Mycorrhizales in vitro:
De la recherche fondamental jusqu'à l'application à grande échelle

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Co-promotor, MYCOVITRO S.L.
Mycorrhizas are mutualistic symbiosis established between 97% of the land plants and some types of soilborne fungi, named "mycorrhizal fungi".

Mycorrhizas establish the primary network for all the other microbial soil communities.
Five types of mycorrhizas, two more abundant

Ectomicorrizas

Arbuscular mycorrhizas
Kingdom Fungi

Arbuscular mycorrhizal fungi

- Monophyletic group, prior to Asco- and Basidiomycota

- Minimal differences between spores; quite recent molecular tools
MYCORRHIZAS:

“...a symbiosis in which an external mycelium of a fungus supplies soil derived nutrients to a plant root.”

(Smith and Read, 1997)

Mycorrhiza formation usually increases plant biomass

Consequently, plant increases its photosynthetic rate in 4 to 20% ...

...meaning that over five billion tons of C per year are fixed by mycorrhizal plants!!!!!
BENEFITS OF APPLYING MYCORRHIZA TO SOILS

- Increases plant root area: Better efficiency in water and nutrients uptake.
- Optimizes Plant Nutrition
- Absorption of less available nutrients, specially PHOSPHORUS
- Stimulates plant growth and survival during stress conditions such as drought, salinity, poor soils or extreme pH, etc.
- Balances other elements absorption: copper, chlorine, etc.
- Better resistance against pathogens: Better nutritional status, cell wall thickening, activation of defense pathways, competition for space.
Arbuscular mycorrhizal fungi: mycelial networking and soil microorganism consortia

- Other fungi (Trichoderma)
- PGPRs
- Nematodes
- Protozoa
- Free N₂-fixing bacteria
- Rhizosphere
- Mycorrhizosphere

Other fungi

PGPRs

Nematodes

Protozoa

Free N₂-fixing bacteria

Rhizosphere

Mycorrhizosphere
If Mycorrhizae are so good, why have we not used them so far?

Because we didn’t know them good enough to produce and use them correctly.
The fungal colony of arbuscular mycorrhizas as we knew it by 1990’s
I. Soil systems:

- Difficult to follow on-site
- Extraction of hyphae is usually destructive
- Most of fine structures cannot be visualized
II. AM monoxenonic cultures:

- Allow continuous, non-destructive measurements and observations
- Compatible with high resolution techniques and *in vivo* microscopy

Bécard & Fortin (1988)
**Monoxenic culture of arbuscular mycorrhizas: historical milestones**

- **1962**  Mosse’s first report of mycorrhiza under “aseptic” conditions
- **1975**  Mosse and Hepper describe the first successful monoxenic culture
- **1986**  Strullu and Romand use intraradical vesicles as starting material
- **1987**  Mugnier & Mosse use ROC as host for mycorrhiza “monoxenic” culture
- **1988**  Bécard and Fortin establish the first successful, continuous, aseptic mycorrhizal monoxenic culture
- **1996**  St-Arnaud et al. develop the two-compartment system. First reports on physiological applications (Bago et al., Villegas et al.)
- **1998**  Use of monoxenic cultures for studies on morphogenesis, biochemistry and genetics widens. Mycorrhizal “gurus” still reluctant.
- **2001**  The Glomeromycota In vitro Collection (Canada, Belgium) is inaugurated as the first bank of in vitro AM germplasm.
- **2000’s**  The dawn of the “mycorrhizal monoxenic age”...
Research on MYCORRHIZAS at Université Laval

A. Fortin

G. Bécard

Y. Piché

L. Simon

H. Vierheilig
Monoxenic culture of mycorrhizas: contribution to basic research
Life cycle

Intraradical
• Homogeneous medium (root cells)

Extraradical
• Heterogeneous medium (soil)

Asymbiotic
• Spore successive regerminations until symbiosis establishment

Appresorium
Germinating spore
Sporulation
Arbuscule
BAS
Entry point with appresorium

Arbuscule

Vesicle

Intraradical

Extraradical

BAS
Asexual spore formation

Crushed spore

Nuclei

DAPI staining
Arbuscular mycorrhizal fungi: coenocytic and multinucleated

Coenocytic hyphae

Lateral nuclei controlling hyphae

Migrating nuclei towards hyphal apex

Nuclei in BAS
Co-evolution of *Glomeromycota* with land plants:

