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Impact of *Rhizophagus* sp. (syn. *Glomus* sp.) and *Trichoderma harzianum* on the potato resistance against *Rhizoctonia solani* and *Phytophthora infestans*, two major potato pathogens

Thèse de doctorat présentée par **Adrien Gallou** en vue de l'obtention du grade de Docteur en Sciences agronomiques et ingénierie biologique

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List of abbreviations

ABA	ABscisic Acid
AM fungi	Arbuscular Mycorrhizal fungi
ANOVA	ANalysis Of VAriance
AUDPC	Area Under Disease Progress Curve
BCF	BioControl Fungi
dap	days after plating
DNA	Deoxyribose Nucleic Acid
dpi	days post inoculation
ERM	Extra-Radical Mycelium
ET	EThylene
GINCO	Glomeromycota *IN vitro* COllection
GO	Gene Ontology
HAM-P system	Half-closed Arbuscular Mycorrhizal-Plant system
HC	Hyphal Compartment
ISR	Induced Systemic Resistance
JA	Jasmonic Acid
MDP	Mycelium Donor Plant
MFC	Mycelium Free Compartment
MIR	Mycorrhiza Induced Resistance
MS medium	Murashige Skoog medium
MSR medium	Modified Strullu-Romand medium
MUCL	Mycothèque de l'Université Catholique de Louvain
P	Phosphorus

PDA	Potato Dextrose Agar
PGPF	Plant Growth Promoting Fungi
PGPR	Plant Growth Promoting Rhizobacteria
PPF	Photosynthetic Photon Flux
PR	Pathogenesis-Related
Q-PCR	Quantitative-Polymerase Chain Reaction
RC	Root Compartment
REST	Relative Expression Software Tool
RNA	RiboNucleic Acid
ROC	Root Organ Culture
RT	Reverse Transcription
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
TF	Transcription Factor

Glossary

Arbuscular mycorrhiza fungal stages: pre-stage refer to the stage *before contact with the root and root colonization*, early stage refer to the stage of *appresorium formation and first hyphal root colonization before arbuscules and vesicles formation* and late stage refer to the stage of *intense root colonization with arbuscules and vesicles formed (i.e. association successfully established and mutualistic exchange between the two partners effective)* (Gallou et al. 2010)

Arbuscular mycorrhiza symbiosis: *a unique association between the mycelium of a soil-borne fungus and more than 80% of higher plants* (Smith and Read 2008)

Biocontrol fungi: *are beneficial organisms that reduce the negative effects of plant pathogens and promote positive responses in the plant* (Soresh et al. 2010)

Induced systemic resistance: *a colonization of plant roots by PGPR or PGPF showing a protection of plant tissues against different types of pathogens* (Van Loon et al. 1998)

Mycorrhiza induced resistance: *a root colonization by AM fungi improving plant resistance/tolerance to biotic stresses* (Pozo et al. 2009)

Plant resistance/tolerance: the resistance is *the ability of plant to reduce the amount of damage* and the tolerance is *the ability of plant to support damage without a reduction of fitness* (Rausher 1992)

Priming: *a faster and/or stronger activation of various cellular defence responses that are induced following attack by pathogens or insects or in response to abiotic stress* (Conrath et al. 2006)

Systemic acquired resistance: *a primary infection with a pathogen rendered non-infected plant tissues more resistant to subsequent pathogen attack* (Ross 1961)

Summary

Potato is the fourth largest food crop cultivated in the world. This crop is susceptible to numerous pests and diseases, which control requires large quantities of pesticides in conventional agriculture. In the last decade, several studies have suggested the use of beneficial microorganisms as promising alternatives to reduce/replace chemicals. Among these microorganisms, the arbuscular mycorrhizal (AM) fungi and the fungal antagonist, *Trichoderma* spp. are the most frequently cited in the literature. Early works on biological control of plant diseases reported the ability of these microorganisms to interact directly with soil pathogens. More recent findings also indicated the ability of these fungi to reprogram plant gene expression and to induce a systemic resistance in plant. The objective of this thesis was to study the biological protection triggered by *Rhizophagus* sp. (syn. *Glomus* sp. MUCL 41833) and *T. harzianum* (MUCL 29707) against two potato crops pathogens, *Rhizoctonia solani* (MUCL 49235) and *Phytophthora infestans* (MUCL 43257). The study was conducted under strict *in vitro* culture conditions allowing to focus directly on the targeted microorganisms by eliminating confounding factors such as other microorganisms. In addition, the *in vitro* systems developed allowed the synchronization of the AM fungal development in the roots and facilitated the investigations during the first stages of the AM fungal establishment. Finally, molecular techniques were used to visualize the plant defence activated during the biological protection process. Our results demonstrated that *Rhizophagus* sp. was able to decrease the disease severity against the two potato pathogens. The

molecular mechanism behind this “protection” seemed principally dependent of the plant hormone signalling pathways. Identically, we demonstrated that *T. harzianum* was able to decrease the impact of the disease caused by *R. solani* in potato roots via the jasmonic acid and salicylic acid signalling pathways. Ours findings supported the beneficial effects of *Rhizophagus* sp. and *T. harzianum* to control, at least partially and during the early phases of pathogen attack, two of the most damaging potato pathogens. It also highlighted the complexity of mechanisms involved and the necessity for further studies on the complex interactions involving a plant, pathogens and beneficial microorganisms.

Outline of the thesis

Within this thesis, different experiments were conducted to answer three main objectives:

(1) Demonstrate the role of *Rhizophagus* sp. as biocontrol fungi (BCF) in potato plants against the soil-borne pathogen *R. solani* and the hemibiotrophic pathogen *P. infestans*.

(2) Increase our knowledge on the molecular mechanisms involved in the mycorrhiza induced resistance (MIR) in potato plants.

(3) Demonstrate the potential of *T. harzianum* as BCF in potato plants against *R. solani* and decipher the molecular base of plant defence involved in this process.

The successive steps of the thesis are presented in this section and schematized in the Figure 1.

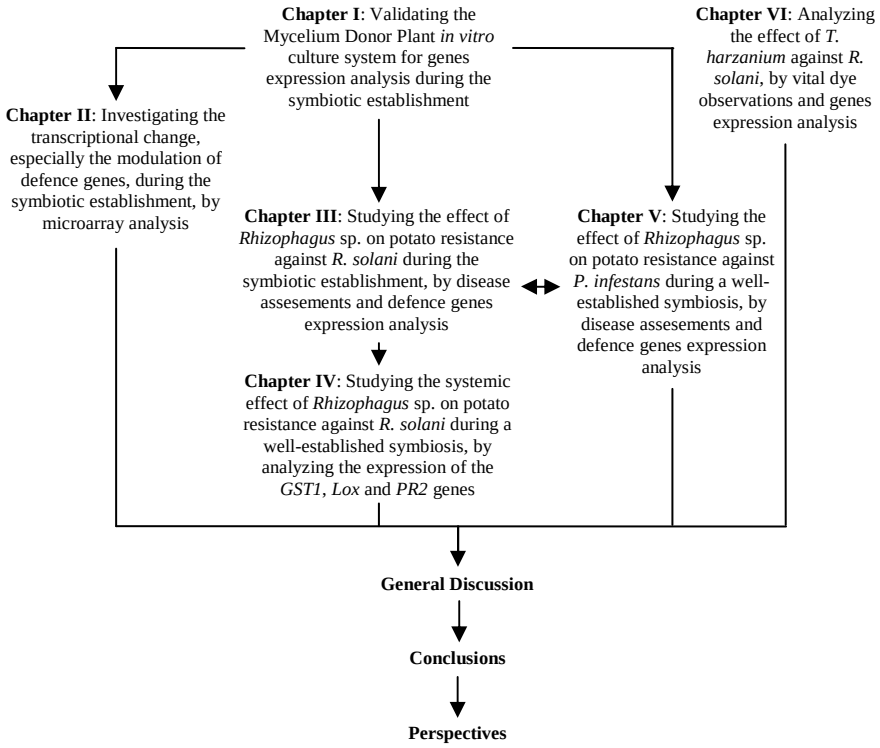


Fig. 1 Outline of the thesis

In the **introduction** section, the subject and general objective of the thesis were presented, while in the **Context of the Study** section, the state of the art relative to the study was reviewed. This chapter summarized the most pertinent and recent literature on two beneficial microorganisms, the AM fungi and *Trichoderma* spp., in the biological protection of pathogens, and reviewed the signalling pathway potentially involved in this mechanism. It also gives a specific attention to two major potato pathogens: the soil-borne fungus *R. solani* and the late blight causal agent *P. infestans*, an Oomycete. The **Materials and Methods** section summarizes the biological materials and briefly describes the most important techniques used in our research.

The major drawback in studying AM symbiosis, especially for molecular approaches, is the difficulty to synchronize the initial developmental events leading to the establishment of the symbiosis (Weidmann et al. 2004). Here, we used the Mycelium Donor Plant (MDP) *in vitro* culture system (Voets et al. 2009) to answer the questions addressed in this work. This system allows to synchronize the development of AM fungi in the roots and the fast/homogenous mycorrhization of seedlings at their early stage of development. The system consists in the plating of plantlets in an actively growing mycelium network supported by a donor mycorrhizal plant under rigorous *in vitro* culture conditions. This system appears therefore particularly adapted to study the molecular events during the AM fungal root colonization and the molecular mechanism of biological protection in the potato plant. This was demonstrated in **Chapter I**.

The MDP *in vitro* culture system was validated for the expression of the phosphate transporter 3 gene and six defence genes expressed in potato plants. These genes were monitored over time by real-time QRT-PCR and paralleled with the root colonization by *Rhizophagus* sp.. Gene expression change was observed in the pre-stage (i.e.before root colonization), early stage (i.e. hyphae root colonization before arbuscules and vesicles formation) and the late stage (i.e. heavy root colonization with arbuscules and vesicles formed) of root colonization.

The results of chapter I were published in Mycorrhiza (Gallou et al. 2010).

After validation of the MDP *in vitro* culture system for gene expression analysis, we studied the global transcriptional change, in particular the regulation of genes related to the defence mechanism, during the three stages of root colonization described above (**Chapter II**). For this study, we performed a microarray experiment using the Potato Array designed by the Potato Oligo Chip Initiative (POCI) (Kloosterman et al. 2008) associated to the MapMan software for gene ontology analysis.

The results of chapter II were accepted for publication in Functional & Integrative Genomics.

The objective of **chapter III**, **IV** and **V** was to investigate the potential of the AM fungus as BCF and to track the expression of

several defence genes involved in different plant hormone pathways, in order to increase our knowledge on the molecular dialogue behind the MIR process. In **chapter I** and **II**, we have demonstrated an induction of numerous plant defence genes, in particular during the pre- and late stage of AM root colonization. In consequence in the first part of this chapter (**Chapter III**), the MDP *in vitro* culture system was used to investigate the effects of *Rhizophagus* sp. on the potato plantlets resistance against *R. solani* during the different stages of root colonization (i.e. (1) the potato plantlets were placed in the ERM network of *Rhizophagus* sp. and were at the same time inoculated with *R. solani* and (2) *R. solani* was inoculated nine days following the contact of the potato plantlets with the ERM network of *Rhizophagus* sp.). In both approaches, the root colonization by *Rhizophagus* sp. and the disease development of *R. solani* were analysed. Moreover, the real-time QRT-PCR was used to assess the expression of defence genes salicylic acid (SA)-, jasmonic acid (JA)-, JA/ethylene (ET)-dependent and a mitogen-activated protein kinase (*MAPK*) gene.

The results of chapter III were submitted to European journal of Plant Pathology.

In **chapter IV**, we developed an *in vitro* split root culture system to investigate the systemic effect of *Rhizophagus* sp. against *R. solani*. The capacity of this *in vitro* culture system was evaluated by measuring the AM fungus development and root colonization. Moreover, the disease assessment and the real-time QRT-PCR, used

to assess the expression of SA-dependent and JA-dependent genes, were analyzed.

The chapter IV is a research paper in preparation.

Following the studies on biological protection triggered by the AM fungus against a soil-borne pathogen in potato, we evaluated the impact of the AM fungus against a hemibiotrophic pathogen inoculated on the leaves of potato. In **Chapter V**, the MDP *in vitro* culture system was used to test the biological protection effect of *Rhizophagus* sp. against *P. infestans*, the causal agent of late blight in potato. As in the previous chapters, the disease assessment and the real-time QRT-PCR, used to assess the expression of SA-, JA-, ET- and JA/ET-dependent genes and *MAPK* gene, were analyzed.

The results of chapter V were published in Physiological and Molecular Plant Pathology (Gallou et al. 2011).

In **Chapter VI**, the Half-closed Arbuscular Mycorrhizal-Plant *in vitro* culture system (HAM-P) (Voets et al. 2005) was used to study the effect of *T. harzianum* against *R. solani* in potato roots. The vital dye (i.e. acridine orange) and the stability of reference genes (i.e. GeNorm software) were evaluated to demonstrate the biological protection effect of *T. harzianum*. Moreover, the time-course expression of defence genes of potato plantlets challenged by the pathogen *R. solani* in the presence/absence of *T. harzianum* was followed during the early stage of the plant-microbes interaction.

The results of the chapter VI were published in European Journal of Plant Pathology (Gallou et al. 2009).

In the **General Discussion** section, the major findings of the thesis were summarized and discussed. In the **Conclusion** section, the major outcomes were given and further commented.

Finally, in the **Perspectives** section, the possible avenues to improve our understanding, on the role of *Rhizophagus* sp. and *T. harzianum* and on the molecular signalling involved during the MIR were presented.

Author contributions

The work presented here was strictly realized during the time-course of my PhD.

The **Introduction** and **Context of the Study** as well as the **General Discussion, Conclusions** and **Perspectives** were written by myself. These parts were not published.

Chapter I is a short note published in Mycorrhiza (2010). My contribution to this chapter was around 65 %. The data collection on AM fungi (i.e. characterisation of the mycelium donor plant (MDP) *in vitro* culture system, estimation of the root colonization and data analysis with SAS Enterprise guide) and the practical work (*in vitro* plant system maintenance and follow-up) of this experiment were performed with the help of Nathalie De Jaeger (PhD student - UCL). The molecular biology experiments, data analysis and writing of the paper were done by myself.

Chapter II is a research paper submitted to Functional & Integrative Genomics. My contribution to this chapter was estimated to 90 %. The practical work of the Agilent "Potato Array POCI" was performed by André Clippe (Technician at Institut of Life Science - UCL) which is acknowledged.

Chapter III is a research paper in preparation for European journal of Plant pathology. The practical work, data analysis and writing of the paper were conducted by myself.

Chapter IV is a research paper in preparation for European journal of Plant pathology. My contribution to this chapter was estimated at 65 %. For the practical work of this experiment, I received the help of Alice Braun (master student at the UCL). The experiment design, data analysis and writing of the paper were conducted by myself.

Chapter V is a research paper submitted to Physiological and Molecular Plant Pathology. This paper is an equal contribution, as first author, with Hernán Patricio Lucero Mosquera (PhD student – UCL in collaboration with "Centro de Biología Celular y Molecular, Universidad Técnica Particular de Loja"). The practical work of this experiment was performed by Hernán Patricio Lucero Mosquera. I have helped in the experimental design, data collection and data analysis. My participation was estimated to 40 %.

Chapter VI is a research paper published in European Journal of Plant Pathology (2009). The practical, data analysis and writing of the paper were conducted by myself.

Special thanks are addressed to the co-authors for their good advices and helps in the revision of the papers.

Introduction

Potato plants are facing numerous pathogens, causing a constant menace to its production worldwide. Among these pathogens, the soil-borne fungus *Rhizoctonia solani* and the late blight causal agent *Phytophthora infestans*, an Oomycete, are among the most damaging. Over the last 15 years, the pressure of *R. solani* has significantly increased in Europe, where the pathogen causes important diseases such as black scurf. Yield losses are estimated to 20 % (Grosch et al. 2006). Potato late bight, caused by the hemibiotrophic pathogen *P. infestans*, is the most destructive potato disease in the world. This microorganism was responsible for the epidemics that resulted in the European potato famine in 1845. Nowadays, the annual losses in Europe (i.e. costs of damage and control) are estimated above 1 billion € (Haverkort et al. 2008).

The control of these two diseases by cultural practices is limited. At the present time, chemicals treatments for *R. solani* and *P. infestans* are the most recommended strategies for their control. In the recent years, microorganisms such as *Pseudomonas*, AM fungi and fungal antagonists (e.g. *clonostachys rosea* and *Trichoderma* spp.) (Whipps 2004; Brewer and Larkin 2005; Grosch et al. 2006) have been considered as promising alternatives to control plant pathogens and to minimise the impact of chemicals on the environment by replacing these molecules or reducing their rate of application (Chet and Inbar 1994; Harman and Björkman 1998).

The AM fungus, an obligate biotroph belonging to the Phylum Glomeromycota (Schüβler et al. 2001), is amongst the most studied beneficial microorganism. It has been frequently reported that root colonization by AM fungi improve plant resistance/tolerance against a

vast array of root pathogens, and under some circumstances on above-ground diseases. Other fungal beneficial microorganisms are *Trichoderma* spp., isolated from soil and present in the rhizosphere. These fungi are opportunistic plant symbionts, and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases (Harman et al. 2004; Lorito et al. 2010).

The protective effects conferred by AM fungi may be localized, i.e. at the site of the host cell containing typical haustoria-like arbuscule structures (Cordier et al. 1998; Azcon-Aguilar et al. 2002; St-Arnaud and Vujanovic 2007) or systemic (Pozo et al. 2002; Khaosaad et al. 2007) with the description of a mycorrhiza induced resistance (MIR) mechanism (Pozo et al. 2010). The mechanisms of MIR remain largely unknown. The establishment of the AM fungus in the roots is a complex and dynamic process involving major changes in plant gene expression (Franken et al. 2007; Reinhardt 2007) among which genes associated with defence mechanisms (Pozo et al. 2010). It is obvious that an in-deep understanding of the mechanisms of defence expressed during AM fungal root colonization will help in the development of control strategies against the major diseases in crop plants.

In addition to the well-known mycoparasitism process (Vinale et al. 2008), *Trichoderma* spp. was also reported to decrease disease caused by different pathogens via the induction of a systemic resistance in the plant. The reaction of the plant to *Trichoderma* spp. was similar to the induced systemic resistance (ISR) triggered by rhizobacteria. However, it has recently been shown that the defence

regulatory protein NPR1 (Non-expresser of *PR1* gene), which plays a key role in SA-dependent SAR (Dong 2004), was essential to the biological protection triggered by *T. harzianum* in *A. thaliana* against *Pythium ultimum*. This result underlined the fact that *Trichoderma* spp. may also use other mechanisms, as systemic acquired resistance (SAR), to induce plant defence (Shoresh et al. 2010).

The soil is a complex and interaction-rich environment. Thus interaction studies between microorganisms under highly controlled conditions (e.g. *in vitro*) are recommended. The use of *in vitro* culture systems provide the possibility to focus directly on the targeted interacting microorganisms by elimination of misleading abiotic and biotic factors. Recently, Voets et al. (2005; 2009) have developed two *in vitro* culture systems (i.e. the half-closed Arbuscular Mycorrhizal-Plant culture system (HAM-P) and the Mycelium Donor Plant culture system (MDP)) adapted for AM fungal studies with whole plants, allowing non-destructive microscope observations. In addition, the HAM-P (Voets et al. 2005), was also demonstrated as a powerful tool for studying the dynamics of others plant-fungal interactions (i.e. *T. harzianum* and *R. solani*) (De Jaeger et al. 2010). In parallel, the MDP (Voets et al. 2009), represented a powerfull tool for improving our knowledge on the AM fungal biological protection by allowing synchronization of the AM fungal development in the roots and facilitating the researches during the first stages of the symbiotic establishment.

In the present thesis, we used these *in vitro* culture systems for investigating the potential of an AM fungus, *Rhizophagus* sp. MUCL 41833, to control two major potato pathogens, *P. infestans* MUCL

43257 and *R. solani* MUCL 49235. Root colonization by the AM fungus, disease development on roots and leaves, plant hormone defence genes and molecular networks changes associated to defence during AM fungal root colonization were considered. In addition, the impact of *T. harzianum* MUCL 29707 against the soil-borne pathogen, *R. solani* MUCL 49235, was evaluated in order to understand the role of this fungus in biotic stress alleviation in potato plants.

Context of the Study

I. Arbuscular Mycorrhiza Fungi

The arbuscular mycorrhiza (AM) symbiosis represents a unique association between the mycelium of a soil-borne fungus and more than 80% of higher plants (Smith and Read 2008). The AM fungus, an obligate biotroph belonging to the Phylum Glomeromycota (Schüβler et al. 2001), receives carbohydrates from the host plant required to complete its life cycle, in exchange of which it provides the plant with nutrients (i.e. such as phosphate, nitrogen and sulphur) and water. The establishment of this successful mutualistic association develops from a complex and dynamic process involving major changes in fungal and plant gene expression (Franken et al. 2007; Reinhardt 2007). During this process, phosphate transporter genes (Liu et al. 1998) as well as transient defence-response genes are activated in the host plant (Liu et al. 2003). As a result of this process, the AM fungus is able to enhance plant growth and production (van der Heijden et al. 2008) as well as increase its resistance/tolerance to biotic and abiotic stresses (Whipps 2004; Pozo et al. 2010).

I.1 The AM fungal life cycle

In nature, the AM fungal mycelium extending from colonized roots represents an important source of inoculum for the colonization of neighbouring plants, due to the several hyphae ramifying from the colony (Friese and Allen 1991). However, AM fungi persist in the soil as spores, isolated vesicles, mycorrhizal root fragments, or fungal

mycelium, which identically could serve as propagules for root colonization. After the development of the extra-radical mycelium (ERM) network from colonized root or isolated propagules (e.g. spores), the AM fungus enters into contact with the root and develops an appressorium at the surface of the epidermic cells before developing within the root. About 4 to 5 h after the development of an appressorium, cellular events strictly coordinated by the nucleus lead to the development of a pre-penetration apparatus that consists of a cytoplasmic column containing microtubules and microfilament bundles, very dense endoplasmic reticulum cisternae, and a central membranous thread. Only after the column has been formed, the fungus grows across the cell within the newly formed membrane tunnel (Genre et al. 2005).

Within the root cortex, the AM fungus develops following two main developmental patterns: the *Arum* type and the *Paris* type (Smith and Read 2008). In the *Arum* type, the fungal hyphae grow in the intercellular spaces between root cortical cells, and penetrate cells to differentiate arbuscules. In the *Paris*-type, the hyphae grow directly from cell to cell so that the intercellular phase of development is highly restricted or completely absent. Fungal penetration of plant cell walls is therefore much more frequent in *Paris*-type than in *Arum*-type. Complex coils develop within colonized cells of *Paris*-type, which may bear arbuscule-like branches (arbusculate coils). In the final step of the root colonization, the members of all genera, with the exception of the *Gigasporaceae*, develop vesicles to varying degrees and in either intercellular or intracellular location in the cortex. The

vesicles also play a role as reproductive and reserve structure (Smith and Read 2008)

Following the root colonization, the AM fungus produces an ERM network in the soil. The ERM develop branching absorbing structures (BAS), which are supposed to be involved in the uptake of minerals (Bago et al. 1998). In the *Gigasporaceae*, the ERM also support auxiliary cells which are believed to play a role as reproductive and reserve structure (Declerck et al. 2004). Finally, external spores are differentiated, acting as reserve and propagation structures (Fig. 2).

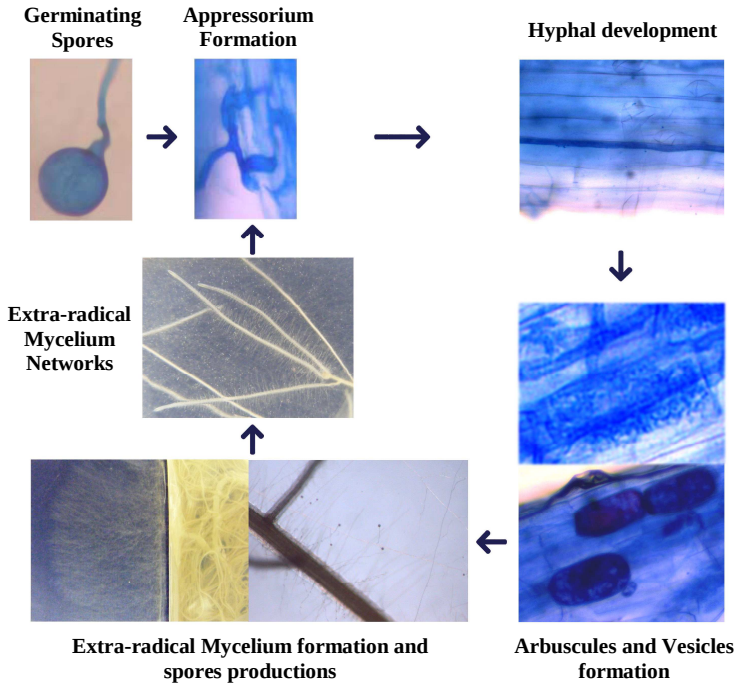


Fig. 2 Life cycle of an arbuscular mycorrhizal fungus of the *Glomeraceae* family

I.2 General benefits of AM fungal symbiosis to plants

The benefits conferred by AM fungi to plants are numerous and diverse. Past studies have reported that AM fungi are able to increase plant nutrition (i.e. growth) and resistance to biotic (i.e. above- and below-ground pathogens) and abiotic (i.e. heavy metal, salinity, drought and soil compaction) stresses (Smith and Read 2008). However, it should be mentioned that high levels of stress may turn the symbiosis between the two partners into a negative relationship, as unfavourable conditions may adversely influence AM fungal performance (Rillig 2004; Hildebrandt et al. 2007; Miransari 2010).

The first well known benefit of AM fungi to plants is the increased growth resulting from and improved nutrient acquisition (Koide and Mosse 2004). Several minerals are transported via the symbiosis to the plant in exchange for carbon (Pearson and Jakobsen 1993). Baylis (1959) was the first to suggest that the beneficial plant growth effect of AM fungi was mediated by the increased accumulation of P. The extra nutrition of P in the mycorrhizal roots was due to the better soil exploration by the ERM network of AM fungi (Li et al. 1991). This ERM is able to take up P and translocate this mineral to the plant (Smith et al. 2000). Additionally, other nutrients such as N, K, Cu and Zn are also transported by the AM fungi to the host plant. The improved plant nutrition generally resulted in an increased biomass production as show with cucumber (Trimble and Knowles 1995), coffee (Siquiera et al. 1998) and cereals (Al-Karaki et al. 2004).

Another beneficial effect of AM fungi is improving soil structure (Ryan and Graham 2002). The AM fungi affect soil structure through (1) binding soil particles on ERM, (2) entanglement of micro-aggregates by hyphae into macro-aggregates, and (3) providing a C source for plant and microorganisms after AM degradation in the soil (Jastrow et al. 1998; Cardoso and Kuyper 2006). In addition, the production of glycoproteins (i.e. glomalin) by AM hyphae has also been reported to improve soil structure (Rillig and Mummey 2006).

Abiotic stresses have important impacts on crop productivity. The AM symbiosis can protect plant under these stresses, thus mitigating the negative impact on plant productivity. The processes involved are numerous. For example under heavy metals stress, the AM fungi induce in plants the expression of specific genes, encoding for metallothioneins, antioxidant enzymes and metal transporters, which induced an increase of tolerance of mycorrhizal plants. Moreover, AM fungi have developed mechanism called phyto-stabilisation increasing the tolerance of plants to heavy metal stresses. The phyto-stabilisation is a mechanism by which the AM fungi increase the plant ability to immobilise heavy metals in the soil via the sequestration in the hyphae and consequently decreasing translocation to plant. In addition, the beneficial effects of AM fungi have been reported under salinity and drought stress conditions by the existence of different mechanisms, such as the increase of osmolytes concentration (i.e. carbohydrates and electrolytes) in plant roots (Ruiz-Lozano et al. 1996; Daei et al. 2009).

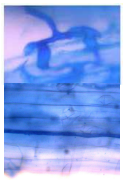
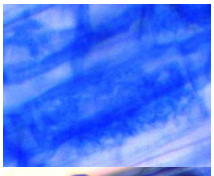
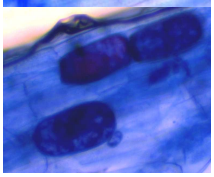
Finally, numerous studies have reported a beneficial effect of AM fungi under biotic stresses, such as the reduction in incidence and/or

severity caused by below-ground pathogens. In contrast, few studies were conducted on the control of above-ground pathogens by AM fungi and results were less conclusive (Whipps 2004; Pozo et al. 2009). The protective effects conferred by AM fungi were principally attributed to the induction of several defence pathways in the mycorrhizal plants, via the MIR mechanism (Pozo et al. 2010) (For more details see paragraph III).

I.3 Plant gene expression in AM symbiotic association

The development of the AM symbiosis involves significant morphological changes in both partners (i.e. the plant and the AM fungus) that are assumed to be coordinated by a reciprocal exchange of signals (Harrison 2005) (See review in Table 1).

Table 1 Overview of plant genes reported to be transcriptionally regulated in a specific stage of AM symbiosis development

Stages	Functional category/Genes	References
Pre-stage (i.e. before contact)	cell-wall modification, signal transduction and translation pathway	Weidmann et al. 2004
	expansin-like (*EXLB1*)	Dermatsev et al. 2010
Early stage (i.e. appressorium and first hyphal development)	germin-like, nodulin 26-like and four other proteins of unknown function	Brechenmacher et al. 2004
	expansin-like (*EXLB1*)	Siciliano et al. 2007; Dermatsev et al. 2010
	putative *avr9/Cf-9*	Siciliano et al. 2007
	Cell wall (*PsENOD12A*)	Albrecht et al. 1998
	Signalling (C1p serine protease)	Roussel et al. 2001
	plant defence	Kapulnik et al. 1996; Ruíz-Lozano et al. 1999; Liu et al. 2003
Late stage (i.e. arbuscules and vesicles formed)	**Cellular modifications genes**	
	Cell wall modification	Grunwald et al. 2004
	MtCel1	Liu et al. 2003; Guether et al. 2009a
	osmotin/thaumatin-like	Hohnjec et al. 2005
	Expansin	Journet et al. 2001; Liu et al. 2004; Balestrini et al. 2005
	Nutrient uptake genes	
	Phosphate transporters	Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002; Karandashov and Bucher 2005, Grunwald et al. 2009
	nitrate transporters	Hildebrandt et al. 2002; Hohnjec et al. 2005; Guether et al. 2009b
	sugar transporters	Harrison 1996; Liu et al. 2003; Hohnjec et al. 2005
	Defence genes	
	plant defence	Salzer et al. 2000; Brechenmacher et al. 2004; Ocon et al. 2007; Requena et al. 2007; Gomez et al. 2009; Guether et al. 2009a; Lopez-Raez et al. 2010
	reactive oxygen species	Fester and Hause 2005; Lanfranco et al. 2005
	jasmonate pathway	Deguchi et al. 2007; Lopez-Raez et al. 2010
	Other plant hormone pathways	Riedel et al. 2008; Fiorilli et al. 2009; Grunwald et al. 2009

I.3.1 Pre- and early stages of AM fungal symbiosis

Before contact with the root (i.e. pre-stage), the induction of genes involved in cell-wall modification, signal transduction and translation pathway has been demonstrated (Weidmann et al. 2004). At the early stage of root colonization, genes encoding a cell wall protein (PsENOD12A; Albrecht et al. 1998) and a C1p serine protease (Roussel et al. 2001) were activated in pea roots during appressorium formation. Plant genes expressed in the early stages of interaction between *Medicago truncatula* and *Funneliformis mosseae* (Syn. *Glomus mosseae*) were detected by non-targeted suppression subtractive hybridization (SSH). Genes encoding germin-like, nodulin 26-like and four other proteins of unknown function were activated at the appressorium stage (Brechenmacher et al. 2004), while other genes encoding an expansin-like protein and a putative avr9/Cf-9 protein were activated during development of the pre-penetration apparatus in *M. truncatula* (Siciliano et al. 2007). Recently, Dermatsev et al. (2010) have demonstrated that the expansin-like gene (*EXLB1*) was involved in the pre- and early stages of AM fungus-tomato association. A number of studies have also reported a transient increased expression of plant defence genes, especially during the early stages of root colonization, followed by a decline in expression (Kapulnik et al. 1996; Ruíz-Lozano et al. 1999; Liu et al. 2003).

It is interesting to note that three genes (*DMI1*, *DMI2*, *DMI3*) involved in the early stages of signal transduction common to both nodulation and AM formation have been identified in *Medicago* sp. by positional cloning in myc− mutants, and homologs have been

described in other plants (Parniske 2004; Oldroyd et al. 2005). In particular, *DMI2*, a receptor-like kinase, and *DMI1*, a predicted ion channel, acting upstream of calcium spiking, were detected. Interestingly, DMI3, a Ca2+ and calmodulin-dependent protein kinase, acts downstream (Endre et al. 2002; Ané et al. 2004; Lévy et al. 2004; Oldroyd et al. 2005). Recently, Genre et al. (2010) have found by *in vivo* confocal microscopy that epidermal cells contacted by AM fungus show repetitive oscillation (spiking) of the nuclear calcium concentration. However, the process of interface construction is initiated after the adhesion of the AM fungal appressorium to the root epidermis showing that the calcium spiking is a secondary signal.

I.3.2 Late stage of AM fungal symbiosis

In the last years, the use of different technique, such as the cDNA arrays (Liu et al. 2003; Grunwald et al. 2004; Hohnjec et al. 2005; Güimil et al. 2005; Fiorilli et al. 2009) and methods to search for differentially expressed transcripts (Wulf et al. 2003; Brechenmacher et al. 2004), have helped to demonstrate the complexity of transcriptional changes in plants during the AM fungal symbiotic phase (Review Table 1).

i. Cellular modification genes

Intra-cellular colonization by AM fungi dramatically changes the morphological organization of the host cells (Bonfante 2001). In particular, a new interface compartment is created, which contains

material similar in composition to the plant cell wall. Grunwald et al. (2004) have identified some Expressed Sequence Tag (ESTs) with gene products involved in cell wall modification. One encodes an extensin-like glycoprotein that belongs to a large protein family characterized by (hydroxy)proline-rich motifs. Members of this family accumulate during both nodule and mycorrhiza development (Journet et al. 2001; Hohnjec et al. 2005). Using cDNA arrays, Liu et al. (2003) observed that the *MtCel1* gene was specifically induced during symbiosis. This result was confirmed by Guether et al. (2009a). The authors observed the induction of Ljwgs_024744.1 (i.e. 78% identity to the *MtCel1* gene) in *Lotus japonicus* roots specifically during symbiosis. The MtCel1 product shares identity with members of the E-type Egase subfamily III, which have been associated with expanding tissues and cellulose synthesis (Brummell et al. 1997).

Cell wall modifications in AM symbiosis are also suggested by the induction of a gene encoding an osmotin/thaumatin-like protein (Hohnjec et al. 2005). These proteins are known to be resistant to proteases and to denaturation, and the corresponding genes are described as being induced in response to osmotic stress or in response to fungi. Moreover, expansins (i.e. extra-cellular proteins) have been located in cucumber mycorrhizal roots where they could be involved in keeping the interfacial material loose and/or in cell wall loosening and cell enlargement (Balestrini et al. 2005). The induction of an expansin gene in mycorrhizal roots has also been found in *M. truncatula* by *in silico* analysis of ESTs (Journet et al. 2001) and by cDNA array experiments (Liu et al. 2004).

ii. Nutrient uptake genes

During the AM symbiotic phase, the nutrient exchange between the two partners is significantly modified. Investigations performed on host plants (e.g. potato, rice, and *M. truncatula*) reported the presence of P transporters exclusively expressed during symbiosis (Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002; Karandashov and Bucher 2005, Grunwald et al. 2009). In particular, *MtPT4* from *M. truncatula* was shown to be located in the peri-arbuscular membrane, at the interface between arbuscules and the invaginated plant cell membrane, generally assumed as the site of nutrient exchanges (Bago et al. 2000; Harrison et al. 2002). Karandashov and Bucher (2005) have shown that the potato phosphate transporter gene *StPT3* is expressed in root cells harbouring various mycorrhizal structures, including thick-coiled hyphae. In addition, mycorrhiza-inducible P transporters have been described in *solanaceous* species (tomato and potato) and rice (Güimil et al. 2005; Nagy et al. 2005).

Moreover, the induction of four genes corresponding to the first AM-related nitrate transporters identified in *M. truncatula* was reported (Hohnjec et al. 2005). Two reports on an AM-induced nitrate transporter in tomato (Hildebrandt et al. 2002) and in *L. japonicus* (Guether et al. 2009b) suggested the involvement of mechanisms supporting ammonium uptake but also nitrate acquisition during AM symbiosis. Recently, the analysis of gene expression in the colonized cortical cell of *M. truncatula* by *R. intraradices* (Syn. *G. intraradices*) revealed a symbiosis-specific ammonium transporter that is likely a candidate for mediating ammonium transport in the AM symbiosis

(Gomez et al. 2009). In AM-colonized roots, enhanced gene expression of sucrose synthase was also observed (Ravnskov et al. 2003; Hohnjec et al. 2003). In the past years, Harrison (1996) reported high levels of expression of a sugar transporter gene (*Mtst1*) in arbuscule-containing cells and in cortical cells surrounding colonized areas. The induction of *Mtst1* was also reported in *F. mosseae*-colonized roots by Hohnjec et al. (2005). In addition, two other sugar transporters (TC77798 and AW584546) were identified in *M. truncatula* colonized by *G. versiforme*[1] using macroarray hybridization (Liu et al. 2003).

iii. Defence genes

Because the AM symbiotic association helps in the improvement of plant health through increased protection against biotic and abiotic stresses (Ocon et al. 2007; Requena et al. 2007), the expression of defence genes and genes associated with defence mechanisms have been extensively investigated. In situ experiments revealed that some transcripts related to plant defence responses were exclusively expressed in arbuscule-containing cells (Balestrini et al. 1997; Blee and Anderson 2000), suggesting a very localized response. For example, the class III of chitinase gene family has been shown to be expressed in cells containing active arbuscules (Salzer et al. 2000) and the glutathione S-transferase transcripts have also been found to accumulate in the late stage of mycorrhizal symbiosis (Brechenmacher et al. 2004). Moreover, recent microarray studies revealed the

[1] Specie of uncertain taxonomic position (Schüβler and Walker 2010)

induction of numerous defence genes related to biotic or abiotic stresses after symbiosis establishment. These studies demonstrated the induction of *PRs*, chitinase, β-1,3-glucanase or peroxidase genes during biotic stresses and numerous heat shock protein genes during abiotic stresses (Gomez et al. 2009; Guether et al. 2009a; Lopez-Raez et al. 2010).

Other studies have also demonstrated the induction of genes associated with the defence during the late stage of AM symbiosis. Fester and Hause (2005) and Lanfranco et al. (2005) detected the induction of genes involved in the detoxification of reactive oxygen species (ROS) during oxidative stress. An increase of genes transcript involved in carotenoid synthesis was associated with mycorrhizal colonization in several plants and suggested their involvement in the efficient protection against ROS generated during the life cycle of arbuscules (Walter et al. 2000; Fester et al. 2002; Hans et al. 2004; Hohnjec et al. 2005). In addition, a number of results revealed in roots of mycorrhizal plants the inductions of numerous genes involved in the different plant hormones. For instance, Deguchi et al. (2007) demonstrated up-regulation of the *jasmonic acid 2* gene in *Lotus japonicus* roots after establishment of the symbiosis with *F. mosseae* and *G. margarita*. Fiorilli et al. (2009) have highlighted the induction of several genes involved in auxin and abscisic acid (ABA) metabolism in the roots of tomato plants colonized by *F. mosseae*, whereas Lopez-Raez et al. (2010) demonstrated the inductions of numerous JA genes in the roots of tomato plants colonized by *F. mosseae* or *R. irregularis*.

I.3.3 Diversity of plant hormones response following AM symbiotic interaction

Despite the high number of experimental studies, the molecular basis and dialogue between the two partners of the symbiosis is still poorly understood. Recent studies have pointed the key role of plant hormones in the regulation of AM symbiosis (Hause et al. 2007). However their roles and functions remain unclear (Riedel et al. 2008). Recently, Lopez-Raez et al. (2010) have shown a regulation of the oxilipin pathway during the AM symbiosis and suggested a key regulatory role for jasmonates. However, these authors demonstrated that only 35 % of the genes were regulated in common, during the interaction between *F. mosseae* or *R. irregularis* and tomato plants. The results suggested a difference in plant response to the colonization by the AM fungi. This observation was also supported by Riedel et al. (2008) and Grunwald et al. (2009). Riedel et al. (2008) have demonstrated that JA levels in mycorrhizal roots compared with non-mycorrhizal controls remained unaltered and that ethylene (ET), rather than JA, signalling played a role in the AM symbiosis between *R. irregularis* and *Nicotiana attenuate*. Grunwald et al. (2009) further revealed, in the AM fungal interaction between *Gigaspora rosea* and *M. truncatula,* only a very small number of genes with a common pattern of regulation in the root colonised by the AM fungus in comparison to roots treated with the two phytohormones, ABA and JA.

II. Plant Immune Response

Within soil, plant roots become quickly colonized by a diverse microflora among which soil-borne bacteria and fungi. These microbes may have either beneficial or detrimental effects on the plant. Beneficial soil-borne microorganisms are diverse. The most commonly reported are the plant growth promoting rhizobacteria (PGPR), the *Rhizobia* (i.e. fixing atmospheric nitrogen for plant), and some fungi such as the AM fungi and *Trichoderma*. These beneficial microorganisms have been reported to stimulate plant growth by, among others, suppressing plant disease (Van Loon et al. 1998; Spaink 2000; Pozo et al. 2002; Harman et al. 2004) or insect herbivory (Van Oosten et al. 2008). This biological protection activity is exerted either directly through antagonism of soil-borne pathogens or indirectly by promoting plant-mediated resistance responses (Vinale et al. 2008; Van der Ent et al. 2009; Pozo et al. 2010).

II.1 Systemic acquired resistance

In the 1960s, Ross (1961) demonstrated that restricted primary infection with a pathogen rendered non-infected plant tissues more resistant to subsequent pathogen attack. This long-lasting and broad spectrum induced disease resistance is referred to as systemic acquired resistance (SAR) (Ross 1961; Durrant and Dong 2004) (Fig. 3). The SAR process is associated with increased levels of SA, and is characterized by the coordinate activation of a specific set of *Pathogenesis-Related* (*PR*) genes, many of which encode PR proteins with antimicrobial activity (Van Loon et al. 2006). Studies with transgenic and mutant plants that are impaired in the production or

perception of SA demonstrated a central role for this phytohormone in SAR (Loake and Grant 2007; Vlot et al. 2008). The regulatory protein NPR1 (Non-expresser of *PR1* gene) emerged as an important transducer of the SA signal, which upon activation by SA acts as a transcriptional co-activator of *PR* gene expression (Dong 2004).

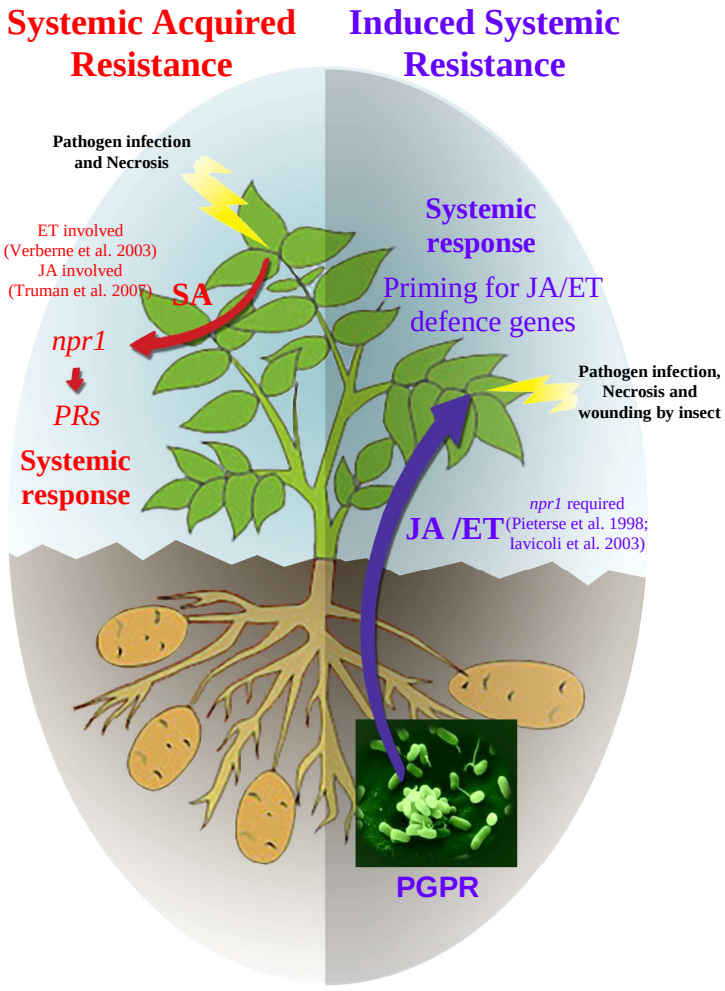


Fig. 3 Systemic Acquired Resistance and Induced Systemic Resistance mechanisms in plants (e.g. potato)

Other hormones are also involved in SAR signalling. In tobacco, Verberne et al. (2003) demonstrated that ET perception is required for the onset of SA-dependent SAR that is triggered upon infection by tobacco mosaic virus. In addition, Truman et al. (2007) showed that the JA-signalling mutants *sgt1b* (*suppressor of g2 allele of SKP1 1b*), *opr3* (*12-oxo-phytodienoate reductase 3*) and *jin1* (*jasmonate insensitive 1*) failed to develop SAR upon leaf infiltration with an avirulent strain of the pathogen *Pseudomonas syringae* pv. *tomato*, suggesting that JAs play a role in SAR as well. However, other JA-signalling mutants such as *jar1* (*jasmonate resistant 1*), *eds8* (*enhanced disease susceptibility 8*), and *coi1* (*coronatine insensitive 1*) were shown to develop normal levels of SAR (Pieterse et al. 1998; Ton et al. 2002; Cui et al. 2005; Attaran et al. 2009). Hence, the exact role of JA signalling in SAR needs to be further explored.

II.2 Induced systemic resistance

Besides pathogens, non-pathogenic microorganisms can also increase the level of disease resistance in plant. It is probable that beneficial microbes and pathogens are recognized in a largely similar manner, ultimately resulting in an enhanced defensive capacity of the plant. However, in plant–beneficial microbe interactions, the microbe molecular response does not ward off the interacting beneficial as it remains accommodated by the plant. This suggests a high degree of coordination and a continuous molecular dialogue between the plant and the beneficial organism. The first evidence of ISR protection was shown by an experiment in which colonization of plant roots by PGPR

resulted in increased resistance of plant tissues against different pathogens (Van Loon et al. 1998) (Fig. 3).

II.2.1 Signalling pathway of ISR

In the past decade, research on the defence signalling pathways that are activated by beneficial microorganisms revealed that JA and ET are central players in the regulation of induced systemic resistance (ISR). In *Arabidopsis*, ISR elicited by *Pseudomonas fluorescence* WCS417r was blocked in the JA-signalling mutants *jar1*, *jin1*, *eds8*, and *coi1* (Pieterse et al. 1998; Ton et al. 2002; Pozo et al. 2008), and in ET signalling mutants such as *etr1* (*ethylene response 1*) and *ein2* (*ethylene insensitive 2*) (Pieterse et al. 1998; Knoester et al. 1999). Identically, for other PGPR (*P. fluorescence* CHAO (Iavicoli et al. 2003); *Serattia marcescens* and *Bacillus pumilus* (Ryu et al. 2004); *P. putida* LSW17S (Ahn et al. 2007), the role of JA and ET in the regulation of the ISR response of *Arabidopsis* was established. Likewise, ISR promoted by the plant growth promoting fungi (PGPF) *Penicillium* sp. GP16-2, *Trichoderma harzianum* T39 and *P. indica* was blocked in JA- and ET-signalling mutants of *Arabidopsis* (Hossain et al. 2008; Korolev et al. 2008; Stein et al. 2008).

In accordance with the role of JA and ET in the regulation of the ISR, transcriptional activity of *PR* genes was not increased in leaf tissue of *Arabidopsis* upon induction of ISR by *P. fluorescence* WCS417r (Pieterse et al. 1996). Furthermore, treatment of the roots of *Arabidopsis* with the PGPR *P. fluorescence* WCS417r was not associated with an increase in SA levels in systemic ISR expressing

leaf tissues (Pieterse et al. 2000). Many examples of SA-independent ISR have been demonstrated in *Arabidopsis* (Van Wees et al. 1997; Ryu et al. 2003; Stein et al. 2008; Segarra et al. 2009) and other plant species, such as tobacco (Zhang et al. 2002), cucumber (Press et al. 1997), tomato (Hase et al. 2008), and rice (De Vleesschauwer et al. 2008). The ability to activate an SA-independent pathway controlling systemic disease resistance seems to be common for beneficial microorganisms and occurs in a broad range of plant species against different types of attackers.

The defence regulatory protein NPR1 plays a key role in SA-dependent SAR, but has also been reported in JA/ET-dependent ISR (Pieterse and Van Loon 2004; Dong 2004). For instance, mutant *Arabidopsis npr1* plants were shown to be blocked in their ability to express ISR upon colonization of the roots by the PGPR *P. fluorescence* WCS417r (Pieterse et al. 1998), and other PGPR or PGPF as *P. fluorescens* CHAO (Iavicoli et al. 2003) and *T. asperellum* T34 (Segarra et al. 2009). In SAR, NPR1 plays an important role as transcriptional co-activator of SA-responsive *PR* genes expression. However, SA-independent ISR is not accompanied by the activation of SA-responsive *PR* genes (Pieterse et al. 1996). Hence, the role of NPR1 in ISR must be different from that in SAR. These different roles of NPR1 are not mutually exclusive, because simultaneous activation of SAR and ISR can lead to an additively enhanced defensive capacity compared to that of SAR and ISR (Van Wees et al. 2000). This suggested that NPR1 is important in regulating and connecting different hormone-dependent induced defence pathways (Dong 2004; Pieterse and Van Loon 2004; Pieterse et al. 2009). However, the exact

molecular mechanisms by which NPR1 exerts its role in these JA/ET-dependent ISR remains to be elucidated.

II.2.2 Local response to beneficial microorganisms

Only few interactions between plants and beneficial microorganisms leading to enhanced systemic resistance have been studied for locally induced changes in plant genes expression or metabolism. In the roots of rice and tomato plants, AM fungi were shown to induce the accumulation of a number of transcripts and proteins, respectively, many of which with a predicted function in plant defence (Güimil et al. 2005; Pozo and Azcon-Aguilar 2007). Likewise, a proteome approach of rice roots colonized by endophytic N2-fixing *Azoarcus* spp. identified an increase in JA-regulated PR-, salt stress-related- and putative receptor like-proteins, especially in less-compatible interactions (Miché et al. 2006). In *M. truncatula* the initial local transcriptional responses to the AM fungus *F. mosseae* showed significant overlap to those initiated by the PGPR *P. fluorescens* C7R12 (Sanchez et al. 2005). Moreover, both beneficial microorganisms were unable to elicit these shared transcriptional responses in the symbiosis-defective mutant *dmi3* (*does not make infections 3*), suggesting that the signalling pathways that are promoted by these different beneficial microorganisms converge (Sanchez et al. 2005).

II.2.3 Priming for enhanced defence

The role of JA and ET in the regulation of PGPR and PGPF to promote systemic defence responses has been mainly established through the analysis of JA- and ET-signalling mutants. However, colonization of the roots by ISR-inducing PGPR is often not associated with an increase in the production of these hormones (Pieterse et al. 2000). Hence, ISR seems to be based on increased sensitivity rather than on increased production of these hormones. Supportive of this notion is the observation that colonization of the roots by beneficial microorganisms is generally not associated with direct activation of JA/ET-responsive genes. As a matter of fact, the transcriptional changes that occur in systemic tissues (i.e. tissues in no direct presence of the beneficial microorganisms) upon colonization of the roots by beneficial microorganisms is in general relatively weak, especially in comparison to the massive transcriptional reprogramming that occurs upon pathogen attack (Verhagen et al. 2004; Fu et al. 2007; Liu et al. 2007). However, upon pathogen or insect attack, ISR-expressing plants display an accelerated defence response (Verhagen et al. 2004; Van Wees et al. 2008). This PGPR-mediated sensitization of the tissue for enhanced defence expression is called ‘priming’ and is characterized by a faster and/or stronger activation of various cellular defence responses that are induced following attack by pathogens or insects or in response to abiotic stress (Conrath et al. 2006). Although priming for enhanced JA/ET-dependent defences is well documented, it should be noted that priming for JA/ET-independent defences by PGPR and PGPF has been reported (Pozo and Azcon-Aguilar 2007; Conn et al. 2008;

Vinale et al. 2008; Van der Ent et al. 2009). However, the molecular mechanisms underlying priming are still poorly understood.

III. Mycorrhiza Induced Resistance (MIR)

Root colonization by AM fungi can improve plant resistance/tolerance to biotic stresses. The bio-protection of AM fungi has been reported in different plants (Review by Whipps 2004). However the mechanisms of bio-protection remain largely unknown. Several studies have reported that improved nutrition and competition within the rhizosphere are central mechanisms. Experimental evidence also supports the involvement of plant defence mechanisms in mycorrhizal plants. During AM fungal root colonization, modulation of plant defence responses occurs upon recognition of the AM fungi. The consequence of this modulation is an effective activation of the plant immune responses. This activation promotes a “primed state” of the plant that allows a more efficient and fast activation of defence mechanisms in response to the attack by a potential pathogen: the so-called mycorrhiza induced resistance (MIR) (Fig. 4).

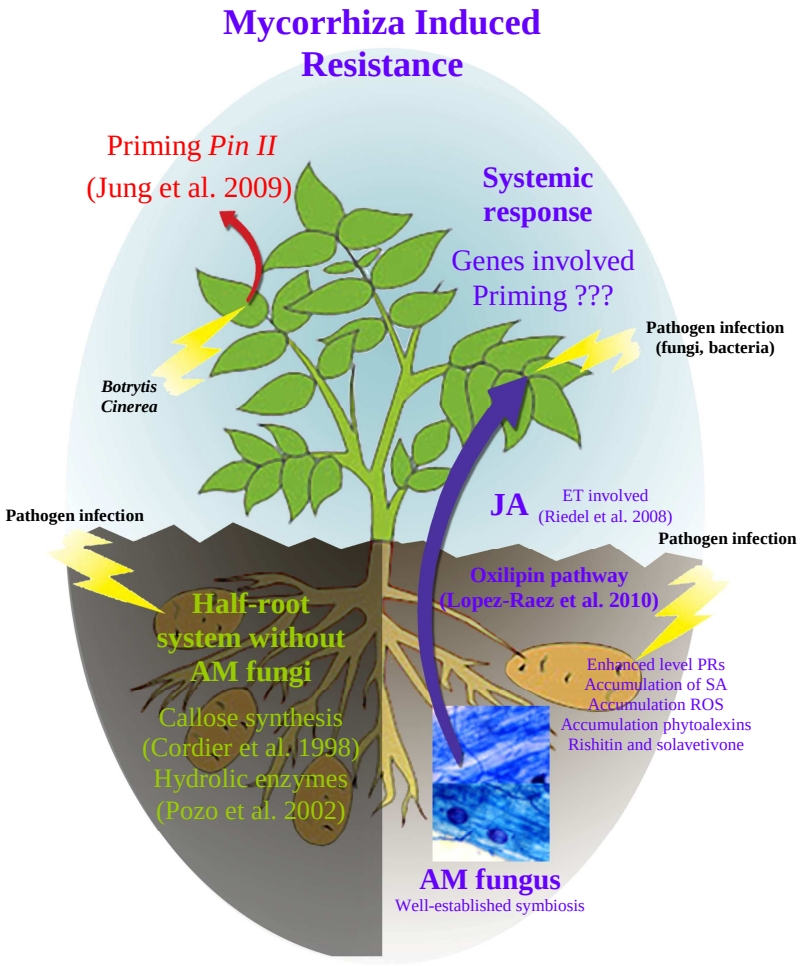


Fig. 4 Mycorrhiza Induced resistance mechanism in plants (e.g. potato)

III.1 Bio-protective effect of AM fungi

Most studies on bio-protection by AM fungi against soil-borne diseases report reductions in incidence and/or severity caused by fungi such as *Rhizoctonia*, *Fusarium* or *Verticillium* and by oomycetes such as *Phytophthora*, *Pythium* or *Aphanomyces* (see Review by Whipps 2004). Similarly, a reduction in detrimental effects caused by parasitic

nematodes, such as *Pratylenchus* and *Meloidogyne*, has been shown in mycorrhizal plants (de la Peña et al. 2006; Li HY et al. 2006; Elsen et al. 2008). In most cases, the local or systemic effect could not been ascertained due to the difficulty to precisely localize the pathogens and AM fungi on the root system. However, split-root experimental systems (i.e. allowing physical separation between AM fungi and pathogens) have revealed reductions in pathogen infection and in disease symptoms in the non colonized parts of root systems of mycorrhizal plants. Systemic protection at the root level has been demonstrated against *Phytophthora* and *Ralstonia* in tomato (Cordier et al. 1998; Pozo et al. 2002), *Gaeumannomyces* in wheat (Khaosaad et al. 2007) and recently against plant parasitic nematodes in bananas (Elsen et al. 2008). Finally, several studies have reported a positive effect of the symbiosis on plant resistance to shoot pathogens. In tomato, AM fungi reduced disease symptoms caused by a phytoplasma and conferred protection against the necrotrophic fungus *Alternaria solani* (Lingua et al. 2002; Fritz et al. 2006; de la Noval et al. 2007). Similarly, Liu and al. (2007) have shown an increased resistance of mycorrhizal *M. truncatula* plants to the shoot bacterial pathogen *Xanthomonas campestris*.

III.2 Mechanism of MIR

There is experimental evidence that plant protection by AM fungi results from a combination of mechanisms acting at different levels (Azcón-Aguilar and Barea 1996). The most widely accepted mechanisms to explain mycorrhiza-induced resistance are the

improvement of plant nutrition and the compensation by the symbiosis of the damage caused by the pathogen. However, studies including nutrient supplemented controls have shown that mycorrhizal effects cannot strictly be regarded as a consequence of improved phosphorus nutrition (Shaul et al. 1999; Fritz et al. 2006; Liu et al. 2007). The protective effects conferred to plants by AM fungi against pathogens may be linked with physiological and biochemical alterations of the host following mycorrhization, e.g. the induction of hydrolic enzymes (Pozo et al. 2002), the enhanced level of pathogenesis related (PR) proteins (Harrison and Dixon 1994), the accumulation of SA (Medina et al. 2003) and reactive oxygen species (Salzer et al. 1999). Some studies also attributed the AM fungal protective effects to a direct competition with the pathogen. Cordier et al. (1998) have suggested a competition effect at the sites of infection. Other studies have mentioned the competition for carbohydrates (Azcon-Aguilar et al. 2002) and the direct competition/inhibition in soil or changes in rhizosphere microbial population (Filion et al. 1999; Barea et al. 2005).

A key factor in the induction of resistance seems to be the extension of root colonization by AM fungi (Pozo et al. 2009). Studies comparing different mycorrhizal colonization levels concluded to the necessity of a well-established AM symbiosis for local and systemic induced resistance (Slezack et al. 2000; Khaosaad et al. 2007). The presence of AM fungi within the root prior to contact with the soil-borne pathogen has also been suggested as an important factor in plant protection (Azcon-Aguilar et al. 2002; Xavier and Boyetchko 2004). This aspect is probably linked with the level of mycorrhizal

colonization (Vierheilig et al. 2008). These authors suggested that the mechanism operating in autoregulation of AM fungal root colonization (i.e. limitation in the root colonization by the AM fungus in plants) may identically impact plant colonization by pathogenic fungi. It seems plausible that a mycorrhizal plant develops a mechanism to hinder further colonization by fungi, not discriminating between AM fungi and soil-borne pathogenic fungi (Vierheilig and Piché 2002; Vierheilig 2004).

III.3 Signalling pathway in MIR

Plant defence mechanism are tightly regulated through an interconnected network of signalling pathways in which JA, ET and SA play major roles (See paragraph II; Conrath et al. 2006; Van Wees et al. 2008). Roots of mycorrhizal plants are associated with higher levels of endogenous JA as compared to non-mycorrhizal plants. The increase occurs after the establishment of the AM fungus, and is probably related to well-established AM fungus symbiosis (Hause et al. 2002). Elevated levels of basal JA production could be related to the increased resistance of mycorrhizal plants to certain pathogens. Moreover, exogenous SA application delays AM fungal colonization, and plant mutants altered in endogenous SA levels point to a role of this pathway in the control of the AM symbiosis (García-Garrido and Ocampo 2002). It is plausible that AM fungi partially repress SA-dependent defence responses in the host in order to achieve a compatible interaction. A suppression of SA responses is also

necessary for the establishment of the *Rhizobium* –legume symbiotic association (Stacey et al. 2006).

Recently, Pozo and Azcon-Aguilar (2007) have proposed that a functional mycorrhiza implies partial suppression of SA-dependent responses in the plant, compensated by an enhancement of JA-regulated responses. This would results in the priming of JA-dependent defence mechanisms and could explain the effectiveness of MIR. This hypothesis was confirmed by the observation of Jung et al. (2009). The authors showed that a transcript profiling of leaves of mycorrhizal and non-mycorrhizal plants 24 h after treatment with JA revealed a stronger induction in mycorrhizal plants of JA-regulated genes, including typical defence-related JA responsive genes such as those coding for proteinase inhibitors (i.e. *Pin II*). However, the importance of each component of the plant resistance response in the protective effect of AM fungi against pathogens and the signalling pathways that control these responses remains mostly speculative.

IV. *Trichoderma* spp.

Trichoderma spp. are amongst the most studied beneficial fungi isolated from soil and present in the rhizosphere. These fungi are opportunistic plant symbionts, which function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases (Harman et al. 2004; Vinale et al. 2008). Depending on the strain, the use of *Trichoderma* spp. in agriculture can provide numerous advantages: (i) control of pathogens and of competitive/deleterious microflora; (ii) improvement of plant health;

(iii) increased plant growth, especially of roots and (iv) improved nitrogen use efficiency (Harman et al. 2004; Vinale et al. 2008; Harman 2011).

It has always been assumed that the beneficial effects of *Trichoderma* spp. for the plants were limited to the biological protection of the pathogens causing disease, particularly in the rhizosphere. It has also been demonstrated that *Trichoderma* is able to systemically activate resistance mechanisms of the plant to pathogen attack (Harman et al. 2004; Vinale et al. 2008) (Fig. 5). Different monocotyledonous and dicotyledonous crops, including Gramineae, Solanaceae and Cucurbitaceae infected with diverse fungi (*R. solani*, *Botrytis cinerea*, *Colletotrichum* spp., *Magnaporthe grisea*, *Phytophthora* spp., *Alternaria* spp. etc.), bacteria (*Xanthomonas* spp., *P. syringae*, etc.) as well as viruses (cucumber mosaic virus), were more resistant to disease development when the plants were treated with *Trichoderma* spp. (Review in Harman et al. 2004). Plant colonization by certain *Trichoderma* spp., reduced disease symptoms caused by one or two different pathogens even when the BCF (*Trichoderma* spp.) was inoculated at a different time (i.e. before or after infection by the pathogen) and at a different location on the plant than the pathogen.

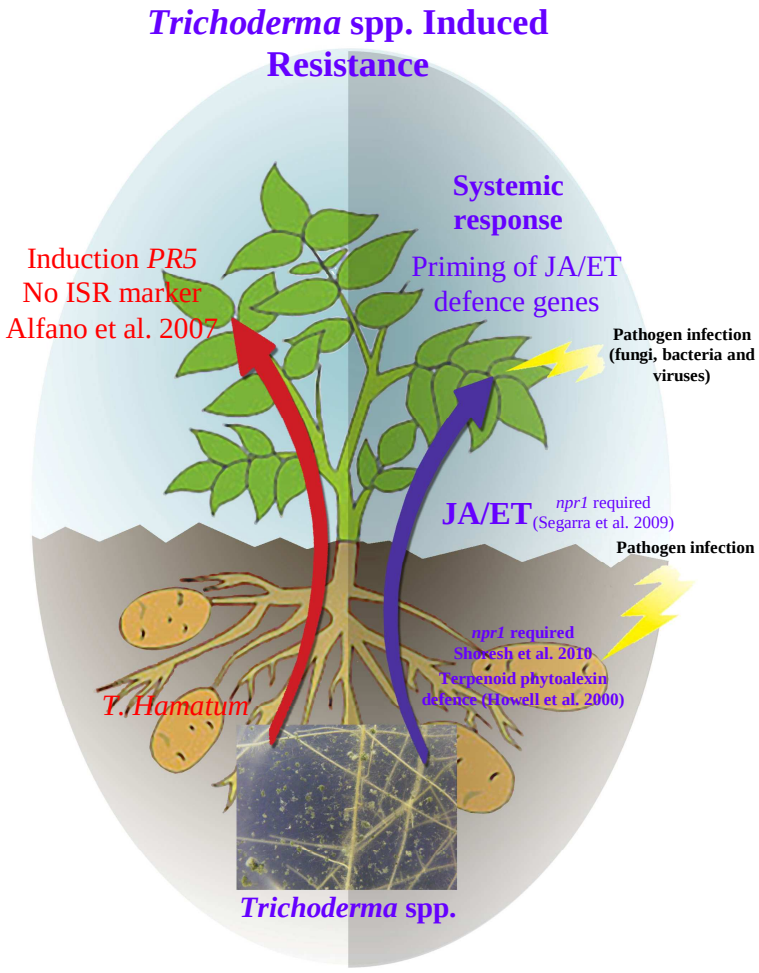


Fig. 5 *Trichoderma* spp. Induced Resistance mechanism in plants (e.g. potato)

These effects on the plant were observed at the molecular level (i.e. genes). For example, extracts from plants subjected to *Trichoderma* root treatments had more anti-microbial activity than extracts derived from untreated plants. This inhibitory effect corresponded to the induction of different endogenous PR and defence-related proteins (chitinases, glucanases, peroxidases and specific phytoalexins) and enzyme activities (HPL, PAL1) in the plant

(Yedidia et al. 1999; Yedidia et al. 2000). Actually, a relatively large number of chemical effectors/elicitors released by the fungi are known (i.e. small proteins, peptides and other metabolites, including volatiles). These effectors/elicitors have been recently reviewed, as signal of transduction in plants (Harman et al. 2004; Lorito et al. 2010; Shoresh et al. 2010). This signalling induced systemic effect in the plants.

The reaction of the plant to *Trichoderma* spp. was similar to the ISR elicited by the interaction of the plant with the PGPR (Van Wees et al. 2000; Yedidia et al. 2003). Lorito et al (2010) and Bae et al. (2011) have demonstrated that both JA and ET signalling may be involved in the systemic resistance process induced by *Trichoderma* spp.. However, it has been shown recently that some *Trichoderma* species could use the SAR to induce plant defence. Alfano et al. (2007) have demonstrated that transcriptomic analysis of tomato plant in presence of *T. hamatum* did not detect induction of ISR markers. The authors observed only the up-regulation of one marker of SAR (i.e. *PR5* gene). This result was confirmed by a recent observation that showed the absence of protection of the *npr1* mutant of *A. thaliana* against *Pythium ultimum* in presence of *T. harzianum*, while protection was observed in the wild-type strains (Shoresh et al. 2010).

The systemic resistance mechanism activated by *Trichoderma* spp. may play a greater role in plant protection than the mechanisms of biological protection. Moreover, the systemic resistance effect appears to be strongly dependent on the *trichoderma* spp. strain used and also on the plant species or cultivar. Clearly, the plants are induced to operate more efficiently and are more resistant to biotic stress if their

roots are colonized by highly effective *Trichoderma* strains (Soresh et al. 2010).

V. Biological control of potato pathogens

Potato (*Solanum tuberosum*) is an herbaceous annual plant. 2008 was acclaimed the year of the potato by the Food and Agriculture Organization (FAO) for (1) its key role in the world global food system as it is the world's fourth most produced food commodity, (2) its ability to grow worldwide, (3) its convenience for farming systems in developing countries – potato crops harbour a high ratio of yield productivity to soil occupation (i.e. 85% of the plant is comestible compared with only 50% in cereals), and (4) its nutritive qualities, with a higher amount of vitamins than grass plants (FAO 2008). Cultivation of potato is promoted by the United Nations for its high development potentialities in Asia and Africa and represents a possible decisive weapon in the fight against starvation.

Despite these numerous advantages, it should be noted that potato plants possesses some naturally occurring toxic compounds called glycoalkaloids (i.e. solanine and chaconine) principally located in the peripheral 1.5 mm layers of the tuber skin. These toxins are probably involved as protective compounds in response to tissue invasion by antimicrobial, insecticidal and fungicidal activities (Nema et al. 2008). The glycoalkaloids have also pharmacological and toxicological effects in humans. Small quantities ($<150 mg.kg^{-1}$/fresh weight) are

known to improve flavour, but these compounds can cause serious illness with possible fatal consequence at concentrations >280mg.kg^{-1}/fresh weight. Glycoalkaloids are not destroyed by cooking, but cutting away green areas and peeling potatoes before cooking ensures healthy eating.

Moreover, potato is susceptible to numerous pathogens for which control methods are lacking. In addition to the different pests (i.e. insects, nematodes), microbial pathogens are annually responsible for the loss of 25 % of worldwide production (FAO 2008). Today, the biological control methods represent a significant complement to other control methods, which are based on prophylactic measures, chemical treatments, genetic approaches or agricultural pratices (e.g. mulch, potato hilling or supression of the foliar phase against *P. infestans*).

The AM fungi are considered as a key functional group of soil biota, which can greatly contribute to crop productivity and ecosystem sustainability in new plant production strategies (Gianinazzi et al. 2010). Moreover, past studies have demonstrated that the inoculation by AM fungi under field conditions could have beneficial effects on potato crops and particularly as beneficial microorganism against two of the major potato disease. Larkin (2008) has demonstrated a diminution (i.e. 17-28 %) of the severity of stem canker and black scurf caused by *R. solani* Kühn, whereas O'Herlihy et al. (2003) have observed a delay in the progression of the late blight disease caused by *P. infestans*.

In addition to the AM fungi, *Trichoderma* spp. are among the most studied BCFs and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman et al. 2004; Lorito et al.

2010). The capacity of this BCF to reduce the disease triggered by *R. solani* on potato plant has been recently shown by Wilson et al. (2008). However, to our knowledge no studies have reported a biological protection of late blight disease on potato plants or on others plants in presence of *Trichoderma* spp..

V.1 *Rhizoctonia solani*

R. solani Kühn (telomorph: *Thanathephorus cucumeris* Frank Donk) is a soil-borne phytopathogenic fungus, which was originally described by Julius Kühn on potato. Black scurf and stem canker caused by *R. solani* Kühn represent economically important diseases. The *Rhizoctonia* disease complex is common and occurs in potato production areas throughout the world (Banville 1989; Powelson et al. 1993; Banville et al. 1996). Like other seed-borne pathogens (Slack 1993), *R. solani* is transmitted by contaminated seed tubers, providing a mechanism for its long-distance dispersal. Once established in soil, the mycelium and sclerotia of the pathogen may provide an additional source of primary infection.

The *Rhizoctonia* disease complex can cause both quantitative and qualitative damage to potato crops. Quantitative losses occur via infection of the stems, stolons and roots, which affects tuber size and number (Carling et al. 1989; Platt 1989), whereas qualitative losses occur mainly through the production of misshapen tubers and the development of sclerotia on the tuber surface (James and McKenzie 1972). Disease severity is not always associated with yield reduction. However, formation of tuber-borne sclerotia downgrades tuber

quality, causing the development of malformed tubers and an alteration in their target size and number (Carling et al. 1989; Jager et al. 1991; Tsror and Peretz-Alon 2005).

The protective effect conferred to potato plants by AM fungi against *R. solani* have been reported by Yao et al. (2002; 2003). In 2002, these authors demonstrated that the micro-propagated potato inoculated with *Claroideoglomus etunicatum* (Syn. *Glomus etunicatum*) exhibited a significant reduction in *R. solani,* ranging between 60.2 and 71.2%, on shoots and crowns, as well as a significant increase in shoot fresh weight, root dry weight and number of tubers produced per plant as compared to the non-mycorrhizal control, in greenhouse experiments. However, with the AM fungal *R. intraradices*, the authors observed only a significant increase of the tubers number as compared to the non-mycorrhizal control. In 2003, the authors noted that the decrease in the severity of symptoms caused by *R. solani* in potato plants associated to *C. etunicatum* was attributed to the accumulation of the phytoalexins rishitin and solavetivone in the mycorrhizal plants in presence of *R. solani*. In addition, the biological protection of *Rhizoctonia* disease was reported on potato under field condition with an inoculum containing eight AM fungal strain (Larkin 2008).

The beneficial effect of *T. harzianum* to control *R. solani* has also been reported recently on potato plants (Wilson et al. 2008). These authors noted a decrease in the severity of symptoms caused by *R. solani* during the first 7 days post-inoculation with *T. harzianum* and attributed this observation to a transient antagonistic effect of this biological control agent at the early stage of the interaction.

V.2 *Phytophthora infestans*

The oomycete *P. infestans* (Mont.) de Bary is a hemibiotrophic pathogen with biotrophic behaviour during early infection and necrotrophic behaviour in the later stage of colonization. Potato late blight is the most important and most destructive disease of potato worldwide. Costs attributed to losses and crop protections against this pathogen are estimated to US $3.25 billion per annum worldwide (Fry and Goodwin 1997). Tuber late blight results in tuber rotting both in the field and later during storage either in tubers used for seeds production or consumption (Kirk et al. 1999; Johnson and Cummings 2009; Olanya et al. 2009). Seed tubers infected with *P. infestans* will either rot during storage, after planting in the field or survive and initiate new epidemics of potato late blight (Dowley and O'Sullivan 1991; Kirk et al. 2009).

The epidemiology of the foliar phase of the disease is correlated to infection in the tuber phase and vice versa (Bain et al. 1997). Tubers are usually infected by inoculum produced on the plant foliage that is subsequently washed down to the soil by water movement resulting from rainfall and irrigation (Porter et al. 2005; Fry 2008). Tubers can become blighted shortly after the disease is established on the foliage. *P. infestans* survive in tubers where it rots tubers intended for commercial use (Niemira et al. 1999) or acts as a primary source of inoculum for infection during the following growing season (Bonde and Schultz 1943).

The protective effect conferred to potato plants by AM fungi against *P. infestans* remains uncertain. To our knowledge, no studies

actually have been published showing a clear positive or negative effect of AM fungi on this disease. Only one study reported a delay in the progression of disease on potato plants colonized with a commercial AM fungus inoculated in the field (O'Herlihy et al. 2003). However, some recent studies suggested a clear relevance of the AM fungus against above-ground pathogens. Lee et al. (2005) have observed a decrease of the anthracnose disease, induced by the hemibiotrophic fungus *Colletotrichum orbiculare*, on the leaves of cucumber plant colonized with *R. intraradices*. More interesting, Jung et al. (2009) have demonstrated that the expansion of necrotic lesions induced by *Botrytis cinerea* was markedly lower in the leaves of mycorrhizal plants colonized with *F. mosseae*. In addition, the authors have related the ISR conferred by the AM fungus to the induction of a JA marker gene, which could underline the existence of a similar mechanism in mycorrhizal potato plants infected by *P. infestans*.

In the case of *Trichoderma* spp., no studies are actually available on the potential of these BCFs to induce a systemic resistance against *P. infestans*, whatever the plants species. However, a study has shown that *T. hamatum* inoculated in compost induced SAR on cucumber, reducing the severity of *Phytophthora* leaf blight (Khan et al. 2004). Moreover, it is well known that *Trichoderma* spp. is able to produce a systemic response in plant during pathogen attack (Vinale et al. 2008; Bae et al. 2011). These results underline the capacity of *Trichoderma* spp. to develop an induced resistance against *P. infestans* disease.

Research objectives

The role of AM fungi and *Trichoderma* spp., in the biological protection of pathogens has been amply described in different plant systems. In the last decade, a particular attention has been addressed to the elicitation of plant defence mechanisms. However, the biological protection mechanisms during the interaction between these two beneficial microorganisms and two of the most important pathogens of potato crops (i.e. *R. solani* and *P. infestans)* remain largely unknown. The plant signalling pathways (i.e. SA, JA and ET) have been proposed to play an important role in the mechanisms of biological protection. In addition, numerous studies have demonstrated the complexity of transcriptional changes in plants during the AM fungal root colonization and underlined that a better knowledge of these molecular changes would result in a better comprehension of the biological protection triggered by the AM fungus.

The main objective of this thesis was to study the effects of *Rhizophagus* sp. MUCL 41833 and *T. harzianum* MUCL 29707 on the potato resistance against *R. solani* MUCL 49235 and *P. infestans* MUCL 43257, as well as to increase our understanding of the molecular mechanism involved in this increased resistance.

To answer this objective, four questions were addressed:

1. Is the Mycelium Donor Plant *in vitro* culture system adequate for plant genes expression analysis during the AM fungal root colonization?

2. Which are the transcriptional changes associated to the biotic stress during the potato root colonization by *Rhizophagus* sp. MUCL 41833?
3. Is *Rhizophagus* sp. MUCL 41833 able to decrease the symptoms caused by *R. solani* MUCL 49235 and *P. infestans* MUCL 43257 in potato plants? Which are the defence genes that could be involved in this process?
    A. Following infection of *R. solani* MUCL 49235 on mycorrhizal potato roots?
    B. Following infection of *R. solani* MUCL 49235 on non-mycorrhizal roots of mycorrhizal potato plants?
    C. Following infection of *P. infestans* MUCL 43257 on the leaves of mycorrhizal potato plants?
4. Is *T. harzianum* MUCL 29707 able to protect the potato plants against *R. solani* MUCL 49235 infection? Which are the defence genes that could be involved in this process?

Materials and Methods

This section summarizes the biological materials and briefly describes the most important techniques used in our research.

I. Biological material

For this research, we have selected two beneficial microorganisms:

***Rhizophagus sp* MUCL 41833:** This obligate biotroph, isolated from a soil of Canary Islands (Spain), was supplied by the Glomeromycota *in vitro* collection (GINCO – http://www.mycorrhiza.be/ginco-bel). This AM fungus formerly named *Glomus* sp. MUCL 41833 has been recently renamed *Rhizophagus* sp. MUCL 41833 according to the new classification of Schüβler and Walker (2010) (http://www.lrz.de/~schuessler/amphylo/ updated 10 March 2011). In addition, this fungus formerly identified as *G. intraradices* (now *R. intraradices*) Schenck and Smith was presently re-classified in a clade that contains the recently described species *G. irregulare* (*R. irregularis*) Błaszk., Wubet, Renker and Buscot (Stockinger et al. 2009).

Rhizophagus sp. MUCL 41833 is the most studied AM fungus under *in vitro* plant culture conditions. Its life cycle and development have been amply described under these conditions (Voets et al. 2009; De Jaeger et al. 2010).

***Trichoderma harzianum* Rifai MUCL 29707:** This opportunistic plant symbiotic fungus has been selected from the database of the Mycothèque de l'Université catholique de Louvain (MUCL - http://bccm.belspo.be/about/mucl.php). This fungal strain has been isolated from a soil of Heverlee (Belgium). Its life cycle was described under *in vitro* plant culture conditions by De Jaeger et al. (2010).

Two fungal plant pathogens have also been selected for this research:

***Rhizoctonia solani* Kuhn MUCL 49235**: This soil-borne phytopathogenic fungus belongs to the anastomosis group 3. This fungus has been selected from the MUCL database and was originally isolated from a potato field culture (Belgium). Moreover, the accomplishment of its life cycle was also confirmed under *in vitro* plant culture conditions (De Jaeger et al. 2010).

***Phytophthora infestans* (Mont.) de Bary MUCL 43257**: This plant pathogen is an Oomycete. It has been selected from the MUCL database and originally isolated from a potato field culture (Belgium).

The four strains were supplied as pure *in vitro* cultures or as *in vitro* produced spore inoculum (i.e. details about the strains are given in the respective chapters).

II. *in vitro* plant culture system

Two *in vitro* plant culture systems were used in this thesis. These *in vitro* culture systems have been developed to study various aspects of the AM fungi and AM symbiosis (e.g. mycelium architecture, anastomoses, healing process …). Recently, these culture systems were used to study plant-fungi interactions, either beneficial or pathogenic (De Jaeger et al. 2010). These systems present several advantages, among which are (a) the use of an autotrophic seedling representing a carbon source for the fungi, (b) the absence of undesirable microorganisms, (c) the possibility to non-destructively monitor the development of the fungal colonies and (d) the pure-high-quality DNA/RNA extraction (Croll et al. 2009).

II.1 Half-closed Arbuscular Mycorrhizal (H-AMP) system

In this *in vitro* culture system (i.e. H-AMP system; Voets et al. 2005), the plant roots were associated to an AM fungus on a gelled medium under strict *in vitro* culture conditions, while the shoot developed under open air conditions (Fig. 6). This autotrophic culture system allowed the AM fungus to develop typical colonization structures and to produce an abundant extra-radical mycelium. Numerous spores were produced, morphologically similar to those observed in the monoxenic culture systems on excised root organs and in pot cultures (Voets et al. 2005). However with this system, the obtention of a high level of AM fungal colonization takes several weeks and the homogeneity between the plants is hardly obtained.

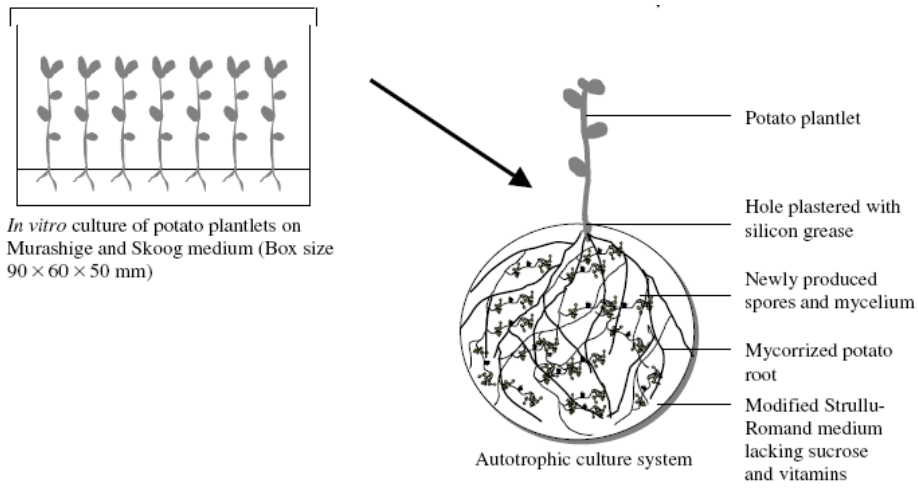


Fig. 6 Schematic representation of the Half-closed Arbuscular Mycorrhizal (H-AMP) culture system used for the *in vitro* mycorrhization of potato plantlets (adapted from Voets et al. 2006)

Briefly, this system consists in making a hole (±2 mm diameter) in the base and the lid of a Petri plate containing 50 ml of Modified Strullu Romand (MSR) medium lacking sucrose and vitamins, and solidified with 3 g l^{-1} Phytagel. A ten-day-old potato plantlet is then transferred to the Petri plate, with the roots placed on the surface of the medium and the shoot extending out of the Petri plate via the hole. Finally, the Petri plate is closed and sealed with Parafilm and the hole cautiously plastered with sterilized silicon grease to avoid contaminations. The Petri plate is finally covered with an opaque plastic bag and incubated vertically.

II.2 Mycelium Donor Plant (MDP) system

This system (i.e. MDP system; Voets et al. 2009) was developed following the same methodology than the H-AMP system and in contrast to the previous allowed to obtained homogenous highly

colonized plants under a short period (i.e. 9 to 12 days). This was realized by using the symbiotic phase of the fungus as inoculum (Voets et al. 2009) (Fig. 7).

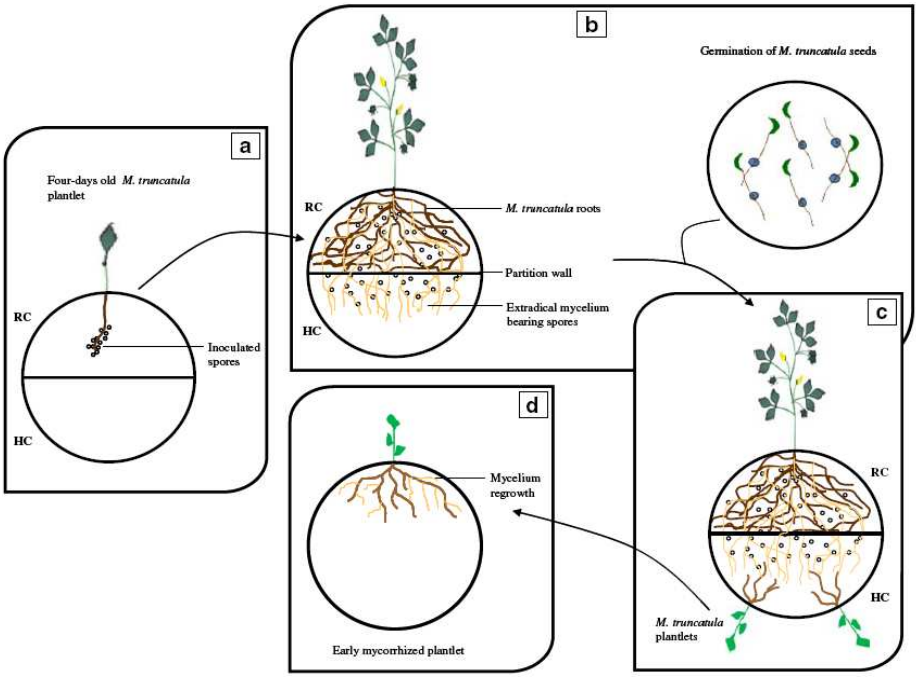


Fig. 7 Schematic representation of the mycelium donor plant (MDP) *in vitro* culture system developed in bi-compartmental Petri plates for the fast and homogenous mycorrhization of seedlings (from Voets et al. 2009). RC (root compartment) and HC (hyphal compartment). Briefly, this system consists of a bi-compartment Petri plate separating a root compartment (RC) from a hyphal compartment (HC)). In the RC, one four-days-old *Medicago truncatula* plantlet is associated with the AM fungus on the MSR medium, lacking sucrose and vitamins (a). After eight weeks, an important fungal network is developed in the RC, extending in the HC as a profuse extra-radical mycelium bearing hundreeds of spores (b). Two four-days-old *M. truncatula* plants are inserted in the HC with their roots placed in contact wit the extra-radical mycelium (c). Finally in the research of Voets et al. (2009), the mycorrhized *M. truncatula* plants were transferred in a mono-compartment Petri plate (H-AMP system) to analyse their capacities to reproduce the fungal life cycle, in order to confirm the functional state of the AM symbiosis (d)

III. Evaluation of root colonization by the AM fungus

The magnified intersections method developed by McGonigle et al. (1990) was used to assess the AM fungal root colonization. This method is nowaday considered as the most adequate to assess the extent of root colonization with arbuscules, vesicles and hyphae.

The experimental procedure consists to align the roots in parallel to the long axis of the slide which are then observed at magnification 10-40x (i.e. generally 2 slides are used for each subsample). The field of view of the microscope is moved using the stage graticule to make ten complete passes across each slide perpendicular to its axis (i.e. number and distance between passes is not critical, but should be constant for a subsample). Except when the cortex is missing, all intersections between roots and the vertical eyepiece crosshair are considered. The position on the root surface at which the centre of the eyepiece crosshairs enters through the side of the root is taken as the point of intersection. Rotation of the vertical crosshair ensures each intersection is at right angles to the axis of the root (Fig. 8). Where the centre of the crosshairs enters a root through an end rather than a side, the point of exit from the root through its side is taken as the point of intersection.

