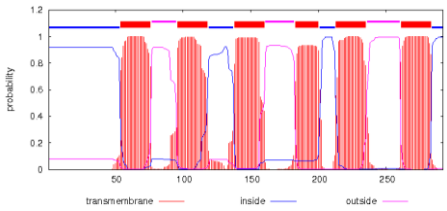
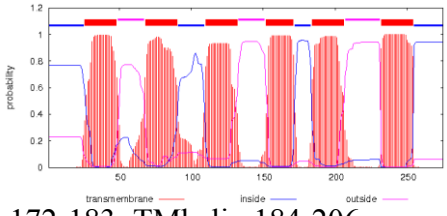
RAGIAEFMATFLFLYITILTVMGVKRSDNGSDGVCTGSVGIQGIAWAFGGMIFC
LVYCTAGISGGHINPAVTFGLFLARKLSLPRAVFYMVCQCLGAICGAGVVKGF
MESEYQMDGGGANVVAPGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARD
SHVPLLAPLPIGFAVFLVHLATIPITGTGINPARSLGAAIIYNRDHAWDDMWIF
WVGPFIGAALAAFYHVIIRAIPFKTRS*
Length: 292; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 130.07592; Exp number, first 60 AAs: 6.79745; Total prob of N-in: 0.91826; Inside 1-53; Mhelix 54-76; Outside 77-95; TMhelix 96-118; Inside 119-137; TMhelix 138-160; Outside 161-182; TMhelix 183-200; Inside 201-212; TMhelix 213-235; Outside 236-260; TMhelix 261-283; Inside 284-292;

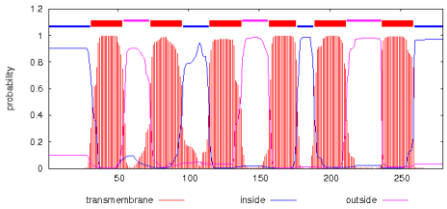


>GQ03010_E09.1
MEMEGGDYEEHPPAPLLDSLELKLWSFYRAVIAEFVATLLFLYITMTTVVENK
QSKGTCGGVGLLGEAWAFGGMIFVLVYCISGISGGHVNPAVTFALFLARKVSL
PRAVLYVVAQCLGAVCGTALVKGIQGSFYASNGGGSNSVSPGYSKGSALLAEI
IGTFVLVYTVFSATDPKRKARDSHVPVLAPLPIGFAVFSIYLATNSITGTGINPA
RSFGPAVIYGHKKSRDDLWIFWIGPLIGAAVATAYHRYLLRAGAFGSKNLGSL
RSQPASAI*
Length: 275; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 130.18762; Exp number, first 60 AAs: 21.69684; Total prob of N-in: 0.76810; Inside 1-25; TMhelix 26-48; Outside 49-67; TMhelix 68-90; Inside 91-109; TMhelix 110-132; Outside 133-151; TMhelix 152-171; Inside 172-183; TMhelix 184-206;



Outside 207-231; TMhelix 232-254; Inside 255-275

>GQ03002_G07.1
MEMEGGEEQTRDYEEHPPAPLLDSLELKLWSFYRAVIAEFVATLLFLYITMTT
VVENKQSKGTCGGVGLLGEAWAFGGMIFVLVYCISGISGGHVNPAVTFALFL
ARKVSLPRAVLYVVAQCLGAVCGTALVKGIQGSFYASNGGGSNSVSPGYSKG
TALLAEIIGTFVLVYTVFSATDPKRKARDSHVPVLAPLPIGFAVFLVHLATIPITG
TGINPARSFGPAVIYGHEKSWDDLWIFWVGPLIGAAVAAAHHQYVLKASGFG
LKNLGSLRSHPASAT*



Length: 280; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 131.162; Exp number, first 60 AAs: 21.39786; Total prob of N-in: 0.90159; Inside 1-30; TMhelix 31-53; Outside 54-72; TMhelix 73-95; Inside 96-114; TMhelix 115-137; Outside 138-156; TMhelix 157-176; Inside 177-188; TMhelix 189-211; Outside 212-236; TMhelix 237-259; Inside 260-280

