Arbuscular mycorrhizal (AM) fungi as an useful biotechnological tool for increasing plant defence mechanisms to alleviate different stresses

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http://archive.bio.ed.ac.uk/jdeacon/microbes/ecto14.jpg


http://sdhydroponics.com/resources/wp-content/uploads/2010/08/mycorrhiza.jpg

- **To contain the increasing impact of human activity** on climate change is required an effort to improve agricultural management strategies with biological alternatives to chemicals
- Arbuscular mycorrhizal fungi (AMF), the **most important terrestrial symbiosis**, represent valuable candidates to develop integrated pest management strategies

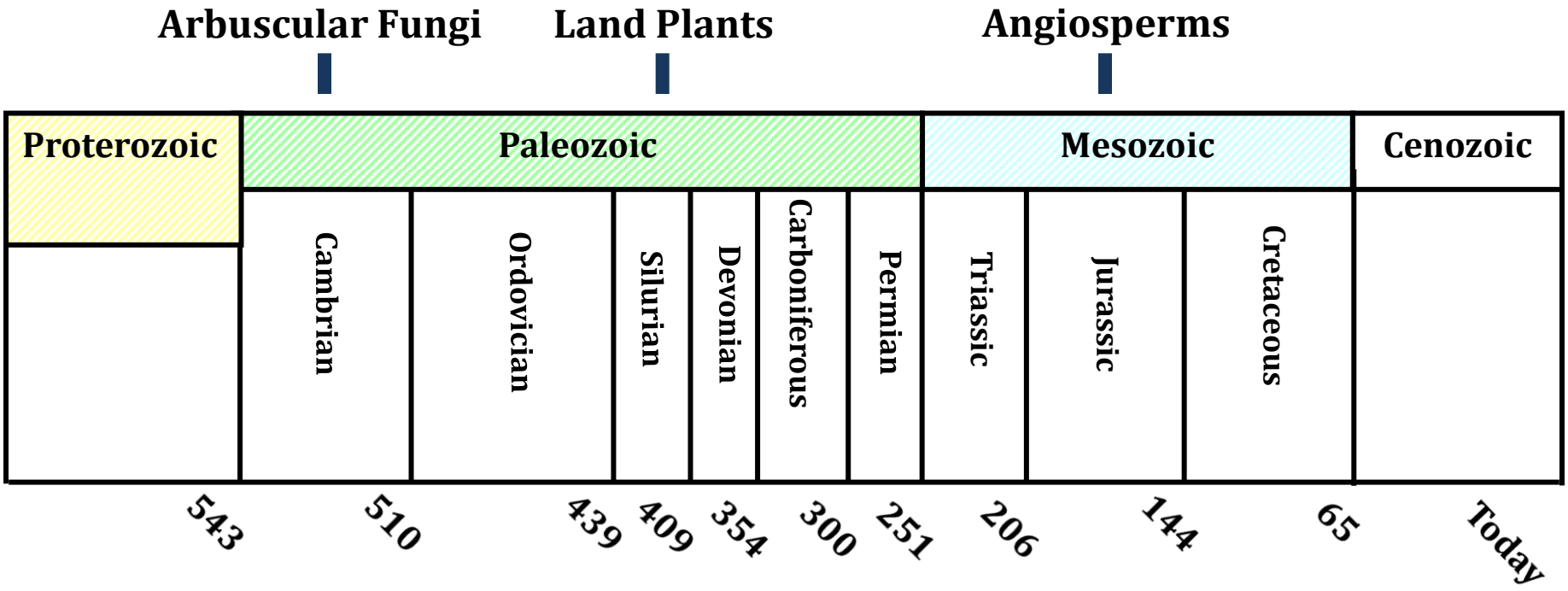

http://www.ictorganics.com/_ccLib/image/articles/DETA1-6.jpg

Under natural conditions, plants strictly speaking do not have roots,
they have mycorrhiza

