ORGANIZATION

Local Coordination:

EU Marie Curie Chair, ICAAM, University of Évora, Alentejo, Portugal

Responsible Coordinator: **Birgit Arnholdt-Schmitt** www.eu_chair.uevora.pt

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COST 870 Coordinators - **Jacqueline Baar - Yoram Kapulnik**
http://www.cost870.eu/

Efficient Plant – AMF interaction

Improving Plant Breeding Initiatives and AMF-Application by Hypothesis-driven Discussions across Disciplines

The meeting aims to advance strategies for plant breeding and commercial applications of arbuscular mycorrhizal fungi (AMF) that enable a higher profit of the interaction between natural and/or commercial AMF in plant nurseries or agricultural field production. To drive a focused discussion, **hypotheses were developed for the meeting that will have to be considered by all oral and poster presentations**. The hypotheses were developed by taking into the account the current state of the scientific knowledge and the actual discussion of the practical problems that need to be faced to gain the benefit from applying AMF. **AMF application aims to improve the robustness of plants to tolerant diverse forms of abiotic and biotic stresses along their life time in view of stable yield production.** Plant breeding on the efficient use of AMF and AMF application is thus a complex approach that has to consider the flexibility of interactions between the diverse biotic and abiotic systems in the environment. This includes not only plant – environment interactions, but also the interaction of the Stakeholders in the field of commercial AMF application. However, **crucial knowledge and steps are getting visible that are restricting at this stage further progress. The meeting tries to open the floor for a focused and relaxed discussion platform.** This will include one day of lectures and discussion on the breeding topics: A) Genotype Differences for efficient plant – AMF interaction; B) Understanding critical events in plant – physiology and C) Functional marker gene approaches followed by a round-table discussion on all topics of Session 1 on the next morning. A second day is dedicated to the highly challenging question how the scientific knowledge can be brought to the fields and how interaction between the involved commercials, farmers and scientists can be improved. **Speakers from all areas are invited to contribute with their experiences and knowledge in science and technologies.** The number of participants is restricted to around 30 to 40.

PROGRAM

3rd to the 4th of July 2010

Workshop
Morphological identification of Arbuscular Mycorrhiza Fungi (AMF) spores
Fritz Oehl, Switzerland and Janusz Blaszkowski, Poland

4th July 2010

(the workshop will end at 4h00 pm)

Get-Together and Evening Lecture in an informal atmosphere:

19h30 Dinner and Traditional Music ('Cantares de Évora') from Alentejo, Portugal

Evening Lecture:

'Challenges for the COST870 meeting in Évora, Portugal, July 2010'

Birgit Arnholdt-Schmitt, Portugal

5th July 2010

SESSION 1: 'Breeding for efficient plant-AMF interaction has a chance?'

A: Genotype differences for efficient plant-AMF interaction – Experiences and Methodologies

Hypothesis: There is genetic variability for efficient plant-AMF interaction in view of yield stability

Chair: Meriel Jones, UK

9h00 – 10h00 **Keynote lecture:**
Ruairidh J.H. Sawers, Switzerland
Characterising plant genetic variability in arbuscular mycorrhizal interactions

10h00 – 10h15 Coffee Break

10h15 – 11h15 **Invited co-lecture:**
Asheesh Singh and Chantal Hamel, Canada
Development of Wheat Cultivars with the Arbuscular Mycorrhizal Symbiosis in Canada

11h15 – 11h35 Isabel Brito, Portugal
Level of AM colonization of two wheat varieties (Triticum aestivum) under different cropping systems

11h35 – 11h55 Carolin Schneider, Germany
Mycorrhiza formation in 88 Kalanchoe blossfeldiana *Poelln. Cultivars*

11h55 – 12h15 Sabine Ravnskov, Denmark
Differential effects of arbuscular mycorrhizal fungi on nutrient uptake, growth and health of varieties of cucumber

12h15 – 12h35 Sandra Varga, Finland
Host sexual and genotypic differences in determining AMF benefit

12h35 – 12h55 Jacqueline Baar, The Netherlands
Application of mycorrhizal fungi from different perspectives

13h00 – 14h30 Lunch

B: Understanding the plant - Physiological processes responsible for efficient plant-AMF interaction in view of yield stability

Hypothesis: There are critical physiological processes that determine efficient plant-AMF interaction during plant development in view of yield stability

Chair: Yoram Kapulnik, Israel

14h30 – 15h15 **Invited lecture:**
Hinanit Koltai, Israel
Effects on plant-mycorrhiza symbiosis in a tomato strigolactones-impaired mutant which is deficient in strigolactones synthesis

15h15 – 15h35 **Michail Orfanoudakis, Greece**
Induced changes on plant root system architecture and root hair frequency after inoculation with arbuscular mycorrhiza fungi. Perspectives on the AMF - application in yield stability

15h35 – 15h55 **José Manuel García, Spain**
Regulation of Arbuscular Mycorrhiza development by Abscisic Acid

15h55 – 16h25 Coffee Break

C: Functional gene marker approaches – Reports about crucial genes and pathways

Hypothesis: Functional markers can be identified for efficient plant-AMF interaction in view of yield stability

Chair: Birgit Arnholdt-Schmitt, Portugal

16h25 – 17h10 Invited lecture:
Natalia Requena, Germany
Sugar transport processes during the arbuscular mycorrhizal symbiosis

17h10 - 17h55 **Daniela Sieh, Germany**
The role of microRNA399 in linking phosphate homeostasis and arbuscular mycorrhizal development

17h55 – 18h30 'Flash Poster Presentations' (2-5 min)

Evening: Free arrangements for visits of historical places (e.g. University), dinner and meetings etc

6th July 2010

9h00 – 10h00 Round-table discussion related to Session 1 A/B/C
Chairs: Birgit Arnholdt-Schmitt, Portugal, Meriel Jones, UK

10h00 – 10h15 Coffee Break

10h15 – 12h30 MC Meeting

For non-MC members:
10h15 – 12h30 Discussions at the Posters

12h30 – 14h00 Lunch

From 14h00: Field Trips: Visits to Companies for olive and grapevine production

7th July 2010

SESSION 2: 'From Science to the Field'

A: Interaction of AMF-Inoculum Producers and Appliers – Experiences

Hypothesis: AMF products and transfer of practical knowledge need to be improved

Chair: Carolin Schneider, Germany

8h30 – 9h30 **Keynote lecture:**
Miroslav Vosatka, Czech Republic
Mycorrhiza expectations: successes and failures related to scientific knowledge and commercial product's quality

9h30 – 10h00 **Invited lecture:**
Tammy Kovar, USA
Think Global

10h00 – 10h15 Coffee Break

10h15 – 10h35 **Armelle Gollotte, France**
Bringing together research scientists, inoculum producers and plant producers: a necessary step for developing applications of AM fungi

10h35 – 10h55 **Susan Rafferty, Ireland**
Specific versus subliminal benefits of inoculants - a case study based on strawberry cultivation in protected cropping

10h55 – 11h15 **Rui Oliveira, Portugal**
Applications of mycorrhizal fungi in forestry: from the nursery to the field

11h15 – 11h35 **Mauritz Vestberg, Finland**
Experiences of using commercial AMF inoculum in Finnish nursery production of woody plant species

11h35 – 11h55 Katarzyna Turnau, Poland
Selection of efficient plant genotypes for heavy metal polluted sites

Short Break (5 min)

12h00 – 13h00 Alok Adholeya, India
Mycorrhiza: Soil to soil

13h00 – 14h30 Lunch

B: Interaction of AMF-Inoculum Producers and Researchers – Experiences

Hypothesis: Better knowledge of the products would support scientific validation of AMF application

***Chair: Miroslav Vosatka*, Czech Republic**

14h30 – 15h00 Invited lecture:
Carolin Schneider, Germany
Statements to the hypothesis of the session

15h00 – 15h20 Alan Cassells, Ireland
Proof of concept – a method to screen inoculants for their host priming activity

15h20 – 15h40 Ibrahim Ortas, Turkey
The Effects of Mycorrhiza Inoculations and P Addition on Gramine Species Growth and Nutrient Uptake

15h40 – 16h00 Graziella Berta, Italy
Arbuscular Mycorrhizae affect melon fruit quality under field conditions.

16h00 – 16h30 'Flash Poster Presentations' (2 – 5 min)

16h30 – 17h15 Coffee Break

17h15 – ca. 18h00 Round-table discussion related to SESSION 2 A/B
Chair: Alan Cassells, Ireland

Ca. 18h00 – 19h00: Final Session to focus on conclusions for the future
Chairs: Jacqueline Baar, The Netherlands, Yoram Kapulnik, Israel, Birgit Arnholdt-Schmitt, Portugal

ABSTRACTS

5th July 2010

SESSION 1:

‘Breeding for efficient plant-AMF interaction has a chance?’