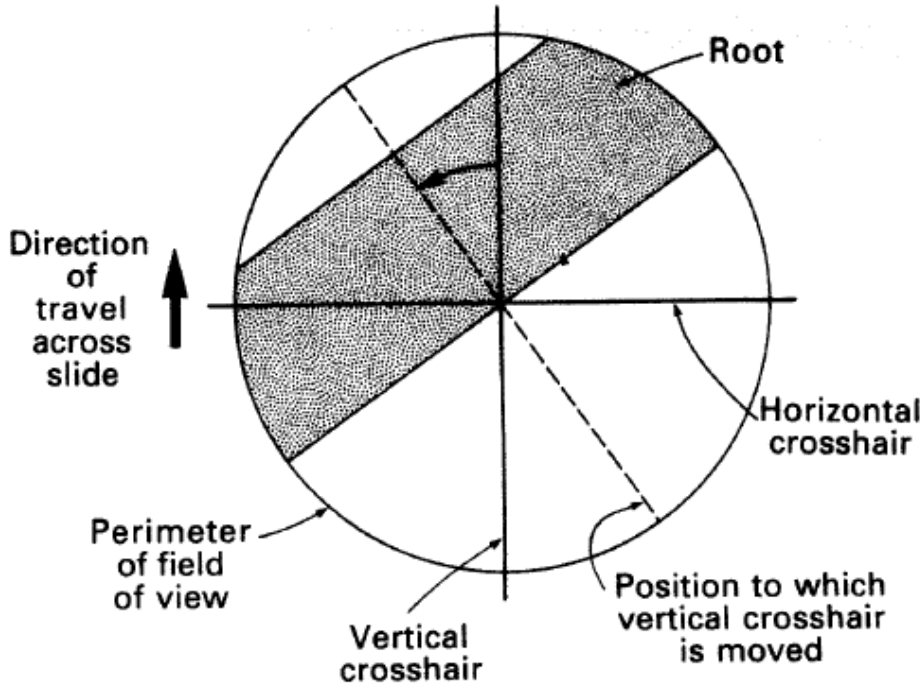


Fig. 8 Diagram showing how a magnified intersection perpendicular to the long axis of the root is made when the root is aligned with its long axis at an angle to the vertical crosshair. The stage is moved until the centre of the crosshairs is contiguous with the first edge of the root reached. To make the perpendicular intersection, the vertical crosshair is then rotated as shown (from McGonigle et al. 1990)

At each intersection, a mark is made if the vertical crosshair crosses an arbuscule, vesicle or hyphae. Intersections are counted in the following categories: “negative” (i.e. no AM fungus observed in root), “hyphae only”, “arbuscules” and “vesicles”. If the vertical crosshair crosses one or more arbuscules or vesicles, the category is incremented by one, and similarly for intersections where hyphae only are observed. When both arbuscules and vesicles are visualized at an intersection, the total number of intersections is only added by one. The arbuscular colonization and vesicles colonization are calculated by dividing the count for the “arbuscules” and “vesicles” categories respectively by the total number of intersections examined. Hyphal

colonization is calculated as the proportion of non-negative intersections. The vizualisation of 100 to 150 intersections per subsample is recommended for this method.

IV. Selection of the reference and targed genes

Throughout the experiments, we have used four reference genes in order to normalize the expression of eight targed genes. The four reference genes were the following:

The elongation factor 1-alpha (*EF1-α* - AB061263): this gene is encoding for a protein which is responsible for the GTP-dependent binding of aminoacyl-tRNAs to the ribosomes. Nicot et al. (2005) have tested the expression of eight reference genes in potato plants exposed to three a/biotic stresses. The authors have shown that the expression of the *EF1-α* gene appeared not influenced during cold, salt, and late blight stresses.

The beta-tubulin (*ß-tub* - Z33382): this gene is encoding for a protein which is a major components of microtubules (i.e. with the alpha-tubulin). The expression of this gene appeared not infuenced during the late blight stress (Nicot et al. 2005).

The ubiquitin conjugating enzyme-like (*Ubc* - DQ241834): this gene is encoding for an enzyme, which has a function in the ubiquitin-mediated protein degradation pathway. This gene is one of the most stable expressed genes in the soybean plants under different conditions and tissues in different (Jian et al. 2008). This

result was alos observed in others plants such as the rice (Jain et al. 2006).

The glyceraldehyde 3-phosphate dehydrogenase (*GAPDH* - AF527779): this gene is encoding for an enzyme involved in glycolysis and glyconeogenesis. This gene is one of the most common genes described for the normalization of gene expression analysis in numerous organisms (Stürzenbaum and Kille 2001).

We decided to select these four genes as reference genes for the normalization of our gene expression analyses by real-time QRT-PCR.

For the targed genes, we decided to select one plant gene marker of the AM fungal colonization and seven genes related to the plant defence mechanism. The targed genes were:

The phosphatase transporter 3 (*PT3* - AJ318822): this gene is encoding for a transmembrane protein, which is specifically induced in arbuscule-containing cells. Moreover, this gene has been shown as a molecular marker to investigate the functional state of the AM symbiosis (Rausch et al. 2001). We used this gene to confirm the establishment of an effective association between the two partners of the symbiosis.

The ethylene response factor 3 (*ERF 3* - EF091875): this gene is encoding for a transcription regulator, formerly called ethylene-responsive element binding proteins (EREBPs), with a promotor element essential for ethylene responsiveness. Moreover this

protein is involved in stress response (Chen et al. 2008). We used this gene as a marker of the ET pathway.

The gluthatione-S-transferase 1 (*GST1* - J03679) (i.e. previously called pathogenesis-related protein; prp1-1): this gene is encoding for an auxin-responsive glutathione-S-transferase 1, which is involved in the cellular detoxification. This gene is also reported to be involved in defence responses (Hahn and Strittmatter 1994). Moreover, recent results provided significant insight into the involvement of gluthatione signalling in NPR1-dependent SA-mediated pathway in mitigating biotic stress (Ghanta et al. 2011). We used this gene as a marker of the SA pathway.

The lipoxygenase (*Lox* - Y18548): this gene is encoding for the lipoxygenase 1 enzyme, which exhibited dual positional specificity and produced roughly equimolar amounts of 13- and 9-hydroperoxides from linoleate (Hughes et al. 2001). Moreover, the immediate products of these enzymes (i.e. multigene families) are involved in defence mechanisms against pathogens (Hwang and Hwang 2010). We used this gene as a marker of the JA pathway.

The mitogen-activated protein kinase (*MAPK* - AB206552): this gene is encoding for a wound-induced protein kinase (i.e. WIPK). The MAPKs are associated with physiological, developmental, hormonal, and stress responses. More particularly, some results indicate that WIPK and (SA)-induced protein kinases are convergent points in the signalling pathway of defence responses in plant-pathogen interactions (Yamamizo et al. 2006).

The phenylalanine ammonia lyase (*PAL* - X63104): this gene is encoding for the phenylalanine ammonia lyase 2 (Joos and Hahlbrock 1991), which is a key enzyme catalyzing the first reaction in the biosynthesis from L-phenylalanine of a wide variety of natural products based on the phenylpropane skeleton. Moreover this protein is believed to be activated by the JA/ET signalling pathway in the context of induced plant defences (Kato et al. 2000). We used this gene as a marker of the JA/ET pathways.

The pathogenesis related 1 (*PR1* - AJ250136): this gene is encoding for the PR1-b protein (i.e. basic form) (Hoegen et al. 2002). This protein may act as an anti-fungal agent and was also shown to be linked to a partial resistance to *P. infestans* in *Solanum* species (Vleeshouwer et al. 2000). This gene (i.e. as *NtPRb-1b* gene; basic form) is associated to the ISR (van Loon and van Strien 1999). We used this gene as a marker of the JA/ET pathways.

The pathogenesis related 2 (*PR2* - AJ009932): this gene is encoding for an acid β-1,3-glucanase protein (Mac et al. 2004). This enzyme is involved in cell wall degradation by digesting β-1,3-glucans in fungal cell walls (Mauch and Staehelin 1989; Chang et al. 1992). This gene is a classical marker of the SAR (van Loon and van Strien 1999). We used this gene as a marker of the SA pathway.

V. Primer design and assay validation

We have designed the best possible primer pairs for each reference and target genes above. A good design is essential for the sensitivity and reproducibility of the real-time QRT-PCR analysis. For the design of each primer pairs, we have used the Light Cycler Probe Design Software 2.0 (Roche, Montreal, QC, Canada) with the following parameters:

- Primer length: 18-23 base pairs (bp) (i.e. this length is long enough for adequate specificity, and short enough for primers to bind easily to the template at the annealing temperature).
- Amplicon length: 90-110 bp.
- Primer melting temperature: 60°C (i.e. Tm above 65°C has a higher potential for secondary annealing).
- GC content: between 40% and 60%

Following the design, all the primer pairs were blasted on the *S. tuberosum* (NCBI database) and on the NCBI nr-database. This is to look for similarities between the target sequence and any closely related family genes or pseudo genes (i.e. non-specific primer pairs were discarded). Then, the primer pairs were checked for the primer-dimer and hairpins formations.

In the next step we confirmed the specificity of the primer on a biological sample (i.e. same material than the experiment). Melting curve analysis was performed in order to display a single peak, which indicates that a single PCR product has been generated. Moreover, the confirmation of the size of the amplicon was realized by gel electrophoresis.

Finally, the primer efficiency was calculated by a standard curve using a serial 10-fold dilution. To allow a good comparison and normalisation, primer efficiency should vary between 80 and 115 %. In this thesis, all the primer efficiencies were between 86% and 114%.

VI. Analysis of the relative gene expression ratio

Throughout this thesis, the relative expression ratios of each targed genes were calculated with the relative expression software tool (REST 2009; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). The REST 2009 Software applies a mathematic model that takes into account the different primer efficiencies and multiple reference genes for normalization. Using multiple reference genes, the geometric mean of all reference gene concentrations was used to calculate the relative expression of individual genes to allow alternative approximations of the true expression values, as following:

$$\text{Concentration} = \text{efficiency}^{\text{average CP (controls)} - \text{average CP (samples)}}$$

$$\text{Relative expression ratio} = \frac{\text{Concentration of targed gene}}{\text{Geometric mean (concentration of reference gene 1, concentration of reference gene 2, …)}}$$

The traditional relative quantitation allows gene expression to be estimated but can not provide statistical information suitable for comparing expression in groups of treated (i.e. treatments) and untreated (i.e. control) samples in a robust manner. The integrated randomization and bootstrapping methods used in REST 2009

Software test the statistical significance of calculated expression ratios.

In the following paragraph, an example of relative expression ratio measured by randomization methods with the REST 2009 (from REST 2009 Software User Guide; http://www.gene-quantification.de/rest-2009.html) (Table 2) is given:

Randomizing for a small set (n=10) produces the relative expression ratio shown in Table 3 (unsorted) and Table 4 (sorted).

Table 2 Example expression data

Index	C_{REF}	S_{REF}	C_{GOI}	S_{GOI}
1	26.74	26.77	27.57	24.54
2	26.85	26.47	27.61	24.95
3	26.83	27.03	27.82	24.57
4	26.68	26.92	27.12	24.63
5	27.39	26.97	27.76	24.66
6	27.03	26.97	27.74	24.89
7	26.78	26.07	26.91	24.71
8	27.32	26.30	27.49	24.9
9		26.14		24.26
10		26.81		24.44

Table 3 Randomization results

j	k	C_{REF}	C_{GOI}	S_{REF}	S_{GOI}	Expression
6	10	27.03	27.74	26.81	24.44	8.625105575
7	8	26.78	26.91	26.30	24.90	2.938192778
1	2	26.74	27.57	26.47	24.95	5.186421266
3	1	26.83	27.82	26.77	24.54	9.480147506
6	6	27.03	27.74	26.97	24.89	7.021676066
1	7	26.74	27.57	26.07	24.71	4.675718457
6	2	27.03	27.74	26.47	24.95	4.797510275
1	2	26.74	27.57	26.47	24.95	5.186421266
1	2	26.74	27.57	26.47	24.95	5.186421266
8	6	27.32	27.49	26.97	24.89	4.844473339

Crossing point values of control (C_{GOI}) and sample (S_{GOI}) for the gene of interest (i.e. targed) and of control (C_{REF}) and sample (S_{REF}) for the reference gene. i: choice of the index for C_{GOI} and C_{REF}. k: choice of the index for S_{GOI} and S_{REF}

These relative expression ratios were sorted. Then, the "true" expression ratio and the standard error range were calculated.

Table 4 Relative expression ratios sorted

Expression
2.938192778
4.675718457
4.797510275
4.844473339
5.186421266
5.186421266
5.186421266
7.021676066
8.625105575
9.480147506

The relative expression ratio is calculated at the position (i.e. median): relative expression ratio sorted at position “0.5 x n” (i.e. n=number of randomizations). For this example, the result was calculated at the fifth position:

Relative expression ratio = 5.186421266

The standard error range, corresponding to the 68% confidence interval, is defined as follows (i.e. $\alpha = 0.32$): minimum = relative expression ratio sorted at position “n x (α/2)” and maximum = relative expression ratio sorted at position “n x (1– α/2)”. For this example the result was:

Confidence68% or standard error range = [4.675718457 - 8.625105575]

Finally, the REST 2009 software determines whether a significant difference exists between samples and controls, while taking issues of reaction efficiency and reference gene normalization into account. Because the normalization and efficiency calculations involve ratios and multiple sources of error, it would be extremely difficult to devise a traditional statistical test, so randomization techniques are employed. The software calculates the 95% confidence interval to perform the hypothesis test: *P(H1)* represents the probability of the alternate hypothesis that the difference between the sample and control groups is due only to chance (i.e. if values in the 95% confidence interval

were greater than 1 with a p-value ≤ 0.05, then the expression of the targed gene in the treated sample was up-regulated).

VII. Design of the potato array

In this thesis, we used the potato array designed by the Potato Oligo Chip Initiative (POCI) (Kloosterman et al. 2008). A set of 246,182 sequence tag (EST) were used to design the array. The ESTs were extracted from approximately 37 libraries derived from various potato tissues, developmental stages, and physiological conditions, including exposure to common scab, *P. infestans*, as well as salt, cold, heat, and drought stress. The EST sequences were produced from the cultivars Bintje, Kennebec, Kuras, Shepody, Agria, and Solara (Table 5).

Table 5 Sources and numbers of potato EST sequences used for the array EST assembly (adapted from Kloosterman et al. 2008)

Institution	Raw ESTs	Cultivars	Reference
TIGR[a]	125927	Kennebec and Bintje	Rensink et al. (2005a, b)
Canadian Potato Genome Project	98976	Shedopy	Flinn et al. (2005); Li et al. (2007)
Aalborg University	12249	Kuras	Crookshanks et al. (2001); Nielsen et al. (2005)
University Erlangen-Nuremberg	6833	Solara	Unpublished
Wageningen University	1824	Bintje	Unpublished
Agriculture and Agri-Food Canada	370	11379-03	Unpublished
Total	246182		

[a] The Institute for Genomic Research

The combined contigs and singletons produced a total of 46345 unique sequences with an average length of 806 bp. From these unigenes, 44K Potato Array, using the Agilent's 60-mer oligos SurePrint technology, were designed for 42034 of the sequences. The

sequences on the POCI array represent a total of 184620 ESTs. Moreover, the POCI array design is based on a large EST collection containing genes from a wide variety of tissues, developmental stages, and treatments allowing for a diverse range of gene transcription studies covering all aspects of potato biology.

VIII. Normalization of the microarray data

In order to minimize the errors introduced by differences in procedures and dye intensity effects between the different arrays, a normalization method was applied. Three algorithms could be used (See Agilent Feature Extraction Software (v9.5) User Guide):

Linear: briefly, **"***this method multiplies the background-subtracted signal by a global constant. The global constant is determined separately for the red channel and the green channel using the selected normalization features. It is calculated using the geometric mean of these features as a value of 1000*" (Agilent Feature Extraction Software (v9.5) User Guide).

LOWESS: "*This method assumes the dye bias is intensity-dependent, and therefore the background-subtracted signal is multiplied by a value that is a function of the feature intensity. The selected normalization features are used to calculate a normalization curve that measures the potential log ratio bias across the entire range of feature intensities. A well-known curve fitting method called LOWESS a "locally weighted linear regression curve fit" is used to fit this curve. Given any feature*

and its intensity, this curve determines the potential dye bias that should be present in the log ratio measurement of the feature. Features are then corrected by multiplying the red and green channels by an adjustment that will remove the bias from the log ratio" (Agilent Feature Extraction Software (v9.5) User Guide).

Linear and LOWESS: "*Dye biases tend to be signal intensity-dependent. A dye normalization method that uses a constant scaling factor can not correct for dye biases across the entire range of signal intensities. This method does a linear normalization across the entire range of data, then applies a non-linear normalization (LOWESS) to the linearized data set*" (Agilent Feature Extraction Software (v9.5) User Guide).

Chapter I

Fast track *in vitro* mycorrhization of potato plantlets allows studies on gene expression dynamics

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Preface

In order to evaluate the effects of the AM fungus[2] *Rhizophagus* sp. MUCL 41833 on the potato resistance against two major potato pathogens and the molecular changes triggered in the plants, we used the Mycelium Donor Plant (MDP) *in vitro* culture system (Voets et al. 2009) rather than the half-closed arbuscular mycorrhizal-plant (HAM-P) *in vitro* culture system (Voets et al. 2005). Both systems were adequate to study the fungal life cycle. However, the MDP *in vitro* culture system allowed to obtain homogenous AM fungal colonization of plantlets within short time (i.e. 9 days) and the synchronization of the AM fungal development in the roots, while with the HAM-P *in vitro* culture system the completion of the life cycle took several weeks and the AM colonization was less homogenous (De Jaeger et al. 2010). This difference is related to the delay for the AM fungal spore to germinate, grow, form appressorium, colonize the roots and develop arbuscules and vesicles with the HAM-P *in vitro* culture system. In addition, the others microorganisms used in our study (i.e. *R. solani* and *T. harzianum*) completed their life cycles after only one week (De Jaeger et al. 2010) and consequently the synchronization and interaction studies of the microorganisms was impossible with the HAM-P *in vitro* culture system.

[2] In this chapter, we took into consideration the AM fungal classification of Schüβler et al. (2001)

Abstract

Root colonization by AM fungi is a dynamic process involving major changes in plant gene expression. Here, the expression of a phosphate transporter gene (*PT3*) and several defence genes, already known to be involved in the various stages of AM establishment, were monitored in the Mycelium Donor Plant (MDP) *in vitro* culture system associating potato plantlets with an AM fungus. This system allows fast and homogenous mycorrhization of seedlings at their early stage of development by growing the plantlets in active mycelial networks but has never been validated for gene expression analysis. Here, real-time QRT-PCR analyses were conducted in parallel to pre- (1 day), early (2 and 3 days) and late stages (6, 9 and 15 days) of root colonization. We observed the induction of a plant gene marker of AM root colonization (*PT3*) at the late stage and the induction of *MAPK* and *PAL* genes at the early and late stages of root colonization. We also demonstrated the induction of *PR1* and *PR2* genes at pre- and late stages and of *GST1* and *Lox* genes at a late stage of root colonization. These results validated the MDP *in vitro* culture system as an optimal tool to study gene expression analysis during the AM fungi establishment. This system further opened the door to investigate gene networks associated with the plants-AM fungi symbiosis.

Keywords: gene expression, *Glomus* sp., *in vitro* system, mycelium network, *Solanum tuberosum*

Introduction

Arbuscular mycorrhizal (AM) symbiosis represents a unique association between the mycelium of a soil-borne fungus and more than 80% of higher plants. The fungus, an obligate biotroph belonging to the Phylum Glomeromycota (Schüβler et al. 2001), receives carbohydrates from the host plant required to complete its life cycle, in exchange, it provides the plant with nutrients, such as phosphate. The establishment of this successful mutualistic association develops from a complex and dynamic process involving major changes in fungal and plant gene expression (Franken et al. 2007; Reinhardt 2007). During this process, phosphate transporter genes (Liu et al. 1998) as well as transient expression of defence related genes are activated in the host plant (Liu et al. 2003).

In the last few years, *in vitro* cultivation systems associating excised root organs with AM fungi have been used to investigate gene expression (Gonzalez-Guerrero et al. 2005; Elfstrand et al. 2005; Waschke et al. 2006). These systems present several advantages among which the absence of undesirable microorganisms and the possibility to monitor non-destructively the development of the fungal colony. However, some limitations are also associated to these systems, materialized by the absence of photosynthetic tissues, a normal hormonal balance and physiological source-sink relationships (Fortin et al. 2002). In addition, the roots used in these experiments are most often transformed organs.

Recently, Voets et al. (2005) and Dupré de Boulois et al. (2006) developed two *in vitro* autotrophic culture systems associating

respectively potato and *Medicago truncatula* to ROC produced AM fungal spores. Mass production of spores (i.e., approx. 12,000 in 22 weeks) was obtained with potato (Voets et al. 2005), and the transport of C from the shoot of *M. truncatula* to the fungus (Voets et al. 2008) and of P and Cs from a root-free labelled compartment to the shoot via the extra-radical mycelium (Dupré de Boulois et al. 2006) was demonstrated. However, with both systems, high-level colonization took several weeks, and produced nearly full-grown mycorrhizal plants before trialling. This may hinder experiments on gene expression analysis in plantlets during the early stages of root colonization. It is obvious that any system allowing the fast and homogenous mycorrhization of seedlings within a few days is highly desirable.

In nature, the AM fungal mycelium growing from colonized roots represents an important source of inoculum for the colonization of neighbouring plants, due to the several hyphae apexes ramifying from the colony (Friese and Allen 1991). Recently, Voets et al. (2009) have developed a new *in vitro* mycorrhization system adapted to seedlings, by using the symbiotic phase of the fungus as inoculum, for fast and homogenous AM colonization. This Mycelium Donor Plant (MDP) *in vitro* culture system was successfully applied to *M. truncatula* plants opening large perspectives to study various aspects of the AM fungi and AM symbiosis.

In this study, we validated the MDP *in vitro* culture system of Voets et al. (2009) for gene expression analysis. The expression of a phosphate transporter gene (*PT3*) and several defence genes, already well known to be involved in the various stages of AM establishment,

were analyzed at six time points, corresponding to the establishment of *Glomus sp.* MUCL 41833 in roots of potato plantlets, by combining the real-time QRT-PCR technology with the assessment of root colonization.

Materials and methods

Biological material

Propagation and maintenance of stock of potato plantlets

Potato plantlets propagated *in vitro* (*Solanum tuberosum* L., var. Bintje) were supplied by the *Station de Haute Belgique* in Libramont, Belgium. Plantlets were micro-propagated every five weeks as described in Voets et al. (2005).

Culture, propagation and maintenance of *Glomus sp.* MUCL 41833

A root organ culture of *Glomus* sp. MUCL 41833, formerly identified as *Glomus intraradices* Schenck and Smith and presently reclassified into a clade contains the recently described species *Glomus irregulare* Blaszk., Wubet, Renker and Buscot (Stockinger et al. 2009) was supplied by GINCO (http://www.mycorrhiza.be/ginco-bel). The spores were extracted by solubilisation of the gellan gel (Doner and Bécard 1991) and approximately 100 were placed in the near vicinity of actively growing transformed carrot (*Daucus carota* L.) roots (approximately 70 mm in length) on Petri plates (90 mm in diameter) containing the Modified Strullu-Romand (MSR) medium

(Declerck et al. 1998 modified from Strullu and Romand 1986), solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA) (Cranenbrouck et al. 2005). The Petri plates were incubated for 3 months in the dark in an inverted position at 27 °C, and several thousand spores were produced during this period.

Medicago truncatula seed disinfection

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A 17 (SARDI, Australia) were surface-sterilized by immersion in sodium hypochlorite (8% active chloride) for 12 min, rinsed three time in de-ionized sterile water and germinated in groups of 25 on Petri plates (90 mm in diameter) filled with 35 ml MSR medium without sucrose or vitamins, and solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA). Petri plates were incubated at 27°C in the dark.

Experimental design

Micro-propagated potato plantlets were plated on actively growing extra-radical mycelium networks of AM fungi developing in the hyphal compartment (HC) of bi-compartmental Petri plates (for details, see Voets et al. 2009). The time-course of gene expression was analyzed during symbiotic establishment in potato roots.

Eight weeks after association, a dense extra-radical mycelium bearing numerous spores developed in the HC. The length of mycelium was 6261 cm ± 2367 and the number of spores 19385 ± 9710 (estimated following the method of Voets et al. 2005).

Two new holes (± 2 mm diameter), separated by 4.5 cm from each other, were then made in the base and the lid of the Petri plates, at the side of the HC. One ten-day old potato plantlet was inserted in each hole following the same methodology described by Voets et al. (2009) with their roots in direct contact with the extra-radical mycelium (Fig. 9). The Petri plates were then sealed carefully and incubated in a growth chamber under controlled conditions, that is 20/16°C (day/night), 70 % relative humidity, photoperiod of 16 h d^{-1} and an average photosynthetic photon flux (PPF) of 225 µmol m-2 s^{-1}. Identically, two micro-propagated potato plantlets were placed in the HC of the control treatment (i.e. non-inoculated four-day old *M. truncatula* seedlings) and grown under the same conditions as described above.

Fig. 9 Mycelium Donor Plant (MDP) *in vitro* culture system, one day after placing two 10-day-old potato plantlets in the hyphal compartment. Root compartment (RC) containing *M. truncatula* roots associated with the AM fungus *Glomus* sp. 41833. Hyphal compartment (HC) containing the extra-radical mycelium of the AM fungus extending from the RC and the root systems of the two potato plantlets

Twenty-four cultural systems were randomly divided into 6 groups of four replicates. Roots were harvested 1, 2, 3, 6, 9 and 15 days after plating (dap) in the extra-radical mycelium network of *Glomus* sp. MUCL 41833. One control plant was also harvested each time. For each cultural system, the two potato plantlets from the HC were pooled to obtain sufficient material for analysis. Half of the material was then used to estimate root colonization while the other half was used for gene expression analysis.

Root colonization

The root colonization was estimated under a dissecting microscope (Olympus SZ40, Olympus Optical GmbH, Hamburg, Germany) at x10 to x40 magnification, according to McGonigle et al. (1990). Roots were cleared in 10% KOH at 50 °C for 90 min, rinsed with distilled water and stained with Trypan blue 1% (Phillips and Hayman 1970) at 50°C for 60 min. Percentages of root colonization were subjected to one-way ANOVA. Tukey Honest Significant Difference (HSD) was conducted to identify significant differences ($P \leq 0.05$) between the groups. Data analysis was performed with the SAS enterprise guide 4.1 (SAS Institute Inc., Cary, USA).

RNA extraction

Total RNA was extracted from 50-100 mg frozen material with Trizol® reagent (Invitrogen, Carlsbad, USA) with an additional chloroform purification step and then purified using the Purelink™

Micro-to-midi total RNA purification system (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The total RNA was subsequently treated with the TURBO DNA-*free*™ kit (Ambion, Austin, USA) according to the manufacturer's instructions. Concentration and purity of total RNA were determined in a NanoDrop®-ND 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA), using a 2 µl aliquot of the total RNA solutions. RNA purity was estimated from the A260/A280 absorbance ratio.

Reverse Transcription

Following total RNA extraction, Reverse transcription (RT) of 500 ng of RNA was performed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Montreal, Canada) in a volume of 20 µl with oligo $(dT)_{18}$ primer at 55°C for 20 min, according to the manufacturer's instructions. For each RNA sample, a reaction without RT was performed as a control for contamination by genomic DNA.

Primer design

Four reference genes: glyceraldehyde phosphate dehydrogenase (*GAPDH*), ubiquitin conjugating enzyme-like (*Ubc*), elongation factor 1-alpha (*EF1-α*) and beta-tubulin (*β-tub*) (Nicot et al. 2005), and seven genes: Gluthatione-S-transferase 1 (*GST1*), Lipoxygenase (*Lox*), MAP kinase (*MAPK*), Pathogenesis Related 1 (*PR1*), Pathogenesis Related 2 (*PR2*), Phenylalanine ammonia lyase (*PAL*) and Phosphate

transporter 3 (*PT3*) were selected. Potato nucleotidic sequences were obtained from the GenBank database. Eleven primer pairs were designed from these sequences (18-23 bp length, temperature of annealing at 60°C, GC% between 40% and 60%) with the Light Cycler Probe Design Software 2.0 (Roche, Montreal, Canada). The forward and reverse primer sets and melting temperatures (in brackets) were as follows: 5'-CCAAGTAACCTCTTGCTAAATGC-3' and 5'-CTGTCATATTCTCGTTCTCTAGG-3 for *MAPK* (79°C); 5'-GCTTTGCTTACTTATTATTGGCG-3' and 5'-GGAAGCAGCCTTAGTAGCATT-3' for *PT3* (82°C); for the nine other primers sets (see Gallou et al. 2009).

Real-Time quantitative PCR

Real-Time PCR analysis was performed using the LightCycler 2.0 (Roche, Montreal, Canada). A set of standard solutions prepared from RT products was included in each run. Reactions were prepared in capillaries using the following concentrations: 7 µl of PCR water, 4µl of 5x LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Mix (Roche, Montreal, Canada), 2 µl of each forward and reverse primer (0.5 µM) and 5 µl of 1:10 diluted cDNA or standard solution as template (For LightCycler experimental run, see Gallou et al. 2009). In order to check PCR efficiency, standard curves (log of cDNA dilution versus C_p) using serial 10-fold dilution of cDNA were created for each pair of selected primers. To obtain good comparison and normalisation, PCR efficiency should range between 80 and 115%. In this study, all the PCRs displayed efficiencies between 91 and 104%.

For the mathematical model it was necessary to determine the crossing point (Cp) for each transcript, defined as the point at which the fluorescence rises appreciably above the background fluorescence. The Fit point method was performed in the LightCycler software 4.1 at which Cp was measured at a constant fluorescence level. The combination of several references genes smoothes out normalisation error due to the small variation in the expression of a single reference gene (Vandesompele et al. 2002). We determined the best combination of reference genes for normalisation of gene expression by using the geNorm software (http://medgen.ugent.be/~jvdesomp/geNorm). The most stable genes expressed in the potato roots during symbiotic establishment with *Glomus* sp. MUCL 41833 were *β-tub* and *EF1-α*. The pairwise variation demonstrated that three reference genes (i.e. *β-tub, EF1-α* and *GAPDH*) were sufficient to normalise gene expression in potato plantlets grown in the extra-radical mycelium network of *Glomus* sp. MUCL 41833 (data not show). Normalisation was achieved using the geometric means of the three reference genes, i.e. *β-tub*, *EF1-α* and *GAPDH*. The data were analysed statistically with software REST 2008 (relative expression software tool; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). Significance values were set at P value < 0.05. P(H1): the probability of alternate hypothesis that differentiate between sample and control groups is due only to chance.

Results

Time course of root colonization of potato plantlets grown in the extra-radical mycelium network of *Glomus* sp. MUCL 41833

The root colonization of potato plantlets was assessed 1, 2, 3, 6, 9 and 15 dap of the root system in the AM fungal extra-radical mycelium network (Table 6). No AM root colonization was observed 1 dap with the extra-radical mycelium network. The first traces of AM fungal root colonization were detected 2 dap (1.9 ± 3 %) and increased slightly (9.9 ± 7 %) 3 dap. However, no significant differences were observed between the values recorded at 1, 2 and 3 dap. Neither arbuscules nor vesicles were observed at 1, 2 and 3 dap. Six dap, the AM root colonization increased markedly (24 ± 24%) and the first arbuscules and vesicles were observed. The percentage of arbuscules and vesicle observed 6 dap was 3.9 and 0.3 respectively. The AM root colonization estimated 9 dap was 48.2 ± 14% and remained almost identical 15 dap (49.9 ± 8%). The values of AM root colonization were significantly higher, 9 and 15 dap as compared to 1, 2 and 3 dap. The 6 dap values recorded were intermediate between those obtained 1, 2, 3 dap and 9, 15 dap. At 9 dap, the percentages of arbuscules and vesicles were 13.6 and 3.5, respectively. At 15 dap, the percentages of arbuscules and vesicles were 8.2 and 5.7 respectively.

Table 6 Root colonization of potato plated on actively growing extra-radical mycelium networks of *Glomus* sp. MUCL 41833

		Root colonization (%) during the time course[a]					
		1	2	3	6	9	15
Structure	Hyphae	0	1.9 a (± 3)	9.9 a (± 7)	24 ab (± 24)	48.2 b (± 14)	49.9 b (± 8)
	Arbuscules	0	0	0	3.9 a (± 1.9)	13.6 b (± 4.6)	8.2 b (± 2.9)
	Vesicles	0	0	0	0.3 a (± 0.5)	3.5 b (± 1.8)	5.7 b (± 2.4)

For each structure, values (other than 0) in a row followed by a different letter differ significantly at $P \leq 0.05$ (one-way ANOVA and Tukey's HSD). The standard error is shown in brackets (Biological replicates = 4). For data of root colonization of 0%, no statistical analyses were done because of the impossibility to calculate the variance

[a] Days after planting of potato plantlets in the network of *Glomus* sp. MUCL 41833

Expression of marker gene of AM root colonization of potato plantlets grown in the extra-radical mycelium network of *Glomus* sp. MUCL 41833

The relative expression ratio of *PT3* genes in potato roots was assessed 1, 2, 3, 6, 9 and 15 dap in the extra-radical mycelium network (Table 7). We observed an induction of *PT3* gene 9 and 15 dap with a maximum of 15 dap [65.30 (19.90 – 191.08)].

Table 7 Relative expression ratio of seven genes (Gluthatione-S-transferase 1 (*GST1*), Lipoxygenase (*Lox*), MAP kinase (*MAPK*), Pathogenesis Related 1 (*PR1*), Pathogenesis Related 2 (*PR2*), Phenylalanine ammonia lyase (*PAL*) and Phoshate transporter 3 (*PT3*)) in potato roots plated on actively growing extra-radical mycelium networks of *Glomus* sp. MUCL 41833, normalized by the geometric mean of the three reference genes (i.e. *β-tub*, *EF1-α* and *GAPDH*)

Gene name	Relative expression ratio during the time course[a]					
	1	2	3	6	9	15
PT3	1.86 (0.44 - 9.71)	0.24 (0.08 - 0.94)	1.81 (1.05 - 2.56)	0.28 (0.05 - 4.00)	**5.51 (2.58 - 16.02)**	**65.30 (19.90 - 191.08)**
GST1	1.40 (1.01 - 2.14)	1.41 (0.93 - 2.70)	*0.30 (0.19 - 0.46)*	**2.72 (2.39 - 3.10)**	**4.48 (2.93 - 7.47)**	**5.68 (4.20 - 8.76)**
Lox	1.00 (0.40 - 3.71)	0.63 (0.10 - 2.08)	0.62 (0.47 - 0.84)	1.80 (1.02 - 4.51)	**3.38 (1.26 - 7.60)**	**6.12 (2.73 - 10.13)**
MAPK	0.64 (0.17 - 2.09)	**5.74 (1.75 - 16.78)**	*0.42 (0.32 - 0.56)*	**2.64 (1.70 - 4.01)**	**2.01 (1.30 - 3.95)**	**8.12 (3.92 - 16.60)**
PAL	1.95 (0.83 - 5.11)	**7.82 (3.22 - 21.04)**	3.88 (1.52 - 14.37)	**12.10 (5.32 - 30.22)**	**3.06 (1.26 - 11.25)**	**28.55 (11.40 - 60.85)**
PR1	**2.50 (1.60 - 4.79)**	0.15 (0.05 - 0.69)	1.05 (0.70 - 1.63)	1.21 (0.55 - 2.31)	**7.35 (1.40 - 57.06)**	**105.31 (60.33 - 229.85)**
PR2	**3.94 (1.57 - 10.30)**	1.63 (0.67 - 3.47)	1.80 (0.98 - 3.27)	**13.39 (6.07 - 29.52)**	**10.51 (1.35 - 194.15)**	**159.94 (85.56 - 330.10)**

Data were analysed statistically by the software REST 2008 (relative expression software tool; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). Up-regulated (**in boldface**) and down-regulated (*in italics*) genes with significance values were set at P value < 0.05 (P(H1): the probability of alternate hypothesis that differentiate between sample and control groups is due only to chance). The standard error range (i.e. refer to 68% confidence interval) is shown in brackets (Biological replicates = 4)
[a] Days after plating of potato plantlets in the extra-radical mycelium network of *Glomus* sp. MUCL 41833

Expression of defence response genes in potato plantlets grown in the extra-radical mycelium network of *Glomus* sp. MUCL 41833

The relative expression ratio of the defence response genes *GST1*, *Lox*, *MAPK*, *PAL*, *PR1* and *PR2* in potato roots was assessed 1, 2, 3, 6, 9 and 15 dap in the extra-radical mycelium network (Table 7). For the *GST1* gene, we observed the repression 3 dap [0.30 (0.19 – 0.46)] and the induction of 6, 9 and 15 dap was detected with a maximum of

15 dap [5.68 (4.20 – 8.76)]. For the *Lox* gene the induction of 9 and 15 dap, was detected with a maximum of 15 dap [6.12 (2.73 – 10.13)]. The *MAPK* and *PAL* genes were induced 2 dap and an induction was noted 6, 9 and 15 dap with a maximum of 15 dap [8.12 (3.92 – 16.60)] for *MAPK* and *PAL* [28.55 (11.40 – 60.85)]. Moreover, we noted the repression of the *MAPK* gene 3 dap [0.42 (0.32 – 0.56)]. For the two Pathogen Related genes (i.e. *PR1* and *PR2*), an induction of 1 dap was detected, and respectively 9 and 15 dap with a maximum of 15 dap [105.31 (60.33 – 229.85)] for *PR1*, and 6, 9 and 15 dap with a maximum of 15 dap [159.94 (85.56 – 330.10)] for *PR2*.

Discussion

Root organ cultures have been considered as suitable systems to investigate various aspects of the AM fungi and AM symbiosis (Fortin et al. 2002). Recently, autotrophic non-transformed plants have been successfully cultured *in vitro* in association with AM fungi (Voets et al. 2005; Dupré de Bulois et al. 2006) and a fast track *in vitro* mycorrhization system (i.e. the MDP *in vitro* culture system) was developed (Voets et al. 2009). This system opens large perspectives for molecular studies but should be validated for gene expression analysis. Here, the expression of a phosphate transporter gene and six defence genes were monitored by real-time QRT-PCR and paralleled with the multi-step process of potato AM fungal root colonization. Gene expression change could be observed in pre-stage (before root colonization – 1 dap), early stage (hyphae root colonization before arbuscules and vesicles formation – 2 and 3 dap) and the late stage of

root colonization (heavy root colonization with arbuscules and vesicles formed – 6, 9 and 15 dap).

During the pre-colonization stage of potato- *Glomus* sp. MUCL 41833 interaction (1 dap), we observed the induction of *PR1* and *PR2* genes, while no induction was noted during the following 2 and 3 dap (early root colonization stage). This change in gene expression between pre- and early stage of AM fungi root colonization was reported earlier by Kapulnik et al. (1996), Ruíz-Lozano et al. (1999) and Liu et al. (2003) and associated with a transient level of defence-gene expression.

During the early stage of potato- *Glomus* sp. MUCL 41833 interaction (2 and 3 dap), we observed the induction of the *MAPK* gene (2 dap). This observation is consistent with the role of MAP kinases in the signalling of plant abiotic stress and pathogen defence (Nagakami et al. 2005) and corroborates the findings of Deguchi et al. (2007). The early stage of root colonization was also characterized by the induction of *PAL* gene as reported by Deguchi et al. (2007).

During the late stage of potato- *Glomus* sp. MUCL 41833 interaction (6, 9 and 15 dap), we observed an induction of *PR1* (9 dap) and *PR2* (6 dap), with a build-up in the level of induction, paralleled thereafter with increased root colonization. *PR1* gene is a PR protein with antifungal properties and an unknown microbial target (Antoniw et al. 1980). This gene is known to respond to fungal infection in potato (Gallou et al. 2009). *PR2* gene is a β-1,3-glucanase induced, among others, in *M. truncatula* roots colonized by *G. intraradices* and *G. mosseae* during the late stage of root colonization (Hohnjec et al. 2005).

We noted the induction of *Lox* gene (i.e. the first enzyme in the biosynthesis pathway of JA gene) after 9 dap. This confirmed earlier result showing that in roots of mycorrhizal plants, the levels of JA were higher as compared to non-mycorrhizal controls (Meixner et al. 2005; Stumpe et al. 2005; Hause et al. 2007). We also observed a significant induction of *PAL* gene 6, 9 and 15 dap, with increased percentages of arbuscules and vesicles in roots. Harrison and Dixon (1994) have demonstrated that transcripts encoding enzymes of the isoflavone biosynthetic pathway, such as *PAL* and chalcone synthase are induced specifically in cells containing arbuscules.

Glutathione-S-transferase transcripts have been found to accumulate in the roots containing arbuscules (Wulf et al. 2003; Brechenmacher et al. 2004). In our study, we observed the induction of *GST1* gene in the potato roots 6, 9 and 15 dap, in parallel with the formation of the first arbuscules and vesicles in the potato roots. We also observed the induction of *MAPK* gene in the late stages of root colonization, as reported by Weidmann et al. (2004) and Grunwald et al. (2004) in *M. truncatula* root colonized by *G. mosseae.*

During the late stage of potato- *Glomus* sp. MUCL 41833 interaction (6, 9 and 15 dap), we observed the induction of the phosphate transporter gene *PT3* (9 dap), with a build-up in the level of induction, 15 dap, paralleled with an increase in root colonization. Rausch et al. (2001) have identified the phosphate transporter gene *StPT3* in potato and localized the *PT3* gene induction specifically in arbuscule-containing cells. Moreover, the authors have demonstrated that this gene could be considered as a molecular marker for investigate the functional state of the AM symbiosis.

During this study on gene expression analysis at the different stages of AM establishment with the MDP *in vitro* culture system, we observed the induction of *PT3* gene at the late stage of potato- *Glomus* sp. MUCL 41833 interaction. Two PR genes (*PR1* and *PR2*) were induced prior to root colonization with a transient expression at 2, 3 and 6 (for *PR1*) dap followed by a continuous build-up in the level of induction. We finally demonstrated the induction of *GST1*, *Lox*, *MAPK* and *PAL* genes at different stages of potato- *Glomus* sp. MUCL 41833 interaction. The result, obtained for *PT3* gene (a plant gene marker of AM root colonization), demonstrated that the potato-*Glomus* sp. MUCL 41833 association was successfully established and the mutualistic exchange between the two partners was effective. The induction of defence genes, well know to be involved at different stages of the AM symbiosis, demonstrated that the potato- *Glomus* sp. MUCL 41833 establishment in the MDP *in vitro* culture system was suitable to study major changes in plant gene expression and corroborated previous results of *in vivo* studies. Our study opened new avenues to investigate the molecular events or gene networks associated with the plants-AM fungi symbiosis, by making it possible to synchronise the development of AM fungi in the roots of plants grown in an established non-perturbed mycelium network under rigorous *in vitro* culture conditions.

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Chapter II

Transcriptional regulation of defence genes and involvement of the WRKY transcription factor in arbuscular mycorrhizal potato root colonization

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Preface

In chapter I, we have described three stages in the development of the AM fungus[3] *Rhizophagus* sp. MUCL 41833 in potato roots. The pre-stage referred to the AM fungus before contact with the root and root colonization, the early stage to the formation of appresorium and first hyphal root colonization and the late stage to the intense root colonization with arbuscules and vesicles formed. In addition, the results obtained on the expression of several plant genes underlined a change in defence response during the AM fungal establishment and principally, an important induction of these genes during the late stage of root colonization. Taken together, these results demonstrated the suitability of the MDP *in vitro* culture system to investigate the molecular events during the symbiotic establishment in potato roots.

Based on these results, we decided in chapter II to perform a genome-wide expression profiling experiment to decipher the regulation of genes associated to biotic stress during the three stages of AM fungal root colonization and to understand the potential of AM fungi to trigger resistance in potato plants.

[3] In this chapter, we took into consideration the AM fungal classification of Schüβler et al. (2001)

Abstract

The establishment of arbuscular mycorrhizal associations causes major changes in plant roots and affects significantly the host in term of plant nutrition and resistance against biotic and abiotic stresses. As a consequence, major changes in root transcriptome, especially in plant genes related to biotic stresses, are expected. Potato microarray analysis, followed by Real-Time quantitative PCR, was performed to detect the wide transcriptome changes induced during the pre-, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833. The microarrays analysis revealed 526 up-regulated and 132 down-regulated genes during the pre-stage, 272 up-regulated and 109 down-regulated genes during the early stage and 734 up-regulated and 122 down-regulated genes during the late stage of root colonization. The most important class of regulated genes was associated to plant stress and in particular to the WRKY transcription factors genes during the pre-stage of root colonization. The expression profiling clearly demonstrated a wide transcriptional change during the pre-, early and late stages of root colonization. It further suggested that the WRKY transcription factor genes could be involved in the mechanisms controlling the arbuscular mycorrhizal establishment by the regulation of plant defence genes.

Key words: arbuscular mycorrhiza fungus, defence genes, gene expression, *in vitro* system, microarray, *Solanum tuberosum*

Introduction

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs belonging to the Phylum Glomeromycota (Schüβler et al. 2001). They form a unique association with the roots of more than 80% of terrestrial plants, including most agricultural and horticultural crop species (Smith and Read 2008). The AM fungi receive carbohydrates from the host plants required to complete their life cycle, in exchange of which they provide the plants with nutrients (e.g. phosphorus) and water.

The establishment of this successful biotrophic association result from a complex, dynamic and still largely unknown signalling mechanism involving major changes in fungal and plant genes expression (Franken et al. 2007; Fiorilli et al. 2009). Many studies have described the colonization process, from the early signalling in non-contacting organisms to root surface contact and development of a pre-penetration apparatus (Genre et al. 2005), followed by fungal penetration and proliferation within and between cells. From a simplified perspective, the AM fungal root colonization may be divided in three main stages, all of which being finely modulated by a signalling dialogue between the two partners of the symbiosis: the pre-stage referring to the stage before contact of the AM fungus with the root, (2) the early stage referring to the stage of hyphopodium formation at the root surface and root colonization before arbuscules/vesicles development and (3) the late stage referring to the stage of intense root colonization with arbuscules and vesicles formed (Gallou et al. 2010).

To understand the complex transcriptome changes, macro- and microarray techniques have been used to decipher the molecular mechanism during the AM root colonization. The first study was conducted by Liu et al. (2003) using a *Medicago truncatula* macroarray of probes for 2268 genes. A number of studies also reported the application of microarrays. These studies demonstrated some transcriptional changes involved in the metabolic pathways that control nutritional exchanges, secondary metabolism, protein turnover, signal transduction, cell wall modification and transcription (Hohnjec et al. 2005; Liu et al. 2007; Gomez et al. 2009). Most of these transcriptional studies were performed with a few plant species, among which *M. truncatula*, *Oryza sativa* (Güimil et al. 2005) and *Lotus japonicus* (Guether et al. 2009). Recently, microarray analyses were also conducted with *Solanum lycopersicum*, an important crop and model species (Dermatsev et al. 2010; Lopez-Raez et al. 2010).

Notwithstanding these studies, gene expression profiling during the successive stages of AM fungal root colonization was only partially resolved. One major obstacle was the difficulty to synchronize the initial developmental events leading to the establishment of the symbiosis (Weidmann et al. 2004). This drawback was recently minimized by Dermatsev et al. (2010). These authors used a semi-synchronized system allowing the synchronization of the AM fungal development. These authors succeeded to investigate the molecular events occurring in tomato roots during the initial stages (i.e. before AM fungus contact with the roots to the development of the first hyphae in the roots). In addition, the study of gene expression profiling was generally focused on a

single developmental stage of the symbiosis and particularly in presence of arbuscules in the roots. However, the recent study of Guether et al. (2009) was the first to compare the transcriptional changes in *L. japonicus* during the early (i.e. first hyphae in the roots) and late (i.e. effective symbiosis with arbuscules in the roots) stages of root colonization.

It is admitted that the changes in plant defence genes expression are essential to the establishment of the AM association and that the biological protection conferred by AM fungi to plants against a broad range of pathogens was probably related to these changes (Pozo and Azcon-Aguilar 2007; Pozo et al. 2010). A number of studies have reported a transient increased expression of plant defence genes, especially during the early stages of root colonization, followed by a decline in expression in the later stages of development (Kapulnik et al. 1996; Ruíz-Lozano et al. 1999; Liu et al. 2003). Moreover, *in situ* experiments have revealed that some transcripts related to plant defence responses were exclusively detected in arbuscules-containing cells (Balestrini et al. 1997; Salzer et al. 2000; Brechenmacher et al. 2004). These results suggested that the localized response may control the development of the fungus inside the roots. More recent transcriptional studies have also demonstrated that plant responses to AM fungi (i.e. obligate biotrophs) present similarities with plant responses to biotrophic pathogens (Sanchez et al. 2004; Güimil et al. 2005). Additionally, numerous studies revealed that in roots of mycorrhizal plants the levels of jasmonic acid (JA) was increased (Meixner et al. 2005; Stumpe et al. 2005; Hause et al. 2007). Recently, Lopez-Raez et al. (2010) have shown the regulation of the oxilipin

pathway and suggested a key regulatory role of jasmonates during the AM symbiosis. Despite these studies, the regulation of defence genes during the AM root colonization remained almost unknown.

Recently, Gallou et al. (2010) investigated the defence genes expression in potato plant during the pre-, early and late stages of root colonization. They used the Mycelium Donor Plant (MDP) *in vitro* culture system (Voets et al. 2009) which allowed to synchronize the development of the AM fungus in the roots, and demonstrated the efficiency of this system to study the changes in defence genes expression during the different stages of AM fungal root colonization in potato plantlets.

In the present study, we investigated the global transcriptional change in the roots of potato plants during the pre-, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833. The MapMan software associated with the Potato Array designed by the Potato Oligo Chip Initiative (POCI) (Kloosterman et al. 2008) was used to distinguish the transcriptional change during the symbiotic establishment. In particular, the modulation of defence genes during these three stages of AM fungal root colonization was investigated with the MapMan Biotic Stress display (Rotter et al. 2007).

Materials and methods

Biological material

In vitro produced potato plantlets (*Solanum tuberosum* L., var. Bintje) were supplied by the *Station de Haute Belgique* in Libramont,

Belgium. Plantlets were micro-propagated every five weeks as described by Voets et al. (2005).

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A 17 (SARDI, Australia) were surface-disinfected by immersion in sodium hypochlorite (8% active chloride) for 10 min, rinsed three time in de-ionized sterilized (121°C for 15 min) water and germinated in groups of 20 on Petri plates (90 mm in diam.) filled with 40 ml Modified Strullu-Romand (MSR) medium (Declerck et al. 1998) without sucrose and vitamins, and solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA). The Petri plates were incubated at 27°C in the dark.

A root organ culture (ROC) of *Glomus* sp. MUCL 41833 was supplied by the Glomeromycota *in vitro* collection (GINCO – http://www.mycorrhiza.be/ginco-bel). The spores were extracted by solubilisation of the gellan gel (Doner and Bécard 1991). An approximate of 100 spores were then placed in the near vicinity of actively growing transformed carrot (*Daucus carota* L.) roots (approximately 70 mm long) on Petri plates (90 mm diam.) containing the MSR medium, following the method described in Cranenbrouck et al. (2005). The Petri plates were incubated for 3 months in the dark in an inverted position at 27 °C. Several thousand spores were produced during this period.

Experimental design

The Mycorrhizal Donor Plant (MDP) *in vitro* culture system (Voets et al. 2009) was used for the microarray analysis of differential

gene expression during the potato root colonization by *Glomus* sp. MUCL 41833. Briefly, four-day-old *M. truncatula* seedlings were transferred to the root compartment (RC) of a bi-compartmented Petri plate (90 mm diam.), with their roots placed on the surface of the medium and the shoot extending outside the Petri plates via a hole. An approximate of 100 spores of *Glomus* sp. MUCL 41833 was inoculated in the vicinity of the roots. The Petri plates were then sealed with Parafilm (Pechiney, Plastic Packaging, Chicago, IL 60631, USA) and the holes plastered with sterilized (121°C for 15 min) silicon grease (VWR International, Belgium). The Petri plates were covered with an opaque plastic bag to keep the AM fungus and plant roots in the dark, while the shoots developed under light conditions. The Petri plates were subsequently transferred to a growth chamber set at 20/16°C (day/night), 70 % relative humidity, with a photoperiod of 16 h d^{-1} and an average photosynthetic photon flux (PPF) of 225 µmol m-2 s^{-1}. After eight weeks of culture, the mycelium crossed the plastic barrier separating the RC form a hyphal compartment (HC). A profuse extra-radical mycelium (ERM) network bearing numerous spores was developed in this HC. At that time, two new holes, separated by 4.5 cm from each other, were made in the base and the lid of the Petri plates, at the side of the HC. One ten-day old potato plantlet was inserted in each hole with the roots in direct contact with the extra-radical mycelium (for details, see Voets et al. 2009). The Petri plates were then sealed carefully and incubated horizontally in a growth chamber under the same conditions as above. Identically, two micro-propagated potato plantlets were placed in the HC of the control

treatment (i.e. non-inoculated MDP *in vitro* culture systems) and grown under the same conditions as described above.

The Petri plates were randomly divided into 3 groups of two replicates. Roots were harvested 1, 3, and 12 days after plating (dap) in the extra-radical mycelium network of *Glomus* sp. MUCL 41833. The same methodology was followed for the control treatment and two replicates were also harvested at each time. For each Petri plate, the roots of the two potato plantlets in the HC were pooled for analysis. Half of the material was then used to estimate the root colonization while the other half was used for RNA extraction and stored at - 80°C until use.

Phosphorus and root colonization analysis

The total phosphorus (P) content was analysed in order to assess the differences in P nutrition between the mycorrhizal and non-mycorrhizal potato plantlets. Shoots were rinsed with deionized water and leaves were oven-dried at 60°C for 72 h and weighed. Tissue P content was determined by inductively coupled plasma atomics emission spectrometry (ICP-AES) (iCAP 6500, Thermo Electron Corporation, Cambridge, UK), after acid (HClO4) digestion. Four biological replicates, each consisting of leaves from the two potato plants of one MDP *in vitro* culture system, were analyzed for each treatment at 12 dap.

The root systems were cleared in 10% KOH at 50 °C for 60 min., rinsed with distilled water and stained with Trypan blue 1% (Phillips and Hayman 1970) at 50°C for 60 min. The root systems were

subsequently mounted on microscope slides and observed under a dissecting microscope (Olympus SZ40, Olympus Optical GmbH, Hamburg, Germany) at X 10-40 magnification. The percentages of root colonization were estimated according to McGonigle et al. (1990).

The total P contents and percentages of root colonization were subjected to one-way ANOVA. Tukey Honest Significant Difference (HSD) was conducted to identify significant differences ($P \leq 0.05$) between the treatments. Data analysis was performed with the SAS enterprise guide 4.1 (SAS Institute Inc., Cary, USA).

RNA extraction

Total RNA was extracted with the RNeasy plant mini kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. The total RNA was treated with the TURBO DNA-*free*™ kit (Ambion, Austin, USA) according to the manufacturer's instructions. Concentration and purity of total RNA were determined in a NanoDrop®-ND 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA) and the total RNA quality was tested using the Agilent 2100 BioAnalyzer as recommended by the manufacturer's protocol (RNA 6000 Nano Assay Protocol) (Agilent Technologies, Santa Clara, USA).

Microarray hybridization and data analysis

The microarray analysis was performed using the 4x44K Potato Array (AMADID#015425) designed by the POCI (Kloosterman et al. 2008) using the Agilent's 60-mer SurePrint technology (Agilent Technologies, Santa Clara, USA). Two independent biological replicates, each consisting of a pool of two potato plants of one MDP *in vitro* culture system, were randomly selected at 1, 3 and 12 dap in the extra-radical mycelium network of *Glomus* sp. MUCL 41833. The microarrays were hybridized, stained, washed and scanned at the Institut of Life Science of the Université catholique de Louvain (Agilent microarray platform, https://www.uclouvain.be/en-276229.html). Synthesis of cDNA and cRNA (i.e. 500 ng of total RNA) was performed with the two-color Quick Amp Labeling Kit and without the two-color RNA spike-in kit as described in the two-color microarray-based gene expression analysis protocol (version 5.7) (Agilent Technologies, Santa Clara, USA). Hybridization and washing of slides were conducted according to the manufacturer's protocols. The hybridized slides were scanned using the scanner Agilent G2505B at high resolution of 5 µm at 532 nm (Cy3) and 633 nm (Cy5) wavelengths (Agilent Technologies, Santa Clara, USA). Microarray images were imported in the Agilent Feature Extraction (FE) software (version 9.1.3.1) and aligned with the appropriate array grid template file (015425_D_F_20061105) (Agilent Technologies, Santa Clara, USA). Data were normalized using the "Linear and LOWESS" algorithm. Unreliable data flagged as absent in all replicate samples by the FE software were discarded. The statistical test for differentially expressed genes was performed using the FE software. The t-statistic test was calculated on the feature and background

signals and their corresponding errors. In order to take account that only two biological replicates have been used in this microarray, we decided to consider the genes as differentially regulated if *P*-values were ≤ 0.01 and the ratio as compared with the controls was ≥ 3.00 or ≤ -3.00. Under this condition, we obtained a good correlation between the two biological replicates (i.e. with a ratio between ≥ 2.00 and ≤ 3.00, we obtained more variability between the two biological replicates). All relevant data concerning sequences on the POCI array were accessible on the POCI database (http://pgrc-35.ipk-gatersleben.de/pls/htmldb_pgrc/f?p=194:1:705382633385532::NO:::). Updated of annotation for the differentially regulated genes was obtained by blastx against the NCBI nr-database (An E-value $<10^{-10}$ was required to take into account the blast result). The data were imported into the MapMan software (http://mapman.gabipd.org/web/guest/mapman) for Gene Ontology (GO) analysis. Updated of GO annotation for the differentially regulated genes was obtained by the web application Mercator Automated Sequence Annotation Pipeline (http://mapman.gabipd.org/web/guest/app/mercator). In order to enable easier data visualization and interpretation of potato genes expression involved in biotic stress (BIN 20.1), these genes were manually subdivided into different subBIN as described by Rotter et al. (2007). The microarray data were submitted to the ArrayExpress public database (E-MEXP-3144).

Real-Time quantitative PCR

Reverse transcription of 500 ng of total RNA was performed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Montreal, Canada) in a volume of 20 µl with oligo $(dT)_{18}$ primer at 55°C for 20 min, according to the manufacturer's instructions. Two independent biological replicates were analysed per treatment. For each RNA sample, a reaction without RT was performed as a control for contamination by genomic DNA. Real-Time PCR analysis was performed using the LightCycler 2.0, the 5x LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Mix and gene-specific primers (Table 8) designed with the Light Cycler Probe Design Software 2.0 (Roche, Montreal, Canada) (For LightCycler 2.0 experimental run, see Gallou et al. 2009). Normalisation was achieved using the geometric means of two potato reference genes (i.e. *βeta-tubulin* and *Elongation Factor-1-alpha*), which showed the most stable expression in potato roots during root colonization by *Glomus* sp. MUCL 41833, determined by the geNorm software (http://medgen.ugent.be/~jvdesomp/geNorm) (See Gallou et al. 2010). The data were analysed statistically with the software REST 2009 (relative expression software tool; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). Significance values were set at P-value < 0.05. P(H1): the probability of alternate hypothesis that differentiate between sample and control groups is due only to chance.

Table 8 Primer sequences and amplification length of the genes used in the Real-Time quantitative PCR analysis

Gene Name or Annotation	Accession number or Probe ID	Primer sequence 5'-3' (Forward)	Primer sequence 5'-3' (Reverse)	Length (bp)
Reference genes				
beta-tubulin [S.tuberosum]	Z33382	ATGTTCAGGCGCAAGGCTT	TCTGCAACCGGGTCATTCAT	101
Elongation factor 1-alpha [S.tuberosum]	AB061263	ATTGGAAACGGATATGCTCCA	TCCTTACCTGAACGCCTGTCA	101
Marker genes of AM fungi				
pathogenesis related protein (PR-1) [S.tuberosum]	AJ250136	GGTGCAGGAGAGAACCTT	GGTACCATAGTTGTAGTTTGGCT	99
phosphate transporter 3 [S.tuberosum]	AJ318822	GCTTTGCTTACTTATTATTGGCG	GGAAGCAGCCTTAGTAGCATT	90
putative DXS-2 [L. hirsutum]	MICRO.13123.C1	CATTATTGGGAAGGCCACAAG	TTCCTCTCGGATGAAGCAG	98
WRKY genes				
WRKY27 [Glycine max]	MICRO.189.C1	ATTGAAGAAGCATCACCAAAGAG	CCACTGAGGTTTGATCGGAG	94
WRKY transcription factor 16 [P. tomentosa x P. bolleana]	MICRO.11686.C1	GAGTTGGAGGTGTTGGATGA	ATCCTCCACTTGAACATTTGTAG	106
WRKY transcription factor 20 [A. thaliana]	MICRO.16616.C1	GGCTGCTGCATTCTTATTGAC	CCACCGGCATATTTGTACT	93
WRKY transcription factor (WRKY3) [S. lycopersicum]	MICRO.16727.C1	GCTCTTCTTCTTGAAGGTGGA	AAAACTTCACTCTTGGGACTAAT	110
putative WRKY transcription factor [N. tabacum]	STMIS76TV	CACCAACACATGTACACATGAAT	GGTCTCAAGTTGTGTCTTTGTTC	110
putative WRKY transcription factor [R. communis]	STMIT91TV	ATGAGCTTGTTTGGACATGC	AATCGCATGATTTGAAATCACG	91
similar to WRKY transcription factor 6 [S.tuberosum]	MICRO.2876.C1	CTCACGTAAAGTACCATTGCAC	GCCTAATAACTCAAACCAAGCC	91
WRKY transcription factor 6 [S. tuberosum]	MICRO.2876.C2	ATGTTATGTCATCTGGGGTTTAC	ATCAAAATTCCAAAGACCCTCC	98
similar to WRKY transcription factor 6 [S. tuberosum]	bf_suspxxxx_0048g10.t7m.scf	GTATGCAGGCACTACTTTAGG	CTTGTGGATGGTCTTGGATT	92
WRKY transcription factor-30 [C. annuum]	MICRO.13165.C1	ACTAAGACGGATCTGTCCTTC	CAAGGGCCCATCTAGCTT	90

Results

Confirmation of the three stages of *Glomus* sp. MUCL 41833 potato root colonization

The potato plantlets were placed in the MDP *in vitro* culture system and the root colonization was assessed 1, 3 and 12 dap of the roots in the AM fungal extra-radical mycelium network (Table 9). As previously described by Gallou et al. (2010), three stages of root colonization (i.e. referring to the pre-, early and late stages, respectively) were observed and further confirmed by molecular analysis (Table 9). In addition, the potato basic Pathogenesis Related 1 (*PR1*) gene (i.e. a marker gene used to identify the three stages of root colonization, Gallou et al. 2010) was up-regulated during the pre- and late stages of root colonization as well as the phosphate transporter gene *StPT3* (i.e. a marker gene induced specifically in arbuscule-containing cells, Rausch et al. 2001) at the late stage of root colonization. However, the absence of these two genes in the array, constructed using expressed sequence tag libraries, did not allow to confirm their patterns of expression during the AM fungal root colonization by microarrays analysis (Kloosterman et al. 2008).

Table 9 Root colonization and expression analysis of the markers genes *PT3* and *PR1* of potato roots plated on actively growing extra-radical mycelium networks of *Glomus* sp. MUCL 41833

	Root colonization (%)		
	Pre-stage	early stage	late stage
Hyphae	0	5.4 a (± 2.4)	33.9 b (± 2.3)
Arbuscules	0	0	11.3 (± 2.6)
Vesicles	0	0	1.3 (± 1.2)
	Relative expression ratio		
	Pre-stage	early stage	late stage
PT3	0.59 (0.32 - 0.74)	0.83 (0.50 - 1.25)	**11.36 (7.54 - 16.86)**
PR1	**201.66 (59.62 – 555.40)**	0.76 (0.48 – 1.21)	**295.68 (161.28 – 535.41)**

For each AM fungal structure, values (other than 0) in a row followed by a different letter differ significantly at $P \leq 0.05$ (one-way ANOVA and Tukey's HSD). The standard error is shown in brackets. For data of root colonization of 0%, no statistical analyses were done because of the impossibility to calculate the variance
Relative expression ratio of *PT3* and *PR1* genes obtained by real-time quantitative PCR was significantly up-regulated (boldface background) with P value < 0.05 (P(H1): the probability of alternate hypothesis that difference between sample and control groups is due only to chance). The standard error range (i.e. refer to 68% confidence interval) is shown in brackets

Global changes in plant genes expression during potato root colonization by *Glomus* sp. MUCL 41833

The genome-wide expression profiling of the potato roots was assessed at the three stages of AM fungal root colonization and the transcriptional responses were interpreted with the MapMan software. The absence of statistical difference in P content between the leaves of non-mycorrhizal and mycorrhizal potato plants suggested that the transcriptional changes was not related to differences in P nutrition

(Table 10). This result was further confirmed by the absence of differences in the expression of P nutrition marker genes present in the array such as the potato phosphate transporter gene *StPT1*, purple and acid phosphatases genes, and other marker genes as the iron deficiency-specific-4 (*IDS4*) and the tomato P starvation-induced (*TPSI1*) genes (Fiorilli et al. 2009; Lopez-Raez et al. 2010).

Table 10 Root colonization and total phosphorus content of the leaves of potato plantlets grown 12 days in presence (i.e. +AMF) or absence (i.e. -AMF) of an actively growing extra-radical mycelium networks of *Glomus* sp. MUCL 41833

Treatments	Roots colonization			Leaves P content (%)
	Hyphal (%)	Arbuscules (%)	Vesicles (%)	
AM-	0	0	0	0.23 a (± 0.02)
AM+	40.5 (± 6.3)	15.4 (± 4.7)	3.6 (± 2.0)	0.20 a (± 0.01)

Values of total phosphorus content, followed by a different letter differ significantly at $P \leq 0.05$ (one-way ANOVA and Tukey's HSD). The standard error is shown in brackets (n = 4)

The microarray analysis revealed 526 up-regulated and 132 down-regulated genes during the pre-stage (Supporting information Table S1), 272 up-regulated and 109 down-regulated genes during the early stage (Supporting information Table S2) and 743 up-regulated and 122 down-regulated genes during the late stage (Supporting information Table S3) of potato root colonization. The total number of genes involved in the three stages was shown in a Venn diagram (Fig. 10). As shown in this Figure, few genes were regulated in the different stages of AM root colonization. During the pre- and early stages of AM fungal root colonization, 28 genes were regulated among which four were up-regulated at these two stages (Table 11). During the early and late stage of AM fungal root colonization, 20 genes were

regulated, among which 19 were up-regulated (Table 12). During the pre- and late stages of AM fungal root colonization, 18 genes were regulated among which 16 were up-regulated (Table 13). Finally, during the three stage of AM fungal root colonization only two genes were regulated (Table 11, 12 and 13). The gene encoding for an allyl alcohol dehydrogenase was up-regulated in the three stages, while the gene encoding for a glucan endo-1,3-beta-D-glucosidase was up-regulated in the pre- and late stages and down-regulated in the early stage of root colonization.

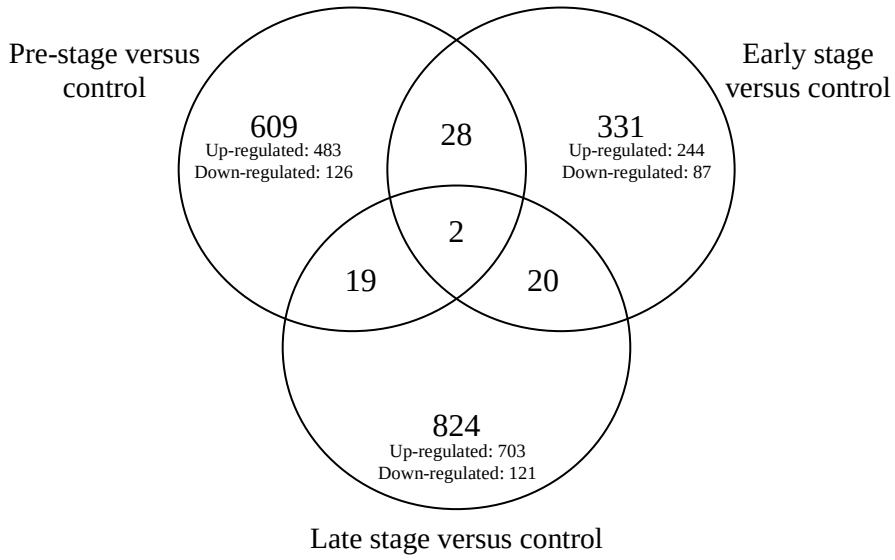


Fig. 10 Venn diagram showing the relationships between genes significantly up- or down-regulated during the three stages (i.e. pre-, early and late) of potato root colonization by *Glomus* sp. MUCL 41833

Table 11 Genes regulated during the pre- and early stages of potato root colonization by *Glomus* sp. MUCL 41833 as compared with the controls treatments, and their changes in expression

Probe ID	ID and Annotation	e-values	Fold change **Pre-**	Fold change **Early**
Metabolism				
MICRO.17922.C1	AAD30613: glucose-1-phosphate adenylyltransferase, putative / ADP-glucose pyrophosphorylase, putative (APS2) [Arabidopsis thaliana]	8E-19	**11.98**	**3.01**
cSTB33G21TH	AAM12777: putative auxin-induced SAUR-like protein [Capsicum annuum]	2E-20	**7.72**	*0.05*
MICRO.10760.C1	NP_188515: At3g18850 [Arabidopsis thaliana] dbj_BAB03094.1_ acyl-CoA:1-acylglycerol-3-phosphate acyltransferase [Arabidopsis thaliana]	3E-45	**4.58**	**18.83**
bf_arrayxxx_0043h04.t3m.scf	ABE73758: 3-hydroxy-3-methylglutaryl CoA synthase [Medicago truncatula]	1E-62	**3.41**	*0.12*
Cell wall				
MICRO.2255.C3	BAC98298: LEXYL1 [Lycopersicon esculentum]	6E-126	**14.17**	*0.12*
MICRO.967.C1	P93218: Polygalacturonase non-catalytic subunit AroGP3 precursor [Lycopersicon esculentum]	0.0	*0.29*	**5.55**
Stress				
MICRO.7372.C1	AAB30525: small heat-shock protein homolog [Solanum tuberosum]	0.0	*0.08*	**4.56**
Enzyme misc				
cPRO29E1TH	NP_175103: aminoacylase/ metallopeptidase [Arabidopsis thaliana]	3E-11	**4.60**	*0.14*
MICRO.1061.C2	NP_001031672: similar to AT4G19880: similar to unknown protein [Arabidopsis thaliana]	2E-110	**3.16**	*0.17*
MICRO.4306.C1	BAD19595: putative lipase [Oryza sativa (japonica cultivar-group)]	4E-12	*0.11*	**7.81**
MICRO.6187.C1	X74906: glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	0.0	**16.22**	*0.20*
MICRO.11037.C3	BAA89423: allyl alcohol dehydrogenase [Nicotiana tabacum]	0.0	**14.32**	**5.69**
RNA				
MICRO.5773.C1	NP_565684: zinc finger (C2H2 type) family protein [Arabidopsis thaliana]	8E-11	**3.06**	*0.07*
MICRO.3896.C2	NP_001062425: putative RNA-binding protein [Oryza sativa (japonica cultivar-group)]	1E-13	*0.28*	**4.91**
DNA				
MICRO.16224.C1	EAY72443: hypothetical protein [Oryza sativa (japonica cultivar-group)]	3E-11	**3.41**	*0.11*
Protein				

071B10AF.esd	AAD20677: putative poly(ADP-ribose) polymerase [Arabidopsis thaliana]	4E-81	**8.86**	*0.16*
STMGQ65TV	NP_051121: Similar to ATCG01280 | Symbols: YCF2.2 | Identical to Protein ycf2 (ycf2-B) [Arabidopsis Thaliana]	4E-11	**7.04**	*0.11*
MICRO.13355.C1	YP_033364: 50S ribosomal protein l9 [Bartonella henselae str. Houston-1]	8E-16	**4.99**	*0.15*
MICRO.5553.C3	AB002695 : aspartic endopeptidase [Cucurbita pepo]	3E-142	**3.88**	*0.20*
Not assigned				
BF_TUBSXXXX_0041C04_T3 M.SCF	No Hits Found		**12.50**	**7.08**
MICRO.4268.C2	NM_126867: adenylate cyclase [Arabidopsis thaliana]	5E-39	**7.81**	*0.32*
BF_TUBSXXXX_0022E02_T3 M.SCF	No Hits Found		**5.76**	*0.13*
MICRO.1142.C1	NP_568340.1: aminoacyl-tRNA hydrolase [Arabidopsis thaliana]	6E-14	**5.24**	*0.16*
bf_cswbxxxx_0007b10.t3m.scf	No Hits Found		**5.09**	*0.16*
bf_mxlfxxxx_0065a05.t3m.scf	No Hits Found		**4.85**	*0.09*
MICRO.15691.C1	No Hits Found		**4.62**	*0.14*
SDBN006L17u.scf	No Hits Found		**3.84**	*0.25*
BF_LBCHXXXX_0024D04_T3 M.SCF	No Hits Found		**3.34**	**3.30**
MICRO.6822.C2	DQ267207 : CA-responsive protein [Brassica oleracea]	4E-70	**3.12**	*0.17*
STDB005N03u.scf	No Hits Found		**3.07**	*0.10*

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the pre- and early stages of AM fungal root colonization versus the controls are ordered according to the gene ontology assignment and the expression fold change during the pre-stage of root colonization. Genes were considered as differentially regulated if *P*-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.0 or ≤ 0.33

Table 12 Genes regulated during the early and late stage of potato root colonization by *Glomus* sp. MUCL 41833 as compared with the controls treatments, and their changes in expression

Probe ID	**ID and Annotation**	**e-values**	Fold change **Early**	Fold change **Late**
Stress				
MICRO.13036.C2	NM_121447: similar to AT5G14430: dehydration-responsive protein-related	4E-11	**3.94**	**3.08**
MICRO.1610.C1	CAB50786: Rx protein [Solanum tuberosum]	6E-14	**3.00**	**5.36**
Enzyme misc				
MICRO.16359.C1	ABD28410: Exostosin-like [Medicago truncatula]	7E-11	**8.11**	**11.10**
MICRO.6187.C1	X74906: glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	0.0	*0.20*	**6.12**
MICRO.11037.C3	BAA89423: allyl alcohol dehydrogenase [Nicotiana tabacum]	0.0	**5.69**	**4.63**

RNA				
MICRO.16550.C1	AAX53089: anthocyanin 1 [Solanum tuberosum]	0.0	**3.08**	**5.98**
DNA				
MICRO.2888.C1	NM_100970: putative endomembrane protein 70 [Arabidopsis thaliana]	9E-45	**3.77**	**18.38**
Protein				
188A11.esd	NM_179025: similar to AT4G10570: ubiquitin carboxyl-terminal hydrolase family protein	1E-16	**5.33**	**5.90**
Signalling				
MICRO.6103.C1	AAT38797: putative Polyprotein [Solanum demissum]	8E-18	**5.29**	**6.58**
MICRO.1716.C1	BAA24382: CLB1 protein - tomato dbj_BAA24382.1_ CLB1 [Lycopersicon esculentum]	0.0	**4.01**	**12.20**
Cell				
cSTD12A19TH	NP_197443: Ran GTPase binding / chromatin binding / zinc ion binding [Arabidopsis thaliana]	6E-102	**5.79**	**21.26**
Transport				
MICRO.9579.C2	XM_002516757: Putative cobalt ion transporter [Ricinus communis]	4E-107	**4.78**	**5.87**
Not assigned				
POCDC04TV	No Hits Found		**9.91**	**58.46**
STMGA05TV	NP_001053463: OSJNBb0038F03.12 [Oryza sativa (japonica cultivar-group)]	3E-12	**6.71**	**37.25**
MICRO.12934.C1	No Hits Found		**6.33**	**83.95**
MICRO.9975.C1	No Hits Found		**5.13**	**4.51**
MICRO.3621.C1	ABI34375: hypothetical protein SDM1_53t00013 [Solanum demissum]	8E-17	**4.91**	**8.88**
POCAA56TP	AAU89759: hypothetical protein [Solanum tuberosum]	3E-13	**4.50**	**6.35**
MICRO.398.C1	No Hits Found		**3.51**	**13.46**
MICRO.1798.C1	No Hits Found		**3.16**	**9.58**
SSBN002C03u.scf	No Hits Found		**3.06**	**4.38**
bf_lbchxxxx_0058b06.t3m.scf	No Hits Found		*0.24*	**6.37**

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the early and late stage of AM fungal root colonization versus the controls are ordered according to the gene ontology assignment and the expression fold change during the early stage of root colonization Genes were considered as differentially regulated if *P*-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.0 or ≤ 0.33

Table 13 Genes regulated during the pre- and late stages of potato root colonization by *Glomus* sp. MUCL 41833 as compared with the controls treatments, and their changes in expression

Probe ID	ID and Annotation	e-values	Fold change **Pre-**	Fold change **Late**
Photosystem				
MICRO.1238.C4	P26320: Oxygen-evolving enhancer protein 1, chloroplast precursor (OEE1) (33 kDa subunit of oxygen evolving system of photosystem II) [Solanum tuberosum]	0.0	**3.65**	*0.12*
Metabolism				
MICRO.13027.C1	AAD18029: lipid transfer protein LTP1 precursor [Capsicum annuum]	6E-169	**3.08**	**7.09**
Stress				
MICRO.12664.C1	AAU95244: putative thaumatin-like protein [Solanum tuberosum]	0.0	**14.41**	**13.01**
Enzyme misc				
MICRO.2286.C17	CAE53273: 1,3-beta-glucan glucanohydrolase [Solanum tuberosum]	0.0	**36.71**	**5.24**
MICRO.2286.C42	AAN78310: beta-1,3-glucanase (PR2) [Solanum tuberosum]	0.0	**3.43**	**3.99**
MICRO.11082.C1	ABE81443: E-class P450, group I [Medicago truncatula]	8E-64	**14.59**	**12.08**
MICRO.6187.C2	CAA52872: glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	0.0	**9.36**	**5.05**
bf_mxlfxxxx_0010b10.t3m.scf	CAI62049: UDP-xylose phenolic glycosyltransferase [Solanum lycopersicum]	1E-62	**6.00**	**9.42**
MICRO.10579.C1	XM_002512090 : putative cytochrome P450 [Ricinus communis]	2E-16	*0.25*	**10.86**
MICRO.6187.C1	X74906: glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	0.0	**16.22**	**6.12**
MICRO.11037.C3	BAA89423: allyl alcohol dehydrogenase [Nicotiana tabacum]	0.0	**14.32**	**4.63**
RNA				
MICRO.14058.C1	Q5NE24: Nodulation-signaling pathway 2 protein [Ricinus communis]	0.0	**10.28**	**27.91**
Signalling				
bf_mxflxxxx_0008b10.t3m.scf	NP_190646: calcium ion binding [Arabidopsis thaliana]	3E-11	**10.70**	**3.91**
Transport				
MICRO.8332.C1	NM_129684: similar to AT2G41190: amino acid transporter family protein	3e-25	**5.65**	**3.35**
Not assigned				
bf_mxflxxxx_0017c01.t3m.scf	No Hits Found		**29.11**	**10.63**

PPCBF91TH	No Hits Found		**8.59**	**3.05**
bf_arrayxxx_0059h11.t7m.scf	No Hits Found		**4.10**	**8.55**
STMIB81TV	NP_001041912: unknown protein [Oryza sativa (japonica cultivar-group)]	8E-13	**3.89**	**18.67**
bf_suspxxxx_0045g12.t7m.scf	No Hits Found		**3.39**	**5.95**
POCCH15TV	No Hits Found		**3.23**	**4.96**
SDBN002E08u.scf	No Hits Found		*0.30*	**9.46**

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the pre- and late stages of AM fungal root colonization versus the controls are ordered according to the gene ontology assignment and the expression fold change during the pre-stage of root colonization. Genes were considered as differentially regulated if *P*-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.0 or ≤ 0.33

The Gene ontology classification obtained with the Mapman software in the three stages of root colonization was presented in Figure 11. During the three stages of root colonization, the most regulated functional assignment was observed in the stress assignment. Following this assignment, we also noted an important regulation of the enzyme miscellaneous (misc), photosystem, development, transport and metabolism assignments However, these results did not reflect the global change in plant gene expression during the three stages of root colonization. For example, only four of the genes involved in the stress assignment were regulated in two different stages. This example emphasized the necessity to take a deeper look at the different processes regulated during root colonization. More particularly, we have decided to focus our analysis on the genes involved or putatively involved in the biotic stress and also in the plant hormone genes which are associated to this assignment.

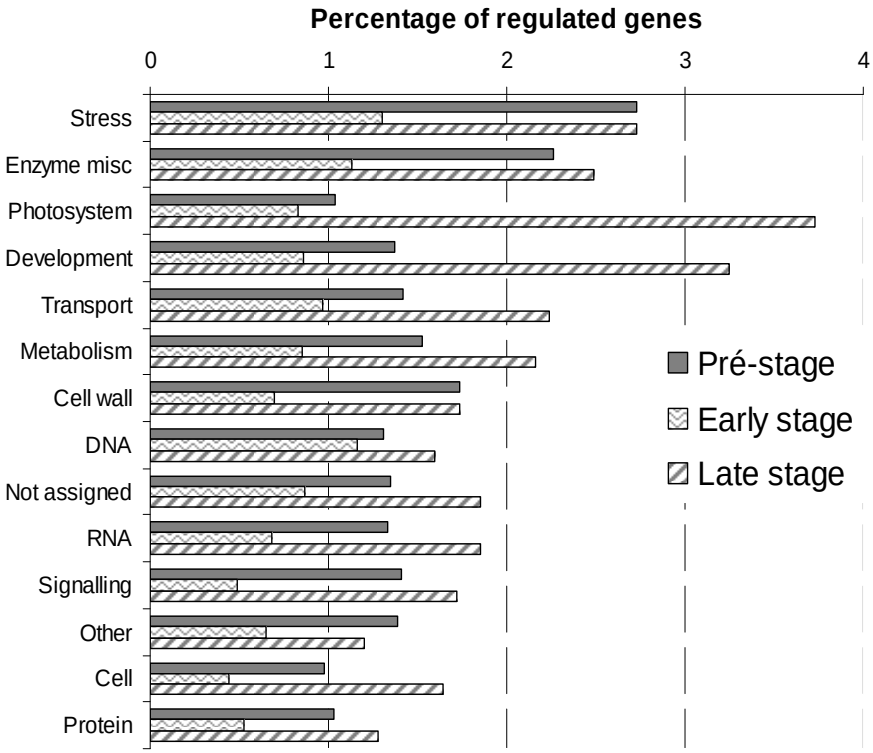


Fig. 11 Comparative analysis of transcription profiles. Functional assignment, determined by the MapMan software, of genes significantly up- or down-regulated during the potato root colonization by *Glomus* sp. MUCL 41833 (i.e. pre-stage, early stage and late stage). The percentage of regulated genes represents the ratio: (number of genes regulated in a functional assignment) / (total number of genes present in the array for this functional assignment)*100

Overview of plant marker genes expressed during the pre, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833

As previously described by Dermatsev et al. (2010), we observed the induction of the *EXBL1* gene (i.e. encoding for an expansin-like protein) during the pre-stage of AM fungal interaction (Supporting information Table S1). During the late stage, the genes previously described as markers of AM symbiosis were up-regulated: patatin

(bf_ivrootxx_0024g04.t3m.scf), chitinase (MICRO.11336.C1 and MICRO.11336.C2), gluthatione S-transferase (MICRO.592.C9) and β-1,3-glucanase (MICRO.2286.C42) (Supporting information Table S3) (Hohnjec et al. 2005; Liu et al. 2007; Siciliano et al. 2007). Among these genes, the MICRO.2286.C42 gene, which has a high homology with *PR2* (AJ009932 – 98%), was also induced during the pre-stage (Gallou et al. 2010). In addition to this gene, it was also interesting to note that some others genes confirmed the earlier gene expression analysis conducted during the AM fungi establishment in potato roots (Gallou et al. 2010). As example, during the late stage we observed the induction of the lipoxygenase gene (*POTLX-3*), which shared 76% of homology with the *Lox* gene (Y18548). At the late stage, we also noted the induction of the MICRO.592.C9 gene, encoding for a probable glutathione S-transferase with showed 86% of homology with the *GST1* gene (J03679) (ratio = 2.89 and *P*-value ≤ 0.01; data not show). In contrast, the induction of the *PAL* gene (X63104) was not confirmed by the MICRO.2219.C8 gene (i.e. 98% of homology with *PAL2*). Unfortunately for the other genes (i.e. *PR1* – AJ250136, *MAPK* – AB206552 and *PT3* – AJ318822), it was not possible to verify their induction with the microarray analysis (i.e. these genes were not present in the array design using expressed sequence tag libraries (Kloosterman et al. 2008)). However, the inductions of two of these genes have been confirmed by Real-Time quantitative PCR (Table 9). Finally, the marker gene 1-deoxy-d-xylulose 5-phosphate synthase 2 (*DXS-2*) (MICRO.13123.C1), encoding for an enzyme of the mevalonate-independent pathway of carotenoid biosynthesis, was also induced at the late stage (i.e.

confirmed by Real-Time quantitative PCR; Table 14; Walter et al. 2007). In addition to this enzyme, others genes related to this biosynthesis pathway were induced at the late stage: 1-deoxy-d-xylulose 5-phosphate reductoisomerase (*DXR*) (MICRO.5323.C2), phytoene desaturase (*PDS*) (MICRO.11694.C2 and MICRO.5233.C2), zeta-carotene desaturase (*ZDS*) (MICRO.4706.C1) and carotenoid isomerase (*CRTISO*) (MICRO.11731.C1) (Fig. 12; Supporting information Table S3).