>GQ03001_P18.1
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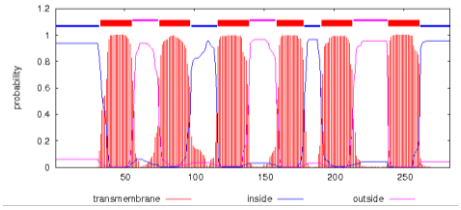
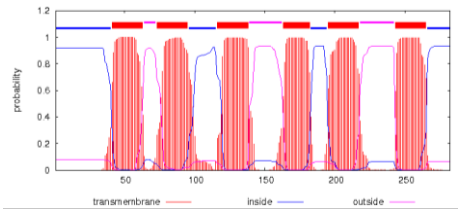
TVVENNRNKVNCSGVGLLGEAWAFGGMIFVLVYCISGISGGHVNPAVTFALFL
ARKVSLPRAVLYIVAQCLGALCGTALVRGIQGSFYASTGGGSNSVSAGYSKGS
ALLAEIIGTFVLVYTVFSATDPKRNARDSHIPVLAPLPIGFAVFLVHLATIPITGT
SINPARSFGPAVIYGHKKSWDDLWIFWVGPLVGAAIAAAYHQYVLRAGGLGL
KSLRSFRSQPTSLAI*



ength: 283; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 131.76741; Exp number, first 60 AAs: 21.34561; Total prob of N-in: 0.93711; Inside 1-32; TMhelix 33-55; Outside 56-77; TMhelix 75-97; Inside 98-116; TMhelix 117-139; Outside 140-158; TMhelix 159-178; Inside 179-190; TMhelix 191-213; Outside 214-238; TMhelix 239-261; Inside 262-283

>GQ03111_E12.1
MTKEERRESEQQGFAPKDYTDPPPAALIETSEFKLWSFYRALIAEFVATLLFLYI
TIATVIGHSRTSTNCGSVGVLGIAWSFGGMIFVLVYCTAGISGGHINPAVTFGLF
LARKVSLPRAILYMIAQCLGAICGTGLVKAFQKSFYDRYGGGANYVHHGYTK
GVGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPI
TGTGINPARSFGAAVIYGHKQSWDDHWIFWVGPFAGAALAAAYHQYILRAAA
IKALGSFRSNANV*



Length: 282; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 131.93621; Exp number, first 60 AAs: 20.17461; Total prob of N-in: 0.91991; Inside 1-40; TMhelix 41-63; Outside 64-72; TMhelix 73-95; Inside 96-115; TMhelix 116-138; Outside 139-162; TMhelix 163-182; Inside 183-194; TMhelix 195-217; Outside 218-242; TMhelix 243-265; Inside 266-282

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GVGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPI
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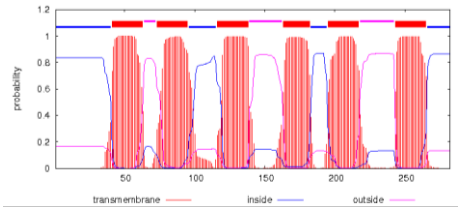

Length: 282; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 132.20968; Exp number, first 60 AAs: 20.38604; Total prob of N-in: 0.83345; Inside 1-40; TMhelix 41-63; Outside 64-72; TMhelix 73-95; Inside 96-115; TMhelix 116-138; Outside 139-162; TMhelix 163-182; Inside 183-194; TMhelix 195-217; Outside 218-242; TMhelix 243-265; Inside 266-282

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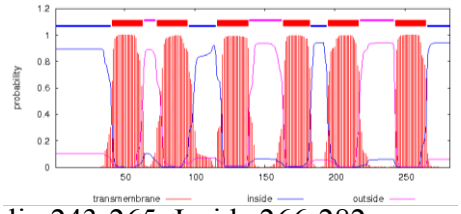

ength: 282; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 131.8592; Exp number, first 60 AAs: 20.19079; Total prob of N-in: 0.89396; Inside 1-40; TMhelix 41-63; Outside 64-72; TMhelix 73-95; Inside 96-115; TMhelix 116-138; Outside 139-162; TMhelix 163-182; Inside 183-194; TMhelix 195-217; Outside 218-242; TMhelix 243-265; Inside 266-282