A: Genotype differences for efficient plant-AMF interaction

– Experiences and Methodologies –

Hypothesis:

There is genetic variability for efficient plant-AMF interaction in view of yield stability

Characterising plant genetic variability in arbuscular mycorrhizal interactions

Ruairidh J. H. Sawers[1], Barbara Wozniak[1], Mesfin N. Gebreselassie[1], Matthias Mueller[1], David P. Janos[2] and Uta Paszkowski[1]

[1]*Department of Plant Molecular Biology, University of Lausanne, Biophore Building, CH-1015 Lausanne, Switzerland.*
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If we hope to optimize mycorrhizas for agricultural benefit, we require the ability to compare the impact of the symbiosis on the performance of different plant varieties. Furthermore, it is important to distinguish a plant's ability to benefit from the symbiosis from dependence on mycorrhiza; increasing mycorrhiza response by decreasing the ability of plants to grow without the symbiosis is clearly of no agronomic interest. In this presentation, the question of quantifying plant genetic variation in mycorrhiza response will be discussed, with special reference to the role molecular studies may have to play in shaping our expectations of the genetic architecture of this trait. On the basis of this discussion, a simple regression procedure will be presented that aims to eliminate variation linked to differences in mycorrhiza dependence and to allow the selection of plant lines that might harbor beneficial genetic variation. The method will be illustrated with reference to both "classic" and novel data sets describing variation in mycorrhiza response among cereal crop varieties. The results of these analyses suggest that modern cereal crop germplasm harbors significant genetic variation in the ability to benefit from mycorrhizal symbiosis and that, consequently, there is potential for plant breeding to optimize the symbiosis.

Development of Wheat Cultivars with the Arbuscular Mycorrhizal Symbiosis in Canada

Asheesh Singh, Chantal Hamel, Ron DePauw, Ron Knox

Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Box 1030 1 Airport Rd., Swift Current, SK, Canada. S9H 3X2

Most plant roots naturally form a symbiosis with arbuscular mycorrhizal (AM) fungi. These AM associations with plant roots enhance absorption, improving plant nutrient uptake and health. Durum genotypes selected for AM symbiotic effectiveness may use soil nutrients more effectively than current cultivars. Selection for 'symbiotic compatibility traits' requires the presence of variation in wheat genotypes. In two greenhouse experiments we discovered that the roots of different durum wheat genotypes develop AM symbioses to different extents and that durum wheat genotypes vary in their biomass and nutrient uptake in response to the AM symbiosis. Experiments involved five genotypes selected from a collection of durum genotypes from Canada and worldwide and which represent distinct population groups as determined by relationship analysis using simple sequence repeat marker evaluation. These genotypes were inoculated with live *Glomus intraradices* formulated as Myke Pro PS3 or inoculated with sterilized inoculant. The first experiment was conducted under poor soil fertility conditions and the second experiment was conducted under conditions of medium soil fertility. The roots of DT710 developed the most extensive AM colonization. Commander expressed the most extreme difference for root colonization between soils of poor and medium fertility. AC Morse and Mongibello showed the least differences among the two soil fertility experiments. There was no growth response to the AM symbioses under conditions of poor soil fertility, but under conditions of medium soil fertility, Mongibello produced more grain and total biomass. The live mycorrhizal treatment produced higher grain Mn concentration and reduced grain cadmium concentration compared to the non mycorrhizal control. The phenotypic variation observed in these experiments indicated potential for breeding for better AM symbiosis in durum wheat using a conventional approach. The next experiments are targeting multiple strains to find a universally compatible wheat cultivar, association mapping and linkage mapping approaches to identify markers with AMF compatibility genes. Ability to screen for AMF compatibility is essential for quicker selection and integration of this trait in elite germplasm.

Level of AM colonization of two wheat varieties (*Triticum aestivum*) under different cropping systems

Isabel Brito, Mário Carvalho, Luís Alho, Filipa Santos

Universidade de Évora - ICAAM, Apartado 94, 7002 - 554 Évora, Portugal

Wheat is known to be a poor mycotrophic host plant, due in some extent, to the elderly and permanent breeding process is has been through, most of the times irrespectively of mycotrophic traits.

Two varieties, corresponding to different epochs of wheat breeding development were tested for AM development: Pirana and Ardila. Pirana is a variety released on the 50's, with a little response to crop inputs and a low yield potential (3.5 ton/ha). Ardila was developed for resistance or tolerance to stem rust (Puccinia graminis) and Septoria diseases and has a yield potential of 7 to 8 ton/ha.

Pirana and Ardila were both tested in two different cropping situations, which lead to different types and amount of AMF inoculum availability.

Results showed that, irrespectively of the cropping system and therefore the availability and type of AMF inoculum, there was no difference in the AM colonization rate of both varieties, contradicting the wide spread idea that old varieties are more mycotrophic than the modern ones and indicating that the yield potential and tolerance to fungal diseases of a particular wheat variety are not related to its ability to form AM.

Beside the genetic potential, the colonization rate of the wheat will depend on the amount of available AM inoculum in the soil. Under modern cropping systems the later one might be an important limitation. When the cropping system favored the development of an intact extraradical mycelium (ERM) previously to the crop planting, the AM colonization rate of wheat was significantly higher in both varieties when compared to the AM colonization rate observed on plant grown in disturbed soil. If the natural AM inoculum of a soil is low, this difference can be larger.

The presented results lead to the conclusion that yield potential of wheat can be increased by the breeding process without losing mycotrophic capacity. Besides, the potential to form AM can be managed within the cropping system by the use of techniques that promote the development of an intact ERM network enabling a faster AM colonization of the crop, from the beginning of its cycle. Therefore for the breading process it is important to understand if there is some kind of interaction between the type of AM inoculum (spores or intact ERM) and the wheat variety.

Mycorrhiza formation in 88 *Kalanchoe blossfeldiana* Poelln. cultivars

Carolin Schneider[1], Imke Hutter[2] and Falko Feldmann[3]

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Mycorrhizal technology developed in the last few years provides agricultural and horticultural practice with suitable commercial mycorrhizal inoculum for the inoculation of f.e. annual vegetables, ornamentals, perennial herbs, shrubs or trees. New inoculation methods for already established plants and the flexibility of modern inoculum products allow the inclusion of mycorrhizal technology within integrated plant production systems as important biological phytosanitary factors. Unfortunately the variability of host dependency on mycorrhizal fungi varies between cultivars. Because of a lack of suitable breeding markers it is discussed whether the degree of mycorrhizal root colonization and the character "mycorrhizal dependency" should be used as breeding markers. A prerequisite is to collect data about mycorrhiza formation of cultivars during breeding processes.

To investigate the importance of the plant genome, a large number of cultivars of *Kalanchoe blossfeldiana* were tested for variation in colonization levels. In *K. blossfeldiana* we found a range of mycorrhiza formation from 0 to 100% of the root system colonized. We are recently analyzing whether clusters of certain proveniences are more colonized than others. Differences of mycorrhization between these cultivars of *K. blossfeldiana* were not due to the influence of environmental differences or variation of the fungal inoculum. Instead, different degrees of root colonization of the cultivars were apparently induced by breeding.

In the speech ist discussed, if it makes sense to breed for better colonization in breeding programmes. The pre-requisit for an effective symbiosis is of course colonization of the roots. Following our experience, even without a strong relationship between degree of colonization and effectiveness, a threshold value of probably 20-30% root colonization should be realized in cultivars otherwise the plant will not or hardly benefit from the mycorrhizal fungi. The mycorrhizal frequency is normally not correlated with the effectiveness. Therefore, it is recently recommended, not *to breed* for higher colonization but *to take care* that plant genotypes are selected during the breeding process with mycorrhizal formation capacity above this threshold.

Differential effects of arbuscular mycorrhizal fungi on nutrient uptake, growth and health of varieties of cucumber

Sabine Ravnskov and John Larsen,

Dept. of Integrated Pest Management, Faculty of Agricultural Sciences, Aarhus University, Flakkebjerg DK-4200 Slagelse, Denmark

Arbuscular mycorrhiza (AM) is known to increase nutrient uptake especially phosphorus (P) in plants, influence on plant growth and to enhance plant tolerance against pathogens. The function of the symbiosis does, however, depend on the combination of species/subspecies/isolates of the organisms involved in the symbiosis.

The influence of the AM fungi, *Glomus mosseae, G. intraradices* and *G. claroideum* on nutritional status of six cultivars of cucumber, and on tolerance of cucumber *cv* Tiffany against *Pythium ultimum* was investigated. Two experiments were conducted; the objective of the first experiment was to study the effect of the three mycorrhizal fungi on growth and nutrient uptake (N, P, K, Mg, Ca, Na, Fe, Zn, Mn and Cu) of six cucumber varieties (Aminex, Langelandskæmpe, Viking, Armada, Tiffany and Megami).The aim of the second study was to examine the effect of *Pythium ultimum* on cucumber *cv* Tiffany inoculated with the three different AM fungi, respectively. Plants were grown for 4-5 weeks in soil:sand mixture (1:1) with low P content (12 ppm). After harvest plants were analysed for dry weight, AM fungal colonisation and signature fatty acid profiles in roots. In Exp. 1, plants were also analysed for N, P, K, Mg, Ca, Na, Fe, Zn, Mn and Cu.

The six varieties of cucumber had significantly different contents of most of the measured nutrients except Fe (P=0.21), and the three AM fungi influenced uptake of all nutrients except N (P=0.11) and Na (P=0,09).

Furthermore, the three AM fungi had differential effect on growth of cucumber infected with *P. ultimum.*

This work underlines the importance of studies of functional compatibility between plant variety and arbuscular mycorrhizal fungal isolate, and of the selection for optimal functional traits before considering plant breeding for the optimal mycorhizal function in plant production.

Host sexual and genotypic differences in determining AMF benefit

Sandra VARGA

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Arbuscular mycorrhizal benefits gained by plants typically depend upon the specific plant and fungal species involved in the symbiosis. Conversely, the growth response of AM fungal species also depends on the associated host-plant species. It is quite well established that the outcome of this relationship between plants and mycorrhizal fungi is further dependent on the environmental conditions experienced by the two partners. What it is less known, however, is how the gender of the host plant as well as its genotype determine the outcome of the symbiotic relationship.

