Università degli Studi di Torino
DIPARTIMENTO di SCIENZE DELLA VITA E
BIOLOGIA DEI SISTEMI

Scientific report on the research activity developed during the project

# "REPROGR_AM - Recruitment of preexisting cell programs in arbuscular mycorrhizas"

Progetti di Ricerca di Ateneo / CSP 2012

Principal Investigator:

Andrea Genre

# Introduction

Arbuscular mycorrhizas (AMs), the most spread mutualistic symbiosis of terrestrial plants, are considered to be essential for optimal plant growth, especially in nutrient-poor soils. The AM symbiosis enhances the capacity of the root to scavenge water and mineral nutrients from the rhizosphere, while providing the fungus with photosynthates that are essential for the completion of its life cycle. The improvement of the plant overall fitness, its enhanced resistance to biotic and abiotic stresses and the increase in crop production, give these beneficial interaction a high relevance in agro-environmental applications, with the consequent economic and social impact.

The first known fossil record of AM fungi dates from the lower Ordovician, around 460 million years ago. This is the period when the first plants appeared on the dry lands, strongly suggesting that AM fungi - with their high efficiency in water and mineral uptake from the soil - have played a crucial role in supporting the plant exit from their original water environment (Bonfante and Genre, 2008). At present, about 80% of the plant species live in symbiosis with AM fungi, which all belong to the phylum Glomeromycota (Schüßler et al., 2001).

AM symbiosis development can be divided in two fundamental steps: presymbiotic phase and symbiotic phase (Gutjahr and Parniske, 2013).

In the earliest stages of the interaction, the presymbiotic phase, a chemical dialogue mediates reciprocal recognition between the two symbionts. During this phase the plant roots exudate strigolactones, plant hormones that were found to be responsible for the induction of hyphal branching and alterations in fungal physiology (Akiyama et al., 2005; Besserer et al., 2006). On the other hand, the perception of fungal secreted signals triggers repetitive oscillations (spiking) in nuclear calcium concentration, a central element in the so-called common SYM pathway that, in legumes, controls the development of both AM and nitrogen-fixing root nodules. In the model legume Medicago truncatula, the SYM pathway includes a leucine-rich repeat (LRR) receptor-like kinase (MtDMI2) and a putative cation channel (MtDMI1) located in the nuclear membrane. The third shared component (MtDMI3) is a nuclear-localized calcium and calmodulin-dependent kinase (CcaMK) which is believed to decipher the oscillating calcium signal, leading to the activation of a regulatory cascade and specific gene transcription. Rhizobia are identified by the host plant through the perception of secreted lipochito-oligosaccharides (LCOs) known as "Nod factors" (Dénarié and Cullimore, 1993) while the recognition of AM fungi is based on undecorated, short-chain chito-oligosaccharides such as chito-tetraose (CO4) as well as Nod factor-like LCOs present in the germinated spore exudates (Genre et al., 2013; Maillet et al., 2011). The calcium spiking responses present different patterns in response to Nod and Myc factors and these differences fall within the so-called calcium signature, according to which an organism discriminates different calcium generated signals (Charpentier and Oldroyd, 2013).

After reciprocal recognition, AM fungi form specialized hyphae, called hyphopodia, on the host root epidermis, marking the initiation of the symbiotic phase. In response to hyphopodium contact, epidermal cells produce a transient intracellular aggregate named the prepenetration apparatus (PPA), which drives fungal colonization and coordinates the biogenesis of the so-called interface compartment where the intracellular hyphae are hosted (Genre et al., 2005). In fact, fungal accommodation involves a proliferation of the host plasma membrane that invaginates around the hyphae, thus creating a specialized apoplastic space. Fungal intracellular accommodation in AM represents an important paradigm for plant biotrophic interactions, since it is the most intimate and extensive example of mutualistic intracellular biotrophy between two eukaryots.

In vivo confocal imaging studies have revealed that the PPA is rich in cytoskeleton, organelles, and secretion-related endomembranes, including the endoplasmic reticulum, the Golgi apparatus and secretory vesicles (Genre et al., 2005; 2008; 2012). PPA development also involves complex nuclear dynamics, including (prior to arbuscule formation) the migration of the nucleus at the centre of the cell and changes in its size and internal organisation (Genre et al., 2008).