>GQ02905_E13.1
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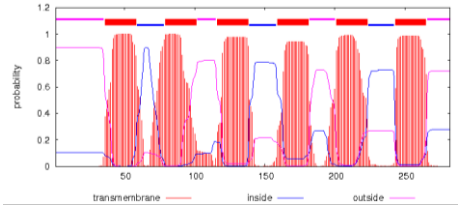

Length: 282; Number of predicted TMHs: 6; Exp number of AAs in TMHs: 133.00058; Exp number, first 60 AAs: 21.29549; Total prob of N-in: 0.10292; Inside 1-35; TMhelix 36-58; Outside 59-78; TMhelix 79-101; Inside 102-115; TMhelix 116-138; Outside 139-158; TMhelix 159-181; Inside 182-200; TMhelix 201-223; Outside 224-242; TMhelix 243-265; Inside 266-282

APPENDIX 6 Analysis of RNAi strains

Because the gene knockdown effect was more pronounced in RNAi1 and RNAi2 strains than in other RNAi strains, they were chosen for further studies (Fig. A6.1). In Southern blot assay, one clear, strong signal of hybridization band using a labeled probe targeting the *hph* gene indicates single copy insertion in the genomic DNA of transgenic strains. It was confirmed that the mock, RNAi1 and RNAi2 strains each harbored a random single insertion of the transgenic cassette, indicated by a single clear hybridization band in Lane 3, 4 and 5, respectively (Fig. A6.2).

No obvious difference in phenotypes was observed between WT, mock and the two RNAi strains at either 20°C or 5°C (Fig. A6.3). Growth at 20°C was significantly faster than at 5°C for both RNAi strains. The dry mass of all the strains was significantly higher at 20°C than at 5°C, but the difference between the strains was not significant at either 20°C or 5°C (Fig. A6.4). The values of Ψ_{turgor} were not significantly different at 20°C and 5°C between WT and two RNAi strains, with exception that at 20°C RNAi2 strain had higher Ψ_{turgor}. (Fig. A6.5).

JQ585595 was the highest expressed MIP in different mycorrhizal root tips at both temperatures. The transcript abundance of JQ585592, JQ585593, JQ585594 and JQ585595 was upregulated at 5°C, whereas JQ585596 and JQ585597 maintained at the same level. Except JQ585594 in RNAi1 at 20°C, and JQ585595 in RNAi2 at 5°C, there was no significant difference in fungal MIP transcript abundance between WT- and RNAi-inoculated root tips (Fig. A6.6). The similar transcript abundance of JQ585595 in WT- and RNAi-inoculated root tips at 20°C showed that the expression of this target gene was not efficiently suppressed in RNAi mycorrhizal tissues, indicating the knockdown effect of RNA interference transgenic construct, as observed in mycelia grown for three weeks on MMN medium, was compensated in the dikaryotic strain mycorrhizated *in vivo* with *P. glauca* roots for months. Gene suppression is particularly challenging in dikaryotic fungi, particularly in case that the transcript abundance of the target gene is high and it tends to be upregulated upon mycorrhization. Consequently, RNAi strains caused an increase in L_{pr} compared with non-inoculated seedlings, but did not cause significant difference in L_{pr} compared with WT at 20°C, 10°C and 5°C (Fig. A6.7). Like WT strain, RNAi strains contributed to the stability of L_{pr} when root temperature dropped to 10°C and 5°C (Fig. A6.7).

