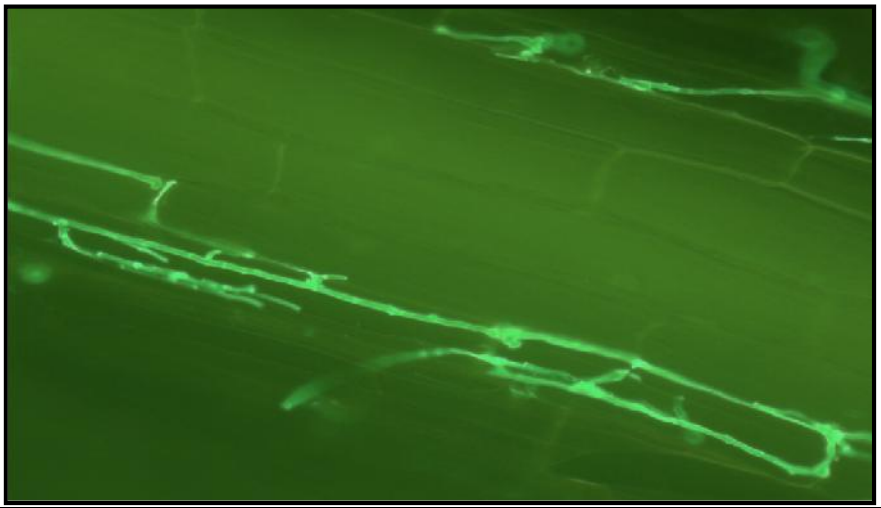
Leibniz-Institut für
Gemüse- und Zierpflanzenbau
Großbeeren & Erfurt
Leibniz Gemeinschaft

# Characterization of phosphate solubilisation and uptake capacity by root fungal endophytes

Diana Rocio Andrade-Linares
Benard Ngwene
Philipp Franken



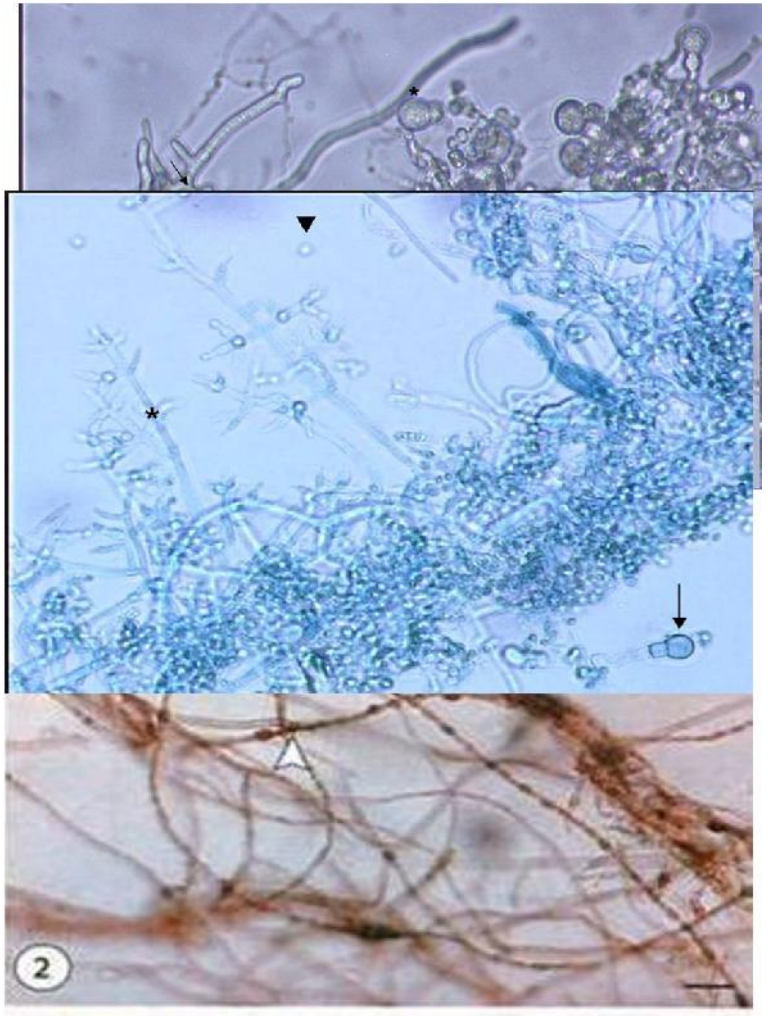
COST FA1103: Endophytes in Biotechnology and Agriculture
14 - 16/11/12, S. Michele all'Adige, Trento, Italy.

# Facultative symbiontes in roots

***Piriformospora indica***

***Trichoderma harzianum***

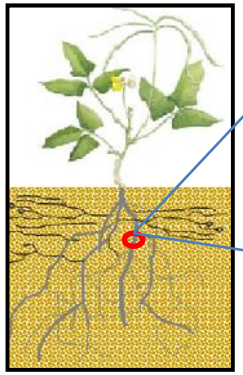
**Dark septate endophytes**
**DSE**

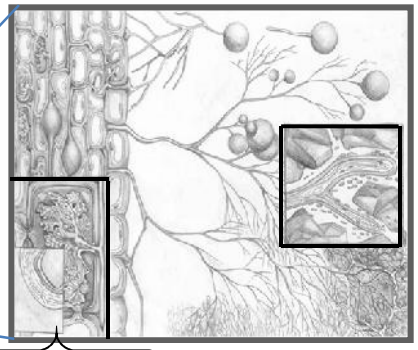


# Fungal endophytes

- Contribution to mineral element uptake and promotion of plant growth

## AM Association

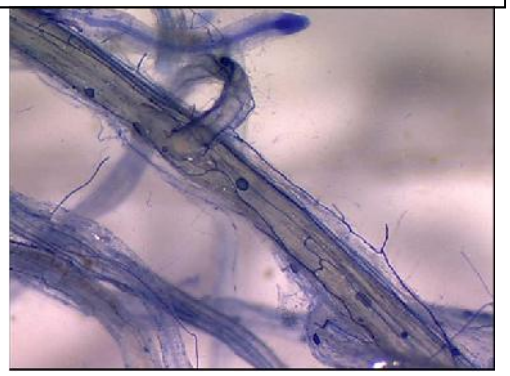




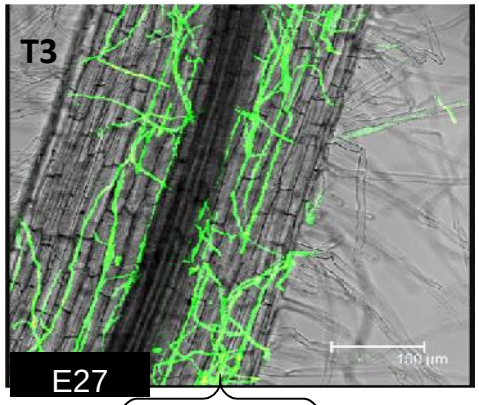
Root cortex: IRM

Soil: ERM

Intra- & extraradical structures

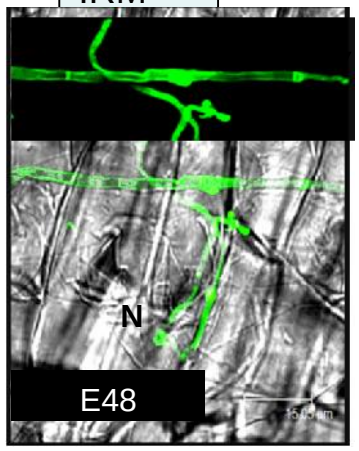


## *RE* association




Root cortex: IRM

Soil: ERM **?**




Intraradical structures Andrade-Linares et al 2011 (Mycology)

# Fungal endophytes

- Research focus

  - To evaluate phosphate solubilization of organic and inorganic phosphate sources and the potential production of phosphatases in liquid medium
  - To test the capability of *Piriformospora indica* to use organic nitrogen in vitro system
  - To evaluate inorganic phosphate transfer, plant growth promotion in tomato plants.
  - To evalute ERM growth of *P. indica* into root inaccessible soil compartments
  - To analyze the interaction between *P. indica* and *Glomus intraradicis* in the symbiosis with tomato plants