Table 14 Expression analyses of the marker gene *DXS-2* for AM symbiosis and *WRKY* genes by real-time quantitative PCR (qPCR) in potato roots plated on an actively growing extra-radical mycelium network of *Glomus* sp. MUCL 41833

Probe ID	Annotation	Microarray			qPCR		
		Pre-	Early	Late	Pre-	Early	Late
MICRO.13123.C1	putative DXS-2 [Lycopersicon hirsutum]	1,00	0,65	4.82[a]	1,55	0,95	16.85[b]
MICRO.2876.C1	similar to WRKY transcription factor 6 [Solanum tuberosum]	4.89[a]	0,58	1,54	4.15[b]	0,46	1,13
MICRO.2876.C2	WRKY transcription factor 6 [Solanum tuberosum]	3.51[a]	0,75	1,23	5.10[b]	0,46	1,00
bf_suspxxxx_0048g10.t7m.scf	similar to WRKY transcription factor 6 [Solanum tuberosum]	4.74[a]	0,65	1,43	4.93[b]	0,41	0,94
MICRO.11686.C1	WRKY transcription factor 16 [Populus tomentosa x P. bolleana]	3.00[a]	0,72	1,34	3.92[b]	0,44	1,67
MICRO.16727.C1	WRKY transcription factor (WRKY3) [Solanum lycopersicum]	16.21[a]	0,78	0,93	10.96[b]	0,64	0,97
MICRO.189.C1	WRKY27 [Glycine max]	10.15[a]	1,39	1,13	54.36[b]	1,78	6.46[b]
STMIS76TV	putative WRKY transcription factor [Nicotiana tabacum]	13.12[a]	0,43	1,33	9.07[b]	0.33[b]	0,95
MICRO.16616.C1	WRKY transcription factor 20 [Arabidopsis thaliana]	12.34[a]	1,33	0,99	8.70[b]	0,94	1,06
MICRO.13165.C1	WRKY transcription factor-30 [Capsicum annuum]	1,47	1,01	4.48[a]	0,94	0,91	2.02[b]
STMIT91TV	putative WRKY transcription factor [Ricinus communis]	7.40[a]	1,00	1,00	26.62[b]	0,69	1,52

[a] Genes expression obtained by microarray analysis were significantly regulated with P-values ≤ 0.01
[b] Genes expression obtained by qPCR analysis on two independent biological replicates were significantly regulated with P value < 0.05

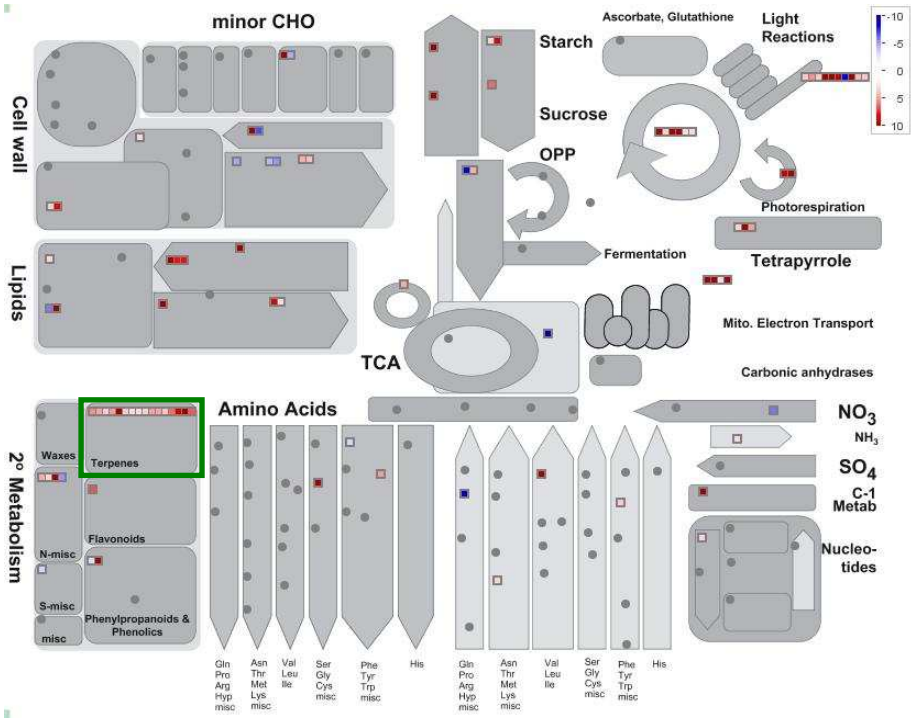


Fig. 12 The MapMan Metabolism Overview display created using the 865 regulated genes identified from the late stage versus the control comparison. This figure shows the impact of *Glomus* sp. MUCL 41833 during the late stage of potato roots colonization on the genes involved in metabolism process. In particular, the induction of the genes related to the biosynthesis of mycorradicin (i.e. 1-deoxy-d-xylulose 5-phosphate synthase 2 (*DXS-2*) (MICRO.13123.C1), 1-deoxy-d-xylulose 5-phosphate reductoisomerase (*DXR*) (MICRO.5323.C2), phytoene desaturase (*PDS*) (MICRO.11694.C2 and MICRO.5233.C2), zeta-carotene desaturase (*ZDS*) (MICRO.4706.C1) and carotenoid isomerase (*CRTISO*)) in the secondary metabolism process of terpenes (represented in the green frame). The fold change is displayed as illustrated in the fold change color bar in the upper right of each panel (blue is repressed and red is induced)

Overview of plant genes pathways commonly regulate during the pre, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833

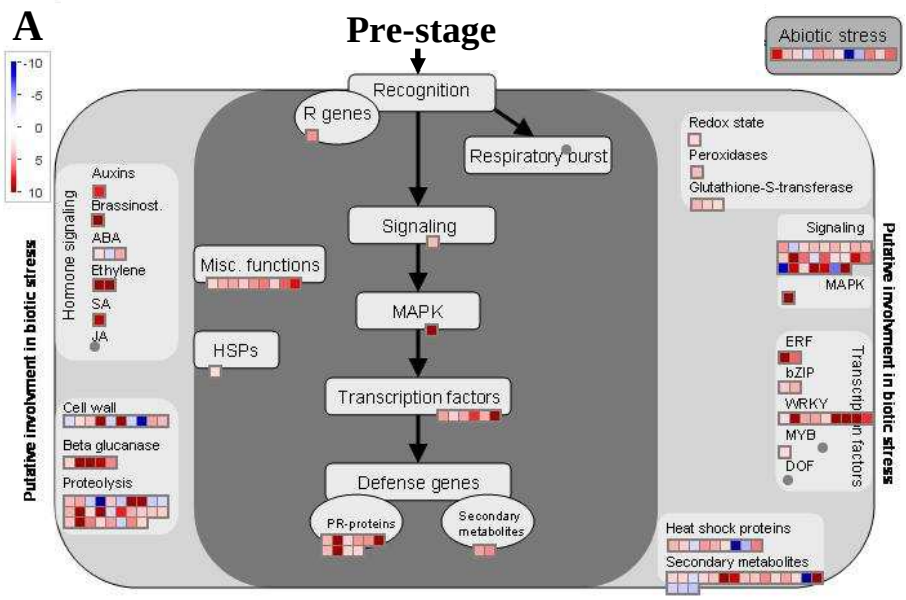
Among the numerous pathways reported in the AM fungal association, the calcium signalling was considered essential for the AM root colonization (Navazio et al. 2007; Kosuta et al. 2008; Chabaud et al. 2011). During the three stages of root colonization, we observed the regulation of genes involved in the calcium signalling. In particular, we noted the induction of the MICRO.12161.C1 and the MICRO.14938.C2 genes encoding for a calmodulin-like protein and a calmodulin-binding protein, respectively (Supporting information Table S1). During root colonization, a large number of genes involved in cell functions, lipid metabolism and protein turnover were regulated (Guether et al. 2009). In our microarray experiment, 27 genes involved in cellular process and lipid metabolism were regulated at the pre-stage and 13 genes at the early stage among which the gene encoding for a β-d-xylosidase (*LEXYL1*; MICRO.2255.C3). During the late stage, we observed 31 genes regulated among which the putative UDP-glucuronate decarboxylase 3 (MICRO.1275.C2). Moreover, we showed a major protein turnover with the regulation of 54, 30 and 69 genes involved in this protein process during the pre-, early and late stages, respectively. This change was characterized by the strong induction (i.e. 218-fold) of the gene encoding for a putative calcium/calmodulin-dependent protein kinase (MICRO.12534.C1) during the late stage (Supporting information Table S3). In addition, the microarray analysis revealed the regulation of numerous genes

involved in transport processes. For instance, the expression of the specific family of ABC transporters and multidrug resistance systems genes was detected during the three stages. Recently, Zhang et al. (2010) have observed that the activities of two *M. truncatula* half-ABC transporters were required for arbuscules development. In addition, we also observed a change in the expression of 16 others transporter genes, among which two metal transporter genes. Finally, we observed the up- or down-regulation of transporters genes involved in nutrient transport during the late stage of root colonization (Supporting information Table S3). These included the strong induction (i.e. 35-fold) of sulphate transporter gene (bf_mxlfxxxx_0068c01.t3m.scf), as well as the down-regulation of one putative sulfate transporter gene (MICRO.7577.C1) and one putative potassium transporter gene (cSTA29C13TH). In addition, we observed the induction of two putative amino acid transporter genes (MICRO.8332.C1 and POAD969TP) and two peptide transporter genes (bf_suspxxxx_0017G02.t3m.scf and MICRO.8650.C1).

Importance of the genes involved in biotic stress during the pre, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833

The overview of the biotic stress pathway with the Mapman software was performed during the three stages of AM root colonization (Fig. 13). The results showed the regulation of an important number of genes involved in biotic stresses. During the pre-stage, we observed a regulation of 124 genes involved in this pathway

and 133 genes during the late stage. Moreover, some of these genes were among the most strongly up-regulated during these two stages, among which the genes encoding for a putative zinc finger family protein (STMHL53TV) and a 1,3-beta-glucan glucanohydrolase (MICRO.2286.C17) during the pre-stage of root colonization. A different situation was observed during the early stage of root colonization with a decrease of the number of genes regulated (i.e. 58 genes) and a weak expression of these genes compared to the two other stages. At this stage, we also noted the down-regulation of some of these genes, as the genes encoding for glutathione S-transferase (MICRO.10085.C1), peroxidase (MICRO.8485.C3) and glucan endo-1,3-beta-D-glucosidase (MICRO.6187.C1). A more detailed description of theses genes is given in the following paragraphs.



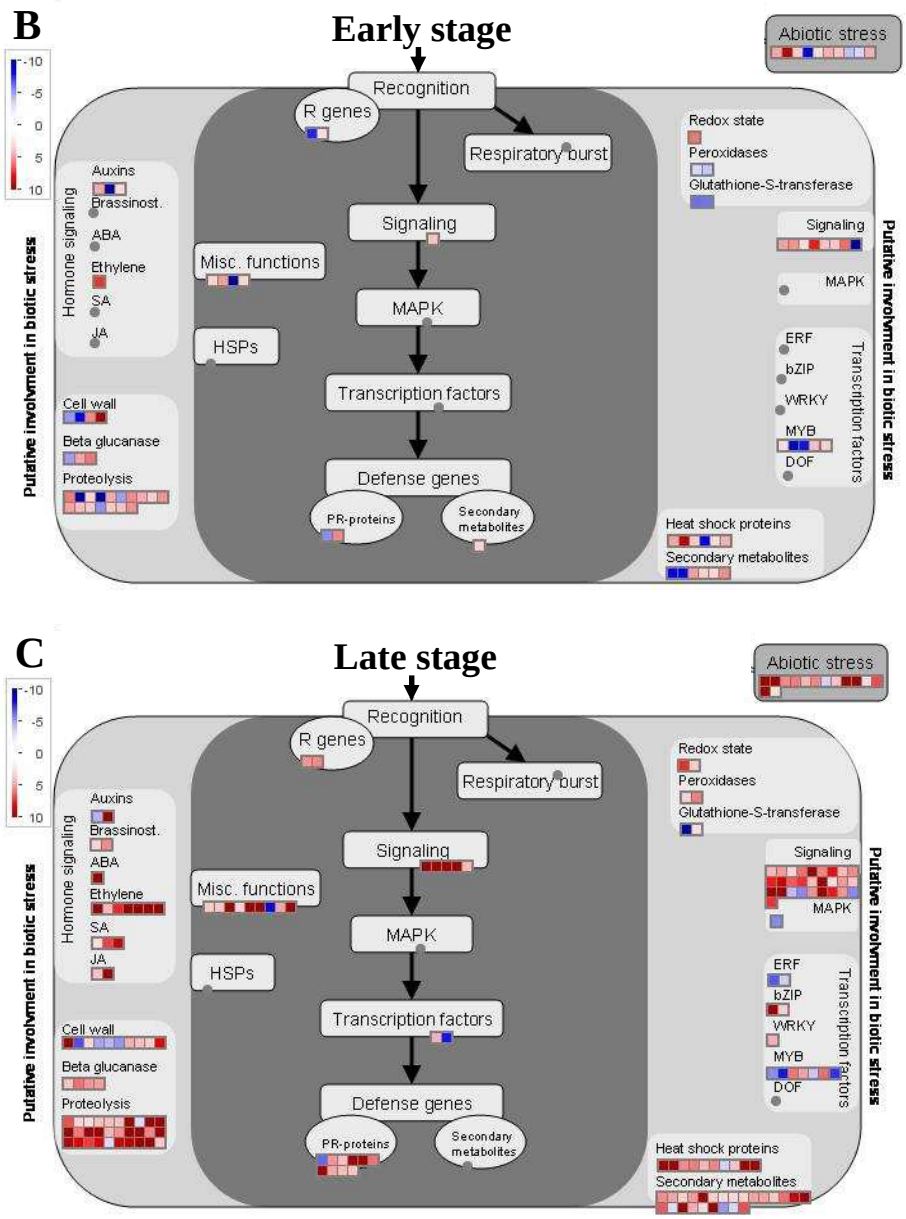


Fig. 13 Impact during the potato root colonization by *Glomus* sp. MUCL 41833 on the genes involved in biotic stress. (A) The MapMan Biotic Stress display created using the 658 regulated genes identified from the pre-stage of the root colonization versus the control. (B) The MapMan Biotic Stress display created using the 381 genes regulated identified from the early stage of root colonization versus the control. (C) The MapMan Biotic Stress display created using the 865 genes regulated identified from the late stage of root colonization versus the control. The fold change is displayed as illustrated in the fold change color bar in the upper left of each panel (blue is repressed and red is induced)

The microarray analysis revealed a change in the regulation of genes involved in the hormones signalling during the AM root colonization (Table 15). During the pre-stage, the genes involved in auxins, brassinosteroid, abscisic acid *(*ABA), ethylene (ET) and acid salicylic (SA) signalling were regulated. The two major genes up-regulated were involved in the process of ethylene synthesis-degradation (MICRO.14687.C1 and POAC667TV). During the early stage, we only showed the regulation of four genes involved in auxins or ET signalling. However, one of theses genes encoding for a putative auxin-induced SAUR-like protein (cSTB33G21TH) was the most down-regulated gene during the early stage. Conversely, in the late stage, the genes involved in the different plant hormone signalling showed an important regulation. Particularly, we observed a strong induction of seven ET signalling genes. Moreover, in this stage, we observed the induction of two jasmonic acid (JA) signalling genes encoding for a lipoxygenase (MICRO.13176.C1) and a 12-oxophytodienoate reductase (bf_mxflxxxx_0006c08.t3m.scf), and the induction of three SA signalling genes.

Table 15 Overview of the markers genes or genes involved in ethylene (ET), acid salicylic (SA) and jasmonic acid (JA) signalling during the pre-, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833 as compared with the controls treatments, and their changes in expression

Probe ID	**ID and Annotation**	**e-values**	Fold change **Pre-**	Fold change **Early**	Fold change **Late**
ET signalling					
MICRO.14687.C1	NM_112126.1: oxidoreductase, 2OG-Fe(II) oxygenase family protein [Arabidopsis thaliana]	5E-108	**26,50**	0,78	1,26
POAC667TV	AAT40509: putative hyoscyamine 6 beta-hydroxylase [Solanum demissum]	0.0	**18,54**	0,68	1,36
bf_mxflxxxx_0040h11.t3m.scf	NM_129381: oxidoreductase, 2OG-Fe(II) oxygenase family protein [Arabidopsis thaliana]	9E-32	1,67	0,81	**13,28**

MICRO.17000.C1	XM_002525784: putative leucoanthocyanidin dioxygenase [Ricinus communis]	9E-73	1,55	0,76	**4,28**
MICRO.2856.C3	XM_002511521: putative Flavonol synthase/flavanone 3-hydroxylase [Ricinus communis]	8E-55	1,00	1,12	**7,55**
MICRO.6939.C1	NM_001035598: universal stress protein (USP) family protein [Arabidopsis thaliana]	8E-12	1,00	1,00	**17,17**
bf_mxflxxxx_0025b06.t3m.scf	BAD16875: putative 1-aminocyclopropane-1-carboxylate deaminase [Oryza sativa (japonica cultivar-group)]	2E-12	1,00	1,00	**10,51**
TBSK03984FD12.t3m.scf	AF348575: putative aminotransferase [Arabidopsis thaliana]	3E-11	0,73	1,12	**11,20**
MICRO.13890.C1	NP_194926: putative protein [Arabidopsis thaliana]	6E-11	0,52	1,52	**38,93**
MICRO.8269.C1	EU003995: gibberellin 2-oxidase 1 [Nicotiana tabacum]	0.0	0,45	**7,37**	0,56
ET marker					
MICRO.2219.C8	X63104: Phenylalanine ammonia-lyase 2 [Solanum tuberosum]	0.0	**4,91**	0,98	0,60
SA signalling					
SDBN001C16u.scf	NM_123239: S-adenosyl-L-methionine:carboxyl methyltransferase family protein [Arabidopsis thaliana]	3E-11	**9,23**	1,00	1,00
MICRO.4827.C2	GU299532: S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase [Solanum lycopersicum]	0.0	1,84	1,12	**7,05**
bf_cswbxxxx_0011d10.t3m.scf	NM_105474: putative S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase [Arabidopsis thaliana]	6E-11	1,42	1,53	**3,03**
MICRO.5928.C1	NM_127124: pentatricopeptide (PPR) repeat-containing protein [Arabidopsis thaliana]	1E-19	0,86	1,00	**9,00**
SA marker					
MICRO.2286.C17	AJ586738: 1,3-beta-glucan glucanohydrolase [Solanum tuberosum]	0.0	**36,71**	1,21	**5,24**
MICRO.6187.C1	X74906: glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	0.0	**16,22**	*0,20*	**6,12**
MICRO.12664.C1	AY737315: putative thaumatin-like protein [Solanum tuberosum]	0.0	**14,41**	1,60	**13,01**
MICRO.6187.C2	AM231409: glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	0.0	**9,36**	0,59	**5,05**
MICRO.12659.C1	AY170827: acidic class II 1,3-beta-glucanase precursor (GluA2) [Solanum tuberosum]	2E-107	**5,61**	0,55	1,96
MICRO.11279.C1	X67693: chitinase; endochitinase [Solanum tuberosum]	0.0	**5,34**	0,57	1,49
MICRO.5426.C4	AJ493450: pathogenesis related protein isoform b1 [Solanum phureja]	0.0	**5,18**	1,03	1,65

MICRO.10439.C1	AF024537: class II chitinase [Solanum tuberosum]	0.0	**4,39**	1,00	0,74
MICRO.11595.C1	AY737317: putative thaumatin-like protein (PR-5/321c) [Solanum tuberosum]	1E-81	**4,32**	0,54	0,73
MICRO.2286.C42	AJ009932: beta-1,3-glucanase (PR2) [Solanum tuberosum]	0.0	**3,43**	1,19	**3,99**
BPLI14P23TH	PR-1a gene for pathogenesis-related protein 1a [Nicotiana tabacum]	7E-75	1,41	1,07	**6,08**
MICRO.11332.C1	NM_001084733: Light Sensitive Hypocotyls 4 (LSH4) [Arabidopsis thaliana]	1E-58	0,82	0,76	*0,16*
MICRO.1528.C1	ABE81419: Thaumatin, pathogenesis-related [Medicago truncatula]	5E-15	0,78	1,61	**12,00**
MICRO.1592.C2	AK229483: putative beta-1,3-glucanase [Arabidopsis thaliana]	1E-93	0,68	**4,86**	1,92
MICRO.1430.C1	X66826: Waxy locus DNA encoding pseudogene similar to beta-1,3-glucanases and beta-1,3-1,4-glucanases [Solanum tuberosum]	0.0	0,66	**6,03**	1,00
JA signalling					
MICRO.13176.C1	U60202: lipoxygenase (POTLX-3) [Solanum tuberosum]	0.0	1,00	1,32	**3,90**
bf_mxflxxxx_0006c08.t3m.scf	AJ242551: 12-oxophytodienoate reductase [Lycopersicon esculentum]	1E-125	1,00	1,00	**15,99**
JA marker					
MICRO.2033.C6	DQ168326: metallocarboxypeptidase inhibitor [Solanum tuberosum]	6E-107	**11,44**	1,00	0,83
cSTD1O5THB	AF459079: putative Kunitz-type tuber invertase inhibitor precursor [Solanum tuberosum]	0.0	**3,36**	1,11	1,82
BF_CSCHXXXX_0042C07.T3M.SCF	U59272: Cysteine protease inhibitor 10 precursor (PCPI-10) (Pcpi10)	0.0	**3,27**	0,83	0,61
MICRO.839.C6	AY089961: putative cysteine proteinase inhibitor precursor 1423 [Solanum tuberosum]	8E-15	1,91	**5,59**	1,40
TBSK02550FE06.t3m.scf	X13181: putative trypsin inhibitor from Potato mRNA for tuber protein p340/p34021	0.0	1,67	1,19	**3,89**
MICRO.6809.C6	DQ168323: proteinase inhibitor II [Solanum tuberosum]	0.0	1,42	*0,18*	0,53
TBSK02874FH06.t3m.scf	L37519: proteinase inhibitor II [Solanum tuberosum]	3E-169	1,39	0,56	**4,82**
MICRO.5302.C3	DQ168314: proteinase inhibitor I precursor [Solanum tuberosum]	2E-107	1,22	1,13	**13,80**
MICRO.4288.C8	U59276: cysteine proteinase inhibitor 5 precursor [Solanum tuberosum]	0.0	1,00	1,55	**4,11**

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the pre-, early and late stages of AM fungal root colonization versus the controls are ordered according to the plant hormone signalling and the expression fold change during the pre-stage of root colonization. Genes were considered as differentially regulated if *P*-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.0 or ≤ 0.33

The secondary metabolism associated with the biotic stress showed also a drastic evolution during the AM root colonization. During the pre-stage, 19 genes of the secondary metabolism process were regulated. More precisely, four genes involved in the isoprenoids process and eleven genes in the phenylpropanoids process (e.g. the gene encoding for the Phenylalanine ammonia-lyase 2 (PAL2; MICRO.2219.C8) involved in the lignin biosynthesis and other processes as the tyrosine and nitrogen metabolism) were regulated. During the early stage, only six genes of the secondary metabolism were regulated. In particular, we observed a down-regulation of two genes involved in the isoprenoids process (bf_arrayxxx_0043h04.t3m.scf and MICRO.8755.C4). During the late stage, we also observed an important regulation of the secondary metabolism process (i.e. 17 genes), with a predominance of the genes involved in the isoprenoids process (i.e. 9 genes) and two genes involved in the phenylpropanoids process. We observed the same profile of response in different processes associated with biotic stress. The proteolysis process showed a strong regulation of these genes during the pre- and late stages of AM root colonization and a low-level of regulation at the early stage. The most important part of genes in the proteolysis process was involved in the process of ubiquitin degradation. The results also showed an identical profile of regulation for the genes involved in the cell wall, beta-glucanase, heat shock protein and signalling pathways.

Another important change in plant genes regulation associated with the biotic stress was the regulation of the transcription factors (i.e. TFs) genes during the three stages of AM root colonization. At

the pre-stage, we observed the up-regulation of 14 TFs genes (two ERF TFs genes encoding for two ethylene-responsive element binding proteins, two bZIP TFs genes, nine WRKY TFs genes and one MYB TF genes). During the early stage, only 4 MYB TFs genes were regulated. Finally at the late stage, a regulation of 11 TFs genes (two ERF TFs genes, two bZIP TFs genes, one WRKY TF gene and six MYB TFs genes) were noted. The WRKY TFs genes were principally up-regulated during the pre-stage. We observed only an up-regulation of one WRKY TF gene at the late stage (MICRO.13165.C1). These results suggested a possible involvement of the WRKY TFs genes during the pre-stage. We decided to confirm the regulation of these ten WRKY TFs genes during the root colonization by *Glomus* sp. MUCL 41833 using Real-Time quantitative PCR (Table 14). The results showed a good correlation between the data obtained by the two methods. This confirmed the regulation of the WRKY TFs genes at the three stages, and particularly, the strong up-regulation of the WRKY TFs genes: MICRO.16727.C1, STMIS76TV, MICRO.16616.C1 and MICRO.189.C1 at the pre-stage. The only difference observed was the up-regulation of the WRKY TF gene (MICRO.189.C1) detected by Real-Time quantitative PCR at the late stage.

Discussion

Numerous studies have demonstrated that root colonization by AM fungi regulates transcriptional changes in the host plant (Liu et al. 2007; Guether et al. 2009; Fiorilli et al. 2009). Defence genes

involved in the control and regulation of the AM symbiosis were particularly important (Lopez-Raez et al. 2010; Pozo et al. 2010). Here we have performed a microarray analysis to reveal significant changes in genes expression, especially of defence genes, during the pre, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833. Microarrays analysis associated to the Mapman software, with a Real-Time quantitative PCR validation step, revealed an important global change in plant gene expression during the three stages of root colonization. In addition, we observed an increase of defence genes at the pre- and late stages. In particular, an induction of nine TFs WRKY genes was noted at the pre-stage. Finally, the similar P content in the leaves of mycorrhizal and non mycorrhizal potato plants and the non-regulation of P nutrition markers genes suggested that the transcriptional changes were not associated to differences in P nutrition.

During the AM fungal potato root colonization, only a few numbers of genes were differentially regulated at the pre, early and late stages. We observed a maximum of 28 genes regulated between the pre- and early stages, and only 2 genes between the three developmental stages. These results supported the wide transcriptional changes occurring during the potato root colonization. These results were corroborated by Dermatsev et al. (2010). These authors only observed a few genes differentially expressed in the AM fungal-tomato interaction during the initial recognition and contact. Guether et al. (2009) also demonstrated a low overlap of expressed genes (i.e. only 7 genes) at the early and late stages of *L. japonicus* root colonization by *Gigaspora margarita*. These results suggested a

dynamic process in AM fungal-potato interaction during the root colonization.

Interestingly, we noted the induction of the majority of the AM symbiosis markers genes during the late stage of root colonization. In particular, the induction of five genes (i.e. *DXS-2*, *DXR*, *PDS*, *ZDS* and *CRTISO*) directly related to the biosynthesis of mycorradicin was observed. This carotenoid pathway is responsible of the yellow coloration of some mycorrhizal roots (Walter et al. 2007) and was considered as a marker of the AM fungal presence in plants roots (Lopez-Raez et al. 2010). In addition, we observed the induction of one expansin-like protein (*EXBL1*) specifically at the pre-stage of root colonization. This gene was reported as an initial host marker of a successful mycorrhizal establishment (Siciliano et al. 2007; Dermatsev et al. 2010). Finally, Dermatsev et al. (2010) have suggested the involvement of the expansin-like proteins in the symbiotic signalling taking place between host and AM fungus during the pre-stage of root colonization.

In this study, we observed the regulation of some genes earlier reported in plant roots in presence of different AM fungi. For example, during the pre-stage of root colonization, we observed some similarity with the pattern of genes expression (.i.e. genes encoding for lipid transfer protein, chloride ion channel or patatin) obtained by Dermatsev et al. (2010). Similarly, the regulation of numerous TFs genes in the three stages of root colonization (e.g. the MYB and GRAS TFs genes) were also reported by Fiorilli et al. (2009), Gomez et al. (2009) and Guether et al. (2009). GRAS are an important family of proteins which play diverse roles in regulation of plant

development. In particular, the Scarecrow and Short Root proteins play a central role in the root development (Bolle 2004). In addition, two GRAS domain proteins, Nodulation signalling pathway 1 (NSP1) and NSP2 have been shown indispensable for Nod-factor signalling and nodule morphogenesis (Smit et al. 2005; Heckmann et al. 2006). MYB proteins are key factors in regulatory networks controlling development, metabolism and responses to biotic and abiotic stresses (Dubos et al. 2010). Moreover, the MYB proteins have been shown up-regulated during nodule development (Colebatch et al. 2004). As a result of their roles in root development and symbiosis, the members of these two families could be good candidates for the regulation of genes expression during the AM fungal root colonization. Finally, during the root colonization, we observed a large induction of genes involved in cell functions, lipid metabolism, protein turnover and transport process, as already reported in the literature (Fiorilli et al. 2009; Gomez et al. 2009; Guether et al. 2009; Lopez-Raez et al. 2010).

Deciphering how the expression of defence genes is modulated during AM fungal root colonization is essential to a better understanding of the mechanisms controlling the symbiotic establishment and the function of AM fungus in the plants. The overview of the biotic stress pathway performed in our study revealed an increased induction of plant defence genes before contact between the AM fungus and the potato roots (i.e. pre-stage), and following the establishment of a functional symbiosis (i.e. late stage). In contrast, the early stage of root colonization was characterized by a lower induction of these defence genes. Moreover, it was interesting to note

that some of these genes are amongst the most down-regulated genes at the early stage. One of the most important category of defence genes are the plant genes hormones for which numerous functions in mycorrhiza formation have been proposed (Hause et al. 2007; Herrera-Medina et al. 2008; Riedel et al. 2008; Lopez-Raez et al. 2010). During the pre-stage, we clearly demonstrated an induction of more than ten genes SA-dependent. Among these, the *PRs* genes were represented by the induction of some beta-1,3-glucanase (*PR2*), chitinase (*PR3*) or thaumatin (*PR5*) genes. Few of these genes were induced in the early stage of root colonization. We only observed the induction of two putative beta-1,3-glucanase and one proteinase inhibitor II classified here in the *PR* genes (i.e. this gene was reported as a marker of the jasmonic acid (i.e. JA) pathway (Wasternack et al. 2006)). These results suggested the transient expression of defence genes before contact between the AM fungus and the host, and in particular of the SA dependent genes. Moreover, this observation supported the hypothesis that AMF are able to repress SA dependent defence responses in the plant to favour a compatible interaction (Pozo and Azcon-Aguilar 2007).

Recently, Lopez-Raez et al. (2010) have demonstrated the regulation of the oxylipin pathway in tomato roots colonized by two AM fungi (*Glomus mosseae* and *Glomus intraradices*). In contrast to this study, which showed the induction of numerous genes involved in the oxylipin pathway, we only observed the induction of two genes involved in this pathway and four markers genes of the JA signalling pathway (i.e. four proteinase inhibitors genes), during the late stage of root colonization. We showed the induction of one lipoxygenase gene

(*POTLX-3*), which shared high similarity with the *Capsicum annuum LOX1* gene encoding for a protein involved in the 9-LOX branch of the oxylipin pathway (Hwang and Hwang 2010), and the 12-oxophytodienoate reductase gene which encode for a protein involved in the 13-LOX branch of the oxylipin pathway (Wasternack et al. 2006). Otherwise, the inductions of the other genes involved in the oxylipin pathway, such as the allene oxide synthase or divinyl ether synthase, were not observed. Consequently, this result may indicate a lower importance of the oxylipin pathway during the symbiotic interaction. In addition to this pathway, recent publications have also described the role of other plant hormones in the control of AM symbiotic establishment, such as ABA and ET (Herrera-Medina et al. 2007; Riedel et al. 2008; Fiorilli et al. 2009; Martin-Rodriguez et al. 2011). For example, Riedel et al. (2008) have shown that JA levels in mycorrhizal roots compared with non-mycorrhizal controls remained unaltered and that ET, rather than JA, signalling played a role in the AM symbiosis between *G. intraradices* and *Nicotiana attenuate*. In our study, during the late stage of root colonization, we observed the induction of numerous genes involved in the hormones signalling, and particularly, the high induction of eight genes of the ET signalling. In addition to the important induction of ET signalling genes, we also noted a down-regulation of the POCC418TP gene, which shared a high homology with the *Nicotiana tabacum* ethylene-responsive transcription factor 3 (NtERF5). This gene has been suggested as a regulator of gene expression during stress and more especially, this gene could acts as a transcriptional repressor. These results suggested

that the ET plays an important function in the interaction between *Glomus* sp. MUCL 41833 and the potato roots.

It was also interesting to note the induction of three genes of the SA signalling, including two salicylic acid carboxyl methyltransferase. This result suggested that the SA pathway could also be involved in the symbiotic interaction, as previously proposed by Medina et al. (2003), Liu et al. (2007) and Pozo and Azcon-Aguilar (2007). In addition to these three plant genes involved in the SA signalling, we observed the regulation of seven defence markers genes of the SA signalling during the late stage of root colonization. For examples, we showed the induction of four beta-glucanase and some chitinase and thaumatin genes. These genes have already been located or induced specifically in the root cells containing arbuscules, such as the class III chitinase gene family (Salzer et al. 2000). Moreover, the beta-1,3-glucanase gene was involved in cell wall degradation by digesting β-1,3-glucans in fungal cell walls (Mauch and Staehelin 1989; Chang et al. 1992) and the chitinase cleaved the fungal elicitors (Kasprzewska 2003). As a consequence, these genes could play a role during arbuscules formation or senescence.

In addition to the plant genes hormones, we also noted the regulation of numerous other defence genes during the late stage of root colonization, among which genes involved in protein degradation (i.e. proteolysis) (Liu et al. 2003; Hohnjec et al. 2005; Guether et al. 2009; Fiorilli et al. 2009; Takeda et al. 2009). In our study, we observed the regulation of 30 genes and particularly the ubiquitin proteolysis genes. The proteolysis process was shown to be involved in the defence responses in a number of plant species (Takizawa et al.

2005) and allowed cells to fine-tune their responses to changing environments (Kurepa and Smalle 2008). The proteolysis genes could play a role in the development of the AM fungus at the level of arbuscule-containing cells.

A remarkable finding, in our research, was the induction of nine TFs WRKY genes during the pre-stage of root colonization. The WRKY TFs are among the largest families of transcriptional regulators in plants and form integral parts of signalling webs that modulate many plant processes (Rushton et al. 2010). The majority of reports concerning the WRKY TFs have indicated that numerous members of this multigene family play central roles in the transcriptional reprogramming associated with the plant immune response (Pandey and Somssich 2009). Three of the WRKY TFs genes regulated (i.e. MICRO.2876.C1, MICRO.2876.C1 and bf_suspxxxx_0048g10.t7m.scf) showed a homology with one of *Arabidopsis* WRKY TF gene (i.e. *AtWRKY70*). *AtWRKY70* has been shown to act at a convergence point determining the balance between SA and JA dependent pathways (Li et al. 2006; Knoth et al. 2007). In relation with the regulation of the SA dependent defence responses during the pre- and early stages of root colonization, we hypothesized that the proteins encoded by these three WRKY TFs genes may control the expression of SA dependent gene during symbiotic establishment. Moreover, two of these WRKY TFs genes (MICRO.11686.C1 and STMIS76TV) were homologue with the *AtWRKY51*, for which the promoter has been identified as a target by the *trans*-Activating TGA (i.e. including glutathione-*S*-transferase and *PR* genes) in a SA dependent response (Thibaud-Nissen et al. 2006).

This observation may indicated that the proteins encoded by these two WRKY TFs genes could control the regulation of some defence genes and suggested also the importance of WRKY TFs genes during the pre-stage of root colonization. During the late stage of root colonization, the results of Real-Time quantitative PCR showed an induction of two WRKY TFs genes, including the *MICRO.13165.C1* which shared homology with *AtWRKY53*. Wang et al. (2006) have demonstrated that the *AtWRKY53* modulate positively the systemic acquired resistance. This observation may indicate that the proteins encoded by these WRKY TFs genes could be involve in the control of defence genes during the late stage of root colonization and also play a role in the control of plant pathogens by AM fungi.

In conclusion, the genome-wide expression profiling performed during the pre-, early and late stages of potato root colonization by *Glomus* sp. MUCL 41833 provided evidence of a global transcriptional change in the potato roots. Particularly, we noticed the importance of the plant defence genes modulation for the mechanisms controlling AM establishment in plant roots. Moreover, an important up-regulation of the WRKY TFs genes during the AM symbiosis was demonstrated for the first time. Different observations suggested that the WRKY TFs proteins could regulate the plant defence genes during the AM establishment, especially at the pre-stage (i.e. before contact) of the symbiotic interaction, and allowed the compatible interaction. In addition, Real-Time quantitative PCR analysis highlighted their regulation during the effective symbiosis, which may underline their involvement in the control of biotic stresses. Further studies are

required to confirm the impacts and functions of WRKY TFs proteins during the AM fungal root colonization.

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Chapter III

Induced resistance against *Rhizoctonia solani* at different developmental stages of the arbuscular mycorrhiza colonization of potato plantlets

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Preface

The studies conducted in chapters I and II revealed a strong induction of several genes putatively involved in the defence mechanism and pathways of plants during the pre- and late stages of AM fungal[4] colonization of potato roots by *Rhizophagus* sp. MUCL 41833. Such observation was already reported in the literature during the late stage and was usually related to an increased resistance of mycorrhizal plants against pathogens infection (Review Pozo et al. 2010). However, the strong induction of defence genes during the pre-stage was not expected. This could be related to the method of AM fungal inoculation. In our study, the MDP *in vitro* culture system allowed the plant to be colonized in a dense/active ERM network of AM fungus which produces multiple and numerous AM fungal contacts with the roots in comparison to the classical "spores" inoculation technique. Thus, the strong induction of defence genes obtained at the pre-stage of root colonization may, as for the late stage, support the potential of the AM fungus to trigger an increased resistance of plant during this developmental stage. However, previous studies have concluded that a well-established AM symbiosis was necessary to induce resistance in colonized plants (Slezack et al. 2000; Khaosaad et al. 2007) and consequently, supported the fact that our hypothesis needed to be confirmed.

In **chapter III**, we decided to compare the effects of *Rhizophagus* sp. MUCL 41833 on the disease development of *R. solani* in potato roots when the plantlets were challenged by both microorganisms at

[4] In this chapter, we took into consideration the AM fungal classification of Schüβler et al. (2001)

the same time (i.e. before roots colonization and contact) or first colonized by *Rhizophagus* sp. MUCL 41833 prior to contact with *R. solani* (i.e. after the AM symbiotic establishment). We discussed the hormonal signalling pathways involved in the biological protection process triggered by *Rhizophagus* sp. MUCL 41833 in both stages.

Abstract

The bio-control of soil-borne pathogens in plants during a well-established arbuscular mycorrhizal (AM) symbiosis has been demonstrated in different studies, while this bio-control before contact and during the AM symbiosis establishment remains controversial. Here, we used the Mycelium Donor Plant *in vitro* culture system to investigate the effects of *Glomus* sp. MUCL 41833 on the control of *R. solani* MUCL 49235 in potato roots before the symbiotic establishment and during a well-established symbiosis. The disease analysis showed a decrease in necrosis caused by *R. solani* in the potato roots during the pre-/early (i.e. before AM fungi contact and first hyphae colonizing roots, respectively) and late (i.e. well-established AM symbiosis) stages of AM symbiosis. Moreover, we observed that *R. solani* had an impact on the AM fungus only when both microorganisms were challenging the root at the same time (i.e. before the symbiotic establishment). In addition, the Real-Time Quantitative PCR analysis of defence genes suggested for the first time that the bio-protection of AM fungi during the pre-/early stages of symbiotic establishment may be associated to a priming of the *MAPK* gene and salicylic acid (SA)-dependent genes. We also showed that the bio-protection during the late stage of symbiotic establishment could be associated to a priming of SA-dependent genes. These results underlined the necessity for further research to elucidate the regulation of the hormone-dependent defence genes during the AM fungal bio-protection.

Keywords arbuscular mycorrhiza fungus, bio-protection, Defence gene expression, *in vitro* system, *Rhizoctonia solani*, *Solanum tuberosum*

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important crop worldwide (FAO 2008) and a precious source of proteins, vitamins, minerals, polyphenols and starch (Morris et al. 2004; Andre et al. 2007). This crop present a high ratio of yield productivity to soil occupation (85 % of the plant is comestible; FAO 2008). Unfortunately, potato is susceptible to numerous pathogens for which control methods are not always efficient (Diallo et al. 2011). One of the most common soil-borne plant pathogen affecting potato is *Rhizoctonia solani* (Fiers et al. 2010). The infected plants develop crown rot, root rot or stem canker, which often leads to wilting and to plant death in the most severe cases. At present, the most widely used method to control this pathogen is via chemicals, which may induce resistances and cause harmful side-effects on the environment (Tsror 2010). In the recent years, beneficial microorganisms such as Pseudomonas sp., arbuscular mycorrhizal (AM) fungi and fungal antagonists (Whipps 2004; Grosch et al. 2006) have been acclaimed as promising alternatives to control plant pathogens among which *R. solani*, and to minimise the impact of chemicals on the environment by replacing these molecules or reducing their rate of application (Harman and Bjorkman 1998).

One of the most common mutualistic association in nature is the AM symbiosis which is formed between soil fungi belonging to the Phylum Glomeromycota (Schüβler et al. 2001) and more than 80% of higher plants including the vast majority of agricultural crops (Smith and Read 2008). The AM fungus, an obligate biotroph, receives

carbohydrates from the host plant required to complete its life cycle, in exchange of which it provides the plant with nutrients (e.g. phosphate, nitrogen and sulphur) and water. The establishment of this symbiosis involves major change in the physiology of the host plant causing a global reprogramming of plant functions and modulating its responses to biotic and abiotic stresses (Liu et al. 2003; Pozo et al. 2010). Numerous studies have reported the reduction in incidence and/or severity caused by soil-borne pathogens in presence of AM fungi (Whipps 2004; Pozo et al. 2010). Among these, Yao et al. (2002; 2003) reported a protective effect conferred by AM fungi against *R. solani* in potato plants. These authors noted a decrease in the severity of symptoms caused by this pathogen in presence of *Glomus etunicatum* and attributed this effect to an increased accumulation of the phytoalexins rishitin and solavetivone in the mycorrhizal potato plants challenged by *R. solani* in comparison to the non-mycorrhizal plants challenged by this pathogen.

The protective effects conferred to plants by AM fungi may be localized, i.e. at the site of the host cell containing arbuscules (Cordier et al. 1998; Azcon-Aguilar et al. 2002) or systemic (Pozo et al. 2002; Khaosaad et al. 2007) via a mycorrhiza induced resistance (MIR) mechanism (Pozo et al. 2009). Plant defence mechanisms are tightly regulated through an interconnected network of signalling pathways in which jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) play major roles (Conrath et al. 2006; Van Wees et al. 2008). Recently, Pozo and Azcon-Aguilar (2007) have suggested that a functional mycorrhiza is linked with a partial suppression of SA-dependent responses in the plant, compensated by an enhancement of JA-

regulated responses. This would result in the priming of JA-dependent defence mechanisms and could explain the effectiveness of MIR. However, the importance of each component of the plant resistance response in the protective effect of AM fungi against soil-borne pathogens and the signalling pathways that control these responses remains poorly understood.

The presence of AM fungi within the roots prior to contact with the soil-borne pathogen seems an important factor in plant protection (Azcon-Aguilar et al. 2002). Recently, Chandanie et al. (2009) confirmed the importance of AM fungal root colonization prior to pathogen infection for the reduction of disease in cucumber plants infected with *R. solani*. These authors demonstrated that the AM fungus was effective in the reduction of damping-off when it was inoculated prior to the pathogen, but not when it was inoculated simultaneously with the pathogen. Moreover, a key factor in the induction of resistance seems also to be the level of root colonization by AM fungi (Pozo et al. 2009). With few exceptions (Caron et al. 1986; Garcia-Garrido and Acampo 1988; St-Arnaud et al. 1997), most studies concluded to the necessity of a well-established AM symbiosis for local and systemic induced resistance (Slezack et al. 2000; Khaosaad et al. 2007).

Recently, Voets et al. (2009) have developed an *in vitro* culture system allowing for the fast and homogenous colonization of seedlings. This system based on the plating of plantlets within an actively growing extra-radical mycelium network (named Mycelium Donor Plant (MDP) *in vitro* culture system), was shown adequate to study the expression of plant defence genes in roots of potato during

the symbiotic establishment of AM fungi (Gallou et al. 2010). An induction of several defence genes during the pre (i.e. before contact of AM fungi with the roots and root colonization), early (i.e. from appresorium formation to the first hyphae colonizing roots, before arbuscules and vesicles formation) and late (i.e. well-established root colonization with arbuscules and vesicles formed) stages of AM symbiotic establishment was reported. This system opened the door to tripartite interactions studies, including the plant (e.g. potato), the AM fungus and the root pathogen (e.g. *R. solani*).

In the present study, we used the MDP *in vitro* culture system to investigate the effects of the AM fungus, *Glomus* sp. MUCL 41833, on the disease caused by *R. Solani* MUCL 49235. We compared the effect of introducing *R. solani* at two different stages of the colonization of the plantlets by the AM fungus: in a first assay, we challenged the plantlets by both microorganisms at the same time, i.e. as from the pre-/early stage of the colonization of the plantlets by *Glomus* sp., while in a second assay, we only introduced *R. solani* after the symbiosis was well established, i.e. in the late stage of the colonization by *Glomus* sp..

In both approaches, we analyzed the root colonization by the AM fungus and the disease development of *R. solani*. In addition, the real-time QRT-PCR was used to assess the expression of defence genes SA-, JA- and ET-dependent, a mitogen-activated protein kinase (*MAPK*) gene encoding for a wound-induced protein kinase which is a convergent point in the signalling pathway of defence responses in plant-pathogen interactions (Yamamizo et al. 2006) and the *PT3* gene, a plant gene marker of functional symbiosis.

Materials and methods

Biological material

In vitro potato plantlets (*Solanum tuberosum* L., var. Bintje) were supplied by the Station de Haute Belgique in Libramont, Belgium. Plantlets were micro-propagated every five weeks as described in Voets et al. (2005).

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A 17 (SARDI, Australia) were surface-disinfected by immersion in sodium hypochlorite (8% active chloride) for 12 min, rinsed three time in de-ionized sterilized (121°C for 15 min) water and germinated in groups of 25 on Petri plates (90 mm in diameter) filled with 35 ml MSR medium without sucrose and vitamins, and solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA). The Petri plates were incubated at 27°C in the dark.

A root organ culture (ROC) of *Glomus* sp. MUCL 41833 was supplied by the Glomeromycota *in vitro* collection (GINCO – http://www.mycorrhiza.be/ginco-bel). The spores were extracted by solubilisation of the gellan gel. An approximate of 100 spores were then placed in the near vicinity of actively growing transformed carrot (*Daucus carota* L.) roots (approximately 70 mm long) on Petri plates (90 mm diam.) containing the Modified Strullu-Romand (MSR) medium (Declerck et al. 1998), solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA), following the method described in Cranenbrouck et al. (2005). The Petri plates were incubated for 3

months in the dark in an inverted position at 27°C. Several thousand spores were produced during this period.

A culture of *R. solani* Kuhn MUCL 49235 was supplied by the Mycothèque de l'Université catholique de Louvain (MUCL – http://bccm.belspo.be/about/mucl.php). A plug of gel containing several bulbils and mycelium was placed on 50 ml Potato Dextrose Agar (PDA) (Scharlau Chemie S.A, Barcelona, Spain) in Petri plates (90 mm diam.). The Petri plates were incubated at 25°C in the dark for seven days.

Experimental design

Mycorrhizal donor plant (MDP) *in vitro* culture systems were set up as described in Voets et al. (2009). Briefly, *M. truncatula* plantlets were inoculated with the AM fungus in the root compartment (RC) of bi-compartmented Petri plates (90 mm diam.). After eight weeks of culture, the mycelium crossed the plastic barrier separating the RC form a hyphal compartment (HC) and a profuse extra-radical mycelium (ERM) network bearing numerous spores developed (for details, see Voets et al. 2009). At that time, two holes (± 2 mm diam.), separated by 4.5 cm from each other, were made in the base and the lid of the Petri plates, at the side of the HC. One ten-day old potato plantlet was inserted in each hole following the same methodology as described in Voets et al. (2009) with their roots in direct contact with the ERM. The Petri plates were then sealed carefully and incubated horizontally (i.e. with the AM fungus, potato and *M. truncatula* roots in the dark) in a growth chamber set at 20/16°C (day/night), 70 %

relative humidity with a photoperiod of 16 h d^{-1} and an average photosynthetic photon flux (PPF) of 300 µmol m^{-2} s^{-1}. In parallel, two micro-propagated potato plantlets were placed in the HC of a control treatment (i.e. non-mycorrhizal *M. truncatula* seedlings) and grown under the same conditions as described above.

In a first experiment, the potato plantlets were placed in the ERM network of the AM fungus and were at the same time inoculated with *R. solani* (i.e. in order to evaluate the effect on the plant roots as from the pre-/early stages of AM symbiosis), while in a second experiment, *R. solani* was inoculated nine days following the contact of the potato plantlets with the ERM network of the AM fungus (i.e. in order to evaluate the effect on the plant roots as from the late stage of AM symbiosis). The inoculation with *R. solani* was performed using a 3 mm diam. plug from a seven day-old culture of *R. solani* containing abundant mycelium and sclerotia. The plug was placed in the close vicinity of the potato roots in the HC. For the two experiments, the same experimental design was used, only the time of inoculation of *R. solani* being different.

For the two experiments, four treatments were considered: (1) a control treatment with potato plantlets plated in the HC without the presence of both fungi (termed Control-1 and Control-2 for experiment 1 and 2, respectively); (2) a treatment with potato plantlets plated in the ERM network of the HC without the presence of *R. solani* (termed AM-1 and AM-2 for experiment 1 and 2, respectively); (3) a treatment with potato plantlets plated in the HC without ERM and inoculated with *R. solani* (termed Rsol-1 and Rsol-2 for experiment 1 and 2, respectively); (4) a treatment with potato plantlets

plated in the ERM of the HC in presence of *R. solani* (termed AM+Rsol-1 and AM+Rsol-2 for experiment 1 and 2, respectively). Roots of the four treatments were harvested 1, 2, 6 and 9 days after plating (dap) of the potato plantlets in the HC, for the first experiment, and 10, 11, 12 and 16 dap of the potato plantlets in the HC, for the second experiment. For each Petri plate, the two potato plantlets from the HC were pooled to obtain sufficient material for analysis. Half of the root systems were used to estimate the root colonization while the other half was used for gene expression analysis. For disease severity assessment, the roots were observed before harvesting. Four biological replicates were used to assess root colonization and disease severity, and three biological replicates (based on the quality and purity of ARN) for gene expression analysis.

Root colonization and disease severity assessment

Half of the root systems in the treatments AM-1, AM-2, AM+Rsol-1 and AM+Rsol-2 was used to estimate the AM fungal root colonization. Roots were cleared in 10% KOH at 50 °C for 90 min., rinsed with distilled water and stained with Trypan blue 1% at 50°C for 60 min. Roots were observed under a dissecting microscope (Olympus SZ40, Olympus Optical GmbH, Hamburg, Germany) at 10-40x magnification, according to McGonigle et al. (1990) with an average number of intersections observed of at least 150.

The root systems in the treatments Rsol-1, Rsol-2, AM+Rsol-1 and AM+Rsol-2 were used to assess the disease severity. Roots were rated visually using a 0–4 scale in which 0 = no lesions, 1 = small

lesions, between 1-3 mm length, 2 = more extensive lesions, up to 5 mm length, 3 = lesions longer than 5 mm and with necrotic areas, 4 = lesions over 50% of the root area with necrosis (Liu and Sinclair 1991). A Disease Index (DI) was calculated using the following equation: % DI = $\sum$ (n × Sn) × 100 / (4N), where n = number of plants with a given disease rating, Sn = disease rating and N = total number of plants rated (Liu and Sinclair 1991).

Disease development was also assessed by the area under disease progress curves (AUDPC). The AUDPC was calculated according to the equation of Campbell and Madden (1990): AUDPC = $\sum^{n-1}$ ((y + y_{+1})/2) x (t_{+1}- t), were n = number of evaluations, y = the disease index and t = the day post inoculation with *R. solani* of each evaluation.

RNA extraction

Total RNA was extracted from 50-100 mg frozen root material with Trizol® reagent (Invitrogen, Carlsbad, USA) with an additional chloroform purification step and then purified using the Purelink™ Micro-to-midi total RNA purification system (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The total RNA was subsequently treated with the TURBO DNA-free™ kit (Ambion, Austin, USA) according to the manufacturer's instructions. Concentration and purity of total RNA were determined in a NanoDrop®-ND 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA), using a 2 µl aliquot of the total

RNA solutions. RNA purity was estimated from the A260/A280 absorbance ratio.

Real-Time quantitative PCR

Following total RNA extraction, reverse transcription (RT) of 500 ng of total RNA for the first experiment and 1 µg for the second experiment was performed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Montreal, Canada) in a volume of 20 µl with oligo $(dT)_{18}$ primer at 55°C for 20 min, according to the manufacturer's instructions. For each RNA sample, a reaction without RT was performed as a control for contamination by genomic DNA. Real-Time PCR analysis was performed using the LightCycler 2.0, the 5X LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Mix and gene-specific primers (For LightCycler 2.0 experimental run, see Gallou et al. 2010). Seven targeted genes were studied (i.e. abbreviations and accession numbers, respectively): Gluthatione-S-transferase 1 (*GST1* - J03679), Lipoxygenase (*Lox* - Y18548), MAP kinase (*MAPK* - AB206552), Phenylalanine ammonia lyase (*PAL* - X63104), basic Pathogenesis Related 1 (*PR1* - AJ250136), acidic Pathogenesis Related 2 (*PR2* - AJ009932) and Phosphatase Transporter 3 (*PT3* - AJ318822) (For primers sequences see Gallou et al. 2010). Normalisation was achieved using the geometric means of three potato reference genes (i.e. *βeta-tubulin* (Z33382), *Elongation Factor-1-alpha* (AB061263) and *Glyceraldehyde 3-phosphate dehydrogenase* (AF527779)), which showed the most stable genes expression in the potato roots during symbiotic establishment of

Glomus sp. MUCL 41833 determined by the geNorm software (http://medgen.ugent.be/~jvdesomp/geNorm) (See Gallou et al. 2010). In order to visualize the up-regulated or down-regulated genes, the data were analysed statistically with the software REST 2009 (relative expression software tool; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). Significance values were set at *P*-value < 0.05. P(H1): the probability of alternate hypothesis that differentiate between sample and control groups is due only to chance. In order to visualize the difference across the treatments for each gene and time point, the data were analysed by the $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001) and were subjected to ANOVA.

Statistical analysis

Percentages of root colonization, disease index, AUDPC and gene expression data (i.e. relative expression ratio) were subjected to one-way ANOVA. Tukey Honest Significant Difference (HSD) was conducted to identify significant differences ($P \leq 0.05$) between the treatments. Data analysis was performed with the SAS enterprise guide 4.1 (SAS Institute Inc., Cary, USA).

Results

Impact of *R. solani* on the AM root colonization in potato roots (1) challenged by the two microorganisms at the same time or (2) first colonized by the AM fungus.

The root colonization of potato plantlets was assessed 1, 2, 6 and 9 dap of the potato plantlets in the ERM network of the AM fungus developing in the HC in absence (AM-1 treatment) or inoculated at the same time with *R. solani* (AM+Rsol-1 treatment) (Table 16). In the AM-1 treatment, the first traces of hyphal colonization were detected 2 dap with few hyphae developing in the cortex. Six dap, the percentage of hyphae increased significantly as compared to 2 dap. The hyphal colonization calculated 9 dap increased up to 32.6 ± 3.1% and significantly differed from 2 and 6 dap. No arbuscules were observed at 1 and 2 dap. The first abuscules were observed 6 dap and significantly increased 9 dap. No vesicles were observed 1 and 2 dap and only few 6 and 9 dap. The percentage of vesicles did not differ between 6 and 9 dap. In the AM+Rsol-1 treatment, the first traces of hyphal colonization were detected 6 dap and increased significantly 9 dap. Neither arbuscules nor vesicles were observed during the time course of the experiment. The values of hyphal colonization were significantly lower in the AM+Rsol-1 treatment at 6 and 9 dap as compared to the values recorded in the AM-1 treatment (Table 16).

Table 16 Root colonization of potato plated on an actively growing extra-radical mycelium network of the AM fungus *Glomus* sp. MUCL 41833 and inoculated (AM+Rsol-1) or not (AM-1) with *R. solani* MUCL 49235, at the same time

Treatment	Structure	Root colonization (%) during the time course[a]			
		1	2	6	9
AM-1	Hyphae	0	7.1 A (± 2.3)	22.9 a/B (± 2.6)	32.6 a/C (± 3.1)
	Arbuscules	0	0	1.5 A (± 1.9)	6.1 B (± 1.6)
	Vesicles	0	0	0.5 A (± 1.0)	1.1 A (± 0.8)

	Hyphae	0	0	3.6 b/A (± 3.2)	13.3 b/B (± 3.8)
AM+Rsol-1	Arbuscules	0	0	0	0
	Vesicles	0	0	0	0

For each structure and time of observation, values (other than 0) in a column followed by different small capitals differed significantly at $P \leq 0.05$. For each structure, values in a row (other than 0) followed by different capitals differed significantly at $P \leq 0.05$. Both analyses were conducted as a one-way ANOVA and Tukey's HSD. The standard error is shown in brackets (Biological replicates = 4). For data of root colonization of 0%, no statistical analyses were done because of the impossibility to calculate the variance

[a] Days after plating (dap) of the potato plantlets in the hyphal compartment supporting the ERM network of the AM fungus

The root colonization of potato plantlets was assessed 10, 11, 12 and 16 dap of the potato plantlets in the ERM network of the AM fungus developing in the HC in absence (AM-2 treatment) or inoculated with *R. solani* nine days following plating in the ERM (AM+Rsol-2 treatment) (Table 17). In the two treatments (AM-2 and AM+Rsol-2), the percentage of hyphae in the potato roots was high at the time of *R. solani* inoculation and did not differ within the treatments during the time course of experiment (i.e. 10, 11, 12 and 16 dap). An identical observation was made for the percentage of arbuscules and vesicles colonization. For each AM root colonization parameter (i.e. hyphae, arbuscules and vesicles), no significant differences were observed between the AM-2 treatment and the AM+Rsol-2 treatment during the time course of the experiment.

Table 17 Root colonization of potato plated on an actively growing extra-radical mycelium network of the AM fungus *Glomus* sp. MUCL 41833 and inoculated (AM+Rsol-2) or not (AM-2) with *R. solani* MUCL 49235, 9 days thereafter

Treatment	Structure	Root colonization (%) during the time course[a]			
		10	11	12	16
AM-2	Hyphae	39.3 a/A (± 0.4)	38.1 a/A (± 7.1)	41.1 a/A (± 1.6)	40.7 a/A (± 7.1)
	Arbuscules	11.5 a/A (± 2.9)	12.5 a/A (± 5.5)	19.7 a/A (± 1.3)	12.0 a/A (± 1.4)
	Vesicles	1.2 a/A (± 1.2)	1.9 a/AB (± 0.8)	4.1 a/AB (± 2.7)	6.6 a/B (± 4.1)
AM+Rsol-2	Hyphae	44.3 a/A (± 1.2)	41.4 a/A (± 9.9)	38.5 a/A (± 4.4)	46.4 a/A (± 9.9)
	Arbuscules	14.8 a/A (± 7.8)	14.8 a/A (± 7.1)	8.8 a/A (± 3.2)	10.8 a/A (± 5.2)
	Vesicles	5.7 a/A (± 4.1)	5.4 a/A (± 3.5)	4.2 a/A (± 3.6)	8.5 a/A (± 4.1)

For each structure and time of observation, values in a column followed by different small capitals differed significantly at $P \leq 0.05$. For each structure, values in a row followed by different capitals differed significantly at $P \leq 0.05$. Both analyses were conducted as a one-way ANOVA and Tukey's HSD. The standard error is shown in brackets (Biological replicates = 4)

[a] Days after plating (dap) of the potato plantlets in the hyphal compartment supporting the ERM network of the AM fungus

Dynamics of disease development in potato roots (1) challenged by the two microorganisms at the same time or (2) first colonized by the AM fungus prior to contact with *R. solani*.

The disease severity recorded on the roots of the potato plantlets was rated visually 1, 2, 6 and 9 dap of the potato plantlets in the HC in absence of ERM network of the AM fungus and inoculated with *R. solani* (Rsol-1 treatment) or in the presence of the ERM network of the AM fungus and inoculated at the same time with *R. solani*

(AM+Rsol-1 treatment) (Table 18). In the Rsol-1 treatment, the first lesions on the roots were observed 2 dap in the HC. Six and 9 dap in the HC, the DI increased significantly (stat. not shown) with values of 57.5 ± 6.4% and 62.2 ± 9.2%, respectively. In the AM+Rsol-1 treatment, the first lesions were also observed 2 dap in the HC. The DI increased significantly (stat. not shown) at 6 and 9 dap in the HC containing the ERM (i.e. 40.7 ± 8.7% and 46.9 ± 8.1 %, respectively) but never exceeded 50%. At 2, 6 and 9 dap, the DI was significantly lower in the AM-Rsol-1 treatment as compared to the Rsol-1 treatment. In addition, the AUDPC value for the AM+Rsol-1 treatment was significantly reduced by 29.9% as compared to the Rsol-1 treatment (Table 18).

Table 18 Disease index (DI) and area under disease progress curves (AUDPC) values of potato roots plated (AM+Rsol-1) or not (Rsol-1) in the extra-radical mycelium of the AM fungus *Glomus* sp. MUCL 41833 and inoculated at the same time with *R. solani* MUCL 49235

Treatment	DI (%)[a] during the time course[b]				AUDPC[c]
	1	2	6	9	
Rsol-1	0	9.0 a (± 3.1)	57.5 a (± 6.4)	62.2 a (± 9.2)	317.0 a (± 18.1)
AM+Rsol-1	0	3.7 b (± 2.7)	40.7 b (± 8.7)	46.9 b (± 8.1)	222.1 b (± 41.1)

Values of DI (other than 0) and AUDPC in the same column, followed by a different letter differed significantly at $P \leq 0.05$ (one-way ANOVA and Tukey's HSD). The standard error is shown in brackets (n = 4). For data of DI of 0%, no statistical analyses were done because of the impossibility to calculate the variance

[a] Disease index was calculated according to the equation of Liu and Sinclair (1991)

[b] Days after plating (dap) of the potato plantlets in the hyphal compartment supporting the ERM network of the AM fungus

[c] Area under disease progress curves (AUDPC) was calculated according to the equation of Campbell and Madden (1990)

The disease severity recorded on the roots of the potato plantlets was rated visually 10, 11, 12 and 16 dap of the potato plantlets in the HC in absence of the ERM network of the AM fungus and inoculated with *R. solani* (Rsol-2 treatment) or in the presence of the ERM network of the AM fungus and inoculated nine days thereafter with *R. Solani* (AM+Rsol-2 treatment) (Table 19). In the Rsol-2 treatment, the first lesions on the roots were observed 11 dap. Twelve and 16 dap in the HC, the DI increased significantly (stat. not shown) to 26.6 ± 9.5%, and 64.3 ± 1.4%, respectively. In the AM+Rsol-2 treatment, the first lesions were also observed 11 dap in the HC. The value of DI increased significantly (stat. not shown) thereafter 12 dap (16 ± 7.4%) and 16 dap (55.2 ± 1.5%). At 11 dap, no significant differences were observed in the DI between the two treatments, while at 12 and 16 dap, this value was significantly lower in the AM+Rsol-2 treatment as compared to the Rsol-2 treatment. In addition, the AUDPC value for the AM+Rsol-2 treatment was reduced by 26.9% as compared to the Rsol-2 treatment (Table 19).

Table 19 Disease index (DI) and area under disease progress curves (AUDPC) values of potato roots plated (AM+Rsol-2) or not (Rsol-2) in the extra-radical mycelium of the AM fungus *Glomus* sp. MUCL 41833 and inoculated 9 days thereafter with *R. solani* MUCL 49235

Treatment	DI (%)[a] during the time course[b]				AUDPC[c]
	10	11	12	16	
Rsol-2	0	12.1 a (± 2.4)	26.6 a (± 9.5)	64.3 a (± 1.4)	207.4 a (± 25.3)
AM+Rsol-2	0	9.6 a (± 2.7)	16.0 b (± 7.4)	55.2 b (± 1.5)	151.5 b (± 9.4)

Values of disease index (other than 0) and AUDPC in the same column, followed by a different letter differed significantly at $P \leq 0.05$ (one-way ANOVA and Tukey's HSD). The standard error is shown in brackets (n = 4). For data of DI of 0%, no statistical analyses were done because of the impossibility to calculate the variance

[a] Disease index was calculated according to the equation of Liu and Sinclair (1991)

[b] Days after plating (dap) of the potato plantlets in the hyphal compartment supporting the ERM network of the AM fungus

[c] Area under disease progress curves (AUDPC) was calculated according to the equation of Campbell and Madden (1990)

Plant defence genes expression in potato roots challenged by the AM fungus and *R. solani* at the same time.

The relative expression ratio of defence response genes (i.e. *GST1*, *Lox*, *MAPK*, *PAL*, *PR1* and *PR2*) and the *PT3* gene in potato roots was assessed 1, 2, 6 and 9 dap of the potato plantlets in the ERM network of the AM fungus developing in the HC in absence (AM-1 treatment) or inoculated at the same time with *R. solani* (AM+Rsol-1 treatment) or in absence of the ERM network of the AM fungus and inoculated with *R. solani* (Rsol-1 treatment) (Fig. 14). In the AM-1 treatment, we observed that the expression profile of defence genes was characterized by an induction of *PR1* and *PR2* genes 1 dap, of *MAPK* and *PAL* genes 2 dap, and by the induction of the majority of defence genes as well as the *PT3* gene 9 dap (Fig. 14). In the Rsol-1 treatment, no induction of the marker gene of AM root colonization (*PT3*) was observed during the time course of the experiment. Moreover, we observed the induction of the *GST1*, *MAPK*, *PAL*, *PR1* and *PR2* genes in the potato roots at different time of the experiment, with a maximum 2 dap in the HC without ERM. Finally, we observed a down-regulation of the *Lox* gene 6 and 9 dap (Fig. 14).

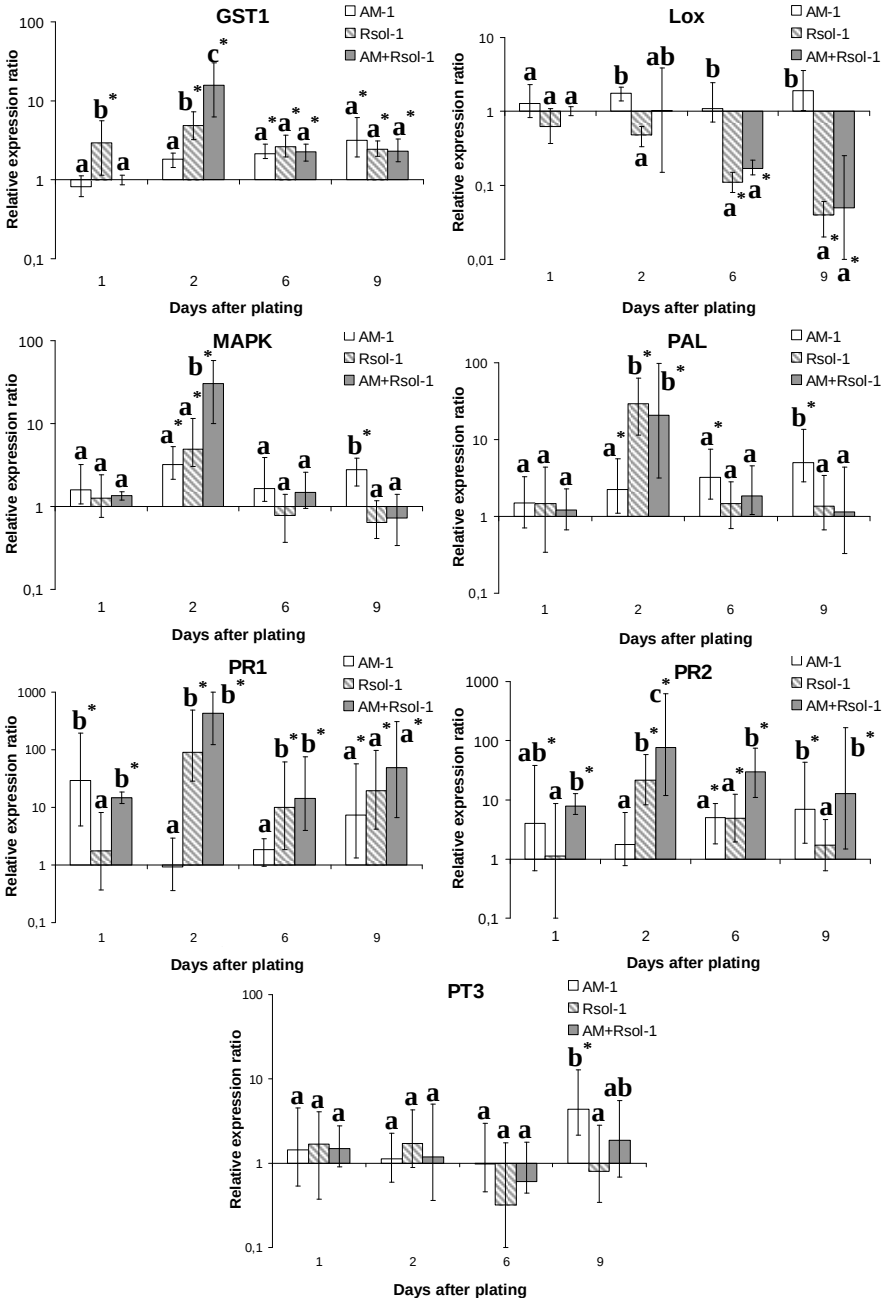

Fig. 14 Relative expression ratio of seven genes (Gluthatione-S-transferase 1 (*GST1*), Lipoxygenase (*Lox*), MAP kinase (*MAPK*), Phenylalanine ammonia lyase (*PAL*), Pathogenesis Related 1 (*PR1*), Pathogenesis Related 2 (*PR2*) and Phosphate transporter 3 (*PT3*)) in potato roots plated in the ERM network of the AM fungus *Glomus* sp. MUCL 41833 developing in the HC in absence (AM-1) or inoculated at the same time with *R. solani* MUCL 49235 (AM+Rsol-1) or in absence of the ERM

network of the AM fungus and inoculated with *R. solani* (Rsol-1). The monitoring of the relative gene expression ratio was done by Real-Time Quantitative PCR at 1, 2, 6 and 9 days after plating the potato plantlets in the hyphal compartment supporting the extra-radical mycelium network of the AM fungus. The data points represent the relative expression ratio of three biological replicates and the standard error range (i.e. refer to 68% confidence interval). Up-regulated genes or down-regulated genes (*****) with significance values were set at P value < 0.05 (P(H1): the probability of alternate hypothesis that difference between sample and control groups is due only to chance) (Software REST 2009). For each time and gene, values of relative expression ratio followed by a different letter differed significantly at $P \leq 0.05$ (Tukey's HSD)

In the AM+Rsol-1 treatment, no induction of the marker gene of AM root colonization (*PT3*) was observed during the time course of the experiment. To the contrary, different induction of defence response genes were observed. For the *Lox* gene, a down-regulation was detected 6 and 9 dap (0.17 (0.14 – 0.22) and 0.05 (0.01 – 0.25), respectively). These values of down-expression were identical to the values of down-expression obtained in the Rsol-1 treatment at 6 and 9 dap. The induction of *GST1* gene was detected 2, 6 and 9 dap in the ERM. Moreover, this gene was observed significantly over-expressed in the AM+Rsol-1 treatment (15.85 (6.27 – 30.07)) as compared with the AM-1 (1.81 (1.44 – 2.20)) and Rsol-1 (4.84 (3.24 – 7.25)) treatments, 2 dap in the ERM. In the case of the *MAPK* and *PAL* genes, we noted their inductions at 2 dap. However, only the *MAPK* gene was significantly over-expressed in the AM+Rsol-1 treatment (30.58 (10.05 – 58.25)) as compared with the AM-1 (3.21 (2.16 – 5.25)) and Rsol-1 (4.92 (3.06 – 11.47)) treatments. The induction observed in the *PAL* gene was identical to the induction observed in the Rsol-1 treatment. Finally at each time of observation, we detected the induction of *PR1* and *PR2* genes, with a maximum 2 dap. For the *PR2* gene, the values in the AM+Rsol-1 treatment at 2 and 6 dap

(75.74 (11.72 – 623.44) and 29.5 (10.83 – 75.13), respectively) were significantly over-expressed as compared with the values obtained in the AM-1 (1.77 (0.77 – 6.18) and 4.99 (1.82 – 8.78), respectively) and Rsol-1 (21.22 (8.34 – 58.54) and 4.97 (1.96 – 12.34), respectively) treatments. For the *PR1* gene at 2 dap, we showed a higher value in the AM+Rsol-1 treatment (426.79 (122.88 – 989.87) as compared with the AM-1 (0.92 (0.36 – 2.95)) and Rsol-1 (91.1 (28.72 – 485.94)) treatments. However, this value was not statistically different to the value obtained in the Rsol-1 treatment.

Plant defence genes expression in potato roots first colonized by the AM fungus prior to contact with *R. solani*.

The relative expression ratio of the defence response genes (i.e. *GST1*, *Lox*, *MAPK*, *PAL*, *PR1* and *PR2*) and *PT3* gene in potato roots was assessed 10, 11, 12 and 16 dap of the potato plantlets in the ERM network of the AM fungus developing in the HC in absence (AM-2 treatment) or inoculated after 9 days with *R. solani* (AM+Rsol-2 treatment) or in absence of the ERM network of the AM fungus and inoculated with *R. solani* (Rsol-2 treatment) (Fig. 15). For the AM-2 treatment, we observed that the expression profile of the defence genes, as well as *PT3* gene, was characterized by an important induction of each gene during the time course of the experiment. We only noted an absence of induction of *Lox* gene 10 dap (Fig. 15). In the Rsol-2 treatment, no induction of the marker gene of AM root colonization (*PT3*) was observed during the time course of the experiment. In addition, we observed the induction of the *GST1*,

MAPK, *PAL*, *PR1* and *PR2* genes in the potato roots, beginning generally 11 dap (i.e. 2 days post inoculation with *R. solani*), with a more or less constant expression 12 and 16 dpi (Fig. 15).

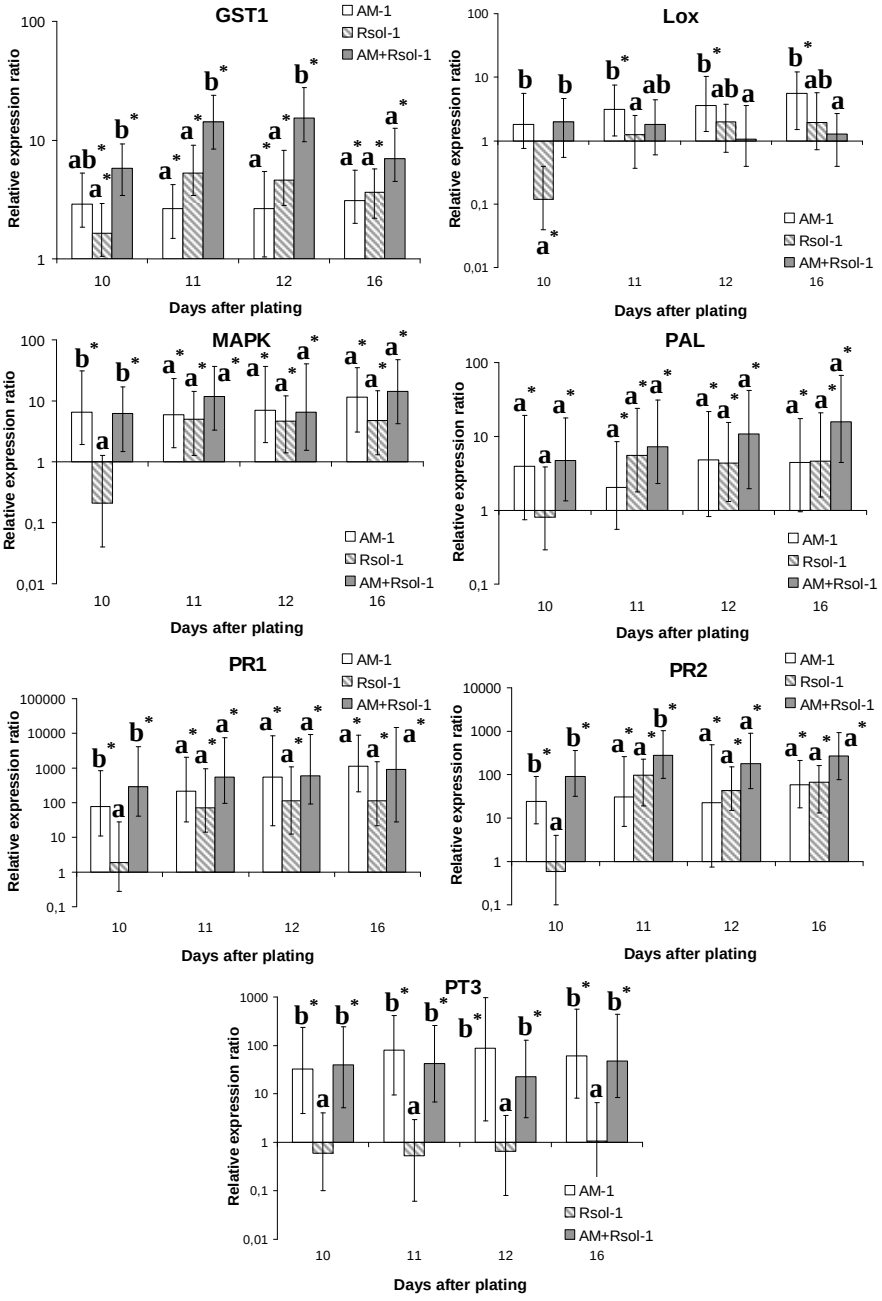

Fig. 15 Relative expression ratio of seven genes (Gluthatione-S-transferase 1

(*GST1*), Lipoxygenase (*Lox*), MAP kinase (*MAPK*), Phenylalanine ammonia lyase (*PAL*), Pathogenesis Related 1 (*PR1*), Pathogenesis Related 2 (*PR2*) and Phosphate transporter 3 (*PT3*)) in potato roots plated in the ERM network of the AM fungus *Glomus* sp. MUCL 41833 developing in the HC in absence (AM-2) or inoculated after 9 days with *R. solani* MUCL 49235 (AM+Rsol-2) or in absence of the ERM network of the AM fungus and inoculated with *R. solani* (Rsol-2). The monitoring of the relative gene expression ratio was done by Real-Time Quantitative PCR at 10, 11, 12 and 16 days after plating the potato plantlets in the hyphal compartment supporting the extra-radical mycelium network of the AM fungus. The data points represent the relative expression ratio of three biological replicates and the standard error range (i.e. refer to 68% confidence interval). Up-regulated genes or down-regulated genes (*****) with significance values were set at P value < 0.05 (P(H1): the probability of alternate hypothesis that difference between sample and control groups is due only to chance) (Software REST 2009). For each time and gene, values of relative expression followed by a different letter differed significantly at P ≤ 0.05 (Tukey's HSD)

For the AM+Rsol-2 treatment, the induction of the marker gene of AM root colonization (*PT3*) was observed at each time of observation (i.e. between 22.41 (3.22 – 261.46) 11 dap and 47.51 (8.38 – 450.50) 16 dap) and these levels of induction were statistically similar to the levels of induction obtained in the AM-2 treatment. No induction of *Lox* gene was detected during the time course of the experiment. Moreover, these relative expression ratio values were significantly lower as compared with the relative expression ratio values obtained in the AM-2 treatment. The five other defence response genes were induced at each time of observation. For the *GST1* gene, we noted the maximum of induction 11 and 12 dap. Additionally, these values (14.18 (8.38 – 23.65) and 15.22 (9.71 – 27.26), respectively) were significantly over-expressed as compared with the values obtained in the AM-2 (2.65 (1.48 – 4.23) and 2.64 (1.04 – 5.47), respectively) and Rsol-2 (5.22 (3.40 – 9.06) and 4.57 (2.79 – 8.17), respectively) treatments. In the case of the *MAPK* and *PAL* genes, no statistical difference was observed between the AM+Rsol-2 treatment and the

AM-2 or Rsol-2 treatments. For the *PR1* gene, we also did not observed significant difference between the AM+Rsol-2 treatment and the AM-2 or Rsol-2 treatments. However as in the AM+Rsol-1 treatment, we noted a higher value in the AM+Rsol-2 treatment (553.59 (95.63 – 7564.75)) as compared with the AM-2 (216.54 (27.52 – 2000.58)) and Rsol-2 (70.69 (14.29 – 958.36)) treatments, 11 dap (i.e. 2 day post inoculation with *R. solani*). Finally, we showed a statistical over-expression of the *PR2* gene in the AM+Rsol-2 treatment (276.64 (80.71 – 1019.13)) as compared with the AM-2 (30.54 (6.38 – 257.76)) and Rsol-2 (95.70 (18.98 – 227.76)) treatments, 11 dap.

Discussion

Numerous studies have reported the role of AM fungi in the control of soil-borne plant pathogens (see review by Pozo et al. 2010). Most suggested that a pre-colonization with AM fungi and a high level of AM fungal root colonization are required to induce resistance as confirmed with *R. solani* in cucumber plants (Chandanie et al. 2009). However, in a recent study, Gallou et al. (2010) demonstrated the induction of two *PR* genes in the pre-stage (i.e. before contact of AM fungi with roots) of symbiotic establishment, suggesting a potential protective effect prior to root colonization. Here we used the Mycelium Donor Plant *in vitro* culture system developed by Voets et al. (2009) to compare the effects of *Glomus* sp. MUCL 41833 on the potato resistance against *R. solani* MUCL 49235. We compared the results when both microorganisms challenged the root system at the

same time (i.e. in order to evaluate the effects as from the pre-/early stages of AM symbiosis) or when the pathogen was inoculated nine days following the contact of the roots with the ERM network of the AM fungus (i.e. in order to evaluate the effects as from the late stage of AM symbiosis). The AM fungal root colonization, disease assessment and the expression of SA-dependent (*GST1* (Hahn and Strittmatter 1994) and acidic *PR2* (Mac et al. 2004)), JA-dependent (*Lox* (Hughes et al. 2001)), JA/ET-dependent (*PAL* (Joos and Hahlbrock 1991) and basic *PR1* (Hoegen et al. 2002)), *MAPK* (Yamamizo et al. 2006) and *PT3* (Rausch et al. 2001) genes, were analyzed.

Potato plantlets grown in the ERM network of the AM fungus and challenged at the same time by *R. solani* had lower AM fungal root colonization levels as compared to plantlets grown in the ERM network in the absence of *R. solani*. Root colonization started 6 dap in the ERM, but neither arbuscules nor vesicles were observed during the 9 days of the experiment. In contrast, in the absence of *R. solani*, root colonization by the AM fungus started 2 dap in the ERM and arbuscules and vesicles were observed 6 and 9 dap in the ERM as earlier reported by Gallou et al. (2010). Interestingly, the disease induced by *R. solani*, evaluated by the AUDPC was reduced by 29.9% in presence of the AM fungus inoculated at the same time to the pathogen as compared to the potato plants grown in absence of the AM fungus. The impact of *R. solani* on the AM fungal root colonization was earlier reported by Kasiamdari et al. (2002) in mung bean inoculated with *Glomus coronatum* and binucleate *Rhizoctonia* sp. These authors observed a decrease in root colonization by *G.*

coronatum when the plants were inoculated at the same time with *R. solani*. The reduced level of lesions by *R. solani* was more unexpected since most studies concluded that a high level of AM colonization is required for resistance (Khaosaad et al. 2007; Chandanie et al. 2009).

However, in the MDP *in vitro* culture system, the plantlets were plated on a dense actively-growing hyphal network bearing thousands of spores. Such dense hyphal network was shown to favour the extensive (> 50%) AM fungus root colonization (Voets et al. 2009) within short time (< 3 days). In our experiment, the root colonization by the AM fungus never reached such value within a period of 3 days. Therefore, the reduced root colonization/infection by the AM fungus and *R. solani,* respectively, clearly suggested either a direct interaction for space and nutrients within the roots, further supporting a competition for colonization/infection sites, or an indirect interaction within the rhizosphere via the massive ERM developing in the vicinity of the roots. This ERM may have impacted the pathogen outside the root as was shown by Filion et al. (1999). Such interaction could possibly explain the reduction of lesions on the root system, even without an early intense AM colonization of the root system. Filion et al. (1999) suggested that the ERM of the AM fungus may directly interact with soil microorganisms, further leading to changes in the microbial equilibrium detrimental to pathogens. In our experiment, *R. solani* had a rapid development, covering the Petri plate in a couple of days, with a slight decrease in colony diameter in presence of an established ERM, as compared to the treatments without the AM fungus (data not shown). Therefore, it was not strictly possible to evaluate whether interaction (via for instance a reduction in the

diameter of the pathogen fungal colony) occurred with the AM fungus, without firmly excluding such hypothesis.

When the potato plantlets were grown in the ERM network nine days prior to the contact with *R. solani*, the root colonization levels were similar to the plantlets grown in the absence of *R. solani* and numerous arbuscules and vesicles were observed. Yao et al. (2002) also mentioned that *G. intraradices* colonization in potato roots was not significantly affected by the presence of *R. solani*, when the AM fungus was inoculated prior to contact with the root pathogen. To the contrary, the AUDPC value due to *R. solani* was reduced by 26.9% in presence of the AM fungus. This result corroborates numerous studies demonstrating a clear protective effect of a high level of AM colonization on the control of soil-borne fungal pathogens (Khaosaad et al. 2007; Chandanie et al. 2009). In our conditions, the reduction of lesions may be explained by several factors among which the competition between both microorganisms for colonization sites as earlier proposed by Cordier et al. (1998). The competition outside the roots may also be an important factor, as mentioned above, presumed to impact the pathogen outside the root as suggested by Filion et al. (1999), although we could not firmly ascertain this last hypothesis.

Defence genes induced in plants by AM fungi (Pozo et al. 2010) may also play a key role in the control of *R. solani*. In particular, the SA-, JA-, ET-dependent genes, which are involved in many plant-pathogen and plant-insect interactions (De Vos et al. 2005; Van Wees 2008), may be promoted in presence of the AM fungus. The real-time QRT-PCR was used to assess the expression of SA- (*GST1* and acidic *PR2*), JA- (*Lox*) and JA/ET- (*PAL* and basic *PR1*) dependent genes as

well as *MAPK* and *PT3* genes in the pre, early and late stages of symbiotic establishment in potato plants challenged by *R. solani*.

During the course of *Glomus* MUCL 41833 association to potato roots in absence of *R. solani* (i.e. from 1 dap to 9 dap in the first experiment and from 10 dap to 16 dap in the second experiment), we observed an induction of the two *PR* genes before root colonization by the AM fungus (i.e. 1 dap), of the *MAPK* and *PAL* genes during the early hyphal root colonization (i.e. 2 dap) and the induction of all the defence genes after the establishment of an effective AM association (i.e. after 9 dap), characterized by the induction of *PT3* gene (i.e. plant gene marker of functional symbiosis). This pattern of genes expression during symbiotic development of AM fungi in potato roots was earlier described by Gallou et al. (2010). In addition, in the roots inoculated by *R. solani*, we observed an induction of *GST1*, *MAPK*, *PAL*, *PR1* and *PR2* genes at 2 days post inoculation of the pathogen in both experiment. This response of plant roots following an attack by *R. solani* was also reported in previous study (Gao et al. 2006).

The potato plantlets plated in the ERM network of the AM fungus and challenged by *R. solani* at the same time showed a priming of defence genes, *GST1* and *MAPK*, 2 dap, and *PR2*, 2 and 6 dap. This mechanism of priming was also observed in the potato plantlets grown 9 days in the ERM network and subsequently challenged by *R. solani*. More specifically, we demonstrated a priming of the two SA-dependent genes (*GST1* and *PR2* genes at 11 and 12 dap and at 11 dap, respectively). Priming has been defined as a unique physiological status of plant materialized by an increased resistance to pathogens which is due to certain beneficial microbes or natural/synthetic

compounds (Conrath et al. 2006). Primed plants display faster and/or stronger activation of various cellular defence responses that are induced following attack by pathogens or insects or in response to abiotic stress (Conrath et al. 2006). The most conclusive finding was the suggestion that the AM fungus in the pre-/early stages was able to induce a priming of the *MAPK* gene and the two SA-dependent genes in the potato roots inoculated with the pathogen and especially before contact of AM fungi with the potato root (i.e. 2 dap the potato plantlets in the ERM network). This result proposed for the first time that the bio-protection of AM fungi during the pre-/early stages of symbiotic association was not only associated to the direct interaction of the ERM of the AM fungus with soil microorganisms (Filion et al. 1999), but also to a priming of defence genes in the plant roots.

In this study, we also observed the priming of the two SA-dependent genes during the late stage of symbiotic establishment in the potato roots inoculated with *R. solani*. The evidence of defence genes primed in well-colonized roots against pathogen infection, has been reported in numerous studies (See review of Pozo et al. 2010). In addition, the jasmonates, which seems to have a crucial role in the AM symbiosis (Hause et al. 2007), have been reported to play an important role in the bio-protection in the late stage of the symbiosis (Pozo et al. 2010). In our experiment, no priming of the JA- (i.e. *Lox*) and JA/ET- (*PAL* and basic *PR1*) dependent genes were observed. However, we observed the repression of the *Lox* gene in the AM+Rsol-2 treatment as compared to the AM-2 treatment and the more pronounced induction of the basic *PR1* gene in the AM+Rsol-1 and AM+Rsol-2 treatments (i.e. 2 dpi with *R. solani* without statistical significance).

These results could anyway suggest a potential role of these genes in the bio-protection triggered by *Glomus* sp. MUCL 41833. In fact, both synergistic and antagonistic crosstalk have been observed between SA- and JA-dependent defences in which the *NPR1* gene and several WRKY transcription factors play important roles in the fine tune of defence genes regulations (Bari and Jones 2009). In addition, it is also interesting to note that recents researches on abscisic acid (ABA) plant mutants have shown a role of the ABA in the establishment of a functional AM symbiosis (Herrera-Medina et al. 2007; Martin-Rodriguez et al. 2011). Moreover, ET and ABA mutually antagonistic are able to modulate some functions in the plant, such as the disease resistance (Anderson et al. 2004).Taken together, these observations suggested that the defence responses triggered by the AM fungus are more complex and need further research to decipher the function of SA-, JA-, ET- and ABA-dependent pathways in their regulations.