Arbuscular mycorrhiza (AM): the mother of plant root endosymbioses

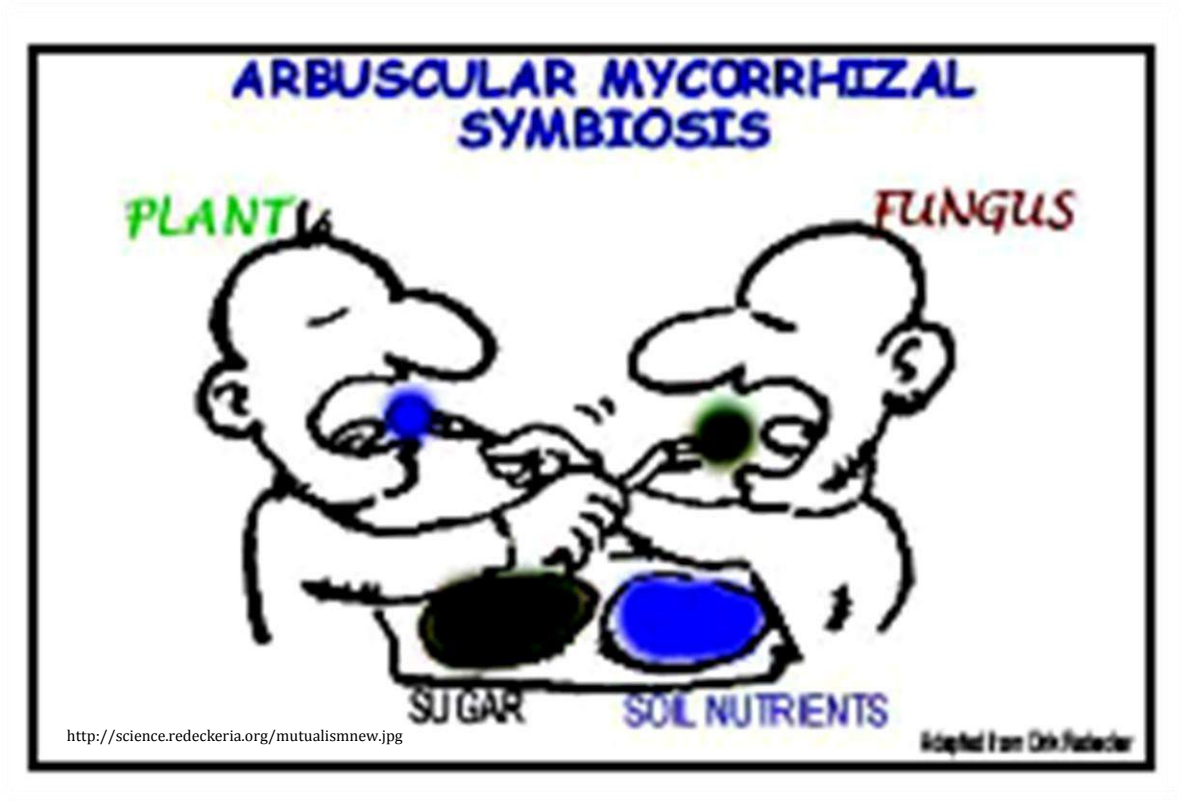
The most widespread terrestrial symbiosis : 70–90% of land plant species

The most ancient terrestrial symbiosis: AM fungi (phylum of Glomeromycota) are unusual organisms because of their age, lifestyle and genetic make-up; they have existed for more than 400 million years morphologically unaltered and could therefore qualify **as living fossils**