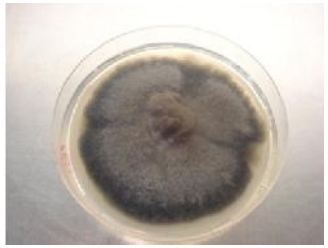
## General methodology

1. Phosphate solubilization
   - Lecithin
   - Phytin
   - Pikovskaya medium
   - Rock Phosphate

→ Agar
Different concentrations

→ Liquid culture
Available Phosphorous and enzimatic activity

DSE48



*L. orchidicola 135*

2. Protease activity in vitro on Casein source

*P. indica*

3. Phosphorous uptake in pot experiments

## 1. Phosphate solubilization

- Lecithin
- Phytin
- Pikovskaya agar

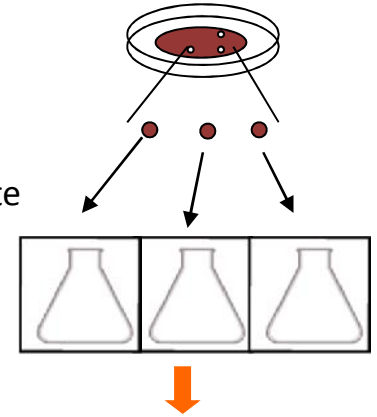
- Phosphate concentrations:
  500, 200, 100, 50, 0 mg/l

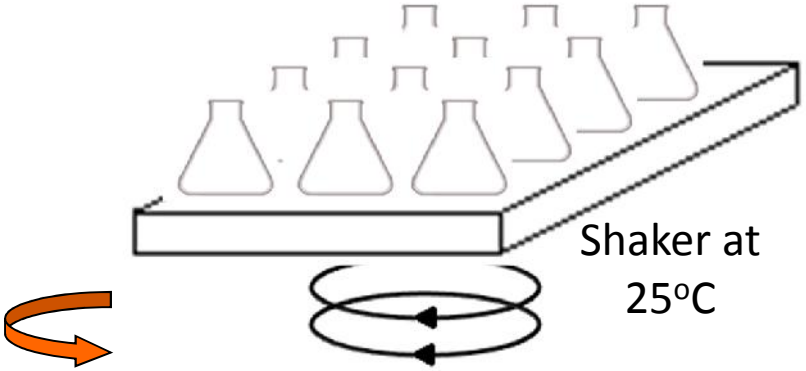
- Growth analysis and hydrolisis during 30 days of incubation

## 2. Available Phosphorous and enzimatic activity

Liquid culture

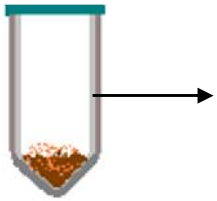
- $KH_2PO_4$ (499 mg/L P)
- Tri-calcium phosphate (TCP, $Ca_3O_8P_2$; 0.25%)
- Rock Phosphate (RP, $P_2O_5$; 0.347% )
- Lecithin
- Phytin