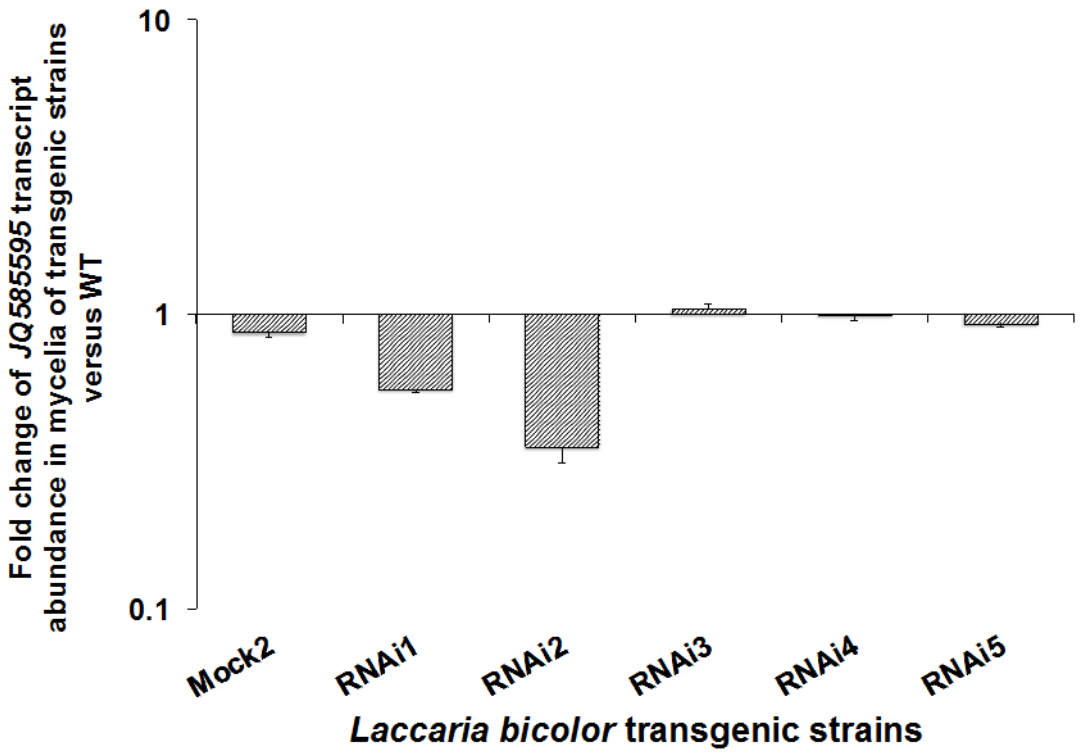

Figure A6.1 Transcript abundance of *JQ585595* in mycelia of *Laccaria bicolor* transgenic RNAi strains. Transcript abundance of *JQ585595* was quantified in transgenic *L. bicolor* mycelia grown on solid MMN medium at 20°C for three weeks using the standard curve method of absolute quantification of qRT-PCR assay. Transcript abundance of *JQ585595* was normalized to the geometric mean of that of reference genes *EF2* and *α-tubulin*. Transcript abundance in transgenic strains was compared with that in wild type (WT)(n = 3 ± SE).

