The arbuscular mycorrhizal symbiosis attenuates symptom severity and reduces virus concentration in tomato infected by Tomato yellow leaf curl Sardinia virus (TYLCSV)

UNIVERSITÀ DEGLI STUDI DI TORINO

Mycorrhiza

The arbuscular mycorrhizal symbiosis attenuates symptom severity and reduces virus concentration in tomato infected by Tomato yellow leaf curl Sardinia virus (TYLCSV)

--Manuscript Draft--

Manuscript Number:	MCOR-D-13-00101R1
Full Title:	The arbuscular mycorrhizal symbiosis attenuates symptom severity and reduces virus concentration in tomato infected by Tomato yellow leaf curl Sardinia virus (TYLCSV)
Article Type:	Original Paper
Keywords:	AM symbiosis; Funneliformis mosseae; viral infection; Tomato; Tomato yellow leaf curl Sardinia virus; geminivirus
Corresponding Author:	Gian Paolo Accotto Consiglio Nazionale delle Ricerche Torino, ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Consiglio Nazionale delle Ricerche
Corresponding Author's Secondary Institution:	
First Author:	Giulia Maffei
First Author Secondary Information:	
Order of Authors:	Giulia Maffei
	Laura Miozzi
	Valentina Fiorilli
	Mara Novero
	Luisa Lanfranco
	Gian Paolo Accotto
Order of Authors Secondary Information:	
Abstract:	The mutualistic association established between arbuscular mycorrhizal (AM) fungi and the majority of land plants has been extensively studied and is considered a natural instrument to improve plant health and productivity, since mycorrhizal plants often show higher tolerance to abiotic and biotic stresses. However, the impact of the AM symbiosis on the infection by viral pathogens is still largely uncertain and little explored. In our study tomato plants were grown under controlled conditions and inoculated with the AM fungus Funneliformis mosseae. Once the colonization had developed, plants were inoculated with Tomato yellow leaf curl Sardinia virus (TYLCSV), a geminivirus causing one of the most serious viral diseases of tomatoes in Mediterranean areas. Four biological conditions were set up: control plants (C), TYLCSV-infected plants (V), mycorrhizal plants (M) and TYLCSV-infected mycorrhizal plants (MV). At the time of analysis, the mycorrhization level, as well as the expression profiles of mycorrhiza-responsive selected genes, were not significantly modified by virus infection, thus indicating that the AM colonization was unaffected by the presence and spread of the virus. On the contrary, in MV plants viral symptoms were milder than in V plants and the concentration of viral DNA was lower in both shoots and roots. Overall F. mosseae colonization appears to exert a beneficial effect on tomato plants attenuating the disease caused by TYLCSV.

The arbuscular mycorrhizal symbiosis attenuates symptom severity and reduces virus concentration in tomato infected by *Tomato yellow leaf curl Sardinia virus* (TYLCSV)

Giulia Maffei, Laura Miozzi, Valentina Fiorilli, Mara Novero, Luisa Lanfranco, Gian Paolo Accotto

Authors affiliations:
G. Maffei, V. Fiorilli, M. Novero, L. Lanfranco
Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
Viale Mattioli 25
10125 Torino, Italy

G. Maffei, L. Miozzi, G.P. Accotto
Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche
Strada delle Cacce 73
10135 Torino, Italy

V. Fiorilli, L. Lanfranco
Istituto per la Protezione delle Piante, Consiglio Nazionale delle Ricerche
Viale Mattioli 25
10125 Torino, Italy

Corresponding author:
Gian Paolo Accotto
e-mail: g.accotto@ivv.cnr.it tel: (+39)0113977910 fax: (+39)011343809

Abstract

The mutualistic association established between arbuscular mycorrhizal (AM) fungi and the majority of land plants has been extensively studied and is considered a natural instrument to improve plant health and productivity, since mycorrhizal plants often show higher tolerance to abiotic and biotic stresses. However, the impact of the AM symbiosis on the infection by viral pathogens is still largely uncertain and little explored. In our study tomato plants were grown under controlled conditions and inoculated with the AM fungus *Funneliformis mosseae*. Once the colonization had developed, plants were inoculated with *Tomato yellow leaf curl Sardinia virus* (TYLCSV), a geminivirus causing one of the most serious viral diseases of tomatoes in Mediterranean areas. Four biological conditions were set up: control plants (C), TYLCSV-infected plants (V), mycorrhizal plants (M) and TYLCSV-infected mycorrhizal plants (MV). At the time of analysis, the mycorrhization level, as well as the expression profiles of mycorrhiza-responsive selected genes, were not significantly modified by virus infection, thus indicating that the AM colonization was unaffected by the presence and spread of the virus. On the contrary, in MV plants viral symptoms were milder than in V plants and the

concentration of viral DNA was lower in both shoots and roots. Overall *F. mosseae* colonization appears to exert a beneficial effect on tomato plants attenuating the disease caused by TYLCSV.

Keywords

AM symbiosis; *Funneliformis mosseae;* viral infection; tomato; Tomato yellow leaf curl Sardinia virus; geminivirus

46

47 **INTRODUCTION**

48 The arbuscular mycorrhizal symbiosis (AM), one of the most widespread mutualistic symbiosis, is formed between
49 fungi belonging to the Glomeromycota phylum and the roots of the majority of land plants, including crops and
50 horticultural species (Bonfante and Genre 2010). AM fungi colonize the root cortex where they form intracellular highly
51 branched structures called arbuscules. The arbusculated cells are considered key structures of the symbiosis and the
52 main site for reciprocal nutrient exchanges: the fungus provides mineral nutrients (phosphorus, nitrogen, sulphur) to the
53 plant and receives carbon compounds derived from photosynthesis (Bonfante and Genre 2010; Lanfranco and Young
54 2012). Beside an improved mineral nutrition, mycorrhizal plants often show a higher tolerance to biotic and abiotic
55 stresses leading, in the end, to an improved plant fitness (Gernns et al. 2001; Van der Heijden and Sanders 2002;
56 Hildebrandt et al. 2007; Pozo and Azcòn-Aguilar 2007; Aroca et al. 2008; Pozo et al. 2010). This symbiotic association
57 is therefore considered a natural instrument that improves the health and productivity of host plants.