Shaker at 25°C

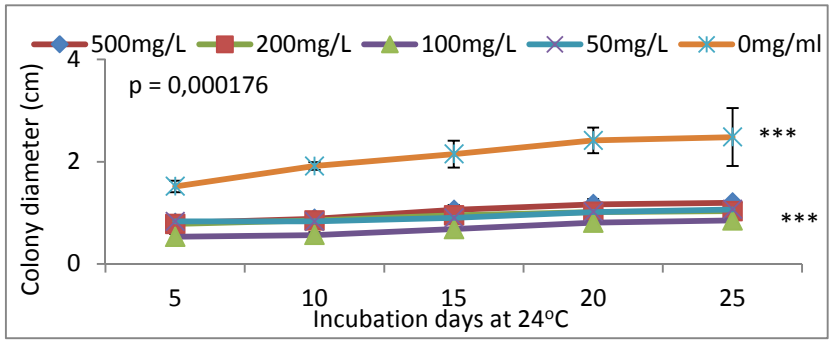
Sampling each 5 days

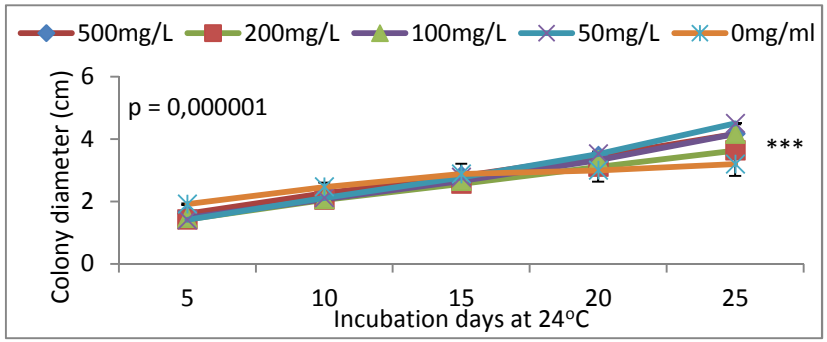


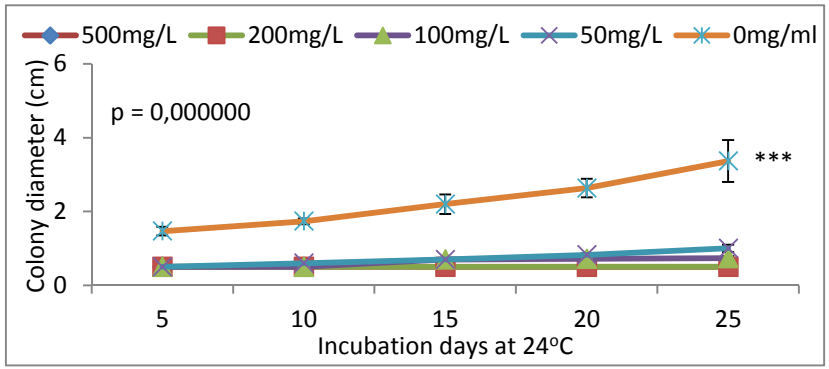
- P mg/L
- pH
- Enzymatic activity

# Assimilation and solubilization of different P- sources

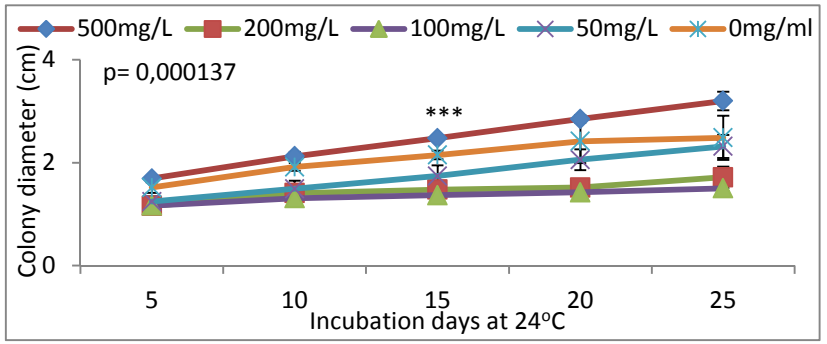
## Growth in Lecithin

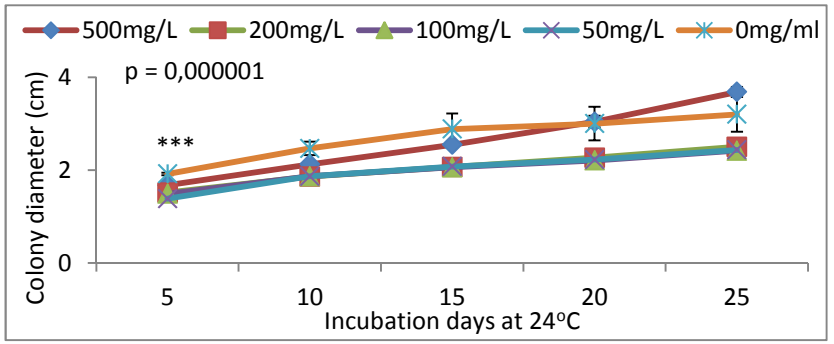


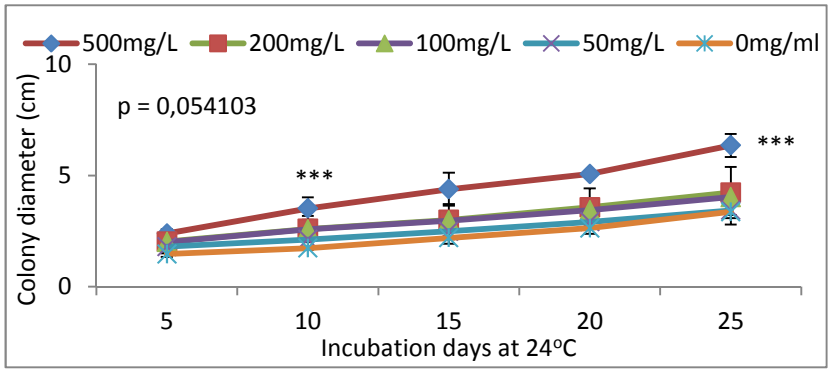




## Growth in Phytin



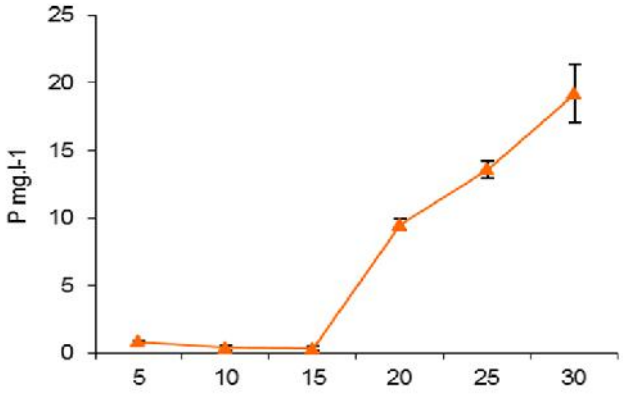

DSE48

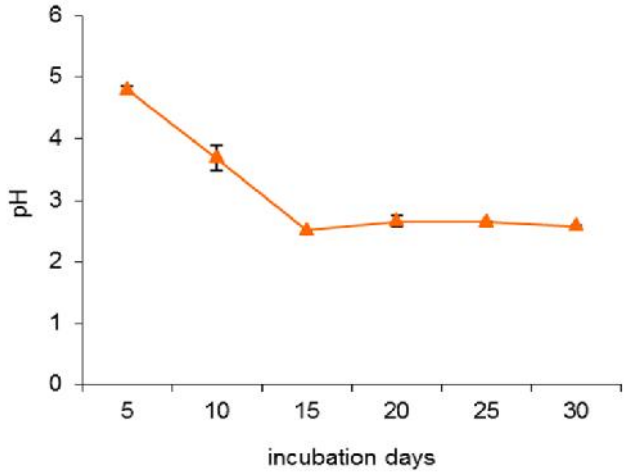
L. o 135

*P. indica*

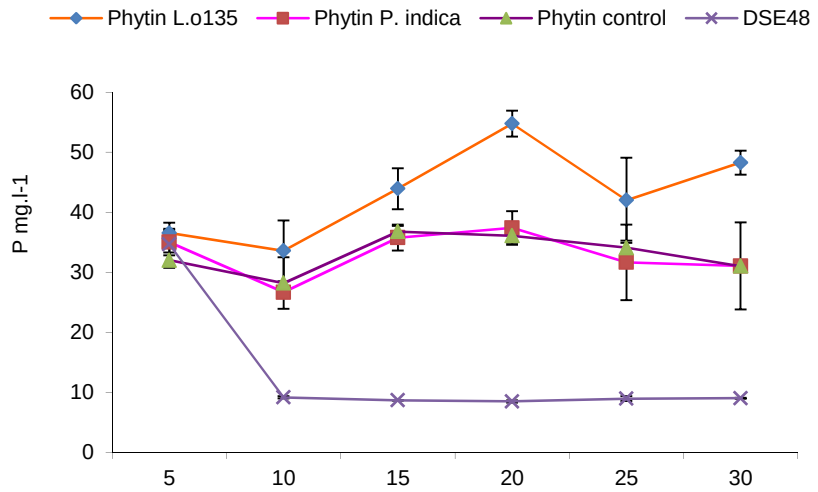
# Available Phosphorous and Enzimatic activity

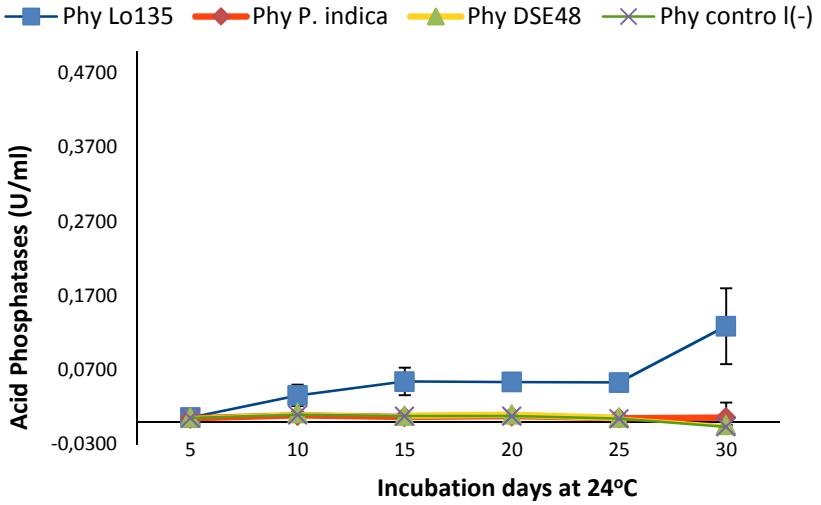
## Lecithin 500 mg/L




## Phytin 150 mg/L

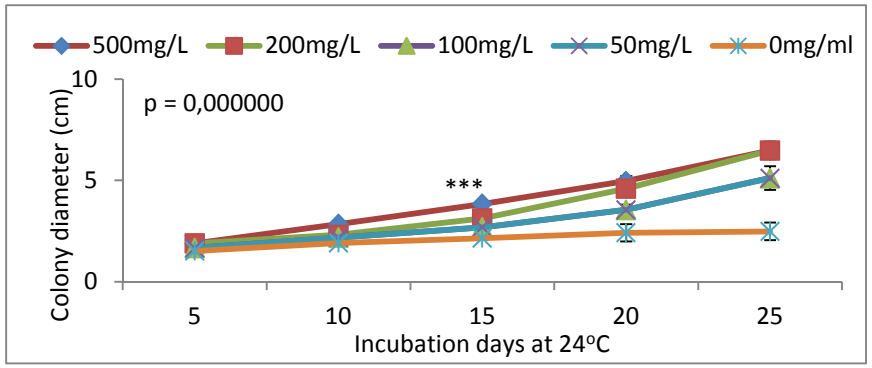


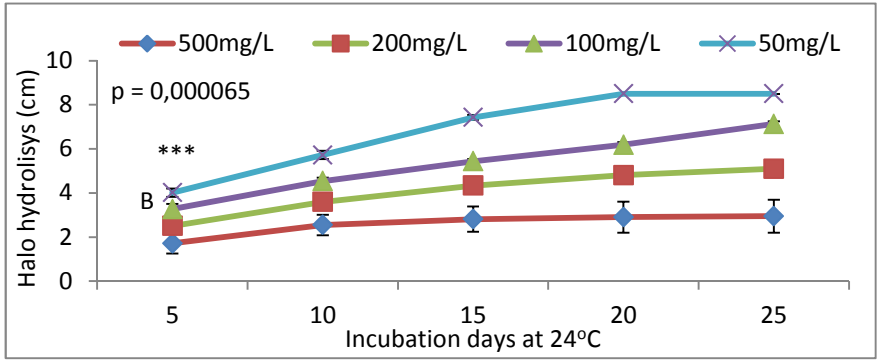



# Assimilation and solubilization of different P- sources

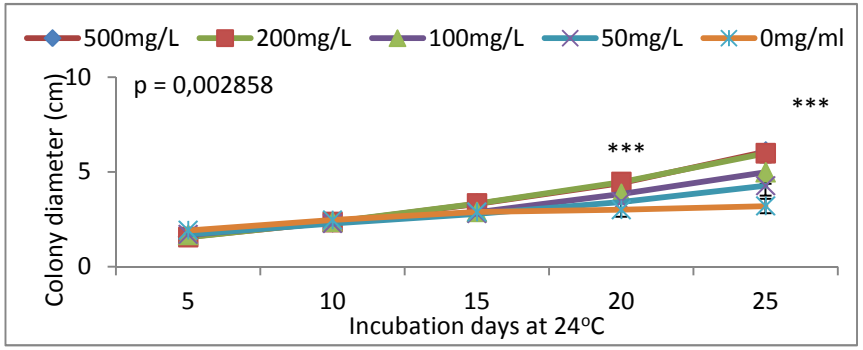
## Pikovaskaya agar with different concentrations of $Ca_3(PO_4)_2$ = ($Ca_3$-P)