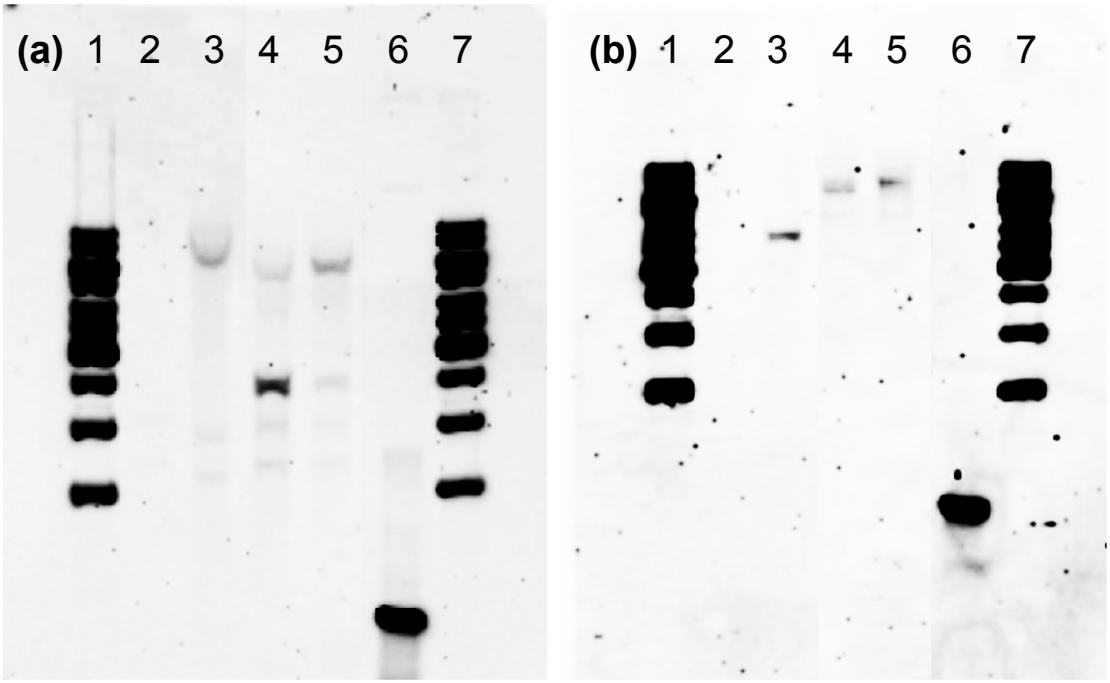

Figure A6.2 Southern blot analysis of *Laccaria bicolor* genomic DNA digested by restriction enzymes *Sac*I (a) or *Bam*HI (b). DNA ladder and digested genomic DNA were loaded as described below: Lane 1 and 7 for 1Kb DNA ladder (GeneRuler™; Fermentas); Lane 2 for WT; Lane 3 for mock; Lane 4 for RNAi1, Lane 5 for RNAi2; Lane 6 for 0.2 ng of 870 bp PCR amplicon of hygromycin phosphotransferase gene as positive control.

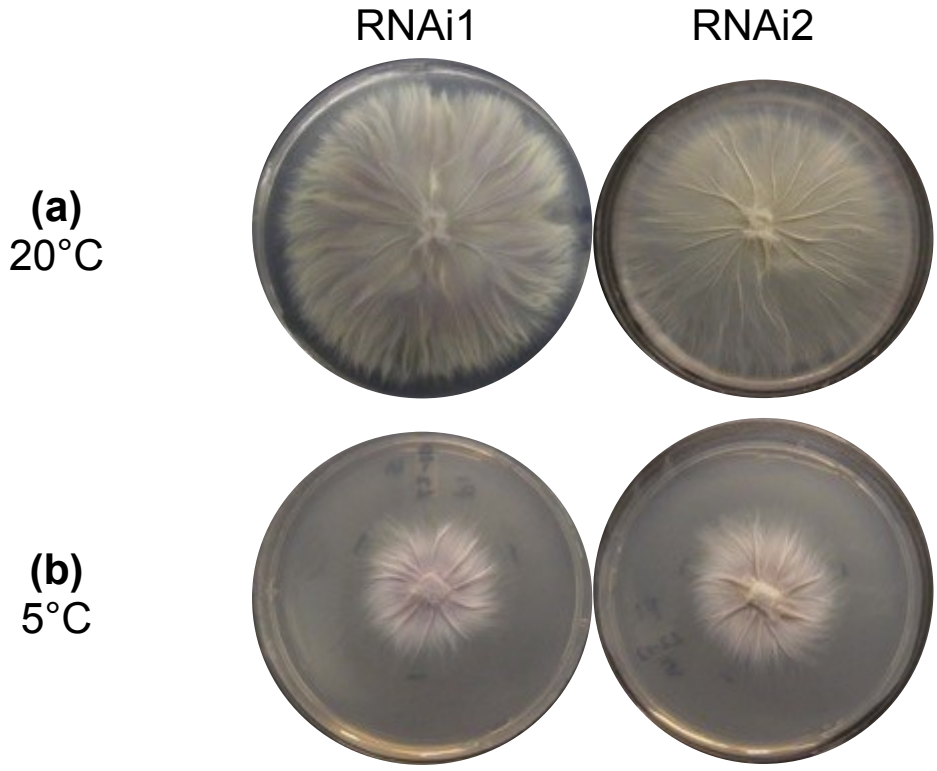

Fig. A6.3 Phenotype of vegetative mycelia of *Laccaria bicolor* RNAi strains grown on MMN medium at 20°C (a) and 5°C (b).