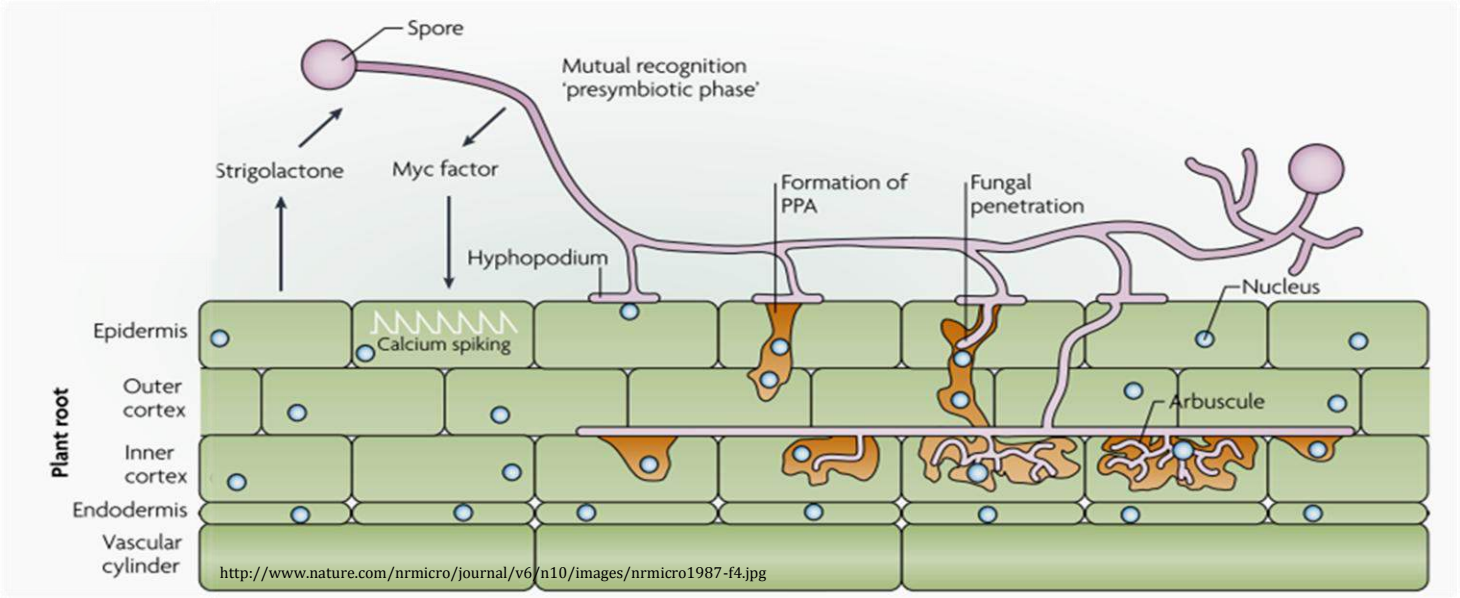


Beneficial effects of AM

The beneficial effects of AM are well known, including enhanced **uptake of water and nutrient** - mostly phosphorus -, moreover increased **resistance to different environmental stresses**



A rapid overview on Arbuscular Mycorrhizal Fungi features following their life cycle: from the soil to the root



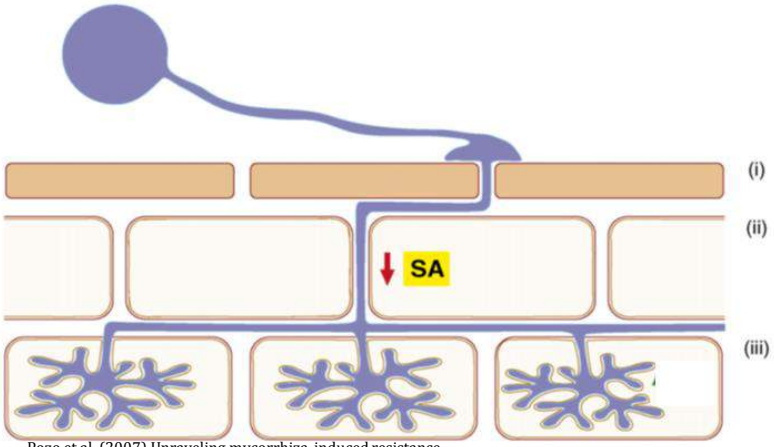
- **Obligate biotrophic organisms** (need their photoautotrophic partner to complete life cycle)
- The step of recogniton is mediated by an **exchange of molecular signals** (strigolactones - branching factors)
- As a consequence of the molecular cross-talking the fungus can produce a penetration apparatus

Plant defence mechanisms are tightly regulated through an interconnected network of signaling pathways

Salicylic Acid (SA) coordinates defence mechanisms generally effective against biotrophic pathogens

Jasmonic Acid (JA) regulates wounding responses and resistance against necrotrophs

AMF induce **partial suppression of SA-dependent responses** in the plant, in order to obtain a compatible interaction



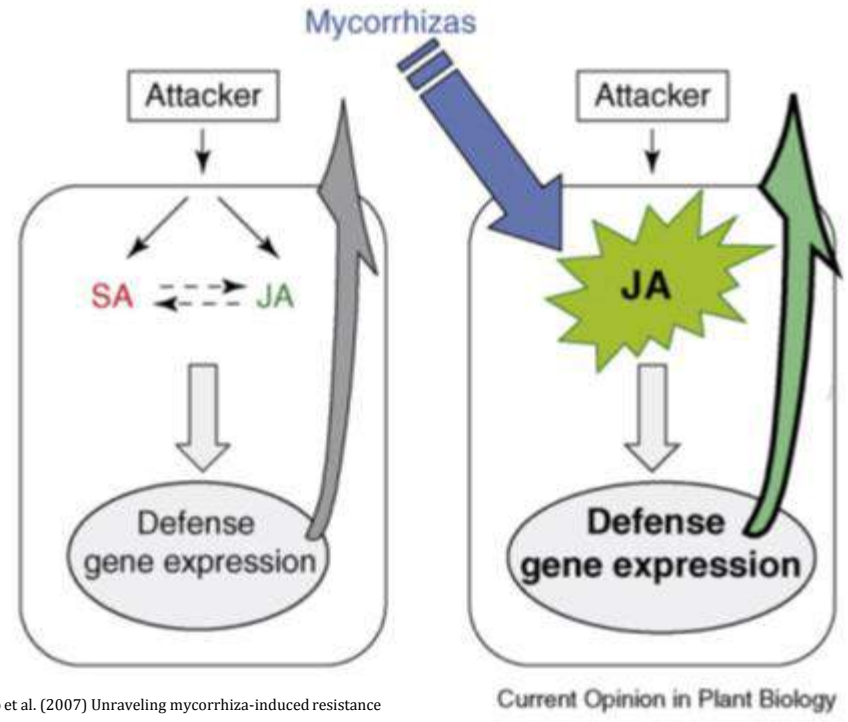
Pozo et al. (2007) Unraveling mycorrhiza-induced resistance