Prepenetration responses are replicated in cortical cells extending the symbiotic interface until the fungus reaches the inner cortex where it forms arbuscules, the highly branched structures that are considered as the main site of nutrient exchange between the symbiotic partners.
PPA observations have highlighted the presence of a few traits of the PPA response which show striking similarities with other basic cell processes. The organization of the secretory membranous compartments, made of a vesicular-tubular structure, closely recalls the pattern observed during cell plate deposition at the end of cell division. In addition to this ultrastructural clue, several morphological and functional analogies can be drawn between PPA assembly and cell division events. Both processes directly involve the whole exocytotic machinery: endoplasmic reticulum, Golgi apparatus, secretory vesicles and cytoskeleton. In both cases de novo cell wall deposition occurs within the cell lumen rather than reinforcing the pre-existing wall. Furthermore, when a differentiated cell undergoes mitosis, a specialized structure is organized, called the phragmosome. Phragmosome formation involves nuclear repositioning and the appearance of broad transvacuolar cytoplasmic strands, which precede mitosis and cell wall deposition within the cell lumen. Similarly, the differentiated cells that are the target of fungal colonization, develop broad cytoplasmic strands, and build a new cell wall compartment within their cell lumen. The thin apoplastic space separating the fungal cell wall from the host perifungal membrane is, in fact, made of amorphous cell wall components of plant origin and very similar in composition to the cell plate (Balestrini & Bonfante, 2005). In addition, several plant genes involved in cell wall synthesis are known to be upregulated in mycorrhizal roots. Events of DNA duplication leading to polyploidy have been reported in mycorrhizal roots (Berta et al., 2000; Fusconi et al., 2005). Changes in the degree of endopolyploidy were strongly correlated with the proportion of root length colonized by arbuscules, across many angiosperms group and this is likely linked to increased metabolism and protein production (Bainard et al.,2011). Nuclear enlargement has also been observed in inner cortical cells containing the pre-penetration apparatus, suggesting endoreduplication is part of pre-invasion cell preparation (Genre et al. 2005, 2008) and could be explained with an early arrest of the cell division programme due to its redirection towards the symbiotic response.
The PPA response also presents a few traits that are characteristic of processes involving cell polarization. A classical example of such polarized responses is apical growth, where focal accumulation of organelles is associated with the local secretion of cell wall components, as opposed to the diffuse cell wall deposition that characterizes the majority of plant cells. Polarization and localized exocytosis are typical features of growing root hairs and pollen tubes. A central actor of these processes is the nucleus, which is always in the vicinity of the growing tip, and an accumulation of cytoplasm, rich in organelles, cytoskeleton and secretory elements, is present in the tip area, feeding the growing cell wall and membrane with new materials. In the case of the PPA, the initial nuclear movement at the future penetration site also suggests the onset of a mechanism of cell polarization.
Cell polarization is also well known to be involved in polar auxin fluxes across the plant tissues. Auxin is not simply transported in a polar fashion, but is itself inducing cell polarity, in a self-regulatory mechanism. Auxin dependence is in fact being observed in an increasing number of cases where plant cells are polarised, including root hairs and pollen tubes.

## Aims of the project

The objectives of the research activity funded by the REPROGR_AM project were both scientific and professional:
Verify the hypothesis that preexisting exocytotic processes related to cell division or polar growth could be recruited during AM development:
- extend the collection of fluorescent constructs and obtain root organ cultures expressing fluorescent constructs for proteins involved in cell division and cell polarization
- apply a confocal in vivo imaging approach to study the localization and role of proteins involved in cell division, cell ploidy and cell polarization processes during AM colonization;
- analyze gene expression levels for the same selected markers of cell division during AM symbiosis.