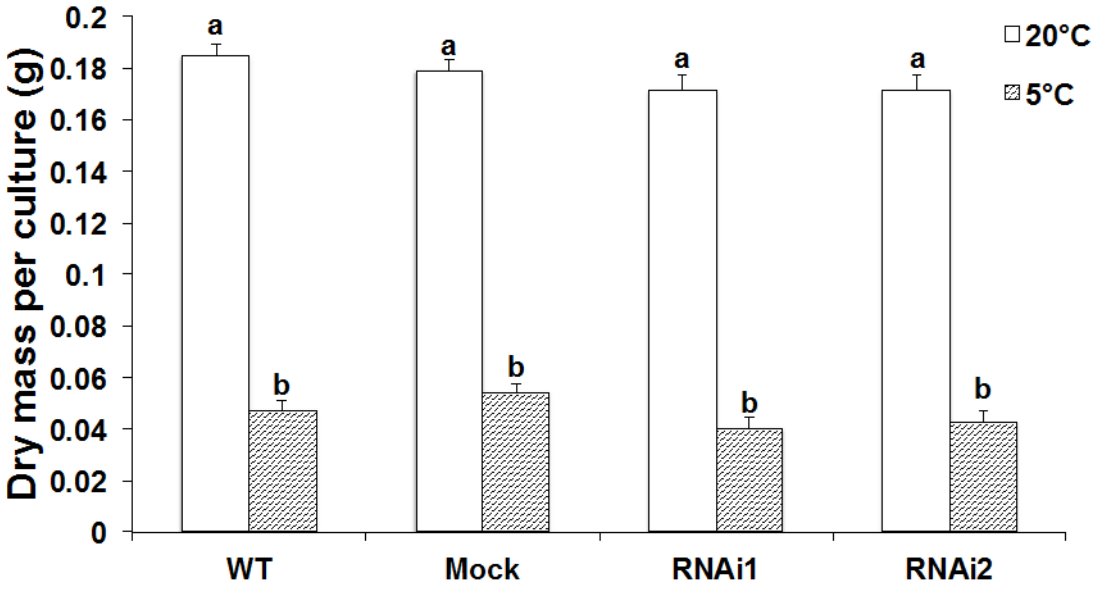

Figure A6.4 Dry mass of mycelia of WT, mock and RNAi strains grown on MMN medium at 20°C and 5°C for three weeks. Different letters indicate significant differences at P ≤ 0.05 determined with ANOVA, Tukey's test (n = 6 ± SE).

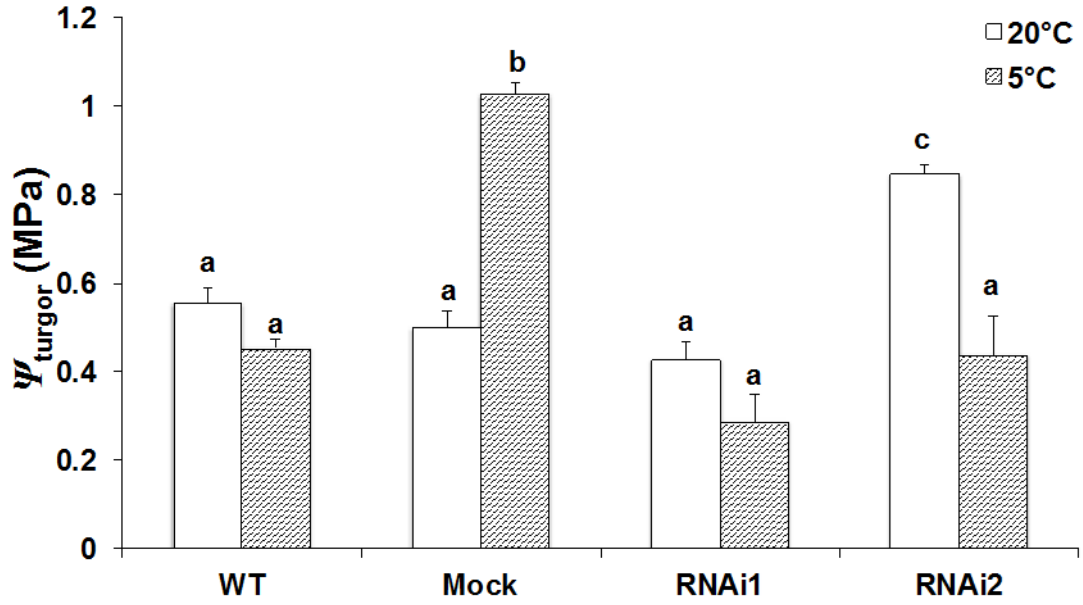

Figure A6.5 Turgor water potential Ψ_{turgor} of mycelia of WT, mock and RNAi strains grown on MMN medium at 20°C and 5°C for three weeks. Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test (n = 3 ± SE).