- **Fossil mycorrhiza** c. 400 M year
- **Contemporary mycorrhiza**

**OBLIGATE SYMBIONTS**
CAUSE OF THE OBLIGATED BIOTROPHISM?
Arbuscular mycorrhizas physiology

Bago (2000) Plant & Soil

Bidirectional transport

Soil

P, N, water

Root

P, N, water

C
Carbon, nitrogen and phosphorus combined fluxes in AMF: an integrative proposal

Bago et al. (2000) New Phytol
Lipid and glycogen fluxes at the origin of massive C intra- to extraradical exportation in AMF

G. intraradices: 0.26 µg TAG/h
Gi. margarita: 1.34 µg TAG/h
AM ecology

Bago et al. (2000)

Stable aggregate formation
Monoxenic culture of mycorrhizas: contribution to inoculant production and large scale use
Cultivation of Mycorrhiza in SOIL or SOLID substrates (conventional methods)

- Being solid:
  - Loss of viable structures of colonization
  - Presence of non-soluble substrates
  - Difficult application by irrigation

- Not being a sterile production process:
  - Presence of other microorganisms
Optimizing mycorrhiza cultivation “IN VITRO” first…

…then making it available!!

CSIC Patent WO/2007/014974:

“Mycorrhizal aseptic inoculant and procedures of application in in-vitro and ex-vitro conditions”

Inventors: Custodia Cano Romero, Alberto Bago Pastor
Ultrapure, autochthonous mycorrhiza gel-type inoculants

• Since it is a GEL...
  ➢ Perfect application by irrigation
  ➢ No insoluble substrates
  ➢ Keeps active all ways of mycorrhization

• Since it is sterile-produced (“in vitro”)...
  ➢ Absence of other microorganisms
vs. OTHER MYCORRHIZAL PRODUCTS

The ONLY mycorrhizae gel product in the world!
Application by Irrigation

(Do not contain clay rests or other non-soluble substances due to its production process)

Totally soluble **semi-liquid gel** vs. competitor products with clay and other non-solubles substances
Certificate of purity
(rg to “in vitro” cultivation, we certify that the product is free of other microorganisms)

Sterile conditions of production from culture to bottling. Solid, conventional formulations don’t allow this process.
• 3 different propagule types:

- **HYPHAE**
  - Faster colonization
  - Only survive in a gel medium

- **ROOTS FRAGMENTS**

- **SPORES**: Long lasting, but slow colonization speed

Colonization time:
- **MYCOGEL**: 2 weeks
- **OTHER**: 4 weeks

(3 different propagation ways are active in the gel, accelerating fungus colonization time)
Autochthonous Fungi:

• Better adaption to soil conditions (physical, chemical and microbiological).

• Better efficacy on plants

• Promote Biodiversity and microbiological recovering/sustainability of soil ("calling effect")

Non Autochthonous Fungi:

• Selection of infective, aggressive fungi

• Substitution of authochtonous species (lost of biodiversity)
1) Soil prospection

2) Mycorrhizal “traps” to multiply fungi

3) Isolation and purification of arbuscular mycorrhizal fungi

4a) In vitro culture

4b) Ex vitro culture

Isolation of autochthonous arbuscular mycorrhizal fungi (c. 1 year each!)
**Propagules concentration**

50,000,000 cfu/l

(Our patented “in vitro” production process allows obtaining ultra-concentrated propagules formulation)

**MYCOGEL**

**OTHER**

100 spores/g

100,000 cfu/l
Effects of applying MYCOGEL in crops

- Higher Plant Vigor
- Higher Root Development
- Earlier and Better Yield
- Better Fruit Quality (flavour, shelf life)
- Increases Plant Resistance to drought and pathogens

- HEALTHIER PRODUCTION
Trials and Results
TRAIL RESULTS

LETTUCE (Granada - SPAIN)