- compare the proteome of AM-inoculated and non-inoculated root segments, to highlight the presence of the same cell division/cell polarity markers as well as other unpredicted proteins of interest.
Maintain and extend the network of international collaborations between the PI and other European research groups
Expand my research group and increase my professional independence
Participate in an ERC call

# 1 Results

1.1 FLUORESCENT CONSTRUCT COLLECTION

During the first months of the project we have extended our collection of fluorescent protein constructs, which were obtained by contacting different researchers in Europe and the US. We have introduced these constructs in Agrobacterium rhizogenes through electroporation and then expressed them in our model plants D.carota (Becard & Fortin, 1988) and M. truncatula (modified from Stougaard, 1995).
These constructs tag specific marker proteins involved in cell division or polar growth processes, and have been used to study the sub-cellular localization of these proteins during AM colonization, to highlight the recruitment of such basic cell mechanisms in symbiosis development and prepenetration responses related to interface biogenesis.

| | | |
|---|---|---|
| Cell polarization and apical growth | | |
| PIP5K3-YFP | key regulator of the machinery that initiates and promotes root hair tip growth | Kusano et al., 2008 |
| RopGEF-GFP | guanine nucleotide exchange factor that activates Rop GTPases to affect tip growth | Riely et al., 2011 |
| DR5rev-GFP | synthetic auxin-sensitive promoter, used as a reporter of auxin accumulation | Liu et al.,2012 |
| DNA duplication and chromatin structure | | |
| T9-GFP | unique marker that associates to centromeric regions of chromatin | Van Damme et al., 2004 |
| H2B-YFP | H2B histone fusion with the yellow fluorescent protein | Boisnard-Lorig et al., 2001 |
| H2B-mEos | photoconvertible tag for the H2B histone allowing the detection of novel DNA synthesis | Wozny et al., 2012 |
| Cell division-related processes | | |
| Tplate-GFP | protein involved in anchoring the cell plate to the parental plasma membrane | Van Damme et al., 2011 |
| Knolle-GFP | protein of the syntaxin family involved in cell plate assembly | Boutté et al., 2010 |
| Tangled-GFP | member of a plant-specific family required for cell plate fusion with the cell wall | Walker et al., 2007 |
| Keule-GFP | Sec1 regulator of vesicle trafficking that binds KNOLLE | Reichardt et al., 2007 |

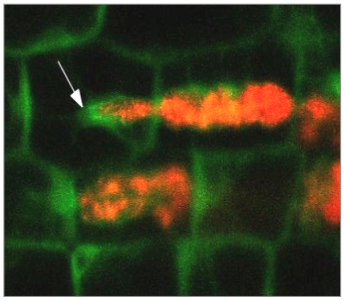
## 1.2 In vivo imaging

For most of the constructs that we have expressed in transgenic root cultures we have also performed confocal in vivo localization analyses during the development of the symbiosis.
These experiments yielded very original results that were of primary importance for the application for an ERC Consolidator Grant (see annex II) and have been presented at international meetings and included in a manuscript currently under preparation. These observations were particularly interesting for Tplate-GFP lines, which are the focus of the next paragraph.

### 1.2.1 CELL DIVISION: TPLATE IS INVOLVED IN AM DEVELOPMENT

Tplate is a plant-specific protein involved in the final steps of cytokinesis, with similarity to the Adaptin/Coatomer proteins. Tplate is recruited at the cortical division zone during anchoring of the cell plate with the parental plasma membrane (Van Damme et al., 2011).

Our in vivo observations in confocal microscopy confirmed the correct localization of Tplate-GFP in the meristem of D.carota, where it labels cell plates and new cell walls. We then colonized these roots with Gigaspora gigantea and observed them during different steps of the colonization process.

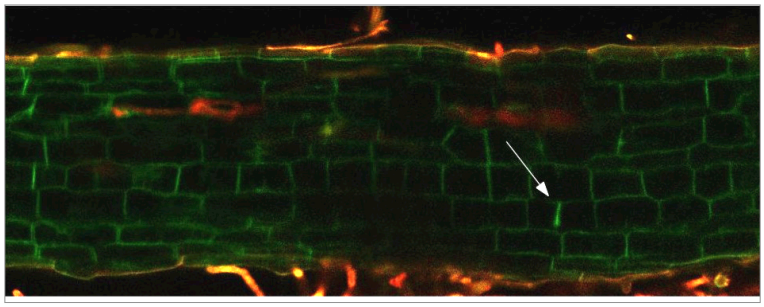


In short, our results strongly suggest that Tplate is involved in interface biogenesis in both epidermal and cortical cells. We could observe a clear labeling of the PPA and surrounding the growing hyphal tip, as shown in Fig.1.