In conclusion, the analysis of disease demonstrated that *Glomus* sp. MUCL 41833 promoted a decrease of necrosis caused by *R. solani* MUCL 49235 in the potato roots, as from a well-established symbiosis, but also as from the pre-/early stages of symbiotic establishment and despite the negative effect of *R. solani* on the AM root colonization. Moreover, our study demonstrated the priming of the *MAPK* gene and the two SA-dependent genes during the pre-/early stages of symbiotic establishment in the potato roots infected by *R. solani*, 2 dap the potato plantlets in the ERM network of the AM fungus. This result suggested for the first time that the AM fungus is able to prime plant defence genes against a soil-borne pathogen during the pre-/early stages of symbiotic establishment. This study also

revealed the priming of the two SA-dependent genes in the potato roots infected by *R. solani* in a well-established AM fungal association. Ours findings and the recent researches on the AM fungal symbiosis underlined the necessity for further research to elucidate the regulation of the hormone-dependent defence genes involved in the AM fungal bio-protection.

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Chapter IV

Systemic resistance in mycorrhizal potato plantlets challenged by *Rhizoctonia solani*

Research paper in preparation

Adrien Gallou, Alice Braun, Sylvie Cranenbrouck and Stéphane Declerck

Preface

The study conducted in chapter III suggested that the AM fungus[5] *Rhizophagus* sp. MUCL 41833 was able to mitigate the disease caused by *R. solani* MUCL 49235 by decreasing the level of necrosis in the potato roots. However, the interaction between the AM fungus and the pathogen was evaluated with the two microorganisms grown in direct contact. As a consequence, the biological protection observed may be attributed to a local mechanism. In addition, the MDP *in vitro* culture system did not allow the inoculation of the two microorganisms at two different locations. Thus, the study of a potential systemic effect of *Rhizophagus* sp. MUCL 41833 to control *R. solani* in the potato roots was not possible with this system.

In chapter IV, we developed a new *in vitro* culture system allowing the fast/homogenous AM fungal colonization of the half of a potato plant roots system to study the systemic biological protection mechanism. It should be note that this research was only the first approach in this thesis to study the systemic effect of the AM fungus under *in vitro* condition. Further experiments and adaptation of the system are required to conclude on the potential of the AM fungus to induce a systemic biological protection. For this work, we evaluated the development of the AM fungus in this culture system and also attempted to measure the disease index in presence of the two microorganisms. Unfortunately, we did not observed visible lesions in presence of the root pathogen. In consequence, we decided to focus our gene expression analysis on the three defence genes which have

[5] In this chapter, took into consideration the AM fungal classification of Schüβler et al. (2001)

displayed the most interesting results in the previous study (Chapter III). We chose the two SA-dependent genes: *GST1* and *PR2*, which are significantly primed during the interaction between the AM fungus and the pathogen, and also the JA-dependent gene: *Lox*, which is significantly repressed during the late stage of AM fungal colonization in presence of the pathogen.

Abstract

The protective effects conferred to plants by AM fungi against soil-borne pathogens have been reported in different studies and attributed to local or systemic mechanisms. If local protection conferred by AM fungi against *R. solani* has been demonstrated, no data reported yet the systemic resistance against this major potato root pathogen. Here, we developed a Mycelium Donor Plant (MDP) *in vitro* split-root culture system to study the existence of a systemic bio-protection effect of *Glomus* sp. MUCL 41833 colonization of potato roots against *R. solani*. Potato plants were grown *in vitro* with half of their root system in contact with the AM fungus and/or *R. solani*. Root colonization and disease severity as well as the expression of SA-dependent (*GST1* and *PR2*) and JA-dependent (*Lox*) genes were assessed. We demonstrated the adequacy of the MDP *in vitro* split-root culture system to produce a dense extra-radical mycelium (ERM) network of AM fungi with high level of root colonization. Ten and 11 days after plating the potato plantlet in the ERM network of AM fungus, an important colonization level was obtained. To the contrary, no lesions were observed with *R. solani*, probably due to the short duration of the experiment. Finally, gene expression analysis suggested the existence of mechanisms of priming and counterbalancing during the systemic interaction between the AM fungus and *R. solani* in the potato plants.

Keywords: AM fungus, *in vitro* split root system, Mycelium network, *Rhizoctonia solani*, *Solanum tuberosum*, Systemic resistance

Introduction

Potato (*Solanum tuberosum*) is the fourth largest food crop cultivated in the world (FAO, 2008). This crop is affected by a cohort of soil-borne pathogens among which *Rhizoctonia solani* is the most important (Banville et al. 1996). The disease caused by this microorganism is a problem occurring throughout the world and its control is at present mainly based on cultural practices and on the use of chemicals. In the last years, alternative control strategies via the utilization of beneficial microorganisms such as Pseudomonas, arbuscular mycorrhizal (AM) fungi and fungal antagonists (Brewer and Larkin 2005; Grosch et al. 2006; St-Arnaud and Vujanovic 2007) have received increased attention. These micoorganisms represent environmental-friendly options to chemical pesticides and promising routes for plant protection against soil-borne diseases (Harman and Bjorkman 1998).

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs belonging to the Phylum Glomeromycota (Schüβler et al. 2001). They colonize the roots of more than 80% of terrestrial plants, including most agricultural and horticultural crop species (Smith and Read 2008). The host plants and AM fungi live in a symbiotic relationship where both partners benefits from the association. The AM fungus receives carbohydrates from the plant in exchange of nutrients, principally phosphorus. In parallel to the improved nutrition, the AM symbiosis protects the plant against a broad range of soil borne microbes (St-Arnaud and Vujanovic 2007) via several mechanisms as reviewed by Vierheilig et al. (2008).

Recently, Pozo and Azcon-Aguilar (2007) disentangled the mechanism of mycorrhiza induced resistance (MIR). These authors explained that the increased resistance of mycorrhizal plants resulted from a combination of local and systemic mechanisms involving salicylic acid (SA) and jasmonate acid (JA) dependent signalling pathways. Although the local protective effects conferred to plants by AM fungi against *R. solani* was evidenced by Kasiamdari et al. (2002), Yao et al. (2002) and Gallou et al. (**Chapter III**), no study yet reported the systemic protection conferred by AM fungi against this root pathogens.

Systemic resistance was reported against *Phytophthora parasitica* (Pozo et al. 2002) and *Gaeumannomyces graminis* (Khaosaad et al. 2007) on tomato and barley grown in split root systems (i.e. divided parts of a root system, allowing physical separation between AM fungi and pathogens). In tomato, Pozo et al. (2002) demonstrated that the root colonization by *Glomus mosseae* reduced the disease symptoms caused by *P. parasitica* in non-colonized root of mycorrhizal plants via an increased lytic activity in these roots, while in barley, Khaosaad et al. (2007) noted that *G. mosseae* root colonization reduced systemically the infection by the root pathogen *G. graminis*. They further suggested that this systemic protection was dependent of the degree of root colonization and was not mediated by the salicylic acid pathway.

Recently, Voets et al. (2009) developed an *in vitro* mycorrhization system allowing for the fast and homogenous colonization of seedlings via actively growing extra-radical hyphae extending form a colonized donor plant. This Mycelium Donor Plant (MDP) *in vitro*

culture system was developed with *M. truncatula* and used to colonize economically-important plants such as potato (Gallou et al. 2010), opening large perspectives to study various aspects of the AM fungi and AM symbiosis. This MDP *in vitro* culture system was recently validated to study gene expression during the AM fungus establishment (Gallou et al. 2010) and to investigate the effects of the AM fungus on the potato resistance against *R. solani* (Gallou et al. **Chapter III**). These authors have demonstrated that root colonization by *Glomus* sp. MUCL 41833 induced a local protection against *R. solani*, further opening the door to the application of *in vitro* split root systems to decipher the systemic resistance conferred by AM fungi against this root pathogen.

In the present study, we developed a MDP *in vitro* split root culture system adapted from the MDP *in vitro* culture system of Voets et al. (2009), to study the systemic resistance in potato plants colonized by *Glomus* sp. MUCL 41833 against the root pathogen *R. solani*. We analyzed the disease development of *R. solani* and used the real-time QRT-PCR technology to assess the expression of three defence response genes dependent on salicylic acid (SA) (i.e. Gluthatione-S-transferase 1 (*GST1*) and Pathogenesis Related 2 (*PR2*)) and on jasmonic acid (JA) (i.e. Lipoxygenase (*Lox*)). These genes are among the most frequently described in this tripartite interaction (Pozo et al. 2002; Pozo and Azcon-Aguilar 2007; Gallou et al. 2010).

Materials and methods

Biological material

Propagation and maintenance of potato plantlets stock

In vitro produced potato plantlets (*Solanum tuberosum* L., var. Bintje) were supplied by the *Station de Haute Belgique* in Libramont, Belgium. Plantlets were micro-propagated every five weeks as described in Voets et al. (2005).

Seed disinfection of *Medicago truncatula*

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A 17 (SARDI, Australia) were surface-disinfected by immersion in sodium hypochlorite (8% active chloride) for 12 min, rinsed three times in de-ionized sterilized (121°C for 15 min) water and germinated in groups of 25 on Petri plates (90 mm diam.) filled with 35 ml MSR medium (Declerck et al. 1998) without sucrose and vitamins, and solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA) (termed MSR[1]). The Petri plates were incubated at 27°C in the dark.

Culture, propagation and maintenance of *Glomus* sp. MUCL 41833 and *R. solani* MUCL 49235

A root organ culture (ROC) of *Glomus* sp. MUCL 41833 was supplied by the Glomeromycota *in vitro* collection (GINCO – http://www.mycorrhiza.be/ginco-bel). Spores were extracted from the culture medium by solubilization of the gelling agent (Doner and

Bécard 1991). An approximate of 100 spores was then selected and placed in the near vicinity of actively growing transformed carrot (*Daucus carota* L.) roots (approximately 70 mm length) on Petri plates (90 mm diam.) containing the MSR medium, solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA), following the method described in Cranenbrouck et al. (2005). The Petri plates were incubated for 3 months in the dark in an inverted position at 27 °C. Several thousand spores were produced during this period.

A culture of *R. solani* Kuhn MUCL 49235 was supplied by the Mycothèque de l'Université catholique de Louvain (MUCL – http://bccm.belspo.be/about/mucl.php). A plug of gel containing several bulbils and mycelium was placed on 50 ml Potato Dextrose Agar (PDA) (Scharlau Chemie S.A, Barcelona, Spain) in Petri plates (90 mm diam.). The Petri plates were incubated at 25 °C in the dark for seven days.

Experimental design

A Mycelium Donor Plant (MDP) *in vitro* split-root culture system was adapted from the MDP *in vitro* culture system developed by Voets et al. (2009). Briefly, tri-compartmented Petri plates (90 mm diam.) were used to physically separate a root compartment (RC) containing *M. truncatula* plants inoculated with *Glomus* sp. MUCL 41833 from a hyphal compartment (HC) exclusively containing the extra-radical mycelium of the AM fungus crossing the plastic barrier separating the RC from the HC, and a mycelium-free compartment (MFC) devoid of AM fungal mycelium (Fig. 16A). A small opening (± 2 mm diam.) was made in the base and the lid of the Petri plates at

the side of the RC using the tip of a heated forceps. The RC and HC of the Petri plates were filled with 20 ml of MSR[1] medium solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA).

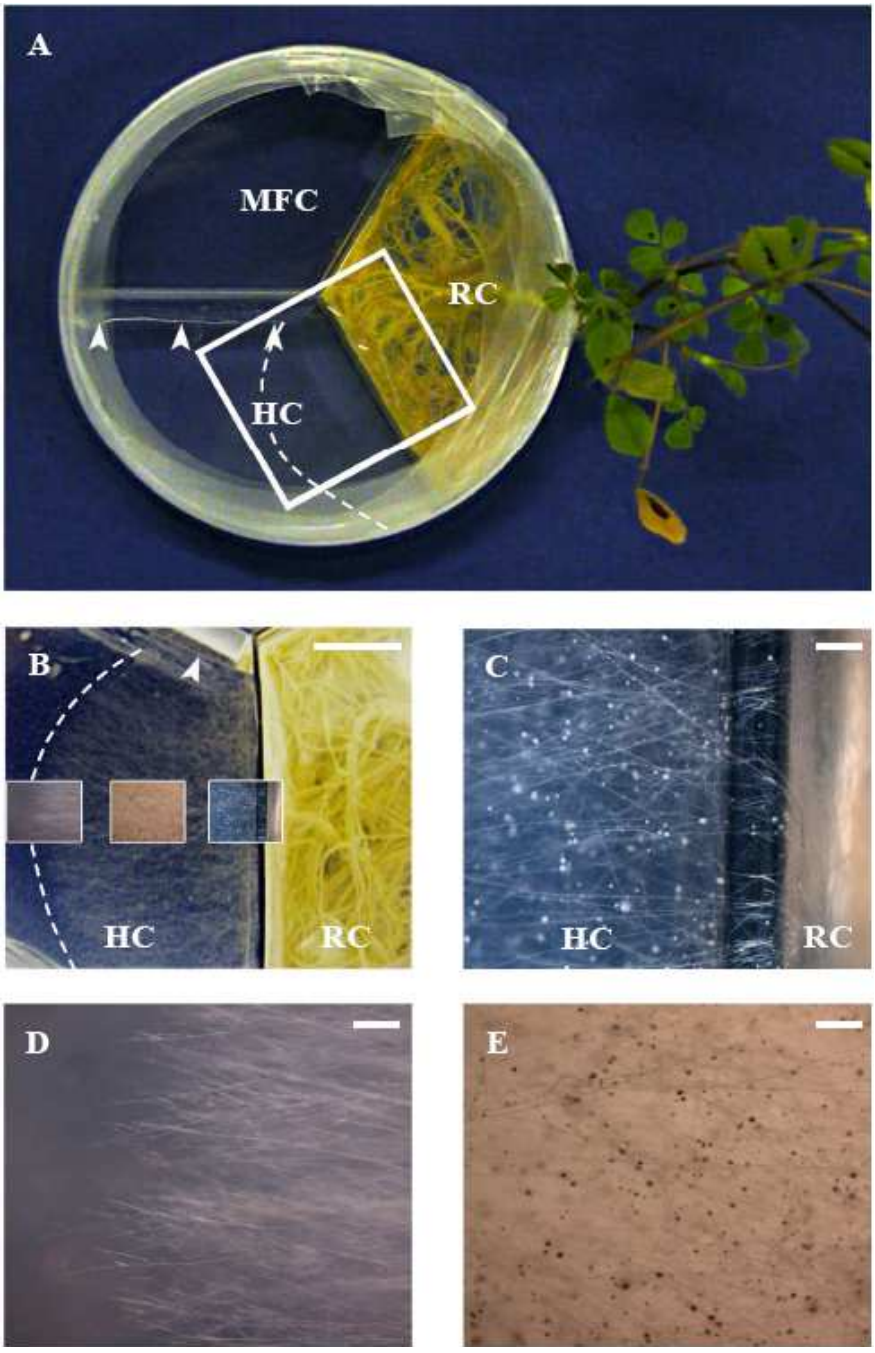


Fig. 16 (A) General view of the Mycelium Donor Plant (MDP) *in vitro* split-root culture system 8 weeks following the association of a *Medicago truncatula* plant with the AM fungus *Glomus* sp. 41833. The system consisted of a root compartment (RC) containing the roots of *M. truncatula* associated with *Glomus* sp. MUCL 41833, a hyphal compartment (HC) containing the extra-radical mycelium of the AM fungus and a mycelium-free compartment (MFC) devoid of AM fungal mycelium. Note the strip of MSR medium (5 mm width) removed from the HC alongside the plastic barrier separating the HC from the MFC (white arrows) and dense extra-radical mycelium front (white dotted curve) developing in the HC and extending from the RC. (B) scaling-up on the mycelium front (white dotted curve), scale bar = 1 cm. The three squares are scaled up in Fig 1C, 1D and 1E. (C) Numerous hyphae crossing the partition wall separating the RC from the HC and extending in the HC, scale bar = 100µm. (D) Close up of the hyphal front showing numerous hyphae extending on the surface of the HC, scale bar = 100 µm. (E) Spores production in the HC, scale bare = 100 µm

Four-day-old *M. truncatula* seedlings were transferred to the RC, with the roots placed on the surface of the MSR[1] medium and the shoot extending outside the Petri plate. Approximately 100 spores of the AM fungus were inoculated in the vicinity of the roots. The Petri plates were then sealed with Parafilm (Pechiney, Plastic Packaging, Chicago, IL 60631, USA) and the openings carefully plastered with sterilized (121°C for 15 min) silicon grease (VWR International, Belgium) to avoid contaminations. Petri plates were subsequently wrapped with opaque plastic bags to keep the AM fungus and *M. truncatula* roots in the dark, while the shoots developed under light conditions. The Petri plates were transferred to a growth chamber under controlled conditions (22/18°C (day/night), 70% relative humidity, photoperiod of 16 h day^{-1} and an average photosynthetic photon flux of 225µmol m^{-2} s^{-1}). Starting from week 3, 10 ml MSR[1] cooled to 40°C in a water bath, was added weekly to the RC. This medium was added to provide the plants with nutrients and to maintain the medium at the top of the partition wall, facilitating

hyphae to cross from the RC to the HC. Roots that passed the partition wall were trimmed.

After a period of 8 weeks, the hyphal length and the number of spores produced in the HC were estimated under a dissecting microscope (Olympus SZ40, Olympus Optical GmbH, Germany) at X 10–40 magnifications following the method detailed in Voets et al. (2005). At that time, the MFC of the Petri plates were filled with 20 ml of MSR^1 medium.

A small opening (±2 mm diam.), was made in the base and the lid of the Petri plates, at the intersection (i.e. at the level of the plastic barrier separating the HC from the MFC) of the HC and MFC (Fig. 16A). To avoid the development of the AM fungus in the MFC, a strip of MSR^1 medium (5 mm width) was removed from the HC alongside the plastic barrier separating the HC from the MFC (Fig. 16A). A 10-day-old potato plantlet was placed on the plastic barrier and half of its root system plated on the HC and the other half on the MFC. The Petri plates were then sealed carefully and incubated in a growth chamber set at 20/16°C (day/night), 70 % relative humidity with a photoperiod of 16 h d^{-1} and an average photosynthetic photon flux (PPF) of 300 μmol m^{-2} s^{-1}. A control treatment was included, consisting of a non-mycorrhizal *M. truncatula* seedling plated in the RC and a 10-day-old potato plantlet placed on the plastic barrier with half of the root system plated on the HC and the other half on the MFC. The Petri plates were placed under the same conditions as described above.

The inoculation with *R. solani* strain was performed using a 3 mm diam. plug from a seven day-old culture of *R. solani* containing abundant mycelium and sclerotia. The plug was placed in the close

vicinity of the potato roots in the HC or MFC. The inoculation of *R. solani* was performed nine days after insertion of the potato plantlets on the plastic barrier separating the HC from the MFC (Fig. 17).

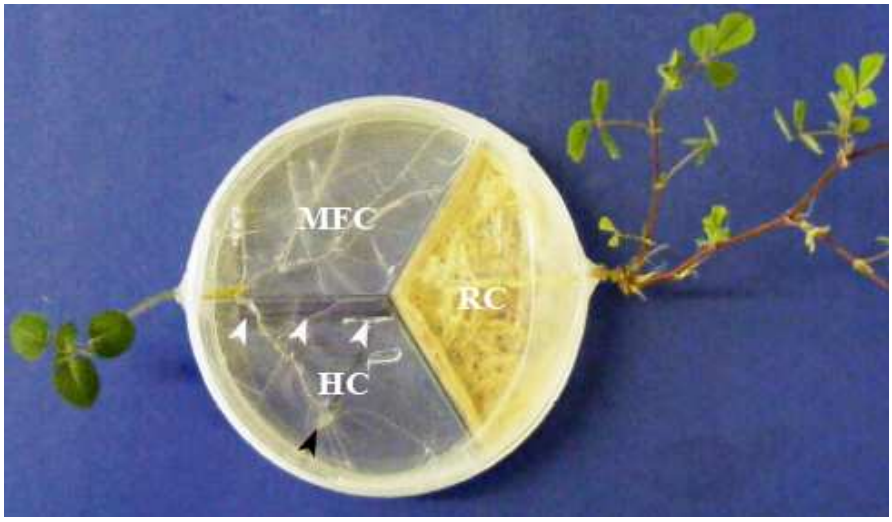


Fig. 17 Mycelium Donor Plant (MDP) *in vitro* split-root culture system, nine days after placing a 10-day-old potato plantlet on the plastic barrier with half of its root system plated on the HC and the other half on the MFC and inoculated with a plug of *R. solani* (black arrow) in the HC. Root compartment (RC) containing *M. truncatula* roots associated with the AM fungus *Glomus* sp. 41833. Hyphal compartment (HC) containing the extra-radical mycelium of the AM fungus extending from the RC and half of the root system of potato plantlet. Note the plug of *R. solani* (black arrow). To avoid the extension of the extra-radical mycelium from the HC to the MFC, a strip of MSR medium (5 mm width) was removed from the HC alongside the plastic barrier separating the HC from the MFC (white arrows). Mycelium free compartment (MFC) devoid of AM fungal mycelium and containing the other half of the potato root system

Five treatments were considered: (1) a control treatment with potato roots plated in the HC and MFC without the AM fungus and *R. solani* (termed Control); (2) a treatment with potato roots plated in the HC and MFC with *Glomus* sp. MUCL 41833 inoculated in the RC and developing exclusively in the HC and without *R. solani* (termed AM^{HC}); (3) a treatment with potato roots plated in the HC and MFC without the presence of the *Glomus* sp. MUCL 41833 in the HC and

inoculated with *R. solani* in the HC (termed $Rsol^{HC}$); (4) a treatment with potato plantlets plated in the HC and MFC with the presence of the *Glomus* sp. MUCL 41833 inoculated in the RC and developing exclusively in the HC and inoculated with *R. solani* in the HC (termed $(AM+Rsol)^{HC}$); (5) a treatment with potato plantlets plated in the HC and MFC with the presence of the *Glomus* sp. MUCL 41833 inoculated in the RC and developing in the HC and inoculated with *R. solani* in the MFC (termed $AM^{HC}+Rsol^{MFC}$).

For each analysis, the HC and MFC of the five treatments were considered separately. The root systems in the HC and MFC of the five treatments were harvested 1 and 2 days post inoculation (dpi) of the potato plantlets with *R. solani* (i.e. 10 and 11 days, respectively after plating of the potato plants in the HC and MFC). Six replicates were considered for each treatment. Three replicates were randomly selected to assess the root colonization and disease severity, and the three other were used for gene expression analysis.

Root colonization and disease severity assessment

Root systems in the HC of the AM^{HC}, $(AM+Rsol)^{HC}$ and $AM^{HC}+Rsol^{MFC}$ treatments were used to estimate the AM fungal root colonization. The root systems were cleared in 10% KOH at 50 °C for 90 min., rinsed with de-ionized water and stained with Trypan blue 1% (Phillips and Hayman 1970) at 50°C for 60 min. The root systems were subsequently mounted on microscopic slides and observed under a dissecting microscope (Olympus SZ40, Olympus Optical GmbH,

Hamburg, Germany) at X 10-40 magnification. The percentage of root colonization was estimated according to McGonigle et al. (1990).

Root systems in the HC of the $Rsol^{HC}$ and $(AM+Rsol)^{HC}$ treatments and in the MFC of the $AM^{HC}+Rsol^{MFC}$ treatment were harvested and the disease severity rated using a 0–4 scale in which 0 = no lesions, 1 = small lesions, between 1-3 mm length, 2 = more extensive lesions, up to 5 mm length, 3 = lesions longer than 5 mm and with necrotic areas, 4 = lesions over 50% of the root area with necrosis (Liu and Sinclair, 1991). A Disease Index (DI) was calculated using the following equation: $\% DI = \sum (n \times Sn) \times 100 / (4N)$, where n = number of plants with a given disease rating, Sn = disease rating and N = total number of plants rated (Liu and Sinclair, 1991).

RNA extraction and Reverse Transcription

Total RNA was extracted from 50 mg frozen root material with Trizol® reagent (Invitrogen, Carlsbad, USA) with an additional chloroform purification step and then purified using the Purelink™ Micro-to-midi total RNA purification system (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The total RNA was subsequently treated with the TURBO DNA-free™ kit (Ambion, Austin, USA) according to the manufacturer's instructions. Concentration and purity of total RNA were determined in a NanoDrop®-ND 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA), using a 2 µl aliquot of the total RNA solutions. RNA purity was estimated from the A260/A280 absorbance ratio. Reverse transcription of 250 ng of total RNA was

performed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Montreal, Canada) in a volume of 20 µl with oligo $(dT)_{18}$ primer at 55°C for 20 min, according to the manufacturer's instructions. For each RNA sample, a reaction without RT was performed as a control for contamination by genomic DNA.

Real-Time quantitative PCR

Real-Time PCR analysis was performed using the LightCycler 2.0, the 5X LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Mix and gene-specific primers (For LightCycler 2.0 experimental run and primer design, see Gallou et al. 2010). Normalisation was achieved using the geometric means of three potato reference genes (i.e. *βeta-tubulin*, *Elongation Factor-1-alpha* and *Glyceraldehyde 3-phosphate dehydrogenase*), which showed the most stable genes expression in the potato roots during symbiotic establishment of *Glomus* sp. MUCL 41833 determined by the geNorm software (http://medgen.ugent.be/~jvdesomp/geNorm) (See Gallou et al. 2010). In order to visualize the up-regulated or down-regulated genes, the data were analysed statistically with software REST 2009 (relative expression software tool; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). Significance values were set at P-value < 0.05. P(H1): the probability of alternate hypothesis that differentiate between sample and control groups is due only to chance. In order to visualize the difference across the treatments for each gene and time point, the data were analysed by the $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001) and were subjected to ANOVA.

Statistical analysis

Root colonization and gene expression data (i.e. relative expression ratio) were subjected to one-way ANOVA. Tukey Honest Significant Difference (HSD) was conducted to identify significant differences ($P \leq 0.05$) between the groups. Data analysis was performed with the SAS enterprise guide 4.1 (SAS Institute Inc., Cary, USA).

Results

Extra-radical mycelium development of *Glomus* sp. MUCL 41833 in the HC of the MDP *in vitro* split root culture system

The AM fungus associated to *M. truncatula* in the RC started to cross the plastic barrier separating the RC from the HC after six weeks (Fig. 16B and 16C) and developed profusely on the surface of the HC within seven weeks (Fig. 16B and 16D). Eight weeks after association, the hyphal length produced in the HC was 5818 ± 2387 cm and the number of spores 4695 ± 2625 (Fig. 16E). At that time, the Petri plates were randomly separated in three groups, i.e. AM^{HC}, $(AM+Rsol)^{HC}$ and $AM^{HC}+Rsol^{MFC}$, each containing 12 Petri plates.

Colonization of the potato root systems plated on the HC containing the extra-radical mycelium of *Glomus* sp. MUCL 41833

The potato plantlets were grown nine days in the MDP *in vitro* split-root culture system with half of the root system developing in the HC in contact with the extra-radical mycelium (ERM) of the AM fungus. The root colonization of the potato plants was subsequently assessed in absence of *R. solani* (AM^{HC} treatment) or 1 and 2 dpi with the root pathogen ($(AM+Rsol)^{HC}$ and $AM^{HC}+Rsol^{MFC}$ treatments) (Table 20). In the HC of the AM^{HC} treatment, the percentage of root colonization was 27.5% at day 10 (1 dpi with *R. solani*) and 22.5% at day 11 (2 dpi with *R. solani*). Identically, the percentage of arbuscules was 5.9% 3.4% at day 10 and 11, respectively. No vesicles were observed at day 10, while the first traces were detected at day 11 (0.6%).

Table 20 Root colonization of potato plantlets grown for 9 days in the extra-radical mycelium of the AM fungus MUCL 41833 in absence (AM^{HC} treatment) or subsequently inoculated for 1 and 2 days with the root pathogen *R. solani* MUCL 49235 ($(AM+Rsol)^{HC}$ and $AM^{HC}+Rsol^{MFC}$ treatments)

Treatment	Structure	Root colonization (%)*	
		1	2
AM^{HC}	Hyphae	27.5 a/A (± 1.5)	22.5 a/A (± 13.0)
	Arbuscules	5.9 a/A (± 0.8)	3.4 a/A (± 3.2)
	Vesicles	0	0.6 a (± 1)
$(AM+Rsol)^{HC}$	Hyphae	25.2 a/A (± 8.5)	25.4 a/A (± 4.2)
	Arbuscules	7.1 a/A (± 4.9)	4.0 a/A (± 4.1)
	Vesicles	0.2 a/A (± 0.4)	1.2 a/A (± 2.4)

AM^{HC}+$Rsol^{MFC}$	Hyphae	22.3 a/A (± 11.2)	23.4 a/A (± 10.3)
	Arbuscules	4.1 a/A (± 3.9)	6.2 a/A (± 5.6)
	Vesicles	1.2 A (± 1.0)	1.2 a/A (± 0.9)

For each structure and time of observation, values (other than 0) in a column followed by different small capitals differed significantly at $P \leq 0.05$. For each structure, values in a row (other than 0) followed by different capitals differed significantly at $P \leq 0.05$. Both analyses were conducted as a one-way ANOVA and Tukey's HSD. The standard error is shown in brackets (Biological replicates = 3). For data of root colonization of 0%, no statistical analyses were done because of the impossibility to calculate the variance

* Days post inoculation of potato plantlets with pathogen: *R. solani*

In the HC of the $(AM+Rsol)^{HC}$ treatment, the percentage of root colonization was 25.2% at day 10 (1 dpi with *R. solani*) and 25.4% at day 11 (2 dpi with *R. solani*). The percentage of arbuscules was 7.1% at 1 dpi with the root pathogen and decreased to 4.0% at 2 dpi. The percentage of vesicles was 0.2% at 1 dpi and increased to 1.2% at 2 dpi.

Finally, in the HC of the AM^{HC}+$Rsol^{MFC}$ treatment, the percentage of root colonization was 22.3% at day 10 (1 dpi with *R. solani*) and increased to 23.4% at day 11 (2 dpi with *R. solani*). The percentage of arbuscules was 4.1% at 1 dpi with the root pathogen and increased to 6.2% at 2 dpi. The percentage of vesicles was 1.2% at 1 and 2 dpi.

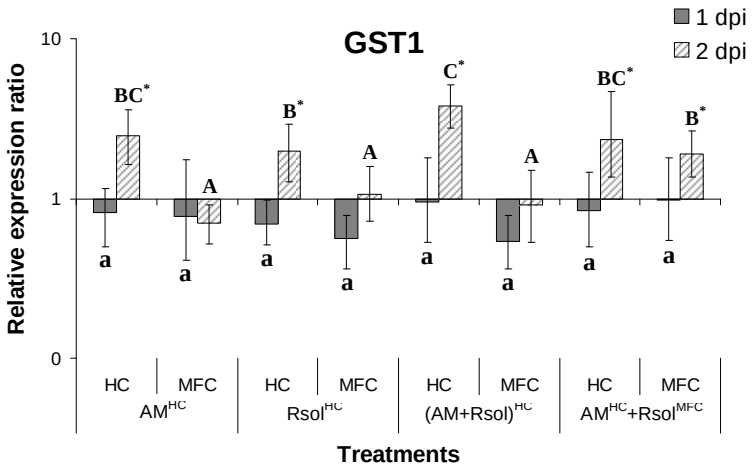
For each structure (i.e. hyphae, arbuscules and vesicles), no significant differences were observed between the treatments and time of observation.

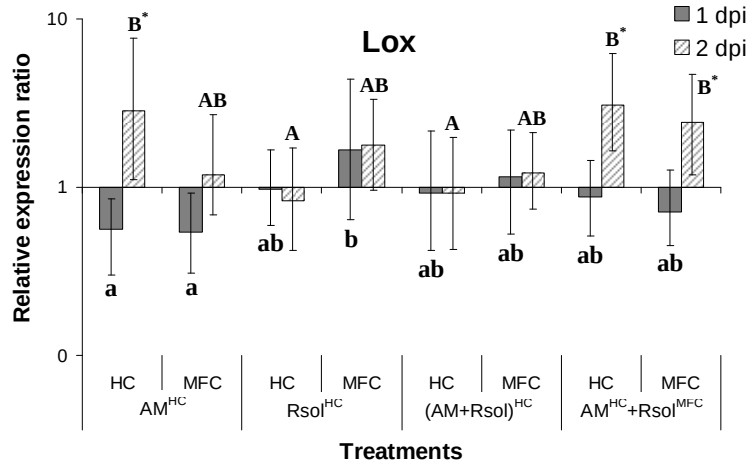
Disease index of potato plantlets grown in the presence of *R. solani*

The potato plantlets were grown nine days in the MDP *in vitro* split-root culture system with half of the root system developing in the HC and the other half in the MFC. The disease severity was subsequently rated visually 1 and 2 dpi of the potato plantlets with *R. solani* in the HC, in presence ($(AM+Rsol)^{HC}$ treatment) or absence ($Rsol^{HC}$ treatment) of the ERM network of the AM fungus, and in the MFC in presence of the ERM network of the AM fungus in the HC ($AM^{HC}+Rsol^{MFC}$ treatment). The disease index was calculated using the equation of Liu and Sinclair (1991) (Data not show). Whatever the treatment, no visible lesions were observed at 1 and 2 dpi of the root pathogen. In addition, the radial growth of *R. solani* was measured in the three treatments (i.e. data not shown). No significant difference was noted between the treatments. At 1 dpi with *R. solani*, the radial growth was 1.1 ± 0.2 cm and doubled (i.e. 2.3 ± 0.3 cm) at 2 dpi with this pathogen.

Expression of defence response genes in potato plantlets in presence/ absence of the AM fungi and *R. solani*

The relative expression ratio of the defence response genes *GST1*, *Lox* and *PR2* was assessed on the root system of the potato plantlets in presence of the ERM of the AM fungus and absence of *R. solani* (in the HC and MFC of the AM^{HC}) or 1 and 2 dpi of the root pathogen in presence (in the HC and MFC of the $AM^{HC}+Rsol^{MFC}$ and $(AM+Rsol)^{HC}$ treatments) or absence (in the HC and MFC of the $Rsol^{HC}$) of the ERM network of the AM fungus (Fig. 18).


GST1
1 dpi
2 dpi
Relative expression ratio
10
1
0
BC*
A
B*
A
C*
A
BC*
B*
a
a
a
a
a
a
a
a
HC
MFC
AMHC
RsolHC
(AM+Rsol)HC
AMHC+RsolMFC
Treatments


Lox
1 dpi
2 dpi
Relative expression ratio
10
1
0
B*
AB
A
AB
A
AB
B*
B*
a
a
ab
b
ab
ab
ab
ab
HC
MFC
AMHC
RsolHC
(AM+Rsol)HC
AMHC+RsolMFC
Treatments

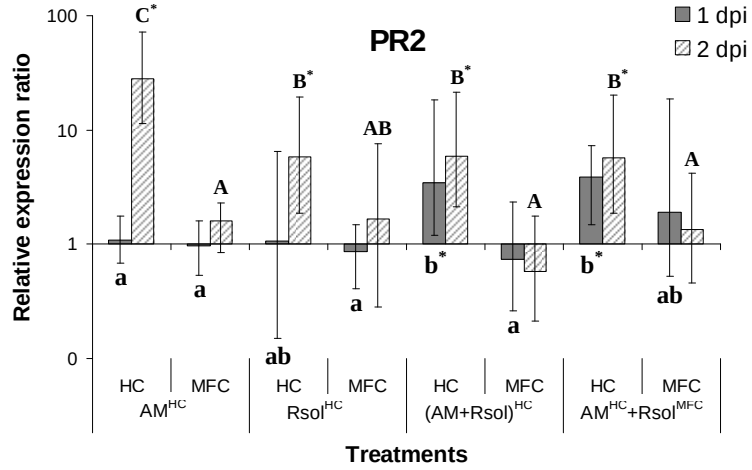

PR2
1 dpi
2 dpi
Relative expression ratio
100
10
1
0
C*
A
B*
AB
B*
B*
A
B*
a
a
ab
a
b*
b*
a
ab
HC
MFC
AMHC
RsolHC
(AM+Rsol)HC
AMHC+RsolMFC
Treatments

Fig. 18 Relative expression ratio of three defence genes (Gluthatione-S-transferase 1 (GST1), Lipoxygenase (Lox) and Pathogenesis Related 2 (PR2)) assessed on the root system of potato plantlets plated in the HC and MFC with the extra-radical mycelium of the AM fungus *Glomus* sp. MUCL 41833 developing exclusively in the HC and in absence (AM^{HC}) or inoculated in the HC ($(AM+Rsol)^{HC}$) or MFC ($AM^{HC}+Rsol^{MFC}$) with *R. solani* MUCL 49235, and in absence of the extra-radical mycelium of the AM fungus developing in the HC and inoculated with *R. solani* in the HC ($Rsol^{HC}$). The monitoring of the relative gene expression ratio was done by Real-Time Quantitative PCR at 1 and 2 days post inoculation (dpi) of the potato plantlets with the root pathogen: *R. solani*. Data were normalized by the geometric mean of the three reference genes (i.e. *EF1-α*, *GAPDH* and *β-tub*). The data points represent the relative expression ratio of three biological replicates and the standard error range (i.e. refer to 68% confidence interval). ***** Up-regulated or down-regulated gene with significance values were set at P value < 0.05 (P(H1): the probability of alternate hypothesis that difference between sample and control groups is due only to chance) (REST 2009). For each gene, values of relative expression ratio, calculated at 1 and 2 dpi with *R. solani* followed by a different small/capital letter differed significantly at $P \leq 0.05$ (Tukey's HSD)

Whatever the treatments considered, no induction or statistical differences was observed in the relative expression ratio of *GST1* gene at day 10 (i.e. 1 dpi with *R. solani*). To the contrary, at day 11 (i.e. 2 dpi with *R. solani*), we showed an induction of *GST1* gene in the compartments containing the AM fungus and/or pathogen. At that time, we noted a statistical higher induction of *GST1* gene in the HC of the $(AM+Rsol)^{HC}$ treatment as compared with the HC of the $Rsol^{HC}$ and MFC of the $AM^{HC}+Rsol^{MFC}$ treatments. In presence of the AM fungus alone (i.e. HC of the AM^{HC} and HC of the $AM^{HC}+Rsol^{MFC}$ treatments) no statistical difference was noted as compared with the presence of both microorganisms together. At day 11, no induction of *GST1* gene was observed in absence of the two microorganisms and their relative expression ratios were significantly lower as compared with the presence of both microorganisms together.

Whatever the treatments considered, no induction was observed in the relative expression ratio of the *Lox* gene, at day 10. Conversely, at day 11, we observed an induction of *Lox* gene in the HC of the AM^{HC}

and HC of the AM^{HC}+$Rsol^{MFC}$ treatments. Interestingly, this gene was not induced in the HC containing both microorganisms (HC of the $(AM+Rsol)^{HC}$ treatment) and this relative expression ratio was significantly lower as compared with the relative expression ratio of the HC of the AM^{HC} and HC of the AM^{HC}+$Rsol^{MFC}$ treatments. For the HC of the $Rsol^{HC}$ treatment and the MFC of the AM^{HC} treatment, no induction of *Lox* gene was observed, whatever the time of observation. Interestingly we noted the induction of *Lox* gene in the HC in presence of *R. solani* while the compartment was colonized by the AM fungus (i.e. MFC of the AM^{HC}+$Rsol^{MFC}$ treatment). Moreover this relative expression ratio was significantly higher compared with the relative expression ratio of the HC of the $Rsol^{HC}$ treatment.

At day 10, we observed the induction of *PR2* gene in the HC of the $(AM+Rsol)^{HC}$ and HC of the AM^{HC}+$Rsol^{MFC}$ treatments, whereas at day 11, we clearly showed the induction of *PR2* gene in both compartments in presence of the AM fungus (i.e. HC of the AM^{HC}, HC of the $(AM+Rsol)^{HC}$ and HC of the AM^{HC}+$Rsol^{MFC}$ treatments), with a higher induction in the HC of the AM^{HC}. The induction of this gene was not observe in the MFC of the AM^{HC} treatment and MFC of the $(AM+Rsol)^{HC}$ treatment. In presence of *R. solani* (i.e. HC of the $Rsol^{HC}$ treatment), we observed the induction of the *PR2* gene at day 11. We did not observe the induction of this gene in the compartment in presence of *R. solani* even with the other compartment colonized by the AM fungus (i.e. MFC of the AM^{HC}+$Rsol^{MFC}$ treatment) at day 11. Moreover, this relative expression ratio was significantly lower compared with the relative expression ratio of the HC of the $Rsol^{HC}$ treatment.

Discussion

The role of AM fungi in the control of root pathogens, among which *R. solani* (Kasiamdari et al. 2002; Yao et al. 2002; Gallou et al. **Chapter III**), has been mentioned in different studies and several mechanisms have been proposed (see review by Pozo and Azcon-Aguilar 2007). In the last decade, Pozo et al. (2002) and Khaosaad et al. (2007) reported on the systemic resistance conferred by AM fungi to mycorrhizal tomato and barley plants attacked by *P. parasitica* and *G. graminis*, respectively. Systemic protection was evidenced by lytic activity noted in the non-mycorrhizal roots of mycorrhizal tomato plants (Pozo et al. 2002) and was dependent on the degree of root colonization (Khaosaad et al. 2007). Both studies were conducted with split root compartment systems under *in vivo* culture conditions. In the recent years, *in vitro* cultures associating an AM fungus and a root organ (Gonzalez-Guerreo et al. 2005; Waschke et al. 2006) or a whole plant (Voets el al. 2009; Gallou et al. 2010) were shown adequate to investigate gene expression and to monitor, non-destructively the development of the AM fungal colony. In the present study, the Mycelium Donor Plant (MDP) *in vitro* culture system, developed by Voets et al. (2009) for the fast and homogenous colonization of autotrophic plants was extended to an MDP *in vitro* split root culture system to investigate the systemic resistance conferred by *Glomus* sp. MUCL 41833 in potato roots challenged by *R. solani*.

Mycelium and spore production of *Glomus* sp. MUCL 41833 in the HC of the MDP *in vitro* split root culture systems was extensive. Above 5800 cm hyphae and 4700 spores were obtained within a

period of 8 weeks, supporting earlier observations by Voets et al. (2009) and Gallou et al. (2010) over the same period of time in bi-compartmented Petri plates. The ERM extending on the HC surface of the MSR medium further contacted the potato roots and, within a period of 10 and 11 days, nearly a quarter of the root system was colonized by the AM fungus with arbuscules and a few vesicles formed. This suggested that the MDP *in vitro* split root culture system was adequate for the development of the extra-radical mycelium (including spore production) and for root colonization.

Root colonization in presence of *R. solani* inoculated (for 1 and 2 days) nine days following plating of the plantlets in the ERM of the AM fungus, was similar to the control (i.e. without the root pathogen). These results corroborated the observations of Gallou et al. (**Chapter III**). These authors demonstrated that *R. solani*, inoculated nine days after plating potato plantlets in the ERM network of *Glomus* sp. MUCL 41833 in the MDP *in vitro* culture system, had not effect on the level of AM fungal root colonization. Identically, in their study conducted *in vivo*, Pozo et al. (2002), showed that root colonization of tomato was not affected by the presence of *P. parasitica*. However, it is to be noted that no lesions were observed in presence as well as in absence of the AM fungus. Therefore, it is doubtful that any impact of the pathogen upon the symbiont would have been observed within this period of time. The absence of root lesions was in disagreement with the observations of Gallou et al. (**Chapter III**). These authors observed the first lesions of *R. solani* in the potato roots 2 dpi with the pathogen (i.e. disease index value of 12.1 ± 2.4 %). Within this period, the root pathogen covered the surface of the entire compartment (HC

or MFC) and the experiment had to be stopped to avoid development in the adjacent compartments. However, some systems were maintained 3 dpi with the pathogen (data not shown) and the first lesions were recorded on the potato roots with values close to those reported by Gallou et al. (2010) after two days. This suggested that the root pathogen had started the process of root infection probably at day 2 without visible lesions on the root surface. Even though the absence of lesions, a number of studies have comforted the role of early root colonization by AM fungi on the control of root pathogens via a mechanism of competition for colonization sites (Cordier et al. 1998). Such hypothesis could not be confirmed in our experiment.

From the recent literature (Pozo et al. 2009), it has been reported that bio-protection may be evidenced by the expression of defence genes induced in plants by AM fungi. In particular, the SA- and JA-dependent genes, which are involved in many plant-pathogen and plant-insect interactions (De Vos et al. 2005), may be promoted in presence of the AM fungus. In addition, it is generally admitted that the lack of lesions caused by a root pathogen does not result in the absence of molecular defence response. For the induced systemic resistance (ISR), Van Wees et al. (1999) have demonstrated that JA-responsive genes displayed a maximum of expression in ISR-expressing plants one day after inoculation by the pathogen. This result was confirmed by Cartieaux et al. (2008), who demonstrated that defence genes were elicited while no disease symptoms or difference in pathogen population were observed in control and ISR-expressing plants. Recently, Gallou et al. (**Chapter III**) have suggested the protective effect conferred by the AM fungus to potato

plants challenged by *R. solani* could be associated with a priming of SA-dependent genes. The highest values of priming of SA-dependent genes were observed 2 dpi with *R. solani*. Therefore, the real-time QRT-PCR technology was considered adequate to assess the expression of SA (*GST1* and *PR2*) and JA-dependent (*Lox*) genes in the AM fungal colonized potato plants, 1 and 2 days after inoculation with the root pathogen even in absence of lesions.

In absence of *R. solani*, the root system of potato plants, grown 10 and 11 days in contact with the ERM of the AM fungus (AM^{HC} treatment), showed an induction of *GST1*, *Lox* and *PR2* genes as earlier reported by Gallou et al. (2010) and Gallou et al. (**Chapter III**). To the contrary, in the mycorrhizal-free roots (i.e. in the MFC of the AM^{HC} treatment), no induction of these genes was observed. These observations were consistent with the results of Pozo et al. (2002). These authors demonstrated that chitinase, β-1,3-glucanase and superoxide dismutase activity in the non mycorrhizal roots of mycorrhizal plants had the same activity than measured in the roots of non-mycorrhizal plants. In presence of *R. solani* in the HC and absence of the AM fungus ($Rsol^{HC}$ treatment) an induction of the two SA-dependent genes (*GST1* and *PR2*) was noted 2 dpi of the pathogen. These observations were confirmed by Gallou et al. (2010) who demonstrated that potato defence response to *R. solani* was dependent on the signal molecules SA and ET but not JA. Identically to the AM^{HC} treatment, no induction of *GST1*, *Lox* and *PR2* genes were observed in the MFC of the $Rsol^{HC}$ treatment at 1 and 2 dpi *of R. solani*. This confirmed previous results reported by Pozo et al. (2002).

When both organisms were present (i.e. the root system of potato plantlets grown nine days in the HC in contact with the ERM of the AM fungus and inoculated in the HC with *R. solani* ($(AM+Rsol)^{HC}$ treatment), we observed a slight priming of *GST1* gene. However, this mechanism was not significant as compared with the HC of the AM^{HC} treatment. In addition in the HC of $(AM+Rsol)^{HC}$ treatment, we also showed the absence of induction of *Lox* gene 2 dpi of the root pathogen as compared to half root system colonized by the AM fungus alone. These observations showed some similarity to those reported by Gallou et al. (**Chapter III**). These authors observed the priming of SA-dependent genes and a repression of the *Lox* gene in the roots of a mycorrhizal potato plant in presence of *R. solani* and have suggested that the bio-protection triggered by the AM fungus in the potato roots against *R. solani* could be attributed to the priming of SA-dependent genes. It is plausible that such mechanism was expressed in our experiment. Conversely, in the MFC of the $(AM+Rsol)^{HC}$ treatment, no induction of *GST1*, *Lox* and *PR2* genes were observed. These results were consistent with the results obtained in the MFC of the AM^{HC} and $Rsol^{HC}$ treatments, where no induction of *GST1*, *Lox* and *PR2* genes were observed.

In presence of both organisms (the AM fungus in the HC and *R. solani* in the MFC (AM^{HC}+$Rsol^{MFC}$ treatment)), we observed the same pattern of genes induction in the HC as in the HC of AM^{HC} treatment. These observations were consistent with the fact that in the root system developing in the HC, only the AM fungus was present, and with the results obtained in the MFC of $Rsol^{HC}$ treatments, in which no effect of *R. solani* on the expression of *GST1*, *Lox* and *PR2* genes

in the non-infected half root system was observed. In the MFC of the AM^{HC}+$Rsol^{MFC}$ treatment, we observed a priming of *Lox* gene 2 dpi with *R. solani* in the non-mycorrhizal roots of the mycorrhizal potato plantlets. Priming has been defined as a unique physiological status of plant materialized by an increased resistance to pathogens which is due to certain beneficial microbes or natural/synthetic compounds. Primed plants display faster and/or stronger activation of various cellular defence responses that are induced following attack by pathogens or insects or in response to abiotic stress (Conrath et al. 2006). Moreover, some studies explained that the protective effect conferred by the AM fungus should be associated with the priming of JA-dependent defence mechanisms (Khaosaad et al. 2007; Pozo et al. 2009). In addition, we observed the counterbalancing of *PR2* gene 2 dpi with *R. solani* in the non-mycorrhizal root system of mycorrhizal potato plantlets. This mechanism of counterbalancing has already been described in plant-fungi interaction (Cartieaux et al. 2008). The authors have shown that among the 707 genes exhibiting transcription changes following pathogen attack (i.e. *Pseudomonas syringae* pv. *tomato* DC3000) in the leaf of *Arabidopsis thaliana* plants, 684 recovered a level of expression similar to that in the control plants when the plants were protected by *Bradyrhizobium* sp. strain ORS278 colonization prior to pathogen challenge. The authors explained that the mechanism of counterbalancing is almost certainly the indication of a successful protection and could operate through priming of defence genes.

In this study, we developed a new MDP *in vitro* split root culture system adapted from the MDP *in vitro* culture system of Voets et al.

(2009) to study the systemic resistance in mycorrhizal potato challenged by the root pathogen *R. solani*. This system allowed for the fast and homogenous colonization of potato plants grown in a dense ERM containing thousand of spores and for gene expression analysis. Even though no lesions were noted on the roots caused by *R. solani*, gene expression analysis showed mechanisms of priming and counterbalancing during the interaction between the AM fungus and the root pathogen. Despite these observations, further researches allowing the clear quantification of the pathogen are required to relate these mechanisms to a systemic control of *R. solani* by the AM fungus. Moreover, a large scale analysis of defence gene expression should be performed to confirm the presence of these two mechanisms during the AM fungus-*R. solani* interaction in the potato roots.

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Chapter V

Mycorrhiza-induced resistance in potato plantlets challenged by *Phytophthora infestans*

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Preface

As in chapters III, numerous studies have reported the biological protection conferred by AM fungi to plants against soil-borne pathogens (Review by Whipps 2004; Pozo et al. 2010). In both chapters, the results supported that the AM fungus[6] *Rhizophagus* sp. MUCL 41833 was able to confer resistance to potato plants against *R. solani*. In contrast, few studies were conducted on the biological protection of above-ground pathogens by AM fungi and the results were less conclusive.

In chapter III.C, we decided to use the MDP *in vitro* culture system to study the biological protection of *Rhizophagus* sp. MUCL 41833 against the late blight disease caused by the hemibiotrophic pathogen *P. infestans*. The biological protection effect of *Rhizophagus* sp. MUCL 41833 was evaluated by measuring the leaf infection index and the expression of defence genes in the leaves of the mycorrhizal potato plants inoculated with *P. infestans*.

[6] In this chapter, we took into consideration the AM fungal classification of Schüβler et al. (2001)

Abstract

Biological control of soil-borne pathogens by arbuscular mycorrhizal (AM) fungi has been repeatedly demonstrated. However, their role in the control of above-ground hemibiotrophic pathogens is less conclusive. Here, we investigated *in vitro* the impact of an AM fungus on *Phytophthora infestans* in potato plants. The leaf infection index was decreased in mycorrhizal potato plants. Real-Time Quantitative PCR revealed the induction of two pathogenesis related genes (*PR1* and *PR2*) in the leaves of mycorrhizal plants shortly after infection with *P. infestans*. These results suggested a systemic resistance in mycorrhizal plants, related to the priming of the two *PR* genes in potato.

Keywords: arbuscular mycorrhiza fungus, defence genes, systemic resistance, *in vitro* system, *Phytophthora infestans*, *Solanum tuberosum*

Introduction

Phytophthora infestans (Mont.) de Bary is a hemibiotrophic pathogen with biotrophic behaviour during early infection and necrotrophic behaviour in the later stage of colonization. This pathogen induces late blight and is considered one of the most devastating microbes that cause disease in potato crops (Erwin and Ribeiro 1996; Kamoun 2003). Annual losses in Europe (i.e. costs of control and damage) are estimated above 1 billion € (Haverkort et al. 2008). Late blight is mainly controlled by repeated pesticide applications, which is time-consuming, costly and harmful for the environment (Bradshaw et al. 1999; Song et al. 2003). The control of this disease is also conducted via integrated management practices including sanitation, utilization of certified seeds, breeding programs for resistant cultivars (Bradshaw et al. 1999; Vleeshouwers et al. 2008) and fertilization management (Ros et al. 2008). In the recent years, natural compounds such as plant extracts as well as microorganisms have been tested in the context of organic potato production (Dorn et al. 2007). Beneficial microorganisms such as *Pseudomonas* species, fungal antagonists and arbuscular mycorrhizal (AM) fungi (O'Herlihy et al. 2003; Andreote et al. 2009; Bae et al. 2010)] have been used against *Phytophthora* disease. For example under greenhouse conditions, Andreote et al. (2009) obtained a diminution of 45 % of the lesion after 5 days of infection by *P. infestans* in potato plants inoculated with *P. putida* strain P9.

AM fungi are soil microorganisms developing obligate symbiotic associations with the roots of more than 80% of higher plants

including the vast majority of agricultural crops (Smith and Read 2008). The fungal symbiont receives carbohydrates from the plant, required to complete its life cycle, in exchange for nutrients (i.e. such as phosphate) and water transferred to the plant (Smith and Read 2008). This bi-directional exchange results in increased growth of the plant and profuse development of the AM fungus. In parallel, AM fungi have been widely reported to confer bio-protection to plants, in particular against soil-borne pathogens. Numerous studies reported reduction in incidence and/or severity caused by fungi such as *Rhizoctonia solani*, *Fusarium oxysporum* or *Verticillium Wilt* and by fungal-like Oomycetes such as *Phytophthora* sp., *Pythium* sp. or *Aphanomyces euteiches* (Whipps 2004; Pozo et al. 2009).

In contrast, few studies were conducted on the above-ground control of pathogens by AM fungi and results were less conclusive (Whipps 2004). Improved tolerance was observed in mycorrhizal plants against biotrophic pathogens such as powdery mildew (*Blumeria* sp.) and rust fungi (*Oidium, Uromyces*) resulting in increased plant mass and yield in comparison to non-mycorrhizal plants (Gernns et al. 2001; Whipps 2004). The positive effect of AM fungi on plant resistance has also been reported against necrotrophic pathogens such as *Alternaria solani* in tomato (Fritz et al. 2006; De La Noval et al. 2007) and *Botrytis cinerea* in roses (Moller et al. 2009) and tomato (Jung et al. 2009). With hemibiotrophic fungi and particularly *Colletotrichum orbiculare*, the impact of AM fungi was more controversial. Lee et al. (2005) observed a decrease of the anthracnose disease on the leaves of cucumber plant colonized with *Glomus intraradices*, whereas, no significant effect on disease

development was observed in cucumber plants inoculated with *G. mosseae* (Chandanie et al. 2006). Finally, in the case of *P. infestans*, a delay in the progression of disease (i.e. during the necrotrophic phase) was observed on potato plants colonized with a commercial AM fungus inoculated in the field (O'Herlihy et al. 2003).

The modulation of plant defence by AM fungi has been proposed as one of the principal mechanism responsible for the control of above-ground pathogens (Pozo et al. 2009). In particular, the signalling pathways of jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) which play major roles in the regulation of plant defence seems to be involved (Van Wees et al. 2008). If the induction of defence mechanism has been clearly observed in AM fungal colonized areas of the roots, and also on the whole root system since long time (Benhamou et al. 1994; Cordier et al. 1998; Pozo et al. 2002), the induction of plant defence has only been recently reported in the leaves of mycorrhizal plants (Liu et al. 2007; Pozo et al. 2009). In a recent study, Jung et al. (2009) demonstrated that the induced systemic resistance conferred by *G. mosseae* against *B. cinerea* in tomato leaves was associated with a marked induction of a JA marker gene (*Pin II*) in the mycorrhizal plants. They argued that the mycorrhiza induced resistance (MIR) against *B. cinerea* could be associated to a priming of JA-dependent defences.

In the present study, the effect of the AM fungus *Glomus* sp. MUCL 41833 on the increased resistance of potato plants against the hemibiotrophic pathogen *P. infestans* MUCL 43257 was evaluated. Analyses on leaf infection index and AM fungal root colonization were coupled to the Real-Time Quantitative PCR technology to

estimate the impact of the AM fungus on the early-development of the late blight disease. In particular, the expression of defence genes dependent on the SA pathway: Gluthatione-S-transferase 1 (*GST1*) and acidic Pathogenesis Related 2 (*PR2*); dependent on the JA pathway: Lipoxygenase (*Lox*); dependent on the ET pathway: Ethylene Response Factor 3 (*ERF 3*); dependent on JA/ET pathways: Phenylalanine Ammonia Lyase (*PAL*) and basic Pathogenesis Related 1 (*PR1*) and a Mitogen-Activated Protein Kinase (*MAPK*) gene encoding for a wound-induced protein kinase which is a convergent point in the signalling pathway of defence responses in plant-pathogen interactions (Yamamizo et al. 2006) were visualised. This study was conducted under *in vitro* culture conditions using the Mycelium Donor Plant (MDP) *in vitro* culture system (Voets et al. 2009), which allowed to synchronize the development of the AM fungus in the potato roots.

Material and methods

Biological Material

A root organ culture (ROC) of *Glomus* sp. MUCL 41833 was supplied by the Glomeromycota *in vitro* collection (GINCO – http://www.mycorrhiza.be/ginco-bel). The strain was grown in association with Ri T-DNA transformed carrot (*Daucus carota* L.) roots clone DC1 on Petri plates (90 mm diam.) containing the Modified Strullu Romand (MSR) medium (Declerck et al. 1998) solidified with 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA),

following the method detailed in Cranenbrouck et al. (2005). The Petri plates were incubated in the dark in an inverted position at 27 °C during several months until thousands of spores were obtained.

A culture of *Phytophthora infestans* (Mont.) de Bary MUCL 43257 was provided by the Mycothèque de l'Université catholique de Louvain (MUCL - http://bccm.belspo.be/about/mucl.php). The strain was cultured on Petri plates (90 mm diam.) containing the V8 medium (Erwin and Ribeiro 1996) and incubated for 7 days at 20 ºC under an average photosynthetic photon flux of 200 µmol m^{-2} s^{-1}. After this period, the cultures were incubated in the dark at 20ºC. After four weeks of cultivation, 100% of the surfaces of the Petri plates were covered by the fungus and an average of 76.5 x 10^3 sporangia was enumerated in each Petri plate (estimated by random counting of several fields of 0.1mm^2 under microscope at magnification X 4.5 and multiplication by the surface of the Petri plate). Sporangia of *P. infestans* were harvested by spraying 5 ml of deionized sterile water over the surfaces of the cultures in the Petri Plates maintained in a slope at 40°. The sporangia accumulated at the bottom of the Petri plates were harvested with a micropipette and placed in a small Petri plate (45 mm diam.). The solution was then adjusted with sterile deionized water in order to obtain 250 sporangia per µl.

In vitro produced potato plantlets (*Solanum tuberosum* L., var. Bintje) were provided by the "Station de Haute Belgique" in Libramont, Belgium. The plantlets were sub-cultured on the Murashige & Skoog (MS) medium. Briefly, nodal cuttings were placed in sterile culture boxes [90 x 60 x 50 mm, (20 cuttings per box)], filled with 50 ml of 4.412 g l^{-1} MS medium, supplemented with

20 g l^{-1} sucrose, 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA) and adjusted to pH 5.9 before sterilization (121°C for 15 min). The plantlets were kept in a growth chamber at a constant temperature of 22 °C and illuminated for 16 h d^{-1} under a photosynthetic photon flux of 225 µmol m^2 s^{-1} (Voets et al. 2005).

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A 17 (SARDI, Australia) were surface-disinfected by immersion in sodium hypochlorite (8% active chloride) for 12 min and then rinsed three times in deionized sterile water. The seeds were germinated in groups of 25 per Petri plate (90 mm in diam.) on the MSR medium, without sucrose and vitamins, solidified with 3 g l^{-1} Phytagel (i.e. MSR^1). The Petri plates were then incubated at 27°C in the dark.

Experimental set-up

Mycelium Donor Plant (MDP) *in vitro* culture systems were set up with *M. truncatula* seedlings (see Voets et al. 2009 for details). Briefly, bi-compartmented Petri plates (90 mm diam.) were used to physically separate a root compartment (RC) from a hyphal compartment (HC). Four days old *M. truncatula* seedlings were transferred to the RC, with the roots placed on the surface of 20 ml MSR^1 medium and the shoot extending outside the Petri plate via a hole. Forty-eight Petri plates were set up and half of them were inoculated with an approximate of 100 spores of *Glomus* sp. MUCL 41833 placed in the close vicinity of the roots. The Petri plates were sealed with Parafilm (Pechiney, Plastic Packaging, Chicago, IL 60631) and the holes carefully plastered with sterilized (121°C for 15

min.) silicon grease (VWR International, Belgium) to avoid contaminations. The Petri plates were subsequently wrapped with opaque plastic bags to keep the AM fungus and *M. truncatula* roots in the dark, while the shoots developed under light conditions. The Petri plates were incubated in a growth chamber set at 22/18°C (day/night), 70% relative humidity, photoperiod of 16 h d^{-1}, and an average photosynthetic photon flux of 225 µmol m^{-2} s^{-1}.

After 6 weeks of incubation, the AM fungus started to cross the partition wall separating the RC from the HC and developed profusely in the HC containing 20 ml MSR[1] medium. After two additional weeks, two new holes were practiced in the HC of the Petri plates to facilitate the emplacement of two (10 days old) *S. tuberosum* plantlets. The roots of the potato plantlets were placed in close contact with the extra-radical mycelium (ERM) of the AM fungus developing on the MSR[1] medium. The Petri plates were sealed following the same procedure as above. The Petri plates were then placed in a growth chamber set at 20/16 ºC (day/night), a RH of 95 %, a photoperiod of 16 h d^{-1} and a photosynthetic photon flux of 225 µmol m^{-2} s^{-1}. Two potato plantlets were also introduced in the control Petri plates (i.e. without the AM fungus associated to *M. truncatula*). The Petri plates were grown under the same conditions as above.

After 12 days, the mycorrhizal and non-mycorrhizal treatments were randomly separated in two groups and the potato plantlets subsequently inoculated or not with *P. infestans*. The inoculated plantlets received 2 µl of the solution of *P. infestans* (i.e. 500 sporangia) placed on the central nerve of the third and fourth leaves starting from the base of each potato plantlet as recommended by

Eucablight (2009). In summary, four treatments were considered consisting of Petri plates containing each two potato plantlets (i.e. considered as one single replicate) plated on the actively growing ERM of *Glomus* sp. MUCL 41833 in the HC and inoculated (+Myc+Pi) or not (+Myc-Pi) with *P. infestans* or grown in absence of the AM fungus and inoculated (-Myc+Pi) or not (-Myc-Pi) with *P. infestans*. Twelve replicates were considered per treatment. Four Petri plates per treatment were harvested 1, 2 and 4 days post inoculation (dpi) with *P. infestans* (corresponding to 13, 14 and 16 days post development of the potato plants in the HC in contact or not with the ERM of the AM fungus) and used to assess the percentage of leaf damage and gene expression analysis. The root systems from the two potato plants of each replicate were used to estimate the root colonization.

Evaluation of root colonization by the AM fungus and leaf infection index by *P. infestans*

The root systems of the two potato plants of each replicate in the treatments +Myc-Pi and +Myc+Pi were harvested, bulked and used to evaluate the root colonization by the AM fungus. Roots were first cleared with 10% KOH at 50 ºC for 60 minutes and then stained with 1% Trypan blue at 50 °C. The roots were then rinsed three times with deionized water and immersed in 50 % Glycerol during 48 h. Roots were observed under a compound microscope (Olympus BH2, Olympus Optical, GmbH, Germany) at 20-40x magnifications, following the method of McGonigle et al. (1990). Root colonization

was assessed by evaluating the total root colonization, percentage of arbuscules (%A), vesicles (%V), and hyphae (%H) with an average number of intersections observed of at least 120.

In each replicate of the four treatments (-Myc-Pi, -Myc+Pi, +Myc-Pi and +Myc+Pi), two of the inoculated leaves (one from each potato plant) was used to assess the surface of leaf damage using Assess 2.0 software (APS 2002-2008). The leaves were placed on a white filter paper in a Petri plate and photographed using a Canon EO5 with an 18-55 mm f 3.5-5.6 objective. The pictures were uploaded to the Assess 2.0 software and the "leaf infection index" calculated as the ratio between the surface of the leaves presenting necrosis to the surface of the leaves without visible damage.

Disease development of the –Myc+Pi and +Myc+Pi treatments was also assessed by the area under disease progress curves (AUDPC). The AUDPC was calculated according to the equation (Campbell and Madden 1990): AUDPC = $\sum^{n-1}$ $((y + y_{+1})/2) \times (t_{+1} - t)$, were n = number of observations, y = the leaf infection index and t = the day post inoculation with *P. infestans* of each observation.

RNA extraction

In each replicate of the four treatments (-Myc-Pi, -Myc+Pi, +Myc-Pi and +Myc+Pi), the two other inoculated leaves (one from each potato plant) were placed in RNAse-free cartridge and immersed in liquid Nitrogen before storage at -80ºC until RNA extraction. Total extraction of RNA was conducted with the Trizol® reagent

(Invitrogen, Carlsbad, USA). An additional chloroform purification step was conducted under chemical flow bench. Then on the open bench, the "Purelink"^{TM} Micro-to-Midi total RNA purification system from Invitrogen, (Carlsbad, USA), was applied following the instructions of the manufacturer. Total RNA was thereafter treated with TURBO DNA-free^{TM} kit (Ambiont, Austin, USA) according to the manufacturer's instructions. Concentrations and purity of total RNA were determined in a NanoDrop®-ND 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA) using a 2-µl aliquot of the total RNA solutions. RNA purity was estimated from the A260/A280 absorbance ratio.

Real-Time Quantitative PCR

Following total RNA extraction, reverse transcription (RT) of 1 µg of total RNA was performed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Montreal, Canada) in a volume of 20 µl with oligo $(dT)_{18}$ primer at 55°C for 20 min, according to the manufacturer's instructions. For each RNA sample, a reaction without RT was performed as a control for contamination by genomic DNA. Real-Time PCR analysis was performed using the LightCycler 2.0, the 5X LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Mix and gene-specific primers (For LightCycler 2.0 experimental run, see Gallou et al. 2009). Four reference genes were selected (i.e. abbreviations and GenBank ID, respectively): glyceraldehyde 3-phosphate dehydrogenase (*GAPDH* - AF527779), ubiquitin conjugating enzyme-like (*Ubc* - DQ241834), elongation factor 1-alpha

(*EF1-α* - AB061263) and beta-tubulin (*β-tub* - Z33382), and seven targeted genes were studied (i.e. abbreviations and accession numbers, respectively): Ethylene Response Factor 3 (*ERF 3* - EF091875), Gluthatione-S-transferase 1 (*GST1* - J03679), Lipoxygenase (*Lox* - Y18548), MAP kinase (*MAPK* - AB206552), Phenylalanine ammonia lyase (*PAL* - X63104), basic Pathogenesis Related 1 (*PR1* - AJ250136) and acidic Pathogenesis Related 2 (*PR2* - AJ009932). The primer pair of *ERF3* was designed with the LightCycler Probe Design Software 2.0 (Roche, Montreal, QC, Canada). The forward and reverse primer set, sequence length and melting temperature (in brackets) were as follows: 5′-AGGAGGAAGATCTGAAATTCCC-3′ and 5′-AACCATAACCGGACTCGAC-3’ (92bp, 81°C); for the ten other primer sets, see Gallou et al. (2010). Normalisation was achieved using the geometric means of three potato reference genes (i.e. *EF1-α*, *GAPDH* and *Ubc*), which showed the most stable genes expression in the potato leaves during the four treatments determined by the geNorm software (http://medgen.ugent.be/~jvdesomp/geNorm) (Vandesompele et al. 2002). In order to visualize the up-regulated or down-regulated genes, the data were analysed statistically with the software REST 2009 (relative expression software tool; Pfaffl et al. 2002; http://rerst.gene-quantification.info/). Significance values were set at *P*-value < 0.05. P(H1): the probability of alternate hypothesis that differentiate between sample and control groups is due only to chance. In order to visualize the difference across the treatments for each gene and time point, the data were analysed by the $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001) and were subjected to ANOVA.

Statistical analysis

Percentages of root colonization, leaf infection index, AUDPC and gene expression data (i.e. relative expression ratio) were subjected to one-way ANOVA. Tukey Honest Significant Difference (HSD) was conducted to identify significant differences ($P \leq 0.05$) between the treatments. Data analysis was performed with the SAS enterprise guide 4.1 (SAS Institute Inc., Cary, USA).

Results

Impact of *P. infestans* MUCL 43257 on the potato root colonization by *Glomus* sp. MUCL 41833

The potato plantlets were grown twelve days in the MDP *in vitro* culture system with theirs roots developing in contact with the ERM of the AM fungus. The root colonization was subsequently assessed 1, 2 and 4 dpi with *P. infestans* (+Myc+Pi) or in absence of the pathogen (+Myc-Pi) (Table 21). Whatever the presence/absence of *P. infestans*, the hyphal colonization of the potato roots was high at the time of the pathogen inoculation and did not significantly differ among treatments during the time course of experiment (i.e. at day 1, 2 and 4). An identical observation was made for the percentage of arbuscules colonization. No significant difference was noted between the treatments whatever the time of observation (Table 21). For the percentage of vesicles, we observed an increase at 4 dpi in the +Myc-Pi treatment (Table 21). This difference was significant with more

vesicles produced in the +Myc-Pi treatment in comparison to the +Myc+Pi treatment (Table 21).

Table 21 Root colonization of potato plantlets plated 12 days on an actively growing extra-radical mycelium network of the AM fungus *Glomus* sp. MUCL 41833, and subsequently inoculated with *P. infestans* MUCL 43257 (+Myc+Pi) or grown in absence of the pathogen (+Myc-Pi)

Treatment	Structure	Root colonization (%)[a]		
		1	2	4
+Myc-Pi	Hyphae	40.1 a/A (± 11.2)	37.6 a/A (± 14.5)	43.2 a/A (± 2.4)
	Arbuscules	11.9 a/A (± 2.8)	16.5 a/A (± 9.7)	12.0 a/A (± 1.8)
	Vesicles	3.6 a/A (± 2.0)	6.2 a/AB (± 3.3)	8.1 a/B (± 0.7)
+Myc+Pi	Hyphae	35.9 a/A (± 2.8)	45.1 a/A (± 9.4)	39.1 a/A (± 3.3)
	Arbuscules	14.1 a/A (± 4.6)	21.2 a/A (± 8.0)	15.5 a/A (± 2.2)
	Vesicles	2.7 a/A (± 0.9)	6.0 a/A (± 2.3)	5.1 b/A (± 1.6)

For each structure and time of observation, values in a column followed by different small capitals differed significantly at $P \leq 0.05$. For each structure, values in a row followed by different capitals differed significantly at $P \leq 0.05$. Both analyses were conducted as a one-way ANOVA and Tukey's HSD. The standard error is shown in brackets (Biological replicates = 4)

[a] Days post inoculation of the potato leaves with *P. infestans*

Impact of *Glomus* sp. MUCL 41833 on the potato leaf infection index induced by *P. infestans* MUCL 43257

The leaf infection index of the four treatments (-Myc-Pi, -Myc+Pi, +Myc-Pi and +Myc+Pi) was recorded on potato plantlets, grown twelve days in the MDP *in vitro* culture system and rated with the Assess 2.0 software at 1, 2 and 4 dpi with *P. infestans* (Table 22 – data

not shown for the -Myc-Pi, +Myc-Pi treatments). In addition, the AUDPC values for the -Myc+Pi and +Myc+Pi treatments were calculated using the equation of Campbell & Madden (1990) (Table 22). In absence of *P. infestans* (-Myc-Pi and +Myc-Pi), no traces of necrosis were observed whatever the time of observation (data not shown). In both treatments inoculated with the pathogen, the first trace of necrosis on the potato leaves were observed at 1 dpi with the pathogen. The leaf infection index was 4.3 ± 0.3 % and 3.2 ± 0.3 % for the -Myc+Pi and +Myc+Pi treatments, respectively. The leaf infection index remained mostly unchanged at 2 dpi and slightly increased at 4 dpi with values of 6.8 ± 0.2 % and 6.4 ± 0.1 % for the -Myc+Pi and +Myc+Pi treatments, respectively. Whatever the time of observation, the leaf infection index was significantly lower in presence of the AM fungus (Table 22). This was further supported by the AUDPC value which was significantly lower in the +Myc+Pi treatment as compared to the -Myc+Pi treatment (Table 22).

Table 22 Leaf infection index and area under disease progress curves (AUDPC) of potato plantlets plated 12 days in the ERM network of the AM fungus *Glomus* sp. MUCL 41833 (+Myc+Pi) or grown in absence of the AM fungus (-Myc+Pi) and subsequently inoculated with *P. infestans* MUCL 43257

Treatment	Leaf infection index (%) during the time course[a]			AUDPC
	1	2	4	
-Myc+Pi	4.3 a (± 0.3)	4.3 a (± 0.4)	6.8 a (± 0.2)	15.5 a (± 0.7)
+Myc+Pi	3.2 b (± 0.3)	2.9 b (± 0.5)	6.4 b (± 0.1)	12.3 b (± 0.7)

Values of leaf infection index and AUDPC in a column, followed by a different letter differed significantly at $P \leq 0.05$ (one-way ANOVA and Tukey's HSD). The standard error is shown in brackets (n = 4)

[a] Days post inoculation of the potato leaves with *P. infestans*

Effect of *Glomus* sp. MUCL 41833 on the plant defence genes expression in the leaves of potato plantlets inoculated with *P. infestans* MUCL 43257

The relative expression ratio of the plant defence genes *ERF3*, *GST1*, *Lox*, *MAPK*, *PAL*, *PR1* and *PR2* were assessed in the leaves of potato plantlets, grown twelve days in the MDP *in vitro* culture system, at 1, 2 and 4 dpi with *P. infestans* or in absence of the pathogen (Fig.19). The Real-Time Quantitative PCR analysis did not reveal any statistical variation in the relative expression ratio of the *MAPK* gene whatever the treatments and time of observation. For the *PAL* gene, we noted a down-regulation in the +Myc-Pi treatment at 4 dpi, whereas no statistical difference in the relative expression ratio was observed between the treatments whatever the time of observation (Fig. 19).

In the two treatments in presence of *P. infestans*, we observed an up-regulation of the *ERF3* and *GST1* genes. The *ERF3* gene was up-regulated in the -Myc+Pi and +Myc+Pi treatments at 1 dpi, while the *GST1* gene was up-regulated in the -Myc+Pi and +Myc+Pi treatments at 4 dpi (Fig. 19). In presence of the AM fungus, we observed an up-regulation of the *Lox* gene at 1 dpi. Moreover, we observed a down-regulation of the *Lox* gene at 4 dpi in the +Myc-Pi treatment, at 2 dpi in the –Myc+Pi treatment, and, at 2 and 4 dpi in the +Myc+Pi treatment (Fig.19). For the *ERF3*, *GST1* and *Lox* genes, the +Myc+Pi treatment did not show statistical difference in the relative expression ratio with the +Myc-Pi or –Myc+Pi treatments.

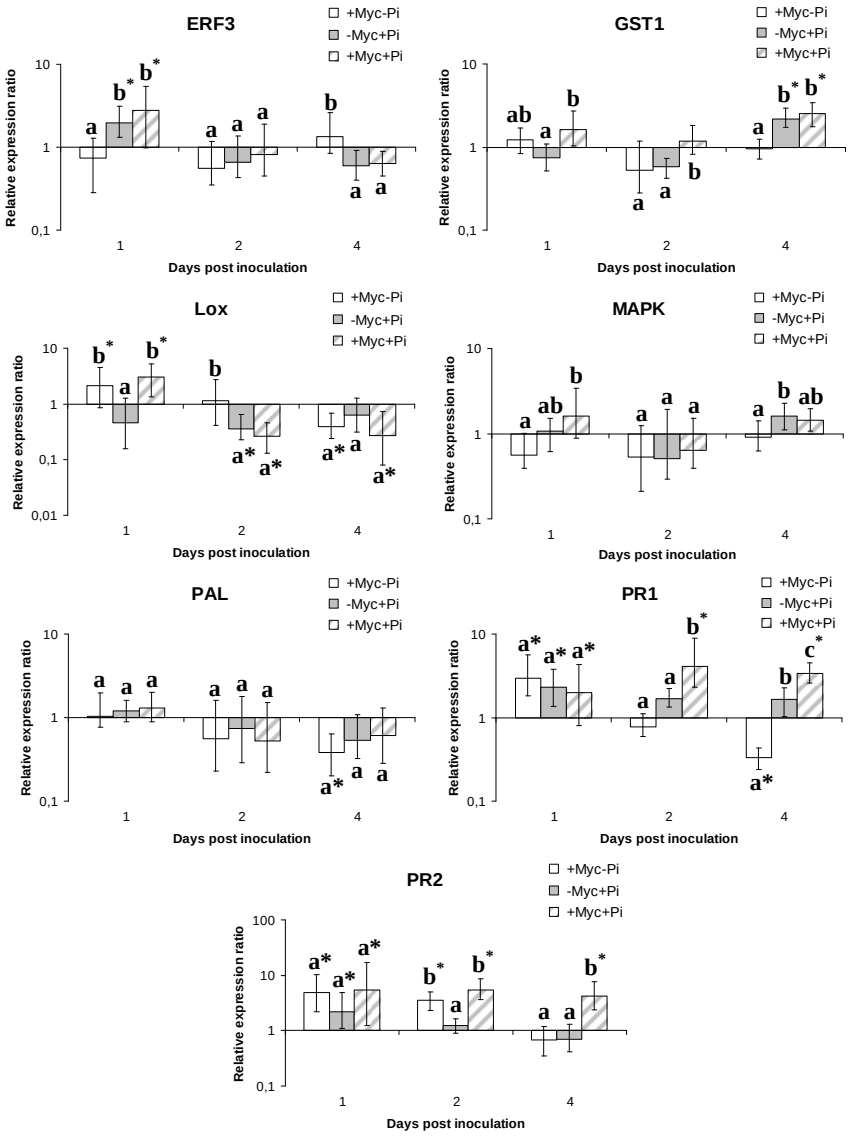

Fig. 19 Relative expression ratio of seven genes (Ethylene Response Factor 3 (*ERF3* - EF091875), Gluthatione-S-transferase 1 (*GST1* - J03679), Lipoxygenase (*Lox* - Y18548), MAP kinase (*MAPK* - AB206552), Phenylalanine ammonia lyase (*PAL* - X63104), basic Pathogenesis Related 1 (*PR1* - AJ250136) and acidic Pathogenesis Related 2 (*PR2* - AJ009932)) in the leaves of potato plantlets plated 12 days in the ERM network of the AM fungus *Glomus* sp. MUCL 41833 or grown in absence of the fungus, and subsequently inoculated or not with *P. infestans* MUCL 43257. The monitoring of the relative gene expression ratio was done by Real-Time Quantitative

PCR at 1, 2 and 4 days post inoculation of the potato leaves with *P. infestans*. Data were compared to the level of expression in the potato control leaves (-Myc-Pi) and normalized by the geometric mean of the three reference genes (i.e. *EF1-α*, *GAPDH* and *Ubc*). The data points represent the relative expression ratio of three biological replicates and the standard error range (i.e. refer to 68% confidence interval). ***** Up-regulated or down-regulated gene with significance values were set at P value < 0.05 (P(H1): the probability of alternate hypothesis that difference between sample and control groups is due only to chance) (REST 2009). For each time and gene, values of relative expression ratio level followed by a different letter differed significantly at $P \leq 0.05$ (Tukey's HSD)

For the two Pathogenesis Related genes, we noticed an up-regulation in the three treatments (Fig. 19). The *PR1* gene was up-regulated at 1 dpi in the +Myc-Pi and –Myc+Pi treatments. The *PR2* gene was up-regulated at 1 and 2 dpi in the +Myc-Pi treatment (i.e. with a maximum value at 1 dpi) and at 1 dpi in the –Myc+Pi treatment. In comparison for the +Myc+Pi treatment, we observed an up-regulation of the *PR1* and *PR2* genes at each time of observation with a maximum of induction for the two genes at 2 dpi. The relative expression ratio of these two *PRs* genes showed a significantly up-regulation in the +Myc+Pi treatment in comparison to the +Myc-Pi and –Myc+Pi treatments at 4 dpi for *PR1*, and at 2 and 4 dpi for *PR2* (Fig. 19).

Discussion

Numerous studies have reported the reduction of disease caused by soil-borne pathogens in plants colonized by AM fungi (Whipps et al. 2004; Pozo et al. 2009). In contrast, the bio-protection conferred by AM fungi against above-ground pathogens is far less documented and results so far seem less conclusive in particular for hemibiotrophs pathogens such as *P. infestans*. In this study, we paralleled leaf

infection index measures with the expression analysis of SA-, JA- and ET-dependent genes to determine the effects of *Glomus* sp MUCL 41833 on the disease expression in potato plantlets challenged by *P. infestans* MUCL 43257. The leaf infection index and AUDPC values showed a decrease of the late blight symptoms in the mycorrhizal plantlets. The Real-Time Quantitative PCR analysis revealed that this reduction of symptoms may be related to a higher induction of *PR1* (i.e. JA/ET-dependent pathway) and *PR2* genes (i.e. SA-dependent pathway) in the AM fungal potato plantlets.

With the exception of a decrease in the percentage of vesicles at day 4 post inoculation with the pathogen, the AM fungal root colonization parameters (i.e. hyphae and arbuscules) of the potato plantlets were similar in presence or absence of *P. infestans*. From an earlier experiment (Voets et al. 2009) measuring the dynamics of root colonization of potato plantlets plated in an actively growing ERM produced in the MDP *in vitro* culture system, it was shown that root colonization reached a plateau within 12 days. The AM root colonization, initiated 12 days prior to *P. infestans* inoculation, combined with the short-duration of contact between the plant and the pathogen (max. 4 days) and limited impact noted on leaf infection index may therefore explain the absence of noticeable effects of the saprotroph on root colonization parameters observed in our experiment.

In contrast, the symptoms of disease observed on the potato leaves were clearly decreased in the mycorrhizal potato plantlets. This impact was particularly marked 1 and 2 dpi by *P. infestans* with a 30 % decrease of the leaf infection index measured in the potato leaves in

presence of *Glomus* sp. MUCL 41833. Although still significant, this decrease was less important at 4 dpi (i.e. 6 %). In addition, the AUDPC values showed a reduction of the late blight disease of 20 % in the leaves of AM potato plants during the four days of experiment. These observations, obtained under a short period of infection and under highly controlled conditions, confirmed previous results of O'Herlihy et al. (2003) under field conditions with pre-mycorrhized (i.e. during three weeks) potato plantlets. These authors observed a delay in the progression of the late blight symptoms over a period of 5 weeks observation. From our results it appeared that the decrease in symptoms was initiated early in the infection process and may be maintained over a long period of time as observed by O'Herlihy et al. (2003).

The decrease in symptoms observed in our study may be related to a systemic resistance expressed in the leaves of AM potato plants during the early developmental stages of *P. infestans* as recently reported by Jung et al. (2009) on tomato plants infected with *B. cinerea*. To confirm this hypothesis, we have performed a Real-Time Quantitative PCR analysis in order to visualize the expression of plant defence genes dependent of SA, JA and ET pathway on the potato leaves.

The first interesting result of this analysis was the induction of the *Lox*, *PR1* and *PR2* genes in the leaves of mycorrhizal potato plants in absence of *P. infestans* (i.e. +Myc-Pi treatment), and particularly at 13 days post development of the plant in the HC in contact with the ERM of the AM fungus (corresponding to 1 dpi in the treatments inoculated with *P. infestans*). This result demonstrated a regulation of SA-, JA-

and JA/ET-dependent genes in presence of *Glomus* sp. MUCL 41833, and confirmed the few previous studies (Liu et al. 2007; Pozo et al. 2009) on the plants leaves transcriptional analysis during AM symbiotic association. Liu et al. (2007) were the first to report the induction of plant defence genes in the shoots of mycorrhizal *M. truncatula* plants and to correlate the induction of defence genes in the shoot with an increase in resistance against the leaf bacterial pathogen, *Xanthomonas campestris*. More recently, two studies have presented transcriptional analysis on leaves of tomato plant colonized by AM fungi. Pozo et al. (2009) confirmed the induction of defence genes in particular of JA-dependent genes, whereas Fiorilli et al. (2009) reported a down-regulation of many defence genes on the leaves of mycorrhizal tomato plants.

In our study, we also observed the induction of the *ERF3*, *GST1*, *PR1* and *PR2* genes in the potato leaves in presence of *P. infestans* (i.e. -Myc+Pi treatment). The induction of SA-, ET- and JA/ET-dependent genes during the early stages of disease development in the leaves was confirmed by previous studies (Beyer et al. 2001; Lindqvist-Kreuse et al. 2010). These authors have clearly demonstrated the implication of numerous *PR* genes, Gluthatione-S-transferase genes and Ethylene Response Factor genes in the potato leaves in response to *P. infestans* infection. However, in their studies, they also mentioned the induction of the *Lox* gene (i.e. JA-dependent). This difference could be explained by the fact that the *Lox* gene (i.e. Y18548) selected in our study was different to the *Lox* gene (i.e. U60202) induced in the study of Beyer et al. (2001).

The most conclusive finding of our Real-Time Quantitative PCR analysis was the up-regulation of the two *PR* genes (i.e. *PR1* and *PR2*) in the leaves of AM potato plantlets at 2 and 4 dpi with *P. infestans*. This result suggested a "priming" of these two *PR* genes in mycorrhizal potato plants infected by *P. infestans*. The priming was defined as a unique physiological status of plant, materialized by an increased resistance to pathogens which is due to certain beneficial microbes or natural/synthetic compounds. Primed plants display faster and/or stronger activation of various cellular defence responses that are induced following attack by pathogens or insects or in response to abiotic stress (Conrath et al. 2006). In addition, *PR1*, which encode for the basic PR1 protein (i.e. JA/ET-dependent), was well known to be link to partial resistance to *P. infestans* in *Solanum* species (Vleeshouwers et al. 2000). Whereas for *PR2* which encode for the acidic β-1,3-glucanase protein (i.e. SA-dependent), Halim et al. (2007) have demonstrated with the use of *NahG* potato plants that SA was an important compound required for basal defence of potato against the late blight disease. Taken together, these results suggested that the systemic resistance induced by *Glomus* sp. MUCL 41833 in the potato plants against *P. infestans* was presumably related to the priming of these two *PR* genes.

Interestingly, it was reported that plants activated distinct defence responses depending on the life-style of the pathogens considered (Spoel et al. 2007). The SA plant defences were primarily involved in the resistance to biotrophic pathogens, whereas the JA plant defences were mostly involved against necrotrophic pathogens and insect infestation (Beckers and Spoel 2006). In earlier experiments it was

reported that *P. infestans* may shift from biotrophic to necrotrophic stage in a short period of time (3 days) (An et al. 2010). Moreover, it has been recently shown that the AM fungus *G. mosseae* was able to induce an induced systemic resistance against the necrotrophic fungus *B. cinerea* associated with a priming of JA plant defence (Jung et al. 2009). In consequence, it seems interesting to study in more details the effects of *Glomus* sp. MUCL 41833 on the JA plant defence genes, despite that the induction of JA defence pathway seems without effect on the resistance level to the pathogen, *P. infestans* (Fauconnier et al. 2008).

In conclusion, the analysis of leaf infection index suggested that *Glomus* sp. MUCL 41833 triggered a systemic resistance in the leaves of potato plantlets, especially, during the first stage of infection with the hemibiotrophic pathogen *P. infestans*. Conversely, the pathogen had no effect on the AM fungal roots colonization within this short period of time. Moreover, we have highlighted the induction of *PR1* and *PR2* genes in the leaves of AM potato plants inoculated with the foliar pathogen, which may indicated that the AM fungal systemic resistance to this pathogen was associated to the priming of these two *PR* genes. However, different observations suggested that the systemic resistance induced by *Glomus* sp. MUCL 41833 was more effective on the first stage of late blight disease (i.e. biotrophic). Further researches are required to elucidate the effect of *Glomus* sp. MUCL 41833 on the late stage of *P. infestans* development (i.e. necrotrophic), especially by the expression analysis of a large set of JA-dependent defence genes and theirs putative effects/functions during the necrotrophic stage of late blight disease.