Many important agronomic plants are sexually dimorphic i.e. having two different types of individuals regarding their sexual expression. Despite its economical importance, relatively little is known about the effects of mycorrhizal symbiosis on host plant reproduction, and this is specially true in sexually dimorphic plants.

Using several greenhouse and common garden experiments, I investigate the relationship between plant sex and mycorrhizal symbiosis using a dioecious (containing female and male individuals within a population) and a gynodioecious (containing female and hermaphrodite individuals within a population) plant species.

Sex-specific mycorrhizal benefits have been observed in important traits such as survival, growth and reproduction. These sex-specific responses to mycorrhizas found are, however, dependent on soil and other plant factors. Evidence suggests that the gender of the host may be important in determining arbuscular mycorrhizal fungal benefits. However, plant genotype seems to be a minor factor in the study species investigated.

B: Understanding the plant
-Physiological processes responsible for efficient plant-AMF interaction in view of yield stability –

Hypothesis:

There are critical physiological processes that determine efficient plant-AMF interaction during plant development in view of yield stability

Effects on plant-mycorrhiza symbiosis in a tomato strigolactones-impaired mutant which is deficient in strigolactones synthesis

Hinanit Koltai, Sivarama P. LekKala, Chaitali Bahattacharya, Einav Mayzlish-Gati, Nathalie Resnick, Smadar Wininger, and Yoram Kapulnik

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Strigolactones are considered a new group of plant hormones. Their role as modulators of plant growth first became evident in Arabidopsis, pea and rice mutants that were flawed in strigolactones production, release or perception. Moreover, strigolactones were shown to be important signals for plant-mycorrhiza interactions. Here we present evidence in tomato (*Solanum lycopersicon*) of strigolactone deficiency. The strigolactone-deficient mutant, *Sl-ORT1* was previously identified as resistant to the parasitic plant *Orobanche*. Biochemical analysis of mutant root extract suggested that it produces only minute amounts of two of the tomato strigolactones: solanacol and didehydro-orobanchol. Accordingly, the transcription level of a key enzyme (CCD7) putatively involved in strigolactones synthesis in tomato was reduced in *Sl-ORT1* in comparison to the wild type (WT). Moreover, *Sl-ORT1* had aberrant symbiosis interaction with the arbuscular mycorrhizal fungus (*Glomus intraradices*): it displayed a reduced ability to induce mycorrhiza hyphal branching. We will discuss these findings as part of our efforts to understanding the plant - physiological processes responsible for efficient plant-AMF interaction. Future prospects as to the enhancement of plant-AMF interaction by strigolactones or their analogous in view of yield stability will be suggested.

Induce changes on plant root system architecture and root hair frequency after inoculation with arbuscular mycorrhiza fungi. Perspectives on the AMF application in yield stability.

Michail Orfanoudakis

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Plant root system is the interaction zone were the arbuscular mycorrhizal fungi and the host plant interact. The effects on plant root system development have been reported in relation with a number of host plants. Changes on shoot :root ratio could have been reported several time as a result of improved nutrition in mineral N, or for plants received supplementary P compared with plants colonised by AMF. Colonisation by AMF could reallocate resources to the root system, in a sophisticate way, causing alterations on the spatial pattern of root branching and significant modifications, including changes to root system to root mortality. Although initially these changes were considered as a result of better nutrition later studies confirmed that root enchantment is a result of AMF symbiosis causing a depression of the mitotic index in apical meristems of AMF-infected roots has been identified as part of the morphogenetic program that, at least in some cases, leads to changes in root morphology . AMF -host interactions could provide benefits beyond those rising from the enchanted nutrition even at low levels of colonisation or at nutrient rich soil environment. AMF could play a critical role on tree species establishment via impacts on root system development. Root hairs are significant anatomical and physiological feature of most plant roots as the a path way of direct plant phosphate absorption, while there is a positive correlation among the available nutrients and number of the root hairs. As it happens with root system architecture root hair development could be controlled to some extend by the soil environment although resent studies identify a group of genes in relation to root hair expression . Although root hair density was associated with nutrient rich environment and with low AMF-host response, recent studies however suggests that root hair density could be a AMF-host response. These changes at the root system are also present in relation to other microorganisms such as Frankia . The effects of AMF on host plant growth, when differ from the benefits rising from the increased nutrition are of a high interest. In particulars changes at the root system could provide the ecological advantage to the plant to compensate harsh growth conditions, particular at the early stages of plant growth.

Regulation of Arbuscular Mycorrhiza development by Abscisic Acid

José Manuel García Garrido[1], José Ángel Martín Rodríguez[1], Rafael Jorge León Morcillo[1], Horst Vierheilig[1], Jutta Ludwig-Müller[2] and Juan Antonio Ocampo Bote[1]

1.-Departamento de Microbiología del suelo y sistemas simbióticos. Estación Experimental del Zaidín, CSIC. Calle Profesor Albareda nº1, 18008 GRANADA, SPAIN. 2.- Institut für Botanik. Technische Universität Dresden. Zellescher Weg 20b 01062 DRESDEN, GERMANY.

The key to understanding the physiological processes responsible for compatibility and efficiency in Arbuscular Mycorrhiza (AM) is to study recognition mechanisms and molecules involved in the AM interaction. In this sense, biochemical and morphogenetic events mediated by plant hormones during AM formation have been suggested and certain roles for phytohormones in ecto- and arbuscular mycorrhiza have been proposed (1). Numerous investigations support the hypothesis that a fine balance between hormones and nutrient availability (phosphorus, carbon or nitrogen) is probably important for the regulation of mycorrhizal formation and functioning. Studies of Abscisic acid (ABA) and AM development have been conducted, mainly in experiments involving measurements of hormone content in AM plants and some contradictory results have been obtained.

Using genetics tools, such as plant mutants defective in hormone production or perception in combination with histochemical and molecular biology techniques for the analysis of AM formation, we studied the mechanism by which ABA determines the susceptibility to AM fungal root colonization. ABA participates in the susceptibility of tomato to infection by AM fungi, and it seems to play an important role in the development of the complete arbuscule and its functionality (2). Microscopic observations and arbuscule quantification showed differences in arbuscule morphology between WT and *sitiens* plants.

The ABA signalling pathway interacts antagonistically with the ethylene signalling pathway and vice versa to modulate plant development. In ours experiments, ethylene perception is crucial to AM regulation and the impairment of mycorrhization in ABA deficient plants is at least partly attributable to ethylene. A double mechanism, dependent an ethylene-independent was suggested for ABA regulation of the AM formation. Altogether, our results indicate different roles for ABA and ethylene during AM formation. ABA affects positively arbuscule formation (determined as percentage of arbuscules in colonized roots) and ethylene mainly regulated the ratio of colonization (determined as mycorrhizal intensity). In accordance whit this, inhibition of ethylene synthesis in ABA-deficient *sitiens* plants specifically increases the intensity of mycorrhiza development whereas ABA application rescues arbuscule abundance in mycorrhizal zones of root.

To identify genes that may play a role in arbuscule functionality we performed transcriptome analysis in response to different mycorrhization status according to the ABA content in the root. Comparative analysis of mycorrhiza up-regulated functional categories revealed significant changes in gene expression associated with the different mycorrhization status according to the ABA content in the roots. The impairment in AM formation in ABA-deficient mutant was associated with up-regulation of genes related to defence and cell wall modification, whereas functional mycorrhization in wild-type plants was associated with activation of genes related to isoprenoid metabolism. The oxylipin pathway was activated in tomato mycorrhizal roots at late stages of interaction, and was related with the control of fungal spread in roots, and not with the establishment of the symbiosis.

1.-**Hause B, Mrosk C, Isayenkov S, Strack D.** 2007. Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 8:101-110.

2.-**Herrera-Medina MJ, Steinkellner S, Vierheilig H, Ocampo JA, García-Garrido JM**. 2007. Abscisic acid determinates arbuscule development and functionality in the tomato arbuscular mycorrhiza. New Phytol 175:554-564.

C: Functional gene marker approaches:

Reports about crucial genes and pathways

Hypothesis:

Functional markers can be identified for efficient plant-AMF interaction in view of yield stability

Sugar transport processes during the arbuscular mycorrhizal symbiosis

Natalia Requena and Nicole Helber

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Hertzstrasse 16 D-76187 Karlsruhe, Germany

One of the central aspects of the nutritional exchange during arbuscular mycorrhizal symbiosis is the transport and allocation of photoassimilates. The fungal partner is a biotroph that develops specialized haustoria, the arbuscules, within inner cortical cells. This crucial structure that gives name to the symbiosis is supposed to be the major place of nutrient exchange between symbiotic partners.

However, given the obligate biotrophism of the AM fungus that it is assumed to obtain carbohydrates only during the in planta phase, it is possible that other locations such as intercellular hyphae or coils might be also places for sugar uptake. The cortical cell harboring an arbuscule has an increased metabolic function and thus a larger sink activity with a higher demand of carbohydrates. It is also believed, based on NMR studies that glucose is the form in which plants provides sugar to their fungal symbionts. However, artificially increased acid invertase activity in the apoplast does not result in an enhanced mycorrhizal phenotype. In addition, the specific fungal transporter responsible for sugar uptake from the apoplast into the fungal cell has not being so far identified. In this presentation we will present some new data about the sugar exchange activities in both directions and the putative role that the genes involved in the sugar uptake might play as markers for the functionality of the symbiosis.