Fig.1: Tplate-GFP labeling (green, arrows) surrounding the growing hypha of G.gigantea (red) during AM colonization in D.carota hairy roots.

Unexpectedly, TPLATE labeling also highlighted the occurrence of ectopic cell divisions in the root cortex in the vicinity of the colonized area. A developing cell plate is visible in Fig.2.

Fig.2: Tplate-GFP labeling highlights cortical cell divisions during AM colonization in D.carota hairy roots. (a) A labeled cell plate is evident in the inner cortical layer (white arrow). (b) 24 hours later, four cells have already divided in the same area (white arrow).



In fact cell divisions are already induced at earlier stages of colonization, before arbuscule formation: colonized roots display a larger number of cells that are wider than long, whereas control roots show a majority of long and narrow cortical cells as shown in Table 1. We interpret this as the effect of cortical cell division induction during AM colonization.

| Control roots | | |
|---|---|---|
| N. of cells | | 103 |
| Longer than wide | 86,41 | 89 |
| Wider than longer | 9,71 | 10 |
| Square cells | 3,88 | 4 |
| Colonized roots | | |
| N. of cells | | 108 |
| Longer than wide | 49,07 | 53 |
| Wider than longer | 47,22 | 51 |
| Square cells | 3,7 | 4 |

Table 1: Measurement of cell dimensions in control and colonized roots of D.carota. The percentage of cells that are wider than long in significantly higher in mycorrhizal roots (pvalue=1.875e-09).

### 1.2.2 TPLATE GENE EXPRESSION ANALYSES

In support of our microscopical observations, we analyzed TPLATE gene expression through quantitative RT-PCR to monitor its regulation in AM symbiosis.

The experiment was done on M.truncatula roots deprived of root apexes, to avoid the overlap between TPLATE expression in root meristems and in colonized area.

In a first experiment TPLATE was more than 3 fold upregulated in mycorrhizal roots compared to controls, although we only analyzed two independent replicates (Fig. 3). These results, although preliminary, directly supported the cellular analyses. TPLATE expression during AM colonization of the root can be related to

both the observed phenomena of protein recruitment at the developing perifungal membrane and cortical cell division reactivation.

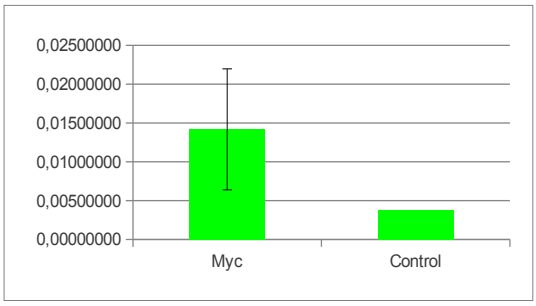


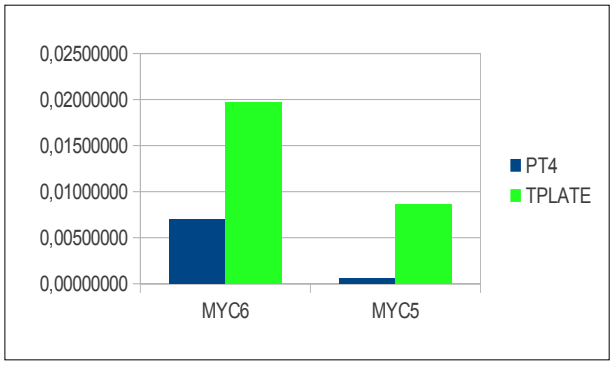

Fig.3: TPLATE gene expression analysis through quantitative RT-PCR. The relative expression levels (2ΔCt) after normalization to MtEf1 transcript levels are shown. Values are means of two biological replicates.

Later experiments, based on a larger set of replicates, confirmed this trend, although a very high degree of variability generated high standard deviations that made the observed differences statistically not significant.