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Chapter VI

Trichoderma harzianum elicits defence response genes in roots of potato plantlets challenged by *Rhizoctonia solani*

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Preface

In the previous chapters, we focused our attention on the role of AM fungi as BCF against *R. solani* and *P. infestans*, two major potato pathogens. However, the most widely used BCF in agricultural systems is the fungal antagonist, *Trichoderma* spp. (Lorito et al. 2010). *Trichoderma* spp. may not produce a significant yield increase of crops, but instead, may protect plants and maintain high yields by buffering the effect of abiotic and biotic stresses affecting the crop. Moreover, *Trichoderma* spp. have been extensively studied using a variety of research approaches, including genomics, transcriptomics, proteomics, metabolomics, etc. As a consequence, the *Trichoderma* spp. appeared to be a promising alternative to reduce the pesticide application in potato crop.

In chapter VI, we evaluated the impact of *T. harzianum* against *R. solani.* Here, the effects of *T. harzianum* on the disease caused by *R. solani* in potato roots, was investigated by a vital dye coupled to an analysis of the expression stability of reference genes and the expression of several defence genes.

It should be note that this chapter was the first work performed during this thesis and that we know the limits of this research. For example, no informations were available about the development of *T. harzianum* or *R. solani* in the potato roots in order to conclude to a clear biological protection effect. Moreover, an interesting perspective to this reseach would be to evaluate the impact of *T. harzianum* on the potato resistance when *R. solani* is inoculated after the development of *T. harzianum*. During my thesis, we have attempted to perform this

experiment. Unfortunately, some problems with the development of both fungi did not allow to conduct this research.

Abstract

Biological control of *Rhizoctonia solani* with *Trichoderma harzianum* has been demonstrated in several studies. However, none have reported the dynamics of expression of defence response genes. Here we investigated the expression of these genes in potato roots challenged by *R. solani* in the presence/absence of *T. harzianum* Rifai MUCL 29707. Analysis of gene expression revealed an induction of *PR1* at 168 h post-inoculation (hpi) and *PAL* at 96 hpi in the plants inoculated with *T. harzianum* Rifai MUCL 29707, an induction of *PR1*, *PR2* and *PAL* at 48 hpi in the plants inoculated with *R. solani* and an induction of *Lox* at 24 hpi and *PR1*, *PR2*, *PAL* and *GST1* at 72 hpi in the plants inoculated with both organisms. These results suggest that in the presence of *T. harzianum* Rifai MUCL 29707, the expression of *Lox* and *GST1* genes are primed in potato plantlets infected with *R. solani* at an early stage of infection.

Keywords: biological control agent, plant defence genes, Quantitative RT-PCR, reference genes, *Rhizoctonia*, *Trichoderma*

Introduction

Rhizoctonia solani is an important soil-borne plant pathogen affecting potato (*Solanum tuberosum*) (Banville et al. 1996). *Rhizoctonia solani*-infected plants may develop crown rot, root rot, or stem canker, which often leads to wilting and to plant death in severe cases. The *Rhizoctonia* disease of potato is a problem occurring throughout the world. Control of this disease has commonly relied on cultural practices and on the use of chemicals. However, cultural practices only are not always efficient and, at the present time, no effective fungicides are available, although some chemicals are recommended. In recent years, efforts have concentrated on the biological control of this pathogen using bacteria and fungal antagonists (Howell 2003; Grosch et al. 2006). These biological control agents (BCA's) represent promising alternatives to minimise the impact of chemicals on the environment by replacing these chemicals or reducing their rate of application to control plant pathogens (Chet and Inbar 1994; Harman and Bjorkman 1998) such as *Rhizoctonia solani*.

Trichoderma spp. are amongst the most studied fungal BCAs, isolated from soil and present in the rhizosphere. These fungi are opportunistic, avirulent plant symbionts, and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases (Harman et al. 2004; Vinale et al. 2008). Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages: (i) control of pathogens and of competitive/deleterious microflora; (ii) improvement of plant health

and (iii) stimulation of root growth (Harman et al. 2004; Vinale et al. 2008). In addition, plant-cell wall-degrading enzymes produced by *Trichoderma*, such as xylanases and cellulases, are able to directly induce ethylene biosynthesis in plants, a well-known response to the presence of pathogens (Harman and Bjorkman 1998).

The beneficial effects of *Trichoderma harzianum* to control *R. solani* have been reported in different studies (Brewer and Larkin 2005; Vinale et al. 2006). Recently, Wilson et al. (2008) investigated the dynamics of *R. solani* on potato plants in the presence of *T. harzianum*. These authors noted a decrease in the severity of symptoms caused by *R. solani* during the first seven days post-inoculation and attributed this observation to a transient antagonistic effect of *T. harzianum* at the early stage of the interaction. However, the molecular basis behind this protective effect remains to be elucidated. Indeed, there is extensive evidence that *Trichoderma* spp. are able to elicit the defence systems of a number of plants (Harman and Bjorkman 1998; Hanson and Howell 2004; Vinale et al. 2008). This evidence was consolidated by studies on the effect of *Trichoderma* spp. inoculations in plant proteome/gene expression by 2d electrophoresis as well as high-density oligo microarrays (Marra et al. 2006; Alfano et al. 2007; Segarra et al. 2007). Therefore, determining how *T. harzianum* affects the expression of defence response genes in potato plantlets challenged by *R. solani* is crucial to understand the mechanism of biological protection against this major pathogen.

In the recent decade, the development of the real-time quantitative reverse transcription-polymerase chain reaction (QRT-PCR) technique

has allowed the accurate expression profiling of RNA transcripts, and has become the most useful method for characterising gene expression in human research (Bustin 2002). This technique was used in plant-microbe interactions by McMaugh and Lyon (2003) to analyse the expression of class II chitinase in Bermudagrass following infection with the root pathogen *Ophiosphaerella narmari*. In this study of plant-microbe interactions, we decided to use the QRT-PCR technique with different SA and JA-dependent genes, among the most frequently described in this tripartite interaction (Yedidia et al. 2003; Shoresh et al. 2005; Gao et al. 2006; Vinale et al. 2008).

In the present study, the time-course expression of defence response genes of potato plantlets challenged by the pathogen *R. solani* in the presence/absence of the BCA *T. harzianum* Rifai MUCL 29707 was followed during the early stage of the plant-microbe(s) interaction. The real-time QRT-PCR technology was used to assess the expression of defence response genes dependent on salicylic acid (SA): Gluthatione-S-transferase 1 (*GST1*) and acidic Pathogenesis Related 2 (*PR2*); dependent on jasmonic acid (JA): Lipoxygenase (*Lox*); and dependent on JA/ethylene (ET): Phenylalanine ammonia lyase (*PAL*), basic Pathogenesis Related 1 (*PR1*). The study was conducted under *in vitro* conditions using a micropropagated potato (*S. tuberosum*) plantlet inoculated with *R. solani* and/or *T. harzianum*.

Materials and methods

Biological material

Propagation and maintenance of potato plantlets stock

In vitro propagated potato plantlets (*S. tuberosum*, var. Bintje) were supplied by the Station de Haute Belgique in Libramont, Belgium. Plantlets were micro-propagated every five weeks as described in Voets et al. (2005). Plantlets were kept in boxes (90x60x50 mm) on the Murashige-Skoog (MS) medium, supplemented with 20 g l^{-1} sucrose, 3 g l^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA) and adjusted to pH 5.9. Boxes were incubated in a growth chamber set at 20/16 °C (day/night) with a photoperiod of 16 h day^{-1} and a photosynthetic photon flux (PPF) of 300 μmol m^{-2} s^{-1}.

Culture, propagation and maintenance of T. harzianum and R. solani

A culture of *T. harzianum* Rifai MUCL 29707 was supplied by the Mycothèque de l'Université catholique de Louvain (MUCL) (http://bccm.belspo.be/about/mucl.php). A plug of gel containing several conidia and mycelium was placed into a sterile 1.5 ml glass tube filled with 0.4 ml of 1% Sterile Distilled Water-Peptone (SDWP) (Duchefa, The Netherlands). The plug was swirled in the SDWP with a vortex mixer (8000 rpm) for 15 s and the suspension was serially diluted to 10^{-2}. From the 10^{-2} dilution, 50 μl was spread in Petri plates (90 mm diam.) on 50 ml Potato Dextrose Agar (PDA) (Scharlau Chemie S.A, Barcelona, Spain). The Petri plates were incubated at 25 °C in the dark for seven days.

A culture of *R. solani* Kuhn MUCL 49235 was supplied by MUCL. A plug of gel containing several bulbils and mycelium was

placed on 50 ml PDA in Petri plates. The Petri plates were incubated at 25 °C in the dark for seven days.

Experimental set-up

Three treatments were considered: potato plantlets inoculated with *T. harzianum* (T-treatment) or with *R. solani* (R-treatment) alone and plants inoculated with *R. solani* and *T. harzianum* at the same time (T+R-treatment). In addition, a control treatment (i.e. potato plantlets grown in the absence of both fungi) was included.

For each treatment, ten day-old potato plantlets were inserted in Petri plates containing 50 ml of the Modified Strullu Romand (MSR) medium (Declerck et al. 1998) without sucrose and vitamins, with the roots placed on the surface of the medium and the shoot extending outside through a hole made at the base and the lid of the Petri plate (for details, see Voets et al. 2005).

The inoculation with *T. harzianum* strain was performed using a 3 mm diam plug from a seven day-old culture of *T. harzianum*. The plug containing abundant mycelium and conidia was placed in the close vicinity of the potato roots. The Petri plates were then sealed with parafilm^{TM} (Pechiney, Plastic Packaging, Chicago, USA, IL 60631) and the hole filled with sterilised (121 °C for 15 min) silicon grease (VWR International, Belgium) to avoid contaminations (Voets et al. 2005).

The inoculation with *R. solani* strain followed the same procedure as above. A 3 mm diam plug from a seven day-old culture of *R. solani*

containing abundant mycelium and sclerotia was placed in the close vicinity of potato roots and the Petri plates were sealed as before.

The inoculation with *T. harzianum* and *R. solani* strains followed the same procedure as above. A 3 mm diam plug from a seven day-old culture of *T. harzianum* containing abundant mycelium and conidia was placed in the close vicinity of the potato roots. In parallel, a three-mm-diameter plug from a seven-day old culture of *R. solani* containing abundant mycelium and sclerotia was placed in the close vicinity of the *T. harzianum* plug and potato roots. The Petri plates were sealed as before.

The control treatment consisted of potato plantlets grown in the absence of both fungi.

In all treatments, the Petri plates were covered with an opaque plastic bag and incubated horizontally in a growth chamber set at 20/16 °C (day/night) with 70% relative humidity, a 16 h day^{-1} photoperiod and a PPF of 300 μmol m^{-2} s^{-1}. All the procedures described above were conducted in sterile conditions under a horizontal laminar hood. Each Petri plate was considered as an experimental unit in two experiments performed in parallel. One experiment was conducted for the analysis of real-time QRT-PCR (stability of reference genes and expression of defence response genes in potato roots challenged by *R. solani* in the presence/absence of *T. harzianum*). The other experiment was conducted by acridine orange observations to detect the presence of DNA and RNA in fresh samples of potato roots challenged by *R. solani* in the presence/absence of *T. harzianum*. Three biological replicates were used for each experiment.

RNA extraction

Three treatments (R-Treatment, T-treatment, T+R-treatment) plus a non-inoculated control were set-up as described above. Roots were harvested at 2, 4, 8, 24, 48, 96 and 168 h post-inoculation (hpi) for the R-treatment and T-treatment, and 24, 48, 72, 96 and 168 hpi for the R+T-treatment. Three samples per treatment were collected at each time. One control plantlet was also harvested at each time, i.e. 2, 4, 8, 24, 48, 72, 96, 168 hpi.

Total RNA was extracted from 50-100 mg of frozen material with Trizol® reagent Invitrogen, Carlsbad, CA, USA) with extra chloroform purification step, and then purified using the Purelink™ Micro-to-midi total RNA purification system (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's instructions. The total RNA was subsequently treated with the TURBO DNA-*free*™ kit (Ambion; Austin, USA) according to the manufacturer's instructions. Concentration and purity of total RNA were determined in a NanoDrop®-ND 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, New Zealand), using a 2 µl aliquot of the total RNA solutions. RNA purity was estimated from the A260/A280 absorbance ratio, which is an estimation of contamination mainly by proteins and phenol.

Reverse transcription

Following total RNA extraction, reverse transcription (RT) was carried out in a final volume of 20 µl, and was started by mixing 1 µg

of total RNA and 0.5 µg of oligo $(dT)_{12-18}$ (Invitrogen; Carlsbad, CA, USA) at 65°C for 15 min. After 5 min incubation at 4°C, 4 µl of 5X RT buffer (250 mM TRIS-HCL, pH 8.3, 250 mM KCl, 2.5 mM spermidine, 50 mM $MgCl_2$, 50 mM DTT), 200 mM of dNTPs, 25 units of RNase inhibitor (Invitrogen; Carlsbad, CA, USA), and 200 units of M-MLV reverse transcriptase (Promega; Madison, WI, USA) were added. RT was incubated at 37°C for 1 h and inactivated at 75°C for 15 min. For each RNA sample, a reaction without RT was performed as a control for contamination by genomic DNA.

Primer design

Four reference genes: glyceraldehyde phosphate dehydrogenase (*GAPDH*), ubiquitin conjugating enzyme-like (*Ubc*), elongation factor 1-alpha (*EF1-α*) and beta-tubulin (*β-tub*) (Nicot et al. 2005), and five defence response genes: Gluthatione-S-transferase 1 (*GST1*), Lipoxygenase (*Lox*), Phenylalanine ammonia lyase (*PAL*), basic Pathogenesis Related 1 (*PR1*) and acidic Pathogenesis Related 2 (*PR2*) were selected. Potato nucleotidic sequences were obtained from GenBank database (Table 23). Nine primer pairs were designed from these sequences (90-110 bp sequence length, optimal Tm at 60°C, GC% between 40% and 60%) with the Light Cycler Probe Design Software 2.0 (Roche, Montreal, QC, Canada) (Table 23). In the calibration step, an experimental control without cDNA was performed to test for primer-dimer formation, primers forming dimers being excluded.

Table 23 Primer sequences of four reference genes and five defence response genes, the amplification length and the melting temperature of the amplified product

Name	Accession number	Primer sequence 5'-3' (Forward)	Primer sequence 5'-3' (Reverse)	Length (bp)	Tm (°C)
β-tub	Z33382	ATGTTCAGGCGCAAGGCTT	TCTGCAACCGGGTCATTCAT	101	84
EF1-α	AB061263	ATTGGAAACGGATATGCTCCA	TCCTTACCTGAACGCCTGTCA	101	84
GAPDH	AF527779	GGACATTGTCTCCAACGC	ATGAGACCCTCCACAATGC	93	84
Ubc	DQ241834	TGATGGTTACCCATTTGAGCC	ACTGGTCCTTCAGGATGTC	110	82
GST1	J03679	TTCTAGCCACCAGATTTGACC	ACATATTCCCTATATTTTTGGAGTGAGTA	97	81
Lox	Y18548	GAGTTCTCCTCATGGTGTTCGTTTA	AGTAGTCTGACACCCAACTT	101	81
PAL	X63104	GGATATGCCATCGAACTTTGAGA	ACAAATAATGGCATGGATGAGG	110	81
PR1	AJ250136	GGTGCAGGAGAGAACCTT	GGTACCATAGTTGTAGTTTGGCT	99	85
PR2	AJ009932	TATCATCAGCAGGGTTGCAAA	TCGCGAAAAATGCTATTTCTAGG	100	81

Real-Time quantitative PCR

Real-Time PCR analysis was performed using the LightCycler 2.0 (Roche, Montreal, QC, Canada). A set of standard solutions prepared from RT products was included in each run. Reactions were prepared in capillaries using the following concentrations: 7 µl of PCR water, 4µL of 5x LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Mix, 2 µL of each forward and reverse primer (0.5 µM) and 5 µl of 1:10 diluted cDNA or standard solution as template. Capillaries were closed, centrifuged and placed into the LightCycler rotor. The following LightCycler experimental run protocol was used: denaturation programme (95°C for 10 min), amplification and quantification programme repeated 40 times (95°C for 10 s, 60°C for 20 s and 72°C for 8 s with a single fluorescence measurement), melting curve programme (60-95°C with a heating rate of 0.1°C sec^{-1} and a continuous fluorescence measurement) and finally a cooling step to 40°C. In order to check PCR efficiency, standard curves (log of cDNA dilution versus C_p) using serial 10-fold dilution of cDNA were created for each pair of selected primers. A 100% PCR efficiency corresponds to a slope of - 3.3. To allow a good comparison and normalisation, PCR efficiency should be between 80 and 115 %. In this study, all the PCRs displayed efficiencies between 86% and 114%. For the mathematical model it was necessary to determine the crossing point (Cp) for each transcript, defined as the point at which the fluorescence rises appreciably above the background fluorescence. The fit point method must be performed in the LightCycler software 4.1 at which Cp will be measured at a constant fluorescence level. The

data were analysed by the $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001) (for details see technical note Roche: No. LC 16/2002). In order to visualize the difference across the treatments for each gene and time point, the data were subjected to one-way ANOVA. Tukey Honest Significant Difference (HSD) was conducted to identify significant differences ($P \leq 0.05$) between the treatments. Data analysis was performed with the SAS enterprise guide 4.1 (SAS Institute Inc., Cary, USA).

Stability of reference genes

To evaluate the effect of T-treatment, R-treatment and T+R-treatment on gene expression in roots of potato plantlets, all samples were normalised to allow comparison by the same reference genes. Normalisation was achieved using four reference genes, i.e. *GAPDH*, *β-tub*, *EF1-α* and *Ubc*. The combination of several references genes smoothes normalisation error due to the small variation in expression of a single reference gene (Vandesompele et al. 2002). The gene expression stability (*M*) was calculated using the geNorm programme (http://medgen.ugent.be/~jvdesomp/geNorm): genes with the lowest M-Value are the most stably expressed (Vandesompele et al. 2002). Furthermore, to estimate the optimal number of internal control genes required for reliable normalisation, normalisation factors (NF*n*) were calculated by stepwise inclusion of the most stably expressed reference genes. Subsequently, pairwise variations (V*n*/*n*+1) were calculated for every series of NF*n* and NF*n*+1 to determine the effect of adding a $(n+1)^{th}$ gene. A great variation indicated that the newly added gene had a significant effect on normalisation and thus should

preferably be included for the calculation of a reliable normalisation factor (Vandesompele et al. 2002). We therefore used the relative expression values for each cDNA sample as input for the geNorm algorithm, from which an M-value was calculated for each reference gene. This M-value reflected the expression stability of the gene compared to the other reference genes; a lower M-value means more stable gene expression and is the basis for the ranking of the genes in order of their expression stability.

Acridine orange observations

Three treatments (R-Treatment, T-treatment, T+R-treatment) plus a control were set-up as described above. Presence of DNA and RNA in fresh samples of potato roots was confirmed using acridine orange (Merck, Whitehouse Station, NJ, USA) staining. Roots were harvested 48, 96 and 168 hpi for the R-treatment, T-treatment, T+R-treatment and control. Three samples per treatment and control were considered at each time. Root systems were harvested and washed in 1% acetic acid (v/v) for 30 s, rinsed with H_2O and incubated successively for 2 min in 0.1 M phosphate buffer (pH 6) and 3 min in 0.01% acridine orange in phosphate buffer (v/v). Samples were then rinsed once in 11% $CaCl_2$ (w/v) and twice in 0.1 M phosphate buffer. Roots were mounted in phosphate buffer and observed using a digital camera (model Leica DFC320; Leica Microsystems Ltd) coupled to an epifluorescence microscope (Nikon type 114) equipped with a mercury lamp (HB-10101 AF) and displayed on a 15-inch hp pavilion

ze4500 screen using the image manager software: Leica IM50, version 4.0 Leica Microsystems Imaging solutions Ltd, Cambridge, UK.

Results

Stability of reference genes in the potato plants inoculated with *R. solani* and/or *T. harzianum*.

GeNorm analysis

In order to determine the best combination of reference genes for normalisation of gene expression, geNorm analysis was used. The geNorm algorithm also determines the M-value and the pairwise variation (Vn/n + 1), which measures the effect of the addition of reference genes on the normalisation factor (equal to the geometric mean of the expression values of the selected reference genes). It is advisable to add additional reference genes to the normalisation factor until the added gene has no significant effect (low V-value). We used the cut-off value 0.15 proposed by Vandensompele et al. (2002) below which the addition of reference genes is not required. The analyses of M-value and pairwise variation with the geNorm software for the reference genes were performed for the three treatments (R-Treatment, T-treatment, T+R treatment) at 48, 96 and 168 hpi (Fig. 20).

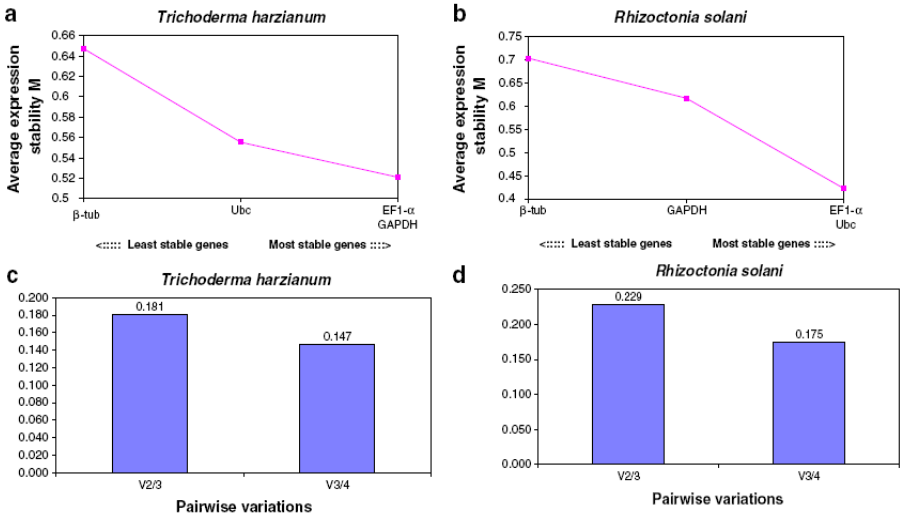

Fig. 20 Average expression stability values of reference genes and the optimal number of reference genes for normalisation by geNorm analysis: (a) and (c) Potato roots inoculated with *T. harzianum*, (b) and (d) Potato roots inoculated with *R. solani*. (a), (b), (c) and (d): relative non-unit data. $V_{(n/n+1)}$ = n reference genes were sufficient for normalisation ($V_{(n/n+1)} < 0.15$) or addition of one reference gene was necessary ($V_{(n/n+1)} > 0.15$)

The most stable genes (genes with the lowest M-value are the most stably expressed, Fig. 20) were *EF1-α* and *GAPDH* (M = 0.521) and *EF1-α* and *Ubc* (M = 0.422) for the T-treatment and R-treatment, respectively. For the T+R-treatment the two most stable genes were *EF1-α* and *GAPDH* (M = 0.164). When considering the three treatments, *EF1-α* and *GAPDH* were the most stable reference genes, followed by *Ubc*.

The V graph (Fig. 20), for the T-treatment, demonstrated that three reference genes were sufficient for normalisation (as $V_{(3/4)} < 0.15$). Similar to the T-treatment, the T+R-treatment had a pairwise variation below the cut-off, so that only three genes were necessary as the internal control. For the R-treatment, the pairwise variation $V_{(3/4)} >$

0.15 (0.175). But we observed for this treatment that the Ct values obtained for all the four reference genes decreased at 168 hpi.

Acridine orange observations

The acridine orange staining allowed the visual detection of DNA and RNA in fresh samples of potato roots. The complete root systems were analysed at 48, 96 and 168 hpi for the three treatments and the control (Fig. 21). At 48 hpi, no visual difference was detected in the level of DNA and RNA staining between the three treatments and the control treatment. No differences were again observed at 96 hpi in the level of DNA and RNA staining, between the T-treatment, the T+R-treatment and the control treatment. However at this sampling time, the potato roots in the R-treatment had a visually similar level of DNA staining, while for RNA it appeared lower. At 168 hpi, only a few nuclei (DNA) were detected, while no RNA was visualised in the R-treatment. To the contrary, no difference was observed in the level of DNA and RNA staining, between the T-treatment and the control treatment at this sampling time, while for the T+R treatment, the presence of DNA and RNA was visually greater than in the R-treatment, but lower than in the control treatment.

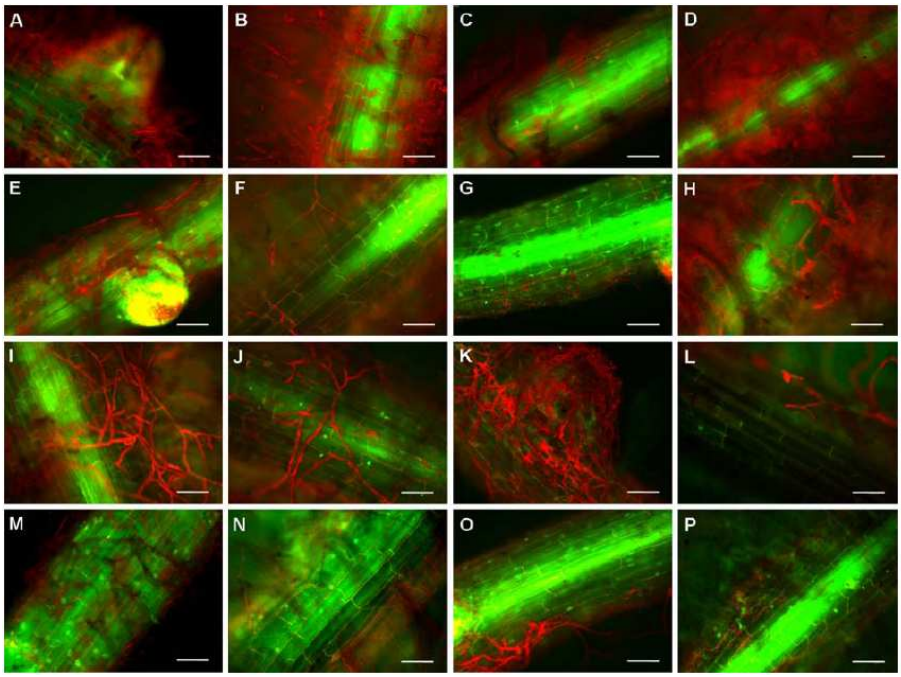

Fig. 21 Observation with the vital dye acridine orange of the potato roots in different treatments. (A), (B), (C) and (D): control. (E), (F), (G) and (H): Potato roots inoculated with *T. harzianum*. (I), (J), (K) and (L): Potato roots inoculated with *R. solani*. (M), (N), (O) and (P): Potato roots inoculated with *T. harzianum* and *R. solani*. (A), (B), (E), (F), (I), (J), (M) and (N): Time-course 96 hpi. (C), (D), (G), (H), (K), (L), (O) and (P): Time-course 168 hpi. Scale bar is 50 µm. Micrographs were taken at ×160 magnification

Following the geNorm analysis and acridine orange observations, we considered that the V-value (0.175) for the R-treatment was adequate for the normalisation with three reference genes (*EF1-α*, *GAPDH* and *Ubc*) for the analysis of defence response genes in potato roots. However, for the R-treatment and the time-course 168 hpi, we decided to discard the relative expression values obtained, because the Ct values obtained for the four reference genes decreased.

Expression of defence response genes in potato plantlets inoculated with *R. solani* or *T. harzianum*

To assess the expression of defence response genes in roots inoculated with *R. solani* or *T. harzianum*, 10 day-old potato plantlets were inoculated with both organisms separately. Roots were harvested at 2, 4, 8, 24, 48, 96 and 168 hpi and the relative expression of *GST1*, *Lox*, *PR1*, *PR2* and *PAL* genes was measured (Table 24).

Table 24 Expression level of the five defence response genes in potato roots during the inoculation with *T. harzianum* (Th) or *R. solani* (Rs), normalised to the three reference genes in the control roots (three biological replicates)

Gene Name	Treatment	Relative expression during the time-course [a]						
		2	4	8	24	48	96	168
GST1	Th	0.81 (0.22)	0.80 (0.19)	0.80 (0.21)	0.83 (0.22)	0.93 (0.27)	1.25 (0.11)	1.55 (0.23)
	Rs	0.57 (0.22)	0.76 (0.21)	0.79 (0.18)	0.74 (0.16)	1.66 (0.93)	1.26 (0.16)	7.5 (4.67) [b]
Lox	Th	0.56 (0.14)	0.74 (0.21)	0.44 (0.08)	0.65 (0.19)	0.6 (0.26)	0.76 (0.14)	0.72 (0.12)
	Rs	0.55 (0.23)	0.59 (0.18)	0.86 (0.21)	0.69 (0.17)	1.09 (0.37)	1.11 (0.11)	1.21 (0.38) [b]
PAL	Th	1.36 (0.5)	1.65 (0.38)	0.89 (0.26)	0.86 (0.17)	0.37 (0.11)	**2.42 (0.18)**	0.99 (0.39)
	Rs	1.49 (0.63)	1.31 (0.52)	1.21 (0.39)	**3.66 (1.38)**	**6.63 (3.87)**	**2.72 (1.02)**	16.09 (11.24) [b]
PR1	Th	1.15 (0.57)	0.76 (0.30)	0.26 (0.03)	1.62 (0.93)	0.83 (0.29)	**2.16 (0.33)**	**22.25 (13.45)**
	Rs	0.41 (0.45)	0.48 (0.18)	0.42 (0.33)	0.25 (0.11)	**37.65 (3.60)**	0.19 (0.17)	5.16 (7.18) [b]
PR2	Th	1.22 (0.37)	1.63 (0.61)	0.79 (0.21)	1.63 (0.74)	2.07 (0.62)	1.98 (0.45)	1.74 (0.04)
	Rs	1.49 (0.62)	1.85 (0.55)	1.01 (0.22)	1.07 (0.24)	**13.26 (12.30)**	**3.19 (0.66)**	24.85 (17.91) [b]

The standard error is shown in brackets. Statistical significance: relative expression ≥ 2

[a] Time after inoculation (h); [b] Relative expression values obtained with Ct values of reference genes not considered because they were too variable for good normalisation

For the T-treatment, no variation in relative expression was observed for the *GST1*, *Lox* and *PR2* genes whatever the time of observation. For the relative expression of *PAL* gene, we noticed a

slight up-regulation at 96 hpi with a relative value of 2.42 ± 0.18. The relative expression of *PR1* gene increased in roots of potato plantlets at 96 hpi, with the maximum expression observed at 168 hpi (22.25 ±13.45).

For the R-treatment, no variation in relative expression was observed for the *GST1* and *Lox* genes whatever the time of observation. For the relative expression of *PR1* and *PR2* genes, we noticed an up-regulation at 48 hpi with a relative value of 37.65 ±3.60 and 13.26 ±12.3, respectively. The relative expression of *PAL* gene increased in roots of potato plantlets at 24 hpi, with a maximum at 48 hpi (6.63 ± 3.87), and decreased at 96 hpi.

We observed for the time-course 168 hpi, an increase of *GST1*, *PAL*, *PR1* and *PR2* gene expression. The relative expression of these genes was probably induced by the decrease of reference gene expression.

Expression of defence response genes in potato plantlets inoculated concomitantly with *R. solani* and T. *harzianum*

To assess the expression of defence response genes in potato roots challenged by *R. solani* in the presence of *T. harzianum*, 10 day-old potato plantlets were inoculated with both organisms concomitantly. Roots were harvested at 24, 48, 72, 96 and 168 hpi and the relative expression of GST1, Lox, PR1, PR2 and PAL genes was measured (Fig. 22).

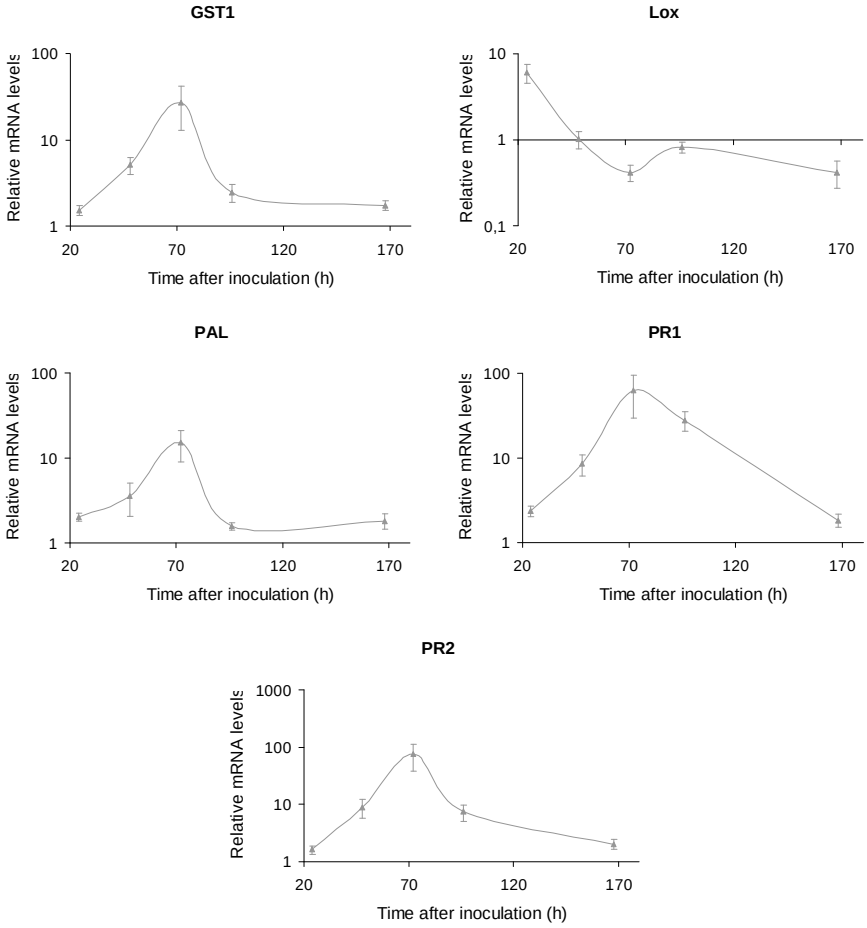

Fig. 22 Time-course of the induction of *GST1*, *Lox*, *PAL*, *PR1* and *PR2* genes. Expression was measured in potato roots inoculated with *T. harzianum* and *R. solani*. The data points represent the means of three biological replicates and the standard error

The results differed between the genes. The expression of *PAL*, *GST1*, *PR1* and *PR2* genes, increased after 48 hpi, with a maximum at 72 hpi, and subsequently (i.e. starting from 96 hpi) decreased. The maximum values obtained were 15.03 ± 5.73, 27.29 ± 15.78, 62.83 ± 36.35 and 79.14 ± 39.75 for *PAL*, *GST1*, *PR1* and *PR2* genes,

respectively. The expression of *Lox* gene was characterised by an induction at 24 hpi with a value of 6.03 ± 1.19.

Moreover, the comparison of the expression level of the five genes during the three treatments demonstrated that the *GST1* and *Lox* genes were up-regulated in the T+R treatment as compared to the T-treatment and R-treatment, with statistical significance at 24 and 48 hpi for *GST1* and 24 hpi for *Lox* (Fig. 23).

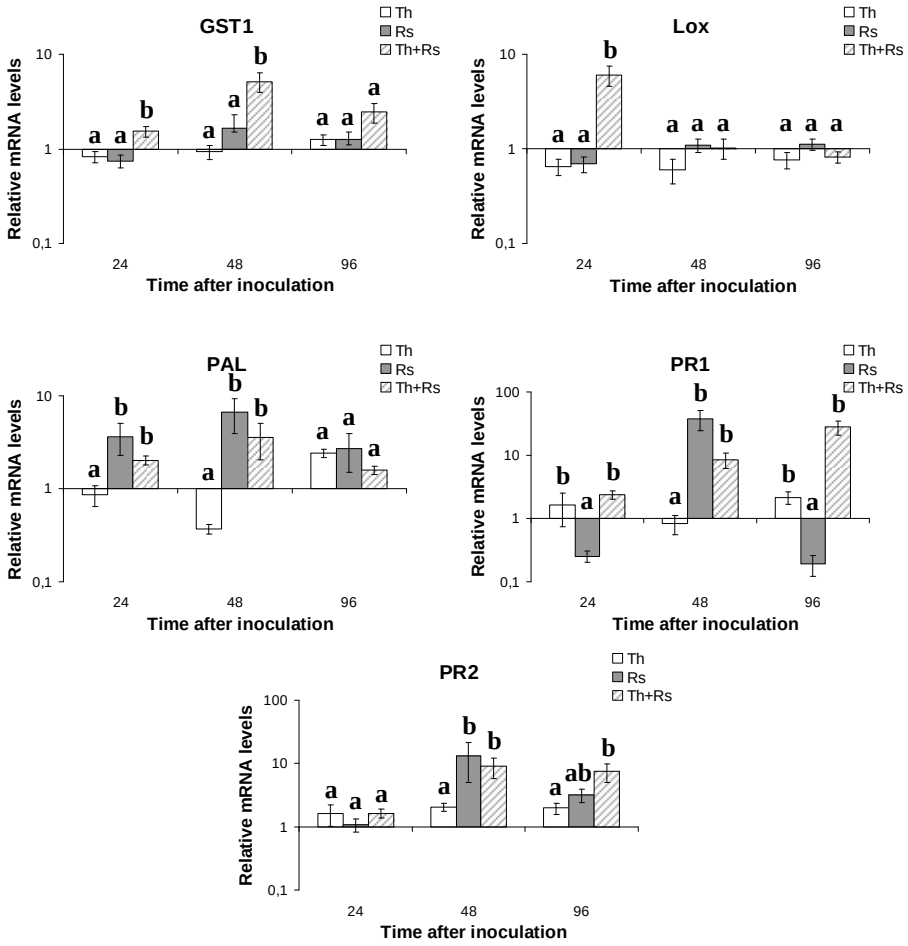

Fig. 23 Expression level of the five defence response genes (Gluthatione-S-transferase 1 (*GST1*), Lipoxygenase (*Lox*), Phenylalanine ammonia lyase (*PAL*), basic Pathogenesis Related 1 (*PR1*) and acidic Pathogenesis Related 2 (*PR2*)) in

potato roots during the inoculation with *T. harzianum* (Th), *R. solani* (Rs) and both (Th+Rs). The monitoring of the relative gene expression level was done by Real-Time Quantitative PCR at 24, 48 and 96 hours after inoculation. The data points represent the means of three biological replicates and the standard error. For each time and gene, values of relative expression level followed by a different letter differed significantly at $P \leq 0.05$ (Tukey's HSD)

Discussion

Biological control (e.g. with *T. harzianum*) of the soil-borne fungal plant pathogen *R. solani* on potato has been reported in many studies over the last few years (Brewer and Larkin 2005; Vinale et al. 2006). Decrease in the severity of symptoms was observed in the early stages of infection (i.e. during the first seven days post-inoculation) and attributed to a transient antagonistic effect of *T. harzianum* (Wilson et al. 2008). However, no studies have investigated the early (i.e. within one week) temporal dynamics of expression of defence gene responses in potato roots challenged by *R. solani* in the presence/absence of *T. harzianum*. This is crucial to understand the mechanism of biological protection conferred by *T. harzianum* and to develop innovative strategies to control *R. solani*. In the present study, we used the QRT-PCR to analyse and compare the expression of defence response genes in roots of *in vitro* produced potato plants inoculated with *R. solani* and/or the BCA, *T. harzianum*. The effect conferred by *T. harzianum* versus *R. solani* was demonstrated by the induction of the *Lox* gene expression at 24 hpi and *GST1* gene expression at 72 hpi in the roots of potato plantlets inoculated with both organisms at the same time. These results were confirmed by the acridine orange observation. Potato plantlets challenged by *R. solani*

had visually greater root DNA and RNA contents in the presence of *T. harzianum* than the plantlets inoculated with *R. solani* alone.

Reliable quantification of gene expression relies on trustworthy normalisation of QRT-PCR data. The best normalisation is obtained using internal reference genes because it takes into consideration variation introduced by RNA sample quality, RNA input quantity and enzymatic efficiency in reverse transcription (Vandesompele et al. 2002). The ideal reference genes have stable expression levels that do not differ in the organs or tissues studied, and that are not influenced by the treatments. Since variation always exists for any reference gene, normalisation of gene expression based on a single reference gene can bias the results. Normalisation with multiple reference genes has become the standard, but reports that identify such genes in plant research are limited, even though algorithms are available to test the expression stability of reference gene candidates (Vandesompele et al. 2002; Pfaffl et al. 2004). For *S. tuberosum*, Nicot et al. (2005) tested seven candidate genes. The *β-tub* and *EF1*-α appeared the best reference genes for normalisation under biotic and abiotic stress conditions. For *Solanum lycopersicum*, Yan and Liou (2006) tested eighteen candidate genes. The *GAPDH* and *Ubc* appeared the most adequate for normalisation with a plant pathogen. Therefore, these four reference genes (i.e. *β-tub*, *EF1-α*, *GAPDH* and *Ubc*) were selected for geNorm software analysis and the expression stability was evaluated in potato roots inoculated with *R. solani* and/or *T. harzianum*. Whatever the treatment, *EF1-α*, *GAPDH*, and *Ubc* were identified as the best candidates for normalisation with multiple reference genes.

The expression stability of the four reference genes was lower in the R-treatment than in the T-treatment and T+R-treatment. In the presence of *T. harzianum* alone, the expression stability of reference genes in potato roots was not affected, while in presence of *R. solani* alone, this stability was markedly affected more so than in the presence of both organisms at the same time. Indeed, the Ct values obtained for all the four reference genes decreased at 168 hpi in the R-treatment, while it remained stable for the T+R-treatment. These observations suggested that *T. harzianum* could confer a protective effect on potato plantlets infected by *R. solani*, at least at the early stage of infection (i.e. within 168 h), confirming the results previously obtained by Wilson et al. (2008) within a similar experimental duration. These results were supported with the vital dye acridine orange. Both single-stranded nucleic acids and double-stranded nucleic acids were detected in root tissues staining orange and green, respectively (Darzynkiewicz 1990). The amount of DNA and RNA was visibly decreased in the root tissues challenged by *R. solani* alone, while only slightly decreased when both organisms where present, and remained unchanged in the presence of *T. harzianum* alone. The reference gene *EF1-α* also supported the increased protection conferred by *T. harzianum* in roots challenged by *R. solani*. The *EF1-α* gene used as a positive control of the vitality (Van Aarle et al. 2007), had decreased expression in the R-treatment at 168 hpi, while it was not affected in the T+R-treatment. The greater impact on gene expression in the roots of plants inoculated with *R. solani* could be attributed to damage caused to the root tissues by *R. solani*, verified at 6 days post-inoculation under the scanning electron microscopes using

the same autotrophic culture system (de la Providencia et al. unpublished).

The induction of plant defence responses mediated by rhizosphere-colonising *Trichoderma* has been well documented (Hanson and Howell 2004; Harman et al. 2004; Shoresh et al. 2005; Vinale et al. 2008). However, in our experiment, we observed only the induction of *PR1* at 168 hpi and a slight induction of *PAL* at 96 hpi, in the plants inoculated by *T. harzianum* alone. This was in apparent contradiction with earlier studies in which a marked induction of *PAL* and *hydroxyperoxide lyase* (Yedidia et al. 2003), and, *Lox*, *PAL*, *ethylene receptor 1*, *ethylene-inducible CTR1-like protein kinase* (Shoresh et al. 2005) were observed after a short time (24 or 48 hpi). We hypothesise such differences to be attributed to the absence of root cell penetration and colonization by the *T. harzianum* strain used in our experiment, verified at 6 days following contact under scanning electron microscope (de la Providencia et al. unpublished). Such invasion was observed in the studies of Yedidia et al. (2003) and Shoresh et al. (2005). However, in both these studies, the fungus was the strain *T. asperellum* T203 (a well-known inducer of plant defence response), considered as *T. harzianum* previously. Different studies have shown that the addition of *Trichoderma* spp. metabolites may act as elicitors of plant resistance, or that their expression in transgenic plants may act also as elicitors and induce the synthesis of phytoalexins, pathogenesis-related (PR) proteins and other compounds (Elad et al. 2000; Dana et al. 2001). This suggested that the induction of *PR1* at 168 hpi in the potato roots inoculated with *T. harzianum* alone, could be due to the production of metabolites by *T. harzianum*.

Plants require a broad range of defence mechanisms to effectively fight invasion by microbial pathogens or attack by herbivorous insects. The signal molecules salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are involved in many plant-pathogen and plant-insect interactions (De Vos et al. 2005; Van Loon 2007). In our study, *R. solani* infection caused an increase in the expression of *PAL* gene in the roots of potato plantlets. The *PAL* gene is activated by the JA/ET signalling pathways in the context of induced plant defences and was shown to be a key regulatory enzyme in the synthesis of SA (Sticher et al. 1997; Kato et al. 2000). In addition, we demonstrated that *PR1* and *PR2* genes were induced at 48 hpi. *PR1* is encoding for the PR1-b protein (i.e. basic form) (Hoegen et al. 2002) and *PR2* is an acidic β-1,3-glucanase (Mac et al. 2004). This PR1-b protein may act as an anti-fungal agent (Vleeshouwer et al. 2000). Moreover this protein is associated to the ISR (van Loon and van Strien 1999). The acid β-1,3-glucanase, involved in fungal cell wall degradation (Mauch and Staehelin 1989; Chang et al. 1992), is induced mainly by systemic acquired resistance (SAR) and SAR inducers, such as SA (Van Wees et al. 1999; Newman et al. 2002). To the contrary, we did not observe the induction of *Lox* gene (i.e. the first enzyme in the biosynthesis pathway of JA is lipoxygenase) in the same roots challenged by *R. solani*. These results were confirmed by Gao et al. (2006) for the induction of *PR1*, one glucanase and different chitinases genes, in tomato roots inoculated by *R. solani*. But, the authors did not observe the induction of *PAL* as in our study. This suggested that potato defence response to *R. solani* attack was dependent on the signal molecules SA and JA/ET.

In the presence of *T. harzianum* and *R. solani*, we observed the up-regulation of the *Lox* gene expression at 24 hpi and *GST1* gene expression at 24 and 48 hpi as compared to the treatments in presence of the two microorganisms alone. This suggested that *T. harzianum* in the presence of *R. solani* induced the *Lox* and *GST1* genes in roots of potato plantlets. A similar observation was made by Yedidia et al. (2003) with the hydroperoxyde lyase gene in cucumber with co-inoculation of *T. asperellum* T-203 and *Pseudomonas syringae* pv. *lachrymans*. A similar response was described with the *Atvsp* gene in rhizobacteria-induced systemic resistance (RISR) in *Arabidopsis* plants and termed potentiation (Van Wees et al. 1999). Taken together, our results confirm that the induction of plant resistance during the *Trichoderma* plant-pathogen interaction is similar to that elicited by rhizobacteria, which enhances the defence system but does not involve the production of PR-proteins. During this mechanism, the defensive capacity of the plant is enhanced through microbial stimulation or similar stresses and the defence responses primed by the BCA in the presence of the pathogen (Harman et al. 2004; Van Loon 2007; Vinale et al. 2008). Moreover, these observations suggest that, in the presence of *T. harzianum*, potato defence response to *R. solani* attack is dependent on the signal molecules JA and SA.

In conclusion, we demonstrated that *T. harzianum* Rifai MUCL 29707 in the presence of *R. solani* induced defence response genes in potato plants grown in an autotrophic *in vitro* culture system. We demonstrated that the *Lox* gene, encoding the first enzyme in the biosynthesis pathway of JA, and the *GST1* gene encoding an auxin-responsive glutathione-S-transferase 1 (Hahn and Strittmatter 1994),

were primed by *T. harzianum* Rifai MUCL 29707 in the presence of *R. solani*. We demonstrated that *T. harzianum* Rifai MUCL 29707 challenged *R. solani* in potato roots by decreasing the damage caused to DNA and RNA in infected roots. Both observations suggest that the signal molecules dependent on JA and SA were activated in potato plantlets infected with *R. solani* in the presence of *T. harzianum* Rifai MUCL 29707, at early stage of infection. Further studies designed to demonstrate whether the activation of these genes is required to protect the potato plantlets at the early stage of the interaction need to be carried out.

Acknowledgements

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General Discussion

Potato occupies a prominent position in the world global food chain, representing a valuable cash crop for millions of farmers around the world (See, FAO 2008). Unfortunately, this culture is susceptible to numerous pathogens. Conventional agriculture mostly relies on the application of chemical pesticides, which are often only partially effective against soil-borne diseases. In addition, repeated applications of pesticides may be detrimental to human health and to the environment. Facing an increased societal pressure for a healthier environment, the European Commission regulation has placed an increased number of molecules on the list to of pesticides to be banned in the coming years. Within this context, alternative or complementary approaches to fight potato diseases, including biological control strategies, are timely and encouraged in modern agriculture.

A number of fungi are known to colonize plant roots without causing disease. These include AM fungi, binucleate *Rhizoctonia* spp., *Piriformospora indica*, various plant growth promoting rhizobacteria (PGPR) and *Trichoderma* spp. (Shoresh et al. 2010). In the last decades, these organisms have been classified as beneficial microorganisms against a wide class of plant diseases. If numerous mechanisms have been reported on the direct impact of these microorganisms against soil borne pathogens (e.g. mycoparasitism caused by *Trichoderma* spp.on *R. solani*), an increasing number of findings nowadays indicate that a primary pathway to control diseases occurs through the ability of these organisms to reprogram plant gene expression.

The ability of beneficial fungi to control plant diseases probably involves the activation of a limited number of general plant pathways.

The hormones signalling pathways, which are central players in the regulation of defence mechanism, are probably the most activated. As demonstrated for *Trichoderma* spp. and PGPR, the JA/ET signalling pathways are essential in the control of pathogens by plants. For AM fungi, experimental evidences suggest a central role of the JA signalling pathway, but the precise function and regulation of this signalling pathway awaits elucidation. Increasing our knowledge on the modifications of plant physiology during the interaction with beneficial microorganisms, as well as in the regulation of plant hormones signalling during infection with pathogen is essential to develop efficient strategies to control pathogens in crops such as potato.

I. Understanding the AM root colonization

In the first step of our research project, the mycorrhizal donor plant (MDP) *in vitro* culture system developed by Voets et al. (2009) was used to investigate gene expression during the AM root colonization in potato (**Chapter I**). This system is based on the plating of young seedlings in an actively growing extra-radical mycelium (ERM) network extending from a donor plant (*M. truncatula*). Colonization of plants via the ERM is a mechanism predominant in nature (Friese and Allen 1991).

In this study, we described three developmental stages of the AM fungal root colonization of potato: (1) the pre-stage referring to the stage before contact of the AM fungus with the root and root colonization, (2) the early stage referring to the stage of appresorium

formation and first hyphal root colonization before arbuscules/vesicles formations and (3) the late stage referring to the stage of intense root colonization with arbuscules and vesicles formed. These three colonization stages were paralleled with a specific induction of several defence genes with a maximum induction observed at the late stage of AM fungus association. This suggested that heavy colonized roots are the most optimal condition for biological protection by AM fungi. In addition, at the pre-colonization stage, we observed an induction of two *PR* genes, which suggested a possible protective effect of AM fungi against pathogen attack at this stage. These two hypotheses were tested in **chapter III**.

Interestingly, our results on root colonization and genes expression confirmed the homogenous AM colonization obtained with the MDP *in vitro* culture system. In the four biological replicates analyzed in **Chapter I**, we observed almost the same pattern of root colonization and genes induction during the AM fungal root colonization. It was also interesting to note that a similar colonization patterns was observed in two other independent experiments (**Chapter II** and **Chapter III**), supporting the suitability of the system to repeat experiments. The synchronization was often reported as a problem to investigate genes expression or genes networks (Balestrini and Lanfranco 2006). This drawback was resolved with the MDP *in vitro* culture system, offering new opportunities to investigate the molecular events or gene networks associated with the plants-AM fungi symbiosis.

In a second step, a genome-wide expression profile was performed on potato roots at the pre-, early and late stage of AM fungal root colonization. Our results highlighted the important modulation of the plant defence genes during the different stages of AM fungal root colonization (**chapter II**). The data were validated by Real-Time quantitative PCR and by the observation of "genes markers" of the AM fungal symbiosis frequently reported in the literature. It is to be noted that the confirmation by the plant genes markers was only possible at the pre- and late stages of root colonization since no markers genes have ever been reported at the early stage (i.e. during the first hyphae development) of root colonization, to our knowledge. In **Chapter II**, we have shown the induction of one expansin-like protein (*EXBL1*), which is a host marker of successful mycorrhization (Siciliano et al. 2007; Dermastev et al. 2010), specifically expressed at the pre-stage of root colonization. The late stage of AM fungal root colonization was also confirmed by different marker genes (i.e. as the *DXS-2* encoding for an enzyme which catalyses the biosynthetic pathway of mycorradicin).

The microarray analysis further showed the induction of a large number of plant genes associated to biotic stresses during the pre- and late stage of AM fungal root colonization. These changes of defence genes expression in the host plant could contribute to an increased resistance against pathogens infections and indicate the potential of AM fungi to trigger the plant defences during these two stages. A similar observation was reported by Liu et al. (2007). These authors have correlated the induction of defence genes in the shoot of *M. truncatula* colonized by *R. intraradices* with an increase in resistance

against the leaf bacterial pathogen, *Xanthomonas campestris*. Consequently as in **chapter I**, the inductions of numerous plant genes associated to biotic stresses during the pre- and late stages of root colonization supported our choice to investigate the role of AM fungi to control *R. solani* during these two stages of root colonization.

II. Specifics remarks on the AM root colonization

In **Chapter I**, it was interesting to note that the level of induction, of the *PT3* gene (i.e. plant gene marker of AM root colonization) could not be paralleled with the level of hyphae colonization. This was also confirmed in the experiments conducted in **Chapter II** and **Chapter III**. To the contrary, it seemed that the level of expression of *PT3* gene increased with the quantity of arbuscules present in the roots (i.e. absolute arbuscule abundance). In other word, an increased density of arbuscules resulted in a higher induction of the *PT3* gene. Although, sometimes the expression was slightly higher in a biological replicates with lower arbuscules abundance. An explanation of this difference could be the experimental design of these researches. In fact, the experiments were not designed in order to demonstrate a putative "correlation" between the arbuscules abundance and the *PT3* expression. In consequence, an experiment, containing enough biological replicates of plant root systems with different percentages of arbuscules colonization, is required to confirm whether a correlation could be expected between both parameters. If such, this experiment could demonstrate the ability of *PT3* gene as molecular marker for estimating the arbuscule abundance, in addition to the establishment of an effective symbiosis

(Rausch et al. 2001). These results were interesting because the quantification of AM fungus in roots by molecular technique (i.e. quantitative PCR) remains still difficult. Gamper et al. (2008) have clearly shown that the unique biological organization of Glomeromycota (i.e. heterogeneity of the nuclear distribution in the AM fungi) imposes restrictions on the applicability of quantitative PCR for the quantification of AM fungus.

Moreover in **Chapter III**, when the potato plantlets were plated in the ERM network of the AM fungus and challenged at the same time with *R. solani*, we showed a priming of two SA-dependent genes (i.e. *GST1* and *PR2*) concomitant to an important decrease of AM colonization level in the potato roots. Recently, de Roman et al. (2011) have also linked a decrease of the AM fungal root colonization in soybean with the induction of the *PR1-a* gene (acidic form and SA-dependent). They have deduced that the activation of SA-dependent defence signalling resulted in an inhibition of AM fungal root colonization. Similarly, we could hypothesize that the induction of *GST1* and *PR2* (two gene markers of SA signalling) during interaction with the soil-borne pathogen (i.e. *R. solani*) could have resulted in a decrease of AM fungal root colonization. Moreover, the SA-dependent defence pathway, effective against biotrophic pathogens (Glazebrook 2005), seems also to impact AM fungi which share similarities with biotrophic pathogens (Guimil et al. 2005; Paszkowki 2006). In addition, we also showed, by the microarray analysis (**chapter II**; Table 15), a marked increase in the SA marker genes induction at the pre-stage followed by a repression at the early stage. This observation suggested the transient expression of SA marker

genes before contact between the AM fungus and the host. Taken together, these results tend to support the hypothesis that the AM fungal root colonization requires the inhibition of SA-dependent defence in the host in order to achieve a compatible interaction (Pozo and Azcon-Aguilar 2007).

III. Understanding the AM fungal and *Trichoderma* spp. induced resistance in potato plants

In **chapters III**, **IV** and **V** (Fig. 24), we demonstrated, under *in vitro* culture conditions, the capacity of the AM fungus to decrease the disease caused by two major potato pathogens (*R. Solani* and *P. infestans*) in short-term experiments. This was materialized by a reduced level of necrosis on the roots and shoots of the plants. Moreover, the gene expression analysis demonstrated that the AM fungus was able to induce changes in the expression of SA-, JA- and ET-dependent genes during this process. These results may therefore support the ability of *Rhizophagus* sp. MUCL 418333 to develop a MIR process in the potato plantlets. The results obtained in **chapter VI** also suggested the ability of *T. harzianum* to develop an induced resistance against *R. solani* in the potato roots. In addition to the previous observations of Wilson et al. (2008) which have attributed the biological protection abilities of *T. harzianum* to a transient antagonistic effect, the analysis of defence genes during this interaction showed that this biological protection was associated with a priming of two genes dependent of the SA (i.e. *GST1*) and JA (i.e. *Lox*) pathways.

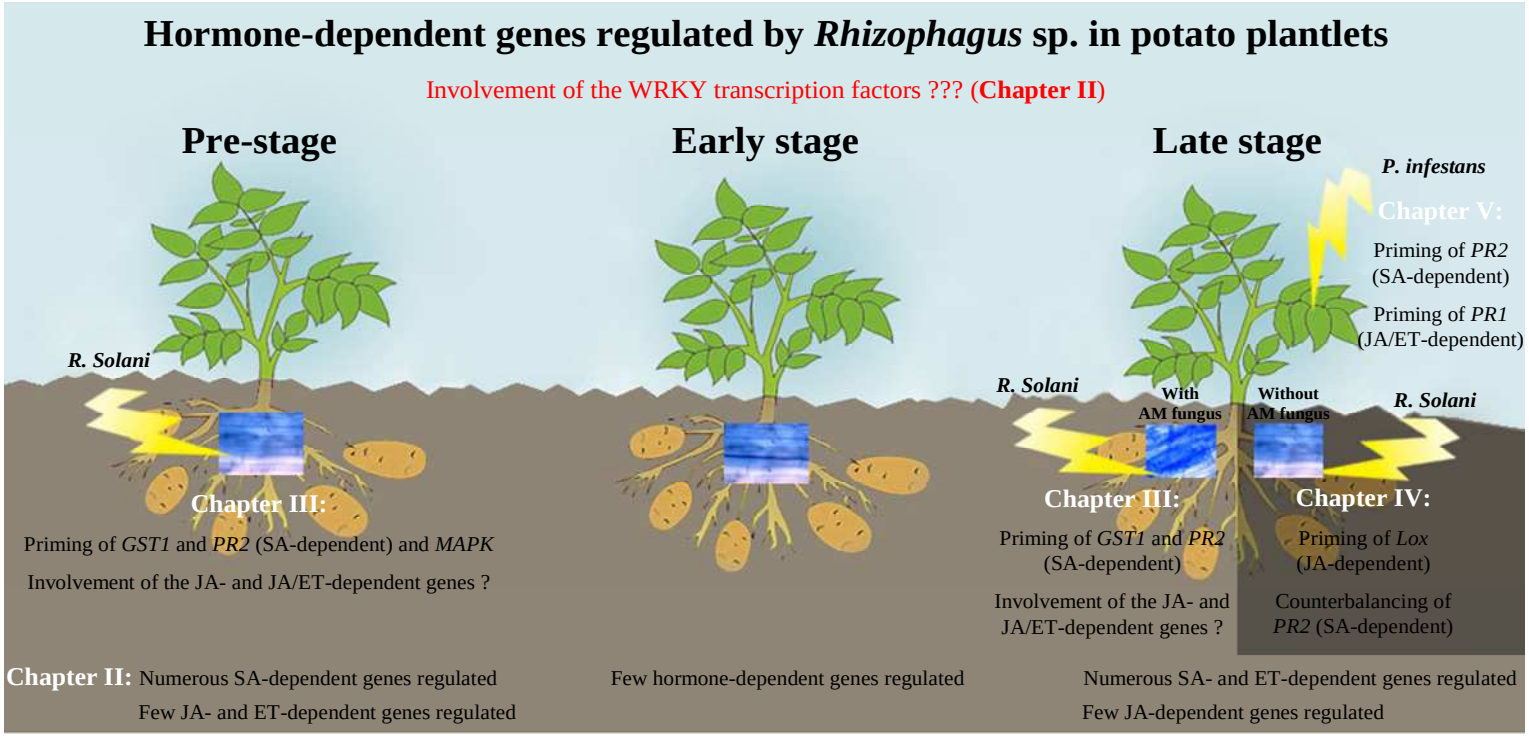

Fig. 24 Overview of the plant hormone-dependent genes regulated by *Rhizophagus* sp. MUCL 41833 in the potato plantlets. The figure summarized the results obtained during this thesis, under *in vitro* condition. Chapter II: hormone-dependent genes regulated in the potato roots during the pre-, early and late stage of root colonization. Chapter III: Hormone-dependent genes regulated in the potato roots after inoculation by *R. solani* at the pre- or late stages of root colonisation. Chapter IV: Hormone-dependent genes regulated in the potato roots after inoculation by *R. solani* on the non-mycorrhizal root part of a mycorrhizal potato plantlet at the late stage of root colonization. Chapter V: Hormone-dependent genes regulated in the leaves of potato after inoculation by *P. infestans* at the late stage of root colonization.

III.1 Local interaction

In **chapter III** (Fig. 24), the results supported that the local protection triggered by *Rhizophagus* sp. MUCL 41833 in potato roots was related with the priming of two SA signalling pathway genes (*GST1* and *PR2*). Both genes were primed in presence of *R. solani* inoculated either at the pre- or late stage of AM fungal root colonization. This result is consistent with the previous observation of Cordier et al. (1998). These authors have shown in mycorrhizal tomato plant a more significant accumulation of PR-1a proteins (i.e. acid form; SA-dependent) than in non-mycorrhizal tomato plants upon *Phytophthora* infection. In contrast, no priming of the JA- (i.e. *Lox*) and JA/ET- (*PAL* and basic *PR1*) dependent genes were observed during this interaction, which is surprising in regard to the recent review of Hause et al. (2007) and Pozo et al. (2010). These authors explained that the biological protection induced by AM fungi may be related to a central role of the JA-dependent defence pathway. Taken together, these results suggested that the local induced resistance observed in our experiment displayed some dissimilarity with the biological protection process described previously.

In addition, the microarray did not reveal a strong induction of the oxilipin pathway during the late stage of root colonization, in comparison to the observations of Lopez-Raez et al. (2010). These authors have demonstrated the strong induction of the oxylipin pathway in the root of tomato plants colonized by two different AM fungi and explained that this expression change in the host could contribute to the biological protection ability of the AM fungi. In

contrast, our microarray experiment revealed a strong induction of the SA signalling pathway during the process of AM fungal root colonization. Therefore, we provided some evidence that the resistance in potato roots triggered by *Rhizophagus* sp. MUCL 41833 against *R. solani* is not only related to a central role of the JA signalling pathway but may also involve the SA signalling pathway.

However, the importance of the SA signalling pathway during the AM fungal protection needs to be considered with care. In fact, the experiment, based on the analysis of a very limited number of defence genes, did not allow to clearly conclude to the implication of the SA pathway rather than the JA or ET pathways in this biological protection. Moreover, it should be kept in mind that plant hormone signalling pathways are not isolated but rather interconnected with a complex regulatory network involving various defence signalling pathways (Singh et al. 2011). Recent studies indicate that ABA, auxin, gibberellic acid, cytokinin, brassinosteroids and peptide hormones are also implicated in plant defence signalling pathways (Review by Bari and Jones 2009). As such, it is interesting to note that, the microarray analysis (**chapter II**) also revealed an important induction of some genes related to the ET pathway during the late stage of AM fungal root colonization. In addition, recent researches on ABA plant mutants have shown a role of the ABA in the establishment of a functional AM symbiosis (Herrera-Medina et al. 2007; Martin-Rodriguez et al. 2011). The ET and ABA, mutually antagonistic, are able to modulate some functions in the plant, such as the disease resistance (Anderson et al. 2004). Taken together, these observations suggested that the defence responses triggered by the AM fungi are more complex and

underlined the necessity of further researches to understand the regulations of the plant hormones signalling pathways during the MIR process.

A possible explanation, for such dissimilarity of hormone signalling pathways during our AM fungal protection, may be the fact that the plant responses to stresses vary depending on the type of interactions/pathosystem studied (Review by Bari and Jones 2009). In previous researches dissimilarities were already observed depending on the AM fungus strains or plants cultivars considered. For example, Yao et al. (2002) have demonstrated that *C. etunicatum* significantly reduced the disease of *R. solani* in potato, whereas no effect was observed with *R. intraradices*. Moreover, the authors also observed that the potato cultivar Goldrush was more influenced (e.g. increase of shoot fresh weight, root dry weight and number of tubers produced per plant) by the AM fungus than the potato cultivar LP89221. In addition, Steinkellner et al. (In press) have recently observed some differences in *F. mosseae* colonization between tomato cultivars and more interesting, some differences in biological protection triggered by the AM fungus against *Fusarium oxysporum* depending on the tomato cultivars. These two researches clearly supported the functional specificity of the AM fungi-plants interaction. Moreover, Lopez-Raez et al. (2010) confirmed also this specificity. These authors observed a significant difference of response of the tomato plant with two different AM fungi. They showed only an overlap of 35% of the regulated genes between the interaction with *R. irregularis*-tomato and *F. mosseae*-tomato. A similar overlap (30%)

was also found in the response of *M. truncatula* to the same fungi (Hohnjec et al. 2005). As a consequence, it might be that these differences in plant response contribute also to the difference in the signalling pathway triggered during the biological protection.

Finally, recent results confirmed that some beneficial microorganisms were able to induce a systemic response by different signalling pathways according to the plant/microorganisms strains combination. For example, it was shown that the ability of *Trichoderma* spp. to induce resistance varied among strains (Shoresh et al. 2010). It has been shown that some *Trichoderma* spp. strains could develop an induced resistance following another pathway than the JA/ET signalling pathways. Alfano et al. (2007) have demonstrated that tomato plant in presence of *T. hamatum* lacked to detect induction of ISR markers, but they observed the up-regulation of one marker of SAR (i.e. *PR5* gene). More recently, Shoresh et al. (2010) showed the absence of protection in the *npr1* mutant of *A. thaliana* against *Pythium ultimum* in presence *T. harzianum* while protection was observed in the wild-type strains. These two researches suggested that the biological protection of some *Trichoderma* spp. may be dependent of the SA signalling pathway.

III.2 Systemic interaction

In contrast to the study conducted in **chapter III**, the studies conducted in **chapter IV** and **V** showed the priming of one SA-dependent gene (i.e. *PR2*) and JA- or JA/ET-dependent genes (i.e. *Lox*

or basic *PR1*) (Fig. 24). These results suggested that the biological protection triggered by the AM fungus in the non-mycorrhizal root part and in the leaves of a mycorrhizal potato plant could also be related to the JA signalling pathway. As a consequence, the induced resistance observed in these two experiments shared some similarities with the MIR process described by Hause et al. (2007) and Pozo et al. (2010) in which the JA signalling pathway may play a central role. Moreover, this mechanism also shared some similarities with the mechanism observed in **chapter VI**. In this chapter, we demonstrated that *T. harzianum* MUCL 29707 in the presence of *R. solani* induced a priming of one JA- and one SA-dependent gene in potato roots, confirming previous observations (Soresh et al. 2010). It was interesting to note that in these experiments, the interactions took place at the same level (i.e. in the roots without direct interaction with the beneficial microorganisms). Indeed, no root cell penetration and colonization were observed by *T. harzianum* MUCL 29707, verified at 6 days following contact under scanning electron microscope (De Jaeger et al. 2010). As a consequence, the priming observed with *T. harzianum* MUCL 29707 was a systemic effect as in the **chapter IV**. Taken together, these results may suggest a similar pattern of defence genes expression during the systemic biological protection of the AM fungus and *T. harzianum* in potato plantlets under *in vitro* conditions.

Curiously, the hormone defence pathway by which the AM fungus could protect the potato plantlets shared some dissimilarity in the different experiments (**chapter III, IV and V**). Moreover, as already explained above, the plant responses to stresses vary depending on the type of interactions/pathosystem studied (Review by Bari and Jones

2009). Therefore, our results suggested that the plant defence response triggered by the AM fungus could differ following a local or systemic infection. However, as in the previous paragraph, this hypothesis needs to be considered with care. The use of a very limited number of markers genes did not allow to demonstrate a major role of a specific hormone pathway rather than another hormone pathway during this biological process. As example, it is well known that the higher plants contains more than one lipoxygenase gene in their genomes. The model plant *A. thaliana* contains 6 lipoxygenase genes and some plants even contain more than 15 different lipoxygenase sequences (Ivanov et al. 2010). Thus, it might be possible that another copy of this gene follows a "priming" pattern during the experiment conducted in **chapter III**.

However, the plant hormone interacts together for defence signal networking to fine tune defence (Singh et al. 2011). As example, several studies indicate that JA- and ET-signalling interact synergistically to induce the expression of defence genes against pathogen infection (Glazebrook 2005). More interesting, it is well known that SA- and JA-mediated defences display antagonistic interactions. The recent literature suggested that the WRKY transcription factors may play an important role in this antagonistic interaction (Review by Bari and Jones 2009). As the *AtWRKY70*, the protein encoded by this gene had been suggested to act as a positive regulator of the SA-dependent defence and a negative regulator of JA-dependent defence. In consequence this protein could be a convergence point for determining the balance between SA and JA

pathways (Li et al. 2006; Knoth et al. 2007). Other WRKY transcription factors have also been suggested to play a role in the mediation of the cross-talk between the SA- and JA-dependent defences (i.e. *AtWRKY62*, *AtWRKY53* …) (Review by Bari and Jones 2009).

In **chapter II**, we observed the induction of several WRKY transcription factor genes during the pre- and late stages of AM fungal root colonization. More interesting, some of these genes shared high homologies with the *AtWRKY70* and *AtWRKY53* transcription factors genes. This result may suggest that the WRKY transcription factors could regulate the SA- and JA-dependent defence in the host during the symbiotic interaction. By extrapolation, we could also suggest that these WRKY transcription factors could be involved in the plant resistance promoted by *Rhizophagus* sp. MUCL 41833. Consequently, these WRKY transcription factors could explained the dissimilarities of responses observed in the different experiments. In fact, during the AM fungal interaction, the WRKY transcription factors could promote one plant hormone pathway rather than another plant hormone pathway, following the interactions encountered. However, this suggestion is highly speculative and needs to be confirmed. Moreover, the results obtained in this thesis did not allow to demonstrate that the plant hormone signalling pathways observed in the three interactions were different.

In this research, we have demonstrated the potential of *Rhizophagus* sp. MUCL 41833 to decrease the symptoms caused by *R.*

solani MUCL 49235 and *P. infestans* MUCL 43257 in potato plants. We have also suggested the ability of *T. harzianum* MUCL 29707 to decrease the symptoms caused by *R. solani* in the potato roots. Therefore, our results supported the potential of these two beneficial microorganisms for the control of potato pathogens. However, it should be kept in mind that these results have been obtained with an "artificial" *in vitro* culture system. Despite the powerful tool represented by this system, the results need to be confirmed under more "natural" conditions (e.g. in pot culture systems).

In addition, the results of gene expression analysis supported the activation of the plant hormone dependent defence genes in the potato plant during the interactions between the beneficial and harmful microorganisms used in our experiments. These results of gene expression provided a large amount of additional information about the biological protection triggered by *Rhizophagus* sp. MUCL 41833 on the potato plant, which allowed to make progress in the understanding of the mycorrhiza induced resistance. Finally, this analysis supported that the plant hormone responses promoted by AM fungi are complex. Further researches are required to understand their regulations during the MIR process, as the use of other AM fungal strains or plant hormone quantifications.

Conclusions

Potato crop faces numerous pathogens, which represent a constant menace to the production worldwide. Large quantities of pesticides are used to control/suppress diseases, but most of these chemicals are harmful to the environment. Therefore there is an urgent need to develop alternatives to control pests and diseases. The AM fungus and *Trichoderma* spp. are nowadays considered as promising beneficial microorganisms. However, the mechanisms by which they improve plant resistance/tolerance to pathogens are yet not fully resolved. Increased knowledge on the defence mechanism involved is thus fundamental to develop/improve strategies for plant protection.

Here, we investigated the effects of *Rhizophagus* sp. MUCL 41833 and *T. harzianum* MUCL 29707 on the potato plants protection against *R. solani* MUCL 49235 and *P. infestans* MUCL 43257. A particular attention was dedicated to the molecular signalling pathway regulated during these processes. Four central questions were addressed:

1. **Is the Mycelium Donor Plant *in vitro* culture system adequate for plant genes expression analysis during the AM fungal root colonization?**

   The MDP *in vitro* culture system developed by Voets et al. (2009) allows the synchronization of the AM fungal development in the roots by plating the seedlings in an actively growing mycelium network extending from a mycorrhizal donor plant. Here, the analysis of defence genes (i.e. *GST1*,

Lox, *MAPK*, *PAL*, *PR1* and *PR2*), as well as the marker gene of a functional symbiosis (i.e. *PT3*), revealed that the MDP *in vitro* culture system was an adequate system to study changes in plant gene expression in potato at the pre-, early and late stage of root colonization by *Rhizophagus* sp. MUCL 41833 (**chapter I**). This study opened new avenues to investigate the molecular events or gene networks associated in the plant-AM symbiosis, especially during the relative inaccessible first stages of the interaction (i.e. pre- and early stages), as well as the molecular signalling involved during biological protection by AM fungi.

2. **Which are the transcriptional changes associated to the biotic stress during the potato root colonization by *Rhizophagus* sp. MUCL 41833?**

The microarray analysis coupled to the MapMan software showed an important global change in plant gene expression during the pre, early and late stages of root colonization and especially of the genes associated to biotic stresses (**chapter II**). This analysis also suggested the possible involvement of WRKY TF genes in the process of AM fungal root colonization. Interestingly, some of the WRKY TFs induced during the pre- and late stages of root colonization shared homology with *Arabidopsis* WRKY TF genes (i.e. *AtWRKY51*, *AtWRKY53* and *AtWRKY70*). These genes are encoding for proteins which play major functions in the mechanism of plant

immune response. This observation would suggest the potential of the AM fungus to promote a plant-mediated resistance response through the WRKY TF proteins. However, further researches are required to confirm the impacts and functions of WRKY TFs proteins during the AM fungal root colonization.

In these two studies (**chapter I** and **chapter II**), an induction of numerous genes related to plant defence was demonstrated during the pre- and late stage of AM fungal root colonization in potato roots. In contrast, only few inductions of defence genes were observed during the early stage. These results may suggest a potential of the AM fungus during the pre- and late stages of root colonization to induce a resistance against pathogen infections.

3. Is *Rhizophagus* sp. MUCL 41833 able to decrease the symptoms caused by *R. solani* MUCL 49235 and *P. infestans* MUCL 43257 in potato plants? Which are the defence genes that could be involved in this process?

The first part of this study (**chapter III**) confirmed the capacity of the AM fungus to protect the potato plant against infection by *R. solani* at the late stage of root colonization. In addition, we also observed a decrease of necrosis at the pre-stage of root colonization suggesting that the AM fungus may either interact with the pathogen in the rhizosphere or induce defence genes expression in the plants prior to colonization. In

the second part (**chapter IV**), the experiment with MDP *in vitro* split root culture system did not allow to clearly evaluate the systemic resistance induced by the AM fungus in the potato roots. However, despite that no lesions caused by *R. solani* were noted on the potato roots, gene expression analysis showed a mechanism of priming and counterbalancing during the systemic interaction between the AM fungus and the root pathogen. Finally, in **chapter V,** we suggested that the AM fungus triggered a systemic resistance in the leaves of potato plantlets during the first stage of infection with the hemibiotrophic pathogen *P. infestans*. Moreover, we have highlighted the priming of the two *PR* genes (i.e. *PR1* and *PR2*) in the leaves of AM potato plants inoculated with the pathogen.

In consequence, these researches supported the ability of *Rhizophagus* sp. MUCL 41833 to protect the potato plantlets against two major potato pathogens. In addition, the gene expression analyses underlined the activation of the hormone dependent defence genes during this process but, as they stand, these results did not allow to associate the MIR process with a hormone signalling pathway rather than another hormone signalling pathway. Further researches are required to understand theirs regulations/functions in the plant during the AM fungal protection.

4. Is *T. harzianum* MUCL 29707 able to protect the potato plants against *R. solani* MUCL 49235 infection? Which

are the defence genes that could be involved in this process?

The vital dye (i.e. acridine orange) analysis and the stability of reference genes (i.e. GeNorm software) suggested the capacity of *T. harzianum* MUCL 29707 to protect the potato plantlets against *R. solani* MUCL 49235 infection (**chapter IV**). In addition, the analysis of defence genes during this interaction suggested that this biological protection could be associated with the priming of JA- and SA-dependent genes. Finally, the absence of root cell penetration and colonization, verified at 6 days following contact under scanning electron microscope (De Jaeger et al. 2010), proved that the defence process involved was systemic.

In conclusion, the results produced by this thesis supported (under strict. *in vitro* culture conditions and in short time disease monitoring) that the two beneficial microorganisms are potentially important microorganisms for the control of potato pathogens. However as previously explained, their roles needs to be confirmed under *in vivo* conditions and, it should also be interesting to quantify the pathogen within the root tissues in order to distinguish if the beneficial microorganisms interact on the plant resistance or tolerance. In addition, the results of gene expression, which provide some evidences on the importance of plant hormone dependent defence in this process, open the way to different researches in the field of the

AM fungal symbiosis or plant bio-protection. As example, it could be interesting to study in detail the importance or not of the WRKY transcription factors during the AM fungal symbiosis and, if these transcription factors are important for the symbiosis, to explore their regulations/functions during the MIR process.

Perspectives

Our research supported the role of the plant hormone signalling pathways in the mycorrhiza induced resistance process. However, our microarray analysis also demonstrated the complexity of the interactions at the level of the plant/AM fungus association. Microarray may represent a key technique for the in-deep comprehension of the mechanisms underlining plant resistance in presence of beneficial microorganisms. This technique has already been used in different studies to discover the molecular changes during the mechanism of ISR. For example, Ahn et al (2007) and Cartieaux et al. (2008), respectively with the PGPR *P. putida* LSW17S and *Bradyrhizobium* sp. strain ORS278, have shown a large number of JA/ET-regulated genes primed during the ISR mechanism. The analysis by microarray could improve our understanding of the molecular change during the MIR. Despite the remarkable efficiency of this technique, the selection of the most relevant observation times for the analysis was really important due to the numerous information generate by the experiment and the complexity of interpretation. In the **chapter III**, we observed the maximum activation of defence gene at the time course 2 and 11 days after plating the potato plantlets in the ERM networks of *Rhizophagus* sp. MUCL 41833 and inoculated with *R. solani*. This time appeared to be the most interesting for the transcriptome analysis. In comparison to the microarray technology, the new high-throughput real-time PCR technology seems a good alternative to analyze the pathways involved in the MIR. This technique allows to analyze a high number of genes or to focus on some specifics pathways (i.e. plant hormone pathways, defence genes

…). Moreover, this technique is fast to produce data expression and easier than microarray technique for the data interpretation.

Despite the powerful tool represented by these techniques, it is impossible to predict the exact protein concentration or activity from the analyses of mRNA levels. In fact, gene expression is a highly regulated and multistep process (e.g. post-transcriptional regulation, translation rate, post-translational modifications, stability of the proteins …). In consequence, proteomics appears as an important tool for further validation of the data generated by gene expression analysis (Baginsky et al. 2010). This technique provides quantitative and structural information about proteins, which are the functional determinants of the cells. However, it should be kept in mind that most of techniques analyze mixtures of tissues containing different cell types. Therefore, it should also be interesting to evaluate the cell-specific change in transcripts or proteins with more accurate methods, as micropipetting or laser capture microdissection.

In the past decade, research on the defence signalling pathways induced by beneficial microorganisms such as PGPR, during the mechanism of ISR have been conducted by the analyse of mutants affected in plant hormone signalling pathways. For example, Pieterse et al. (1998) have shown that mutants of *A. thaliana* defective in JA-signalling (*coi* and *jar1*) and in ET-signalling (*etr1*) were unable to induce ISR in presence of the PGPR WCS417r. These studies allowed to directly relating the signalling pathway involved in the defence mechanism. However, the same studies with the AM fungus were really difficult to conduct. In fact, the association between the AM

fungus and the plant showed a high complexity in the recognition and in the molecular program during the symbiosis, in which the plant hormone signalling pathways were involved.

Reviews (e.g. Hause et al. 2009; Pozo et al. 2010) explained that plant hormone signalling pathways play an important role in the control of AM symbiosis. These observations were confirmed by Herrera-Medina et al. (2008) and Tejeda-Sartorius et al. (2008). Herrera-Medina et al. (2008) showed that the tomato mutant *jai-1* insensitive increased the frequency and intensity of AM fungus colonization. To the contrary, Tejeda-Sartorius et al. (2008) showed that the tomato mutant *spr2* (JA signalling) was severely decreased in AM fungi colonization. The real impact of the SA and JA signalling pathway was yet unknown, but their effects on AM fungal colonization by direct modification of AM symbiosis underlined the difficulty to use these signalling plant mutants to study the MIR process. However, these plant mutants were particularly interesting for a deeper knowledge of the mechanisms controlling symbiotic establishment, as proved recently with tomato mutant affected in ABA and ET pathways (Martin-Rodriguez et al. 2011). A coherent perspective, following the result of the **chapter II** and this remark, should be to obtain *WRKY TF* potato mutants and analyse their effects on the AM symbiosis.

To investigate the role of the hormone signalling pathway and the gene expression pattern associated to the MIR, a solution could be to use the high-throughput expression technology in association with the application of different defence-related stimuli, as the methyl

jasmonate, acibenzolar-S-methyl, 1-aminocyclopropane-1-carboxylic acid … For example, Pozo et al. (2009) compared the response of non-mycorrhizal plants or plants colonised by either *F. mosseae* or *R. irregularis* to the application in the shoots of different defence-related stimuli. The leaves transcription profiling of mycorrhizal and non-mycorrhizal plants 24h after treatment with methyl jasmonate revealed a stronger induction in mycorrhizal plants, particularly in *F. mosseae* colonised plants, of JA-regulated genes. These results supported a possible prominent role of the JA signalling pathway in the MIR. Moreover, de Roman et al. (2011) have used this technique to demonstrate that the activation of the SA signalling pathway upon acibenzolar-S-methyl treatment resulted in an inhibition of AM root colonization. In addition, this technique was also applied to decipher the pathways involved in the ISR (Pieterse et al. 1998; Van der Ent et al. 2009) and seems also a coherent perspective to our research.

Similar to the experiment of Pozo et al. (2009) which showed a stronger induction of JA-regulated genes in the leaves of tomato plants colonized by *F. mosseae* in comparison to tomato plants colonized by *R. irregularis*, a number of studies have demonstrated a difference in plant response according to the AM fungal strain used. For example, Yao et al. (2002) have shown a stronger reduction of mortality caused by *R. solani* in potato plants colonized by *C. etunicatum*, compared to potato plants colonized by *R. intraradices*. Recently, these differences in plant response were demonstrated by the observation of a stronger induction of the oxylipin pathway in the tomato roots colonized by *F. mosseae* compared to tomato roots colonized by *R. irregularis*

(Lopez-Raez et al. 2010). As a consequence, this difference in plant response could contribute to difference in the biological protection ability of the AM fungi.

These observations underlined the importance in future studies to test different AM fungi for their potential to induce the MIR. However, the complexity of AM fungus-plants association (Smith and Read 2008) make necessary to verified the effect for each interaction. It was likely that the biological protection by AM fungi differed with the pathogen considered and the location of infection (see review Pozo et al. 2010). In addition, the review of Wehner et al. (2010) explained the functional complementarities among AM fungal species in regard to processes leading to pathogen tolerance or resistance, both among and within groups of mechanisms. The authors expected that the plant protection triggered by AM fungi against pathogens could increase with an enhancement of the AM fungal diversity, because an AM fungal assemblage provided more/different benefits in comparison to single AM species (i.e. nutrient uptake, competition with pathogen, lignifications of roots, etc...).

In the study (**Chapter VI**) on the biological protection of *R. solani* by *T. harzianum*, the results have shown a divergence of gene expression in the potato roots in comparison to previous studies (Yedidia et al. 2003; Shoresh et al. 2005). The differences observed were mainly attributed to the absence of root cell penetration and colonization of potato plants by the *Trichoderma* strain used in our experiment, verified at 6 days following contact under scanning electron microscope (De Jaeger et al. 2010). In fact, Yedidia et al.

(2003) and Shoresh et al. (2005) used the strain *T. asperellum* T203 and demonstrated its presence in the cucumber roots, restricted mainly to the epidermis and outer cortex. In addition, it was admitted that each *Trichoderma* strain has specificity in their abilities to trigger biological control, reprogram plant genes expression, alleviate physiological and abiotic stress (Shoresh et al. 2010) … As a consequence, a comparison of the different abilities between the two strains could be an interesting perspective.

So far, our thesis clearly supported the positive impact of the two beneficial microorganisms against two major potato pathogens and the possible key role of the plant hormone signalling pathways in these biological protections. Moreover, the work underlined the fundamental necessity to understand the regulation of plant hormone signalling pathways during these processes, in order to develop efficient strategies to control pathogens in potato crop. However, it seems important before further researches to validate the ecological significance of these mechanisms of plant protection under more "natural" conditions. In consequence, the first step could be to validate the efficiency of these beneficial microorganisms under *in vivo* conditions and/or to select the more relevant species for further molecular and proteomic studies in order to decipher their mechanisms of plant protection.

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Overview of the Scientific Achievements

I. Scientific publications

I.1 Research articles published

- **Gallou A**, Cranenbrouck S, Declerck S (2009) *Trichoderma harzianum* elicit defence response genes in roots of potato plantlets challenged by *Rhizoctonia solani*. European Journal of plant pathology 124:219–230

- **Gallou A**, De Jaeger N, Cranenbrouck S, Declerck S (2010) Fast track *in vitro* mycorrhization of potato plantlets allows studies on gene expression dynamics. Mycorrhiza 20:201–207

- **Gallou A**, Lucero-Mosquera HP, Cranenbrouck S, Suárez JP, Declerck S (2011) Mycorrhiza-induced resistance in potato plantlets challenged by *Phytophthora* infestans. Physiological and Molecular Plant Pathology, in press, doi:10.1016/j.pmpp.2011.06.005

- **Gallou A**, Declerck S, Cranenbrouck S (accepted: 17 July 2011) Transcriptional regulation of defence genes and involvement of the WRKY transcription factor in arbuscular mycorrhizal potato root colonization. Functional & Integrative Genomics

I.2 Research articles submitted

– **Gallou A**, Declerck S, Cranenbrouck S (submitted to European journal of Plant Pathology) Induced resistance against *Rhizoctonia solani* at different developmental stages of the arbuscular mycorrhiza colonization of potato plantlets

I.3 Research articles in preparation

– **Gallou A**, Braun A, Cranenbrouck S, Declerck S (In preparation) Systemic resistance in mycorrhizal potato plantlets challenged by *Rhizoctonia solani*

II. Conference participation

– **Gallou A**, Cranenbrouck S, Declerck S (2008) Reference gene expression in potato roots challenged with *Rhizoctonia solani* in presence of the bio-control agent *Trichoderma harzianum*. Poster at the Conference on Plant-Microbial Interaction 2008, Krakow, Poland, 2–6 July 2008

– **Gallou A**, Cranenbrouck S, Declerck S (2009) *In vitro* plant culture systems for studying plant-fungi interactions. Poster at the 7ème Colloque National de la Société Française de Phytopathologie 2009, Lyon, France, 8–11 June 2009

– **Gallou A**, Cranenbrouck S, Declerck S (2010) Biocontrol effect of *Glomus* sp. MUCL 41833 on potato plantlets against *Rhizoctonia solani* using a fast track *in vitro* mycorrhization

system. Poster at the 9th International Mycological Congress on the Biology of Fungi, Edinburgh, Scotland, 1–6 August 2010

- **Gallou A**, Braun A, Cranenbrouck S, Declerck S (2010) Effets d'un champignon mycorhizien à arbuscules sur l'induction d'une résistance chez des plantules de pomme de terre en présence de *Rhizoctonia solani*. Poster at the Secondes Journées Francophones Mycorhizes, Bruxelles, Belgique, 15-17 septembre 2010

III. Teaching and Student supervision

- International Training on *in vitro* Culture of Arbuscular Mycorrhizal Fungi: *Plant-systems* training. Louvain la Neuve, Belgium (2009-2011).

