DEFENCE-RELATED ENZYMES IN PEPPER ROOTS DURING INTERACTIONS WITH ARBUSCULAR MYCORRHIZAL FUNGI AND/OR *Verticillium dahliae*

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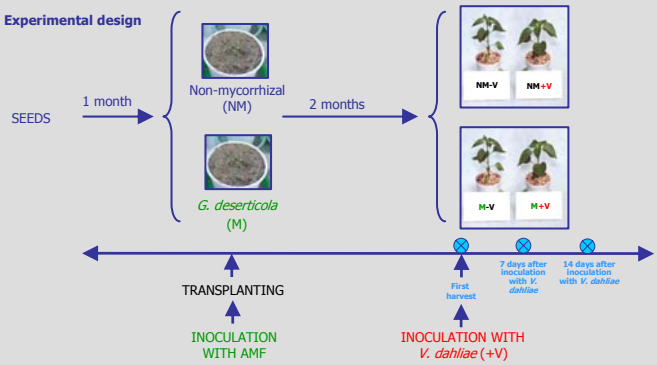
INTRODUCTION

The active defence responses of plants include the activation of genes that are coding for enzymes of the phenylpropanoid pathway or for pathogenesis-related (PR) proteins, some of them with chitinase, chitosanase and β-1,3-glucanase activities (Pozo et al., 2002) that degrade wall of many pathogenic fungi (Van Loon and Van Strien, 1999).
Previous studies have described that the arbuscular mycorrhizal fungus (AMF) *Glomus deserticola* (Trappe, Bloss and Menge) can reduce the deleterious effect of *Verticillium dahliae* Kleb. on *Capsicum annuum* L. cv. Piquillo growth and yield (Garmendia et al., 2004). The establishment of the mycorrhizal association involves the production of plant defence related proteins that could contribute to the bioprotection (Lambais, 2000).

Our **first objective** was to study if *V. dahliae* induced defence mechanisms in roots before infected-pepper developed visible symptoms of disease. The **second aim** was to determine if AMF induced defence-related enzymatic activities in pepper roots before or after pathogen's attack.

MATERIAL AND METHODS

• **Experimental design**



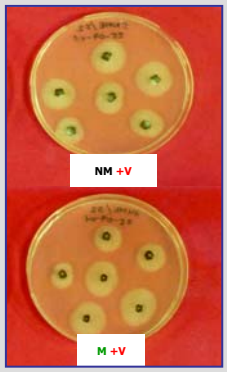
Growth conditions:
25/15ºC day/night
14h photoperiod
Soil-sand-vermiculite (1:2.5:2.5 v/v/v)
Nutrition: LANS solution

Treatments:
NM-V: non-mycorrhizal healthy plants
NM+V: non-mycorrhizal plants inoculated with *V. dahliae*
M-V: mycorrhizal healthy plants
M+V: mycorrhizal plants inoculated with *V. dahliae*

DETERMINATIONS

- Disease assessment
- AMF colonization
- Plant growth parameters
- Root enzymatic activities:

  Phenylalanine ammonia-lyase (PAL) (EC 4.1.1.5)
  Chitinase (EC 3.2.1.14)
  β-1,3-glucanase (EC 3.2.1.39)
  Chitosanase (EC 3.2.1.99)
  Peroxidase (EC 1.11.1.7)
  Superoxide dismutase (SOD) (EC 1.15.1.1)



Photograph 1. Isolation of *V. dahliae* from cross–stem sections of inoculated non-mycorrhizal (NM) and mycorrhizal (M) pepper plants on Messiaen culture medium 14 days after the pathogen inoculation. *Verticillium*-infected plants did not show any visible disease symptom.

Table 1. Mycorrhizal colonization (%), shoot dry matter (DM) (g plant^{-1}) and root DM (g plant^{-1}) in non-mycorrhizal (NM) and mycorrhizal (M) plants just before inoculating *Verticillium dahliae*. Means ± SD (n = 3 plants) were compared with Student's *t*-test within each column. Values followed by a common letter are not significantly different ($p \leq 0.05$).

Treatments	Mycorrhizal colonization (%)	Shoot DM (g plant^{-1})	Root DM (g plant^{-1})
NM	-	0.87 ± 0.26 a	0.47 ± 0.14 a
M	25.32 ± 6.76	0.90 ± 0.29 a	0.43 ± 0.13 a

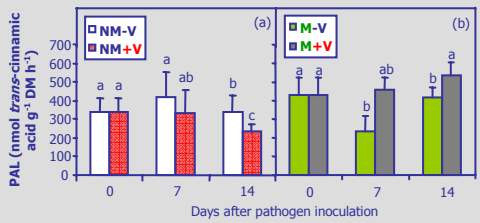


Figure 1. **Phenylalanine ammonia-lyase (PAL)** activities (nmol *trans*-cinnamic acid g^{-1} DM h^{-1}) in roots from non-mycorrhizal (NM) (a) and mycorrhizal (M) (b) plants inoculated (+V) or not (-V) with *Verticillium dahliae*. Means ± SD (n = 3 plants) just before inoculating *Verticillium* were compared with Student's *t*-test and data on days 7 and 14 after pathogen inoculation were analysed by Tukey-b-test. Histograms followed by a common letter are not significantly different ($p \leq 0.05$). PAL activities were assayed according to Dunn et al. (1998) with slight modifications.