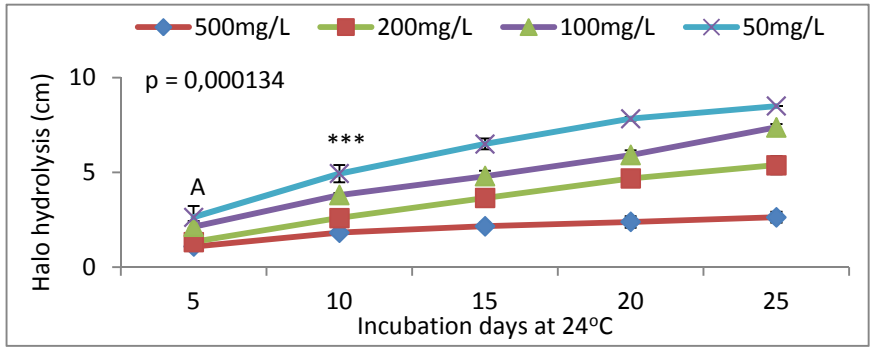
DSE48



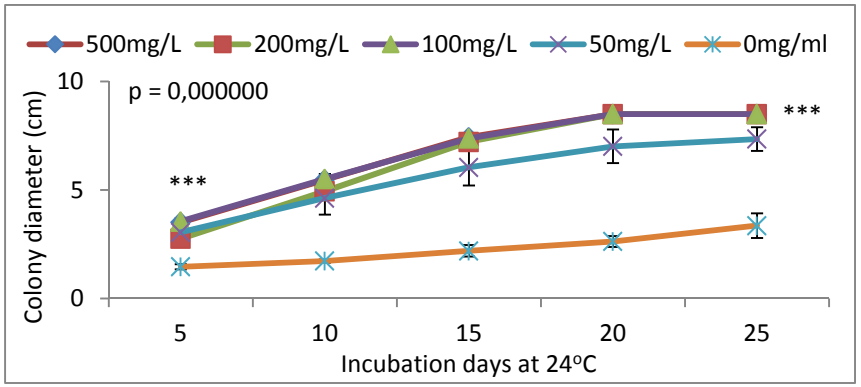




L. o 135



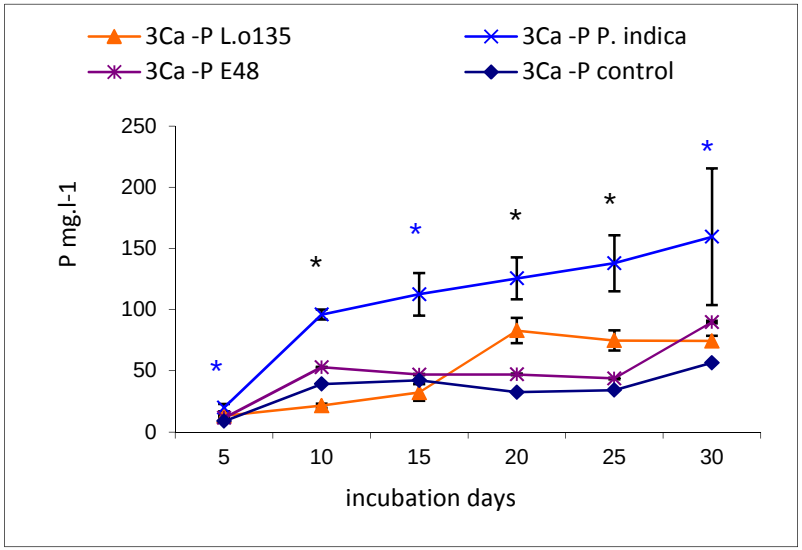




*P. indica*

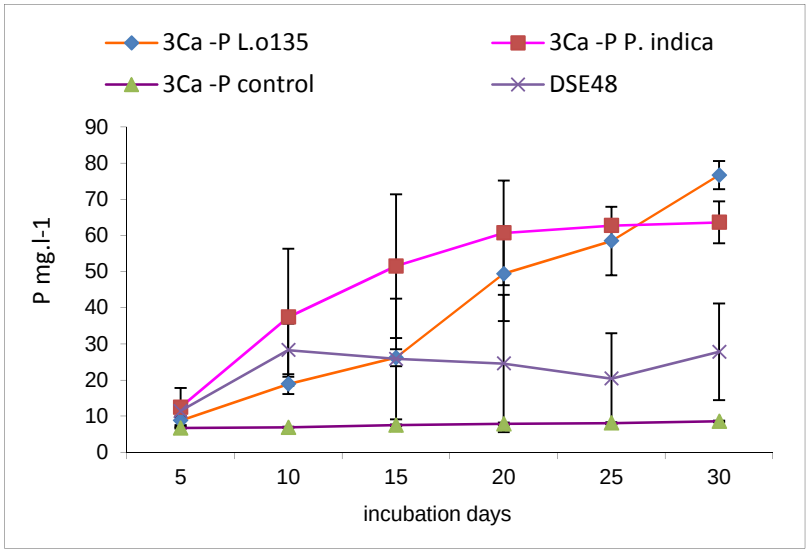


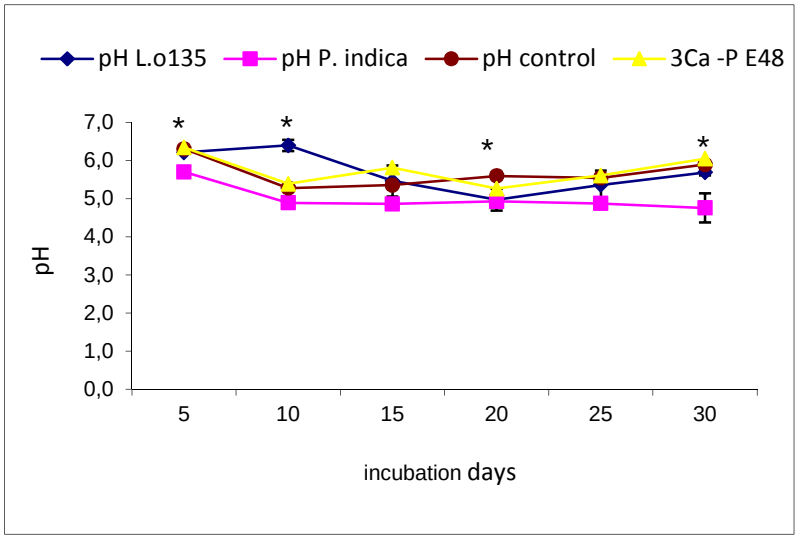
# Available Phosphorous and Enzimatic activity