Current Opinion in Plant Biology

AMF induce partial suppression of SA-dependent responses in the plant, **compensated by an enhancement of JA-regulated responses**

JA-responsive genes and genes involved in JA biosynthesis are expressed in arbuscule-containing cells

Mycorrhizal roots are associated with increased levels of endogenous JA



Pozo et al. (2007) Unraveling mycorrhiza-induced resistance

Increased susceptibility to biotrophs

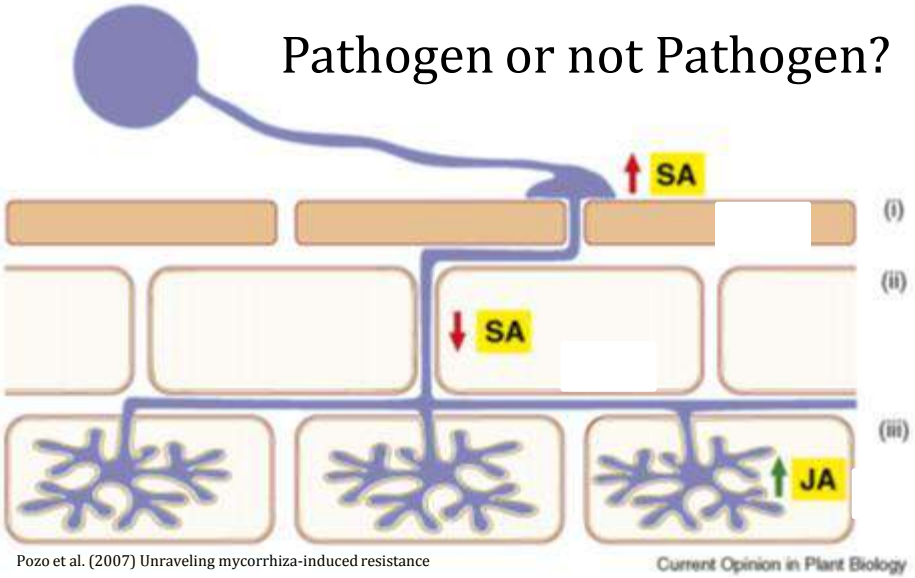
Increased resistance to necrotrophs and generalist chewing insects

Modulation of plant defense mechanisms

During the interaction a **molecular dialogue between plants and microorganisms** occurs in order to determinate the final outcome of the relationship:

parasitism ⟷ mutualism

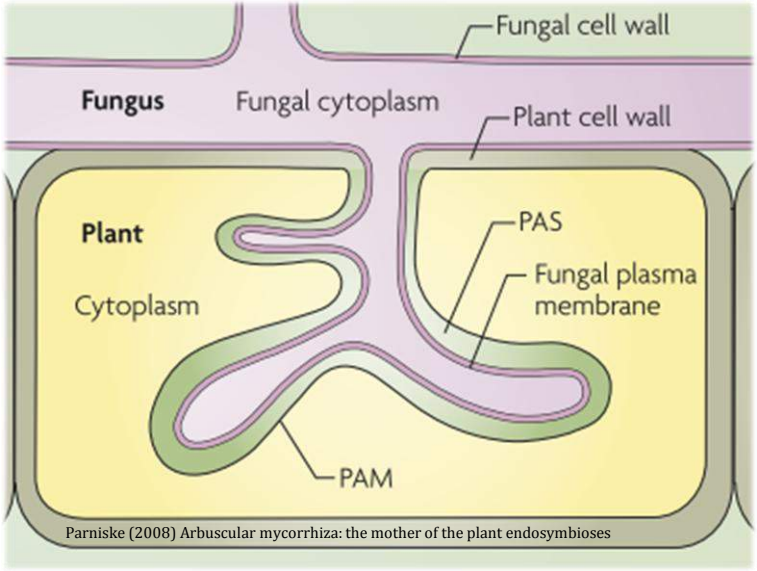
through highly coordinated cellular processes



During the phase of colonization the **arbuscular fungi can be "confused" as a biotrophic pathogen** because these organisms share some characteristics as the production of elicitors (signals for the defence reaction by the plant)

Inside the root cells

Symbiotic development results in the formation of tree-shaped subcellular structures within plant cells, known as **arbuscules** (from the Latin *arbusculum*)