- International Training on *in vitro* Culture of Arbuscular Mycorrhizal Fungi: *ROC system* training. Louvain la Neuve, Belgium (2008).

- Supervision of one student (**Alice Braun**) from the Biology faculty (UCL), who performed her master thesis at the laboratory of Prof. S. Declerck.

Alice Braun (Biologist UCL, 2009-2010)

Effet d’un champignon mycorhizien à arbuscules sur l’induction d’une résistance systémique chez la plantule de pomme de terre cultivée en présence de *Rhizoctonia solani*.

Recent titles in the collection

2009

N°148, Pokrzywa Wojcieh, Targeting of the yeast Sna3p and Sna4p to the endosomal pathway depends on their interaction with ubiquitin ligase Rsp5p (Promoteur: Morsomme P. / février 2009)

N°149, de Lespinay Alexis, Study of seed priming mechanisms of three plant species used in revegetation of industrial sites (Promoteurs: Lutts S., Declerck S. / mai 2009)

N°150, Hoton Florence, Insights into the Ecology and Genetic Diversity of Cereulide-producing Bacillus cereus strains (Promoteur: Mahillon J. / mai 2009)

N°151, Fornelos Martins Nadine, GIL01 and relatives, a new generation of tectiviruses infecting the *Bacillus cereus* group (Promoteur: Mahillon J. / mai 2009)

N°152, Marghadi Saïd, Intégration des méthodes d'aide à la décision dans l'aménagement multifonctionnel des forêts au Maroc (Promoteurs: Devillez F., Farcy Ch. / juillet 2009)

N°153, Harahagazwe Dieudonné, Heat tolerance assessment of the potato crop: evaluation of CIP clones in the lowlands of Burundi (Promoteurs: Ledent J-F., Rusuku G. / août 2009)

N°154, Godet Marie, Survey of filamentous fungi and development of molecular tools for their rapid and accurate identification, in advanced life support systems like the International Space Station (Promoteurs: Declerck S., Munaut F. / septembre 2009)

N°155, Verbelen Claire, Nanoscale probing of the mycobacterial cell wall (Promoteur: Dufrêne Y. / octobre 2009)

N°156, Gaidashova Svetlana, Effect of plant parasitic nematodes and arbuscular mycorrhizal fungi on banana (*Musa* spp.) in the East African highland cropping systems (Promoteurs: Declerck S., De Waele D. / octobre 2009)

N°157, Gyuricza Veronika, Influence of arbuscular mycorrhizal fungi on the uptake and accumulation of radiocesium by plants and on its redistribution between plants (Promoteur: Declerck S. / novembre 2009)

N°158, Stenuit Benoît, Biological Degradation of 2,4,6-Trinitrotoluene (TNT): Bioprospecting for TNT-Denitrating Bacteria and Deciphering of Multiple TNT Denitration Pathways (Promoteur: Agathos S. / octobre 2009)

N°159, De Muynck Benoit, Expression of a functional antibody in *Nicotiana tabacum* plants and culture cells: analysis of proteolytic products and identification of putative extracellular peptidases involved (Promoteurs: Boutry M., Navarre C. / décembre 2009)

N°160, Mattern Samuel, Mapping and source identification of groundwater pollution by nitrate: Theory and application to the Brusselian sand groundwater body (Promoteur: Bogaert P. / décembre 2009)

N°161, Mehrvar Mohsen, Harahagazwe, Dieudonné, Diversity of soil-borne sugar beet viruses in Iran (Promoteur: Bragard C. / novembre 2009)

2010

N°162, Radoux Julien, Updating lan cover maps by GIS-driven analysis of very high resolution satellite images (Promoteur: Defourny P. / 13 janvier 2010)

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N°168, Hochstenbach Jean-François, The yeast proteolipid Pmp3p is a new component of the plasma membrane microdomains associated with the eisosomes (Promoteur: Morsomme P. / 4 mai 2010)

N°169, Laloy Eric, Measuring and modeling the impact of intercrop management on plotscale runoff and erosion in a continuous maize cropping system (Promoteur: Bielders C. / 31 mai 2010)

N°170, Debecker Damien, MoO3-based heterogeneous catalysts for the metathesis of propene (Promoteur: Gaigneaux E. / 28 mai 2010)

N°171, Cornelis Jean-Thomas, Impact of tree species on silicon cycling in temperate soil-tree systems (Promoteur: Delvaux B. / 25 mai 2010)

N°172, Bontemps Sophie, Towards an automated satellite-based alarm system for global forest monitoring (Promoteur: Defourny P. / 17 juin 2010)

Supporting information

Table S1 The 658 genes regulated during the pre-stage of potato root colonization by Glomus sp. MUCL 41833 as compared with the control treatment, and their changes in expression

Probe ID	Annotation	Ratio Pre-
ACDA01291D07.T3m.scf	putative GTP-binding protein DRG [Oryza sativa (japonica cultivar-group)] dbj_BAC79856.1_ putative GTP-binding protein DRG [Oryza sativa (japonica cultivar-group)]	**38,46**
STMHL53TV	At5g41350/MYC6_6 [Arabidopsis thaliana] gb_AAK82555.1_ AT5g41350/MYC6_6 [Arabidopsis thaliana] ref_NP_568590.1_ zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana]	**37,57**
MICRO.2286.C17	1,3-beta-glucan glucanohydrolase [Solanum tuberosum]	**36,71**
MICRO.2134.C1	exostosin family protein [Arabidopsis thaliana]	**34,72**
MICRO.14132.C2	hydroxyproline-rich glycoprotein family protein [Arabidopsis thaliana] NP_177422.1	**33,29**
ACDA00273G09.T3m.scf	No Hits Found	**31,20**
MICRO.3782.C1	TRZ3 (TRNASE Z 3); 3'-tRNA processing endoribonuclease/ catalytic [Arabidopsis thaliana] NP_175628.2	**29,79**
bf_mxflxxxx_0017c01.t3m.scf	No Hits Found	**29,11**
POABD13TP	transporter-related [Arabidopsis thaliana]	**28,28**
STMEF60TV	No Hits Found	**28,15**
MICRO.4334.C1	Similar to gb|Z84386 anthranilate N-hydroxycinnamoyl/benzoyltransferase from Dianthus caryophyllus [Arabidopsis thaliana]	**27,82**
MICRO.10096.C1	MutT domain protein-like [Oryza sativa (japonica cultivar-group)] dbj_BAD37816.1_ MutT domain protein-like [Oryza sativa (japonica cultivar-group)]	**27,12**
MICRO.14687.C1	hypothetical protein [Arabidopsis thaliana] ref_NP_187896.1_ oxidoreductase, 2OG-Fe(II) oxygenase family protein [Arabidopsis thaliana]	**26,50**
MICRO.12402.C1	chloroplast outer envelope protein OEP86 homolog T10P11.19 - Arabidopsis thaliana gb_AAC19285.1_ Arabidopsis putative chloroplast outer envelope 86-like protein T10P11.19 (GB: AC002330) [Arabidopsis thaliana] ref_NP_567242.2_ chloroplast outer ...	**23,76**
POAC056TP	No Hits Found	**23,26**
MICRO.5099.C3	predicted protein [Populus trichocarpa] XP_002304277.1	**22,31**
SSBN001C23u.scf	ATP-dependent clp protease ATP-binding subunit clpA homolog CD4A, chloroplast precursor pir__A35905 endopeptidase Clp (EC 3.4.21.-) ATP-binding chain cd4A, chloroplast [similarity] - tomato gb_AAA34160.1_ ATP-dependent protease (CD4A)	**21,57**
MICRO.7627.C1	putative protein [Arabidopsis thaliana] gb_AAL38360.1_ putative protein [Arabidopsis thaliana] ref_NP_974403.1_ XH/XS domain-containing protein / XS zinc finger domain-containing protein [Arabidopsis thaliana] ref_NP_190436.2_ XH/XS domain-containin ...	**20,62**
MICRO.5171.C1	unknown [Arabidopsis thaliana] gb_AAK96447.1_ AT3g57280/F28O9_130 [Arabidopsis thaliana] gb_AAK62581.1_ AT3g57280/F28O9_130 [Arabidopsis thaliana]	**19,66**
cSTA5C20TH	dynamin family protein [Arabidopsis thaliana]	**19,20**
MICRO.16902.C1	No Hits Found	**19,19**
POAC667TV	putative hyoscyamine 6 beta-hydroxylase [Solanum demissum]	**18,54**
MICRO.13429.C1	similar to AT2G30780: pentatricopeptide (PPR) repeat-containing protein	**18,35**
SDBN005N10u.scf	OSJNBb0039L24.13 [Oryza sativa (japonica cultivar-group)] emb_CAE04574.1_ OSJNBb0039L24.13 [Oryza sativa (japonica cultivar-group)]	**17,50**

MICRO.17481.C1	No Hits Found	**16,43**
MICRO.6187.C1	glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	**16,22**
MICRO.16727.C1	WRKY transcription factor (WRKY3) [Solanum lycopersicum]	**16,21**
MICRO.830.C2	ribosomal protein L27a [Petunia x hybrida]	**15,82**
MICRO.7250.C1	Cyclin-like F-box; FBD [Medicago truncatula]	**15,71**
MICRO.7460.C1	unknown protein [Arabidopsis thaliana] pir__H84857 hypothetical protein At2g42760 [imported] - Arabidopsis thaliana ref_NP_181804.1_ expressed protein [Arabidopsis thaliana]	**15,46**
bf_suspxxxx_0025E04.t3m.scf	Deoxyhypusine synthase gb_AAG53641.1_ deoxyhypusine synthase [Lycopersicon esculentum]	**15,26**
MICRO.2961.C1	No Hits Found	**15,00**
MICRO.5283.C1	cytochrome P450 [Panax ginseng]	**14,87**
cSTA34J19TH	beta-glucosidase 01 [Solanum lycopersicum]	**14,84**
MICRO.11082.C1	CYP71D10p [Glycine max] pir__T05939 cytochrome P450 monooxygenase 71D10p [soybean sp]	**14,59**
cSTA4B19TH	No Hits Found	**14,51**
bf_mxflxxxx_0071b02.t3m.scf	No Hits Found	**14,46**
MICRO.12664.C1	putative thaumatin-like protein [Solanum tuberosum]	**14,41**
SDBN002L17u.scf	No Hits Found	**14,41**
MICRO.633.C3	mitogen-activated protein kinase 3 [Lycopersicon esculentum]	**14,38**
MICRO.11037.C3	allyl alcohol dehydrogenase [Nicotiana tabacum]	**14,32**
MICRO.11559.C1	zinc finger (CCCH-type) protein-like [Oryza sativa (japonica cultivar-group)]	**14,31**
MICRO.2255.C3	LEXYL1 [Lycopersicon esculentum]	**14,17**
MICRO.8673.C1	tRNA intron endonuclease [Arabidopsis thaliana] dbj_BAA88627.1_ tRNA intron endonuclease [Arabidopsis thaliana]	**14,08**
MICRO.7771.C1	reversibly glycosylated polypeptide [Triticum aestivum]	**13,90**
BF_LBCHXXXX_0042G06_T3M.SCF	putative disease resistant protein rga4 [Solanum bulbocastanum]	**13,81**
SDBN002J05u.scf	SAR8.2 protein precursor [Capsicum annuum]	**13,61**
bf_mxflxxxx_0029a03.t3m.scf	No Hits Found	**13,56**
MICRO.1569.C1	putative protein [Arabidopsis thaliana] pir__T45956 hypothetical protein F7J8.140 - Arabidopsis thaliana ref_NP_195736.1_ e-cadherin binding protein-related [Arabidopsis thaliana]	**13,12**
STMIS76TV	putative WRKY transcription factor [Nicotiana tabacum]	**13,12**
SSBN002I15u.scf	No Hits Found	**12,99**
POCD018TP	AT3g20810/MOE17_10 [Arabidopsis thaliana] gb_AAM10317.1_ AT3g20810/MOE17_10 [Arabidopsis thaliana] ref_NP_850617.1_ transcription factor jumonji (jmjC) domain-containing protein	**12,90**
cSTA33F16TH	No Hits Found	**12,75**
cPRO14A17TH	No Hits Found	**12,57**
BF_TUBSXXXX_0041C04_T3M.SCF	No Hits Found	**12,50**
POCC282TV	putative copia-like polyprotein [Ipomoea trifida]	**12,40**
MICRO.16616.C1	WRKY transcription factor 20 [Arabidopsis thaliana]	**12,34**
MICRO.10430.C1	hypothetical protein [Nicotiana tabacum]	**12,19**
MICRO.3948.C1	Is a member of the PF_01553 Acyltransferase family. [Arabidopsis thaliana] pir__E96842 hypothetical protein F23A5.31 [imported] - Arabidopsis thaliana	**12,16**
MICRO.573.C1	putative serine carboxypeptidase precursor [Gossypium hirsutum]	**12,15**
bf_mxflxxxx_0023g11.t3m.scf	No Hits Found	**12,07**
MICRO.17922.C1	glucose-1-phosphate adenylyltransferase, putative / ADP-glucose pyrophosphorylase, putative (APS2) [Arabidopsis thaliana]	**11,98**

bf_stolxxxx_0038c01.t3m.scf	No Hits Found	**11,87**
STMCF57TV	unknown protein [Arabidopsis thaliana] gb_AAK64148.1_ unknown protein [Arabidopsis thaliana] ref_NP_568585.1_ expressed protein [Arabidopsis thaliana]	**11,75**
BF_TUBSXXXX_0012C02_T3M.SCF	No Hits Found	**11,68**
MICRO.2033.C6	metallocarboxypeptidase inhibitor [Solanum tuberosum]	**11,44**
cSTE9D8TH	S-adenosyl-methionine-sterol-C- methyltransferase [Nicotiana tabacum] pir__T03848 probable sterol 24-C-methyltransferase (EC 2.1.1.41) - common tobacco	**11,26**
MICRO.15989.C1	ABC transporter-like protein [Arabidopsis thaliana]	**11,11**
bf_acdcxxxx_0014c05.t3m.scf	No Hits Found	**11,03**
bf_arrayxxx_0102f02.t7m.scf	UDP-glucose:salicylic acid glucosyltransferase [Nicotiana tabacum]	**10,99**
MICRO.14424.C1	OSJNBa0079M09.4 [Oryza sativa (japonica cultivar-group)] emb_CAE05335.2_ OSJNBa0079M09.4 [Oryza sativa (japonica cultivar-group)]	**10,92**
MICRO.14983.C1	unnamed protein product [Arabidopsis thaliana] ref_NP_200032.1_ sugar isomerase (SIS) domain-containing protein [Arabidopsis thaliana]	**10,81**
MICRO.9928.C1	cystathionine beta-lyase [Solanum tuberosum]	**10,72**
111G12AF.esd	No Hits Found	**10,68**
bf_mxflxxxx_0002b09.t3m.scf	No Hits Found	**10,41**
MICRO.14066.C1	ethylene responsive protein [Mesembryanthemum crystallinum] gb_AAF63205.1_ AP2-related transcription factor [Mesembryanthemum crystallinum]	**10,32**
MICRO.14058.C1	similar to AT4G08250: scarecrow transcription factor family protein	**10,28**
MICRO.7765.C2	OSJNBa0096F01.6 [Oryza sativa (japonica cultivar-group)]	**10,26**
MICRO.4142.C3	putative photosystem I reaction centre PSI-D subunit precursor (psaD gene) [Solanum tuberosum]	**10,20**
MICRO.189.C1	WRKY27 [Glycine max]	**10,15**
MICRO.835.C1	hydrogen peroxide-induced 1 [Nicotiana tabacum]	**10,05**
MICRO.973.C3	No Hits Found	**9,98**
STDB002J08u.scf	NBS-coding resistance gene protein [Nicotiana tabacum] ACF22002.1	**9,89**
MICRO.4408.C1	transducin family protein / WD-40 repeat family protein [Arabidopsis thaliana]	**9,73**
bf_mxflxxxx_0035c10.t3m.scf	No Hits Found	**9,45**
MICRO.6187.C2	glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	**9,36**
SDBN001C16u.scf	similar to S-adenosyl-L-methionine:carboxyl methyltransferase family protein [Arabidopsis thaliana]	**9,23**
POCCK05TV	No Hits Found	**9,15**
MICRO.17483.C1	hypothetical protein [Arabidopsis thaliana] gb_AAD15316.1_ hypothetical protein [Arabidopsis thaliana] pir__F85045 hypothetical protein AT4g03600 [imported] - Arabidopsis thaliana	**9,09**
bf_suspxxxx_0007C10.t3m.scf	No Hits Found	**9,06**
MICRO.2416.C1	putative CCR4-associated factor [Arabidopsis thaliana] gb_AAK93623.1_ putative CCR4-associated factor [Arabidopsis thaliana] gb_AAD15397.2_ putative CCR4-associated factor [Arabidopsis thaliana] ref_NP_565735.1_ CCR4-NOT transcription complex ...	**9,02**
MICRO.14714.C1	orcinol O-methyltransferase [Rosa hybrid cultivar] emb_CAD29458.1_ orcinol O-methyltransferase [Rosa chinensis] emb_CAH05077.1_ orcinol O-methyltransferase 1 [Rosa chinensis]	**8,99**
bf_suspxxxx_0024F06.t3m.scf	putative GTP-binding protein [Arabidopsis thaliana] ref_NP_179816.1_ Ras-related GTP-binding protein, putative [Arabidopsis thaliana] pir__H84610 probable GTP-binding protein [imported]	**8,95**
MICRO.1089.C3	Vacuolar ATP synthase subunit C (V-ATPase C subunit) (Vacuolar proton pump C subunit) gb_AAC83084.1_ vacuolar adenosine triphosphatase subunit C [Mus musculus]	**8,92**

MICRO.14938.C2	calmodulin-binding protein [Beta vulgaris]	**8,89**
071B10AF.esd	putative poly(ADP-ribose) polymerase [Arabidopsis thaliana]	**8,86**
ACDA00586A10.T3m.scf	No Hits Found	**8,81**
bf_ivrootxx_0031a12.t3m.scf	No Hits Found	**8,78**
MICRO.7547.C2	unknown protein [Oryza sativa (japonica cultivar-group)] dbj_BAC10134.1_ unknown protein [Oryza sativa (japonica cultivar-group)] sp_Q8LHP0_U222_ORYSA Hypothetical UPF0222 protein P0519E12.3	**8,75**
MICRO.6335.C1	ankyrin-like protein [Arabidopsis thaliana] pir__E84725 ankyrin-like protein [imported] - Arabidopsis thaliana ref_NP_180741.1_ ankyrin repeat family protein [Arabidopsis thaliana]	**8,70**
MICRO.12229.C1	unknown protein [Oryza sativa (japonica cultivar-group)]	**8,69**
MICRO.1831.C5	plastidic ATP/ADP-transporter [Solanum tuberosum] pir__T07420 ATP/ADP-transporter, chloroplast - potato sp_O24381_TLC1_SOLTU PLASTIDIC ATP/ADP-TRANSPORTER	**8,65**
PPCBF91TH	No Hits Found	**8,59**
ACDA03632G08.T3m.scf	No Hits Found	**8,55**
cSTS27E21TH	No Hits Found	**8,50**
bf_lbchxxxx_0060b01.t3m.scf	No Hits Found	**8,45**
bf_mxflxxxx_0011c07.t3m.scf	No Hits Found	**8,39**
MICRO.1115.C1	AMMECR1 family [Arabidopsis thaliana] NP_565894.1	**8,34**
STDB005P06u.scf	starch phosphorylase (EC 2.4.1.1) precursor - potato sp_P04045_PHS1_SOLTU Alpha-1,4 glucan phosphorylase, L-1 isozyme, chloroplast precursor (Starch phosphorylase L-1) dbj_BAA00407.1_ alpha-glucan phosphorylase precursor [Solanum tuberosum]	**8,31**
bf_mxflxxxx_0071a11.t3m.scf	No Hits Found	**8,29**
MICRO.2104.C1	unknown protein [Arabidopsis thaliana] gb_AAL36347.1_ unknown protein [Arabidopsis thaliana] gb_AAF66132.1_ hypothetical protein	**8,25**
bf_suspxxxx_0032d03.t3m.scf	hypothetical protein isoform 1 [Vitis vinifera] XP_002265949.1	**8,19**
MICRO.1588.C7	osmotin-like protein [Solanum tuberosum]	**8,15**
MICRO.7553.C1	similar to AT2G32630: pentatricopeptide (PPR) repeat-containing protein	**8,03**
160F12AF.esd	patatin [Solanum tuberosum] pir__A26017 patatin T5 precursor - potato sp_P15478_PAT5_SOLTU PATATIN T5 PRECURSOR (POTATO TUBER PROTEIN) prf__1301309A patatin	**8,01**
MICRO.7968.C1	No Hits Found	**7,95**
STMHQ94TV	putative urease accessory protein F [Lycopersicon esculentum]	**7,87**
STMCY49TV	No Hits Found	**7,86**
MICRO.4268.C2	adenylate cyclase [Arabidopsis thaliana] NP_565353.1	**7,81**
MICRO.2386.C1	putative protein [Arabidopsis thaliana] pir__T51260 hypothetical protein T8M16_50 - Arabidopsis thaliana	**7,79**
BF_LBCHXXXX_0005D01_T3M.SCF	No Hits Found	**7,77**
cSTB33G21TH	putative auxin-induced SAUR-like protein [Capsicum annuum]	**7,72**
MICRO.11781.C1	F6N18.12 [Arabidopsis thaliana]	**7,70**
bf_mxlfxxxx_0042d12.t3m.scf	No Hits Found	**7,68**
bf_ivrootxx_0056h10.t3m.scf	No Hits Found	**7,60**
MICRO.529.C1	No Hits Found	**7,60**
cSTD1H12TH	hypothetical protein [Vitis vinifera] XP_002265699.1	**7,55**
ACDA02225B05.T3m.scf	No Hits Found	**7,49**
MICRO.259.C2	auxin responsive transcription factor [Arabidopsis thaliana]	**7,45**

STMIT91TV	putative WRKY transcription factor [Ricinus communis]	**7,40**
bf_arrayxxx_0078e12.t3m.scf	No Hits Found	**7,39**
MICRO.1238.C1	Oxygen-evolving enhancer protein 1, chloroplast precursor (OEE1) (33 kDa subunit of oxygen evolving system of photosystem II) (OEC 33 kDa subunit) (33 kDa thylakoid membrane protein)	**7,35**
bf_arrayxxx_0059h11.t7m.scf	No Hits Found	**7,29**
MICRO.6948.C1	No Hits Found	**7,25**
cSTB3N9TH	No Hits Found	**7,20**
MICRO.12288.C1	Similar to gb_U85207 snRNP core Sm protein homolog Sm-X5 from Mus musculus. EST gb_AA612141 comes from this gene. [Arabidopsis thaliana] pir__H86164 hypothetical protein F15K9.7 - Arabidopsis thaliana	**7,11**
MICRO.2688.C1	At3g46740/T6H20_230 [Arabidopsis thaliana] gb_AAM83239.1_ AT3g46740/T6H20_230 [Arabidopsis thaliana] emb_CAB51191.1_ chloroplast import-associated channel homolog [Arabidopsis thaliana] pir__T12975 outer envelope membrane protein homolog T6H20.230 - ...	**7,09**
STMGQ65TV	Similar to ATCG01280 | Symbols: YCF2.2 | Identical to Protein ycf2 (ycf2-B) [Arabidopsis Thaliana]	**7,04**
MICRO.7475.C1	unnamed protein product [Arabidopsis thaliana]	**7,01**
MICRO.9600.C1	No Hits Found	**6,99**
MICRO.4106.C1	No Hits Found	**6,95**
SSBN001H04u.scf	No Hits Found	**6,92**
cSTA36F13TH	chloroplast malate dehydrogenase [Lycopersicon esculentum]	**6,88**
POCAM80TV	No Hits Found	**6,81**
bf_cswbxxxx_0059d01.t3m.scf	No Hits Found	**6,79**
MICRO.12161.C1	calmodulin-like protein [Arabidopsis thaliana] pir__T08398 calmodulin homolog F18B3.50 - Arabidopsis thaliana ref_NP_190646.1_ calmodulin-related protein, putative [Arabidopsis thaliana]	**6,76**
BF_LBCHXXXX_0051C01_T3M.SCF	No Hits Found	**6,72**
MICRO.9665.C1	Contains similarity to aminoacylase from Sus scrofa domestica gi_S27010 and contains a peptidase M20 PF_01546 domain. ESTs gb_H76043, gb_AA394953, gb_AI995115, gb_AA651481 come from this gene. [Arabidopsis thaliana] pir__C96507 hypothetical protein ...	**6,59**
BF_TUBSXXXX_0054D12_T3M.SCF	No Hits Found	**6,55**
MICRO.12238.C1	hypothetical protein F9E11.7 [imported] - Arabidopsis thaliana gb_AAG51873.1_ disease resistance protein, putative	**6,52**
bf_acdcxxxx_0057a11.t3m.scf	No Hits Found	**6,49**
STMCK61TV	cytochrome P450 [Panax ginseng]	**6,42**
MICRO.8396.C1	unknown protein [Arabidopsis thaliana] gb_AAK59597.1_ unknown protein [Arabidopsis thaliana] pir__H86427 unknown protein [imported] - Arabidopsis thaliana gb_AAG51102.1_ unknown protein [Arabidopsis thaliana] ref_NP_564354.1_ early-responsive to ...	**6,38**
bf_arrayxxx_0040d02.t7m.scf	putative AP2/EREBP transcription factor [Arabidopsis thaliana] pir__H96837 unknown protein T21F11.9 [imported] - Arabidopsis thaliana gb_AAF27133.1_ unknown protein	**6,35**
cSTB1D11TV	No Hits Found	**6,31**
MICRO.12707.C1	No Hits Found	**6,30**
POCDL86TV	tRNA methyl transferase family protein [Arabidopsis thaliana] pir__H96550 hypothetical protein F11M15.16 [imported] - Arabidopsis thaliana gb_AAD30642.1_ Unknown protein [Arabidopsis thaliana]	**6,29**
bf_swstxxxx_0050c08.t3m.scf	No Hits Found	**6,25**
TBSK03589FD01.t3m.scf	No Hits Found	**6,21**
bf_ivrootxx_0009h07.t3m.scf	regulator of gene silencing [Nicotiana tabacum]	**6,18**
MICRO.11217.C1	40S ribosomal protein S21 (RPS21C) [Arabidopsis thaliana]	**6,16**

bf_mxflxxxx_0008b10.t3m.scf	calcium ion binding [Arabidopsis thaliana]	**6,13**
SDBN001J19u.scf	At1g22970/F19G10_8 [Arabidopsis thaliana] gb_AAL49950.1_ At1g22970/F19G10_8 [Arabidopsis thaliana] pir__G86363 F19G10.8 protein - Arabidopsis thaliana gb_AAB72164.1_ unknown protein	**6,11**
MICRO.11792.C1	flavin monoxygenase-like protein floozy [Petunia x hybrida]	**6,11**
PPCCP91TH	No Hits Found	**6,10**
BF_TUBSXXXX_0047C11_T3M.SCF	No Hits Found	**6,09**
MICRO.17844.C1	PREDICTED: hypothetical protein XP_515510 [Pan troglodytes]	**6,05**
cSTB34D23TH	probable CCR4-associated factor [imported] - Arabidopsis thaliana	**6,03**
MICRO.15319.C1	unnamed protein product [Arabidopsis thaliana] ref_NP_200912.1_ expressed protein [Arabidopsis thaliana]	**6,00**
SDBN005O14u.scf	No Hits Found	**5,98**
MICRO.915.C2	Disease resistance protein [Medicago truncatula]	**5,96**
PPCAG93TH	No Hits Found	**5,93**
MICRO.1309.C2	putative protein [Arabidopsis thaliana] pir__T49184 hypothetical protein MAA21.60 - Arabidopsis thaliana ref_NP_191902.1_ expressed protein [Arabidopsis thaliana]	**5,91**
BF_TUBSXXXX_0030D05_T3M.SCF	No Hits Found	**5,90**
MICRO.15253.C1	putative Avr9 elicitor response protein [Oryza sativa (japonica cultivar-group)] dbj_BAD05427.1_ putative Avr9 elicitor response protein [Oryza sativa (japonica cultivar-group)]	**5,88**
MICRO.4740.C2	flavonoid 3*-hydroxylase [Petunia x hybrida] sp_Q9SBQ9_F3PH_PETHY Flavonoid 3*-monooxygenase (Flavonoid 3*-hydroxylase) (Cytochrome P450 75B2)	**5,86**
MICRO.3062.C2	similar to heat shock transcription factor B2B [Arabidopsis thaliana]	**5,84**
cSTA40K19TH	No Hits Found	**5,81**
MICRO.14001.C2	No Hits Found	**5,80**
MICRO.17965.C1	cytosolic NADP-malic enzyme [Lycopersicon esculentum] pir__T06402 malate dehydrogenase (oxaloacetate-decarboxylating) (NADP) (EC 1.1.1.40) 2, cytosolic - tomato	**5,79**
MICRO.17624.C1	Cyclin-like F-box; F-box protein interaction domain; Galactose oxidase, central [Medicago truncatula]	**5,79**
bf_stolxxxx_0009f08.t3m.scf	No Hits Found	**5,77**
BF_TUBSXXXX_0022E02_T3M.SCF	No Hits Found	**5,76**
MICRO.5482.C1	lipid transfer protein 2 [Lycopersicon pennellii]	**5,75**
ACDA03840H12.T3m.scf	No Hits Found	**5,72**
bf_cswcxxxx_0007f05.t3m.scf	No Hits Found	**5,70**
MICRO.4759.C1	No Hits Found	**5,69**
bf_arrayxxx_0014f07.t3m.scf	No Hits Found	**5,68**
bf_mxflxxxx_0051a07.t3m.scf	hypothetical protein [Ricinus communis] XP_002522866.1	**5,66**
MICRO.8332.C1	similar to AT2G41190: amino acid transporter family protein	**5,65**
MICRO.8058.C1	No Hits Found	**5,63**
bf_suspxxxx_0048f12.t7m.scf	No Hits Found	**5,62**
MICRO.12659.C1	glucan endo-1,3-beta-D-glucosidase (EC 3.2.1.39) acidic precursor (clone gI9) - common tobacco (cv. Samsun NN) gb_AAA63542.1_ acidic beta-1,3-glucanase sp_P23547_E13G_TOBAC Glucan endo-1,3-beta-glucosidase, acidic isoform GI9 precursor ...	**5,61**
BF_TUBSXXXX_0029A05_T3M.SCF	No Hits Found	**5,60**
MICRO.13306.C1	iron-regulated transporter 1 [Lycopersicon esculentum] gb_AAF97509.1_ iron-regulated transporter 1 [Lycopersicon esculentum]	**5,60**

cSTB47L21TH	ubiquitin-specific protease (AtUBP3) [Arabidopsis thaliana] emb_CAB38904.1_ ubiquitin-specific protease (AtUBP3) [Arabidopsis thaliana] gb_AAL79594.1_ AT4g39910/T5J17_80 [Arabidopsis thaliana] gb_AAL24275.1_ AT4g39910/T5J17_80 [Arabidopsis thaliana] ...	**5,58**
POCCO23TV	No Hits Found	**5,57**
MICRO.347.C53	unknown [Solanum tuberosum] ABC01914.1	**5,55**
MICRO.958.C2	benzoyl coenzyme A: benzyl alcohol benzoyl transferase [Nicotiana tabacum]	**5,54**
bf_suspxxxx_0039b02.t3m.scf	No Hits Found	**5,53**
bf_mxlfxxxx_0066b03.t3m.scf	No Hits Found	**5,53**
bf_arrayxxx_0061d05.t7m.scf	polyphenol oxidase pir__T07098 catechol oxidase (EC 1.10.3.1) (allele POT72) - potato (fragment)	**5,52**
POADV29TP	No Hits Found	**5,51**
POCCH29TP	Nadrin [Homo sapiens] ref_NP_001006635.1_ nadrin [Homo sapiens]	**5,50**
MICRO.8600.C1	No Hits Found	**5,49**
MICRO.17667.C1	alcohol dehydrogenase (EC 1.1.1.1) - wheat (cv. Millewa)	**5,48**
bf_ivrootxx_0055d11.t3m.scf	unknown [Solanum phureja x Solanum stenotomum]	**5,47**
bf_suspxxxx_0064b07.t3m.scf	No Hits Found	**5,46**
BF_TUBSXXXX_0046A04_T3M.SCF	No Hits Found	**5,44**
MICRO.16334.C1	No Hits Found	**5,43**
bf_mxflxxxx_0033a08.t3m.scf	No Hits Found	**5,42**
MICRO.13629.C1	At2g36970/T1J8.15 [Arabidopsis thaliana] gb_AAD31582.1_ putative glucosyltransferase [Arabidopsis thaliana] gb_AAL06924.1_ At2g36970/T1J8.15 [Arabidopsis thaliana] ref_NP_181234.1_ UDP-glucoronosyl/UDP-glucosyl transferase family protein [Arabidopsi ...	**5,41**
cSTB43C21TH	No Hits Found	**5,41**
MICRO.713.C2	cytochrome P450 [Arabidopsis thaliana] gb_AAO30051.1_ cytochrome P450 [Arabidopsis thaliana] gb_AAL61910.1_ cytochrome P450 [Arabidopsis thaliana] ref_NP_188079.1_ cytochrome P450, putative [Arabidopsis thaliana]	**5,40**
MICRO.9429.C1	hypothetical protein [Vitis vinifera] XP_002266635.1	**5,39**
SSBN002H19u.scf	No Hits Found	**5,38**
MICRO.17961.C1	pre-mRNA cleavage complex-related [Arabidopsis thaliana]	**5,37**
MICRO.8847.C1	No Hits Found	**5,36**
BPLI8C14TH	glucose-6-phosphate/phosphate-translocator precursor [Solanum tuberosum] pir__T06997 probable glucose-6-phosphate/phosphate-translocator precursor - potato (fragment)	**5,35**
MICRO.11279.C1	chitinase; endochitinase [Solanum tuberosum]	**5,34**
MICRO.17959.C1	No Hits Found	**5,32**
MICRO.17448.C1	hypothetical protein CNBF0780 [Cryptococcus neoformans var. neoformans B-3501A]	**5,32**
bf_ivrootxx_0063b01.t3m.scf	No Hits Found	**5,31**
MICRO.13609.C1	leucine-rich repeat receptor-like protein kinase 1 [Populus nigra]	**5,30**
MICRO.6714.C1	unknown protein [Arabidopsis thaliana] gb_AAM66017.1_ unknown [Arabidopsis thaliana] gb_AAM10307.1_ AT3g10020/T22K18_16 [Arabidopsis thaliana] gb_AAL06834.1_ AT3g10020/T22K18_16 [Arabidopsis thaliana] gb_AAK97732.1_ AT3g10020/T22K18_16 [Arabidopsis ...	**5,30**
MICRO.12947.C1	unknown protein [Arabidopsis thaliana] gb_AAL38810.1_ unknown protein [Arabidopsis thaliana] emb_CAB81913.1_ putative protein [Arabidopsis thaliana] pir__T48152 hypothetical protein T10O8.10 - Arabidopsis thaliana ref_NP_195750.1_ ...	**5,29**
MICRO.5864.C1	similar to AT2G03550: hydrolase	**5,29**

bf_suspxxxx_0007E10.t3m.scf	No Hits Found	**5,28**
MICRO.3818.C1	MATE efflux protein-related [Arabidopsis thaliana]	**5,27**
STMIR15TV	ABC transporter, putative [Arabidopsis thaliana] ref_NP_174330.1_ ATP-binding cassette transport protein, putative [Arabidopsis thaliana] pir__E86428 probable ABC transporter [imported]	**5,26**
bf_mxlfxxxx_0030h03.t3m.scf	NTGP4 [Nicotiana tabacum]	**5,25**
MICRO.1142.C1	aminoacyl-tRNA hydrolase [Arabidopsis thaliana] NP_568340.1	**5,24**
MICRO.9952.C2	unknown [Arabidopsis thaliana] dbj_BAB01994.1_ unnamed protein product [Arabidopsis thaliana] dbj_BAD44537.1_ unknown protein [Arabidopsis thaliana] dbj_BAD44457.1_ unknown protein [Arabidopsis thaliana] ref_NP_566844.1_ expressed protein ...	**5,22**
MICRO.8555.C2	probable peptide transporter [imported] - Arabidopsis thaliana gb_AAF29404.1_ peptide transporter, putative [Arabidopsis thaliana] ref_NP_175630.1_ proton-dependent oligopeptide transport (POT) family protein [Arabidopsis thaliana]	**5,21**
MICRO.2055.C3	No Hits Found	**5,21**
MICRO.3003.C1	At1g60080 [Arabidopsis thaliana] gb_AAD14485.1_ Similar to gb_AF025438 Opa-interacting protein (OIP2) from Homo sapiens. [Arabidopsis thaliana] pir__D96625 hypothetical protein T2K10.14 [imported] - Arabidopsis thaliana ref_NP_176216.1_ 3* ...	**5,20**
MICRO.5426.C4	pathogenesis related protein isoform b1 [Solanum phureja]	**5,18**
bf_mxflxxxx_0027d09.t3m.scf	No Hits Found	**5,17**
bf_stolxxxx_0054c05.t3m.scf	No Hits Found	**5,16**
MICRO.10935.C1	No Hits Found	**5,15**
MICRO.7189.C1	Small heat shock protein, chloroplast precursor pir__T06324 heat shock protein 21, chloroplast - tomato gb_AAB07023.1_ heat shock protein	**5,15**
MICRO.4350.C1	Unknown protein [Arabidopsis thaliana] gb_AAL34184.1_ unknown protein [Arabidopsis thaliana] gb_AAK59507.1_ unknown protein [Arabidopsis thaliana] pir__H96814 hypothetical protein T30F21.5 [imported] - Arabidopsis thaliana ref_NP_565184.1_ integral ...	**5,13**
SDBT002G23x.scf	No Hits Found	**5,12**
ACDA01761C09.T3m.scf	No Hits Found	**5,11**
MICRO.13039.C1	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]	**5,10**
bf_cswbxxxx_0007b10.t3m.scf	No Hits Found	**5,09**
cSTC5K5TH	No Hits Found	**5,09**
MICRO.1992.C2	gb protein [Sorghum bicolor]	**5,08**
bf_mxlfxxxx_0040f07.t3m.scf	No Hits Found	**5,06**
MICRO.5148.C2	hypothetical protein PGEC568D21.10 [Solanum demissum]	**5,05**
bf_swstxxxx_0037f06.t3m.scf	No Hits Found	**5,04**
STMJD44TV	putative translation transactivator/inclusion body protein [Nicotiana tabacum]	**5,03**
MICRO.17754.C1	putative salt tolerance protein 5 [Oryza sativa (japonica cultivar-group)]	**5,01**
MICRO.11371.C1	No Hits Found	**5,01**
ACDA04816B04.T3m.scf	No Hits Found	**5,00**
MICRO.13355.C1	50S ribosomal protein l9 [Bartonella henselae str. Houston-1]	**4,99**
MICRO.13304.C1	beta-fructofuranosidase	**4,98**
BF_LBCHXXXX_0022B06_T3M.SCF	ENSANGP00000010437 [Anopheles gambiae]	**4,98**
bf_ivrootxx_0021f12.t3m.scf	No Hits Found	**4,96**
MICRO.8980.C2	Vacuolar protein-sorting protein 33 homolog (AtVPS33) gb_AAK48903.1_ VPS33 [Arabidopsis thaliana] ref_NP_567009.1_ vacuolar protein sorting protein, putative [Arabidopsis thaliana]	**4,95**

cSTC6K15TH	No Hits Found	**4,94**
MICRO.17120.C1	unknown protein [Arabidopsis thaliana]	**4,94**
MICRO.11018.C1	tumor-related protein [Nicotiana glauca x Nicotiana langsdorffii], putative EXLB1 [Arabidopsis thaliana]	**4,92**
MICRO.2219.C8	Phenylalanine ammonia-lyase 2 [Solanum tuberosum]	**4,91**
MICRO.8516.C1	similar to AT1G29370: kinase-related	**4,91**
BF_LBCHXXXX_0054H09_T3M.SCF	No Hits Found	**4,90**
MICRO.2876.C1	similar to WRKY transcription factor 6 [Solanum tuberosum]	**4,89**
MICRO.538.C1	No Hits Found	**4,87**
STMHL93TV	No Hits Found	**4,86**
bf_mxflxxxx_0075h12.t3m.scf	basic chitinase [Arabidopsis thaliana] gb_AAL90922.1_ AT3g16920/K14A17_4 [Arabidopsis thaliana] gb_AAL06524.1_ AT3g16920/K14A17_4 [Arabidopsis thaliana] ref_NP_188317.1_ glycoside hydrolase family 19 protein [Arabidopsis thaliana]	**4,86**
bf_mxlfxxxx_0065a05.t3m.scf	No Hits Found	**4,85**
MICRO.10369.C1	subtilisin-like protease [Lycopersicon esculentum] emb_CAA06997.1_ subtilisin-like protease [Lycopersicon esculentum] pir__T07169 subtilisin-like proteinase (EC 3.4.21.-) 3 - tomato	**4,84**
MICRO.4080.C2	No Hits Found	**4,83**
MICRO.331.C95	No Hits Found	**4,83**
MICRO.14477.C1	abscisic acid insensitive 5 (ABI5) [Arabidopsis thaliana] gb_AAK19599.1_ bZIP protein [Arabidopsis thaliana] ref_NP_565840.1_ bZIP transcription factor family protein / ABA-responsive element-binding protein, putative [Arabidopsis thaliana]	**4,82**
bf_mxlfxxxx_0032f01.t3m.scf	No Hits Found	**4,81**
SDBN002H02u.scf	hypothetical protein LES1_20t00003 [Solanum lycopersicum] ABI34272.1	**4,81**
MICRO.17709.C1	No Hits Found	**4,80**
cSTA14C9TH	No Hits Found	**4,80**
TBSK01135FG07t3m.scf	hypothetical protein PGEC542.21 [Solanum demissum]	**4,77**
MICRO.10815.C1	endonuclease/exonuclease/phosphatase family protein [Arabidopsis thaliana]	**4,77**
MICRO.7884.C2	protein kinase-like protein [Capsicum annuum]	**4,75**
bf_suspxxxx_0048g10.t7m.scf	similar to WRKY transcription factor 6 [Solanum tuberosum]	**4,74**
SSBT001M18x.scf	No Hits Found	**4,72**
POCAH90TV	meprin and TRAF homology domain-containing protein / MATH domain-containing protein [Arabidopsis thaliana]	**4,70**
MICRO.9126.C1	patatin-like protein 1 [Nicotiana tabacum]	**4,69**
MICRO.7173.C2	hypothetical protein isoform 1 [Vitis vinifera] XP_002264446.1	**4,67**
cSTA20C1TH	No Hits Found	**4,66**
bf_acdaxxxx_0063e08.t3m.scf	hypothetical protein PGEC858M02.5 [Solanum demissum]	**4,66**
bf_suspxxxx_0033h10.t3m.scf	gag-pol polyprotein-like [Solanum tuberosum]	**4,65**
MICRO.4083.C7	No Hits Found	**4,64**
SSBT004G08x.scf	hydrogen peroxide-induced 1 [Nicotiana tabacum]	**4,63**
MICRO.15691.C1	No Hits Found	**4,62**
MICRO.8547.C1	hypothetical protein F13K9.3 - Arabidopsis thaliana gb_AAG51477.1_ hypothetical protein [Arabidopsis thaliana] ref_NP_174113.1_ microtubule associated protein (MAP65/ASE1) family protein [Arabidopsis thaliana]	**4,62**
cSTB45B9TH	No Hits Found	**4,61**
MICRO.6780.C4	Hsp20.0 protein [Lycopersicon peruvianum]	**4,61**

MICRO.18121.C1	No Hits Found	**4,61**
cPRO29E1TH	Contains similarity to aminoacylase from Sus scrofa domestica gi_S27010 and contains a peptidase M20 PF_01546 domain. ESTs gb_H76043, gb_AA394953, gb_AI995115, gb_AA651481 come from this gene. [Arabidopsis thaliana] pir__C96507 hypothetical protein ...	**4,60**
MICRO.11618.C1	PREDICTED OJ1117_F10.8 gene product [Oryza sativa (japonica cultivar-group)]	**4,60**
MICRO.1010.C1	unnamed protein product [Arabidopsis thaliana]	**4,59**
cSTB31N13TH	No Hits Found	**4,59**
MICRO.12087.C1	putative retroelement pol polyprotein-like [Solanum tuberosum]	**4,58**
MICRO.10760.C1	At3g18850 [Arabidopsis thaliana] dbj_BAB03094.1_ acyl-CoA:1-acylglycerol-3-phosphate acyltransferase [Arabidopsis thaliana] gb_AAM20700.1_ unknown protein [Arabidopsis thaliana] ref_NP_974335.1_ phospholipid/glycerol acyltransferase family protein ...	**4,58**
MICRO.10440.C1	Cf-2.2 [Lycopersicon pimpinellifolium]	**4,57**
BF_LBCHXXXX_0019F11_T3M.SCF	No Hits Found	**4,56**
bf_mxlfxxxx_0019h04.t3m.scf	ubiquitin-conjugating enzyme 7 (UBC7) [Arabidopsis thaliana]	**4,55**
MICRO.11677.C3	P450 hydroxylase [Solanum melongena] pir__S36807 cytochrome P450 71A3 - eggplant (fragment) sp_P37119_C713_SOLME CYTOCHROME P450 71A3 (CYPLXXIA3) (P-450EG3)	**4,53**
cSTS11P21TH	unknown [Arabidopsis thaliana]	**4,52**
MICRO.7474.C4	NPR1-interactor protein 1 [Lycopersicon esculentum]	**4,52**
bf_mxflxxxx_0073g07.t3m.scf	putative polyprotein [Solanum tuberosum]	**4,50**
STMGC88TV	hypothetical protein [Arabidopsis thaliana] gb_AAB88637.1_ hypothetical protein [Arabidopsis thaliana] pir__T00921 hypothetical protein At2g42110 [imported] - Arabidopsis thaliana ref_NP_181741.1_ expressed protein [Arabidopsis thaliana]	**4,49**
MICRO.3667.C1	unknown protein [Arabidopsis thaliana]	**4,48**
MICRO.14232.C1	PREDICTED: similar to 60S acidic ribosomal protein P1 [Pan troglodytes] ref_NP_000994.1_ ribosomal protein P1 isoform 1 [Homo sapiens] gb_AAH07590.1_ Ribosomal protein P1, isoform 1 [Homo sapiens] gb_AAH03369.1_ Ribosomal protein P1, isoform 1 [Homo ...	**4,48**
MICRO.4654.C1	alcohol oxidase-related [Arabidopsis thaliana]	**4,46**
MICRO.6910.C1	lipase class 3 family protein [Arabidopsis thaliana]	**4,45**
bf_lbchxxxx_0061f09.t3m.scf	No Hits Found	**4,43**
MICRO.906.C1	glutathione-s-transferase, putative [Arabidopsis thaliana] gb_AAN76990.1_ MAPEG-like protein [Arabidopsis thaliana] gb_AAL47424.1_ At1g65820/F1E22_4 [Arabidopsis thaliana] gb_AAK62622.1_ At1g65820/F1E22_4 [Arabidopsis thaliana] ref_NP_176758.1_ ...	**4,42**
MICRO.9542.C2	No Hits Found	**4,41**
MICRO.10439.C1	class II chitinase [Solanum tuberosum]	**4,39**
cSTS10N24TH	Similar to Kin17 protein [Arabidopsis thaliana] gb_AAK93688.1_ unknown protein [Arabidopsis thaliana] gb_AAK25842.1_ unknown protein [Arabidopsis thaliana] pir__H96596 hypothetical protein T5A14.13 [imported] - Arabidopsis thaliana ref_NP_564690.1_ ...	**4,38**
MICRO.16637.C1	No Hits Found	**4,37**
BF_TUBSXXXX_0057C01_T3M.SCF	No Hits Found	**4,36**
PotatoF1489.scf	small heat-shock protein homolog [Solanum tuberosum] pir__T07031 low molecular weight heat shock protein homolog - potato	**4,36**
MICRO.11840.C1	ferric reductase [Medicago truncatula]	**4,35**
MICRO.6379.C1	lipase-like protein [Arabidopsis thaliana] gb_AAK62388.1_ lipase-like protein [Arabidopsis thaliana] ref_NP_567350.1_ hydrolase, alpha/beta fold family protein [Arabidopsis thaliana]	**4,33**
SSBT005N13x.scf	No Hits Found	**4,32**

MICRO.11595.C1	pathogenesis-related protein 5-1 [Helianthus annuus]	**4,32**
BF_TUBSXXXX_0048H01_T3M.SCF	No Hits Found	**4,31**
MICRO.4255.C1	similar to AT1G76990 | Symbols: ACR3 | ACR3 (ACT Domain Repeat 3)	**4,30**
bf_lbchxxxx_0059e02.t3m.scf	No Hits Found	**4,29**
MICRO.17541.C1	Hypothetical protein CBG12710 [Caenorhabditis briggsae]	**4,28**
MICRO.5923.C2	unknown protein [Arabidopsis thaliana] ref_NP_187168.1_ phosphoglycerate/bisphosphoglycerate mutase family protein [Arabidopsis thaliana]	**4,27**
STMGT91TV	homeobox leucine-zipper protein [Zinnia elegans]	**4,27**
MICRO.15066.C1	receptor-like protein kinase [Nicotiana tabacum]	**4,25**
MICRO.6245.C2	putative pectinesterase [Arabidopsis thaliana] gb_AAK28637.1_ putative pectinesterase [Arabidopsis thaliana] dbj_BAB09799.1_ pectinesterase [Arabidopsis thaliana] ref_NP_200149.1_ pectinesterase family protein [Arabidopsis thaliana]	**4,24**
bf_arrayxxx_0035b05.t7m.scf	GTP cyclohydrolase II/3,4-dihydroxy-2-butanone 4-phosphate synthase [Malus x domestica]	**4,24**
BF_LBCHXXXX_0013F08_T3M.SCF	No Hits Found	**4,22**
bf_mxflxxxx_0009b12.t3m.scf	myosin II heavy chain-like [Oryza sativa (japonica cultivar-group)]	**4,21**
BF_LBCHXXXX_0022F02_T3M.SCF	No Hits Found	**4,20**
STDB005O19u.scf	AT3g52570/F22O6_50 [Arabidopsis thaliana] gb_AAL67113.1_ AT3g52570/F22O6_50 [Arabidopsis thaliana] ref_NP_190825.2_ expressed protein [Arabidopsis thaliana]	**4,19**
bf_suspxxxx_0008H07.t3m.scf	class III peroxidase [Gossypium hirsutum]	**4,18**
ACDA04576F04.T3m.scf	TSJT1 [Nicotiana tabacum] sp_P24805_TSJT_TOBAC Stem-specific protein TSJT1 pir__S13551 stem-specific protein - common tobacco	**4,18**
MICRO.8260.C1	putative poly(ADP-ribose) glycohydrolase [Arabidopsis thaliana] gb_AAK72256.1_ poly(ADP-ribose) glycohydrolase [Arabidopsis thaliana] ref_NP_565730.1_ poly (ADP-ribose) glycohydrolase (PARG) family protein [Arabidopsis thaliana]	**4,17**
STMGS16TH	DNA-binding protein [Daucus carota]	**4,16**
MICRO.11238.C1	cytochrome P450 [Lotus corniculatus var. japonicus]	**4,15**
ACDA03767B11.T3m.scf	No Hits Found	**4,15**
bf_acdcxxxx_0057f01.t3m.scf	No Hits Found	**4,15**
MICRO.1835.C1	putative rubisco expression protein [Arabidopsis thaliana] gb_AAK28639.1_ putative rubisco expression protein [Arabidopsis thaliana] ref_NP_566752.1_ AAA-type ATPase family protein / ankyrin repeat family protein [Arabidopsis thaliana]	**4,13**
ACDA04577F05.T3m.scf	No Hits Found	**4,12**
091B08AF.esd	No Hits Found	**4,11**
cSTE13L10TH	P0581F09.11 [Oryza sativa (japonica cultivar-group)] dbj_BAB64214.1_ P0581F09.11 [Oryza sativa (japonica cultivar-group)] dbj_BAB39954.1_ contains ESTs AU062927(C51629),AU030693(E60120)~similar to Arabidopsis thaliana chromosome 1, F14P1.8~unknown ...	**4,10**
ACDA02472F12.T3m.scf	No Hits Found	**4,10**
bf_mxlfxxxx_0010b10.t3m.scf	UDP-glucose:salicylic acid glucosyltransferase [Nicotiana tabacum]	**4,08**
STMCF48TH	unnamed protein product [Arabidopsis thaliana] ref_NP_198887.1_ zinc finger (C2H2 type) family protein [Arabidopsis thaliana]	**4,07**
BF_TUBSXXXX_0039C11_T3M.SCF	No Hits Found	**4,06**
bf_swstxxxx_0045a02.t3m.scf	No Hits Found	**4,06**
STMHC24THB	BEL1-related homeotic protein 5 [Solanum tuberosum]	**4,05**
cSTB21E19TH	putative bHLH transcription factor [Arabidopsis thaliana]	**4,05**

bf_ivrootxx_0024d02.t3m.scf	No Hits Found	**4,04**
BF_LBCHXXXX_0039A03_T3M.SCF	No Hits Found	**4,03**
bf_mxlfxxxx_0034d02.t3m.scf	orcinol O-methyltransferase [Rosa hybrid cultivar]	**4,02**
MICRO.4165.C12	ribulose bisphosphate carboxylase [Solanum tuberosum] sp_P26575_RBSA_SOLTU Ribulose bisphosphate carboxylase small chain 2A, chloroplast precursor (RuBisCO small subunit 2A) pir__RKPOS2 ribulose-bisphosphate carboxylase (EC 4.1.1.39) precursor small ...	**4,02**
cSTA4J24TH	unknown [Arabidopsis thaliana]	**4,01**
MICRO.9129.C1	At5g10910/T30N20_180 [Arabidopsis thaliana] emb_CAB96846.1_ putative protein [Arabidopsis thaliana] gb_AAL24223.1_ AT5g10910/T30N20_180 [Arabidopsis thaliana] pir__T50800 hypothetical protein T30N20_180 - Arabidopsis thaliana ref_NP_196652.1_ mraW ...	**4,00**
MICRO.9429.C3	hypothetical protein [Vitis vinifera] XP_002266635.1	**3,99**
SDBN004E04u.scf	No Hits Found	**3,98**
MICRO.16990.C1	No Hits Found	**3,97**
MICRO.1612.C1	hypothetical protein [Oryza sativa (japonica cultivar-group)] gb_AAP54875.1_ hypothetical protein [Oryza sativa (japonica cultivar-group)] gb_AAK20044.1_ hypothetical protein [Oryza sativa (japonica cultivar-group)]	**3,96**
SDBN004B18u.scf	No Hits Found	**3,95**
cSTB6M18TH	acyltransferase-like [Solanum melongena]	**3,95**
MICRO.14283.C1	P-450 IIA3 protein (1 is 3rd base in codon) [Homo sapiens]	**3,94**
TBSK02852FF08.t3m.scf	No Hits Found	**3,94**
MICRO.14266.C2	zinc finger (MYND type) family protein [Arabidopsis thaliana]	**3,93**
STDB005M08u.scf	No Hits Found	**3,92**
MICRO.8569.C2	putative lipid transfer protein [Solanum tuberosum]	**3,92**
bf_suspxxxx_0040b06.t3m.scf	NADPH-ferrihemoprotein reductase (EC 1.6.2.4) - mung bean	**3,91**
POCAW44TP	unnamed protein product [Arabidopsis thaliana] ref_NP_188563.1_ leucine-rich repeat family protein [Arabidopsis thaliana]	**3,90**
STMIB81TV	unknown protein [Oryza sativa (japonica cultivar-group)]	**3,89**
MICRO.5553.C3	aspartic endopeptidase [Cucurbita pepo]	**3,88**
MICRO.3742.C1	similar to AT2G38670 | Symbols: PECT1 | PECT1 (PHOSPHORYLETHANOLAMINE CYTIDYLYLTRANSFERASE 1); ethanolamine-phosphate cytidylyltransferase	**3,87**
MICRO.12431.C1	No Hits Found	**3,86**
SSBT003I15x.scf	No Hits Found	**3,85**
cSTB20H9TH	No Hits Found	**3,85**
SDBN006L17u.scf	No Hits Found	**3,84**
MICRO.381.C1	Isocitrate lyase (Isocitrase) (Isocitratase) (ICL) pir__T06353 isocitrate lyase (EC 4.1.3.1) - tomato gb_AAA82738.1_ isocitrate lyase	**3,82**
cSTS11N3TH	pyrophosphate-dependent phosphofructo-1-kinase [Prunus armeniaca]	**3,81**
MICRO.11519.C1	No Hits Found	**3,80**
MICRO.10581.C1	putative histidine-containing phosphotransfer protein 4 [Populus x canadensis]	**3,79**
MICRO.8006.C1	hypothetical protein [Vitis vinifera] CAN79287.1	**3,79**
BPLI6C22TH	putative S-receptor kinase homolog 2 precursor [Oryza sativa (japonica cultivar-group)] dbj_BAD05462.1_ putative S-receptor kinase homolog 2 precursor [Oryza sativa (japonica cultivar-group)] dbj_BAD01294.1_ putative S-receptor kinase homolog 2 ...	**3,79**
MICRO.5528.C2	No Hits Found	**3,78**
bf_stolxxxx_0032A06.t3m.scf	No Hits Found	**3,77**

POCA938TP	putative polyprotein [Solanum demissum]	**3,76**
MICRO.592.C16	Probable glutathione S-transferase (Pathogenesis-related protein 1) pir__T07595 glutathione transferase (EC 2.5.1.18) homolog GST1 - potato gb_AAA68430.1_ glutathione S-transferase	**3,75**
bf_suspxxxx_0011e01.t3m.scf	Similar to fumarylacetoacetate hydrolase, gb_L41670 from Emericella nidulans. [Arabidopsis thaliana] pir__F86255 hypothetical protein [imported] - Arabidopsis thaliana	**3,74**
bf_ivrootxx_0064g07.t3m.scf	putative copper transport protein [Arabidopsis thaliana] gb_AAO50473.1_ putative copper transport protein [Arabidopsis thaliana] ref_NP_850091.1_ copper transporter, putative [Arabidopsis thaliana]	**3,74**
bf_suspxxxx_0015d03.t3m.scf	No Hits Found	**3,73**
cSTB37F21TH	Transcriptional activator TAF-1 pir__S16346 hypothetical protein - common tobacco	**3,72**
cSTB39B13TH	No Hits Found	**3,71**
cSTA18O9TH	predicted protein of unknown function [Arabidopsis thaliana] gb_AAD17338.1_ F15P23.2 gene product [Arabidopsis thaliana] pir__B85010 hypothetical protein AT4g00740 [imported] - Arabidopsis thaliana	**3,71**
BPLI18D5TH	No Hits Found	**3,70**
MICRO.12390.C1	ferric-chelate reductase [Lycopersicon esculentum]	**3,68**
MICRO.10561.C1	ring domain containing protein [Capsicum annuum]	**3,67**
MICRO.8524.C3	At5g19860 [Arabidopsis thaliana] ref_NP_568383.1_ expressed protein [Arabidopsis thaliana]	**3,67**
bf_suspxxxx_0036c08.t3m.scf	glutamate decarboxylase isozyme 1 [Nicotiana tabacum]	**3,66**
MICRO.1238.C4	Oxygen-evolving enhancer protein 1, chloroplast precursor (OEE1) (33 kDa subunit of oxygen evolving system of photosystem II) (OEC 33 kDa subunit)	**3,65**
bf_mxlfxxxx_0023a07.t3m.scf	No Hits Found	**3,63**
MICRO.3583.C2	putative disease resistance protein [Solanum tuberosum]	**3,62**
MICRO.17395.C1	cytochrome P450 [Solanum tuberosum]	**3,62**
MICRO.5552.C1	putative protein kinase Xa21 [Oryza sativa (japonica cultivar-group)] dbj_BAD34191.1_ putative protein kinase Xa21 [Oryza sativa (japonica cultivar-group)]	**3,62**
MICRO.17114.C1	zinc finger-like protein [Arabidopsis thaliana] pir__T50806 zinc finger-like protein - Arabidopsis thaliana ref_NP_196658.1_ zinc finger (C2H2 type) family protein [Arabidopsis thaliana]	**3,61**
MICRO.15534.C1	phosphoribosylanthranilate transferase-like protein [Arabidopsis thaliana] ref_NP_197299.1_ C2 domain-containing protein [Arabidopsis thaliana]	**3,60**
cPRO8G23TH	histone H2B1 [Gossypium hirsutum] pir__T09722 histone H2B1 - upland cotton sp_O22582_H2B_GOSHI Histone H2B	**3,58**
bf_mxflxxxx_0021d04.t3m.scf	No Hits Found	**3,57**
MICRO.1081.C9	No Hits Found	**3,57**
bf_cswcxxxx_0002b01.t3m.scf	luminal binding protein (BiP) [Nicotiana tabacum] pir__S21880 dnaK-type molecular chaperone blp5 precursor - common tobacco sp_Q03685_BIP5_TOBAC Luminal binding protein 5 precursor (BiP 5) (78 kDa glucose-regulated protein homolog 5) (GRP 78-5)	**3,56**
POCDF37TP	No Hits Found	**3,56**
bf_ivrootxx_0055e06.t3m.scf	putative Ligatin (Hepatocellular carcinoma-associated antigen 56) [Oryza sativa (japonica cultivar-group)] dbj_BAD16422.1_ putative Ligatin (Hepatocellular carcinoma-associated antigen 56) [Oryza sativa (japonica cultivar-group)]	**3,55**
cPRO9O19TH	No Hits Found	**3,54**
MICRO.7301.C1	unknown protein [Arabidopsis thaliana] gb_AAK92723.1_ unknown protein [Arabidopsis thaliana] dbj_BAB02262.1_ unnamed protein product [Arabidopsis thaliana] ref_NP_566858.1_ expressed protein [Arabidopsis thaliana]	**3,53**
STMDA89TV	No Hits Found	**3,52**

MICRO.2876.C2	WRKY transcription factor 6 [Solanum tuberosum]	**3,51**
MICRO.14106.C2	unknown protein [Arabidopsis thaliana] pir__F86340 protein F2D10.34 [imported] - Arabidopsis thaliana ref_NP_173506.1_ zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana]	**3,51**
STMJA76TV	Contains similarity to receptor protein kinase-like protein from Arabidopsis thaliana gb_AL161513. It contains a eukaryotic protein kinase domain PF_00069. EST gb_AI997574 comes from this gene pir__B86479 hypothetical protein F14D7.1 - Arabidopsis ...	**3,50**
SDBN002L14u.scf	No Hits Found	**3,47**
MICRO.7843.C2	Unknown protein [Arabidopsis thaliana] gb_AAM63885.1_ unknown [Arabidopsis thaliana] gb_AAL66928.1_ unknown protein [Arabidopsis thaliana] gb_AAK62422.1_ Unknown protein [Arabidopsis thaliana] pir__C86158 hypothetical protein F22D16.19 - Arabidopsis ...	**3,46**
cSTA27M14TH	No Hits Found	**3,46**
BF_TUBSXXXX_0054A12_T3M.SCF	No Hits Found	**3,46**
MICRO.7548.C1	expressed protein [Arabidopsis thaliana]	**3,45**
SSBT002M13x.scf	No Hits Found	**3,43**
MICRO.13345.C1	NIMIN2c protein [Nicotiana tabacum]	**3,43**
MICRO.2286.C42	beta-1,3-glucanase (PR2) [Solanum tuberosum]	**3,43**
bf_stolxxxx_0040c06.t3m.scf	No Hits Found	**3,43**
BF_LBCHXXXX_0022A08_T3M.SCF	No Hits Found	**3,43**
MICRO.5979.C1	putative protein [Arabidopsis thaliana] pir__T47428 hypothetical protein T22K7.60 - Arabidopsis thaliana ref_NP_190024.1_ expressed protein [Arabidopsis thaliana]	**3,42**
cPRO7F2TH	AT5g59960/mmn10_180 [Arabidopsis thaliana] gb_AAK32906.1_ AT5g59960/mmn10_180 [Arabidopsis thaliana]	**3,42**
MICRO.16224.C1	hypothetical protein [Oryza sativa (japonica cultivar-group)]	**3,41**
SDBN003H19u.scf	putative short-chain alcohol dehydrogenase [Oryza sativa (japonica cultivar-group)]	**3,41**
bf_arrayxxx_0043h04.t3m.scf	hydroxymethylglutaryl coenzyme A synthase [Hevea brasiliensis]	**3,41**
MICRO.3873.C1	IQD12 IQD12 (IQ-domain 12); calmodulin binding [Arabidopsis thaliana]	**3,41**
bf_mxlfxxxx_0022d10.t3m.scf	No Hits Found	**3,40**
MICRO.3672.C1	No Hits Found	**3,40**
bf_suspxxxx_0045g12.t7m.scf	No Hits Found	**3,39**
bf_mxflxxxx_0036f07.t3m.scf	No Hits Found	**3,39**
SDBN005E19u.scf	geranylgeranyl pyrophosphate synthase 1 (GGPS1) [Solanum lycopersicum]	**3,37**
MICRO.11948.C1	No Hits Found	**3,36**
cSTD1O5THB	putative Kunitz-type tuber invertase inhibitor precursor [Solanum tuberosum]	**3,36**
cSTB40C24TH	No Hits Found	**3,35**
bf_acdcxxxx_0018h01.t3m.scf	unknown [Arabidopsis thaliana] ref_NP_564349.1_ expressed protein [Arabidopsis thaliana]	**3,35**
BF_LBCHXXXX_0024D04_T3M.SCF	No Hits Found	**3,34**
PPCBA67TH	unknown protein [Arabidopsis thaliana] gb_AAF79235.1_ F10B6.27 [Arabidopsis thaliana] gb_AAL62389.1_ unknown protein [Arabidopsis thaliana] ref_NP_172940.1_ expressed protein [Arabidopsis thaliana]	**3,34**
STMHI78TV	resistance protein SlVe1 precursor [Solanum lycopersicoides] gb_AAP20228.1_ resistance protein SlVe1 precursor [Solanum lycopersicoides]	**3,33**
MICRO.13746.C1	No Hits Found	**3,33**
MICRO.18314.C1	Malonyl-CoA-[acyl-carrier-protein] transacylase [Bacillus cereus ATCC 14579] gb_AAP10772.1_ Malonyl-CoA-[acyl-carrier-protein] transacylase [Bacillus cereus ATCC 14579]	**3,33**

cSTA27E5TH	putative NADH-ubiquinone oxidoreductase [Oryza sativa (japonica cultivar-group)] gb_AAT44268.1_ putative NADH-ubiquinone oxidoreductase [Oryza sativa (japonica cultivar-group)]	**3,32**
STMCS09TV	No Hits Found	**3,32**
cSTB15N23TH	reverse transcriptase like protein [Arabidopsis thaliana] emb_CAB10226.1_ reverse transcriptase like protein [Arabidopsis thaliana] pir__H71406 probable reverse transcriptase-like protein - Arabidopsis thaliana	**3,32**
BF_TUBSXXXX_0033G12_T3M.SCF	No Hits Found	**3,32**
BF_LBCHXXXX_0037E03_T3M.SCF	No Hits Found	**3,31**
BF_LBCHXXXX_0013C03_T3M.SCF	No Hits Found	**3,31**
bf_swstxxxx_0022c10.t3m.scf	No Hits Found	**3,31**
cSTA3G10TH	transferase family protein [Arabidopsis thaliana]	**3,31**
STMCO35TH	carbonic anhydrase [Vigna radiata]	**3,31**
MICRO.13567.C1	No Hits Found	**3,30**
076A07AF.esd	No Hits Found	**3,30**
BF_TUBSXXXX_0051F09_T3M.SCF	No Hits Found	**3,30**
bf_cswcxxxx_0007c09.t3m.scf	No Hits Found	**3,29**
MICRO.7075.C1	unknown protein [Arabidopsis thaliana] gb_AAO42331.1_ unknown protein [Arabidopsis thaliana] gb_AAD24394.2_ expressed protein [Arabidopsis thaliana] ref_NP_565462.1_ expressed protein [Arabidopsis thaliana]	**3,29**
MICRO.13012.C1	sesquiterpene synthase 2 [Lycopersicon hirsutum]	**3,29**
MICRO.13269.C1	unknown protein [Arabidopsis thaliana] gb_AAK76650.1_ unknown protein [Arabidopsis thaliana] emb_CAB87916.1_ putative protein [Arabidopsis thaliana] pir__T49284 hypothetical protein T21J18.150 - Arabidopsis thaliana ref_NP_190457.1_ F-box family ...	**3,27**
ACDA04111G07.T3m.scf	At1g08780 [Arabidopsis thaliana] ref_NP_563827.1_ prefoldin, putative [Arabidopsis thaliana]	**3,27**
BF_CSCHXXXX_0042C07.T3M.SCF	Cysteine protease inhibitor 10 precursor (PCPI-10) (Pcpi10) gb_AAB63099.1_ cysteine proteinase inhibitor 10 precursor pir__T07746 probable cysteine proteinase inhibitor 10 - potato (fragment)	**3,27**
bf_lbchxxxx_0055d07.t3m.scf	No Hits Found	**3,27**
cSTD2A13TH	26S proteasome subunit 8	**3,26**
BF_TUBSXXXX_0035H01_T3M.SCF	No Hits Found	**3,26**
POAE531TV	glycosyl transferase, putative [Arabidopsis thaliana] gb_AAO42776.1_ At3g02350/F11A12_103 [Arabidopsis thaliana] gb_AAL84957.1_ AT3g02350/F11A12_103 [Arabidopsis thaliana] gb_AAG12603.1_ unknown protein	**3,26**
cSTB25G2TH	similar to 4CL2_TOBAC4-coumarate--CoA ligase 2 [Nicotiana tabacum]	**3,25**
POCCH15TV	No Hits Found	**3,23**
MICRO.12866.C1	hypothetical protein [Arabidopsis thaliana] dbj_BAD43156.1_ hypothetical protein [Arabidopsis thaliana] dbj_BAD43152.1_ hypothetical protein [Arabidopsis thaliana] ref_NP_180594.2_ endo/excinuclease amino terminal domain-containing protein ...	**3,21**
MICRO.2536.C1	No Hits Found	**3,21**
POADG91TV	No Hits Found	**3,20**
bf_ivrootxx_0026f04.t3m.scf	putative retroelement pol polyprotein-like [Solanum tuberosum]	**3,20**
MICRO.10710.C2	putative protein [Arabidopsis thaliana] emb_CAB45904.1_ putative protein [Arabidopsis thaliana] pir__T10675 hypothetical protein F3L17.20 - Arabidopsis thaliana	**3,19**
BF_TUBSXXXX_0041H09_T3M.SCF	No Hits Found	**3,17**

ACDA03014D02.T3m.scf	No Hits Found	**3,16**
STDB005G20u.scf	No Hits Found	**3,16**
MICRO.1061.C2	similar to AT4G19880: similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G45020.1); similar to Intracellular chloride channel [Medicago truncatula] (GB:ABC75353.2); contains InterPro domain Thioredoxin-like fold (InterPro:IPR012336); contains InterPro domain Glutathione S-transferase, C-terminal-like (InterPro:IPR010987); contains InterPro domain Glutathione S-transferase, predicted (InterPro:IPR016639)	**3,16**
SSBN003A23u.scf	unnamed protein product [Arabidopsis thaliana] dbj_BAC41882.1_ unknown protein [Arabidopsis thaliana] gb_AAO63337.1_ At5g58530 [Arabidopsis thaliana] ref_NP_200661.1_ glutaredoxin family protein [Arabidopsis thaliana]	**3,15**
bf_arrayxxx_0010f05.t7m.scf	No Hits Found	**3,15**
POCAC26TP	No Hits Found	**3,15**
MICRO.10445.C1	putative protein [Arabidopsis thaliana] ref_NP_568279.1_ fringe-related protein [Arabidopsis thaliana]	**3,13**
MICRO.6822.C2	CA-responsive protein [Brassica oleracea]	**3,12**
030C09AF.esd	patatin [Solanum tuberosum] pir__S51596 patatin precursor, non-sucrose-inducible - Solanum brevidens gb_AAA66198.1_ patatin precursor	**3,12**
bf_arrayxxx_0059c12.t7m.scf	At1g11170/T28P6_16 [Arabidopsis thaliana] gb_AAL77664.1_ At1g11170/T28P6_16 [Arabidopsis thaliana] ref_NP_172583.2_ expressed protein [Arabidopsis thaliana]	**3,11**
MICRO.10103.C1	putative V-type ATPase 116kDa subunit family [Solanum demissum]	**3,11**
bf_arrayxxx_0034e11.t7m.scf	high molecular weight heat shock protein [Malus x domestica]	**3,11**
072B08AF.esd	No Hits Found	**3,10**
STMEH39TH	hypothetical protein PGEC542.21 [Solanum demissum]	**3,10**
bf_cswbxxxx_0025c08.t3m.scf	No Hits Found	**3,10**
bf_lbchxxxx_0062f02.t3m.scf	No Hits Found	**3,09**
MICRO.17719.C1	putative calcium-binding protein [Arabidopsis thaliana]	**3,09**
BF_LBCHXXXX_0019H07_T3M.SCF	No Hits Found	**3,09**
MICRO.193.C2	No Hits Found	**3,08**
MICRO.9205.C1	similar to AT3G09040: pentatricopeptide (PPR) repeat-containing protein	**3,08**
MICRO.13027.C1	lipid transfer protein LTP1 precursor [Capsicum annuum]	**3,08**
STDB005N03u.scf	No Hits Found	**3,07**
bf_cswbxxxx_0023h12.t3m.scf	No Hits Found	**3,07**
MICRO.5773.C1	similar to AT2G29660: zinc finger (C2H2 type) family protein	**3,06**
MICRO.3851.C1	No Hits Found	**3,06**
MICRO.14246.C2	No Hits Found	**3,05**
PotatoF0718.scf	No Hits Found	**3,05**
MICRO.18040.C1	No Hits Found	**3,04**
cSTA28H16TH	No Hits Found	**3,04**
STMIG72TV	putative protein [Arabidopsis thaliana] emb_CAB38915.1_ putative protein [Arabidopsis thaliana] pir__T06108 hypothetical protein T5J17.190 - Arabidopsis thaliana ref_NP_195712.1_ hypothetical protein [Arabidopsis thaliana]	**3,03**
bf_ivrootxx_0042a11.t3m.scf	No Hits Found	**3,03**
bf_cswbxxxx_0060b03.t3m.scf	No Hits Found	**3,02**
MICRO.4372.C1	No Hits Found	**3,02**

MICRO.13617.C1	crocetin dialdehyde-like [Oryza sativa (japonica cultivar-group)]	**3,02**
MICRO.7927.C1	No Hits Found	**3,01**
TBSK01009FE01t3m.scf	No Hits Found	**3,01**
BF_LBCHXXXX_0023G06_T3M.SCF	No Hits Found	**3,00**
SDBN005E15u.scf	No Hits Found	**3,00**
SSBN002H13u.scf	unknown protein [Arabidopsis thaliana] gb_AAK68784.1_ Unknown protein [Arabidopsis thaliana] ref_NP_564113.1_ expressed protein [Arabidopsis thaliana]	**3,00**
bf_mxlfxxxx_0013h03.t3m.scf	No Hits Found	**3,00**
MICRO.11686.C1	WRKY transcription factor 16 [Populus tomentosa x P. bolleana]	**3,00**
MICRO.17559.C1	putative calcium-binding protein [Arabidopsis thaliana]	**3,00**
MICRO.7880.C2	beta-carotene hydroxylase [Lycopersicon esculentum]	*-3,00*
bf_arrayxxx_0034b06.t7m.scf	No Hits Found	*-3,00*
MICRO.16439.C1	translation initiation factor (eIF-1A) [Beta vulgaris]	*-3,02*
cSTA1M6TH	similar to AT1G74580: pentatricopeptide (PPR) repeat-containing protein	*-3,02*
MICRO.2763.C1	putative nucleotide sugar epimerase [Arabidopsis thaliana] pir__A86152 hypothetical protein F22M8.13 [imported] - Arabidopsis thaliana gb_AAF76478.1_ Contains similarity to CAPI protein from Staphylococcus aureus gi_P39858 and contains a NAD ...	*-3,02*
MICRO.1675.C1	No Hits Found	*-3,03*
MICRO.7281.C1	P-Protein precursor [Solanum tuberosum] pir__T07826 aminomethyltransferase (EC 2.1.2.10) precursor - potato sp_O49954_GCSP_SOLTU Glycine dehydrogenase [decarboxylating], mitochondrial precursor (Glycine decarboxylase) (Glycine cleavage system P-protein)	*-3,04*
MICRO.3107.C1	cell cycle switch protein [Arabidopsis thaliana]	*-3,04*
cSTB18B6TH	putative RNA-binding protein [Arabidopsis thaliana]	*-3,04*
MICRO.874.C1	nitrate transporter [Nicotiana tabacum]	*-3,05*
MICRO.10110.C1	integral membrane protein, putative	*-3,06*
STDB001B16u.scf	FtsH-like protein Pftf precursor-like [Arabidopsis thaliana] pir__T49960 FtsH-like protein F8M21.140 [similarity] - Arabidopsis thaliana ref_NP_568311.1_ FtsH protease, putative [Arabidopsis thaliana]	*-3,07*
084B04AF.esd	unnamed protein product [Oryza sativa (japonica cultivar-group)] dbj_BAA88534.1_ unnamed protein product [Oryza sativa (japonica cultivar-group)]	*-3,07*
MICRO.10978.C1	putative calmodulin-binding heat-shock protein [Arabidopsis thaliana] gb_AAP04168.1_ putative calmodulin-binding heat-shock protein [Arabidopsis thaliana] emb_CAB62005.1_ calmodulin-binding heat-shock-like protein [Arabidopsis thaliana] pir__T46125 ...	*-3,09*
cSTS8N10TH	unknown protein [Arabidopsis thaliana] gb_AAM14077.1_ unknown protein [Arabidopsis thaliana] emb_CAB77956.1_ putative protein [Arabidopsis thaliana] emb_CAB45801.1_ putative protein [Arabidopsis thaliana] pir__T10558 hypothetical protein T12G13.150 ...	*-3,09*
MICRO.11321.C1	9-cis-epoxy-carotenoid dioxygenase 2 [Solanum tuberosum]	*-3,11*
MICRO.7180.C1	lysine-ketoglutarate reductase/saccharopine dehydrogenase bifunctional enzyme [Arabidopsis thaliana]	*-3,13*
MICRO.10593.C1	arm repeat-containing protein [Nicotiana tabacum]	*-3,15*
MICRO.8307.C3	protein product [Vitis vinifera] CBI39183.3	*-3,16*
MICRO.8590.C1	unknown protein [Arabidopsis thaliana] gb_AAL32522.1_ Unknown protein [Arabidopsis thaliana]	*-3,18*
ACDA04543C07.T3m.scf	hypothetical protein [Vitis vinifera] XP_002267265.1	*-3,19*
MICRO.6520.C1	squamosa promoter binding protein 2-like [Oryza sativa (japonica cultivar-group)]	*-3,20*
bf_ivrootxx_0038h09.t3m.scf	At3g18370/MYF24_8 [Arabidopsis thaliana] gb_AAL10490.1_ AT3g18370/MYF24_8 [Arabidopsis thaliana] ref_NP_566607.1_ C2 domain-containing protein [Arabidopsis thaliana]	*-3,21*

MICRO.1781.C2	At2g39570/F12L6.23 [Arabidopsis thaliana] gb_AAK74028.1_ At2g39570/F12L6.23 [Arabidopsis thaliana]	*-3,25*
bf_cswcxxxx_0011b06.t3m.scf	putative pumilio/Mpt5 family RNA-binding protein [Oryza sativa (japonica cultivar-group)] dbj_BAB89558.1_ putative pumilio/Mpt5 family RNA-binding protein [Oryza sativa (japonica cultivar-group)]	*-3,26*
MICRO.5228.C1	No Hits Found	*-3,26*
MICRO.2137.C1	adenylyl-sulfate reductase [Lycopersicon esculentum]	*-3,27*
MICRO.5679.C1	prefoldin subunit family protein [Arabidopsis thaliana] NP_171872.2	*-3,29*
MICRO.107.C5	similar to Mus musculus (Mouse). 16 days embryo head cDNA, RIKEN full-length enriched library, clone:C130090E04 product:succinate-Coenzyme A ligase, ADP-forming, beta subunit, full insert sequence [Dictyostelium discoideum]	*-3,29*
MICRO.12451.C1	No Hits Found	*-3,31*
STMCW13TV	putative phosphate/phosphoenolpyruvate translocator [Arabidopsis thaliana]	*-3,31*
MICRO.8560.C1	unnamed protein product [Arabidopsis thaliana]	*-3,33*
SDBN002E08u.scf	No Hits Found	*-3,33*
MICRO.7367.C1	At5g63160 [Arabidopsis thaliana] gb_AAQ87004.1_ BTB and TAZ domain protein 1 [Arabidopsis thaliana] dbj_BAB10558.1_ unnamed protein product [Arabidopsis thaliana] ref_NP_201121.1_ speckle-type POZ protein-related [Arabidopsis thaliana]	*-3,33*
bf_acdcxxxx_0006d08.t3m.scf	No Hits Found	*-3,34*
MICRO.3684.C3	ribosomal protein L24 precursor - common tobacco sp_Q02764_RK24_TOBAC 50S ribosomal protein L24, chloroplast precursor (CL24) gb_AAA34114.1_ ribosomal protein L24 gb_AAA34086.1_ ribosomal protein L24	*-3,34*
MICRO.787.C1	At3g15820/MSJ11_22 [Arabidopsis thaliana] gb_AAK49592.1_ AT3g15820/MSJ11_22 [Arabidopsis thaliana]	*-3,35*
MICRO.1155.C1	No Hits Found	*-3,35*
MICRO.5312.C1	mini-chromosome maintenance 7 [Pisum sativum]	*-3,36*
POAED79TP	CPuORF37 (Conserved peptide upstream open reading frame 37) [Arabidopsis thaliana] NP_001119403.1	*-3,36*
MICRO.14121.C4	unknown protein [Arabidopsis thaliana] gb_AAL24407.1_ Unknown protein [Arabidopsis thaliana] ref_NP_566627.1_ expressed protein [Arabidopsis thaliana]	*-3,38*
MICRO.1844.C1	No Hits Found	*-3,38*
MICRO.16388.C1	vicilin seed storage protein [Juglans nigra]	*-3,38*
MICRO.2654.C1	2OG-Fe(II) oxygenase [Medicago truncatula]	*-3,39*
MICRO.967.C1	probable polygalacturonase (EC 3.2.1.15) 1 - tomato gb_AAB39557.1_ AROGP3	*-3,41*
MICRO.3331.C1	aconitase [Lycopersicon pennellii]	*-3,41*
MICRO.4642.C1	P450 hydroxylase [Solanum melongena] sp_P37122_C762_SOLME Cytochrome P450 76A2 (CYPLXXVIA2) (P-450EG7) pir__S38534 cytochrome P450 76A2 - eggplant	*-3,41*
MICRO.4434.C1	beta-D-glucosidase [Gossypium hirsutum]	*-3,43*
MICRO.15769.C1	phosphatidylinositol transfer-like [Oryza sativa (japonica cultivar-group)] dbj_BAD01712.1_ phosphatidylinositol transfer-like [Oryza sativa (japonica cultivar-group)]	*-3,43*
MICRO.12312.C1	glossy1 homolog - rice (fragment) gb_AAB87722.1_ glossy1 homolog [Oryza sativa]	*-3,44*
bf_mxflxxxx_0005b04.t3m.scf	Gty37 protein [Gerbera hybrid cultivar]	*-3,46*
cSTA26M2TH	putative cytochrome P450 [Glycine max] sp_O49858_C823_SOYBN Cytochrome P450 82A3 (P450 CP6) pir__T07748 probable cytochrome P450 - soybean	*-3,47*
STMHS77TV	putative pollen thioesterase [Petunia integrifolia subsp. inflata]	*-3,49*
MICRO.6456.C1	serine protease [Lycopersicon esculentum]	*-3,50*
MICRO.3347.C1	aspartokinase-homoserine dehydrogenase [Glycine max] pir__T06242 aspartate kinase (EC 2.7.2.4) / homoserine dehydrogenase (EC 1.1.1.3) precursor - soybean chloroplast	*-3,54*

BF_CSCHXXXX_0010A03.T3M.SCF	No Hits Found	*-3,55*
cSTA6G24TH	putative cytochrome P450 [Arabidopsis thaliana] ref_NP_180803.1_ ent-kaurenoic acid hydroxylase, putative / cytochrome P450, putative [Arabidopsis thaliana] pir__B84733 probable cytochrome P450 [imported] - Arabidopsis thaliana sp_Q9C5Y2_KAO2_ARATH ...	*-3,55*
MICRO.8820.C1	vesicle-associated membrane family protein / VAMP family protein [Arabidopsis thaliana]	*-3,55*
MICRO.16611.C1	hypothetical protein [Arabidopsis thaliana] gb_AAD15353.1_ hypothetical protein [Arabidopsis thaliana] pir__H85035 hypothetical protein AT4g02820 [imported] - Arabidopsis thaliana ref_NP_192191.1_ pentatricopeptide (PPR) repeat-containing protein ...	*-3,56*
MICRO.3874.C1	putative alcohol dehydrogenase [Lycopersicon esculentum]	*-3,56*
MICRO.15654.C1	At1g28110 [Arabidopsis thaliana] gb_AAL24336.1_ serine carboxypeptidase II, putative [Arabidopsis thaliana] ref_NP_564298.1_ serine carboxypeptidase S10 family protein [Arabidopsis thaliana] ref_NP_973926.1_ serine carboxypeptidase S10 family protei ...	*-3,59*
MICRO.13775.C1	No Hits Found	*-3,60*
MICRO.3896.C2	putative RNA-binding protein [Oryza sativa (japonica cultivar-group)]	*-3,62*
bf_suspxxxx_0056g10.t3m.scf	wax synthase isoform 1 [Vitis vinifera]	*-3,63*
MICRO.2571.C1	unknown protein [Oryza sativa (japonica cultivar-group)] gb_AAP68395.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	*-3,63*
071C12AF.esd	leucine-rich repeat family protein / protein kinase family protein [Arabidopsis thaliana]	*-3,63*
POABJ64TP	aspartic proteinase 2 [Glycine max]	*-3,67*
bf_swstxxxx_0009h06.t3m.scf	putative protein [Arabidopsis thaliana] gb_AAM13260.1_ putative protein [Arabidopsis thaliana] emb_CAB85560.1_ putative protein [Arabidopsis thaliana] gb_AAK32900.1_ AT5g04550/T32M21_140 [Arabidopsis thaliana] gb_AAL32555.1_ putative protein ...	*-3,68*
bf_mxlfxxxx_0016f06.t3m.scf	No Hits Found	*-3,71*
MICRO.5306.C1	No Hits Found	*-3,71*
cSTB46O11TH	No Hits Found	*-3,71*
MICRO.17914.C1	glycosyl transferase family 8 protein [Arabidopsis thaliana]	*-3,73*
MICRO.16247.C1	hydroxyproline-rich glycoprotein [Medicago truncatula] ABY48133.1	*-3,73*
MICRO.17750.C1	F2K11.25 [Arabidopsis thaliana]	*-3,74*
MICRO.2177.C1	protein kinase-like protein [Arabidopsis thaliana] gb_AAL91617.1_ AT5g59010/k19m22_210 [Arabidopsis thaliana] ref_NP_200709.2_ protein kinase-related [Arabidopsis thaliana]	*-3,75*
MICRO.2342.C2	photosystem II reaction center PsbP family protein [Arabidopsis thaliana]	*-3,79*
MICRO.2878.C1	OSJNBb0011N17.8 [Oryza sativa (japonica cultivar-group)] emb_CAD41091.2_ OSJNBb0011N17.8 [Oryza sativa (japonica cultivar-group)]	*-3,80*
MICRO.15.C1	glycosyl hydrolase family 79 N-terminal domain-containing protein [Arabidopsis thaliana]	*-3,86*
MICRO.4006.C1	similar to AT3G29575: similar to TMAC2 (TWO OR MORE ABRES-CONTAINING GENE 2) [Arabidopsis thaliana]	*-3,87*
BF_TUBSXXXX_0063F11_T3M.SCF	No Hits Found	*-3,88*
MICRO.13469.C1	unknown protein [Arabidopsis thaliana] dbj_BAA98205.1_ unnamed protein product [Arabidopsis thaliana] gb_AAL91173.1_ unknown protein [Arabidopsis thaliana] sp_Q9LHS0_CA10_ARATH Putative clathrin assembly protein At5g35200 ref_NP_198370.1_ epsin ...	*-3,89*
STMDN36TH	NAD(P)H oxidoreductase, isoflavone reductase homologue [Solanum tuberosum] sp_P52578_IFRH_SOLTU Isoflavone reductase homolog (CP100) pir__T07386 2*-hydroxyisoflavone reductase (EC 1.3.1.45) - potato	*-3,91*
MICRO.17661.C1	expressed protein [Arabidopsis thaliana]	*-3,93*
bf_suspxxxx_0046a03.t7m.scf	glucosyl transferase, jasmonate-induced - common tobacco dbj_BAA19155.1_ glucosyl transferase [Nicotiana tabacum]	*-3,94*
MICRO.11679.C2	hypothetical protein [Ricinus communis] XP_002524491.1	*-3,97*