The role of microRNA399 in linking phosphate homeostasis and arbuscular mycorrhizal development

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Many plants improve their phosphate (Pi) availability by forming mutualistic associations with arbuscular mycorrhizal (AM) fungi. Pi-replete plants are much less colonized by AM fungi than Pi-deplete plants. This indicates a link between plant Pi-signaling and AM development. Recently, components of phosphate homeostasis signaling pathway in plants have been identified. We showed by using phosphite as a structural analog of phosphate that this signaling pathway is also involved in regulating the AM symbiosis. MicroRNAs (miRs) of the 399-family are systemic Pi-starvation signals important for maintenance of Pi-homeostasis in *Arabidopsis thaliana*, and might also qualify as signals regulating AM development in response to Pi availability. We investigated the miR399 family in AM-capable *Medicago truncatula* and experimentally confirmed ten new miR399-family genes. The 15 miR399 genes found in this species are in obvious contrast to only six genes in non AM-capable Arabidopsis. Expression of the miR399 genes was studied in shoots and roots of *M. truncatula* plants in dependence of Pi-status and AM symbiosis with *Glomus intraradices*. Pi-deplete plants showed increased expression of mature miR399 and miR399 primary transcripts (pri-miR399s), and unexpectedly, some pri-miR399 species were higher in leaves of mycorrhizal plants than in leaves of non-mycorrhizal plants. Compared to non-mycorrhizal Pi-deplete roots, mycorrhizal roots of Pi-deplete *M. truncatula* and tobacco plants had increased Pi-contents due to symbiotic Pi-uptake, but displayed higher mature miR399 levels, which accumulated in arbuscule containing cells. Expression levels of *MtPho*2 remained low and PHO2-dependent Pi-stress marker transcript levels remained high in these mycorrhizal roots. Hence, an AM symbiosis-related signal appears to increase miR399 expression and thereby decrease PHO2 activity in arbuscule-containing cells, indicating a role of miR399 in the cellular response to increased phosphate due to symbiotic phosphate transport.

POSTERS

Testing different varieties of *Zea mays* L. as host plant for inoculum production of arbuscular mycorrhizal fungi

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In Germany, the variety "Blizzard" of *Zea mays* was used for inoculum production of arbuscular mycorrhizal fungi (AMF) for years. Unfortunately it can not be found on the market anymore. Currently the variety "Gelber Badischer Landmais" is used, but this variety will be not present in future, either. So we are looking for another maize variety, which is suitable for mass inoculum production of AMF. Hereby we tested 6 different maize varieties ("Magister", "Santiago", "Goldoli", "Ravenna", "Monitor", "Castilla") with AMF in 3 subsequent years.

Maize plants were cultivated in soil substrate with fungal inoculum "Agri" (90 infection units/cm^3) under greenhouse conditions. Every 2 weeks the plants were fertilized with NPK (13+5+20) fertilizer. The roots for measurement of root length, root fresh weight and fungal colonisation were collected after 9 weeks. We used trypan blue staining method after Phillips and Hayman (1970) for visualization of fungal structures in the roots and the method of Trouvelot et al. (1986) for analyse of colonisation of roots by AMF.

There were no significant differences in root length and root fresh weight of mycorrhizated maize varieties. On the other hand the frequency of mycorrhiza in root system variegated between the varieties. The variety "Magister" showed the highest colonisation rate of AMF in the roots. The root colonisation rate of AMF of the same maize variety showed differences between the years. The inoculation experiments resulted in no correlation between root biomass and mycorrhization frequency. Environmental factors could have more impact on the mycorrhization rate than culture variety. For the future we recommend to use the maize variety "Magister" for inoculum production.

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Selection of *Allium cepa* cultivars for secondary metabolite production and dependency on mycorrhiza

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Vegetative propagules of five popular cultivars of *Allium cepa* L. obtained from polish producers were cultivated on sterile substrate that was inoculated or not with several strains of mycorrhizal fungi. The experiment showed very low dependency of plants at this stage on mycorrhizal fungi. Among AMF strains used the most effective was *G. intraradices*. No arbuscules were found in case of three varieties inoculated with *G. mosseae*. The highest mycorrhizal colonization was noted in case of cv. sochaczewska. In a few cases the vitality index (PI) was higher in mycorrhizal than in nonmycorrhizal plants, although, the differences were not statistically significant. The differences in secondary metabolites were observed only between plant cultivars. The highest quercetin concentration was found in cv. wenta and sochaczewska while the highest kaempferol was detected in cv. sochaczewska and stutgarter. No influence of AMF was found.

In another experiment onion seeds of twelve cultivars were introduced into non-sterile commercial substrate which was either inoculated or not with *G. intraradices*. Although the control substrate contained mycorrhizal fungi, the effect of inoculation with *G. intraradices* was obvious, showing that plants from seeds are highly responsive to properly selected AMF strains. Not only the biomass but also the seed survival of inoculated plants was higher. Following the inoculation the best development of plants was noticed in case of cv. ishikura, augusta, globo and inga. The positive effect of AMF on production of ascorbic acid by plants was found in case of cv. inga, ishikura, augusta and wolska. Preliminary data show that, although the concentration of P, N, Ca and Fe was not increased in mycorrhizal plants, the total content of these substances were increased.

These investigations were carried out under financial support of the Ministry of Science and Higher Education, project 197/N-COST/2008/0.

Community Structure of AM Fungi Colonizing Roots of Different Varieties of *Hordeum vulgare*

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The host dependent population growth rates of AM fungi are explained as a possible mechanism for the maintenance of the diversity of fungal species. In the present studies we are investigating the influence of different genotypes of one plant species on the AM fungal community in various soils. Twenty one varieties of *Hordeum vulgare* from the gene bank of Latvia were analyzed concerning their influence on the community structure of AM fungi colonizing their roots. The average of colonization varied widely between the different *Hordeum* varieties, whereas the oldest variety Vairogs from 1930, the average mycorrhizal colonization was approximately 72% and the variety Ansis, selected in 2001, 54 % respectively. Mycorrhizal colonization of the six in Latvia most commonly grown cultivars was comparatively high

Are these differences in mycorrhizal colonization explained by changes in the composition of the fungal community in the root system in response to the different plant genotypes? This problem has been evaluated using a nested PCR approach based on 25S rDNA polymorphism and taxon specific primers. Furthermore, we analyze the distribution of AM fungi in the field with respect to the different varieties.

The most effective colonizers of the five identified fungi were *G. mosseae, G. geosporum* and *G. rosea*. These fungi were each detected with an average frequency of 45-47%. *G. claroideum* was slightly less common in the roots of the *Hordeum vulgare* cultivars (37%), and the frequency of *G. intraradices* was the lowest (26 %).

Additional soil analysis shows presence of .*Acaulospora* sp. and *Scutellospora sp*.

Genetic traits determining mycorrhizal responsiveness in maizze

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Phosphate is an essential nutrient for normal plant growth. Most of the phosphate in the soil is not available to plants due to its immobilization in the soil. One of the ways that plants facilitate phosphate uptake is by forming symbioses with arbuscular mycorrhizal fungi (AMF). During this mutualistic relation the fungus improves the nutrition of the plant, notably by increasing phosphate uptake, and in return it receives photosynthate. Plant varieties are known to vary in the extent of symbiotic benefit, suggesting genetic diversity underlying phenotypic variation.

In our study a core maize diversity panel consisting of 25 lines (Flint-Garcia et al., 2005 Plant J. 44, 1054-1064) were examined for their responsiveness to mycorrhizal colonization. Shoot and root dry weight, and total tissue phosphorus content were measured from mycorrhizal and non-mycorrhizal plants to calculate responsiveness to AM colonization. Furthermore the effect of different phosphorus concentrations on the level of colonization and plant performance were quantified. Moreover to unravel the molecular mechanisms determining mycorrhizal responsiveness we characterized the maize Pht1-like transporters. A total of 14 Pht1-like maize genes were identified in the maize genome and their expression characterized, in the presence or absence of mycorrhizal fungi, under different phosphate levels, and in different cultivars that vary in responsiveness to colonization by AMF.

Characterization of plant-mycorrhiza symbiosis and plant-*Orobanche* parasitism in a tomato strigolactone-impaired mutant which is deficient in strigolactones synthesis

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Strigolactones, the new group of plant hormones, were shown to be important signals for plant-mycorrhiza interactions as inducers of hyphae branching of the arbuscular mycorrhiza fungi (AMF), and as essential signals for seed germination of the parasitic plant *Orobanche*. We have characterized the effects of strigolactones deficiency on plant-interactions in the tomato (*Solanum lycopersicon*) mutant *Sl-ORT1*. The strigolactone-deficient mutant, *Sl-ORT1* was identified as resistant to *Orobanche*. The resistance was due to decrease in the ability of *Sl-ORT1* to induce *Orobanche* seed germination. Moreover, *Sl-ORT1* was shown to have aberrant symbiosis interaction with AMF (*Glomus intraradices*): it displayed a reduced ability to induce mycorrhiza hyphal branching. Analysis of grafted plants showed that roots rather than shoots are responsible for the resistance phenotype of *Sl-ORT1* to both AMF and *Orobanche*. Moreover, double grafting of WT interstock in *Sl-ORT1* mutant plants, although restored shoot phenotype to that of the WT, did not restore root phenotype to that of the WT (i.e., *Orobanche* and AMF sensitivity). The results suggest that strigolactones are important plant-interaction signaling molecules also in tomato and that strigoalctones signaling is moving mainly upwards, towards the shoot apex, rather than downwards, towards the root apex.