Parniske (2008) Arbuscular mycorrhiza: the mother of the plant endosymbioses

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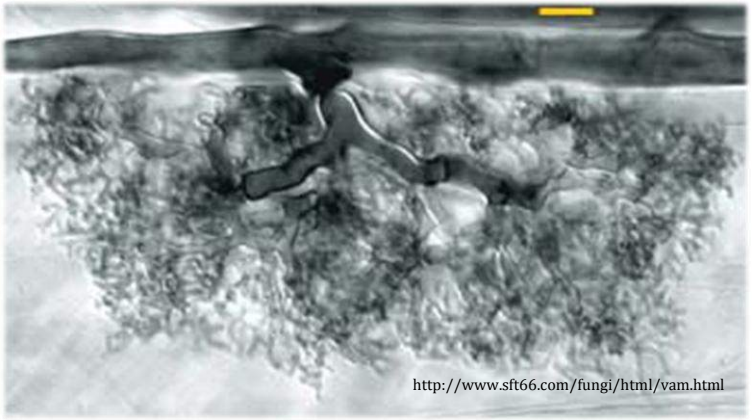


- Each fungal branch is surrounded by a **periarbuscular membrane (PAM)**
- Apoplastic interface between the fungal plasma membrane and the plant-derived PAM is called **periarbuscular space (PAS)**

- Some biotic and abiotic stress conditions generate **reactive oxygen species (ROS)** in plant tissues causing damage to proteins, lipids and photosynthetic pigments as well

- **Plants can detoxify** these **oxidative molecules** through elevating antioxidant activity secreting ROS-scavenging enzymes such as catalase, superoxide dismutase and peroxidase

Mycorrhizal Induced Resistance (MIR)

- Several studies suggest that **AM symbiosis helps plants to alleviate biotic and abiotic stresses**
- Symbiont stimulates a **systemic pre-alert condition** in the plant, but is able to modulate the defending answers to establish the symbiosis
- Instead of constitutive activation of plant defence, MIR is associated with priming for an efficient activation of **JA-dependent system**
- **Higher levels of endogenous JA**: quick and more effective activation of defence (JA-regulated defence genes) upon attack

Mycorrhizal Induced Resistance (MIR)

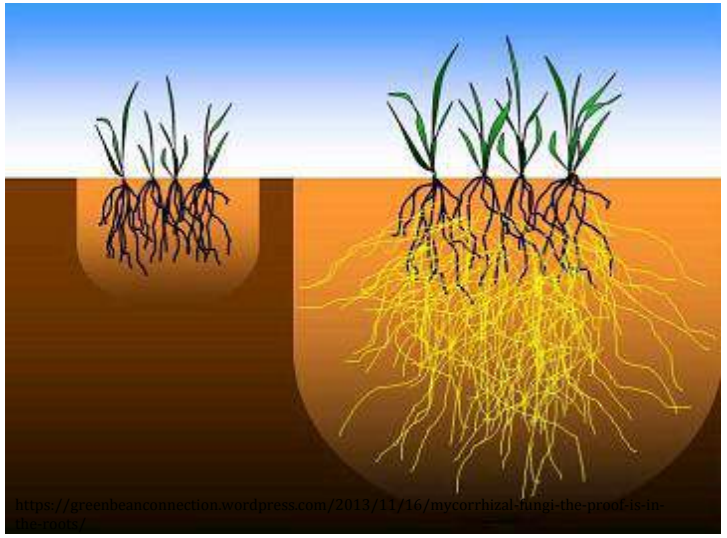
- The **mycorrhiza-induced resistance (MIR) provides systemic protection** against a wide range of plant pathogens

- Moreover some works show clearly that the amelioration of stress resistance by AM symbiosis is often related to the enhancement of antioxidant levels or activities in mycorrhizal plants

No direct activation but PRIMING:
precondition of plant tissues for a more effective activation of defenses

Aims of the research

- Investigate the effects of **high** and **low temperatures,** as well as **mechanical stress,** on inoculated and non-inoculated sunflower plants



- Measure the effect of AMF inoculation on the activity of **polyphenol oxidase (PPO)** and **guaiacol peroxidase (POX)** and on the expression of **glutathione-S-transferase (GST)** under stress conditions

At early stage of mycorrhization

Material and Methods

Plant material and growth conditions

- 4-day old pre-germinated (*Helianthus annuus* L.) seeds were sown in plastic pots. Substrate = soil:sand 1:1, v/v

- 15 g of inoculum Symbivit® (80 propagules g^{-1}):
  - *Rhizophagus intraradices* BEG140,
  - *Funneliformis mosseae* BEG95,
  - *Claroideoglomus etunicatum* BEG92,
  - *Claroideoglomus claroideum* BEG96,
  - *Glomus microaggregatum* BEG56,
  - *Funneliformis geosporum* BEG199

Material and Methods

- 9 days-old seedlings (inoculated and non-inoculated) were selected and exposed to different stress conditions
  - incubation **at 38 °C for 24 h** (High Temperature stress, HT)
  - incubation **at 4 °C for 24 h** (Low Temperature stress, chilling stress, LT)
  - **hypocotyls injured** by nicking (Mechanical Wounding stress, MW)
- After 24 h the leaves were collected and analyzed
- All treatments were replicated 5 times