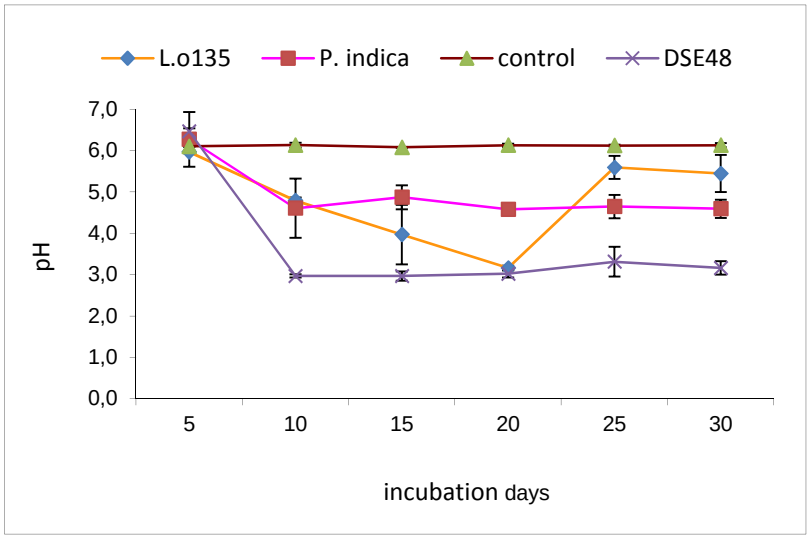
## $Ca_3$-P 500 mg/L




## $Ca_3$-P 150 mg/L

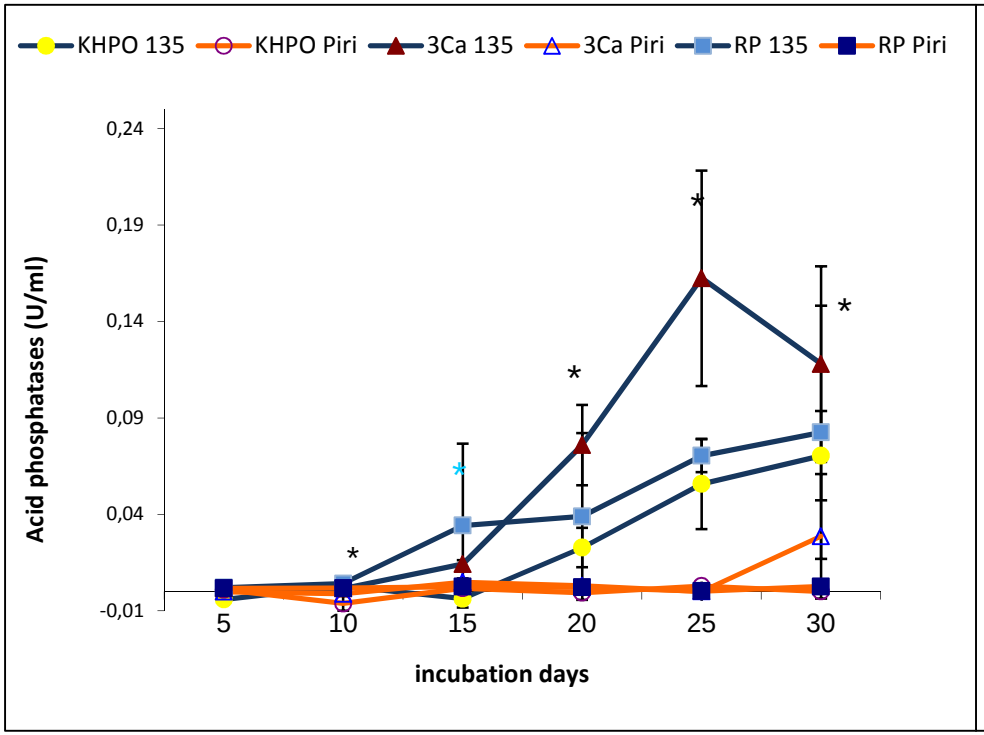


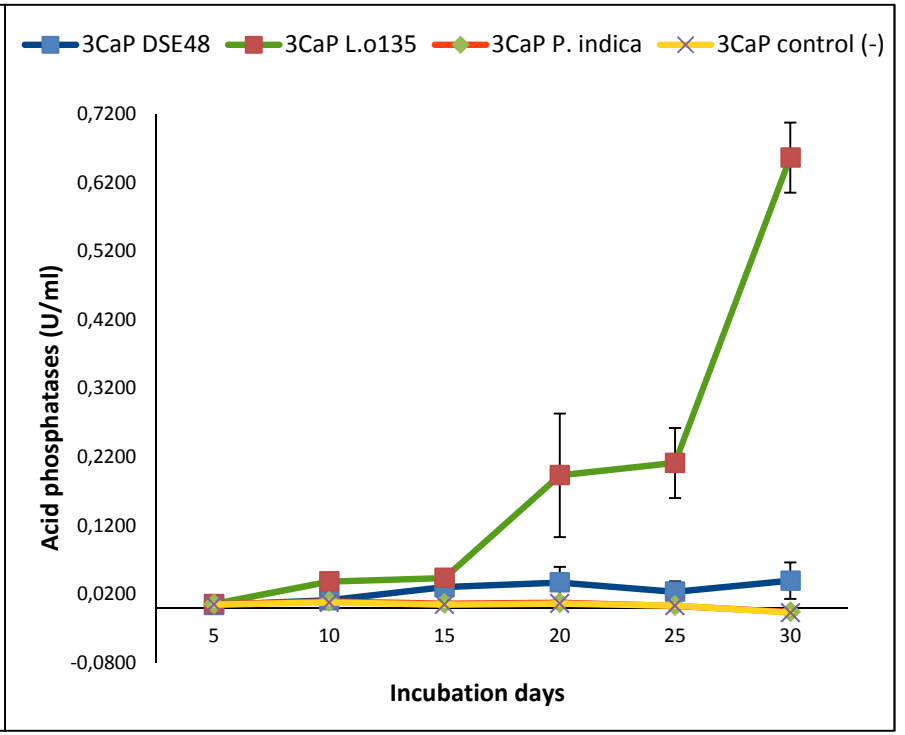



# Available Phosphorous and Enzimatic activity

Ca3-P 500 mg/L

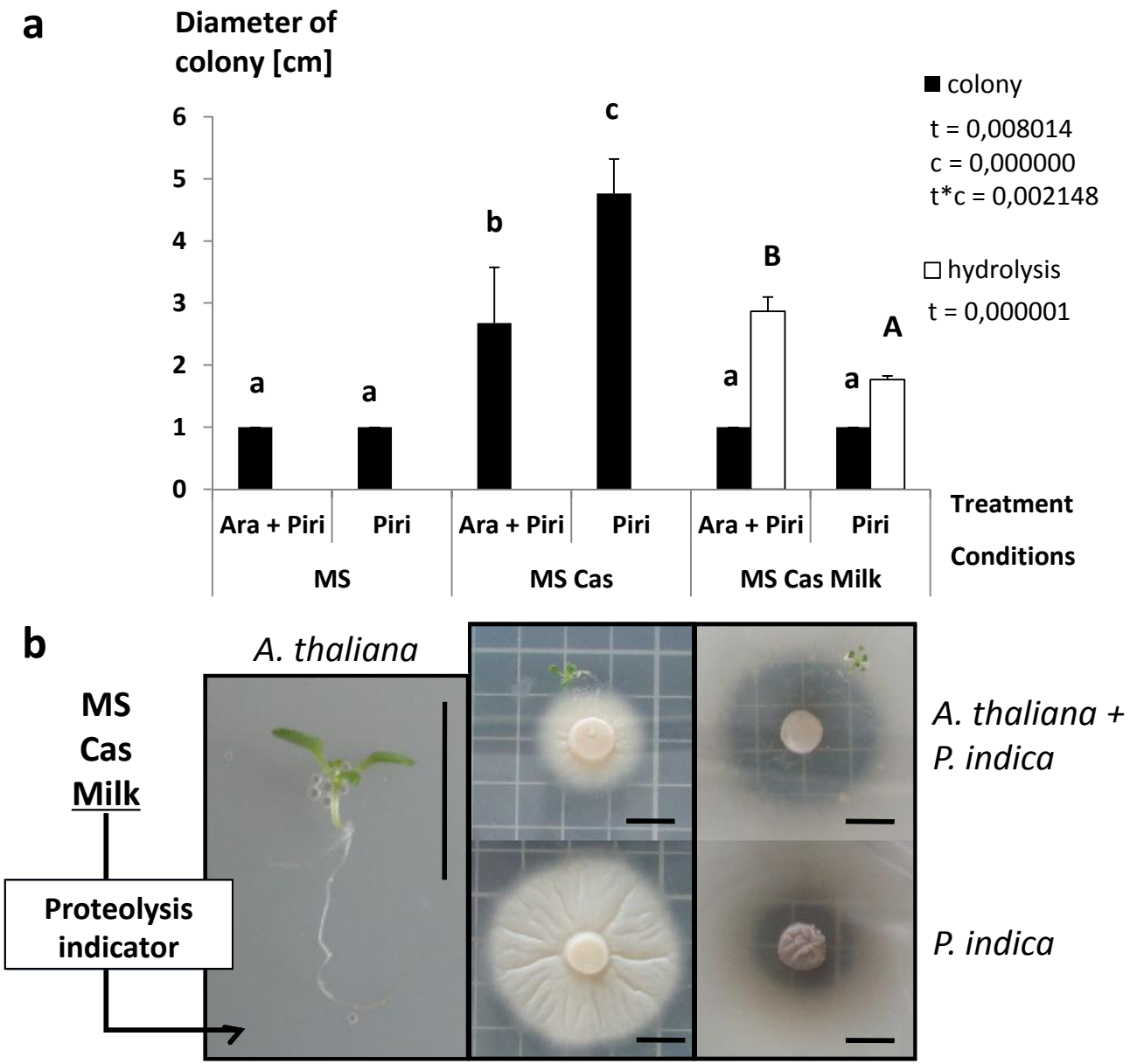



Ca3-P 150 mg/L



## 2. Protease activity

(Brunel, J. et al. 2012)



# Conclusions

- Lecithin inhibits growth of the DSE48 and *P. indica*, while *L. orchidicola* 135 is able to growth up to a concentration of 500 mg/L, however the release of $P_i$ is low (20mg/L) and after 20 days of incubation.

- The endophytes growth in high concentration of Phytin, specially *P. indica*, however it did not release $P_i$ and not present enzymatic activity in contrast to *L. orchidicola* 135.

- The fungal endophytes grow better at concentrations higher than 100 mg/L in Pikovskaya ($Ca_3$-P) agar but the solubilization was less evident at 500 mg/L $(PO_4)_{2.}$

- *P. indica* does not produce hydrolysis halo in comparison to the DSE in $Ca_3$-P but it is able to release $P_i$ in liquid medium and seems to be more efficient at higher concentration (500 mg/L) in contrast to *L. orchidicola* 135. However *P. indica* does not have extracellular phosphatase activity.

- *P. indica* does produce hydrolysis halo in casein agar which indicate the production of proteases which seems to be regulated by the host plant.