Comparison of phenotypic, rDNA and FAME analysis of in vitro grown selected gene pool of Glomeromycota.

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Arbuscular mycorrhizal fungi (AMF) are important symbionts of plants that improve plant nutrient uptake and in turn help in plant growth promotion. Genetic analysis of arbuscular mycorrhizal fungi has been a complicated task because of the difficulty faced in obtaining pure axenic cultures and the presence of heterogeneity in rDNA sequence within a single arbuscular mycorrhizal spore. As available methods of genotyping were not sufficient for identification upto species level, a new polyphasic approach was adopted. Ten arbuscular mycorrhizal fungi collected from Centre for Mycorrhizal Culture Collection (CMCC), TERI New Delhi, India were grown in isolated root organ culture. A comparative characterization of each strain was done using fatty acid methyl ester profile (FAME), partial sequencing of small subunit–internal transcribed spacer (SSU-ITS) and large subunit (LSU) rRNA gene in combination with morphological examination of arbuscular mycorrhizal spores. Neighbor joining tree (NJ) obtained from SSU-ITS rDNA sequences were broadly similar to the NJ tree obtained from LSU rDNA sequences. FAME profiles obtained using fatty acid data sets and results were compared to a neighbor joining tree of rDNA sequence within the same species. We recommend that a combination of the morphological, biomarker (FAME) and molecular (sequencing of highly variable D1-D2 region of LSU and ITS rRNA gene) method could be employed for phylogenetic analysis and species level resolution of Glomeromycota.

Key word – Mycorrhiza fungus, Glomeromycota, FAME profiles

7th July 2010

SESSION 2:

'From Science to the Field'

A: Interaction of AMF- Inoculum Producers and Appliers – Experiences

Hypothesis:

AMF products and transfer of practical knowledge need to be improved

Mycorrhiza expectations: successes and failures related to scientific knowledge and commercial product's quality (Keynote lecture)

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During the last decade, the production of mycorrhizal fungi has entered its booming phase and it is gradually becoming an industry. The commercial competition increases at least in Europe but more and more also globally and markets have started to overlap significantly since few years ago. Though there are still limitations in market expansion and it is mainly due to failures of commercial products to meet expectations of the users.

Attitude of end users has been, however, changing recently and they emphasize more non-nutritional aspects of mycorrhizal symbiosis, for example, increased erosion control due to soil aggregation and glomalin production, CO_2 sequestration due to C allocation in fungal mycelium networks, effects of mycorrhiza on plant hormonal balance and on production of secondary metabolites.

There are still numerous commercial products labeled as containing 'mycorrhizal fungi with efficient nutrition effects' but in fact unable to form mycorrhiza sufficiently thus relying on non-mycorrhizal nutrients component in their formulation. Nevertheless, the market is becoming more educated and end users want to make sure that the product, they buy, contains efficient and sufficient number of fungal propagules to form mycorrhizal colonization reliably.

There is still an open question as the regulatory issues have not been sorted out internationally, it is, however, clear that the claims on biopesticide effects of mycorrhiza are better to avoid in Europe due to high costs of biopesticide registration.

The old mantra remains, what is the further challenge for science: among others it is to bring solid scientific evidence to establish new unique selling points of mycorrhiza and to develop internationally recognized methodology for quality control. The main challenge for scientific community is also to support performance of mycorrhiza application in the case studies, which reflect more closely real conditions of plant commercial cultivation and to make efforts to facilitate new joint projects with mycorrhiza producers. Independent quality control, product certification and international recognition of product quality remained a wish of producers and probably also some scientists who are aware that successful commercial applications will also result in more funding of fundamental science.

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Tammy Kovar
Owner – Think Global, LLC
Doing business as

- Biological Tree Services, residential and commercial landscape services
- Sustainable Landscape Supply, product sales to homeowners and commercial landscape providers who are working to create sustainable landscapes in the US and around the world

Tammy Kovar is currently the owner of a successful landscaping company which uses biologically active and environmentally friendly products to increase the fertility of her client's landscapes while still acting in the best interest of the environment. Many of the products and services Tammy provides, largely her tree recovery programs for all species, as well as programs to increase agricultural production, utilize various types of mycorrhizal fungi. Her clientele range from private home owners to commercial installations and golf courses, to large scale agriculturalists.

Even in our tumultuous economic environment, Tammy's businesses have still flourished largely thanks to the ever-expanding trend for individuals and companies to implement environmentally friendly programs.

Tammy is also a distributor of many various biological products, which she uses in the service portion of her company. She is constantly vetting new products and technologies to expand the scope of her client base, and has recently designed a fully organic fertilizer line which incorporates an array of beneficial bacteria to aid in nutrient uptake efficiency, and sequestration of materials already present in the environment.

Tammy will be discussing how the utilization of biologically active technologies, in particular mycorrhizae, has aided in the success of her business, and how to strategize a business development program when launching a company which specializes in biological products.

Bringing together research scientists, inoculum producers and plant producers: a necessary step for developing applications of AM fungi

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Fundamental research has clearly demonstrated the central role plaid by AM fungi in the rhizosphere and their beneficial effects on plant growth and health. As a consequence, AM fungi have potentially important applications in plant production. However the beneficial effects observed by researchers under very controlled conditions are more difficult to reproduce under real production conditions in the greenhouse or in the field, because inoculation strategies and growing conditions may not be compatible with development and efficient functioning of AM fungi. Even good quality commercial inocula may not have the formulation adapted to a particular plant production system. In addition, traditional plant cultural practices are usually incompatible with the use of AM fungi. As a consequence, plant producers do not often observe beneficial effects of AM fungi on plant growth and they do not continue using AM fungi.

Therefore, in order to obtain significant beneficial effects on plant growth and health in the greenhouse or in the field under real production conditions, communication is essential between scientists, inoculum producers and plant producers. Scientists and inoculum producers have to be in close contact with plant producers and give them the appropriate advice adapted to the cultivated crops. They have to be aware of the cultural conditions and problems associated with each plant production system. In order to optimise the use of AM fungi, researchers, inoculum producers and plant producers should work together to design experiments for the identification of growing conditions compatible with efficient activity of AM fungi and good plant development. In this context, it is necessary to have the proper controls and use enough replicates for proper statistical analysis. Experiments should include non-inoculated and inoculated plants as well as plants grown under conventional conditions. It is necessary to show plant producers that there are advantages in using AM fungi and that this can result in an economical benefit. Benefit can be obtained by improved yield, increased tolerance to biotic and abiotic stresses or enhanced production of secondary metabolites beneficial to human health. Therefore, it is necessary to run demonstration experiments including estimation of the associated costs and benefits in order to convince plant producers to adopt mycorrhizal biotechnologies.

Finally scientists and inoculum producers really need to improve inoculation strategies in terms of AM fungal species / isolates and formulation. For example, even if the use of AM fungal spores is safer to ensure the absence of pathogens in inocula, they may not always be as efficient as a whole inoculum containing a growth substrate, root fragments and mycelium. In addition, commercial distribution and market pressure can lead to bad inoculum formulation and bad advice to customers.
Moreover if an inoculum producer uses the same strain for all applications under different climates and soil conditions, this may not give the best results and may not be good for biodiversity in the long term. In these conditions, using locally-adapted isolates may be preferable but it is not always easy to register many isolates in some European countries. In some circumstances, the other option of AM fungal population restoration in the soil through rotations for example, should be considered. Although this is not directly profitable to inoculum producers, this may create new business opportunities.

These considerations will be exemplified based on our experience of introducing AM fungal inoculation during commercial production of plants in the greenhouse and in particular of raspberry plants.

Specific versus subliminal benefits of inoculants - a case study based on strawberry cultivation in protected cropping

Dr Susan Rafferty-McArdle, Alan C. Cassells

There is widespread acceptance that mycorrhizal fungi and plant growth-promoting bacteria, possibly acting in unison, are beneficial for plant growth. These benefits include protection against abiotic stress ('growth promotion') and/or protection against disease. These benefits have been demonstrated in trials on the establishment of microplants, seedlings and cuttings (assisting in combating putative abiotic and biotic establishment stress) and in longer term trials on plant field performance, crop yield and crop quality. However, the results of such trials have been variable with inconsistent positive growth effects and/or disease protection observed. Here, we report on the prophylactic use of biological treatments for in-season (early summer) and out-of-season (late summer) strawberry crops in protected cropping. The cropping system experiences transient abiotic stress (heat and water stress) in the in-season crop and endemic development of powdery mildew in the out-of-season crop.

Trials were carried out using arbuscular mycorrhizal fungi (AMF -*Glomus* spp.), PGPR (*Bacillus subtilis*) and the plant defence primer chitosan; singly and in combinations, on two crops of strawberry plants grown from runners, namely, early summer and late summer plantings. The crop was monitored for disease progression based on a powdery mildew disease assessment key and plants were captured using thermal imaging. Fruit yield and runner production were monitored until the end of fruit harvesting. The persistence of the inoculants was confirmed by re-isolation at the end of the season.