Material and Methods

RNA isolation and cDNA synthesis

- Vantage Total RNA Purification Kit (Origene, USA)
- First-strand cDNA Synthesis for Quantitative RT-PCR kit (Origene, USA)

RT-PCR (Glutathion S-transferase gene expression)

Primers

- *Ha*-GST-f (5'-CCTCAGGATGCTTACGAGAAGG-3')
- *Ha*-GST-r (5'-GCAGAAATATCAACCAGGTTGATG-3') (Radwan et al. 2005)

The PCR amplification conditions

94 °C for 3 min
94 °C for 15 s
61 °C for 15 s } 26x cycle
72 °C for 20 s
72 °C for 5 min

PCR were analyzed by electrophoresis on 2 % agarose gels

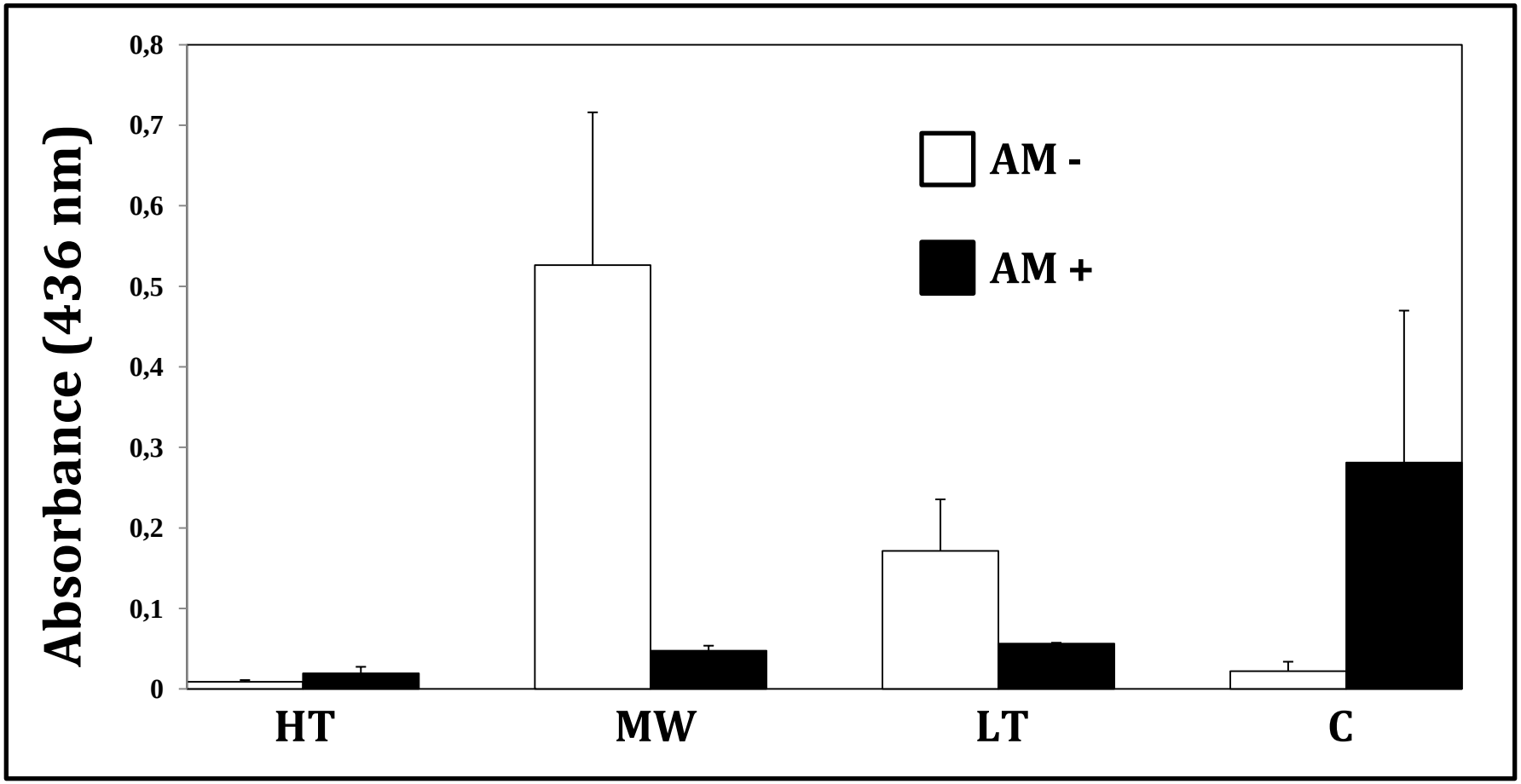
Material and Methods

Enzyme assays

- Half gram of fresh leaves and hypocotyl segments were ground under liquid nitrogen
- Extraction buffer was added to the tissue powder and centrifuged; supernatant was used for further measurements