MICRO.8310.C1	No Hits Found	*-3,99*
MICRO.17529.C1	No Hits Found	*-3,99*
MICRO.10579.C1	cytochrome P450 [Arabidopsis thaliana]	*-4,00*
MICRO.2264.C3	No Hits Found	*-4,02*
MICRO.3428.C1	putative sialin [Oryza sativa (japonica cultivar-group)]	*-4,04*
cSTB35P16TH	hypothetical protein [Vitis vinifera] XP_002268453.1	*-4,05*
STMCG25TV	putative myb-like DNA-binding protein [Solanum demissum]	*-4,09*
MICRO.7402.C1	predicted protein [Arabidopsis thaliana] emb_CAA16542.1_ predicted protein [Arabidopsis thaliana] pir__T04506 hypothetical protein F8F16.210 - Arabidopsis thaliana	*-4,10*
MICRO.3274.C4	No Hits Found	*-4,11*
MICRO.11045.C2	gast1 [Lycopersicon esculentum] sp_P27057_GST1_LYCES GAST1 protein precursor pir__S22151 gibberellin-regulated protein GAST1 - tomato	*-4,18*
MICRO.3426.C8	No Hits Found	*-4,26*
cSTA3J19TH	Highly similar to phosphoribosylanthranilate transferase [Arabidopsis thaliana] pir__F86359 hypothetical protein F12K8.4 - Arabidopsis thaliana ref_NP_173675.1_ C2 domain-containing protein [Arabidopsis thaliana]	*-4,35*
MICRO.3470.C2	heat shock transcription factor 8 [Lycopersicon peruvianum] pir__S25481 heat shock transcription factor HSF8 - Peruvian tomato sp_P41153_HSF8_LYCPE HEAT SHOCK FACTOR PROTEIN HSF8 (HEAT SHOCK TRANSCRIPTION FACTOR 8) (HSTF 8) (HEAT STRESS TRANSCRIPTIO ...	*-4,41*
POABI73TV	SNF2 domain-containing protein / helicase domain-containing protein / RING finger domain-containing protein [Arabidopsis thaliana]	*-4,51*
MICRO.9680.C1	glutamate/malate translocator [Nicotiana tabacum]	*-4,62*
MICRO.4845.C1	Potyvirus VPg interacting protein [Nicotiana benthamiana]	*-4,79*
cSTB31P20TH	No Hits Found	*-4,89*
MICRO.9595.C1	GDSL-motif lipase/hydrolase-like protein [Arabidopsis thaliana] ref_NP_197672.1_ GDSL-motif lipase, putative [Arabidopsis thaliana]	*-4,96*
bf_stolxxxx_0056g12.t3m.scf	No Hits Found	*-5,01*
BF_LBCHXXXX_0026E09_T3M.SCF	No Hits Found	*-5,18*
BPLI5E20TH	No Hits Found	*-5,29*
bf_cswbxxxx_0063c05.t3m.scf	No Hits Found	*-5,39*
MICRO.17620.C1	No Hits Found	*-5,52*
MICRO.9078.C1	No Hits Found	*-5,68*
MICRO.17626.C1	histone H3 [Trichinella spiralis]	*-5,96*
MICRO.2749.C1	Rho guanyl-nucleotide exchange factor/ protein binding [Arabidopsis thaliana]	*-6,10*
MICRO.12730.C1	No Hits Found	*-6,24*
52-xl_pcrli45-87.e_G12_061.scf	D12 oleate desaturase [Solanum commersonii] pir__T10480 Delta12 fatty acid desaturase (EC 1.14.99.-) [imported] - Commerson*s wild potato	*-6,33*
bf_suspxxxx_0011h04.t3m.scf	No Hits Found	*-6,75*
MICRO.5515.C1	Ammonium transporter 1, member 3 (LeAMT1	*-7,16*
bf_suspxxxx_0049c02.t3m.scf	putative glucosyltransferase [Phaseolus lunatus]	*-7,66*
MICRO.17064.C1	unknown protein [Arabidopsis thaliana] pir__E84780 hypothetical protein At2g36420 [imported] - Arabidopsis thaliana ref_NP_181183.1_ expressed protein [Arabidopsis thaliana]	*-7,85*
MICRO.16615.C1	putative protein [Arabidopsis thaliana] gb_AAL38610.1_ AT3g52740/F3C22_140 [Arabidopsis thaliana] gb_AAK96599.1_ AT3g52740/F3C22_140 [Arabidopsis thaliana] pir__T49027 hypothetical protein F3C22.140 - Arabidopsis thaliana ref_NP_566972.1_ expressed ...	*-8,17*

MICRO.18269.C1	ribosomal protein L2 [Lycopersicon esculentum] pir__R5TOL8 ribosomal protein L8, cytosolic - tomato sp_P29766_RL2_LYCES 60S ribosomal protein L2 (L8) (Ribosomal protein TL2)	*-8,35*
MICRO.4306.C2	putative lipase [Oryza sativa (japonica cultivar-group)] dbj_BAD20155.1_ putative lipase [Oryza sativa (japonica cultivar-group)] dbj_BAD19595.1_ putative lipase [Oryza sativa (japonica cultivar-group)]	*-8,55*
bf_mxlfxxxx_0062e11.t3m.scf	No Hits Found	*-9,06*
MICRO.2328.C1	putative calmodulin-binding protein [Oryza sativa (japonica cultivar-group)]	*-9,14*
cSTD17P3TH	At4g20170/F1C12_90 [Arabidopsis thaliana] gb_AAL24253.1_ AT4g20170/F1C12_90 [Arabidopsis thaliana]	*-9,34*
MICRO.4306.C1	putative lipase [Oryza sativa (japonica cultivar-group)]	*-9,43*
MICRO.6.C1	putative multiple stress-responsive zinc-finger protein [Oryza sativa (japonica cultivar-group)] dbj_BAD35521.1_ putative multiple stress-responsive zinc-finger protein [Oryza sativa (japonica cultivar-group)]	*-9,49*
cSTB47C24TH	xyloglucan endotransglycosylase [Lycopersicon esculentum]	*-9,49*
STMGT54TV	No Hits Found	*-9,77*
MICRO.12814.C1	CG6141-PB, isoform B [Drosophila melanogaster] gb_AAF53048.2_ CG6141-PA, isoform A [Drosophila melanogaster]	*-10,12*
MICRO.8284.C1	acetyltranferase-like protein [Arabidopsis thaliana]	*-11,18*
MICRO.12244.C1	cysteine proteinase Mir3 [Zea mays]	*-11,34*
MICRO.7372.C1	small heat-shock protein homolog [Solanum tuberosum]	*-12,76*
bf_ivrootxx_0058c11.t3m.scf	hypothetical protein AT4G33590 [Arabidopsis thaliana]	*-92,97*

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the pre-stage of the symbiotic interaction of AM fungus in the potato roots versus control comparison are ordered according to the expression fold change. Genes were considered as differentially regulated if P-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.00 or ≤ -3.00

Table S2 The 381 genes regulated during the early stage of potato root colonization by Glomus sp. MUCL 41833 as compared with the control treatment, and their changes in expression

Probe ID	Annotation	Ratio Early
MICRO.7349.C2	AGR_C_5039p, putative, expressed [Oryza sativa (japonica cultivar-group)]	**218,03**
POCAR86TV	No Hits Found	**22,22**
MICRO.9776.C1	hypothetical protein [Ricinus communis] XP_002523315.1	**20,31**
MICRO.10760.C1	At3g18850 [Arabidopsis thaliana] dbj_BAB03094.1_ acyl-CoA:1-acylglycerol-3-phosphate acyltransferase [Arabidopsis thaliana] gb_AAM20700.1_ unknown protein [Arabidopsis thaliana] ref_NP_974335.1_ phospholipid/glycerol acyltransferase family protein ...	**18,83**
MICRO.14089.C1	xyloglucan endotransglucosylase-hydrolase XTH6 [Lycopersicon esculentum]	**12,14**
bf_acdcxxxx_0052g07.t3m.scf	putative gag-pol protein [Solanum demissum]	**11,02**
POCDC04TV	No Hits Found	**9,91**
bf_mxflxxxx_0025a02.t3m.scf	hypothetical protein [Vitis vinifera] XP_002264841.1	**9,87**
STDB004K07u.scf	Small heat shock protein, chloroplast precursor pir__T06324 heat shock protein 21, chloroplast - tomato gb_AAB07023.1_ heat shock protein	**9,41**
MICRO.7849.C3	adenosine-5*-phosphosulfate-kinase [Catharanthus roseus] pir__T08076 adenylyl-sulfate kinase (EC 2.7.1.25) precursor - Madagascar periwinkle sp_O49204_KAPS_CATRO Adenylyl-sulfate kinase, chloroplast precursor (APS kinase) (Adenosine-5*phosphosulfate ...	**8,63**
ACDA02091G03.T3m.scf	No Hits Found	**8,55**
MICRO.6396.C2	putative protein [Arabidopsis thaliana] pir__T48012 hypothetical protein T17J13.160 - Arabidopsis thaliana ref_NP_191779.1_ expressed protein [Arabidopsis thaliana]	**8,39**
MICRO.16359.C1	putative pectin-glucuronyltransferase [Oryza sativa (japonica cultivar-group)]	**8,11**
STMCX94TH	GTP cyclohydrolase II	**7,92**
MICRO.4306.C1	putative lipase [Oryza sativa (japonica cultivar-group)]	**7,81**
cSTA8N10TH	unknown protein [Arabidopsis thaliana] ref_NP_849485.1_ calcineurin B-like protein 10 (CBL10) [Arabidopsis thaliana] gb_AAO14864.2_ calcineurin B-like protein [Arabidopsis thaliana]	**7,75**
MICRO.2286.C100	hypothetical protein [Solanum demissum]	**7,63**
MICRO.4735.C3	No Hits Found	**7,52**
MICRO.8269.C1	gibberellin 2-oxidase 1 [Nicotiana tabacum]	**7,37**
MICRO.5158.C2	AT3g52570/F22O6_50 [Arabidopsis thaliana] gb_AAL67113.1_ AT3g52570/F22O6_50 [Arabidopsis thaliana] ref_NP_190825.2_ expressed protein [Arabidopsis thaliana]	**7,25**
bf_cswbxxxx_0063d11.t3m.scf	No Hits Found	**7,23**
MICRO.7661.C1	putative protein [Arabidopsis thaliana] pir__T49929 hypothetical protein F17I14.120 - Arabidopsis thaliana	**7,11**
BF_TUBSXXXX_0041C04_T3M.SCF	No Hits Found	**7,08**
MICRO.17165.C1	LEA protein [Cicer arietinum] sp_O49816_LEA1_CICAR Late embryogenesis abundant protein 1 (CapLEA-1)	**6,99**
POAD681TP	No Hits Found	**6,85**

bf_mxflxxxx_0022h04.t3m.scf	unknown [Arabidopsis thaliana] gb_AAO63896.1_ unknown protein [Arabidopsis thaliana] gb_AAO42204.1_ unknown protein [Arabidopsis thaliana] ref_NP_564140.1_ nodulin MtN3 family protein [Arabidopsis thaliana]	**6,79**
STMGA05TV	OSJNBb0038F03.12 [Oryza sativa (japonica cultivar-group)]	**6,71**
MICRO.6718.C1	No Hits Found	**6,63**
MICRO.12902.C1	sulfate transporter [Arabidopsis thaliana]	**6,55**
POCD701TP	No Hits Found	**6,44**
ACDA04414H10.T3m.scf	No Hits Found	**6,38**
bf_swstxxxx_0018d01.t3m.scf	No Hits Found	**6,36**
MICRO.12934.C1	No Hits Found	**6,33**
bf_mxflxxxx_0008a03.t3m.scf	No Hits Found	**6,30**
MICRO.3417.C1	P0460H02.3 [Oryza sativa (japonica cultivar-group)] dbj_BAB61227.1_ putative Dephospho-CoA kinase (27.4 kD) (3K840) [Oryza sativa (japonica cultivar-group)]	**6,28**
MICRO.2600.C3	No Hits Found	**6,26**
SSBN002H23u.scf	hypothetical protein F22M8.9 - Arabidopsis thaliana gb_AAF76474.1_ Contains similarity to a guanine nucleotide exchange factor from Homo sapiens gb_AF111162 and contains a Sec7 PF_01369 domain. [Arabidopsis thaliana] ref_NP_171698.1_ guanine ...	**6,25**
MICRO.7926.C1	unknown protein [Arabidopsis thaliana] dbj_BAB01110.1_ unnamed protein product [Arabidopsis thaliana] gb_AAM20514.1_ unknown protein [Arabidopsis thaliana] ref_NP_188473.1_ expressed protein [Arabidopsis thaliana]	**6,23**
SDBN005C04u.scf	putative callose synthase catalytic subunit [Gossypium hirsutum]	**6,22**
PPCCB33TH	protein phosphatase 2A regulatory subunit B* [Arabidopsis thaliana] gb_AAL15383.1_ AT3g26020/MPE11_17 [Arabidopsis thaliana] gb_AAK56256.1_ AT3g26020/MPE11_17 [Arabidopsis thaliana]	**6,19**
MICRO.9692.C1	type-A response regulator [Catharanthus roseus]	**6,18**
POACH39TP	No Hits Found	**6,16**
MICRO.15257.C1	No Hits Found	**6,15**
bf_mxlfxxxx_0061e01.t3m.scf	ribosomal protein L24 precursor - common tobacco sp_Q02764_RK24_TOBAC 50S ribosomal protein L24, chloroplast precursor (CL24) gb_AAA34114.1_ ribosomal protein L24 gb_AAA34086.1_ ribosomal protein L24	**6,11**
MICRO.5123.C1	No Hits Found	**6,08**
STMDZ42TV	At5g22100 [Arabidopsis thaliana] emb_CAC34505.1_ putative protein [Arabidopsis thaliana] gb_AAM10395.1_ At5g22100/At5g22100 [Arabidopsis thaliana] dbj_BAD43201.1_ unknown protein [Arabidopsis thaliana] dbj_BAD43181.1_ unknown protein [Arabidopsis ...	**6,07**
MICRO.1430.C1	Waxy locus DNA encoding pseudogene similar to beta-1,3-glucanases and beta-1,3-1,4-glucanases [Solanum tuberosum]	**6,03**
MICRO.16302.C1	At4g15700 [Arabidopsis thaliana] emb_CAB78612.1_ glutaredoxin homolog [Arabidopsis thaliana] emb_CAB10348.1_ glutaredoxin homolog [Arabidopsis thaliana] ref_NP_193305.1_ glutaredoxin family protein [Arabidopsis thaliana] gb_AAR92328.1_ At4g15700 ...	**5,98**
bf_cswbxxxx_0042e01.t3m.scf	No Hits Found	**5,97**
cSTS22L23TH	AT4g13930/dl3005c [Arabidopsis thaliana] emb_CAB78435.1_ hydroxymethyltransferase [Arabidopsis thaliana] emb_CAB10172.1_ hydroxymethyltransferase [Arabidopsis thaliana] gb_AAK32757.1_ AT4g13930/dl3005c [Arabidopsis thaliana] ref_NP_193129.1_ glycine	**5,96**
MICRO.3335.C2	putative gamma-glutamyl hydrolase [Arabidopsis thaliana] pir__D96815 probable gamma-glutamyl hydrolase [imported] - Arabidopsis thaliana ref_NP_177987.1_ gamma-glutamyl hydrolase, putative / gamma-Glu-X carboxypeptidase, putative / conjugase, ...	**5,92**
MICRO.6854.C1	unknown protein [Arabidopsis thaliana] gb_AAL07177.1_ unknown protein [Arabidopsis thaliana] pir__B86157 hypothetical protein [imported] - Arabidopsis thaliana ref_NP_563661.1_ expressed protein [Arabidopsis thaliana] gb_AAG10623.1_ Unknown protein ...	**5,89**

MICRO.3483.C1	BEL1-related homeotic protein 5 [Solanum tuberosum]	**5,88**
MICRO.7366.C1	unknown protein [Arabidopsis thaliana] gb_AAK93687.1_ unknown protein [Arabidopsis thaliana] ref_NP_567819.1_ expressed protein [Arabidopsis thaliana] ref_NP_974633.1_ expressed protein [Arabidopsis thaliana]	**5,86**
MICRO.6632.C1	No Hits Found	**5,81**
cSTD12A19TH	zinc finger protein, putative / regulator of chromosome condensation (RCC1) family protein [Arabidopsis thaliana]	**5,79**
POACY26TP	No Hits Found	**5,76**
bf_stolxxxx_0057d04.t3m.scf	OSJNBa0079M09.13 [Oryza sativa (japonica cultivar-group)] emb_CAD40495.2_ OSJNBa0079M09.13 [Oryza sativa (japonica cultivar-group)] dbj_BAA84779.1_ nonphototrophic hypocotyl 1b [Oryza sativa (japonica cultivar-group)]	**5,73**
POCCJ58TV	No Hits Found	**5,72**
MICRO.11037.C3	allyl alcohol dehydrogenase [Nicotiana tabacum]	**5,69**
MICRO.740.C1	zinc-binding protein-like [Arabidopsis thaliana] dbj_BAB10724.1_ zinc-binding protein-like [Arabidopsis thaliana] dbj_BAD44301.1_ zinc-binding protein-like [Arabidopsis thaliana] sp_Q9FN32_YPL6_ARATH Yippee-like protein At5g53940 ref_NP_200205.1_ ...	**5,68**
MICRO.67.C2	remorin family protein [Arabidopsis thaliana]	**5,66**
cSTD9N2TH	unnamed protein product [Arabidopsis thaliana] gb_AAO11550.1_ At5g44930/K21C13_11 [Arabidopsis thaliana] gb_AAK82521.1_ AT5g44930/K21C13_11 [Arabidopsis thaliana] ref_NP_199306.1_ exostosin family protein [Arabidopsis thaliana] ref_NP_851132.1_ ...	**5,64**
bf_mxflxxxx_0002g10.t3m.scf	No Hits Found	**5,64**
bf_ivrootxx_0044a08.t3m.scf	unknown [Glycine max] ACU24519.1	**5,63**
cSTA45E10TH	No Hits Found	**5,60**
MICRO.839.C6	putative cysteine proteinase inhibitor precursor 1423 [Solanum tuberosum]	**5,59**
MICRO.4809.C1	unnamed protein product [Arabidopsis thaliana]	**5,58**
MICRO.967.C1	probable polygalacturonase (EC 3.2.1.15) 1 - tomato gb_AAB39557.1_ AROGP3	**5,55**
MICRO.14214.C1	putative nucleoid DNA-binding protein [Cicer arietinum]	**5,55**
cSTB30J8TH	No Hits Found	**5,53**
MICRO.6824.C1	No Hits Found	**5,51**
cPRO5C2TH	laccase-like protein [Arabidopsis thaliana] pir__T48484 laccase-like protein - Arabidopsis thaliana	**5,49**
MICRO.18224.C1	No Hits Found	**5,48**
POCDM37TP	zinc finger (C3HC4-type RING finger) protein-like [Oryza sativa (japonica cultivar-group)] dbj_BAD45526.1_ zinc finger (C3HC4-type RING finger) protein-like [Oryza sativa (japonica cultivar-group)]	**5,45**
MICRO.2642.C1	putative ubiquitin carboxyl terminal hydrolase [Arabidopsis thaliana] gb_AAK26025.1_ putative ubiquitin carboxyl terminal hydrolase [Arabidopsis thaliana] gb_AAD23896.2_ putative ubiquitin carboxyl terminal hydrolase [Arabidopsis thaliana] ...	**5,42**
ACDA03298C10.T3m.scf	similar to AT1G17370| Symbols: UBP1B | UBP1B (OLIGOURIDYLATE BINDING PROTEIN 1B)	**5,40**
bf_mxlfxxxx_0057f03.t3m.scf	hypothetical protein [Ricinus communis] XP_002519109.1	**5,38**
SDBT002E15x.scf	early light inducible protein [Lycopersicon esculentum]	**5,37**
bf_mxlfxxxx_0012g07.t3m.scf	No Hits Found	**5,36**
188A11.esd	similar to AT4G10570: ubiquitin carboxyl-terminal hydrolase family protein	**5,33**
cPRO30I22TH	putative protein [Arabidopsis thaliana] pir__T50803 hypothetical protein T30N20_210 - Arabidopsis thaliana	**5,30**
POCA693TP	No Hits Found	**5,29**
MICRO.6103.C1	putative Polyprotein [Solanum demissum]	**5,29**

SDBN002P15u.scf	No Hits Found	**5,27**
SDBN006M22u.scf	similar to AT2G02650: reverse transcriptase-related	**5,26**
MICRO.3132.C1	No Hits Found	**5,25**
MICRO.3960.C1	No Hits Found	**5,25**
MICRO.4970.C1	No Hits Found	**5,23**
MICRO.3474.C1	Protein-tyrosine phosphatase mitochondrial 1, mitochondrial precursor, putative [Ricinus communis] XP_002531467.1	**5,22**
MICRO.7513.C2	isoamylase isoform 1 [Solanum tuberosum]	**5,21**
MICRO.11440.C1	No Hits Found	**5,19**
BF_LBCHXXXX_0046H01_T3M.SCF	No Hits Found	**5,18**
MICRO.13235.C2	putative aminoalcoholphosphotransferase [Arabidopsis thaliana] gb_AAL86327.1_ putative aminoalcoholphosphotransferase [Arabidopsis thaliana] gb_AAC61768.1_ aminoalcoholphosphotransferase [Arabidopsis thaliana] pir__F86268 ...	**5,18**
POADK14TV	similar to AT3G63510: FAD binding / catalytic/ tRNA dihydrouridine synthase	**5,16**
MICRO.11949.C1	Ubiquinone biosynthesis protein COQ9, mitochondrial precursor, putative [Ricinus communis] XP_002516247.1	**5,14**
MICRO.9975.C1	No Hits Found	**5,13**
MICRO.9208.C1	No Hits Found	**5,13**
bf_cswbxxxx_0033f03.t3m.scf	No Hits Found	**5,11**
MICRO.7543.C1	unknown [Arabidopsis thaliana]	**5,09**
MICRO.10581.C2	putative His-Asp phosphotransfer protein [Pisum sativum]	**5,08**
MICRO.5360.C1	No Hits Found	**5,06**
MICRO.7581.C1	Tetratricopeptide repeat protein, putative [Ricinus communis]	**5,03**
MICRO.17123.C1	hydroxycinnamoyl transferase [Nicotiana tabacum]	**5,03**
PotatoF1153.scf	No Hits Found	**5,01**
cSTA6E7TH	expressed protein [Arabidopsis thaliana] ref_NP_565626.1_ adhesion regulating molecule family [Arabidopsis thaliana]	**5,00**
MICRO.2827.C1	Hcr2-0B [Lycopersicon esculentum]	**4,99**
MICRO.14540.C1	No Hits Found	**4,97**
cSTE26P22TH	putative ionotropic glutamate receptor homolog GLR4 [Oryza sativa (japonica cultivar-group)]	**4,97**
MICRO.14962.C1	No Hits Found	**4,95**
cSTA35O21TH	probable peptide transporter [imported] - Arabidopsis thaliana gb_AAF29404.1_ peptide transporter, putative [Arabidopsis thaliana] ref_NP_175630.1_ proton-dependent oligopeptide transport (POT) family protein [Arabidopsis thaliana]	**4,94**
MICRO.6051.C1	unnamed protein product [Arabidopsis thaliana] gb_AAF73130.1_ cyclic nucleotide-binding transporter 1 [Arabidopsis thaliana] gb_AAF73128.1_ cyclic nucleotide-binding transporter 1 [A. thaliana]	**4,93**
MICRO.3896.C2	putative RNA-binding protein [Oryza sativa (japonica cultivar-group)]	**4,91**
MICRO.3621.C1	hypothetical protein SDM1_53t00013 [Solanum demissum] ABI34375.1	**4,91**
MICRO.8402.C1	No Hits Found	**4,89**
cSTB38P12TH	histone H3	**4,88**
bf_ivrootxx_0041e12.t3m.scf	AT3g09100/MZB10_13 [Arabidopsis thaliana] ref_NP_187522.2_ mRNA capping enzyme family protein [Arabidopsis thaliana]	**4,87**
MICRO.1592.C2	putative beta-1,3-glucanase [Arabidopsis thaliana] gb_AAM15281.1_ putative beta-1,3-glucanase [Arabidopsis thaliana] pir__E84471	**4,86**
MICRO.7163.C2	probable beta-1,3-glucanase [imported] - Arabidopsis thaliana At1g66140/F15E12_19 [Arabidopsis thaliana] gb_AAL24195.1_ At1g66140/F15E12_19 [Arabidopsis thaliana] gb_AAL06967.1_ sp_Q39263_ZFP4_ARATH Zinc finger protein 4 pir__B96686 probable C2H2-type zinc finger ...	**4,86**

bf_mxlfxxxx_0014c09.t3m.scf	Hsp19.9 protein [Lycopersicon peruvianum]	**4,85**
MICRO.9192.C1	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor (NADP-dependent glyceraldehydephosphate dehydrogenase subunit A) gb_AAA34075.1_ glyceraldehyde-3-phosphate dehydrogenase A-subunit precursor	**4,83**
MICRO.5365.C1	No Hits Found	**4,81**
MICRO.15639.C1	MED21 (MEDIATOR 21) [Arabidopsis thaliana] NP_001078352.1	**4,79**
MICRO.9579.C2	similar to AT3G21580: cobalt ion transporter	**4,78**
MICRO.17169.C1	AT3g57890/T10K17_100 [Arabidopsis thaliana] gb_AAK96632.1_ AT3g57890/T10K17_100 [Arabidopsis thaliana] ref_NP_567059.1_ tubulin-specific chaperone C-related [Arabidopsis thaliana]	**4,75**
MICRO.16094.C1	No Hits Found	**4,74**
MICRO.11710.C1	cysteine proteinase precursor [Ipomoea batatas]	**4,73**
MICRO.8608.C1	putative serine/threonine protein kinase [Oryza sativa (japonica cultivar-group)] gb_AAV43835.1_ putative serine/threonine protein kinase [Oryza sativa (japonica cultivar-group)]	**4,71**
MICRO.9052.C2	transport inhibitor response 1 (TIR1) [Arabidopsis thaliana] gb_AAN71945.1_ putative transport inhibitor response TIR1, AtFBL1 protein [Arabidopsis thaliana] pir__T48087 transport inhibitor response protein TIR1 [imported] - Arabidopsis thaliana ...	**4,70**
MICRO.16381.C1	oleosin-like protein [Citrus sinensis] pir__T10121 oleosin-like protein, salt stress-induced - sweet orange prf__2119230A oleosin homolog	**4,68**
SSBN002A13u.scf	putative zinc protease PQQL [Oryza sativa (japonica cultivar-group)] dbj_BAB85389.1_ similar to zinc protease PQQL [Oryza sativa (japonica cultivar-group)] dbj_BAC00679.1_ putative zinc protease PQQL [Oryza sativa (japonica cultivar-group)]	**4,66**
MICRO.15820.C1	similar to AT3G46790 | Symbols: CRR2 | CRR2 (CHLORORESPIRATORY REDUCTION 2)	**4,66**
BF_LBCHXXXX_0048G07_T3M.SCF	No Hits Found	**4,65**
cSTA1D11TH	unnamed protein product [Arabidopsis thaliana] ref_NP_200022.1_ tRNA-splicing endonuclease positive effector-related [Arabidopsis thaliana]	**4,64**
MICRO.1669.C1	putative cyclophylin protein [Arabidopsis thaliana] emb_CAB87793.1_ cyclophylin-like protein [Arabidopsis thaliana] pir__T49181 cyclophylin-like protein - Arabidopsis thaliana ref_NP_191899.1_ peptidyl-prolyl cis-trans isomerase cyclophilin-type ...	**4,62**
POCD322TP	hypothetical protein [Ricinus communis] XP_002528894.1	**4,61**
SDBN005P19u.scf	No Hits Found	**4,60**
MICRO.9134.C1	50S Ribosomal protein L13 [Arabidopsis thaliana] gb_AAL34279.1_ putative ribosomal protein L13 [Arabidopsis thaliana] gb_AAK44133.1_ putative ribosomal protein L13 [Arabidopsis thaliana] emb_CAA60775.1_ ribosomal protein L13 [Arabidopsis thaliana] ...	**4,58**
MICRO.7089.C1	No Hits Found	**4,57**
MICRO.7372.C1	small heat-shock protein homolog [Solanum tuberosum] pir__T07031 low molecular weight heat shock protein homolog - potato	**4,56**
MICRO.6336.C2	At5g14680 [Arabidopsis thaliana] emb_CAB87635.1_ putative protein [Arabidopsis thaliana] pir__T48641 hypothetical protein T15N1.170 - Arabidopsis thaliana gb_AAS65947.1_ At5g14680 [Arabidopsis thaliana] ref_NP_196972.1_ universal stress protein (USP ...	**4,55**
SSBN003F04u.scf	putative c-type cytochrome synthesis protein [Oryza sativa (japonica cultivar-group)] gb_AAR87329.1_ putative c-type cytochrome biogenesis protein [Oryza sativa (japonica cultivar-group)]	**4,53**
SSBT005C10x.scf	No Hits Found	**4,52**
POCAA56TP	hypothetical protein [Solanum tuberosum]	**4,50**
bf_lbchxxxx_0066h06.t3m.scf	No Hits Found	**4,49**
BF_TUBSXXXX_0034C08_T3M.SCF	No Hits Found	**4,48**
MICRO.6916.C1	unknown protein [Arabidopsis thaliana] NP_172086.1	**4,45**
MICRO.2286.C18	No Hits Found	**4,45**

MICRO.16195.C1	No Hits Found	**4,44**
bf_lbchxxxx_0057a07.t3m.scf	No Hits Found	**4,42**
MICRO.6470.C1	oxidoreductase, acting on the CH-CH group of donors [Arabidopsis thaliana] NP_974140.1	**4,41**
SSBN001P05u.scf	No Hits Found	**4,40**
MICRO.7597.C1	At3g59630 [Arabidopsis thaliana] emb_CAB75460.1_ putative protein [Arabidopsis thaliana] gb_AAL91627.1_ AT3g59630/T16L24_180 [Arabidopsis thaliana] pir__T49304 hypothetical protein T16L24.180 - Arabidopsis thaliana ref_NP_191522.1_ diphthamide ...	**4,39**
bf_stolxxxx_0040c03.t3m.scf	No Hits Found	**4,37**
MICRO.15510.C1	No Hits Found	**4,36**
MICRO.2557.C1	prolyl 4-hydroxylase [Nicotiana tabacum]	**4,33**
SDBN003M02u.scf	No Hits Found	**4,31**
BF_TUBSXXXX_0047A06_T3M.SCF	No Hits Found	**4,31**
bf_mxflxxxx_0055a05.t3m.scf	No Hits Found	**4,30**
MICRO.12553.C1	unknown protein [Arabidopsis thaliana] gb_AAN86203.1_ unknown protein [Arabidopsis thaliana] ref_NP_568500.1_ expressed protein [Arabidopsis thaliana]	**4,29**
MICRO.15261.C2	cold-regulated LTCOR12 [Lavatera thuringiaca]	**4,28**
bf_cswbxxxx_0061e09.t3m.scf	No Hits Found	**4,26**
MICRO.8896.C1	At5g22210 [Arabidopsis thaliana] emb_CAC34514.1_ putative protein [Arabidopsis thaliana] dbj_BAD43915.1_ unknown protein [Arabidopsis thaliana] ref_NP_974820.1_ expressed protein [Arabidopsis thaliana] ref_NP_680207.1_ expressed protein [Arabidopsis ...	**4,26**
MICRO.154.C4	Ipomoea nil Pn47p	**4,25**
cSTD8J16TH	OSJNBb0065L13.3 [Oryza sativa (japonica cultivar-group)] emb_CAE03360.1_ OSJNBb0065L13.3 [Oryza sativa (japonica cultivar-group)]	**4,22**
MICRO.9590.C1	putative retrotransposon protein [Solanum demissum]	**4,21**
STMHS91TV	No Hits Found	**4,18**
MICRO.436.C1	Similar to CCS1 [Arabidopsis thaliana] dbj_BAC41965.1_ unknown protein [Arabidopsis thaliana] pir__B96530 Similar to CCS1 [imported] - Arabidopsis thaliana ref_NP_564544.1_ cytochrome c biogenesis protein family [Arabidopsis thaliana]	**4,15**
cSTD4N24TH	similar to AT3G49710: pentatricopeptide (PPR) repeat-containing protein	**4,13**
MICRO.15587.C1	RNA-directed RNA polymerase [Solanum tuberosum]	**4,12**
POCCF66TP	zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana]	**4,10**
SDBN003J04u.scf	P0489B03.9 [Oryza sativa (japonica cultivar-group)] dbj_BAB90560.1_ P0489B03.9 [Oryza sativa (japonica cultivar-group)] dbj_BAB91753.1_ P0679C12.2 [Oryza sativa (japonica cultivar-group)]	**4,08**
MICRO.14071.C3	putative homeobox-leucine zipper protein [Oryza sativa (japonica cultivar-group)] dbj_BAD17827.1_ putative homeobox-leucine zipper protein [Oryza sativa (japonica cultivar-group)]	**4,07**
ACDA03951B03.T3m.scf	No Hits Found	**4,05**
MICRO.1716.C1	CLB1 protein - tomato dbj_BAA24382.1_ CLB1 [Lycopersicon esculentum]	**4,01**
MICRO.4165.C17	Ribulose bisphosphate carboxylase small chain C, chloroplast precursor (RuBisCO small subunit C) pir__RKPOSC ribulose-bisphosphate carboxylase (EC 4.1.1.39) precursor small chain rbcS-c - potato gb_AAA33838.1_ ribulose bisphosphate carboxylase (EC ...	**4,00**
MICRO.6022.C1	Cyclin-like F-box; FBD [Medicago truncatula]	**3,98**
MICRO.13036.C2	similar to AT5G14430: dehydration-responsive protein-related	**3,94**
cSTB37A20TH	No Hits Found	**3,93**

cPRO23H8TH	No Hits Found	**3,90**
MICRO.14563.C1	F10O3.1 [Arabidopsis thaliana] pir__G86162 protein F10O3.1 [imported] - Arabidopsis thaliana	**3,89**
MICRO.12200.C1	glutathione reductase (NADPH) [Nicotiana tabacum] pir__S38908 glutathione-disulfide reductase (EC 1.8.1.7) - common tobacco (fragment) sp_P80461_GSHC_TOBAC Glutathione reductase, chloroplast precursor (GR) (GRase)	**3,85**
MICRO.3352.C1	No Hits Found	**3,83**
MICRO.7107.C8	No Hits Found	**3,81**
MICRO.14060.C3	similar to AT2G40090 | Symbols: ATATH9 | ATATH9 (ABC2 homolog 9)	**3,80**
cSTB38N12TH	No Hits Found	**3,79**
POCCM96TP	No Hits Found	**3,78**
MICRO.2888.C1	putative endomembrane protein 70 [Arabidopsis thaliana]	**3,77**
bf_arrayxxx_0015d05.t3m.scf	No Hits Found	**3,75**
cSTD14L16TH	putative DnaJ domain containg protein, 3*-partial [Oryza sativa]	**3,74**
cSTB45F20TH	potyviral helper component protease-interacting protein 1 [Solanum tuberosum subsp. andigena]	**3,73**
MICRO.3722.C1	putative leucyl-tRNA synthetase [Oryza sativa (japonica cultivar-group)] dbj_BAD26302.1_ putative leucyl-tRNA synthetase [Oryza sativa (japonica cultivar-group)]	**3,72**
BF_TUBSXXXX_0028D11_T3M.SCF	No Hits Found	**3,71**
bf_mxflxxxx_0069h01.t3m.scf	No Hits Found	**3,70**
STMHT53TV	unknown protein [Arabidopsis thaliana] pir__A86242 hypothetical protein [imported] - Arabidopsis thaliana gb_AAD31330.1_ EST gb_F13926 comes from this gene. [Arabidopsis thaliana] ref_NP_563879.1_ sodium symporter-related [Arabidopsis thaliana]	**3,68**
bf_acdcxxxx_0001a08.t3m.scf	No Hits Found	**3,67**
MICRO.4505.C1	At5g51950/MSG15_3 [Arabidopsis thaliana] gb_AAK56275.1_ AT5g51950/MSG15_3 [Arabidopsis thaliana] ref_NP_200008.1_ glucose-methanol-choline (GMC) oxidoreductase family protein [Arabidopsis thaliana]	**3,66**
STMDP74TH	hexose transporter [Nicotiana tabacum]	**3,65**
MICRO.2247.C1	No Hits Found	**3,64**
cSTS3A22TH	No Hits Found	**3,64**
MICRO.1681.C2	No Hits Found	**3,63**
cSTB41F2TH	No Hits Found	**3,61**
MICRO.2270.C1	AAA-ATPase-like protein [Oryza sativa (japonica cultivar-group)]	**3,60**
bf_suspxxxx_0038b11.t3m.scf	No Hits Found	**3,59**
MICRO.180.C4	tobacco nucleolin [Nicotiana tabacum]	**3,58**
MICRO.11770.C1	cycloidea [Lycopersicon esculentum]	**3,57**
ACDA04707A03.T3m.scf	No Hits Found	**3,57**
MICRO.6650.C2	amino acid transporter c [Vicia faba]	**3,56**
MICRO.3225.C2	flavin containing monooxygenase 3 -like [Oryza sativa (japonica cultivar-group)]	**3,55**
MICRO.6591.C1	myb family transcription factor [Arabidopsis thaliana]	**3,53**
MICRO.9064.C1	alcohol NADP+ oxidoreductase (drd-1 gene) [Solanum tuberosum]	**3,52**
MICRO.398.C1	No Hits Found	**3,51**
BF_LBCHXXXX_0050E08_T3M.SCF	expressed protein [Arabidopsis thaliana]	**3,51**

MICRO.17219.C1	No Hits Found	**3,50**
MICRO.4476.C1	No Hits Found	**3,49**
MICRO.6570.C1	No Hits Found	**3,47**
bf_stolxxxx_0061b03.t3m.scf	No Hits Found	**3,46**
MICRO.18153.C1	No Hits Found	**3,45**
bf_arrayxxx_0075b03.t3m.scf	No Hits Found	**3,44**
MICRO.7834.C1	acylaminoacyl-peptidase like protein [Arabidopsis thaliana] emb_CAB10236.1_ acylaminoacyl-peptidase like protein [Arabidopsis thaliana] pir__B71408 probable acylaminoacyl-peptidase - Arabidopsis thaliana	**3,43**
bf_swstxxxx_0035e01.t3m.scf	No Hits Found	**3,43**
POADF30TP	glycerol-3-phosphate acyltransferase [Lycopersicon esculentum]	**3,42**
bf_ivrootxx_0016h12.t3m.scf	No Hits Found	**3,40**
MICRO.7940.C1	No Hits Found	**3,39**
MICRO.592.C15	S-formylglutathione hydrolase [Arabidopsis thaliana] gb_AAB84335.1_ putative esterase D [Arabidopsis thaliana] gb_AAL38594.1_ At2g41530/T32G6.5 [Arabidopsis thaliana] gb_AAK73263.1_ putative esterase D [Arabidopsis thaliana] gb_AAK55678.1_ ...	**3,39**
bf_ivrootxx_0021h02.t3m.scf	No Hits Found	**3,37**
SDBN006O11u.scf	putative transposon MuDR mudrA-like protein [Solanum demissum]	**3,36**
bf_mxflxxxx_0008d05.t3m.scf	No Hits Found	**3,35**
MICRO.17904.C1	P0007F06.8 [Oryza sativa (japonica cultivar-group)] dbj_BAB84369.1_ P0007F06.8 [Oryza sativa (japonica cultivar-group)]	**3,34**
MICRO.6805.C1	putative HYS1 [Oryza sativa (japonica cultivar-group)] dbj_BAD16917.1_ putative HYS1 [Oryza sativa (japonica cultivar-group)]	**3,34**
MICRO.6258.C2	protein phosphatase-like [Arabidopsis thaliana]	**3,33**
SSBN003C24u.scf	DC1 domain containing protein [Nicotiana tabacum]	**3,31**
BF_LBCHXXXX_0049G03_T3M.SCF	No Hits Found	**3,30**
BF_LBCHXXXX_0024D04_T3M.SCF	No Hits Found	**3,30**
MICRO.14504.C1	putative retrotransposon protein [Solanum demissum]	**3,29**
MICRO.13448.C1	No Hits Found	**3,28**
MICRO.4371.C1	unnamed protein product [Petunia x hybrida] emb_CAA32729.1_ unnamed protein product [Petunia x hybrida] sp_P11650_CFIA_PETHY Chalcone--flavonone isomerase A (Chalcone isomerase A) pir__ISPJCA chalcone isomerase (EC 5.5.1.6) A - garden petunia	**3,27**
POAC302TV	No Hits Found	**3,26**
MICRO.3765.C1	hexokinase 5 [Nicotiana tabacum]	**3,25**
MICRO.2120.C3	hypothetical protein [Arabidopsis thaliana] pir__T02502 hypothetical protein At2g38430 [imported] - Arabidopsis thaliana	**3,25**
bf_ivrootxx_0023d11.t3m.scf	No Hits Found	**3,24**
MICRO.4162.C3	No Hits Found	**3,22**
MICRO.4170.C3	Carbonic anhydrase, chloroplast precursor (Carbonate dehydratase) pir__T02936 carbonate dehydratase (EC 4.2.1.1) precursor, chloroplast - common tobacco gb_AAA34065.1_ chloroplast carbonic anhydrase prf__1909357A carbonic anhydrase	**3,22**
MICRO.5319.C5	Polyprotein, putative [Solanum tuberosum]	**3,21**
BF_LBCHXXXX_0044B10_T3M.SCF	No Hits Found	**3,20**

BF_LBCHXXXX_0018H07_T3M.SCF	auxin response factor 19 (ARF19) [Solanum lycopersicum]	**3,19**
STMCQ36TV	No Hits Found	**3,17**
MICRO.1798.C1	No Hits Found	**3,16**
MICRO.940.C1	No Hits Found	**3,15**
MICRO.1329.C3	putative gamma TIP [Nicotiana glauca]	**3,14**
bf_cswbxxxx_0022a08.t3m.scf	No Hits Found	**3,13**
MICRO.4718.C1	At5g57670 [Arabidopsis thaliana]	**3,13**
MICRO.712.C12	No Hits Found	**3,11**
SDBN005G06u.scf	nucleotide binding / ubiquitin-protein ligase [Arabidopsis thaliana] NP_850206.4	**3,10**
MICRO.6828.C1	No Hits Found	**3,09**
MICRO.18213.C1	No Hits Found	**3,08**
MICRO.16550.C1	anthocyanin 1 [Lycopersicon esculentum]	**3,08**
cSTA17K10TH	dihydropterin pyrophosphokinase /dihydropteroate synthase [Pisum sativum] pir__T06595 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase (EC 2.7.6.3) - garden pea	**3,07**
SSBN002C03u.scf	No Hits Found	**3,06**
MICRO.15057.C1	small heat shock protein [Lycopersicon esculentum] gb_AAM96944.1_ small heat shock protein [Lycopersicon esculentum]	**3,06**
bf_cswbxxxx_0032e09.t3m.scf	No Hits Found	**3,05**
bf_mxflxxxx_0036h11.t3m.scf	putative retrotransposon protein [Solanum demissum]	**3,04**
MICRO.12207.C1	At3g23510/MEE5_5 [Arabidopsis thaliana] gb_AAL38380.1_ AT3g23510/MEE5_5 [Arabidopsis thaliana] ref_NP_188995.2_ cyclopropane fatty acid synthase, putative / CPA-FA synthase, putative [Arabidopsis thaliana]	**3,04**
SSBN002I07u.scf	No Hits Found	**3,03**
MICRO.6172.C4	No Hits Found	**3,03**
MICRO.5193.C1	No Hits Found	**3,02**
MICRO.17898.C1	At2g27385 [Arabidopsis thaliana] gb_AAS65937.1_ At2g27385 [Arabidopsis thaliana] ref_NP_850100.1_ expressed protein [Arabidopsis thaliana]	**3,02**
MICRO.6375.C1	putative wall-associated serine/threonine kinase [Oryza sativa (japonica cultivar-group)] dbj_BAD22233.1_ putative wall-associated serine/threonine kinase [Oryza sativa (japonica cultivar-group)] dbj_BAD22232.1_ putative wall-associated ...	**3,01**
MICRO.17922.C1	glucose-1-phosphate adenylyltransferase, putative / ADP-glucose pyrophosphorylase, putative (APS2) [Arabidopsis thaliana]	**3,01**
MICRO.16424.C1	No Hits Found	**3,00**
MICRO.1610.C1	Rx protein [Solanum tuberosum]	**3,00**
bf_ivrootxx_0017h09.t3m.scf	No Hits Found	**3,00**
MICRO.4189.C1	hypothetical protein [Arabidopsis thaliana] gb_AAM63007.1_ unknown [Arabidopsis thaliana] dbj_BAC42160.1_ unknown protein [Arabidopsis thaliana] gb_AAO50707.1_ unknown protein [Arabidopsis thaliana] ref_NP_566393.1_ expressed protein [Arabidopsis ...	*-3,00*
097E08AF.esd	maoC-like dehydratase domain-containing protein [Arabidopsis thaliana] NP_200842.1	*-3,01*
MICRO.3598.C4	unknown protein [Oryza sativa (japonica cultivar-group)]	*-3,03*
MICRO.8485.C3	peroxidase	*-3,06*
POABH03TV	unknown protein [Arabidopsis thaliana] gb_AAO42117.1_ unknown protein [Arabidopsis thaliana] ref_NP_180444.2_ expressed protein [Arabidopsis thaliana]	*-3,11*
MICRO.4268.C2	adenylate cyclase [Arabidopsis thaliana] NP_565353.1	*-3,15*

MICRO.17192.C1	No Hits Found	*-3,19*
MICRO.8382.C12	germin-like protein GLP2a copy1 [Arabidopsis thaliana] dbj_BAB09370.1_ oxalate oxidase (germin protein)-like protein [Arabidopsis thaliana] gb_AAN72181.1_ germin-like protein GLP2a copy1 [Arabidopsis thaliana] ref_NP_198732.2_ germin-like protein ...	*-3,25*
MICRO.135.C16	Alcohol dehydrogenase 3 gb_AAA33808.1_ alcohol dehydrogenase 3 (EC 1.1.1.1)	*-3,29*
cSTB47P14TH	No Hits Found	*-3,33*
MICRO.3063.C1	cytochrome P450 [Pisum sativum]	*-3,39*
MICRO.13158.C4	No Hits Found	*-3,45*
STMHW01TV	No Hits Found	*-3,52*
MICRO.4956.C2	prosystemin [Solanum tuberosum] pir__T06993 prosystemin PRO1 - potato	*-3,60*
ACDA01448A08.skm.scf	Rieske iron sulphur protein [Solanum tuberosum] sp_P37841_UCRI_SOLTU Ubiquinol-cytochrome c reductase iron-sulfur subunit, mitochondrial precursor (Rieske iron-sulfur protein) (RISP)	*-3,68*
SDBN002L22u.scf	No Hits Found	*-3,74*
MICRO.991.C2	secretory peroxidase [Nicotiana tabacum]	*-3,83*
MICRO.13154.C1	At1g77260/T14N5_19 [Arabidopsis thaliana] gb_AAK56248.1_ At1g77260/T14N5_19 [Arabidopsis thaliana] ref_NP_565153.1_ dehydration-responsive protein-related [Arabidopsis thaliana]	*-3,97*
SDBN006L17u.scf	No Hits Found	*-4,02*
bf_lbchxxxx_0058b06.t3m.scf	No Hits Found	*-4,11*
BF_LBCHXXXX_0004E01_T3M.SCF	No Hits Found	*-4,19*
bf_arrayxxx_0083g03.t3m.scf	No Hits Found	*-4,23*
POAD618TP	unknown protein [Arabidopsis thaliana] gb_AAL34281.1_ unknown protein [Arabidopsis thaliana] gb_AAK59421.1_ unknown protein [Arabidopsis thaliana] gb_AAM47948.1_ unknown protein [Arabidopsis thaliana] gb_AAL32518.1_ Unknown protein [Arabidopsis ...	*-4,31*
ACDA03576B12.T3m.scf	No Hits Found	*-4,36*
MICRO.1770.C3	TSI-1 protein [Lycopersicon esculentum] pir__T07403 TSI-1 protein - tomato	*-4,39*
bf_acdcxxxx_0042g01.t3m.scf	TSI-1 protein [Lycopersicon esculentum] pir__T07403 TSI-1 protein - tomato	*-4,44*
MICRO.9296.C1	No Hits Found	*-4,49*
MICRO.10609.C1	NIM1-like protein 2 [Lycopersicon esculentum]	*-4,56*
MICRO.4933.C1	At2g31130/T16B12.6 [Arabidopsis thaliana] gb_AAC63838.2_ expressed protein [Arabidopsis thaliana] gb_AAL27517.1_ At2g31130/T16B12.6 [Arabidopsis thaliana] ref_NP_565715.1_ expressed protein [Arabidopsis thaliana]	*-4,61*
MICRO.10308.C2	twi1 [Lycopersicon esculentum] pir__T07404 probable glucosyltransferase twi1 (EC 2.4.1.-) - tomato (fragment)	*-4,68*
MICRO.16908.C1	No Hits Found	*-4,81*
MICRO.5553.C3	aspartic endopeptidase [Cucurbita pepo]	*-4,96*
MICRO.6187.C1	glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	*-5,02*
MICRO.13234.C1	No Hits Found	*-5,07*
MICRO.14186.C1	At2g32990 [Arabidopsis thaliana] gb_AAB91971.1_ putative glucanse [Arabidopsis thaliana] gb_AAL32517.1_ putative glucanse [Arabidopsis thaliana] pir__T01108 cellulase (EC 3.2.1.4) T21L14.7 - Arabidopsis thaliana ref_NP_180858.1_ glycosyl hydrolase ...	*-5,14*
bf_cswcxxxx_0002f12.t3m.scf	ribonuclease P [Arabidopsis lyrata subsp. lyrata] XP_002880292.1	*-5,23*
cSTA43B1TH	putative protein [Arabidopsis thaliana] pir__T47503 hypothetical protein F9K21.210 - Arabidopsis thaliana ref_NP_190149.1_ RNA recognition motif (RRM)-containing protein [Arabidopsis thaliana]	*-5,28*

MICRO.16621.C1	Contains similarity to a jasmonate inducible protein from Brassica napus gb_Y11483 and contains a Jacalin-like lectin PF_01419 domain. EST gb_AI998212 comes from this gene. [Arabidopsis thaliana] gb_AAF14583.1_ RTM1 [Arabidopsis thaliana] pir__A8619 ...	*-5,36*
MICRO.6809.C6	proteinase inhibitor II [Solanum tuberosum] sp_Q00782_IP2X_SOLTU Proteinase inhibitor type II precursor pir__T07597 proteinase inhibitor II - potato	*-5,44*
bf_mxlfxxxx_0038d10.t3m.scf	No Hits Found	*-5,61*
bf_mxflxxxx_0055h12.t3m.scf	glucuronosyl transferase-like protein [Arabidopsis thaliana] ref_NP_191130.1_ UDP-glucoronosyl/UDP-glucosyl transferase family protein [Arabidopsis thaliana] pir__T47710 glucuronosyl transferase-like protein - Arabidopsis thaliana	*-5,69*
MICRO.5326.C1	salt tolerance protein 4 [Beta vulgaris]	*-5,71*
MICRO.3031.C1	Unknown protein [Arabidopsis thaliana] gb_AAS99692.1_ At1g10020 [Arabidopsis thaliana] pir__T00621 hypothetical protein T27I1.4 - Arabidopsis thaliana ref_NP_172473.1_ expressed protein [Arabidopsis thaliana] gb_AAR92283.1_ At1g10020 [Arabidopsis ...	*-5,80*
MICRO.6822.C2	CA-responsive protein [Brassica oleracea]	*-5,88*
MICRO.12567.C1	No Hits Found	*-5,96*
MICRO.1061.C2	similar to AT4G19880: similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G45020.1); similar to Intracellular chloride channel [Medicago truncatula] (GB:ABC75353.2); contains InterPro domain Thioredoxin-like fold (InterPro:IPR012336); contains InterPro domain Glutathione S-transferase, C-terminal-like (InterPro:IPR010987); contains InterPro domain Glutathione S-transferase, predicted (InterPro:IPR016639)	*-6,02*
MICRO.10085.C1	glutathione S-transferase GST 12 [Glycine max]	*-6,11*
MICRO.17012.C1	No Hits Found	*-6,26*
bf_cswbxxxx_0007b10.t3m.scf	No Hits Found	*-6,34*
MICRO.12570.C2	unknown protein [Arabidopsis thaliana] gb_AAK92812.1_ unknown protein [Arabidopsis thaliana] ref_NP_851167.1_ expressed protein [Arabidopsis thaliana] ref_NP_568754.1_ expressed protein [Arabidopsis thaliana]	*-6,37*
071B10AF.esd	putative poly(ADP-ribose) polymerase [Arabidopsis thaliana]	*-6,41*
MICRO.1142.C1	aminoacyl-tRNA hydrolase [Arabidopsis thaliana] NP_568340.1	*-6,46*
SSBN003H18u.scf	No Hits Found	*-6,58*
MICRO.13355.C1	50S ribosomal protein l9 [Bartonella henselae str. Houston-1]	*-6,59*
BF_LBCHXXXX_0050B04_T3M.SCF	No Hits Found	*-6,66*
MICRO.8761.C1	putative protein [Arabidopsis thaliana] emb_CAA19754.1_ putative protein [Arabidopsis thaliana] pir__T05101 hypothetical protein F28M20.120 - Arabidopsis thaliana ref_NP_194897.1_ transcriptional factor B3 family protein [Arabidopsis thaliana]	*-6,69*
cSTA1M14TH	putative NAC domain protein [Solanum tuberosum]	*-6,73*
BF_LBCHXXXX_0012F09_T3M.SCF	gag-pol polyprotein [Phaseolus vulgaris]	*-6,81*
cSTB46H13TH	No Hits Found	*-7,03*
MICRO.4165.C9	No Hits Found	*-7,14*
MICRO.2711.C1	putative transposase [Arabidopsis thaliana] gb_AAO24589.1_ At3g42170 [Arabidopsis thaliana] pir__T46111 probable transposase - Arabidopsis thaliana	*-7,26*
cPRO29E1TH	Contains similarity to aminoacylase from Sus scrofa domestica gi_S27010 and contains a peptidase M20 PF_01546 domain. ESTs gb_H76043, gb_AA394953, gb_AI995115, gb_AA651481 come from this gene. [Arabidopsis thaliana] pir__C96507 hypothetical protein ...	*-7,29*
bf_mxlfxxxx_0041e02.t3m.scf	predicted protein [Populus trichocarpa] XP_002307383.1	*-7,33*
MICRO.12700.C2	hypothetical protein At1g64065 [Arabidopsis thaliana] gb_AAT68728.1_ hypothetical protein At1g64065 [Arabidopsis thaliana] ref_NP_974086.1_ expressed protein [Arabidopsis thaliana]	*-7,39*

MICRO.15691.C1	No Hits Found	*-7,41*
MICRO.17766.C1	similar to AT4G36500: similar to unknown protein [Arabidopsis thaliana]	*-7,48*
SDBN003H17u.scf	P450 hydroxylase [Solanum melongena] sp_P37117_C714_SOLME Cytochrome P450 71A4 (CYPLXXIA4) (P-450EG2) pir__S36805 cytochrome P450 71A4 - eggplant	*-7,49*
MICRO.112.C6	NL27 [Solanum tuberosum]	*-7,51*
MICRO.395.C1	acetyltransferase 1-like [Oryza sativa (japonica cultivar-group)]	*-7,58*
bf_mxflxxxx_0069c07.t3m.scf	zinc ion binding [Arabidopsis thaliana] NP_191849.2	*-7,62*
bf_mxlfxxxx_0055h11.t3m.scf	pentatricopeptide (PPR) repeat-containing protein-like [Oryza sativa (japonica cultivar-group)]	*-7,81*
MICRO.15146.C2	hypothetical protein [Vitis vinifera] XP_002267969.1	*-7,89*
BF_TUBSXXXX_0022E02_T3M.SCF	No Hits Found	*-7,99*
BF_TUBSXXXX_0009H09_T3M.SCF	No Hits Found	*-8,00*
bf_arrayxxx_0043h04.t3m.scf	hydroxymethylglutaryl coenzyme A synthase [Hevea brasiliensis]	*-8,05*
MICRO.5844.C2	No Hits Found	*-8,15*
bf_lbchxxxx_0057b05.t3m.scf	No Hits Found	*-8,19*
BF_LBCHXXXX_0002F06_T3M.SCF	No Hits Found	*-8,28*
MICRO.2255.C3	LEXYL1 [Lycopersicon esculentum]	*-8,29*
MICRO.8755.C4	terpene synthase [Vitis vinifera]	*-8,38*
SDBT002K03x.scf	Hsp20.1 protein [Lycopersicon peruvianum]	*-8,39*
bf_mxlfxxxx_0063d12.t3m.scf	No Hits Found	*-8,43*
MICRO.16391.C1	hypothetical protein SDM1_46t00004 [Solanum demissum] ABI34352.1	*-8,61*
bf_arrayxxx_0040c04.t3m.scf	galactokinase-like protein [Arabidopsis thaliana]	*-8,88*
MICRO.132.C1	similar to AT2G42570: similar to unknown protein [Arabidopsis thaliana]	*-8,91*
SSBN003C19u.scf	similar to AT1G74630: pentatricopeptide (PPR) repeat-containing protein	*-8,99*
bf_cswbxxxx_0040a03.t3m.scf	No Hits Found	*-9,05*
POADZ22TV	putative diacylglycerol kinase [Arabidopsis thaliana]	*-9,09*
MICRO.16224.C1	hypothetical protein [Oryza sativa (japonica cultivar-group)]	*-9,15*
MICRO.4960.C1	No Hits Found	*-9,33*
BF_TUBSXXXX_0036D09_T3M.SCF	No Hits Found	*-9,48*
SDBN006K05u.scf	No Hits Found	*-9,49*
STMGQ65TV	Similar to ATCG01280 | Symbols: YCF2.2 | Identical to Protein ycf2 (ycf2-B) [Arabidopsis Thaliana]	*-9,56*
STDB005N03u.scf	No Hits Found	*-9,77*
bf_arrayxxx_0078h06.t3m.scf	No Hits Found	*-10,31*
MICRO.8628.C1	subtilisin-like protease [Glycine max]	*-10,56*
MICRO.2317.C1	unknown protein [Arabidopsis thaliana]	*-10,59*
bf_mxlfxxxx_0065a05.t3m.scf	No Hits Found	*-10,94*
MICRO.13253.C2	NBS-LRR disease resistance protein homologue [Hordeum vulgare]	*-10,99*
bf_swstxxxx_0046c11.t3m.scf	putative galactokinase [Arabidopsis thaliana] gb_AAL59925.1_ putative galactokinase [Arabidopsis thaliana] ref_NP_187681.2_ GHMP kinase family protein [Arabidopsis thaliana]	*-11,03*

bf_swstxxxx_0050b12.t3m.scf	No Hits Found	*-11,08*
MICRO.13478.C1	putative chalcone isomerase [Lycopersicon esculentum]	*-11,16*
POCAK50TP	hypothetical protein [Arabidopsis thaliana] gb_AAL15227.1_ unknown protein [Arabidopsis thaliana] gb_AAK44045.1_ unknown protein [Arabidopsis thaliana] ref_NP_566205.1_ Ku70-binding family protein [Arabidopsis thaliana]	*-11,45*
MICRO.1262.C1	similar to AT3G08640: alphavirus core protein family	*-11,92*
STDB004H01u.scf	No Hits Found	*-12,23*
cSTD17F23TH	No Hits Found	*-12,29*
MICRO.5773.C1	similar to AT2G29660: zinc finger (C2H2 type) family protein	*-13,88*
MICRO.14122.C1	Chain P, Crystal Structure Of Human Thimet Oligopeptidase.	*-20,11*
cSTB33G21TH	putative auxin-induced SAUR-like protein [Capsicum annuum]	*-21,46*

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the early stage of symbiotic establishment of AM fungus in the potato roots versus control comparison are ordered according to the expression fold change. Genes were considered as differentially regulated if P-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.00 or ≤ -3.00

Table S3 The 865 genes regulated during the late stage of potato root colonization by Glomus sp. MUCL 41833 as compared with the control treatment, and their changes in expression

Probe ID	Annotation	Ratio Late
POCAO75TV	No Hits Found	**557,39**
STMHW15TV	No Hits Found	**372,44**
099E10AF.esd	auxin response factor 1 [Solanum lycopersicum]	**295,03**
MICRO.12609.C1	CDPK adapter protein 1 [Mesembryanthemum crystallinum]	**252,97**
STMCV27TV	No Hits Found	**227,63**
MICRO.12534.C1	putative calcium/calmodulin-dependent protein kinase kinase [Ricinus communis]	**218,00**
STMHK88TV	No Hits Found	**192,08**
ACDA02636D08.T3m.scf	No Hits Found	**161,55**
STMHB06TV	FCLY (FARNESYLCYSTEINE LYASE); prenylcysteine oxidase [Arabidopsis thaliana]	**142,64**
MICRO.5735.C1	CPD photolyase [Cucumis sativus] dbj_BAB39480.1_ CPD photolyase [Cucumis sativus]	**135,50**
POABX60TV	No Hits Found	**133,19**
cSTD13H3TH	Hsp90-2-like [Solanum tuberosum]	**133,06**
bf_mxlfxxxx_0017a02.t3m.scf	No Hits Found	**120,71**
POCC267TP	vacuolar protein sorting 28 isoform 1 [Homo sapiens] gb_AAH06485.1_ Vacuolar protein sorting 28, isoform 1 [Homo sapiens] gb_AAH19321.1_ Vacuolar protein sorting 28, isoform 1 [Homo sapiens] gb_AAF00499.1_ VPS28 protein [Homo sapiens] ...	**114,89**
ACDA01778E02.T3m.scf	No Hits Found	**106,38**
POCBP75TV	No Hits Found	**103,11**
STMGQ11TV	leaf senescence protein-like [Oryza sativa (japonica cultivar-group)]	**99,33**
PotatoF1087.scf	No Hits Found	**96,12**
TBSK04474FE10.t3m.scf	No Hits Found	**95,88**
MICRO.12240.C1	unknown protein [Arabidopsis thaliana] gb_AAN12986.1_ unknown protein [Arabidopsis thaliana] ref_NP_566364.1_ calcineurin-like phosphoesterase family protein [Arabidopsis thaliana]	**94,51**
PotatoF0700.scf	No Hits Found	**90,74**
PotatoF1116.scf	No Hits Found	**86,45**
STMIH92TH	guanine nucleotide exchange family protein [Arabidopsis thaliana]	**86,00**
MICRO.12934.C1	No Hits Found	**83,95**
PotatoF1180.scf	No Hits Found	**83,02**
MICRO.2326.C2	At2g34770/T29F13.2 [Arabidopsis thaliana] gb_AAC16270.1_ fatty acid hydroxylase (FAH1) [Arabidopsis thaliana] gb_AAK91343.1_ At2g34770/T29F13.2 [Arabidopsis thaliana] pir__T01359 fatty acid hydroxylase (EC 1.14.15.-) FAH1 - Arabidopsis thaliana ...	**69,53**
MICRO.11687.C1	At4g25340/T30C3_20 [Arabidopsis thaliana] gb_AAL09783.1_ AT4g25340/T30C3_20 [Arabidopsis thaliana] ref_NP_567717.1_ immunophilin-related / FKBP-type peptidyl-prolyl cis-trans isomerase-related [Arabidopsis thaliana]	**67,66**
MICRO.13672.C1	No Hits Found	**65,72**

bf_arrayxxx_0060e11.t7m.scf	No Hits Found	**64,99**
bf_mxflxxxx_0012c09.t3m.scf	No Hits Found	**64,87**
MICRO.3155.C2	At1g31300/T19E23_12 [Arabidopsis thaliana] gb_AAL24160.1_ At1g31300/T19E23_12 [Arabidopsis thaliana] ref_NP_564377.1_ expressed protein [Arabidopsis thaliana]	**64,70**
cSTA20E18TH	putative membrane import protein [Arabidopsis thaliana] gb_AAK93602.1_ putative membrane import protein [Arabidopsis thaliana] ref_NP_188634.1_ porin family protein [Arabidopsis thaliana] sp_Q9LHE5_OM40_ARATH Probable mitochondrial import receptor ...	**63,04**
SSBN002E02u.scf	No Hits Found	**60,59**
bf_mxflxxxx_0042h11.t3m.scf	No Hits Found	**59,81**
POCDC04TV	No Hits Found	**58,46**
MICRO.1575.C1	similar to AT5G43560: meprin and TRAF homology domain-containing protein / MATH domain-containing protein	**58,30**
bf_swstxxxx_0057h03.t3m.scf	No Hits Found	**54,77**
POCA468TV	At2g41370 [Arabidopsis thaliana] gb_AAC78536.1_ hypothetical protein [Arabidopsis thaliana] pir__H84840 hypothetical protein At2g41370 [imported] - Arabidopsis thaliana ref_NP_181668.1_ ankyrin repeat family protein / BTB/POZ domain-containing ...	**53,80**
MICRO.8649.C3	elicitor-inducible protein EIG-J7 [Capsicum annuum]	**53,64**
MICRO.11749.C1	No Hits Found	**52,45**
MICRO.4606.C1	putative transcription factor [Oryza sativa (japonica cultivar-group)] gb_AAP54297.1_ putative transcription factor [Oryza sativa (japonica cultivar-group)] gb_AAK21342.1_ putative transcription factor [Oryza sativa (japonica cultivar-group)]	**50,97**
POAC118TV	PERK1-like protein kinase [Nicotiana tabacum]	**49,81**
119B06AF.esd	hypothetical protein At2g31380 [imported] - Arabidopsis thaliana	**49,56**
MICRO.1179.C1	protoporphyrinogen oxidase [Solanum tuberosum] pir__T07121 protoporphyrinogen oxidase (EC 1.3.3.4) IX, mitochondrial - potato (fragment)	**48,62**
ACDA03993E09.T3m.scf	No Hits Found	**48,59**
MICRO.7586.C1	putative clathrin coat assembly protein [Arabidopsis thaliana] gb_AAL38763.1_ putative clathrin coat assembly protein [Arabidopsis thaliana] ref_NP_190655.2_ clathrin adaptor complex small chain family protein [Arabidopsis thaliana]	**47,52**
MICRO.1917.C1	At1g72880 [Arabidopsis thaliana] ref_NP_849880.1_ acid phosphatase survival protein SurE, putative [Arabidopsis thaliana] ref_NP_177431.1_ acid phosphatase survival protein SurE, putative [Arabidopsis thaliana]	**46,67**
MICRO.652.C1	No Hits Found	**46,48**
POCD177TP	putative polyprotein [Solanum demissum]	**45,31**
MICRO.4276.C2	putative ripening-related P-450 enzyme [Vitis vinifera]	**45,28**
MICRO.12703.C1	nectarin 5 [Nicotiana langsdorffii x Nicotiana sanderae]	**45,23**
STMIZ22TV	No Hits Found	**44,55**
MICRO.1446.C2	expressed protein [Arabidopsis thaliana] ref_NP_974429.1_ expressed protein [Arabidopsis thaliana]	**41,71**
bf_arrayxxx_0093h04.t7m.scf	No Hits Found	**41,48**
bf_cswbxxxx_0008b12.t3m.scf	No Hits Found	**41,15**
MICRO.4318.C2	similar to eyes absent protein [Arabidopsis thaliana]	**40,66**
SDBN006K20u.scf	putative retroelement pol polyprotein-like [Solanum tuberosum]	**39,78**
STMCT95TV	putative protein kinase APK1A [Trifolium pratense]	**39,49**
bf_arrayxxx_0051h03.t7m.scf	No Hits Found	**39,16**

MICRO.13890.C1	putative protein [Arabidopsis thaliana] emb_CAA16591.1_ putative protein [Arabidopsis thaliana] pir__T04647 hypothetical protein F10N7.210 - Arabidopsis thaliana ref_NP_194926.1_ expressed protein	**38,93**
SSBT005G22x.scf	No Hits Found	**38,74**
MICRO.7828.C1	unknown protein [Arabidopsis thaliana]	**37,99**
cPRO18P12TH	No Hits Found	**37,42**
STMGA05TV	OSJNBb0038F03.12 [Oryza sativa (japonica cultivar-group)]	**37,25**
MICRO.1275.C2	putative UDP-glucuronate decarboxylase 3 [Nicotiana tabacum]	**37,08**
POAD616TV	No Hits Found	**35,97**
bf_lbchxxxx_0058h06.t3m.scf	No Hits Found	**35,62**
MICRO.10499.C2	unknown [Solanum tuberosum] ABA40464.1	**34,94**
bf_mxlfxxxx_0068c01.t3m.scf	sulfate transporter 2 [Lycopersicon esculentum]	**34,63**
POCC517TV	similar to AT1G73480: hydrolase, alpha/beta fold family protein	**34,56**
MICRO.15827.C1	No Hits Found	**34,51**
MICRO.4172.C1	Hcr9-OR3A [Lycopersicon pimpinellifolium]	**33,91**
bf_swstxxxx_0043b02.t3m.scf	No Hits Found	**33,68**
POCAE23TV	No Hits Found	**33,64**
MICRO.5412.C1	No Hits Found	**33,04**
cSTS3E17TH	No Hits Found	**32,89**
cPRO15G9TH	No Hits Found	**32,74**
MICRO.13264.C1	branched chain alpha-keto acid dehydrogenase E1-alpha subunit [Lycopersicon esculentum] pir__T06589 3-methyl-2-oxobutanoate dehydrogenase (lipoamide) (EC 1.2.4.4) E1-alpha chain precursor, mitochondrial - tomato	**32,23**
BF_CSCHXXXX_0027C12.T3M.SCF	No Hits Found	**32,17**
cSTA25E13TH	hypothetical protein [Arabidopsis thaliana] pir__T47972 hypothetical protein F15G16.190 - Arabidopsis thaliana ref_NP_191739.1_ expressed protein [Arabidopsis thaliana]	**31,91**
cPRO1F18TH	Remorin (pp34) pir__T07780 remorin - potato gb_AAB49425.1_ remorin [Solanum tuberosum]	**31,87**
bf_suspxxxx_0060a10.t3m.scf	zinc finger (ubiquitin-hydrolase) domain-containing protein [Arabidopsis thaliana]	**31,74**
MICRO.9963.C1	putative polyprotein [Solanum tuberosum]	**31,56**
POAB109TF	zinc-finger protein [Oryza sativa (indica cultivar-group)]	**30,75**
MICRO.7203.C1	secondary cell wall-related glycosyltransferase family 8 [Populus tremula x Populus tremuloides]	**30,16**
POCC042TV	No Hits Found	**30,12**
bf_arrayxxx_0008h01.t3m.scf	No Hits Found	**29,92**
MICRO.12080.C1	putative actin related protein 2 [Oryza sativa (japonica cultivar-group)] dbj_BAD03538.1_ putative actin related protein 2 [Oryza sativa (japonica cultivar-group)] dbj_BAD03487.1_ putative actin related protein 2 [Oryza sativa (japonica cultivar-group)]	**29,78**
cPRO3P16TH	No Hits Found	**29,51**
ACDA04926C06.T3m.scf	No Hits Found	**28,25**
POCAJ71TP	No Hits Found	**28,16**
MICRO.14058.C1	similar to AT4G08250: scarecrow transcription factor family protein	**27,91**
MICRO.5867.C1	4-coumarate CoA ligase isoform 4 [Arabidopsis thaliana] gb_AAQ56837.1_ At5g63380 [Arabidopsis thaliana] gb_AAM97124.1_ 4-coumarate-CoA ligase-like protein [Arabidopsis thaliana] ref_NP_201143.1_ 4-coumarate--CoA ligase family protein / ...	**27,56**

MICRO.9327.C1	No Hits Found	**27,01**
bf_arrayxxx_0077h04.t3m.scf	No Hits Found	**25,84**
POCC910TV	GAL83 protein [Solanum tuberosum]	**25,34**
POABH36TV	RRM-containing RNA-binding protein-like protein [Solanum tuberosum]	**25,07**
cPRO20B10TH	ubiquitin thiolesterase/ zinc ion binding (AT3G47890) [Arabidopsis thaliana]	**25,03**
MICRO.7128.C1	No Hits Found	**24,68**
cPRO20J10TH	bZIP transcription factor (BZI-1) [Nicotiana tabacum]	**24,61**
bf_suspxxxx_0051f03.t3m.scf	No Hits Found	**23,74**
PotatoF0403.scf	hypothetical protein [Vitis vinifera] XP_002282213.1	**23,36**
POCBQ63TV	Heat shock protein DnaJ [Medicago truncatula]	**22,68**
SSBN001D23u.scf	No Hits Found	**22,58**
SSBT001N21x.scf	No Hits Found	**22,45**
TBSK00866FA02t3m.scf	At1g51580 [Arabidopsis thaliana] gb_AAG50879.1_ hypothetical protein [Arabidopsis thaliana] pir__D96554 hypothetical protein F19C24.19 [imported] - Arabidopsis thaliana gb_AAG52626.1_ hypothetical protein	**22,22**
bf_cswbxxxx_0051d10.t3m.scf	No Hits Found	**21,74**
MICRO.10302.C1	Cytochrome P450-like protein [Arabidopsis thaliana] emb_CAA16595.1_ Cytochrome P450-like protein [Arabidopsis thaliana] ref_NP_194922.1_ cytochrome P450, putative [Arabidopsis thaliana] pir__T04651 cytochrome P450 F10N7.250 - Arabidopsis thaliana	**21,63**
MICRO.4199.C1	putative GDSL-motif lipase/acylhydrolase [Arabidopsis thaliana] gb_AAO50514.1_ unknown protein [Arabidopsis thaliana] gb_AAO42146.1_ unknown protein [Arabidopsis thaliana] ref_NP_198322.1_ GDSL-motif lipase/hydrolase family protein [Arabidopsis thal ...	**21,49**
MICRO.3961.C1	No Hits Found	**21,46**
cPRO24I15TH	F21D18.16 [Arabidopsis thaliana] pir__D96521 protein F21D18.16 [imported] - Arabidopsis thaliana	**21,38**
MICRO.5292.C1	At1g17330 [Arabidopsis thaliana] gb_AAO00736.1_ unknown protein [Arabidopsis thaliana] ref_NP_173176.1_ metal-dependent phosphohydrolase HD domain-containing protein-related [Arabidopsis thaliana]	**21,34**
MICRO.5225.C1	No Hits Found	**21,30**
cSTD12A19TH	zinc finger protein, putative / regulator of chromosome condensation (RCC1) family protein [Arabidopsis thaliana]	**21,26**
bf_lbchxxxx_0064c02.t3m.scf	No Hits Found	**21,25**
MICRO.6804.C1	No Hits Found	**21,23**
cSTA19G13TH	phospholipid/glycerol acyltransferase family protein [Arabidopsis thaliana] NP_177990.1	**21,04**
POCAR48TV	unknown [Arabidopsis thaliana]	**20,91**
MICRO.17189.C1	unknown [Solanum lycopersicum] AAW22882.1	**20,86**
SDBN004I17u.scf	No Hits Found	**20,85**
STDB004D20u.scf	No Hits Found	**20,74**
MICRO.6657.C1	unnamed protein product [Arabidopsis thaliana]	**20,67**
MICRO.17938.C2	AAA-type ATPase family protein [Arabidopsis thaliana]	**20,63**
bf_arrayxxx_0051h09.t3m.scf	Hsp90-2-like [Solanum tuberosum]	**20,56**
SSBN003O12u.scf	unknown protein [Arabidopsis thaliana] gb_AAO63435.1_ At1g76250 [Arabidopsis thaliana] ref_NP_177752.1_ expressed protein [Arabidopsis thaliana]	**20,21**

bf_mxlfxxxx_0057b05.t3m.scf	No Hits Found	**19,58**
BF_TUBSXXXX_0065A08_T3M.SCF	No Hits Found	**19,41**
bf_stolxxxx_0051g03.t7m.scf	No Hits Found	**19,39**
MICRO.7781.C1	No Hits Found	**19,35**
MICRO.6947.C1	No Hits Found	**18,95**
STMIB81TV	unknown protein [Oryza sativa (japonica cultivar-group)]	**18,67**
cSTS19G2TH	similar to AT5G53390: similar to unknown protein [Arabidopsis thaliana]	**18,56**
MICRO.15368.C1	zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana]	**18,44**
MICRO.2888.C1	putative endomembrane protein 70 [Arabidopsis thaliana]	**18,38**
bf_mxlfxxxx_0071g09.t3m.scf	No Hits Found	**18,04**
MICRO.14508.C1	Selenoprotein W sp_P63302_SELW_HUMAN Selenoprotein W	**17,76**
PotatoF1106.scf	No Hits Found	**17,45**
POAB159TF	plastidic aldolase NPALDP1 [Nicotiana paniculata]	**17,32**
MICRO.6939.C1	universal stress protein (USP) family protein [Arabidopsis thaliana]	**17,17**
ACDA01308E12.T3m.scf	No Hits Found	**16,97**
SDBN003N13u.scf	No Hits Found	**16,72**
099H08AF.esd	No Hits Found	**16,67**
POCDK67TV	regulator of chromosome condensation like [Arabidopsis thaliana] gb_AAO63928.1_ putative regulator of chromosome condensation (cell cycle regulatory protein) [Arabidopsis thaliana] ref_NP_199644.2_ regulator of chromosome condensation (RCC1) family ...	**16,66**
MICRO.2138.C2	gamma response I protein [Arabidopsis thaliana] pir__T49091 gamma response I protein - Arabidopsis thaliana	**16,58**
MICRO.7772.C1	No Hits Found	**16,55**
SDBN005P23u.scf	No Hits Found	**16,28**
MICRO.8037.C1	No Hits Found	**16,23**
MICRO.3610.C1	similar to AT2G38320: similar to unknown protein [Arabidopsis thaliana]	**16,19**
POAE055TV	unknown [Arabidopsis thaliana] ref_NP_567058.1_ OTU-like cysteine protease family protein [Arabidopsis thaliana] ref_NP_850716.1_ OTU-like cysteine protease family protein [Arabidopsis thaliana]	**16,14**
MICRO.15713.C1	No Hits Found	**16,11**
bf_cswbxxxx_0035h04.t3m.scf	unknown protein [Arabidopsis thaliana] gb_AAL49877.1_ unknown protein [Arabidopsis thaliana] ref_NP_191639.2_ zinc finger (DHHC type) family protein [Arabidopsis thaliana]	**16,02**
bf_mxflxxxx_0006c08.t3m.scf	12-oxophytodienoate reductase [Lycopersicon esculentum]	**15,99**
MICRO.8183.C1	Similar to CCS1 [Arabidopsis thaliana] dbj_BAC41965.1_ unknown protein [Arabidopsis thaliana] pir__B96530 Similar to CCS1 [imported] - Arabidopsis thaliana ref_NP_564544.1_ cytochrome c biogenesis protein family [Arabidopsis thaliana]	**15,93**
MICRO.1125.C3	No Hits Found	**15,88**
MICRO.12028.C1	No Hits Found	**15,84**
SDBN003E19u.scf	No Hits Found	**15,77**
MICRO.1735.C1	unknown [Arabidopsis thaliana]	**15,62**
STMGM44TV	At3g28050/MMG15_6 [Arabidopsis thaliana] gb_AAK50076.1_ AT3g28050/MMG15_6 [Arabidopsis thaliana] ref_NP_566831.1_ nodulin MtN21 family protein [Arabidopsis thaliana]	**15,58**
MICRO.6401.C2	AT5g52970/MNB8_3 [Arabidopsis thaliana] gb_AAK82479.1_ AT5g52970/MNB8_3 [Arabidopsis thaliana] ref_NP_568781.1_ thylakoid lumen 15.0 kDa protein [Arabidopsis thaliana]	**15,55**

MICRO.17780.C1	No Hits Found	**15,41**
MICRO.13861.C1	No Hits Found	**15,34**
POCBD63TV	No Hits Found	**15,19**
MICRO.4393.C5	expressed protein [Arabidopsis thaliana] gb_AAR97892.1_ [4Fe-4S] cluster assembly factor [Arabidopsis thaliana]	**15,06**
MICRO.13072.C1	No Hits Found	**14,99**
cSTC2L3TH	No Hits Found	**14,86**
MICRO.5423.C1	ultraviolet-B-repressible protein [Gossypium hirsutum]	**14,79**
STMHT46TV	protein binding / ubiquitin-protein ligase/ zinc ion binding [Arabidopsis thaliana]	**14,72**
SDBN002C10u.scf	No Hits Found	**14,64**
186A10.esd	No Hits Found	**14,58**
PPCBV43TH	Similar to Arabidopsis thaliana DNA chromosome 4, BAC clone F25I24 (AL049525) [Oryza sativa (japonica cultivar-group)] dbj_BAA83361.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	**14,46**
POACT93TP	Oxygen-evolving enhancer protein 1, chloroplast precursor (OEE1) (33 kDa subunit of oxygen evolving system of photosystem II) (OEC 33 kDa subunit) (33 kDa thylakoid membrane protein)	**14,40**
MICRO.12684.C1	unknown [Arabidopsis thaliana] gb_AAM91732.1_ unknown protein [Arabidopsis thaliana] gb_AAK64145.1_ unknown protein [Arabidopsis thaliana] gb_AAC27838.2_ PsbP domain protein, putative [Arabidopsis thaliana] ref_NP_565906.1_ photosystem II reaction ...	**14,35**
bf_mxlfxxxx_0056b11.t3m.scf	osmotin-like protein [Solanum tuberosum]	**14,28**
MICRO.5847.C1	rubisco activase precursor sp_Q40460_RCA1_TOBAC Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplast precursor (RuBisCO activase 1) (RA 1)	**14,19**
cPRO8I21TH	No Hits Found	**14,11**
MICRO.646.C1	polyphenol oxidase pir__T07096 catechol oxidase (EC 1.10.3.1) (allele POT32) - potato	**14,02**
POCCT75TP	No Hits Found	**13,93**
cSTD11G22TH	molecular chaperone Hsp90-1 [Lycopersicon esculentum]	**13,88**
MICRO.5302.C3	proteinase inhibitor I precursor [Solanum tuberosum]	**13,80**
MICRO.11933.C1	putative protein [Arabidopsis thaliana] emb_CAB38304.1_ putative protein [Arabidopsis thaliana] gb_AAF75234.1_ short-root protein [Arabidopsis thaliana] pir__T04722 hypothetical protein F19F18.140 - Arabidopsis thaliana ref_NP_195480.1_ short-root ...	**13,74**
bf_suspxxxx_0024A03.t3m.scf	unknown [Lycopersicon esculentum]	**13,68**
PPCCK68TH	ribulose bisphosphate carboxylase [Solanum tuberosum] sp_P26575_RBSA_SOLTU Ribulose bisphosphate carboxylase small chain 2A, chloroplast precursor (RuBisCO small subunit 2A) pir__RKPOS2 ribulose-bisphosphate carboxylase (EC 4.1.1.39) precursor small ...	**13,62**
bf_mxlfxxxx_0073g08.t3m.scf	adenylate cyclase [Arabidopsis thaliana] NP_565353.1	**13,56**
MICRO.6770.C3	sucrose phosphate synthase [Lycopersicon esculentum]	**13,52**
MICRO.8816.C1	Hypothetical protein [Arabidopsis thaliana] gb_AAO64041.1_ unknown protein [Arabidopsis thaliana] gb_AAO42291.1_ unknown protein [Arabidopsis thaliana] pir__H86272 hypothetical protein F7A19.6 - Arabidopsis thaliana ref_NP_172850.2_ expressed protei ...	**13,47**
MICRO.398.C1	No Hits Found	**13,46**
MICRO.6200.C1	jasmonic acid-amino acid-conjugating enzyme (JAR4) [Nicotiana attenuata]	**13,42**
MICRO.1081.C2	No Hits Found	**13,39**
MICRO.15334.C1	ASYMMETRIC LEAVES2-like gene 4 protein [Arabidopsis thaliana] dbj_BAB10551.1_ unnamed protein product [Arabidopsis thaliana] gb_AAL40346.1_ LOBa [Arabidopsis thaliana] sp_Q9FML4_LOB_ARATH LATERAL ORGAN BOUNDARIES protein ref_NP_851253.1_ LOB domain ...	**13,36**

bf_acdcxxxx_0059c03.t3m.scf	ARF [Oryza sativa (japonica cultivar-group)]	**13,31**
bf_mxflxxxx_0040h11.t3m.scf	leucoanthocyanidin dioxygenase-like protein [Arabidopsis thaliana]	**13,28**
MICRO.17591.C1	OSJNBa0086O06.18 [Oryza sativa (japonica cultivar-group)] emb_CAE04870.2_ OSJNBa0086O06.18 [Oryza sativa (japonica cultivar-group)]	**13,26**
STMHP88TV	No Hits Found	**13,24**
bf_mxflxxxx_0006d12.t3m.scf	flower-specific gamma-thionin-like protein/acidic protein precursor [Solanum lycopersicum]	**13,19**
108D09AF.esd	No Hits Found	**13,16**
MICRO.3978.C3	basic helix-loop-helix (bHLH) family protein [Arabidopsis thaliana]	**13,11**
BF_LBCHXXXX_0037A03_T3M.SCF	No Hits Found	**13,06**
MICRO.12664.C1	putative thaumatin-like protein [Solanum tuberosum]	**13,01**
MICRO.8650.C1	LeOPT1 [Lycopersicon esculentum]	**12,99**
bf_mxlfxxxx_0071h07.t3m.scf	No Hits Found	**12,95**
MICRO.7881.C1	No Hits Found	**12,88**
MICRO.842.C2	putative RNA-binding protein [Arabidopsis thaliana] gb_AAL49941.1_ AT3g04610/F7O18_9 [Arabidopsis thaliana] ref_NP_187112.1_ KH domain-containing protein [Arabidopsis thaliana]	**12,81**
cSTS30C5TH	putative RNA helicase [Arabidopsis thaliana] gb_AAL67014.1_ putative RNA helicase [Arabidopsis thaliana] ref_NP_174527.2_ RNA helicase, putative [Arabidopsis thaliana]	**12,75**
bf_stolxxxx_0044a05.t3m.scf	No Hits Found	**12,69**
bf_arrayxxx_0068h09.t3m.scf	No Hits Found	**12,63**
SSBN001N17u.scf	No Hits Found	**12,59**
MICRO.7731.C1	No Hits Found	**12,55**
MICRO.925.C2	No Hits Found	**12,46**
MICRO.2095.C2	pepper esterase [Capsicum annuum]	**12,41**
bf_mxflxxxx_0020d07.t3m.scf	No Hits Found	**12,38**
MICRO.6543.C2	No Hits Found	**12,31**
bf_mxflxxxx_0065f11.t3m.scf	No Hits Found	**12,26**
MICRO.1716.C1	CLB1 protein - tomato dbj_BAA24382.1_ CLB1 [Lycopersicon esculentum]	**12,20**
MICRO.13827.C1	At3g52450 [Arabidopsis thaliana] gb_AAU90053.1_ At3g52450 [Arabidopsis thaliana] emb_CAB43434.1_ putative protein [Arabidopsis thaliana] pir__T08454 hypothetical protein F22O6.170 - Arabidopsis thaliana ref_NP_190813.1_ U-box domain-containing ...	**12,19**
MICRO.7391.C1	No Hits Found	**12,16**
BF_TUBSXXXX_0033F08_T3M.SCF	No Hits Found	**12,16**
MICRO.3560.C1	No Hits Found	**12,10**
MICRO.11082.C1	CYP71D10p [Glycine max] pir__T05939 cytochrome P450 monooxygenase 71D10p [soybean sp]	**12,08**
MICRO.416.C21	No Hits Found	**12,05**
MICRO.1528.C1	Thaumatin, pathogenesis-related [Medicago truncatula]	**12,00**
MICRO.8900.C1	No Hits Found	**11,99**
bf_stolxxxx_0037f08.t3m.scf	hypothetical protein F28B23.15 - Arabidopsis thaliana gb_AAG50680.1_ hypothetical protein [Arabidopsis thaliana] ref_NP_173942.1_ importin beta-2 subunit family protein [Arabidopsis thaliana]	**11,93**