In the early season crop, the treatments were found to have no significant effects on fruit yield, fruit number, fruit weight or runner production but there were, arguably, economic benefits to some treatments. In the late season crop, both fruit yield and runner production were lower than in the early crop due to powdery mildew disease. Several of the treatments significantly increased fruit yield and runner production. The significance of these results is discussed in relation to 'insurance' crop protection strategies and product claims.

Applications of mycorrhizal fungi in forestry: from the nursery to the field

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Mycorrhizal fungi can improve plant survival and growth by stimulating the uptake of soil nutrients and water, and by increasing plant resistance against biotic (e.g. plant pathogens) and abiotic (e.g. presence of toxic elements) stresses. Thus, the application of mycorrhizal fungi in forest nursery production may help to reduce the input of chemical fertilisers and pesticides. Although potentially beneficial, nursery inoculation is not always straightforward, and requires the selection of compatible and efficient mycorrhizal fungal isolates tuned for specific target plant and growth conditions. Positive effects of inoculation with selected mycorrhizal fungi on seedling growth under nursery conditions have been commonly reported. However, in most studies the performance of inoculated seedlings is not evaluated after transplanting to the field.

In the present study, selected mycorrhizal fungal isolates were inoculated under nursery conditions. Plant and mycorrhizal development were assessed. After 12 months, plants were transplanted to the field and their performance evaluated.

The application of mycorrhizal fungi inocula increased the growth and vigour of seedlings and mycorrhiza development under nursery conditions and improved the performance of outplanted seedlings.

Mycorrhizal inoculation can be an efficient biotechnological tool and should be regarded as a good-practice management in forestry.

Experiences of using commercial AMF inoculum in Finnish nursery production of woody plant species

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One aim of this study was to find out whether woody plant species commonly used in nursery production and landscaping in cold temperate regions of Northern Europe are colonized after AMF inoculation in peat substrates, and if there is a benefit from inoculation. The AMF product "Myko-Ymppi" produced by MTT was added into light sphagnum peat growing medium as 5% (v;v) during potting or cutting propagation in spring. Plant species studied were *Syringa vulgaris, Dasiphora fructicosa, Viburnum opulus, Prunus padus 'colorata', Prunus sargentii, Prunus domestica and Hippophae rhamnoides.* AMF inoculation caused root colonisation in every plant species which varied from 2% in *S. vulgaris* and *P. domestica* to 62% in *H. rhamnoides.* However, AMF inoculation had no significant impact on growth in any of the plant species and cultivars studied.

Another aim of this study was to find out whether AMF inoculation have an impact on rooting, subsequent growth and winter hardening of woody cuttings propagated in nursery field soil. Such cuttings are propagated for one year in the soil whereafter they are lifted in late autumn and taken to a storage house with a temperature of zero degrees C for overwintering. Proper hardening in autumn is crucial for survival in storage during winter. Plant species studied were *Rosa pimpinellifolia* and *Diervilla lonicera*. Cutting beds were inoculated with Myko-Ymppi at the rate of 500 g m^{-2}. AMF inoculation slightly increased root colonisation in both *Rosa* and *Diervilla* measeured in summer and in autumn, but the impact of inoculation on growth attributes was negligible. An exception was the rose cultivar Plena, in which inoculation slightly decreased shoot growth of cuttings. In *Diervilla*, inoculation had no effect on starch accumulation of stems measured in late autumn. However, saccarose accumulation in stems was slightly decreased due to AMF inoculation. After winter storage, the amount of starch in stems of *Diervilla* was slightly higher in AMF treated than in control plants. Significantly higher chlorophyll fluorescence was also observed in the stems of AMF inoculated plants, indicating that AMF treated plants were less stressed after winter storage than non-inoculated plants. Growth initiation in spring also tended to be faster in AMF treated than in non-treated plants.

It can be concluded that the woody plants chosen for this study did not react, or reacted only slightly positively to AMF inoculation in the prevailing nursery conditions. All plant species and cultivars however got colonized by AMF, which is a prerequisite for AMF inoculated plants to stand abiotic and biotic stress at their final locality. Studies over several years are needed for evaluating the real value of AMF inoculation in the long run!

Selection of efficient plant genotypes for heavy metal polluted sites

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Phytoremediation of heavy metal rich areas is not an easy task. In places where there are still valuable metals such as Ni or Au the best solution is to use plants to extract them. On the contrary, in places such as Pb-Zn wastes the most important are plants that exclude metals from their shoots and do not transfer the metals from roots to shoots during the whole vegetation period including senescing leaves. Mycorrhizal plants seem to be the most effective for phytoremediation as many of them show better tolerance/resistance to soil toxicity, drought and nutrient deficiency. Still this is not enough to be a good candidate in phytoremediation. Usually, the annual grasses of eco-tolerant cultivars are the first choice in such cases. Experiments carried out in industrial wastes clearly show, however, that these plants are easily whipped out from such areas as the new seedlings that have to develop each year hardly survive under strong stress. Perennial grasses could be much better. Plants such as *Miscanthus giganteus* might be a good choice and the experiments using fertilization, mycorrhizal inoculation and the use of flavonoids to improve mycorrhizal activity was carried out. Plant vitality was monitored using Handy PEA, although, the real improvement by mycorrhizal fungi was found in root development in the first year of cultivation. Further investigation will be carried out using other methods that were shown to be valuable while studying plants that are available in higher number of cultivars. This include the use of the refractometer that gives fast and reliable estimation of macronutrinets such as K, N, P and Ca as well as concentration of antioxidants such as ascorbic and malic acid.

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Mycorrhiza: Soil to soil

Adholeya, Alok

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The soil around the roots of the majority of land plants is teeming with the spores of arbuscular mycorrhiza fungi. These spores are the initiators of colonization, an event which leads to myriad benefits to the host plant. It was of great interest and benefit to screen out AMF with high efficacy and introduce them into the root organ culture (ROC). This being done successfully the next step was to produce the desired culture on a large scale. The effort to upscale yielded the in *vitro* based mass production technology. This technology resulted in inoculum from an efficient isolate, which was axenically produced, was of known efficacy with a known number of propagules. The attraction of the system was also production of a large quantity of inoculum using comparatively smaller infrastructure. The next challenge lay in involving the industry and the farmers in parallel. It was imperative to generate their interest and this was achieved by field applications in demonstration trials on a large magnitude. These have been done on different cropping systems, in different soil types and different agro climatic zones. Such trials and field demonstrations generated immense data, which has contributed in enhancing our knowledge especially in the application of mycorrhiza. The scientific learning has also been the holistic benefits accrued. Thus from the humble beginning of a spore from the soil, the journey has resulted in the return of the spore to the soil as a product harnessed to endow benefits and gains to the soil and the crop.

B: Interaction of AMF-Inoculum Producers and Researchers – Experiences

Hypothesis:

Better knowledge of the products would support scientific validation of AMF application

Statements to the Hypothesis:
'Better knowledge of the products would support scientific validation of AMF application'

Falko Feldmann

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Would standardized labels on AM products support scientific and practical validation of AMF application? Since 1997 a standardized label for mycorrhizal inocula exists issued by the Committee of Mycorrhiza Application in Germany (CMAG). This was discussed in COST Action 838 and supported by leading AMF companies in the EU in 2002.
These standards are widely accepted by AM producers sustainably on the market but not by the majority of retailers. What may be the reasons? Which quality control procedures underlie the labels? What is the biological reason why labeling is so difficult?

Thirteen years after the first labeling proposal we will recommend a reduction of labeling to absolute essential factors which allow scientific and practical comparison of AM products.

Proof of concept – a method to screen inoculants for their host priming activity

Alan Cassells

AMF and PGPR inoculants have been extensively investigated for their application in controlling plant disease. The commercial exploitation of inoculants, however, has been undermined by reports, of success and failure, and irreproducibility. The latter is attributed to the influence of altered environmental factors, and specific inoculants-plant genotype interaction. A novel aseptic (monoxenic) method has been developed to test the hypothesis that inoculants function in disease control by priming the host's biotic defences.

The model system is based on aseptic microhydroponics - a modification of micropropagation where the nutrient rich, high-salt plant growth medium of Murashige and Skoog is replaced by a Hoagland -derived simple mineral salt solution and were the agar plant support is replaced by polyurethane foam. Established microplants are inoculated with aseptic AMF or bacterial spores. The microplants are harvested over a time course; proteins are extracted and run gels in 1-dimension. Bands of target molecular weights are excised. A modification of the MALDI quadrupole time-of-flight mass spectrometric protocol of Shevchenko et al. (2000) is used to produce and identify tryptic peptides derived from the protein band. Raw data files are analysed using ProteinLynx Globalserver 2 (Waters) in order to assess the identities of the proteins present in the digest. The peak file list generated from PLGS2 analysis is used in search engines accepting this format of data file (e. g. Mascot, Matrix Science).

Representative results will be presented showing specificity in the host defence pathway primed by specific inoculant isolates and demonstrating the efficacy of priming in controlling model diseases. The hypothesis will be discussed that 'more durable disease resistance in plants may be conferred by strategic use of inoculant isolates (AMF and/or PGPR) and plant activators, appropriate to the specific disease(s) of the crop cultivar'.