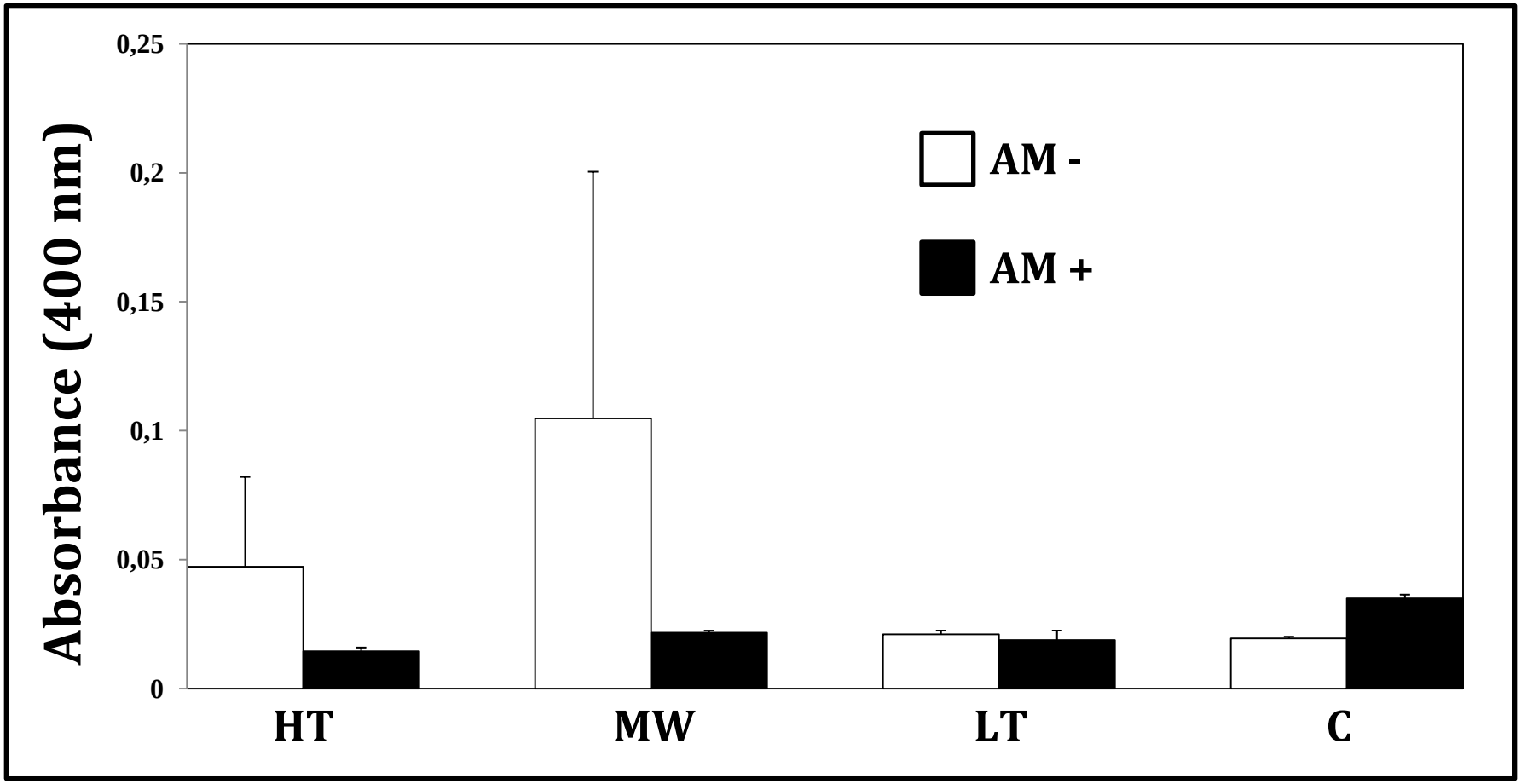
- The level of **guaiacol peroxidase** activity was tested according to Rathmell and Sequera (1974), the absorbance was recorded at 436 nm
- The activity of **polyphenol oxidase** was determined as described by Fehrmann and Dimond (1967), the polyphenol oxidase activity was measured by the increase in the absorbance at 400 nm

Results – Guaiacol peroxidase (POX)