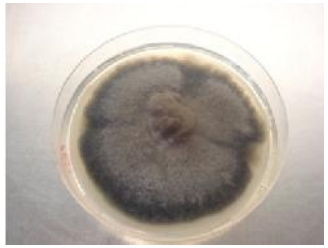
## General methodology

1. Phosphate solubilization
   - Lecithin
   - Phytin
   - Pikovskaya medium
   - Rock Phosphate

→ Agar
Different concentrations

→ Liquid culture
Available Phosphorous and enzimatic activity

DSE48



*L. orchidicola 135*

2. Protease activity in vitro on Casein source

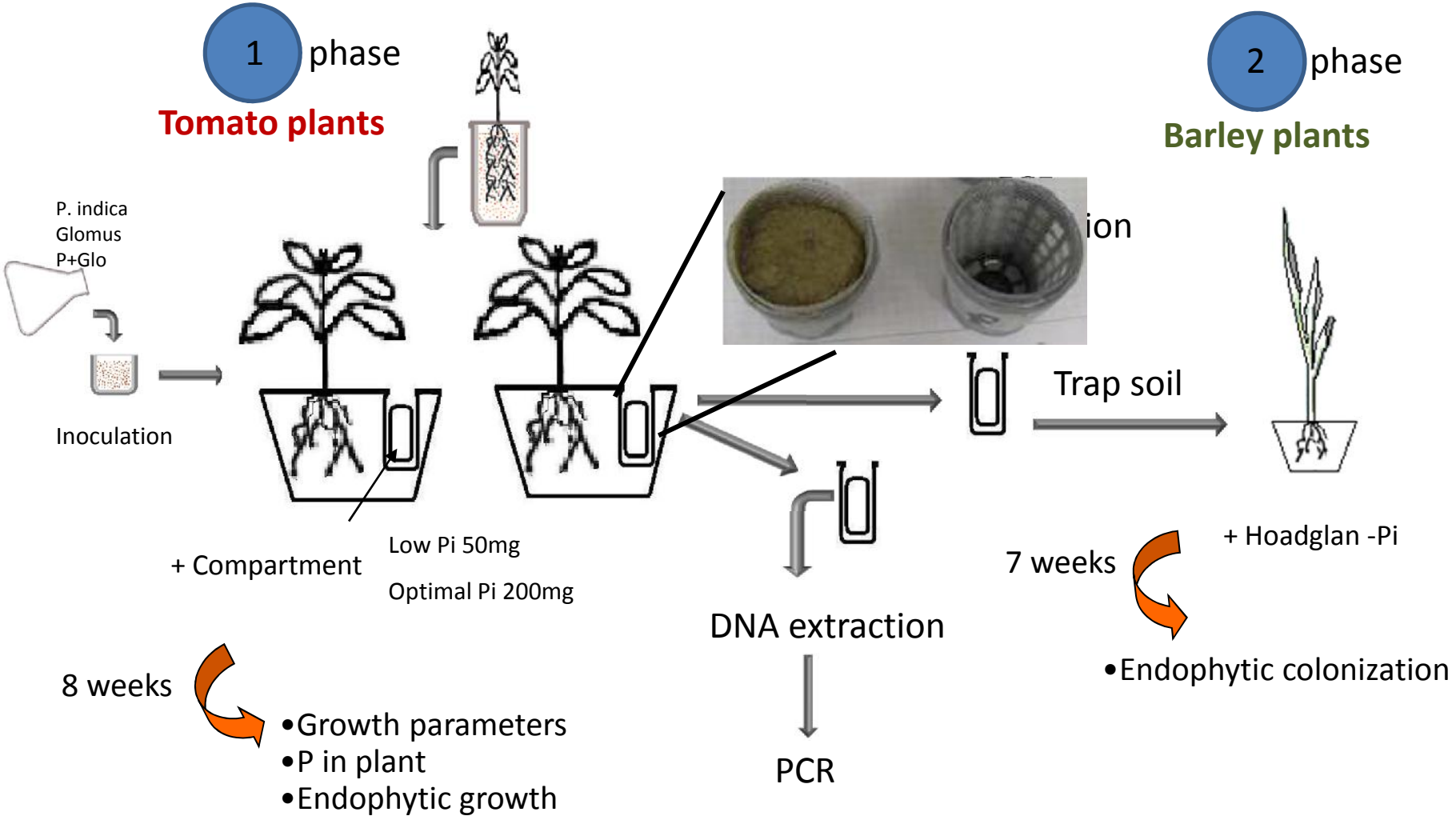
*P. indica*

3. Phosphorous uptake in pot experiments

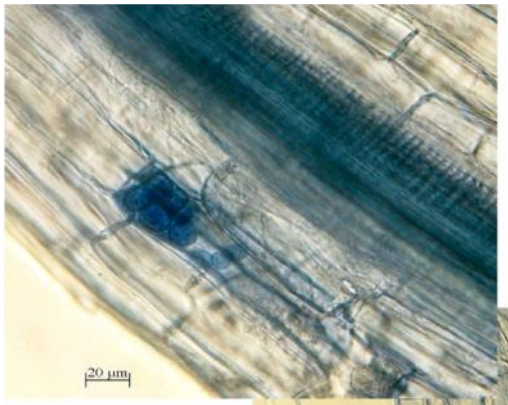
3. Phosphorous uptake: Pi content in plant