SDBN005H14u.scf	serine/threonine-protein kinase bri1, putative [Ricinus communis] XP_002514937.1	**11,88**
MICRO.8309.C2	disease resistance-like protein [Oryza sativa (japonica cultivar-group)] gb_AAO72679.1_ unknown [Oryza sativa (japonica cultivar-group)]	**11,81**
POCBB56TV	similar to AT3G42860: zinc knuckle (CCHC-type) family protein	**11,78**
MICRO.12630.C1	41 kD chloroplast nucleoid DNA binding protein (CND41) [Nicotiana sylvestris]	**11,72**
MICRO.9057.C1	cc-nbs-lrr resistance protein [Populus trichocarpa] XP_002332999.1	**11,69**
POAB291TF	ubiquitin-specific protease 6 [Arabidopsis thaliana]	**11,61**
PPCAN68TH	No Hits Found	**11,57**
MICRO.17716.C1	vetispiradiene synthase [Solanum tuberosum]	**11,50**
bf_mxflxxxx_0064a03.t3m.scf	putative receptor kinase [Ricinus communis]	**11,46**
MICRO.14312.C1	apolipoprotein E	**11,41**
MICRO.5178.C1	unknown protein [Arabidopsis thaliana] gb_AAO50598.1_ unknown protein [Arabidopsis thaliana] gb_AAD17434.2_ hypothetical protein [Arabidopsis thaliana] ref_NP_565301.1_ expressed protein [Arabidopsis thaliana]	**11,39**
bf_mxlfxxxx_0058b08.t3m.scf	No Hits Found	**11,36**
STMHN08TH	unknown protein [Arabidopsis thaliana] pir__C84615 hypothetical protein At2g22660 [imported] - Arabidopsis thaliana ref_NP_179851.1_ glycine-rich protein [Arabidopsis thaliana]	**11,34**
MICRO.3160.C2	putative phosphate translocator [Oryza sativa (japonica cultivar-group)]	**11,29**
POCAA10TO	No Hits Found	**11,26**
bf_suspxxxx_0054d09.t3m.scf	No Hits Found	**11,23**
TBSK03984FD12.t3m.scf	aminotransferase [Arabidopsis thaliana] gb_AAP68345.1_ At1g62960 [Arabidopsis thaliana] gb_AAF75807.1_ Strong similarity to ACS5 from Lupinus albus gb_AF119414, and contains an Aminotransferase-classI domain PF_00155. [Arabidopsis thaliana] ...	**11,20**
POCC056TV	No Hits Found	**11,18**
POCCI94TP	No Hits Found	**11,16**
bf_lbchxxxx_0062c05.t3m.scf	cytoplasmic dynein heavy chain 2 protein [Tetrahymena thermophila]	**11,15**
MICRO.16359.C1	putative pectin-glucuronyltransferase [Oryza sativa (japonica cultivar-group)]	**11,10**
146C04AF.esd	No Hits Found	**11,07**
MICRO.15779.C1	At5g09390/T5E8_190 [Arabidopsis thaliana] gb_AAM62448.1_ unknown [Arabidopsis thaliana] gb_AAM74500.1_ AT5g09390/T5E8_190 [Arabidopsis thaliana] ref_NP_568211.1_ CD2-binding protein-related [Arabidopsis thaliana]	**11,06**
MICRO.11136.C1	chalcone reductase [Sesbania rostrata]	**11,03**
POCC537TV	No Hits Found	**11,01**
bf_mxflxxxx_0005d08.t3m.scf	similar to AT2G30900: similar to unknown protein [Arabidopsis thaliana]	**10,99**
MICRO.3936.C1	DNA topoisomerase II [Nicotiana tabacum] gb_AAN85207.1_ DNA topoisomerase II [Nicotiana tabacum]	**10,95**
MICRO.13361.C1	No Hits Found	**10,89**
MICRO.10579.C1	cytochrome P450 [Arabidopsis thaliana]	**10,86**
MICRO.12691.C1	beta-ketoacyl-ACP synthase II [Perilla frutescens]	**10,83**
MICRO.15185.C2	cytochrome c biogenesis [Beta vulgaris subsp. vulgaris] dbj_BAA99300.1_ cytochrome c biogenesis protein [Beta vulgaris subsp. vulgaris] ref_NP_063988.1_ cytochrome c biogenesis protein [Beta vulgaris subsp. vulgaris]	**10,82**
MICRO.7042.C1	hypothetical protein [Ricinus communis] XP_002532208.1	**10,77**
STMGN54TV	amidase-like protein [Arabidopsis thaliana] emb_CAB45449.1_ amidase-like protein [Arabidopsis thaliana] ref_NP_195214.1_ amidase family protein [Arabidopsis thaliana]	**10,76**

cSTB41N22TH	No Hits Found	**10,72**
MICRO.17380.C1	F23M19.3 [Arabidopsis thaliana] pir__D86467 protein F23M19.3 [imported] - Arabidopsis thaliana	**10,71**
bf_mxlfxxxx_0022e10.t3m.scf	No Hits Found	**10,67**
bf_mxflxxxx_0017c01.t3m.scf	No Hits Found	**10,63**
MICRO.14712.C1	No Hits Found	**10,56**
bf_mxflxxxx_0025b06.t3m.scf	putative 1-aminocyclopropane-1-carboxylate deaminase [Oryza sativa (japonica cultivar-group)] dbj_BAD16875.1_ putative 1-aminocyclopropane-1-carboxylate deaminase [Oryza sativa (japonica cultivar-group)]	**10,51**
MICRO.5483.C1	No Hits Found	**10,46**
MICRO.10980.C1	No Hits Found	**10,45**
MICRO.4139.C1	OSJNBa0091C07.4 [Oryza sativa (japonica cultivar-group)] emb_CAE05842.2_ OSJNBa0091C07.4 [Oryza sativa (japonica cultivar-group)]	**10,41**
MICRO.414.C1	No Hits Found	**10,38**
cSTB36L11TH	ADP /ATP translocator [Solanum tuberosum] sp_P25083_ADT1_SOLTU ADP,ATP carrier protein, mitochondrial precursor (ADP/ATP translocase) (Adenine nucleotide translocator) (ANT) pir__S21974 ADP,ATP carrier protein, ant - potato	**10,36**
MICRO.4218.C1	No Hits Found	**10,35**
bf_mxlfxxxx_0032a04.t3m.scf	unknown [Arabidopsis thaliana]	**10,31**
MICRO.3523.C1	No Hits Found	**10,28**
cSTE8O2TH	No Hits Found	**10,23**
STMIV75TV	chromosome condensation protein [Arabidopsis thaliana] ref_NP_199671.1_ structural maintenance of chromosomes (SMC) family protein [Arabidopsis thaliana]	**10,18**
MICRO.7632.C1	T5E21.6 [Arabidopsis thaliana]	**10,15**
MICRO.8255.C1	carbonyl reductase-like protein [Arabidopsis thaliana] ref_NP_199916.1_ short-chain dehydrogenase/reductase (SDR) family protein [Arabidopsis thaliana]	**10,04**
MICRO.11021.C1	41 kD chloroplast nucleoid DNA binding protein (CND41) [Nicotiana sylvestris]	**9,96**
BF_LBCHXXXX_0003C09_T3M.SCF	No Hits Found	**9,93**
bf_mxlfxxxx_0061g03.t3m.scf	hypothetical protein [Vitis vinifera] XP_002284432.1	**9,89**
MICRO.5163.C1	protein kinase-like protein [Arabidopsis thaliana]	**9,86**
MICRO.9105.C5	tyramine hydroxycinnamoyl transferase [Solanum tuberosum]	**9,82**
POCCG03TP	hypothetical protein [Arabidopsis thaliana] ref_NP_187066.1_ expressed protein [Arabidopsis thaliana]	**9,80**
bf_stolxxxx_0029g07.t3m.scf	No Hits Found	**9,75**
MICRO.17574.C1	At3g29770 [Arabidopsis thaliana] gb_AAG12619.1_ hypothetical protein	**9,71**
POCBA73TV	No Hits Found	**9,69**
MICRO.9163.C2	hypothetical protein [Vitis vinifera] XP_002285425.1	**9,67**
bf_mxflxxxx_0019c08.t3m.scf	No Hits Found	**9,65**
BF_TUBSXXXX_0016D12_T3M.SCF	No Hits Found	**9,62**
STMHQ44TV	florfenicol resistance protein-like [Oryza sativa (japonica cultivar-group)]	**9,60**
bf_ivrootxx_0025e11.t3m.scf	F16L1.9 protein - Arabidopsis thaliana ref_NP_173637.3_ SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein [Arabidopsis thaliana] gb_AAF87855.1_ Contains similarity to a KIAA0420 protein from Homo sapiens gi_2887415 and ...	**9,59**

MICRO.1798.C1	No Hits Found	**9,58**
bf_stolxxxx_0011H10.t3m.scf	No Hits Found	**9,56**
BPLI7L4TH	unknown protein [Arabidopsis thaliana] gb_AAL36261.1_ unnamed protein product [Arabidopsis thaliana] ref_NP_200445.1_ zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana]	**9,53**
cSTB24K8TH	No Hits Found	**9,50**
MICRO.3773.C3	No Hits Found	**9,48**
SDBN002E08u.scf	No Hits Found	**9,46**
bf_mxlfxxxx_0058e07.t3m.scf	hypothetical protein [Vitis vinifera] XP_002284679.1	**9,45**
bf_mxlfxxxx_0010b10.t3m.scf	UDP-glucose:salicylic acid glucosyltransferase [Nicotiana tabacum]	**9,42**
MICRO.273.C1	unknown [Arabidopsis thaliana] ref_NP_567185.1_ F-box family protein [Arabidopsis thaliana] ref_NP_849277.1_ F-box family protein [Arabidopsis thaliana]	**9,40**
POCCE89TV	No Hits Found	**9,38**
MICRO.5295.C1	putative oxygen evolving enhancer protein [Solanum demissum]	**9,36**
bf_swstxxxx_0007b05.t3m.scf	No Hits Found	**9,35**
SSBN003L05u.scf	No Hits Found	**9,32**
ACDA03870C06.T3m.scf	No Hits Found	**9,31**
bf_mxlfxxxx_0010e06.t3m.scf	No Hits Found	**9,29**
PotatoF1059.scf	No Hits Found	**9,29**
MICRO.17846.C1	No Hits Found	**9,27**
MICRO.11055.C1	nodulin-related protein-like [Oryza sativa (japonica cultivar-group)] dbj_BAD11650.1_ nodulin-related protein-like [Oryza sativa (japonica cultivar-group)]	**9,26**
MICRO.701.C2	putative protein [Arabidopsis thaliana] emb_CAB80631.1_ putative protein [Arabidopsis thaliana] pir__T05005 hypothetical protein T19P19.70 - Arabidopsis thaliana ref_NP_195678.1_ SAP domain-containing protein [Arabidopsis thaliana]	**9,22**
bf_mxlfxxxx_0067c09.t3m.scf	No Hits Found	**9,20**
MICRO.14215.C1	putative histone deacetylase [Arabidopsis thaliana] gb_AAM65853.1_ histone deacetylase [Arabidopsis thaliana] gb_AAL60022.1_ putative histone deacetylase [Arabidopsis thaliana]	**9,18**
bf_mxflxxxx_0074h08.t3m.scf	OSJNBa0019K04.7 [Oryza sativa (japonica cultivar-group)] emb_CAD41660.3_ OSJNBa0019K04.7 [Oryza sativa (japonica cultivar-group)]	**9,15**
MICRO.11401.C1	No Hits Found	**9,15**
MICRO.5499.C1	glycolate oxidase [Arabidopsis thaliana] gb_AAO22568.1_ putative glycolate oxidase [Arabidopsis thaliana] ref_NP_188029.1_ (S)-2-hydroxy-acid oxidase, peroxisomal, putative / glycolate oxidase, putative / short chain alpha-hydroxy acid oxidase, ...	**9,13**
SDBN002O09u.scf	glucuronosyl transferase homolog, ripening-related - tomato (fragment)	**9,12**
MICRO.6087.C2	flavonoid 1-2 rhamnosyltransferase [Citrus maxima]	**9,10**
bf_ivrootxx_0035d07.t3m.scf	No Hits Found	**9,09**
PotatoF1362.scf	No Hits Found	**9,08**
PotatoF1544.scf	No Hits Found	**9,07**
MICRO.9343.C1	No Hits Found	**9,05**
STMHK42TV	cytochrome P450-like [Arabidopsis thaliana] ref_NP_198460.1_ cytochrome P450 family protein [Arabidopsis thaliana]	**9,04**
POCBC12TV	F-box protein ORE9, AtFBL7 [Arabidopsis thaliana] gb_AAK97303.1_ F-box containing protein ORE9 [Arabidopsis thaliana] ref_NP_565979.1_ F-box family protein (ORE9) [Arabidopsis thaliana]	**9,03**

MICRO.13913.C1	vetispiradiene synthase [Solanum tuberosum]	**9,01**
MICRO.1293.C1	F5O8.34 [Arabidopsis thaliana] pir__B86372 protein F5O8.34 [imported] - Arabidopsis thaliana ref_NP_173791.2_ expressed protein [Arabidopsis thaliana]	**9,01**
MICRO.5928.C1	similar to AT2G15630: pentatricopeptide (PPR) repeat-containing protein	**9,00**
bf_mxlfxxxx_0010b09.t3m.scf	polyprotein [Arabidopsis thaliana]	**8,99**
MICRO.2552.C1	No Hits Found	**8,98**
bf_mxflxxxx_0051a05.t3m.scf	No Hits Found	**8,96**
cSTB40G1TH	No Hits Found	**8,92**
SDBN003K01u.scf	No Hits Found	**8,90**
MICRO.701.C1	acinusL protein-like [Oryza sativa (japonica cultivar-group)] dbj_BAC79704.1_ acinusL protein-like [Oryza sativa (japonica cultivar-group)]	**8,89**
MICRO.3621.C1	hypothetical protein SDM1_53t00013 [Solanum demissum] ABI34375.1	**8,88**
MICRO.1707.C8	membrane located receptor-like protein [Nicotiana tabacum]	**8,84**
SDBN005F10u.scf	serine-threonine protein kinase, plant-type, putative [Ricinus communis] XP_002533280.1	**8,82**
RA1nr054.scf	No Hits Found	**8,80**
BF_LBCHXXXX_0045B11_T3M.SCF	No Hits Found	**8,79**
POAE880TV	No Hits Found	**8,79**
MICRO.7520.C1	No Hits Found	**8,77**
MICRO.6479.C2	DNA-directed RNA polymerase beta chain (PEP) (Plastid-encoded RNA polymerase beta subunit) (RNA polymerase beta subunit) pir__RNNTB DNA-directed RNA polymerase (EC 2.7.7.6) beta chain - common tobacco chloroplast emb_CAA77346.1_ RNA polymerase beta ...	**8,76**
bf_cswbxxxx_0020a06.t3m.scf	hypothetical protein [Vitis vinifera] XP_002271238.1	**8,75**
MICRO.2095.C1	pepper esterase [Capsicum annuum]	**8,73**
MICRO.7904.C2	No Hits Found	**8,68**
MICRO.984.C1	starch phosphorylase (EC 2.4.1.1) precursor - potato sp_P04045_PHS1_SOLTU Alpha-1,4 glucan phosphorylase, L-1 isozyme, chloroplast precursor (Starch phosphorylase L-1) dbj_BAA00407.1_ alpha-glucan phosphorylase precursor [Solanum tuberosum]	**8,65**
bf_suspxxxx_0041c06.t3m.scf	No Hits Found	**8,65**
cSTB45B11TH	No Hits Found	**8,61**
MICRO.1081.C8	unknown protein [Arabidopsis thaliana] ref_NP_200878.2_ glycosyltransferase family protein 2 [Arabidopsis thaliana]	**8,59**
MICRO.12251.C1	At1g63840/T12P18_14 [Arabidopsis thaliana] gb_AAL25547.1_ At1g63840/T12P18_14 [Arabidopsis thaliana] pir__E96663 probable RING zinc finger protein T12P18.14 [imported] - Arabidopsis thaliana gb_AAG52454.1_ putative RING zinc finger protein	**8,58**
bf_arrayxxx_0059h11.t7m.scf	No Hits Found	**8,55**
MICRO.1668.C1	zinc finger (DHHC type) family protein [Arabidopsis thaliana]	**8,50**
STDB004G12u.scf	No Hits Found	**8,49**
POCC837TP	AT5g61030/maf19_30 [Arabidopsis thaliana] dbj_BAB10366.1_ unnamed protein product [Arabidopsis thaliana] gb_AAL31194.1_ AT5g61030/maf19_30 [Arabidopsis thaliana] ref_NP_200911.1_ RNA-binding protein, putative [Arabidopsis thaliana]	**8,45**
MICRO.12076.C1	similar to AT1G55900 | Symbols: EMB1860, TIM50 | TIM50 (EMBRYO DEFECTIVE 1860)	**8,41**
POCDF88TV	cotton fiber expressed protein 1 [Gossypium hirsutum] pir__T09820 fiber protein 1 [imported] - upland cotton	**8,35**
MICRO.10942.C1	expansin precursor [Lycopersicon esculentum]	**8,34**

cSTS11F13TH	Beta tubulin 1 [Lupinus albus] pir__S35142 tubulin beta chain - white lupine sp_P37392_TBB1_LUPAL Tubulin beta-1 chain (Beta-1 tubulin)	**8,27**
MICRO.9569.C1	At1g69340/F10D13.28 [Arabidopsis thaliana] gb_AAK50063.1_ At1g69340/F10D13.28 [Arabidopsis thaliana] ref_NP_564960.1_ appr-1-p processing enzyme family protein [Arabidopsis thaliana]	**8,22**
SDBN003D20u.scf	No Hits Found	**8,20**
cSTB17F23TH	No Hits Found	**8,18**
cSTS11F17TH	No Hits Found	**8,12**
MICRO.17872.C1	ZPT2-13 [Petunia x hybrida]	**8,11**
POCCJ51TV	putative translation initiation factor eIF3 [Arabidopsis thaliana]	**8,08**
POCCH66TP	No Hits Found	**8,06**
MICRO.14428.C1	ATNAP4 (Arabidopsis thaliana non-intrinsic ABC protein 4) NP_563693.1	**8,02**
BPLI1E21TH	putative anthocyanidin-3-glucoside rhamnosyltransferase [Arabidopsis thaliana] dbj_BAA98157.1_ anthocyanidin-3-glucoside rhamnosyltransferase-like [Arabidopsis thaliana] gb_AAO63454.1_ At5g49690 [Arabidopsis thaliana] ref_NP_199780.1_ ...	**7,98**
MICRO.9407.C2	serine/threonine kinase [Brassica oleracea] pir__T14450 serine/threonine kinase (EC 2.7.1.-) BRLK - wild cabbage	**7,95**
MICRO.2868.C3	microsomal omega-6-desaturase [Nicotiana tabacum]	**7,93**
MICRO.10599.C2	unknown [Arabidopsis thaliana] gb_AAM16187.1_ At1g31940/F5M6.6 [Arabidopsis thaliana] gb_AAL47448.1_ At1g31940/F5M6.6 [Arabidopsis thaliana] ref_NP_564386.1_ expressed protein [Arabidopsis thaliana]	**7,92**
POCC626TP	PREDICTED: similar to host cell factor C1 regulator 1 (XPO1 dependant) isoform 1	**7,92**
MICRO.10642.C1	DNA-binding family protein [Arabidopsis thaliana]	**7,90**
MICRO.1889.C7	cytochrome P450 [Arabidopsis thaliana]	**7,86**
POCBC09TV	No Hits Found	**7,85**
SDBN005B07u.scf	No Hits Found	**7,84**
cPRO1E20TH	transducin family protein / WD-40 repeat family protein (AT4G07410) [Arabidopsis thaliana]	**7,80**
STMGR94TV	No Hits Found	**7,78**
MICRO.17974.C1	At1g22630/F12K8_2 [Arabidopsis thaliana] gb_AAK73963.1_ At1g22630/F12K8_2 [Arabidopsis thaliana] gb_AAF18516.1_ Unknown protein [Arabidopsis thaliana]	**7,78**
MICRO.14590.C1	No Hits Found	**7,76**
MICRO.3382.C1	transformer-SR ribonucleoprotein [Nicotiana tabacum] pir__T03986 transformer-SR ribonucleoprotein - common tobacco (fragment)	**7,75**
MICRO.17259.C1	No Hits Found	**7,72**
MICRO.17382.C1	putative retroelement pol polyprotein-like [Solanum tuberosum]	**7,69**
POCB071TV	hypothetical protein [Ricinus communis] XP_002525993.1	**7,68**
MICRO.2439.C2	protein prenyltransferase alpha subunit-related [Arabidopsis thaliana]	**7,66**
MICRO.4760.C2	protein phosphatase-2C	**7,65**
cSTE27K19TH	No Hits Found	**7,62**
cSTS11B3TH	No Hits Found	**7,60**
PotatoF0515.scf	No Hits Found	**7,59**
bf_mxflxxxx_0014e03.t3m.scf	No Hits Found	**7,58**
MICRO.2856.C3	putative Flavonol synthase/flavanone 3-hydroxylase [Ricinus communis]	**7,55**
SSBN001M16u.scf	No Hits Found	**7,51**
MICRO.11515.C1	glutaredoxin family protein [Arabidopsis thaliana]	**7,49**
POCAY80TV	No Hits Found	**7,45**
POCBI64TP	putative protein [Arabidopsis thaliana] ref_NP_196631.1_ expressed protein [Arabidopsis thaliana]	**7,42**

SDBN006C23u.scf	No Hits Found	**7,40**
bf_cswbxxxx_0039b06.t3m.scf	No Hits Found	**7,37**
SDBN004H05u.scf	putative 40S ribosomal protein S19 [Arabidopsis thaliana] gb_AAM65679.1_ putative 40S ribosomal protein S19 [Arabidopsis thaliana] gb_AAL34186.1_ putative 40S ribosomal protein S19 [Arabidopsis thaliana] gb_AAK44092.1_ putative 40S ribosomal protein ...	**7,36**
bf_ivrootxx_0029a05.t3m.scf	ATP binding / protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase/ sugar binding [Arabidopsis thaliana]	**7,35**
cSTE25G10TH	hypothetical protein [Ricinus communis]	**7,31**
MICRO.7857.C1	No Hits Found	**7,29**
bf_ivrootxx_0008f05.t3m.scf	putative membrane transporter [Arabidopsis thaliana] pir__G84864 probable membrane transporter [imported] - Arabidopsis thaliana	**7,26**
MICRO.16775.C1	hypothetical protein [Vitis vinifera] XP_002282448.1	**7,23**
STMJG25TH	No Hits Found	**7,22**
POCAL26TV	No Hits Found	**7,21**
MICRO.12203.C2	RING-H2 subgroup RHE protein [Populus alba x Populus tremula]	**7,19**
MICRO.13462.C1	hypothetical protein PGEC542.21 [Solanum demissum]	**7,15**
MICRO.18099.C2	No Hits Found	**7,12**
POADC56TV	hypothetical protein [Oryza sativa (japonica cultivar-group)] dbj_BAD13231.1_ hypothetical protein [Oryza sativa (japonica cultivar-group)]	**7,11**
MICRO.13027.C1	lipid transfer protein LTP1 precursor [Capsicum annuum]	**7,09**
bf_mxlfxxxx_0015h05.t3m.scf	No Hits Found	**7,07**
MICRO.331.C153	No Hits Found	**7,06**
MICRO.4827.C2	S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase [Solanum lycopersicum]	**7,05**
POAB119TF	unnamed protein product [Vitis vinifera] CBI26997.3	**7,02**
bf_stolxxxx_0021g10.t3m.scf	No Hits Found	**6,99**
POCAD35TV	No Hits Found	**6,98**
POCBU64TP	PREDICTED: hypothetical protein XP_028217 [Homo sapiens]	**6,97**
STDB005G09u.scf	No Hits Found	**6,96**
bf_mxflxxxx_0025b02.t3m.scf	No Hits Found	**6,92**
bf_mxlfxxxx_0016d01.t3m.scf	fiber protein Fb2 [Gossypium barbadense]	**6,89**
bf_mxflxxxx_0066f07.t3m.scf	No Hits Found	**6,87**
BF_TUBSXXXX_0044D07_T3M.SCF	No Hits Found	**6,86**
bf_ivrootxx_0011g05.t3m.scf	putative dipeptidyl peptidase IV [Arabidopsis thaliana] dbj_BAB10391.1_ dipeptidyl peptidase IV-like protein [Arabidopsis thaliana] ref_NP_197814.1_ prolyl oligopeptidase family protein [Arabidopsis thaliana]	**6,83**
POAE242TV	No Hits Found	**6,80**
MICRO.6561.C1	putative UDP rhamnose--anthocyanidin-3-glucoside rhamnosyltransferase [Arabidopsis thaliana] gb_AAO63434.1_ At4g27570 [Arabidopsis thaliana] emb_CAB81407.1_ UDP rhamnose--anthocyanidin-3-glucoside rhamnosyltransferase-like protein [Arabidopsis ...	**6,79**
POCA641TV	unknown protein [Oryza sativa (japonica cultivar-group)] dbj_BAC45146.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	**6,78**
ACDA01431H03.T3m.scf	No Hits Found	**6,75**

MICRO.94.C4	Elongation factor 1-beta (EF-1-beta) ref_NP_174314.2_ elongation factor 1-beta / EF-1-beta [Arabidopsis thaliana]	**6,75**
bf_mxflxxxx_0004e10.t3m.scf	No Hits Found	**6,73**
MICRO.331.C46	No Hits Found	**6,71**
bf_suspxxxx_0022E09.t3m.scf	No Hits Found	**6,68**
MICRO.2636.C2	No Hits Found	**6,66**
SSBT004J24x.scf	No Hits Found	**6,66**
SSBN002I10u.scf	reverse transcriptase related protein	**6,65**
MICRO.14412.C1	elicitor-inducible cytochrome P450 [Nicotiana tabacum] dbj_BAA35080.1_ putative cytochrome P450 [Nicotiana tabacum]	**6,62**
MICRO.14017.C1	putative protein [Arabidopsis thaliana] emb_CAB56391.1_ putative protein [Arabidopsis thaliana] sp_Q8L936_CA16_ARATH Putative clathrin assembly protein At4g40080 pir__A85475 hypothetical protein AT4g40080 [imported] - Arabidopsis thaliana ...	**6,61**
MICRO.3652.C4	sesquiterpene synthase 2 [Lycopersicon hirsutum]	**6,60**
cSTA24H23TH	unknown protein [Arabidopsis thaliana] gb_AAO64886.1_ At4g13370 [Arabidopsis thaliana] emb_CAB40772.1_ putative protein [Arabidopsis thaliana] emb_CAB78379.1_ putative protein [Arabidopsis thaliana] pir__T06294 hypothetical protein T9E8.110 - ...	**6,59**
MICRO.6103.C1	putative Polyprotein [Solanum demissum]	**6,58**
MICRO.12901.C2	No Hits Found	**6,54**
PPCCS03TH	No Hits Found	**6,53**
STDB001I18u.scf	cyclic nucleotide-gated calmodulin-binding ion channel [Nicotiana tabacum]	**6,50**
MICRO.2286.C37	No Hits Found	**6,48**
SDBN003B12u.scf	No Hits Found	**6,46**
072A09AF.esd	protein kinase (EC 2.7.1.-) - common ice plant (fragment)	**6,45**
bf_mxlfxxxx_0020h06.t3m.scf	No Hits Found	**6,41**
bf_mxflxxxx_0050f11.t3m.scf	putative UDP-galactose/UDP-glucose transporter [Solanum demissum]	**6,40**
BPLI4I9TH	probable zinc finger protein, 58191-56692 [imported] - Arabidopsis thaliana gb_AAG51898.1_ zinc finger protein, putative	**6,38**
bf_lbchxxxx_0058b06.t3m.scf	No Hits Found	**6,37**
POCAA56TP	hypothetical protein [Solanum tuberosum]	**6,35**
MICRO.12036.C1	No Hits Found	**6,35**
SDBN002B10u.scf	No Hits Found	**6,34**
bf_mxflxxxx_0051g06.t3m.scf	No Hits Found	**6,31**
bf_stolxxxx_0070b02.t3ma.scf	No Hits Found	**6,29**
bf_mxflxxxx_0057h03.t3m.scf	No Hits Found	**6,28**
MICRO.14.C1	fructokinase 3 [Lycopersicon esculentum]	**6,26**
MICRO.2253.C1	No Hits Found	**6,26**
bf_arrayxxx_0033g05.t3m.scf	No Hits Found	**6,23**
MICRO.12201.C1	hypothetical protein F19K23.18 [imported] - Arabidopsis thaliana gb_AAB60776.1_ F19K23.18 gene product [Arabidopsis thaliana] ref_NP_176416.1_ pentatricopeptide (PPR) repeat-containing protein [Arabidopsis thaliana]	**6,20**
SDBN003B13u.scf	putative receptor kinase [Oryza sativa (japonica cultivar-group)] dbj_BAD26280.1_ putative receptor kinase [Oryza sativa (japonica cultivar-group)] dbj_BAD26041.1_ putative receptor kinase [Oryza sativa (japonica cultivar-group)]	**6,18**

MICRO.8464.C3	unknown protein [Oryza sativa (japonica cultivar-group)] ref_XP_506269.1_ PREDICTED B1120F06.103 gene product [Oryza sativa (japonica cultivar-group)] dbj_BAC84531.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	**6,18**
MICRO.5349.C4	sesquiterpene synthase 2 [Lycopersicon hirsutum]	**6,15**
STMIP13TV	mitochondrial phosphate translocator [Betula pendula]	**6,13**
MICRO.6187.C1	glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	**6,12**
MICRO.8553.C1	hypothetical protein [Ricinus communis] XP_002527480.1	**6,11**
MICRO.5825.C1	No Hits Found	**6,09**
cSTA32D7TH	putative protein [Arabidopsis thaliana] emb_CAB45062.1_ putative protein [Arabidopsis thaliana] pir__T09890 hypothetical protein T22A6.100 - Arabidopsis thaliana	**6,09**
BPLI14P23TH		**6,08**
MICRO.12038.C1	No Hits Found	**6,06**
STMCU53TV	AT4g00870 [Arabidopsis thaliana] emb_CAD58596.1_ putative bHLH transcription factor [Arabidopsis thaliana] pir__T01559 hypothetical protein A_TM018A10.7 - Arabidopsis thaliana gb_AAB62853.1_ similar to the myc family of helix-loop-helix transcriptio ...	**6,05**
bf_ivrootxx_0033a07.t3m.scf	blight resistance protein RGA3 [Solanum bulbocastanum]	**6,04**
POCAA21TV	No Hits Found	**6,01**
POACJ81TV	putative ABC1 protein [Arabidopsis thaliana]	**5,99**
MICRO.16550.C1	anthocyanin 1 [Lycopersicon esculentum]	**5,98**
bf_suspxxxx_0045g12.t7m.scf	No Hits Found	**5,95**
bf_mxflxxxx_0041g10.t3m.scf	No Hits Found	**5,93**
bf_slmwxxxx_0015h06.t3m.scf	No Hits Found	**5,93**
MICRO.9042.C1	similar to AT5G11540: FAD-binding domain-containing protein	**5,91**
188A11.esd	similar to AT4G10570: ubiquitin carboxyl-terminal hydrolase family protein	**5,90**
MICRO.14370.C1	heat shock protein-related (AT2G29970) [Arabidopsis thaliana]	**5,89**
POCCM11TP	hypothetical protein LOC284361 isoform 2 [Homo sapiens] gb_AAV30545.1_ hematopoietic signal peptide-containing membrane domain-containing 1 [Homo sapiens]	**5,88**
MICRO.9579.C2	similar to AT3G21580: cobalt ion transporter	**5,87**
MICRO.14349.C1	PREDICTED: similar to Translationally controlled tumor protein (TCTP) (p23) (Histamine-releasing factor) (HRF) [Pan troglodytes] ref_NP_003286.1_ tumor protein, translationally-controlled 1 [Homo sapiens] ref_NP_999538.1_ translationally controlled ...	**5,86**
MICRO.14070.C1	salt-inducible protein-like [Arabidopsis thaliana] ref_NP_197146.1_ pentatricopeptide (PPR) repeat-containing protein [Arabidopsis thaliana]	**5,84**
MICRO.13108.C2	putative elicitor-inducible cytochrome P450 [Oryza sativa (japonica cultivar-group)] dbj_BAD36163.1_ putative elicitor-inducible cytochrome P450 [Oryza sativa (japonica cultivar-group)]	**5,82**
MICRO.7497.C6	cation diffusion facilitator 8 [Stylosanthes hamata]	**5,81**
BF_TUBSXXXX_0060C05_T3M.SCF	peroxidase (TPX1) [Lycopersicon esculentum]	**5,79**
108B12AF.esd	No Hits Found	**5,77**
cSTA43E9TH	No Hits Found	**5,75**
cSTB44H15TH	No Hits Found	**5,74**
bf_ivrootxx_0019d12.t3m.scf	Protein kinase [Medicago truncatula]	**5,73**
cPRO7L13TH	F27F5.26 [Arabidopsis thaliana] ref_NP_175132.1_ zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana]	**5,70**
MICRO.4364.C1	putative C2H2-type zinc finger protein [Arabidopsis thaliana] gb_AAO64832.1_ At2g02080 [Arabidopsis thaliana] ref_NP_178317.2_ zinc finger (C2H2 type) family protein [Arabidopsis thaliana]	**5,70**

cSTS11L19TH	unknown [Medicago truncatula] ACJ83275.1	**5,69**
MICRO.4562.C9	chloroplast small heat shock protein class I [Capsicum frutescens]	**5,68**
MICRO.17903.C1	eukaryotic translation initiation factor 3 subunit (eIF-3)-like [Oryza sativa (japonica cultivar-group)] dbj_BAD12895.1_ eukaryotic translation initiation factor 3 subunit (eIF-3)-like [Oryza sativa (japonica cultivar-group)] dbj_BAD09184.1_ ...	**5,65**
MICRO.10671.C1	unknown protein [Oryza sativa (japonica cultivar-group)] gb_AAO73284.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	**5,65**
MICRO.7757.C1	No Hits Found	**5,64**
MICRO.12680.C1	No Hits Found	**5,63**
MICRO.12751.C1	hypothetical protein [Vitis vinifera] XP_002272678.1	**5,62**
MICRO.695.C8	No Hits Found	**5,61**
MICRO.4082.C8	TIR-NBS disease resistance-like protein [Populus trichocarpa]	**5,59**
cSTB37C5TH	putative receptor-like serine-threonine protein kinase [Solanum tuberosum]	**5,59**
MICRO.7993.C1	At1g18700/F6A14_19 [Arabidopsis thaliana] gb_AAL47463.1_ At1g18700/F6A14_19 [Arabidopsis thaliana] ref_NP_173305.2_ DNAJ heat shock N-terminal domain-containing protein [Arabidopsis thaliana]	**5,56**
bf_mxlfxxxx_0001f02.t3m.scf	No Hits Found	**5,55**
MICRO.1096.C3	HOBBIT protein [Arabidopsis thaliana] ref_NP_849994.1_ cell division cycle family protein / CDC family protein [Arabidopsis thaliana]	**5,53**
bf_arrayxxx_0078f01.t7m.scf	putative BRASSINAZOLE-RESISTANT 1 protein [Ricinus communis]	**5,53**
POCCU69TV	No Hits Found	**5,51**
cSTD12H8TH	mitochondrial carrier protein-like protein [Oryza sativa (japonica cultivar-group)]	**5,50**
PotatoF0599.scf	No Hits Found	**5,48**
bf_arrayxxx_0022e11.t7m.scf	putative Rab geranylgeranyltransferase, beta subunit [Oryza sativa (japonica cultivar-group)] dbj_BAD10061.1_ putative Rab geranylgeranyltransferase, beta subunit [Oryza sativa (japonica cultivar-group)]	**5,47**
PotatoF1717.scf	No Hits Found	**5,46**
bf_cswbxxxx_0001d03.t3m.scf	CDPK adapter protein 1 [Mesembryanthemum crystallinum]	**5,43**
MICRO.7467.C1	similar to dynamin-like protein encoded by GenBank Accession Number X99669 [Arabidopsis thaliana]	**5,42**
PPCAN65TH	No Hits Found	**5,42**
cSTB18J15TH	No Hits Found	**5,40**
bf_suspxxxx_0053f07.t3m.scf	ARI1 (ARIADNE); protein binding / zinc ion binding (ARI1) [Arabidopsis thaliana]	**5,39**
PotatoF0497.scf	No Hits Found	**5,38**
MICRO.1610.C1	Rx protein [Solanum tuberosum]	**5,36**
MICRO.1690.C2	cytochrome P450 [Helianthus tuberosus]	**5,35**
MICRO.331.C76	Chlorophyll a-b binding protein 3C, chloroplast precursor (LHCII type I CAB-3C) (LHCP) pir__CDTO3C chlorophyll a/b-binding protein 3C precursor - tomato prf__1204205G protein 3C,chlorophyll binding	**5,35**
MICRO.11330.C1	putative salt-inducible protein [Arabidopsis thaliana] pir__D84531 probable salt-inducible protein [imported] - Arabidopsis thaliana ref_NP_179165.1_ pentatricopeptide (PPR) repeat-containing protein [Arabidopsis thaliana]	**5,33**
SSBN001F02u.scf	Ras-related protein Rab11A pir__T03625 GTP-binding protein Rab11a - common tobacco gb_AAA74115.1_ Nt-Rab11a gene product	**5,31**
MICRO.12827.C1	putative ABC transporter [Oryza sativa (japonica cultivar-group)] dbj_BAD22238.1_ putative ABC transporter [Oryza sativa (japonica cultivar-group)]	**5,30**
STMGV55TV	No Hits Found	**5,29**
MICRO.16245.C1	Na H-antiportor [Petunia x hybrida]	**5,28**

MICRO.1816.C1	RNA recognition motif (RRM)-containing protein [Arabidopsis thaliana]	**5,26**
BF_TUBSXXXX_0003H10_T3M.SCF	No Hits Found	**5,26**
MICRO.2286.C17	1,3-beta-glucan glucanohydrolase [Solanum tuberosum]	**5,24**
055D06AF.esd	putative cysteine proteinase inhibitor precursor 1423 [Solanum tuberosum]	**5,22**
MICRO.3710.C1	DNA (cytosine-5)-methyltransferase [Lycopersicon esculentum] pir__T07757 probable DNA (cytosine-5-)-methyltransferase (EC 2.1.1.37) - tomato	**5,21**
SDBN002M04u.scf	No Hits Found	**5,20**
POAD763TV	PAR-1c [Nicotiana tabacum] pir__S62700 photoassimilate-responsive protein PAR-1c precursor - common tobacco	**5,19**
BPLI14D4TH	No Hits Found	**5,18**
MICRO.8264.C1	At5g15430 [Arabidopsis thaliana] gb_AAT71961.1_ At5g15430 [Arabidopsis thaliana] emb_CAC01742.1_ putative protein [Arabidopsis thaliana] pir__T51521 hypothetical protein T20K14_40 - Arabidopsis thaliana ref_NP_197047.1_ calmodulin-binding ...	**5,17**
MICRO.1210.C1	flavin monoxygenase-like protein floozy [Petunia x hybrida]	**5,14**
bf_mxlfxxxx_0041d12.t3m.scf	Hcr2-5D [Lycopersicon esculentum] pir__T30553 disease resistance protein Hcr2-5D - tomato	**5,13**
MICRO.11916.C1	putative polyprotein [Solanum demissum]	**5,11**
SSBN003H07u.scf	No Hits Found	**5,11**
MICRO.511.C3	No Hits Found	**5,10**
MICRO.5323.C2	1-deoxy-D-xylulose-5-phosphate reductoisomerase [Lycopersicon esculentum]	**5,09**
MICRO.16169.C1	No Hits Found	**5,09**
SSBN001N05u.scf	hypothetical protein [Vitis vinifera] XP_002278940.1	**5,08**
bf_suspxxxx_0031e04.t3m.scf	At2g26710 [Arabidopsis thaliana] gb_AAB95305.1_ putative cytochrome P450 [Arabidopsis thaliana] dbj_BAD42995.1_ putative cytochrome P450 [Arabidopsis thaliana] pir__H84663 probable cytochrome P450 [imported] - Arabidopsis thaliana ref_NP_180239.1_ ...	**5,06**
MICRO.6187.C2	glucan endo-1,3-beta-D-glucosidase [Lycopersicon esculentum]	**5,05**
MICRO.11745.C1	MSH4 [Arabidopsis thaliana]	**5,04**
110G11AF.esd	No Hits Found	**5,03**
MICRO.8362.C1	No Hits Found	**5,03**
POAD969TP	unnamed protein product [Arabidopsis thaliana] ref_NP_188589.1_ amino acid permease family protein [Arabidopsis thaliana]	**5,01**
cPRO1K16TH	similar to AT4G11610: C2 domain-containing protein	**5,00**
MICRO.1130.C1	putative leucine zipper protein [Gossypium hirsutum]	**4,99**
bf_mxlfxxxx_0020b06.t3m.scf	vetispiradiene synthase [Solanum tuberosum]	**4,98**
POCCH15TV	No Hits Found	**4,96**
MICRO.6361.C1	pTOR [Arabidopsis thaliana] ref_NP_175425.2_ target of rapamycin protein (TOR) [Arabidopsis thaliana]	**4,94**
MICRO.8164.C1	putative protein [Arabidopsis thaliana] pir__G85092 hypothetical protein AT4g09150 [imported] - Arabidopsis thaliana ref_NP_192654.1_ T-complex protein 11 [Arabidopsis thaliana]	**4,94**
SDBN003G15u.scf	DYW9 protein [Arabidopsis thaliana] pir__T52645 hypothetical protein DYW9 [imported] - Arabidopsis thaliana (fragment)	**4,93**
MICRO.14584.C1	proline rich 7 (synaptic) [Pan troglodytes] XP_518131.2	**4,91**
bf_mxlfxxxx_0053b07.t3m.scf	No Hits Found	**4,90**
MICRO.7759.C1	putative (R)-limonene synthase [Ricinus communis]	**4,88**
MICRO.17009.C1	No Hits Found	**4,87**
BF_LBCHXXXX_0045E12_T3M.SCF	No Hits Found	**4,85**

bf_mxflxxxx_0073g05.t3m.scf	No Hits Found	**4,83**
MICRO.13123.C1	putative 1-deoxy-D-xylulose 5-phosphate synthase 2 [Lycopersicon hirsutum]	**4,82**
TBSK02874FH06.t3m.scf	proteinase inhibitor II [Solanum tuberosum] emb_CAA27408.1_ unnamed protein product [Solanum tuberosum] prf__1301308A proteinase inhibitor II	**4,82**
SDBN003D01u.scf	defensin [Nicotiana attenuata]	**4,81**
SSBN002F06u.scf	No Hits Found	**4,79**
MICRO.10807.C1	protein kinase CK2 regulatory subunit CK2B3 [Zea mays]	**4,77**
MICRO.6946.C1	No Hits Found	**4,76**
bf_acdcxxxx_0022g09.t3m.scf	COG0770: UDP-N-acetylmuramyl pentapeptide synthase [Clostridium thermocellum ATCC 27405]	**4,75**
bf_mxflxxxx_0006b11.t3m.scf	No Hits Found	**4,73**
cSTA25G21TH	No Hits Found	**4,73**
MICRO.9727.C1	expressed protein [Arabidopsis thaliana]	**4,72**
MICRO.1143.C1	mitochondrial malate dehydrogenase [Lycopersicon esculentum]	**4,70**
MICRO.11724.C1	No Hits Found	**4,69**
MICRO.15575.C1	putative protein [Arabidopsis thaliana] pir__T49940 hypothetical protein F17I14.230 - Arabidopsis thaliana ref_NP_196520.1_ expressed protein [Arabidopsis thaliana]	**4,68**
SSBN001N07u.scf	No Hits Found	**4,66**
MICRO.10112.C1	No Hits Found	**4,65**
MICRO.11037.C3	allyl alcohol dehydrogenase [Nicotiana tabacum]	**4,63**
054B06AF.esd	Deoxyhypusine synthase gb_AAG53641.1_ deoxyhypusine synthase [Lycopersicon esculentum]	**4,62**
MICRO.3810.C1	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein [Arabidopsis thaliana]	**4,61**
MICRO.9684.C1	No Hits Found	**4,60**
MICRO.794.C1	rRNA methylase-like protein [Arabidopsis thaliana] pir__T49974 rRNA methylase-like protein - Arabidopsis thaliana ref_NP_197043.1_ tRNA/rRNA methyltransferase (SpoU) family protein [A. thaliana]	**4,59**
bf_mxflxxxx_0020a08.t3m.scf	unknown [Arabidopsis thaliana] gb_AAF79252.1_ F12K21.2 [Arabidopsis thaliana] ref_NP_564450.1_ expressed protein [Arabidopsis thaliana]	**4,57**
MICRO.6024.C3	No Hits Found	**4,56**
BF_LBCHXXXX_0050D04_T3M.SCF	No Hits Found	**4,53**
PPCBI28TH	Glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor (NADP-dependent glyceraldehydephosphate dehydrogenase subunit B) gb_AAA34076.1_ glyceraldehyde-3-phosphate dehydrogenase B-subunit precursor	**4,52**
MICRO.9975.C1	No Hits Found	**4,51**
cSTE26D7TH	No Hits Found	**4,49**
MICRO.13165.C1	WRKY transcription factor-30 [Capsicum annuum]	**4,48**
MICRO.333.C1	breast cancer susceptibility protein 2b [Arabidopsis thaliana] ref_NP_195783.3_ BRCA2 repeat-containing protein [Arabidopsis thaliana]	**4,47**
MICRO.14723.C3	BEL1-related homeotic protein 29 [Solanum tuberosum]	**4,46**
cPRO1D21TH	40S ribosomal protein S16 [Euphorbia esula]	**4,44**
MICRO.15614.C1	No Hits Found	**4,42**
POAB903TV	ABC transporter-like protein [Arabidopsis thaliana] pir__T07717 probable ABC-type transport protein T23J7.110 - Arabidopsis thaliana	**4,41**
MICRO.7313.C1	heat stress transcription factor 8 [Lycopersicon esculentum] pir__S25478 heat shock transcription factor HSF8 - tomato sp_Q40152_HSF8_LYCES Heat shock factor protein HSF8 (Heat shock transcription factor 8) (HSTF 8) (Heat stress transcription factor)	**4,41**

MICRO.10196.C2	putative ubiquitin-conjugating enzyme [Oryza sativa (japonica cultivar-group)] gb_AAP54809.1_ putative ubiquitin-conjugating enzyme [Oryza sativa (japonica cultivar-group)] gb_AAL58113.1_ putative ubiquitin-conjugating enzyme [Oryza sativa (japonica ...	**4,39**
SSBN002C03u.scf	No Hits Found	**4,38**
POCBU74TP	general transcription factor IIF, polypeptide 2, 30kDa [Homo sapiens] gb_AAH01771.1_ General transcription factor IIF, polypeptide 2, 30kDa [Homo sapiens] emb_CAH72459.1_ OTTHUMP00000040988 [Homo sapiens] emb_CAH72032.1_ general transcription factor ...	**4,36**
SDBN005C16u.scf	No Hits Found	**4,35**
MICRO.1075.C6	phi-1 [Nicotiana tabacum]	**4,33**
MICRO.12195.C1	No Hits Found	**4,33**
POCA375TF	unknown protein [Arabidopsis thaliana] gb_AAL38254.1_ unknown protein [Arabidopsis thaliana]	**4,31**
bf_mxlfxxxx_0074c12.t3m.scf	No Hits Found	**4,30**
MICRO.5148.C1	No Hits Found	**4,29**
MICRO.17000.C1	leucoanthocyanidin dioxygenase-like protein [Arabidopsis thaliana] gb_AAS49108.1_ At3g13610 [Arabidopsis thaliana] ref_NP_187970.1_ oxidoreductase, 2OG-Fe(II) oxygenase family protein [A.thaliana]	**4,28**
BF_TUBSXXXX_0022E01_T3M.SCF	No Hits Found	**4,27**
bf_arrayxxx_0015f06.t7m.scf	No Hits Found	**4,26**
cSTA37J9TH	hypothetical protein [Vitis vinifera] XP_002267812.1	**4,25**
MICRO.2616.C1	Avr9/Cf-9 rapidly elicited protein 256 [Nicotiana tabacum]	**4,23**
bf_swstxxxx_0045c01.t3m.scf	No Hits Found	**4,22**
bf_cswcxxxx_0006g04.t3m.scf	At5g16070 [Arabidopsis thaliana] gb_AAM91565.1_ TCP-1 chaperonin-like protein [Arabidopsis thaliana] ref_NP_197111.2_ chaperonin, putative [Arabidopsis thaliana]	**4,20**
PotatoF1558.scf	plasma membrane intrinsic protein PIP2 [Solanum chacoense]	**4,19**
STMDB53TV	No Hits Found	**4,18**
bf_suspxxxx_0017G02.t3m.scf	putative peptide transport protein [Oryza sativa (japonica cultivar-group)] gb_AAP54224.1_ putative peptide transport protein [Oryza sativa (japonica cultivar-group)] gb_AAG21898.1_ putative peptide transport protein [Oryza sativa]	**4,16**
MICRO.3206.C2	putative photosystem I subunit III precursor [Nicotiana tabacum]	**4,15**
SSBT001K11x.scf	hypothetical protein [Oryza sativa (japonica cultivar-group)] dbj_BAB64132.1_ hypothetical protein [Oryza sativa (japonica cultivar-group)]	**4,14**
MICRO.7212.C2	No Hits Found	**4,13**
MICRO.4288.C8	cysteine proteinase inhibitor 5 precursor [Solanum tuberosum]	**4,11**
MICRO.15141.C1	putative polygalacturonase [Arabidopsis thaliana] ref_NP_181916.1_ polygalacturonase, putative / pectinase, putative [Arabidopsis thaliana] pir__T00668 probable polygalacturonase [imported] - Arabidopsis thaliana	**4,09**
MICRO.9044.C1	C2 domain-containing protein / GRAM domain-containing protein [Arabidopsis thaliana] NP_191525.2	**4,08**
MICRO.4830.C2	serine protease [Lycopersicon esculentum]	**4,06**
bf_mxflxxxx_0048b04.t3m.scf	T1N15.2 [Arabidopsis thaliana]	**4,05**
MICRO.6985.C2	acylamino acid-releasing enzyme [Arabidopsis thaliana] ref_NP_193193.2_ acylaminoacyl-peptidase-related [Arabidopsis thaliana]	**4,03**
MICRO.4830.C1	P69E protein [Lycopersicon esculentum] pir__T06579 subtilisin-like proteinase (EC 3.4.21.-) p69e - tomato	**4,03**
MICRO.17358.C1	zinc finger (CCCH-type) family protein [Arabidopsis thaliana]	**4,02**
MICRO.16919.C1	unknown protein [Oryza sativa (japonica cultivar-group)] gb_AAP06874.1_ unknown protein [O. sativa (japonica cultivar-group)] gb_AAP06842.1_ unknown protein [O. sativa (japonica cultivar-group)]	**4,01**

MICRO.2286.C42	beta-1,3-glucanase (PR2) [Solanum tuberosum]	**3,99**
MICRO.4497.C3	probable epoxide hydrolase (EC 3.3.2.3) (clone EH10.1) - potato gb_AAA81892.1_ epoxide hydrolase	**3,99**
bf_ivrootxx_0056d01.t3m.scf	hypothetical protein At1g69900 [Arabidopsis thaliana]	**3,98**
POCCT18TP	No Hits Found	**3,96**
MICRO.6149.C2	molybdopterin cofactor synthesis protein A, putative [Ricinus communis] XP_002524317.1	**3,95**
bf_mxlfxxxx_0001e11.t3m.scf	No Hits Found	**3,94**
MICRO.6030.C2	At2g15020 [Arabidopsis thaliana] gb_AAD03359.1_ hypothetical protein [Arabidopsis thaliana] gb_AAL91197.1_ unknown protein [Arabidopsis thaliana] pir__A84524 hypothetical protein At2g15020 [imported] - Arabidopsis thaliana ref_NP_179106.1_ expressed ...	**3,93**
MICRO.12104.C2	heat shock protein binding / unfolded protein binding [Arabidopsis thaliana]	**3,92**
bf_mxflxxxx_0008b10.t3m.scf	calcium ion binding [Arabidopsis thaliana]	**3,91**
MICRO.13176.C1	lipoxygenase (POTLX-3) [Solanum tuberosum]	**3,90**
068E01AF.esd	No Hits Found	**3,90**
TBSK02550FE06.t3m.scf	putative trypsin inhibitor from Potato mRNA for tuber protein p340/p34021	**3,89**
MICRO.3652.C3	germacrene C synthase [Lycopersicon esculentum] pir__T06266 germacrene C synthase - tomato	**3,87**
MICRO.5478.C1	No Hits Found	**3,86**
POCCJ07TV	No Hits Found	**3,85**
SDBN002J03u.scf	NtPRp27 [Nicotiana tabacum]	**3,82**
STDB002H09u.scf	P0512C01.35 [Oryza sativa (japonica cultivar-group)] dbj_BAB92380.1_ P0512C01.35 [Oryza sativa (japonica cultivar-group)]	**3,81**
bf_ivrootxx_0026h03.t3m.scf	No Hits Found	**3,80**
POAD870TV	putative vacuolar protein sorting	**3,80**
POCB845TP	No Hits Found	**3,79**
MICRO.12637.C4	unknown protein [imported] - Arabidopsis thaliana gb_AAG51180.1_ unknown protein [Arabidopsis thaliana] ref_NP_175481.1_ expressed protein [A. thaliana] gb_AAF87869.1_ Unknown protein [A. thaliana]	**3,78**
STMIY72TV	glucosyltransferase [Nicotiana tabacum]	**3,77**
MICRO.1887.C1	At1g79210/YUP8H12R_1 [Arabidopsis thaliana] gb_AAM19806.1_ At1g79210/YUP8H12R_1 [Arabidopsis thaliana] ref_NP_178042.1_ 20S proteasome alpha subunit B, putative [Arabidopsis thaliana]	**3,76**
MICRO.12660.C2	unknown protein [Arabidopsis thaliana] gb_AAK76639.1_ unknown protein [A. thaliana] ref_NP_567899.1_ ferredoxin-related [A. thaliana]	**3,75**
MICRO.8305.C1	No Hits Found	**3,73**
BF_LBCHXXXX_0020C09_T3M.SCF	No Hits Found	**3,71**
PPCCC05TH	No Hits Found	**3,71**
MICRO.207.C2	unknown [Glycine max] ACU23324.1	**3,70**
ACDA01928A08.T3m.scf	No Hits Found	**3,69**
MICRO.331.C89	Chlorophyll a-b binding protein 3C, chloroplast precursor (LHCII type I CAB-3C) (LHCP) pir__CDTO3C chlorophyll a/b-binding protein 3C precursor - tomato prf__1204205G protein 3C,chlorophyll binding	**3,68**
bf_mxlfxxxx_0019f08.t3m.scf	No Hits Found	**3,67**
MICRO.4590.C1	xyloglucan endotransglucosylase-hydrolase XTH7 [Lycopersicon esculentum]	**3,65**
bf_mxlfxxxx_0014b07.t3m.scf	chlorophyll a/b binding protein (124 AA) [Raphanus sativus] sp_P14584_CB21_RAPSA Chlorophyll a-b binding of LHCII type I protein (CAB) (LHCP)	**3,64**

MICRO.4653.C1	No Hits Found	**3,63**
MICRO.15940.C1	beta-adaptin-like protein A [Arabidopsis thaliana] gb_AAF61671.1_ beta-adaptin-like protein A [Arabidopsis thaliana] ref_NP_196710.1_ adaptin family protein [Arabidopsis thaliana] pir__T48508 beta-adaptin-like protein A - Arabidopsis thaliana	**3,62**
MICRO.11885.C1	No Hits Found	**3,61**
MICRO.11694.C2	4-hydroxyphenylpyruvate dioxygenase (4HPPD) (HPD) (HPPDase) gb_AAC49815.1_ 4-hydroxyphenylpyruvate dioxygenase [Daucus carota] pir__T14353 probable 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) - carrot	**3,61**
cSTE25C10TH	superoxide dismutase [Fe] [Lycopersicon esculentum]	**3,60**
SSBN001L23u.scf	No Hits Found	**3,59**
MICRO.5321.C1	No Hits Found	**3,58**
POCD051TV	No Hits Found	**3,57**
MICRO.5337.C1	putative 1-deoxy-D-xylulose 5-phosphate synthase 2 [Lycopersicon hirsutum]	**3,56**
TBSK03621FF09.t3m.scf	No Hits Found	**3,56**
MICRO.13314.C1	No Hits Found	**3,55**
SSBN002B15u.scf	No Hits Found	**3,54**
MICRO.7335.C3	At5g44170 [Arabidopsis thaliana] dbj_BAB10984.1_ unnamed protein product [Arabidopsis thaliana] gb_AAL62395.1_ putative protein [Arabidopsis thaliana] ref_NP_199230.1_ expressed protein [Arabidopsis thaliana]	**3,53**
bf_arrayxxx_0047h05.t7m.scf	No Hits Found	**3,53**
086B01AF.esd	similar to AT1G74900 | Symbols: OTP43 | OTP43 (ORGANELLE TRANSCRIPT PROCESSING DEFECT 43)	**3,51**
MICRO.16497.C1	unknown protein [Arabidopsis thaliana] ref_NP_849520.1_ bZIP protein [Arabidopsis thaliana]	**3,50**
bf_mxflxxxx_0069f01.t3m.scf	No Hits Found	**3,49**
BPLI15I14TH	ribulose bisphosphate carboxylase [Solanum tuberosum] sp_P26575_RBSA_SOLTU Ribulose bisphosphate carboxylase small chain 2A, chloroplast precursor (RuBisCO small subunit 2A) pir__RKPOS2 ribulose-bisphosphate carboxylase (EC 4.1.1.39) precursor small ...	**3,48**
MICRO.11500.C1	unknown protein [Arabidopsis thaliana] dbj_BAC42859.1_ unknown protein [Arabidopsis thaliana] ref_NP_850037.1_ expressed protein [Arabidopsis thaliana]	**3,47**
SDBN002P21u.scf	No Hits Found	**3,45**
POCAH82TV	unnamed protein product [Arabidopsis thaliana]	**3,44**
bf_ivrootxx_0024g04.t3m.scf	patatin [Solanum tuberosum] pir__A26017 patatin T5 precursor - potato sp_P15478_PAT5_SOLTU PATATIN T5 PRECURSOR (POTATO TUBER PROTEIN) prf__1301309A patatin	**3,43**
POCAD80TV	S-adenosyl-methionine-sterol-C-methyltransferase homolog [Nicotiana tabacum] pir__T03845 probable sterol 24-C-methyltransferase (EC 2.1.1.41) - common tobacco (fragment)	**3,43**
MICRO.5233.C2	phytoene desaturase [Lycopersicon esculentum]	**3,42**
BF_TUBSXXXX_0064B09_T3M.SCF	unknown protein [Arabidopsis thaliana]	**3,42**
bf_cswcxxxx_0002h07.t3m.scf	No Hits Found	**3,41**
MICRO.229.C1	unknown protein [Arabidopsis thaliana] gb_AAL66967.1_ unknown protein [Arabidopsis thaliana] gb_AAL09789.1_ AT4g29060/F19B15_90 [Arabidopsis thaliana]	**3,40**
MICRO.499.C2	peroxidase (EC 1.11.1.7) TPX2 precursor - tomato gb_AAA65636.1_ peroxidase	**3,39**
MICRO.4881.C2	similar to AT3G53450, similar to unknown protein [Arabidopsis thaliana]; similar to Conserved hypothetical protein 730 [Medicago truncatula]; contains InterPro domain Conserved hypothetical protein CHP00730	**3,39**

MICRO.3971.C4	No Hits Found	**3,38**
MICRO.10979.C1	hypothetical protein [Ricinus communis] XP_002518699.1	**3,37**
cSTB45P17TH	No Hits Found	**3,36**
cSTE5G17TH	ara4-interacting protein [Ricinus communis]	**3,36**
MICRO.8332.C1	similar to AT2G41190: amino acid transporter family protein	**3,35**
MICRO.945.C1	unknown protein [Arabidopsis thaliana] gb_AAL32564.1_ Unknown protein [Arabidopsis thaliana]	**3,34**
bf_mxlfxxxx_0036f05.t3m.scf	No Hits Found	**3,33**
BF_TUBSXXXX_0041H03_T3M.SCF	No Hits Found	**3,33**
POADD78TV	aminolevulinate dehydratase	**3,32**
cSTA20K21TH	osmotin-like protein precursor [Solanum nigrum]	**3,31**
MICRO.2277.C1	UMP synthase [Nicotiana plumbaginifolia]	**3,30**
MICRO.5811.C1	NAD synthetase [Nicotiana tabacum]	**3,29**
bf_mxflxxxx_0052f03.t3m.scf	No Hits Found	**3,27**
MICRO.13092.C2	unknown protein [Arabidopsis thaliana] gb_AAO24547.1_ At1g11700 [. thaliana] gb_AAD30253.1_ ESTs gb_R65381 and gb_T44635 come from this gene. [A. thaliana] pir__F86250 hypothetical protein	**3,26**
TBSK03136FF04.t3m.scf	No Hits Found	**3,26**
bf_lbchxxxx_0061d01.t3m.scf	No Hits Found	**3,25**
MICRO.4622.C1	similar to AT1G65920: regulator of chromosome condensation (RCC1) family protein / zinc finger protein-related	**3,25**
MICRO.14505.C1	similar to AT1G79990: coatomer protein complex, subunit beta 2 (beta prime), putative	**3,24**
MICRO.12385.C1	NtEIG-E80 [Nicotiana tabacum]	**3,23**
POCC683TV	No Hits Found	**3,22**
bf_arrayxxx_0047b05.t7m.scf	Putative histone H2A [Oryza sativa (japonica cultivar-group)] gb_AAN06860.1_ Putative histone H2A [Oryza sativa (japonica cultivar-group)]	**3,21**
POCBO38TV	hsr203J [Solanum tuberosum]	**3,21**
bf_cswcxxxx_0002d08.t3m.scf	P0460C04.11 [Oryza sativa (japonica cultivar-group)] dbj_BAB92919.1_ P0460C04.11 [Oryza sativa (japonica cultivar-group)]	**3,20**
BPLI18M23TH	alpha-amylase (EC 3.2.1.1) precursor - southern Asian dodder (fragment) gb_AAA16513.1_ alpha amylase precursor	**3,19**
MICRO.123.C1	No Hits Found	**3,19**
MICRO.7427.C4	tropinone reductase I [Solanum tuberosum]	**3,18**
cSTC9F16TH	No Hits Found	**3,17**
BF_LBCHXXXX_0003A09_T3M.SCF	No Hits Found	**3,16**
MICRO.13961.C1	pSTH-21 protein [Solanum tuberosum] pir__S35162 STH-21 protein - potato sp_P17641_PRS1_SOLTU Pathogenesis-related protein STH-21 gb_AAA02829.1_ STH-21 protein	**3,16**
MICRO.4706.C1	zeta-carotene desaturase [Lycopersicon esculentum]	**3,15**
STMGQ31TV	No Hits Found	**3,15**
bf_arrayxxx_0063h02.t3m.scf	No Hits Found	**3,14**
STMHH29TV	No Hits Found	**3,14**
bf_cswcxxxx_0011c11.t3m.scf	isoamylase N-terminal domain containing protein [Oryza sativa (japonica cultivar-group)]	**3,13**
MICRO.14423.C1	Williams Beuren syndrome chromosome region 22 protein [Homo sapiens] gb_AAH11696.2_ Williams Beuren syndrome chromosome region 22 protein [Homo sapiens]	**3,13**

SSBN003G18u.scf	cytochrome P450 [Panax ginseng]	**3,12**
MICRO.11336.C2	chitinase (EC 3.2.1.14) 2 - cone shell (Conus tulipa) sp_Q7M443_CHI2_TULBA Chitinase 2 (Tulip bulb chitinase-2) (TBC-2)	**3,11**
MICRO.7962.C1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase/ sugar binding [Arabidopsis thaliana]	**3,11**
MICRO.13690.C1	Similar to gb_AF099053 phosphatidylserine synthase-2 from Mus musculus. EST gb_N96271 comes from this gene. [Arabidopsis thaliana] pir__G86284 F9L1.4 protein - Arabidopsis thaliana	**3,10**
MICRO.4848.C3	small blue copper protein Bcp1 [Boea crassifolia]	**3,09**
MICRO.13036.C2	similar to AT5G14430: dehydration-responsive protein-related	**3,08**
MICRO.14275.C1	unknown protein [Arabidopsis thaliana] gb_AAO42280.1_ unknown protein [Arabidopsis thaliana] ref_NP_172832.3_ expressed protein [Arabidopsis thaliana]	**3,08**
MICRO.9644.C1	glutamate dehydrogenase, putative [Arabidopsis thaliana]	**3,07**
ACDA03729G09.T3m.scf	No Hits Found	**3,06**
POADI66TP	Quinonprotein alcohol dehydrogenase-like [Medicago truncatula] ABN08942.1	**3,06**
PPCBF91TH	No Hits Found	**3,05**
MICRO.9095.C1	proline-rich protein [Nicotiana alata] pir__S31096 proline-rich protein - Persian tobacco	**3,05**
MICRO.592.C9	Probable glutathione S-transferase (Pathogenesis-related protein 1) pir__T07595 glutathione transferase (EC 2.5.1.18) homolog GST1 - potato gb_AAA68430.1_ glutathione S-transferase	**3,04**
cSTD20A15TH	unknown protein [Arabidopsis thaliana] dbj_BAC42931.1_ unknown protein [Arabidopsis thaliana] gb_AAO63991.1_ putative RRM-containing protein [Arabidopsis thaliana] ref_NP_187100.1_ RNA recognition motif (RRM)-containing protein [Arabidopsis thaliana]	**3,04**
bf_mxflxxxx_0018h10.t3m.scf	No Hits Found	**3,04**
bf_cswbxxxx_0011d10.t3m.scf	putative S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase	**3,03**
MICRO.11698.C1	ENSANGP00000011122 [Anopheles gambiae str. PEST]	**3,03**
bf_arrayxxx_0057c05.t3m.scf	No Hits Found	**3,02**
MICRO.11691.C1	proteinase IV F2P9.14 [imported] - Arabidopsis thaliana gb_AAG52522.1_ putative protease IV	**3,02**
MICRO.11336.C1	chitinase (EC 3.2.1.14) 2 - cone shell (Conus tulipa) sp_Q7M443_CHI2_TULBA Chitinase 2 (Tulip bulb chitinase-2) (TBC-2)	**3,02**
STMIT79TV	acyl CoA reductase [synthetic construct] gb_AAD38039.1_ acyl CoA reductase [Simmondsia chinensis]	**3,02**
MICRO.11731.C1	carotenoid isomerase [Lycopersicon esculentum]	**3,01**
cPRO23B9TH	No Hits Found	**3,01**
POCCL69TV	N2, N2-dimethylguanosine tRNA methyltransferases-like protein [Arabidopsis thaliana] pir__T47750 N2,N2-dimethylguanosine tRNA methyltransferases-like protein - Arabidopsis thaliana ref_NP_191192.1_ N2,N2-dimethylguanosine tRNA methyltransferase ...	**3,01**
bf_mxflxxxx_0071d02.t3m.scf	No Hits Found	**3,00**
MICRO.11751.C1	No Hits Found	**3,00**
MICRO.11352.C3	DEMETER protein [Arabidopsis thaliana] sp_Q8LK56_DME_ARATH Transcriptional activator DEMETER (DNA glycosylase-related protein DME)	**3,00**
bf_mxflxxxx_0036a04.t3m.scf	4-coumarate--CoA ligase family protein / 4-coumaroyl-CoA synthase family protein (AT4G19010) [Arabidopsis thaliana]	*-3,00*
MICRO.282.C1	putative aspartic proteinase nepenthesin [Oryza sativa (japonica cultivar-group)] dbj_BAD21978.1_ putative aspartic proteinase nepenthesin [Oryza sativa (japonica cultivar-group)]	*-3,01*
bf_arrayxxx_0080d10.t7m.scf	No Hits Found	*-3,03*

MICRO.331.C51	No Hits Found	*-3,05*
MICRO.4816.C1	unknown protein [Arabidopsis thaliana]	*-3,06*
bf_mxlfxxxx_0042g07.t3m.scf	unknown [Lycopersicon esculentum]	*-3,08*
SDBN002I21u.scf	hypothetical protein [Arabidopsis thaliana] gb_AAP04120.1_ unknown protein [Arabidopsis thaliana] gb_AAO42310.1_ unknown protein [Arabidopsis thaliana] ref_NP_187664.2_ transducin family protein / WD-40 repeat family protein [Arabidopsis thaliana]	*-3,10*
bf_acdcxxxx_0018h08.t3m.scf	No Hits Found	*-3,11*
SSBN001N10u.scf	No Hits Found	*-3,13*
bf_swstxxxx_0004d10.t3m.scf	No Hits Found	*-3,14*
bf_ivrootxx_0037g07.t3m.scf	hypothetical protein PGEC542.21 [Solanum demissum]	*-3,17*
MICRO.4641.C1	No Hits Found	*-3,19*
MICRO.12041.C1	glycosyl transferase family 17 protein [Arabidopsis thaliana]	*-3,20*
SDBN005F03u.scf	chorismate synthase 2 [Lycopersicon esculentum] sp_Q42885_ARC2_LYCES Chorismate synthase 2, chloroplast precursor (5-enolpyruvylshikimate-3-phosphate phospholyase 2) pir__S40409 chorismate synthase (EC 4.2.3.5) 2 precursor - tomato	*-3,21*
BPLI1E24TH	ILP1 (increased level of polyploidy1-1D); translation repressor [Arabidopsis thaliana]	*-3,23*
MICRO.15902.C1	unnamed protein product [Arabidopsis thaliana]	*-3,26*
MICRO.11404.C1	putative alliin lyase [Oryza sativa (japonica cultivar-group)]	*-3,28*
SSBT005J19x.scf	putative ribosomal protein S29 [Oryza sativa (japonica cultivar-group)] gb_AAP68880.1_ putative ribosomal protein S29 [Oryza sativa (japonica cultivar-group)]	*-3,30*
cSTA2D7TH	kinesin-like protein [Arabidopsis thaliana] pir__T46242 kinesin-like protein - Arabidopsis thaliana ref_NP_190534.1_ kinesin motor protein-related [Arabidopsis thaliana]	*-3,32*
STMIB36TV	No Hits Found	*-3,33*
MICRO.17348.C1	unknown protein [Arabidopsis thaliana]	*-3,36*
MICRO.3711.C1	bHLH transcription factor PTF1-like [Oryza sativa (japonica cultivar-group)]	*-3,38*
SSBN001O11u.scf	At5g64156 [Arabidopsis thaliana] dbj_BAB10283.1_ unnamed protein product [Arabidopsis thaliana] gb_AAK96773.1_ Unknown protein [Arabidopsis thaliana] ref_NP_201220.1_ methylase family protein [Arabidopsis thaliana]	*-3,41*
MICRO.6780.C1	Hsp20.0 protein [Lycopersicon peruvianum]	*-3,43*
MICRO.10241.C1	At1g12740 [Arabidopsis thaliana] dbj_BAD44087.1_ hypothetical protein [Arabidopsis thaliana] ref_NP_172734.2_ cytochrome P450 family protein [Arabidopsis thaliana]	*-3,44*
MICRO.5672.C1	alcohol oxidase-related [Arabidopsis thaliana] NP_171895.2	*-3,46*
cSTA2E9TH	putative glucosyltransferase [Phaseolus lunatus]	*-3,47*
TBSK00120FB12.t3m.scf	No Hits Found	*-3,48*
bf_mxflxxxx_0054g04.t3m.scf	At1g28060/F13K9_16 [Arabidopsis thaliana] pir__C86406 88.6K hypothetical protein F13K9.16 - Arabidopsis thaliana gb_AAG51492.1_ hypothetical protein [Arabidopsis thaliana] ref_NP_174127.1_ small nuclear ribonucleoprotein family protein / snRNP famil ...	*-3,50*
MICRO.15621.C1	expressed protein [Arabidopsis thaliana] gb_AAO11614.1_ At2g05170/F5G3.7 [Arabidopsis thaliana] gb_AAL32006.1_ At2g05170/F5G3.7 [Arabidopsis thaliana] pir__F84465 hypothetical protein At2g05170 [imported] - Arabidopsis thaliana ref_NP_027676.1_ ...	*-3,53*
POCC418TP	Ethylene-responsive transcription factor 3 (Ethylene-responsive element-binding factor 3 homolog) (EREBP-5) (NtERF5) [Nicotiana tabacum]	*-3,55*
MICRO.4671.C1	hypothetical protein [Oryza sativa (japonica cultivar-group)] dbj_BAD31099.1_ hypothetical protein [Oryza sativa (japonica cultivar-group)] dbj_BAC15471.1_ hypothetical protein [Oryza sativa (japonica cultivar-group)]	*-3,56*

bf_mxflxxxx_0072f05.t3m.scf	similar to AT1G26740: structural constituent of ribosome	*-3,58*
cSTE9F17TH	putative SSR alpha subunit [Oryza sativa (japonica cultivar-group)]	*-3,61*
MICRO.1522.C1	putative protein [Arabidopsis thaliana] ref_NP_851016.1_ SAC3/GANP family protein [Arabidopsis thaliana]	*-3,63*
bf_suspxxxx_0012f07.t3m.scf	similar to AT3G11590: similar to unknown protein [Arabidopsis thaliana]; similar to unnamed protein product [Vitis vinifera]; contains domain Bcr-Abl oncoprotein oligomerization domain	*-3,66*
MICRO.1218.C2	putative proline-rich protein APG isolog [Cicer arietinum]	*-3,67*
MICRO.2697.C1	RNA recognition motif (RRM)-containing protein-like [Oryza sativa (japonica cultivar-group)] dbj_BAD10531.1_ RNA recognition motif (RRM)-containing protein-like [Oryza sativa (japonica cultivar-group)]	*-3,70*
STMIW86TV	No Hits Found	*-3,72*
cSTB30E5TH	pentatricopeptide (PPR) repeat-containing protein [Arabidopsis thaliana]	*-3,76*
MICRO.5509.C1	unknown protein [Oryza sativa (japonica cultivar-group)] gb_AAU10797.1_ unknown protein [Oryza sativa (japonica cultivar-group)] gb_AAT07648.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	*-3,77*
bf_ivrootxx_0022h01.t3m.scf	hypothetical protein [Vitis vinifera] XP_002277435.1	*-3,81*
MICRO.3952.C1	unnamed protein product [Arabidopsis thaliana]	*-3,83*
cSTA35F22TH	putative kinesin-like protein [Arabidopsis thaliana]	*-3,87*
cSTA29C13TH	putative potassium transporter HAK1p [Mesembryanthemum crystallinum]	*-3,90*
bf_arrayxxx_0071f04.t7m.scf	No Hits Found	*-3,92*
cSTB48K8TH	unknown [Arabidopsis thaliana] pir__T51158 hypothetical protein [imported] - Arabidopsis thaliana ref_NP_190755.1_ mitochondrial substrate carrier family protein [Arabidopsis thaliana]	*-3,93*
MICRO.15836.C1	unknown protein [Arabidopsis thaliana] gb_AAM20565.1_ unknown protein [Arabidopsis thaliana] ref_NP_188894.2_ senescence-associated protein-related [Arabidopsis thaliana]	*-3,96*
bf_ivrootxx_0040e05.t3m.scf	unknown protein [Arabidopsis thaliana] gb_AAO63361.1_ At5g48850 [Arabidopsis thaliana] ref_NP_199696.2_ male sterility MS5 family protein [Arabidopsis thaliana]	*-4,00*
MICRO.7345.C1	No Hits Found	*-4,02*
bf_suspxxxx_0049g12.t3m.scf	No Hits Found	*-4,03*
MICRO.7768.C1	No Hits Found	*-4,08*
POABZ48TV	unknown protein [Arabidopsis thaliana] gb_AAL24150.1_ unknown protein [Arabidopsis thaliana] ref_NP_568558.1_ proline-rich spliceosome-associated (PSP) family protein / zinc knuckle (CCHC-type) family protein [Arabidopsis thaliana]	*-4,11*
MICRO.976.C1	putative phosphatidylinositol-4-phosphate 5-kinase [Arabidopsis thaliana] gb_AAL69491.1_ putative phosphatidylinositol-4-phosphate 5-kinase [Arabidopsis thaliana] ref_NP_176286.2_ phosphatidylinositol-4-phosphate 5-kinase family protein [Arabidopsis ...	*-4,13*
MICRO.3300.C1	hypothetical protein [Vitis vinifera] XP_002280344.1	*-4,18*
MICRO.15448.C1	expressed protein [Arabidopsis thaliana]	*-4,21*
bf_mxflxxxx_0010h01.t3m.scf	STIG1 [Lycopersicon esculentum]	*-4,23*
cSTA23L3TH	At4g38650 [Arabidopsis thaliana] ref_NP_195577.2_ glycosyl hydrolase family 10 protein [Arabidopsis thaliana]	*-4,29*
MICRO.18202.C1	auxin-responsive-like protein [Arabidopsis thaliana] dbj_BAB17304.1_ auxin-responsive GH3 homologue [Arabidopsis thaliana] ref_NP_200262.1_ auxin-responsive GH3 protein, putative (DFL-1) [Arabidopsis thaliana]	*-4,33*
MICRO.15417.C1	UvrD/REP helicase family protein [Arabidopsis thaliana]	*-4,39*
MICRO.3570.C1	similar to AT1G60770: pentatricopeptide (PPR) repeat-containing protein	*-4,44*
cSTA9J19TH	unknown protein T5M16.5 [imported] - Arabidopsis thaliana gb_AAG51678.1_ unknown protein	*-4,47*

STMHR71TV	No Hits Found	*-4,53*
bf_ivrootxx_0018a02.t3m.scf	putative translation initiation factor 2B beta subunit [Nicotiana tabacum]	*-4,56*
MICRO.6495.C1	putative aldo/keto reductase family protein [Oryza sativa (japonica cultivar-group)] gb_AAR00611.1_ putative aldo/keto reductase family protein [Oryza sativa (japonica cultivar-group)]	*-4,58*
MICRO.12249.C1	putative acid phosphatase [Arabidopsis thaliana] ref_NP_181394.1_ acid phosphatase class B family protein [Arabidopsis thaliana] pir__A84807 probable acid phosphatase [imported] - Arabidopsis thaliana	*-4,60*
MICRO.2581.C1	predicted protein [Populus trichocarpa] XP_002321872.1	*-4,67*
cSTB16D12TH	similar to AT1G60770: pentatricopeptide (PPR) repeat-containing protein	*-4,71*
cSTA44E6TH	exo-1,3-beta-glucanase [Lilium longiflorum]	*-4,76*
bf_swstxxxx_0005d01.t3m.scf	No Hits Found	*-4,79*
MICRO.8151.C1	unknown protein [Oryza sativa (japonica cultivar-group)] dbj_BAD22126.1_ unknown protein [Oryza sativa (japonica cultivar-group)]	*-4,81*
095C01AF.esd	putative pentatricopeptide repeat containing protein [Oryza sativa (japonica cultivar-group)]	*-4,89*
MICRO.13676.C1	putative exonuclease [Oryza sativa (japonica cultivar-group)] gb_AAP53700.1_ putative exonuclease [Oryza sativa (japonica cultivar-group)] gb_AAK98687.1_ Putative exonuclease [Oryza sativa]	*-4,93*
bf_mxflxxxx_0073g11.t3m.scf	unknown [Solanum chacoense]	*-4,98*
MICRO.11318.C1	hypothetical protein [Vitis vinifera] XP_002283784.1	*-5,01*
cSTA18G13TH	Strong similarity to gi_4734005 F3L12.7 hypothetical protein from Arabidopsis thaliana BAC gb_AC007178 pir__E86285 hypothetical protein F9L1.10 - Arabidopsis thaliana	*-5,06*
MICRO.8007.C2	proline-rich family protein [Arabidopsis thaliana]	*-5,07*
cSTS2F14TH	No Hits Found	*-5,10*
MICRO.8885.C1	At1g30760/T5I8_22 [Arabidopsis thaliana] gb_AAL15318.1_ At1g30760/T5I8_22 [Arabidopsis thaliana] ref_NP_174363.1_ FAD-binding domain-containing protein [Arabidopsis thaliana]	*-5,16*
cSTB32A2TH	similar to AT4G38300: glycosyl hydrolase family 10 protein	*-5,22*
bf_mxflxxxx_0037f09.t3m.scf	Gip1-like protein [Petunia x hybrida]	*-5,28*
STMDJ47TV	putative RNA-binding protein [Arabidopsis thaliana]	*-5,33*
cSTB46N2TH	MYB2 [Dendrobium sp. XMW-2002-2]	*-5,42*
MICRO.16870.C1	SWIB complex BAF60b domain-containing protein / plus-3 domain-containing protein / GYF domain-containing protein [Arabidopsis thaliana]	*-5,46*
092G06AF.esd	phospholipase C [Arabidopsis thaliana]	*-5,49*
MICRO.9420.C1	GTPase, putative	*-5,52*
MICRO.12493.C1	expressed protein [Arabidopsis thaliana]	*-5,53*
MICRO.1908.C1	sarcoplasmic reticulum histidine-rich calcium-binding protein [Zea mays] NP_001149915.1	*-5,60*
MICRO.14329.C1	No Hits Found	*-5,68*
bf_arrayxxx_0087h07.t3m.scf	putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase [Oryza sativa (japonica cultivar-group)]	*-5,73*
MICRO.1643.C2	At2g03760/F19B11.21 [Arabidopsis thaliana] gb_AAD20078.1_ putative steroid sulfotransferase [Arabidopsis thaliana] gb_AAK53042.1_ At2g03760/F19B11.21 [Arabidopsis thaliana] pdb_1Q44_A Chain A, Crystal Structure Of An Arabidopsis Thaliana	*-5,77*
MICRO.12815.C1	Cytochrome b5	*-5,86*
MICRO.8263.C1	No Hits Found	*-5,93*
MICRO.4874.C2	putative glucosyltransferase [Phaseolus lunatus]	*-5,98*
MICRO.4348.C1	unknown [Arabidopsis thaliana] ref_NP_566294.1_ expressed protein [Arabidopsis thaliana]	*-6,03*

MICRO.2511.C2	methyltransferase-like [Oryza sativa (japonica cultivar-group)] dbj_BAD37452.1_ methyltransferase-like [Oryza sativa (japonica cultivar-group)]	*-6,08*
MICRO.14463.C1	putative DNA replication licensing factor, mcm5 [Arabidopsis thaliana] pir__G84487 probable DNA replication licensing factor, mcm5 [imported] - Arabidopsis thaliana ref_NP_178812.1_ minichromosome maintenance family protein / MCM family protein ...	*-6,16*
MICRO.2558.C1	similar to AT1G06920 | Symbols: ATOFP4, OFP4 | ATOFP4/OFP4 (ARABIDOPSIS THALIANA OVATE FAMILY PROTEIN 4)	*-6,23*
MICRO.11332.C1	expressed protein [Arabidopsis thaliana] gb_AAL87358.1_ At2g31160/T16B12.3 [Arabidopsis thaliana] gb_AAL08239.1_ At2g31160/T16B12.3 [Arabidopsis thaliana] pir__C84717 hypothetical protein At2g31160 [imported] - Arabidopsis thaliana ref_NP_565716.1_ ...	*-6,27*
cSTA12B11TH	No Hits Found	*-6,33*
MICRO.6680.C1	No Hits Found	*-6,46*
bf_mxflxxxx_0056g11.t3m.scf	Pathogenesis-related transcriptional factor and ERF [Medicago truncatula]	*-6,55*
STMEI81TV	hypothetical protein [Arabidopsis thaliana] gb_AAC78695.1_ hypothetical protein [Arabidopsis thaliana] pir__T01504 hypothetical protein T10M13.4 - Arabidopsis thaliana ref_NP_192112.1_ expressed protein [Arabidopsis thaliana]	*-6,64*
MICRO.7577.C1	Probable sulfate transporter 3.3 (AST91) pir__B86365 probable sulphate transporter protein [imported] - Arabidopsis thaliana ref_NP_173722.1_ sulfate transporter, putative [Arabidopsis thaliana] gb_AAC00610.1_ Putative sulphate transporter ...	*-6,68*
MICRO.2069.C2	putative NAD dependent epimerase [Arabidopsis thaliana] gb_AAK44025.1_ putative NAD dependent epimerase [Arabidopsis thaliana] dbj_BAB03000.1_ nucleotide sugar epimerase-like protein [Arabidopsis thaliana] gb_AAL32703.1_ nucleotide sugar ...	*-6,75*
MICRO.4095.C2	putative DEAD/DEAH box RNA helicase protein [Arabidopsis thaliana] emb_CAB80208.1_ RNA helicase (RH16) [Arabidopsis thaliana] emb_CAB45452.1_ RNA helicase (RH16) [Arabidopsis thaliana] gb_AAO22635.1_ putative DEAD/DEAH box RNA helicase protein ...	*-6,89*
STMEE35TH	unknown protein [Arabidopsis thaliana] dbj_BAC42316.1_ unknown protein [Arabidopsis thaliana] emb_CAB72163.1_ putative protein [Arabidopsis thaliana] pir__T47753 hypothetical protein F24I3.10 - Arabidopsis thaliana ref_NP_191252.1_ zinc finger (DHHC ...	*-7,07*
MICRO.7777.C1	hypothetical protein AT4G06676 [Arabidopsis thaliana]	*-7,23*
bf_mxflxxxx_0018e09.t3m.scf	MYB transcription factor [Hevea brasiliensis]	*-7,29*
bf_arrayxxx_0084b12.t3m.scf	No Hits Found	*-7,34*
bf_mxflxxxx_0038h01.t3m.scf	No Hits Found	*-7,56*
MICRO.1229.C1	phantastica [Lycopersicon esculentum]	*-7,78*
MICRO.15271.C2	No Hits Found	*-8,06*
MICRO.1238.C4	Oxygen-evolving enhancer protein 1, chloroplast precursor (OEE1) (33 kDa subunit of oxygen evolving system of photosystem II) (OEC 33 kDa subunit)	*-8,23*
MICRO.4191.C1	leucine-rich repeat protein (LRR1) [Nicotiana tabacum]	*-8,38*
MICRO.8678.C1	sieve element occlusion b [Solanum phureja]	*-8,46*
MICRO.11716.C1	EEF48 [Solanum melongena]	*-8,79*
STMHG75TV	No Hits Found	*-8,81*
MICRO.3065.C1	fis1 [Linum usitatissimum] sp_Q40255_DHAL_LINUS Probable aldehyde dehydrogenase (Flax inducible sequence 1)	*-9,02*
MICRO.10754.C1	No Hits Found	*-9,42*
MICRO.9812.C1	glutathione S-transferase [Cucurbita maxima]	*-10,03*
SDBN002O14u.scf	ATP citrate lyase b-subunit [Lupinus albus]	*-13,88*

Genes significantly up- (**in boldface**) or down-regulated (*in italics*) during the late stage of symbiotic establishment of AM fungus in the potato roots versus control comparison are ordered according to the expression fold change. Genes were considered as differentially regulated if P-values ≤ 0.01 and the values of fold change compared with the controls was ≥ 3.00 or ≤ -3.00