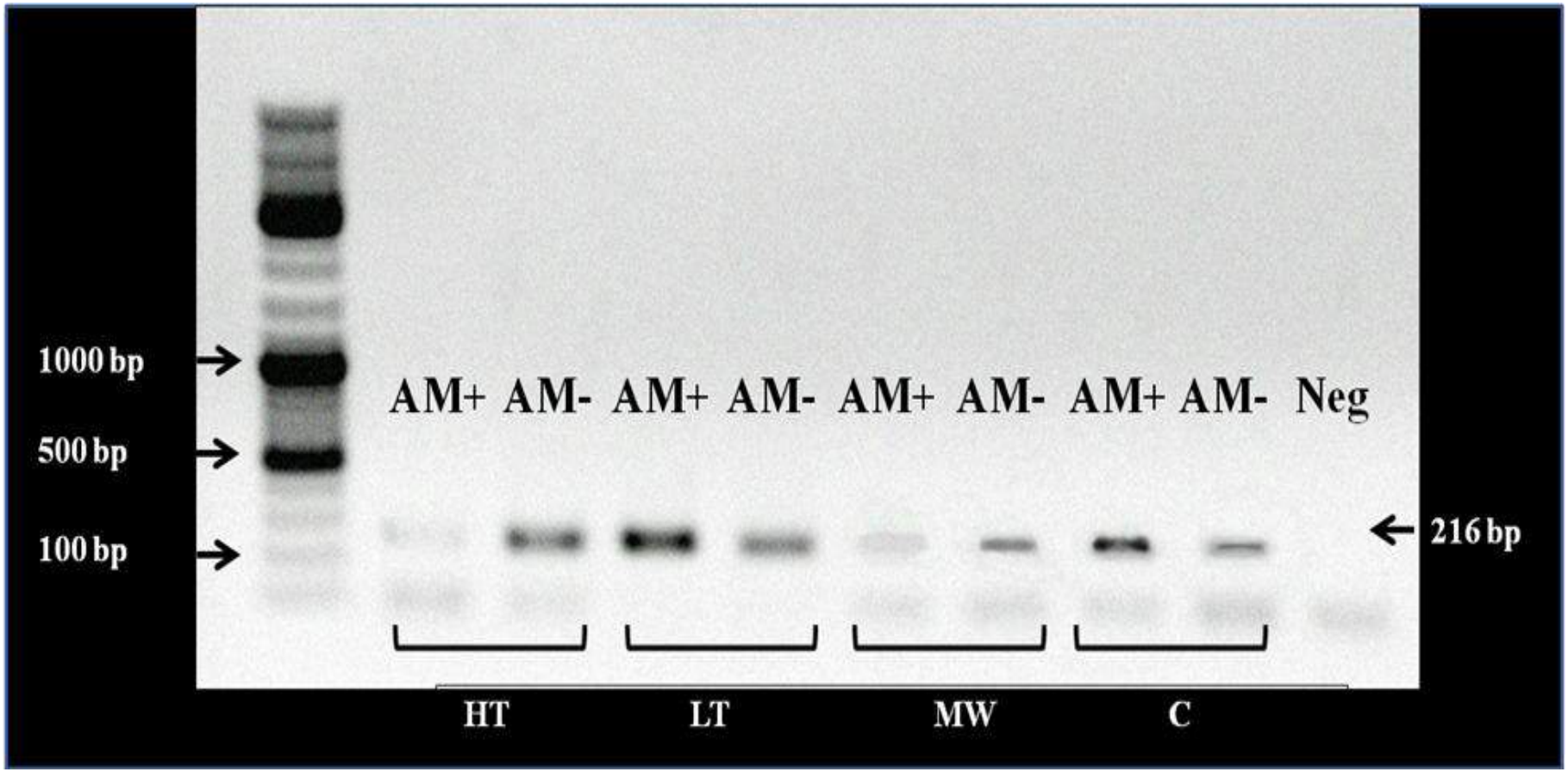
Effect of mycorrhizal inoculation on POX activity in leaves of sunflower seedlings under different stress [High Temperature stress (HT), Mechanical Wounding stress (MW), Low Temperature stress (LT), Control (C, no stress), non-inoculated plants (AM-), AMF inoculated plants (AM+)]

Results – Polyphenol oxidase (PPO)



Effect of mycorrhizal inoculation on PPO activity in leaves of sunflower seedlings under different stress [High Temperature stress (HT), Mechanical Wounding stress (MW), Low Temperature stress (LT), Control (C, no stress), non-inoculated plants (AM-), AMF inoculated plants (AM+)]

Results - Glutathion S-transferase (GST)



Effect of mycorrhizal inoculation on GST expression in leaves of sunflower seedlings under different stress [High Temperature stress (HT), Mechanical Wounding stress (MW), Low Temperature stress (LT), Control (C, no stress), non-inoculated plants (AM-), AMF inoculated plants (AM+)]

Conclusions

- Our results demonstrate that **mycorrhizal inoculation at seedling stage stimulates abiotic stress responses** in the plants

- On the basis of these preliminary data **we provided a deeper insight into the role of arbuscular mycorrhiza in arresting reactive oxygen species and strengthening antioxidant defense in the host plants**

- Although our results could help a better understanding of various stresses resistance mechanisms, **additional experiments are required to clarify the molecular basis** underlying the **regulation processes of the enzymes involved**

Thank you for your attention!