Root colonization

Extraradical Mycelium



# Tomato colonized roots (frequency and intensity)

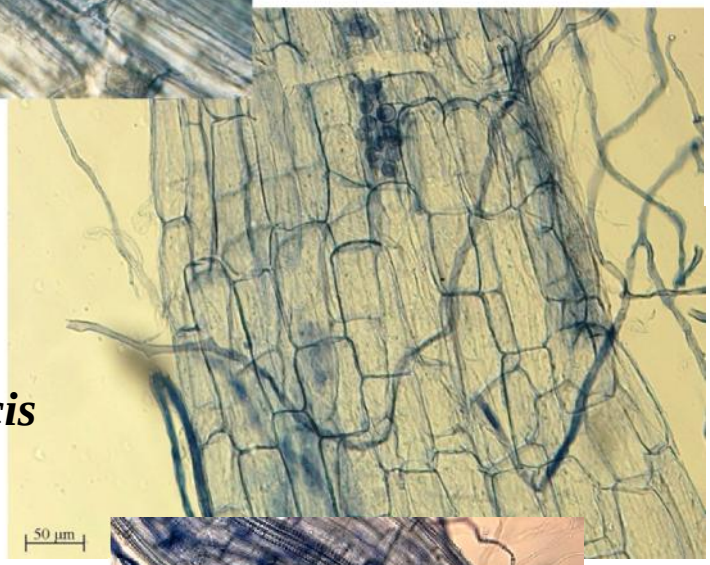


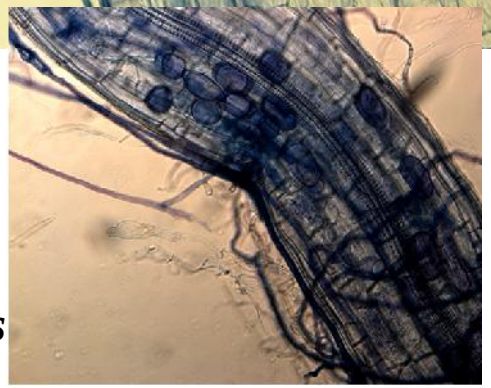

***P. indica***

***P. Indica***

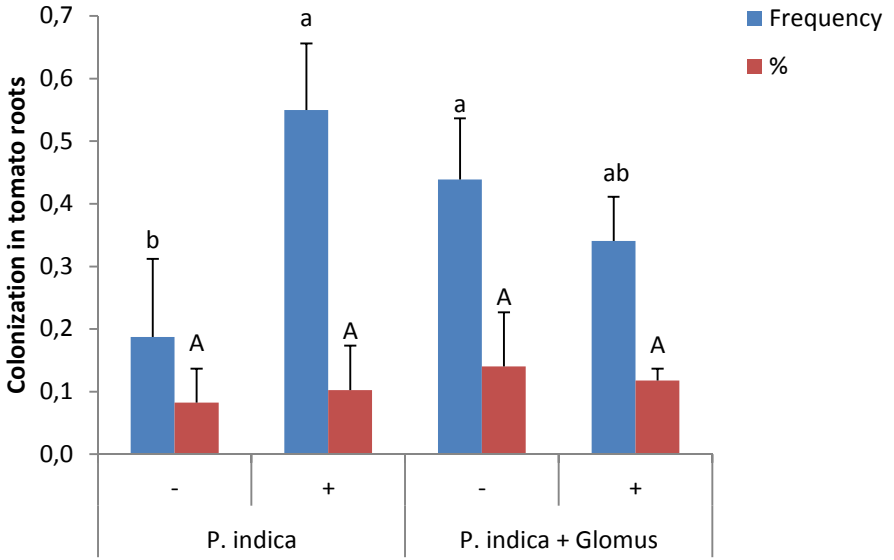
**+**

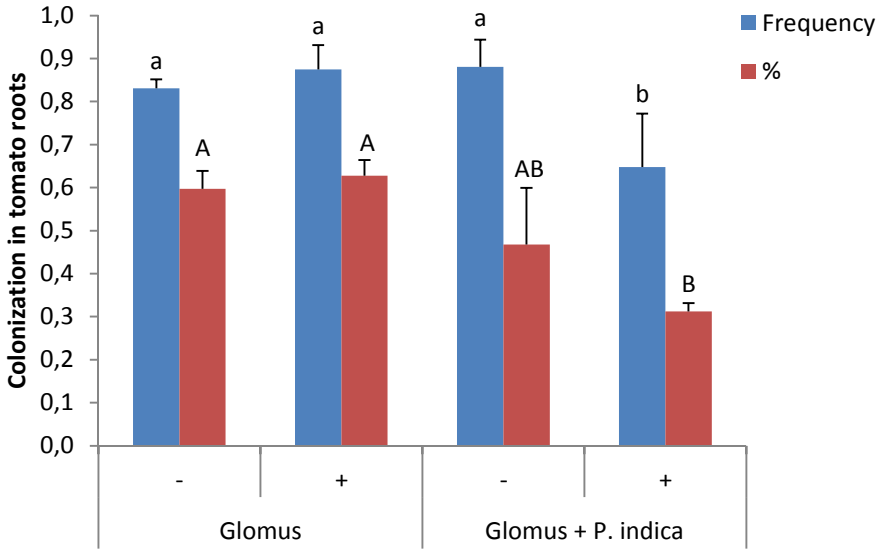
***G. intraradicis***



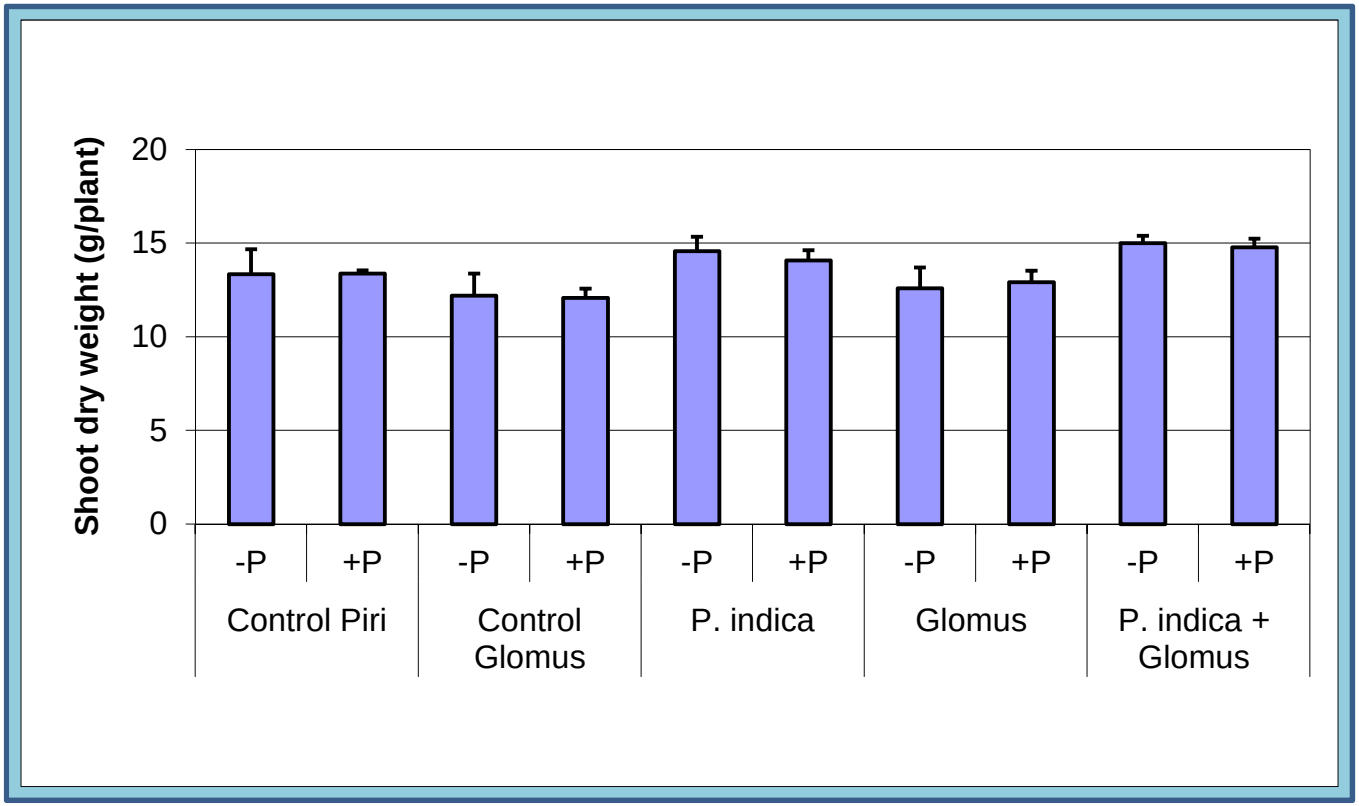



***G. intraradicis***



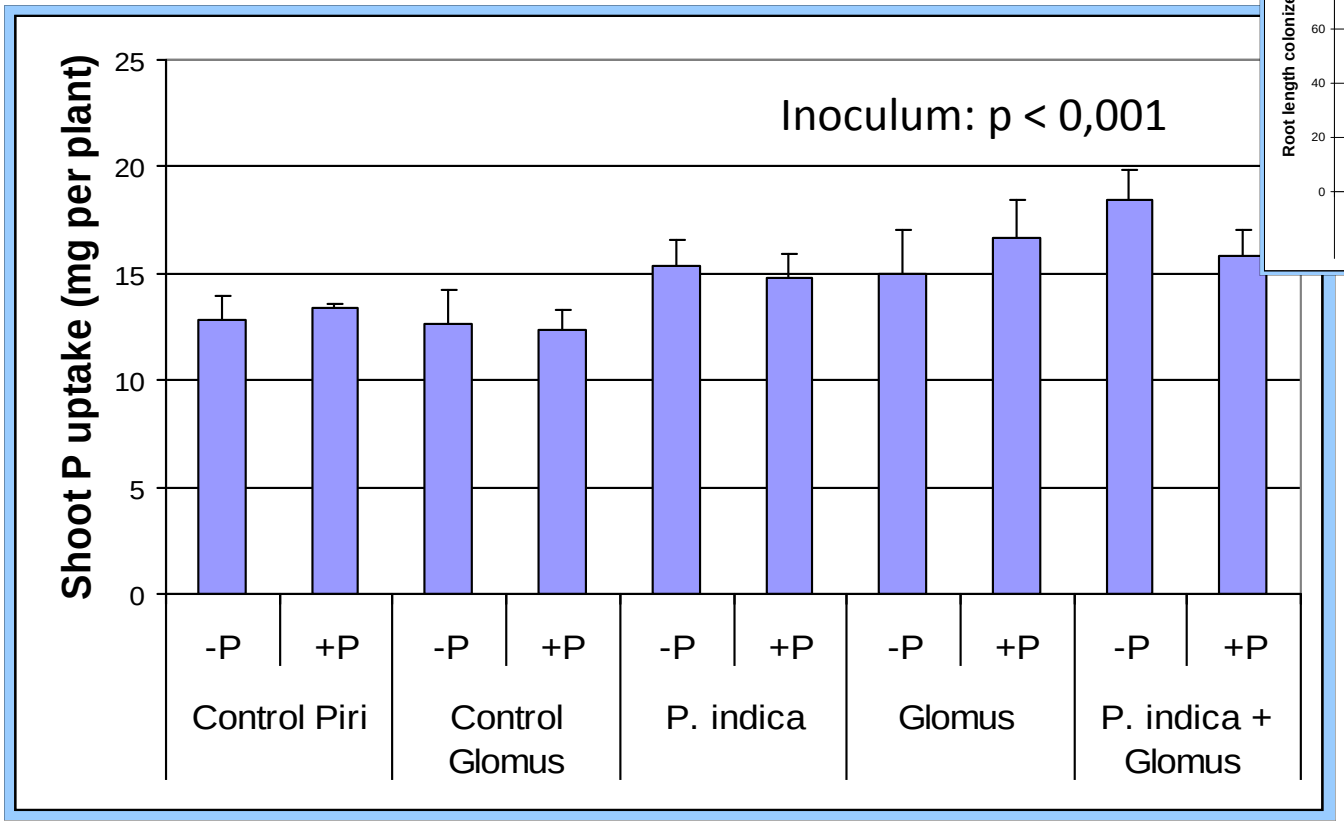



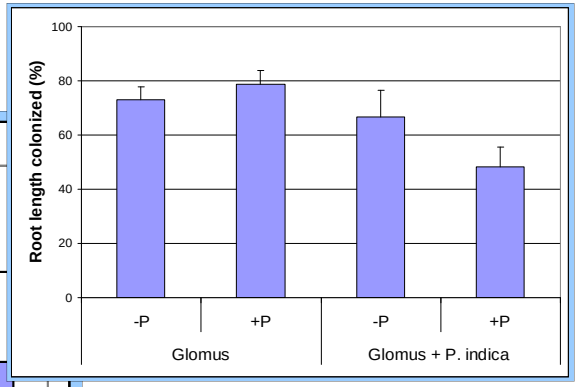
## Shoot dry weight after harvest (g per plant)