To better characterize TPLATE expression patterns, we compared the expression levels of this gene with those of the MtPT4, an inducible phosphate transporter considered a marker of active arbusculated cells in Medicago (Javot et al., 2007).

We found a strong correlation in the expression levels of the two genes (Fig.4). This suggests that the expression of TPLATE correlates with the intensity of mycorrhizal colonization.

Fig.4: TPLATE and PT4 gene expression analysis through quantitative RT-PCR. Relative expression levels were measured using gene-specific primers and cDNA roots of M.truncatula colonized by Glomus intraradices (Myc) in two independent replicates (MYC6 and MYC5). All plants were harvested 2 months after inoculation with Glomus intraradices. The relative expression levels (2ΔCt) after normalization to MtEf1 transcript levels (Wulf et al., 2003) are shown.




## 1.2.3 MEDICAGO TRUNCATULA TPLATE PROMOTER ISOLATION THROUG TAIL-PCR

This encouraging result stimulated the development of more targeted analyses: we are currently developing a GUS assay to visualize the activity of TPLATE promoter in situ, during the development of the symbiosis.

In order to express a TPLATE:GUS construct in the model plant Medicago truncatula we tried to isolate the promoter sequence of the MtTPLATE gene (which is not present in M. truncatula genomic sequences yet).

We first of all performed a BLAST search to identify a nucleotide sequence on M. truncatula genome showing good similarity with the Arabidopsis thaliana TPLATE gene sequence and found a region of interest on Mt chromosome 7 (MTR_7g031450).

We then used the TAIL-PCR technique to recover DNA fragments adjacent to the known gene sequence. Two arbitrary degenerate primers AD1 and AD2 were used in combination with specific primers designed on the nucleotide sequence of MtTPLATE gene (Table 2), and three consecutive PCR reactions were carried out using a Phusion Taq DNA polymerase. The amplified fragments were run on a 1.2% agarose gel (Fig.6A), purified and then cloned into the pCR2.1-TOPO vector (TOPO TA cloning, Invitrogen) and the recombinant plasmids were transformed into TOP10-E. coli (Invitrogen) using electroporation. Positive clones were sequenced and resulting sequences (~800bp) were used to design new specific primers and carry on a second round of TAIL-PCR reactions with the same thermal protocol in order to isolate at least 2000 nucleotide to recover the full MtTPLATE promoter.

While the first round of TAIL-PCR proceeded smoothly, the second round generated a number of false positives. We decided to design a GUS construct containing the available promoter sequence (circa 800bp), introduced it in A. rhizogenes and generated transformed roots in M. truncatula. Unfortunately, the resulting

roots did not show any GUS reaction in the root meristems, indicating that the partial promoter sequence was not sufficient to drive GUS transcription.
Eventually we chose to switch to the model legume Lotus japonicus, whose genome is completely sequenced and where the design of specific primer pairs for the amplification and cloning of the whole T-PLATE promoter is more straightforward.
Indeed, the full T-PLATE promoter has recently been isolated and cloned, and we are currently running a transformation experiment in both L. japonicus and M. truncatula. The results of these experiments are expected to clarify the local upregulation of T-PLATE in the few root cells that respond to fungal colonization by activating cell division (as observed in T-PLATE-GFP lines).

### 1.2.4 CELL PLOYDY: H2BmEos PHOTO-CONVERTIBLE CONSTRUCT

To better understand the occurrence of endoreduplication events during the development of AM symbiosis we selected a photo-convertible tag that binds histones and allows the detection of novel DNA synthesis. The construct H2BmEos (Wozny et al., 2012), kindly provided to our laboratory by Dr. Jaideep Mathur (University of Guelph, Canada) encodes a chimeric protein generated from the fusion of the Arabidopsis thaliana histone H2B with the photoconvertible fluorescent protein mEos. The latter emits green fluorescence when excited with blue light, but when it is exposed for few seconds to UV light, changes its wavelength of emission from green to red. H2BmEos can therefore be used to highlight the events of DNA endoreduplication: in fact, the chimeric protein replaces the H2B native histones, and accumulates in the nucleus in an amount proportional to the amount of DNA. After the photo-conversion of a group of cells to the red fluorescence, the onset of endoreduplication events can be highlighted through the re-emergence of proteins with green fluorescence, due to the incorporation of newly synthesized proteins in the nocleosomes. The combined occurrence of green and red emitting fluorescent proteins give a yellow fluorescence in the nuclei that have duplicated their DNA.
We have successfully expressed H2BmEos in Daucus carota root organ cultures and developed an efficient photoconversion protocol, derived from the ones available in literature.