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The Effects of Mycorrhiza Inoculations and P Addition on Gramine Species Growth and Nutrient Uptake

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Since world fertilizer sources, especially P, are very limited and costly it is reasonable to use natural organic sources such as mycorrhizal inoculation compared to manufactured fertilizers. Also using heavy fertilizer caused soil pollution consequentially environmental pollutant and soil quality is great concern. In order to use less fertilizer it is sound to search and screen the effect of mycorrhizal inoculation on several gamine species for better mycorrhiza management. Wheat and barley are important crops grown widely in the Mediterranean region. Wheat is one of the strategically crop in Mediterranean societies food chain. One experiment was set up and repeated several times to search the effect of mycorrhizal inoculation under field condition on Menzilat soil series (*Typic Xerofluvents*) which is located in the Research Farm, Faculty of Agriculture, University of Çukurova, (Eastern Mediterranean region) Turkey. Mcorrhizae inoculum was used approximately 50 mm under seeds. Chemical phosphate fertilizer used in 0 and 100 kg P_2O_5 ha^{-1}. In this experiment two wheat genotypes (Genç99, Atilla), Triticale and Barley plant species were grown during 2001, 2003, 2005 and 2007 with and without phosphorus treatments. Yield, P, Zn, Cu, Fe, Mn concentration in shoot tissue and seed were analyzed. Also root colonization was determined.

The results have shown that in four years experiment during 2001, 2003, 2005 and 2007, mycorrhiza inoculation significantly increased germinal species yield. Mycorrhiza inoculation also increased root colonization and nutrient uptake especially P, Zn, Cu uptake.

Root colonization has been effect with high P addition. In low P addition root colonization was higher than high P addition treatments. Positive response of mycorrhizal inoculation was found on seed yield under low P application.

Arbuscular Mycorrhizae affect melon fruit quality under field conditions.

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Previous works have suggested that arbuscular mycorrhizal (AM) fungi can enhance the quality of plant products. Therefore, the aim of the present study was the investigation of possible effects of AM fungi on melon growth, yield and fitness.

Plants belonging to two cultivars of *Cucumis melo* (Expò and Baggio), exposed or not to an inoculum of various AM fungal species, were transplanted and grown, and analyzed in order to assess shoot, leaf, flower and fruit development. The size, weight, sugar (sucrose, glucose and fructose) and carotenoid (carotens and xantophylls) concentration of melon fruits were recorded. Seeds collected from mycorrhizal and non-mycorrhizal plants were counted, measured and tested for germination and first root elongation.

Inoculated melons flowered earlier and produced a significantly higher number of flowers than uninoculated ones. Moreover, AM colonization affected shoot morphology increasing both shoot diameter and internode length. In mycorrhizal plants, fruit quality was enhanced, as testified by the increased concentration of sugars, carotenoids and electric conductivity in the pulp, without any yield loss. At the same time, mycorrhizal colonization significantly improved the size (length and width) and the total weight of the seeds, as well as both their germination and root elongation in the first stages of growth.

In conclusion, this study shows that AM colonization can improve melon fruit quality under field conditions. In addition, it suggests that AM fungi might contribute to increase the fitness of the host plant, directing the production of seeds with higher germination rate and faster growth of the main root.

POSTERS

Development of a next generation inoculum with broad spectrum application

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It has always been of interest and a need to develop an inoculum which would deliver a diverse nutrient requirement to numerous plant species in a single formulation. Such an inoculum would cater to a range of hosts from herbs to forestry species from different agro-climatic zones. One such inoculum would be a formulation comprising of arbuscular mycorrhiza (AM) and ectomycorrhiza (EM) together. Earlier studies have shown that the two can form symbiosis together in a single host without antagonism when cultured in *in vitro* conditions. The development of such an *in vitro* based inoculum would ensure its purity and quality. The challenge lies in culturing all three together with all the three coexisting without suppressing each other. An initiative to meet this end has been taken up by us for developing an AM-EM inoculum in the root organ culture (ROC). A dual culture of ectomycorrhiza fungi and transformed root was initially set up to establish a media, which would support their respective growth as an ROC. AMF was then introduced to this dual culture for analyzing the growth parameters of all three. The isolates were procured from Centre for Mycorrhizal Culture Collection (CMCC), New Delhi. The efficiency of production as well as the efficacy of the inoculum produced by this *in vitro* method will be compared with that produced by the conventional method. Cost effieciency comparisons too will be a parameter for consideration in the complete feasibility of the production of such an inoculum.

Mycorrhizal and saprophytic fungi based inocula in organic cultivation og vegetative crops.

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Nowadays many growers in Europe seek alternative organic solutions for their crop production providing sustainable schemes which allow achieving similar yield and food quality as the conventional ones. The alternative technology based on using unique combination of selected organic matter inputs together with fungal inoculants (mycorrhizal and saprophytic) is being tested within the framework of the EU Eurostars MICROFRUIT project and the COST project OC09057 funded by the Ministry of Education, Youth and Sports of the Czech Rep. in the fruit and vegetable productions in the Czech Rep. and Hungary. Pot experiments done on leek (*Allium porum* var. Starozagorsky Kamus), onion (*Allium cepa* L. var. Alice), tomato (*Lycopersicon lycopersicum* var. Darinka F1) and field trial done on Hungarian spice peppers (*Capsicum annuum* L. var. longum cv. Szegedi and *Capsicum annuum* L. var. longum cv. Kalocsai) showed positive effects of fungi based microbial inocula (single or combined inocula) on yield parameters (increase by 10% in Kalocsai pepper, by 65% in Szegedi pepper, by 74% in onion, by 108% in leek, by 455% in tomato) and proved synergistic effect between AM fungi and saprophytic fungi retrieving nutrients from decomposition of wood chips organic matter in promoting plant growth. However, fungal inoculants affected not only plant growth parameters but also improved target food health properties such as content of minerals (K, Mg and Ca) or antioxidant compounds. The promising results could enable to introduce the tested biotechnological way of organic growing into food crop production on country or even European scale.

Support of the project Mykotech2, funded by the Ministry of Education, Youth and Sports of the Czech Republic, grant no. OC09057, and Eurostars Project Microfruit E!4366 is acknowledged.

Effects of arbuscular mycorrhizae on the establishment of psammophylic plants on a Mediterranean coastal sand dune

Nogales, A., Calvet, C., Estaún, V., Camprubí, A.

Sand dune systems are characterized by a low organic matter content and low nutrient availability. Their stabilization is linked to the survival of a vegetation cover. In the restoration of low-fertility soils the use of arbuscular mycorrhiza has clear benefits, both by increasing plant growth and as a step towards the establishment of a more sustainable system that preserves a life-supporting soil matrix. In this work, we studied whether inoculation of psammophylic plants seedlings with an effective arbuscular mycorrhizal fungus favours plant development under the harsh conditions of a coastal sandy soil.

In a field experiment we assessed the contribution of the AM fungus to the survival and growth of *Ammophila arenaria* (L.) Link, *Lotus creticus* L. and *Pancratium maritimum* L. transplanted in a sand dune located in the coastal area of Northeastern Spain. Plants were inoculated under greenhouse conditions and transplanted after the establishment of the symbiosis. The experimental plots were replicated within the revegetated area. Plant survival and growth were periodically determined for each plant species during the first year and compared with the field performance of non mycorrhizal plants.

Improvement of the nutritional quality of lettuce by applying a commercial inoculum of arbuscular mycorrhizal fungi (AMF)

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Lettuce is considered a major food crop within the European Union and it is the most used food crop for the called "Fourth Range" of vegetables. The healthy properties of lettuce are attributed to a large supply of antioxidant compounds and fiber content (Serafini et al. 2002, Llorach et al. 2008) presumably involved in the beneficial effect on lipid metabolism and protection against cardiovascular diseases (Nicolle et al. 2004). The establishment of the mutualistic association between arbuscular mycorrhizal fungi (AMF) and the root of a host plant involves a continuous cellular and molecular dialogue between both symbionts that includes, for example, the production of plant defense related proteins and/or enzymes belonging to the phenylpropanoid pathway (Azcón-Aguilar et al. 2002). Therefore, the symbiosis of lettuce with AMF may enhance the synthesis of antioxidant compounds of interest for human health thus improving the quality of lettuce. Our objective was to test if the inoculation of two varieties of lettuce (the green variety "Batavia" and the red variety "Maravilla") with a commercial inoculum (AEGIS Endo Gránulo, Atens, a mixture of *Glomus intraradices* and *G. mosseae*) induced the accumulation of antioxidant compounds and other substances of nutritional interest for human diet - such as sugars and proteins - in the outer and/or inner leaves. Results showed that the inoculation of lettuce with the commercial inoculum enhanced plant growth, being the mycorrhizal efficiency index (MEI) similar for both varieties of lettuce. In addition, mycorrhizal plants accumulated higher amount of soluble sugars and proteins in the internal leaves and greater contents of phenolic compounds in the external leaves than non-mycorrhizal plants. The presence of AMF also induced the accumulation of phenolics in the inner leaves of the variety Maravilla. The application of the commercial inoculum enhanced the content of total chlorophylls in both the outer and the inner leaves in the green and the red varieties of lettuce. Moreover, mycorrhizal plants accumulated higher amount of carotenoids than non-mycorrhizal ones in the external leaves and, in the case of the red variety Maravilla, the increase in carotenoids was also observed in the internal leaves.