GARLIC (Granada - SPAIN)
<table>
<thead>
<tr>
<th>TOMATO</th>
<th>Campaign 09/10</th>
<th>Campaign 10/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface (Ha)</td>
<td>0,6</td>
<td>0,6</td>
</tr>
<tr>
<td>Number of plants</td>
<td>3.500</td>
<td>3.500</td>
</tr>
<tr>
<td>Variety</td>
<td>Torry</td>
<td>Torry</td>
</tr>
<tr>
<td>Production (Kg)</td>
<td>40.000</td>
<td>60.000</td>
</tr>
<tr>
<td>Fertilization cost N</td>
<td>29 €</td>
<td>--</td>
</tr>
<tr>
<td>Fertilization cost P</td>
<td>100 €</td>
<td>--</td>
</tr>
<tr>
<td>MYCOGEL cost</td>
<td>--</td>
<td>40 €</td>
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<table>
<thead>
<tr>
<th>EGGPLANT</th>
<th>Campaign 09/10</th>
<th>Campaign 10/11</th>
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<tbody>
<tr>
<td>Surface (Ha)</td>
<td>0,4</td>
<td>0,4</td>
</tr>
<tr>
<td>Number of plants</td>
<td>2.200</td>
<td>2.200</td>
</tr>
<tr>
<td>Variety</td>
<td>Cristal</td>
<td>Cristal</td>
</tr>
<tr>
<td>Production (Kg)</td>
<td>29.400</td>
<td>32.000</td>
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<tr>
<td>Fertilization cost N</td>
<td>29 €</td>
<td>--</td>
</tr>
<tr>
<td>Fertilization cost P</td>
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<tr>
<td>MYCOGEL cost</td>
<td>--</td>
<td>20 €</td>
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Data obtained under agronomic conditions. El Ejido (Almería, Spain)
## Trail Results

<table>
<thead>
<tr>
<th>TON/Ha</th>
<th>1ª</th>
<th>2ª</th>
<th>3ª</th>
<th>1ª+2ª</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0Kg/Ha</td>
<td>3,3</td>
<td>2,3</td>
<td>0,8</td>
<td>5,6</td>
<td>6,4</td>
</tr>
<tr>
<td>MYCOGEL® 0Kg/Ha</td>
<td>4,1</td>
<td>1,7</td>
<td>0,7</td>
<td>5,8</td>
<td>6,5</td>
</tr>
<tr>
<td>Control 50Kg/Ha</td>
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<td>2,5</td>
<td>1,4</td>
<td>8,6</td>
<td>10,0</td>
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<tr>
<td>MYCOGEL® 50Kg/Ha</td>
<td>8,6</td>
<td>3,1</td>
<td>0,9</td>
<td>11,7</td>
<td>12,6</td>
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<tr>
<td>Control 130Kg/Ha</td>
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<td>2,6</td>
<td>1,1</td>
<td>11,7</td>
<td>12,8</td>
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<tr>
<td>MYCOGEL® 130Kg/Ha</td>
<td>10,4</td>
<td>3,3</td>
<td>0,8</td>
<td>13,7</td>
<td>14,5</td>
</tr>
</tbody>
</table>

**Potato**

Trial carried out by Universidad Nacional (COLOMBIA)
TRIAL RESULTS

Oil Palm Tree

0 P + MYCOGEL®

50% P Control

Trial carried out by Universidad Nacional (COLOMBIA)
Melon

**TRIAL RESULTS**

**MYCOGEL®**  
**MYCOGEL® + Humic acids**  
**CONTROL -**

<table>
<thead>
<tr>
<th></th>
<th>Total Weight (Ton/Ha)</th>
<th>Nº fruits / line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38,4</td>
<td>7,8</td>
</tr>
<tr>
<td>MYCOGEL®</td>
<td>46,5</td>
<td>9,3</td>
</tr>
<tr>
<td>MYCOGEL® + HUmic</td>
<td>40,8</td>
<td>8,3</td>
</tr>
</tbody>
</table>

Trial carried out by Independent consultant Valorhiz, (FRANCE)
TRIAL RESULTS

Green Beans

**Total fresh weight (with pod)**

- Weight (g)

**Number of fruit/ plant**

- Number of fruit/plant

Trial carried out by Independent consultant Valorhiz, (FRANCE)
TRIAL RESULTS

Vineyard

- CaCO$_3$

Control  MYCOGEL

+ CaCO$_3$

Control  MYCOGEL

Olive tree

Control  Foreigner  Autochthonous

Trial carried out by CSIC (Granada, SPAIN)
TRIAL RESULTS

Banana

Trial carried out by ICIA (Canary Islands, SPAIN)

Avocado

Trial carried out by IFAPA (Malaga, SPAIN)
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MERCI!
¡GRACIAS!

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