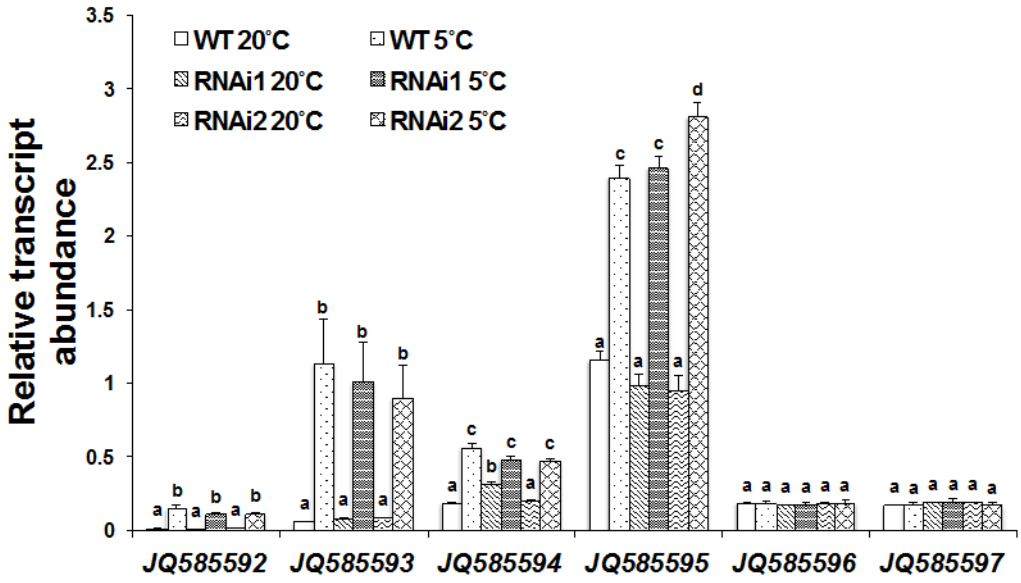

Figure A6.6 The relative transcript abundance of *Laccaria bicolor* MIPs in roots of *Picea glauca* mycorrhized with the wild-type (WT) and two RNAi strains (RNAi1 and RNAi2) of *L. bicolor* and exposed to root temperature of 20°C and 5 °C. The transcript abundance of target MIPs was normalized to that of the reference gene EF2. Different letters indicate significant differences at $P \leq 0.05$ determined with ANOVA, Tukey's test ($n = 3 \pm$ SE).

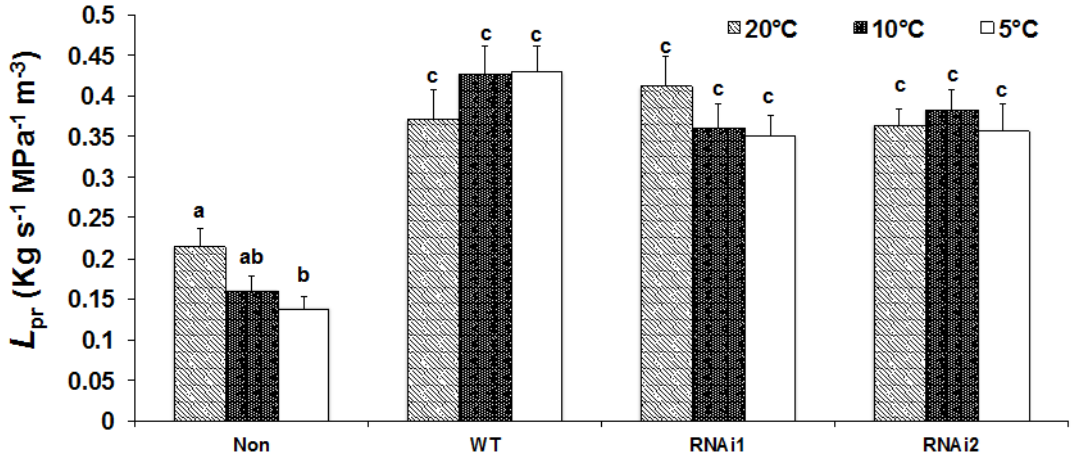

Figure A6.7 Root hydraulic conductivity (L_{pr}) in non-inoculated (Non) *Picea glauca* seedlings and in seedlings inoculated with the wild-type (WT) and two RNAi strains (RNAi1 and RNAi2) of *Laccaria bicolor*. Means ($n = 6$) ± SE are shown. Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey's test).