### 1.2.5. CELL POLARITY: LOCALIZATION OF GFP MARKERS DURING AM COLONIZATION

GFP fusions for two unrelated cell polarity markers were succesfully expressed in D. carota: ROPGEF2-GFP and PIP5K3-YFP. Control observations confirmed the localization of both constructs at the tip of growing root hairs, while prolonged investigation of roots colonizaed by G. gigantea never showed any fluorescent signal in association with intraradical fungal structures. Albeit further confirmations will have to be searched for, these results seem to exclude the involvement of cell polarization processes during fungal accommodation by the host root.

## 1.3. PROTEOMIC ANALYSES

Our goal was to identify proteins localized in the PPA assembly and in the proliferating perifungal membrane. The project was technically challenging, since AM fungi colonize only part of the root system and, in addition, PPAs are short-lived structures that independently develop in single cells within the colonized regions; these cells inevitably account for a small percentage of the root material at any given sampling time. As a consequence, trying to purify such rare proteins from whole root extractions would lead to excessive dilution. We used different experimental setups to addresses this problem. The first approach, consisted in inoculating root organ cultures growing in petri dishes and sampling short root segments that were contacted by the fungus. We had already successfully applied this procedure for RNA extraction from root organ cultures (ROC), with the successful identification of PPA-related transcripts. The second approach was based on an experimental setup aimed at synchronizing spore germination and intensifying root colonization. The last approach used Millipore filters to restrict hyphal and root growth to a thin pocket, where the frequency of infection units is increased. In all cases we harvested samples of short root segments where fungal adhesion structures were detected on the epidermis; apexes were removed and control samples were collected from non inoculated plants.

After one year of sample collection we harvested: ~1g of root material from targeted inoculation experiments, ~3g of root material from filter experiments and ~200mg of root material from sandwich assemblies. Since previous studies have highlighted the accumulation of exocytotic membranes in the PPA we expected that most of the interesting targets should be membrane proteins. To this aim, the protocol developed by Dr. Chiapello in maize and rice was applied to Medicago truncatula.
Proteins were extracted from each sample, enriched for membrane proteins, purified and digested through filter aided sample preparation (FASP) method.
Protein sequencing and characterization was approved by the consortium Prime-XS that provides European Union funded access to an infrastructure of state-of-the-art proteomics technology to the biological and biomedical research community in Europe (http://www.primexs.eu). In our case the analyses were done in ETH Zurich (CH).
We received the first proteomic data in December 2014, and a new set of samples has been sent to ETH for analysis, whose results are expected in the coming weeks.
Samples included control (MOCK) and treated (MYC) samples for the three test experiments, as presentd in the following table:

| Name | Sample | Group1 | Group2 | concentration | Volume ul | total ug |
|---|---|---|---|---|---|---|
| 20141017_MPM_03 | Filter | A | mock | 1.01 | 400 | 162 |
| 20141017_APM_04 | Filter | A | myc | 0.6 | 400 | 96 |
| 20141021_MPM_03 | Contact | B | mock | 0.3 | 400 | 48 |
| 20141021_APM_04 | Contact | B | myc | 0.43 | 400 | 69 |
| 20141024_MPM_05 | Sandwich | C | mock | 0.2 | 400 | 32 |
| 20141024_APM_06 | Sandwich | C | myc | 0.18 | 400 | 29 |

One first interesting data is that the peptide quantification nicely mirror the starting material amount. Each sample has been split in 6 fractions, using a HILIC column, before being analyzed by Orbitrap Velos mass spectrometer. Protein identification and quantification, using the intensity precursor ion label-free approach, was carried out in the Functional Genomic Center of Zurich.