Effect of inoculation treatment

# Shoot P uptake after harvest (mg per plant)



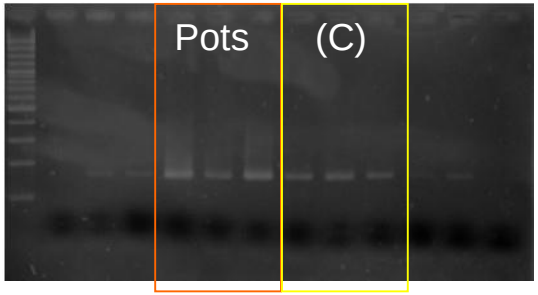




Effect of inoculation treatment

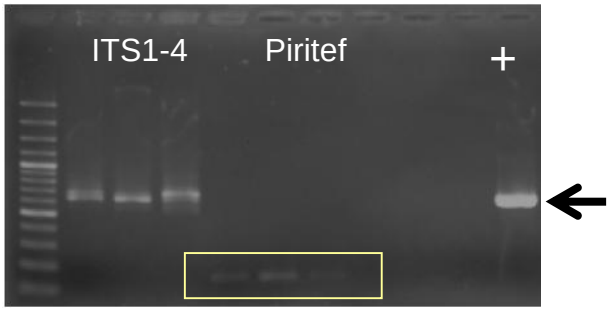
# Detection of extraradical mycelium of *P. indica*

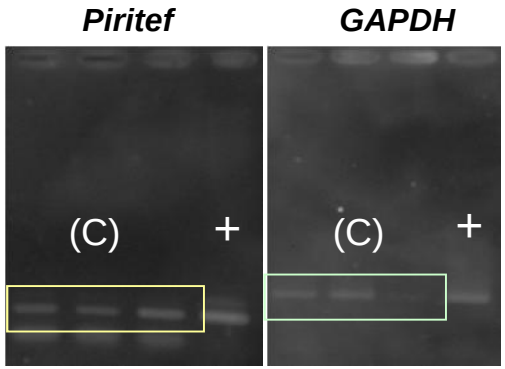
Soil of compartments

*P. Indica* in:




Soil for trap pots with barley



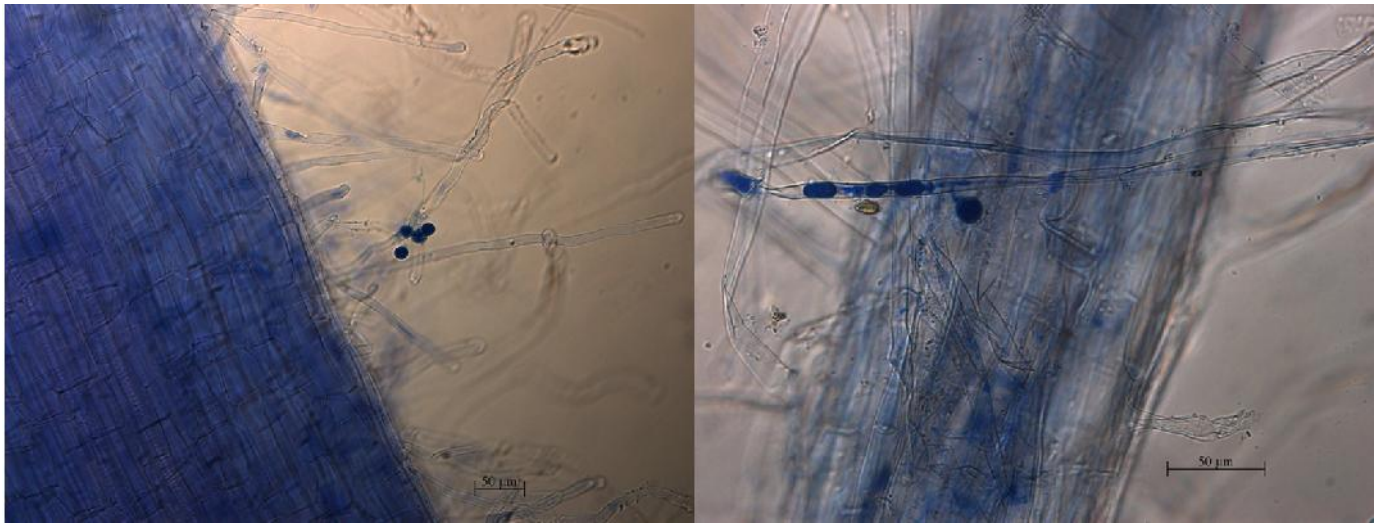




Primer pair for the translation elongation factor 1–α gene (TEF) : ***Piritef***

Primer pair for the glyceraldehyde-3-phosphate dehydrogenase gen (***GAPDH***)

*P. indica* and *G. intraradicis* in Compartment (C)

## Colonization of trap plant roots

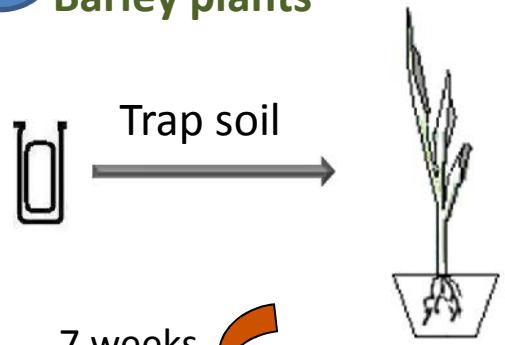


*P. indica* spores and hyphae attaching root hair of barley plant

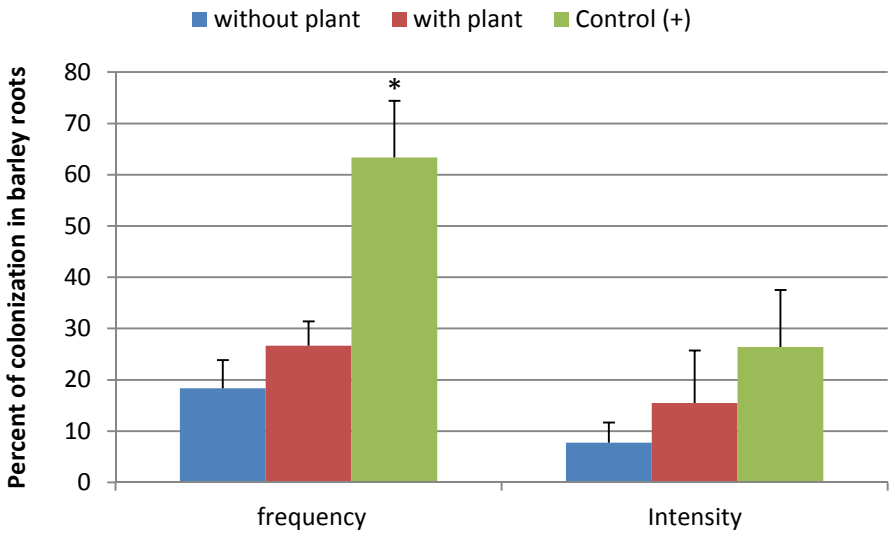
*P. indica* spores inside root hair of barley plant

2

**Barley plants**




- Endophytic colonization



# Conclusions

- Tomato root plants were colonized at the same time by *P. indica* and *G. intraradicis*. This colonization seems to be lower for *P. indica* than for *G. intraradicis*.

- However the AMF colonization was negatively affected in the combination treatment (Piri + Glomus) at high concentration of $P_i$ in the compartment.

- The inoculation with the endophyte *P. indica* showed a significant effect on plant dry weight as well as on P uptake and this effect is higher with the presence of the AMF in roots.

- *P. indica* is able to produce ERM which was detected by PCR in soil compartments and was also recovered in the roots of trap plants.

- Phosphorous and nitrogen uptake from organic sources and interaction witn AMF.

- Quantification of extraradical mycelium and root colonization at saprophytic and endophytec stages in soil.

- Regulation of phosphate transportes

- Influence of endohyphal bacteria on fungal and plant nutrition.

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