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Influence of fertilization on photosynthetic performance, mycorrhizal colonization, ascorbic acid production and nutrient concentration in *Verbascum thapsus* under high stress conditions

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Bioremediation is a well known, non-invasive method of heavy metal–rich substrate rehabilitation. To achieve the goal plants and associating microorganisms have to be established in the area of interest. The focus of this study is to find optimal composition of fertilizers for the establishment of plant cover on mine tailing of Trzebionka industrial plant in Poland. *Verbascum thapsus* was selected during the long term field experiment concerning introduction of plants from xerothermic grasslands into the industrial wastes. The plants were inoculated with *Glomus clarum*, a mycorrhizal fungus isolated from the area and were cultivated under greenhouse conditions. The pots were treated with: 1. water; 2. LA nutrient solution (40 ml per pot, containing 0.8 g of K kg^{-1}) and, 3. LA solution with additional dose of potassium (1.2 g kg^{-1}). The growth, photosynthesis and leaf nutrient composition of the plants were compared. The experimental data showed that plants that received LA with extra potassium had higher concentration of ascorbic acid, potassium, iron, nitrate and nitrite in plant leaves than those that were treated only with LA, although the biomass of plants was lower in the first case. Plants receiving only water were the smallest, and had lower concentration of ascorbic acid, nitrite and Ca than plants treated with LA or LA plus K. Measurement of photosynthesis gave similar picture, although, leaf hairs interfered with the measurements. These data show that fertilization with additional potassium might stimulate production of antioxidative substances although the growth of plants is lower. Thus the treatment can enhance vitality of the plant.

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Microscopy and PCR detection: Two methods for confirmatory assays on arbuscular mycorrhizal fung infection

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Arbuscular Mycorrhizal (AM) Fungi are obligate symbiothrophic microorganisms, prevalent in almost 90% of the higher plants. The beneficial role in plant development make them suitable for including in bioinoculans for agriculture, either alone or combined with different other type of microorganisms.

Due to its biotrofic nature, all assays of AM fungi are aggravate by the impossibility of cultivation under axenical conditions. The most common technique used to characterize the symbiosis with AM fungi is microscopical examination. Modern, molecular methods that analyze DNA sequence of a group or a species, can also be applied to confirm the infection.

Different fungal structures (apressoria, vesicles, internal and external spores and hyphae) have been observed under the microscope, unstained or following a cotton-blue staining. Structural details of spores and hyphae can be used to distinguish the AM Fungi from other contaminant fungi. Infection was further confirmed by Polymerase Chain Reaction (PCR) targeting two ubiquitar species of *Glomus*: *G. intraradices* and *G. mosseae*.

The two methods are complementary and can be used to asses the performance of bioinoculants, as well as for estimating the symbiotical potential of the inoculum.

Development of a next generation inoculum with broad spectrum application

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It has always been of interest and a need to develop an inoculum which would deliver a diverse nutrient requirement to numerous plant species in a single formulation. Such an inoculum would cater to a range of hosts from herbs to forestry species from different agro-climatic zones. One such inoculum would be a formulation comprising of arbuscular mycorrhiza (AM) and ectomycorrhiza (EM) together. Earlier studies have shown that the two can form symbiosis together in a single host without antagonism when cultured in in vitro conditions. The development of such an in vitro based inoculum would ensure its purity and quality. The challenge lies in culturing all three together with all the three coexisting without suppressing each other. An initiative to meet this end has been taken up by us for developing an AM-EM inoculum in the root organ culture (ROC). A dual culture of ectomycorrhiza fungi and transformed root was initially set up to establish a media, which would support their respective growth as an ROC. AMF was then introduced to this dual culture for analyzing the growth parameters of all three. The isolates were procured from Centre for Mycorrhizal Culture Collection (CMCC), New Delhi. The efficiency of production as well as the efficacy of the inoculum produced by this in vitro method will be compared with that produced by the conventional method. Cost effieciency comparisons too will be a parameter for consideration in the complete feasibility of the production of such an inoculum.

Lysimeter experiment on the influence of plant cover and microorganisms on heavy metal mobility in metal rich soil

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Due to the multitude of metal polluted soils good and widely applicable bioremediation procedures are needed. But, before good solutions for the removal of metals are found, stabilizing the metals within the substrate will prevent further pollution via runoff. Plants and microorganisms play a crucial role in metal stabilization through changing the substrate properties and metal immobilization.

To study the effect of plants and microorganisms on metal movements in the soil we have set up a lysimeter experiment. The lysimeters consisted of undisturbed soil columns sampled from the Pantelimon area, near Bucharest, Romania, the site of a former battery plant. The soil is very rich in metals with concentrations of around 2000 mg/Kg for lead, 250 mg/Kg for copper and 500 mg/Kg zinc, with the highest concentrations at a depth of 0-10 cm.

The lysimeters have been planted with Helianthus annuus L. and, within different experimental variants, amended with mycorrhizal fungi and actinomycetes.

By measuring the variation of soil parameters, metal concentration in soil water and plant health we could study the influence of plants and microorganisms on heavy metal mobility in the soil and accumulation in plants.

Key words: lysimeter, heavy metals, Helianthus annuus L., bioremediation

ADDITIONAL POSTERS
OF MYCORRHIZA RESEARCH
AT THE UNIVERSITY OF EVORA

In vitro interactions between ectomycorrhizal fungi from cork-oak trees and the pathogen *Phytophthora cinnamomi*

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The root pathogen *P. cinnamomi* is associated to decline disease in Cork oak (*Quercus suber)* trees. The protective effect of ectomycorrhizal (ECM) fungi against root pathogens are well demonstrated on several experiments in greenhouse and field conditions. ECM fungi may protect roots directly by providing a physical barrier and/or secreting antibiotics.

In this study, we tested the development in co-culture of six mycorrhizal fungi collected from rhizosphere of cork-oak forest (*Pisolithus tinctorius, Scleroderma citrinum*, *Suillus bovinus, Amanita rubescens, Paxillus atrotomentosus* and *Xerocomus subtomentosus*) against *Phytophthora cinnamomi*. The goal was to analyze antibiosis effects of the selected ECM against *P. cinnamomi*.

Inoculation of *P. cinnamomi* with one week delay was tested. Controls were made by confrontation of species with themselves. Co-cultures were established on 90 mm Petri dishes with PDA and BAF medium, with 3 cm distance between inoculums, incubated at 25ºC in the dark. Culture development was measured daily until colonies attaint the hedge of plates.

Results in PDA medium showed that *P. tinctorius*, *A. rubescens* and *S. citrinum* had no direct antibiosis effect on *P. cinnamomi*, but *S. bovinus* was able to reduce *P. cinnamomi* development. In BAF medium *P. atrotomentosus* and *P. tinctorius* reduced P. *cinnamomi* development.

Exploring the antagonistic activity of ECM fungal community present in cork oak forest rhizosphere can lead to an adequate selection of strains for use as biological control agents against the root pathogens *P. cinnamomi*.

Misleading mycorrhiza?
Latest developments and new insights

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Stone pine (*Pinus pinea* L.) is an important Mediterranean forest tree, mostly due to its edible seeds which constitute a relevant resource for the portuguese economy. The improvement of in vitro rhizogenesis of microshoots of *P. pinea* was developed in our laboratory using a co-culture system using fungal symbionts known to form ectomycorrhizas with stone pine.

In the acclimation phase in mixed substrates, or in rhizotrons, anatomical and morphological studies were done to observe the development of the root system in microshoots from the co-culture system vs. control plants. Surprisingly, extensive dichotomous and coralloid branching of lateral roots was seen to occur spontaneously in inoculated, as well in control and, moreover, similar branching occurred in liquid culture of excised seedling roots without the presence of ECM fungi, both with, and without hormone induction. These organs striking similarity with pine ectomycorrhizas prompted their anatomical analysis since dichotomous branching of short lateral roots and the formation of coralloid organs are diagnostic of ectomycorrhizas. Histological cuts performed on the root samples revealed anatomical differences between the fungal induced and control plants formations and revealed other anatomical differences at a much smaller scale.

Currently new culture media and other ECM simbionts are beeing tested in order to validate our initial findings and to understand the physiological mechanisms related with the production of these interesting structures, as a survival mechanism for pine trees and as a launching pad for fungi colonization of the root systems.

Phylogenetic and inorganic profile of Mediterranean mycorrhizal *Amanita ponderosa* mushrooms

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Keywords: *Amanita ponderosa*, mycorrhizal edible mushrooms, RAPD-PCR, M13-PCR, inorganic analyses.

Amanita ponderosa are wild mushroom eatable, growing spontaneously in some Mediterranean microclimates, namely in Alentejo and Andaluzia, in the Iberian Peninsula. The nutritional values of these fungi make them highly exportable. Due to the wide diversity of mushrooms in nature, it is essential to differentiate and to identify the various edible species. Mushrooms can accumulate high concentrations of some elements, namely toxic metals, because the symbiotic relation between these macrofungi and some plants in its habitats. The aim of this study was to access molecular biomarker to characterize mycorrhizal *Amanita ponderosa* collected from six different regions of the southwest of the Iberian Peninsula and to evaluate their inorganic composition.

Some RAPD-PCR and M13-PCR primers were successfully used to establish different genetic fingerprinting profile of these edible mushrooms. The inorganic analyses showed that mineral composition of these mushrooms depends on the ecosystem where they grow. Principal components analyses of inorganic profile show three different clusters according geographic origin. Ca, Na and Mg were the elements that mainly contribute to the heterogenic inorganic profile between studied strains.

Levels of trace metals are considerably lower, acceptable to human consumption at nutritional and low toxic levels. It was not found a direct correlation between the cluster forming by molecular patterns and by mineral contents, however each study evidences the influence of different factors and both approaches are essentials for certification processes of wild eatable mushrooms.

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