### 1.4.1 STATISTICAL SAMPLE ANALYSIS

In total we identified 4093 proteins, using at least 2 peptides and keeping the FDR below 1%. Figure 9 reports the sample distribution.
The number of proteins identified in the different samples are reported below.

| Condition | MOCK | MYC | UNION | JOIN | Qualitative reproducibility | Quantitative correlation |
|---|---|---|---|---|---|---|
| *Contact* | 4078 | 4084 | 4087 | 4075 | 99.7% | 0.883 |
| *Filter* | 4053 | 4057 | 4082 | 4028 | 98.7% | 0.661 |
| *Sandwich* | 4070 | 4055 | 4091 | 4034 | 98.6% | 0.726 |

Numbers for reproducibility and correlation are reported. Qualitative reproducibility: reflects the reproducibility between the mock and myc condition in terms of presence/absence. Quantitative reproducibility: reflects the reproducibility between the mock and myc condition in terms of protein quantifications.

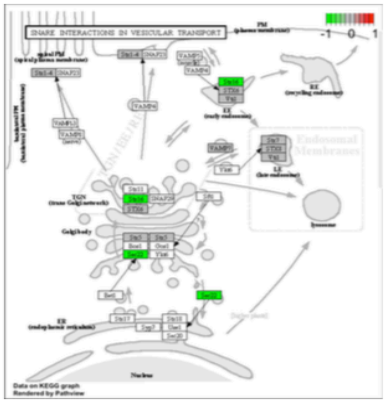
The number of proteins identified in all samples is very similar due to the fact that we used the same amount of protein concentration to allow the protein quantification. Qualitative and quantitative reproducibility shows that even if we identified the same proteins in MOCK and MYC conditions they have a different expression level. As expected we do not have in this phase a massive synthesis of new proteins, but a regulation of constitutive proteins.

### 1.4.2. PRINCIPAL COMPONENT ANALYSIS AND CLUSTER ANALYSIS

In order to understand how samples correlate, we did a simple PCA. PCA reveals that the 2 conditions (MOCK – MYC) of each experiment are more similar than the different treatments across the experiments. Cluster analysis revealed a few interesting trends to further investigate, like cluster14: very high protein expression in both conditions, cluster3 and cluster7: show very similar protein expression, slightly higher in MYC. ANOVA test revealed that only 63 proteins were differentially regulated between MOCK and MYC considering all the experiments.

### 1.4.3. INTRA-EXPERIMENT ANALYSIS

*Target inoculation analysis* - Even if the correlation between MOCK and MYC samples is 0.88, the analysis of variance reveals statistical significant differences in proteins quantification.
Two lists have been produced: one with 509 proteins up regulated in MYC condition and a second one with 580 proteins down regulated. We considered regulated proteins with log2 fold change of at least 1.
*Filter analysis* - Analysis of variance revealed statistical significant differences in proteins quantification. Two lists have been produced: one with 1248 proteins up regulated in MYC condition and a second one with 1030 proteins down regulated.
*Sandwich analysis* - Analysis of variance reveals statistical significant differences in proteins quantification. Two lists have been produced: one with 861 proteins up regulated in MYC condition and a second one with 974 proteins down regulated.



Using Blast2GO software and Fischer's test we characterized which biological processes and molecular function are up- or down-regulated in target inoculated samples. The biological analyses are still in progress in this moment and the new set of data will provide essential information under this respect.

Figure shows two interesting pathways: "SNARE interaction in vesicular transport" and " Plant-Pathogen interaction". Green represents the MYC upregulated proteins, while red downregulated proteins.

Despite the fact that the analyses have been not yet completely carried out, the statistical analysis already revealed some interesting information for the project aims. We identified an impressive number of proteins considering that we started from grams or milligrams of plant root pieces and we enriched from membrane proteins, excluding from the analysis the cytosolic proteins.