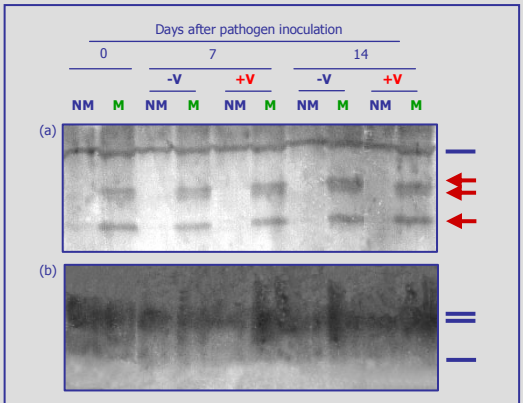


Figure 2. Acidic (a) and basic (b) **chitinase** activities after separation of proteins under native conditions using the Davis and the Reisfeld systems, respectively, in 15% (w/v) polyacrylamide gels containing 0.01% (w/v) glycol chitin as substrate. Roots extracts in McIlvaine buffer (7 µg of protein per sample) from non-mycorrhizal (NM) and mycorrhizal (M) plants inoculated (+V) or not (-V) with *Verticillium dahliae* were analysed just before inoculating the pathogen as well as 1 and 2 weeks later. Bars indicate constitutive isoforms and arrows mark additional isoforms.

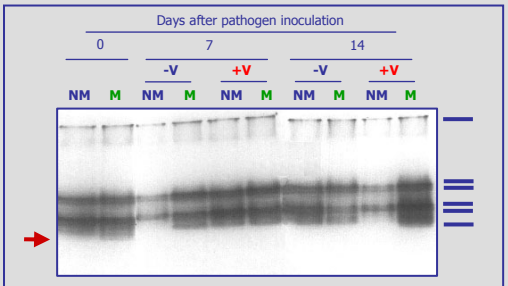


Figure 4. **Peroxidase** activity after separation of proteins under native conditions using the Davis system in 15% (w/v) polyacrylamide gels. Root protein extracts in McIlvaine buffer (7 µg of protein per sample) from non-mycorrhizal (NM) and mycorrhizal (M) plants inoculated (+V) or not (-V) with *Verticillium dahliae* were analysed just before inoculating the pathogen as well as 1 and 2 weeks later. After protein separation, gels were incubated in 0.1 M Tris-HCl (pH 7.6) with 4-chloro-1-naphthol and H_2O_2. Bars indicate constitutive isoforms and arrows mark additional isoforms.

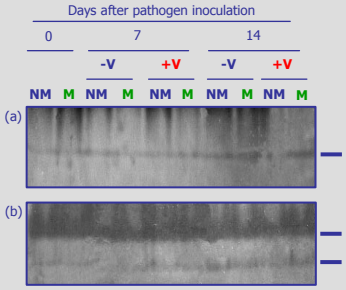


Figure 3. Acidic (a) and basic (b) **β-1,3-glucanase** activities after separation of proteins under native conditions using the Davis and the Reisfeld systems, respectively, in 15% (w/v) polyacrylamide gels containing β-glucans as substrate. Roots extracts in McIlvaine buffer (7 µg of protein per sample) from non-mycorrhizal (NM) and mycorrhizal (M) plants inoculated (+V) or not (-V) with *Verticillium dahliae* were analysed just before inoculating the pathogen as well as 1 and 2 weeks later. Bars indicate constitutive isoforms and arrows mark additional isoforms.

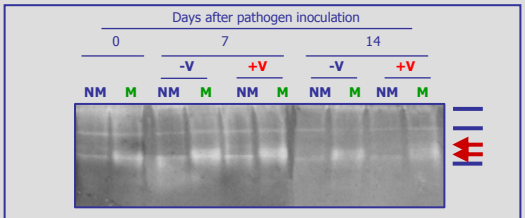


Figure 5. **Superoxide dismutase (SOD)** isoenzymes after separation of proteins under native conditions using the Davis system in 15% (w/v) polyacrylamide gels. Root protein extracts in McIlvaine buffer (7 µg of protein per sample) from non-mycorrhizal (NM) and mycorrhizal (M) plants inoculated (+V) or not (-V) with *Verticillium dahliae* were analysed just before inoculating the pathogen as well as 1 and 2 weeks later. Bars indicate constitutive isoforms and arrows mark additional isoforms.

CONCLUSIONS

- Mycorrhizal-specific induction of new isoforms of acidic chitinases and SOD together with enhanced PAL and peroxidase activities two weeks after pathogen inoculation could be involved in the biocontrol of *Verticillium*-induced wilt in pepper (*C. annuum* L. cv. Piquillo) by *Glomus deserticola*.
- *Verticillium dahliae* neither stimulated the phenylpropanoid pathway nor elicited hydrolytic activities in non-mycorrhizal pepper roots one and two weeks after its inoculation.
- No signal corresponding to chitosanase activity was detected in any treatment.

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