## 2 International collaborations

In this period I have maintained a long established and fruitful collaboration with the group headed by **Dr David Barker at LIPM (CNRS/INRA), in Toulouse (France)**, which was further formalized in a **Galileo grant** by the Université Franco-Italienne, covering the costs for short visits between the two groups. More recently, a joint application for a **PICS grant** from the CNRS has been submitted and is currently under evaluation.
I have also established new collaborations. One with the group of **Prof. Jens Stougaard, at the University of Aarhus, Denmark**: I was selected for an **EMBO short-term fellowship** that covered the costs for a two-month visit to the lab in Aarhus, where he conducted cellular analyses. Within the same collaboration, a PhD student from the Danish lab came visiting our group in March-April 2014, thanks to another EMBO fellowship, and another member of the staff in Aarhus came to Turin in July 2014.
Another collaboration was established with **Dr. Ulrike Mathesius, at ANU University in Canberra, Australia**. My PhD student, Dr Giulia Russo visited her lab for 7 months from October 2013 to April 2014, also supported by a **CIB fellowship**.

## 3 Expanding the research team

Thanks to the REPROGR_AM grant I was able to **expand my research group** to include Dr. Marco Chiapello as a Post-doc researcher. Dr. Chiapello is a very talented young scientist who gave a major contribution in the molecular analyses performed so far and in the undergoing development of the GUS constructs.
He gave a fundamental help to my PhD student and myself and applied to our samples a novel protein extraction protocol that he has developed over the last few years. its efficiency was extremely high and we expect the conclusion of the bioinformatics analyses to provide direct evidence for the involvement of our candidate cell division markers in AM presymbiotic cellular responses.

## 4 Result presentation

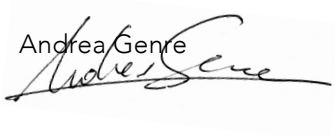
The results obtained by the REPROGR_AM project were presented in the following forms:
- PhD Thesis by Dott. Giulia Russo (discussed June 26 2015): Presymbiotic signalling and cooptation of basic exocytotic processes in arbuscular mycorrhizas
- Presentations at international meetings:
    - 11th Fungal Biology Conference, karlsruhe Germany September 30 - October 2 2013
    - 18th International Microscopy Congress, Prague Czeck Republic September 7-12 2014
    - 2nd Adam Kondorosi Symposium: Frontiers in Legume Biology, Gif-sur-Yvette France December 11-12 2014
    - 8th Congress of the International Symbiosis Society, Lisbon Portugal July 12-18 2015
    - 2nd International Molecular Mycorrhiza Meeting, Cambridge UK September 2-5 2015
    - Spotlight on microbiology, Marnurg Germany September 16 2015
- Two manuscripts are in preparation

## 5 Views and perspectives

The REPROGR_AM grant has been an essential step towards the application for an **ERC Consolidator Grant** (Annex II). The proposal **passed the first selection step** (September 2014) and was only rejected after the **Panel Interview** in Brussels (December 2014).
As the attached proposal shows (Annex II), the DReAM project represents an update and the natural evolution of the REPROGR_AM project: the preliminary results obtained so far have been invaluable in directing the research lines of the DReAM project. Furthermore, the presence of Dr. Chiapello in the team allowed me to introduce two new research lines based on proteomics analyses, that were not included in the original REPROGR_AM project.
REPROGR_AM allowed me to expand my **network of international contacts** and collaborations, which is an important prerequisite for competing for a EU grant. Being the PI in charge of the REPROGR_AM project surely increased my chances of being selected for the **EMBO fellowship** that funded my stay in Denmark last year and contributed to the acceptance of my PhD student in Dr. Mathesius lab team, in ANU, one of the most prestigious universities in the world. Such collaborations are going to be the foundation of my research for the coming years. Furthermore, thanks to the Unito/CSP grant, I obtained a good degree of **independence** in my research work, having the possibility to hire a young scientist and cover the costs of my own small – but growing – research group.
Altogether, this experience has been **extremely positive** under any respect and I want to take this opportunity for **thanking the Compagnia di San Paolo for its support to local research in such a critical period**. I believe the format of the 2012 grant application was very efficient in pushing the grantees into the competition for EU funds, and the layout of the project proposal – largely replicating the form of European grant applications – was of great help when I started writing the ERC application.

Torino, 29.09.2015

Andrea Genre



## Annex I - References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# Annex II - ERC Consolidator Grant project proposal

ERC-CoG-2014 Proposal n° 648376 submitted on: *20/5/2014*