58 The AM symbiosis can influence the outcome of plant-pathogen interactions (Pozo and Azòn-Aguilar 2007; Pozo et al.
59 2010). A well established symbiosis prior the challenge with the attacker seems a requirement for mycorrhizal
60 protection against pathogens (Rosendahl 1985; Cordier et al. 1998; Slezack et al. 2000; Khaosaad et al. 2007). Although
61 the effect may also depend by the specific plant-fungus combination and by environmental factors, mainly beneficial
62 effects have been observed in the case of soil-borne pathogens: alleviation of damage was shown for diseases caused by
63 fungi such as *Rhizoctonia*, *Fusarium* and *Verticillum* spp., by oomycetes including *Phytophtora*, *Pythium* and
64 *Alphanomices* spp. (Pozo et al. 2010; Whipps 2004) and parasitic nematodes such as *Pratylenchus* and *Meloidogyne*
65 spp. (de la Pena et al. 2006, Li et al. 2006; Vos et al. 2013). The bioprotective role may rely on different mechanisms but
66 is surely not exclusively dependent on an improved mineral nutrition. To control root pathogens competition for
67 colonization sites or photosynthates, changes in the root apparatus and/or in the microbial rhizosphere communities and
68 also activation of plant defence have been proposed (Whipps 2004; Pozo et al. 2002; Pozo et al. 2010; Ismail and Hijri
69 2012; Vos et al. 2013).

70 Information on the effects of the AM symbiosis on pathogens attacking the epigean parts of plants, i.e. the parts that
71 grow above the ground surface (hereafter called shoot pathogens) are scarce and more controversial. The effects on
72 shoot-targeting organisms seem to greatly depend on the attacker life style (Pozo and Azòn-Aguilar 2007). Enhanced
73 susceptibility to biotrophic pathogens such as powdery mildew and rust fungi was observed (Whipps et al. 2004;
74 Gernns et al. 2009). However, colonization by AM fungi has been reported to reduce symptoms caused by phytoplasma
75 (Lingua et al. 2002; Garcia-Chapa et al. 2004; D'Amelio et al. 2007) the necrotrophs *Alternaria solani* (Fritz et al. 2006)
76 and *Botrytis cinerea* (Pozo et al. 2010; Fiorilli et al. 2011), as well as the bacterial pathogen *Xanthomonas campestris*
77 (Liu et al. 2007).

78 Very little information is available about the interaction with viral infections. Mycorrhizal colonization was shown to
79 increase the multiplication of some viruses (Daft and Okusanya 1973; Dehne 1982; Miozzi et al. 2011). Shaul et al.
80 (1999) focused on disease symptoms and demonstrated that mycorrhizal tobacco plants infected by *Tobacco mosaic*
81 *virus* (TMV) showed an enhanced disease severity compared to non-mycorrhizal plants. More recently, Miozzi et al.
82 (2011) analysed the interactions between *Funneliformis mosseae* and *Tomato spotted wilt virus* (TSWV) in tomato and
83 observed that regulation of plant genes responding to virus infection was attenuated by mycorrhization, causing, in the
84 long term, a higher virus titer in mycorrhizal than non mycorrhizal plants.

With the aim to extend our knowledge on the impact of the AM colonization on viral infection we focused our attention on a virus family, the *Geminiviridae*, not yet explored, with characteristics different from those examined so far. They are important pathogens on many crops in tropical and sub-tropical regions, and their economic relevance is constantly growing (Varma and Malathi 2003; Mansoor et al. 2006; Jeske 2009). Several of them infect tomato (*Solanum lycopersicum*), one of the most important vegetable crops worldwide and currently used as a model plant for basic research with an increasing number of genomics and functional genomics tools available (The Tomato Genome Consortium 2012).

Among the members of the genus *Begomovirus*, *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV) are the cause of one of the most serious diseases of tomato cultivation (Accotto et al. 2000). Like all geminiviruses, they have a single-stranded DNA genome and replicate in the cell nucleus. They colonize the phloem vessels (Morilla et al. 2004) and the typical disease symptoms they induce consist in leaf curling and yellowing of leaf edges, plant growth reduction, flower abortion and drastic loss of fruit production (Czosnek 2007).

It this work we have examined the impact of colonization by the AM fungus *Funneliformis mosseae* on infection by TYLCSV in tomato plants and showed that the AM symbiosis exerts a positive systemic effect leading to an attenuation of the disease.

MATERIALS AND METHODS

Biological material

Solanum lycopersicum cv. Moneymaker seeds were surface-sterilized by washing in 70% ethanol with a few drops of Tween 20 for 3 min and in sodium hypochlorite 5% for 13 min, and rinsed three times in distilled water for 10 min each time. The seeds were placed in Petri dishes containing H_2O:agar (0.6%), incubated for one week in the dark (25 °C) and then exposed to light for another week with a photoperiod of 14/10 light/dark. Seedlings were then transferred to pots containing sterile quartz sand. Altogether, the experiment aiming to study the impact of colonization by the AM fungus *F. mosseae* on infection by TYLCSV in tomato consisted in four groups of plants: control plants (C), TYLCSV-infected plants (V), mycorrhizal plants (M), TYLCSV-infected mycorrhizal plants (MV). Inoculation of *Funneliformis mosseae* (Syn. *Glomus mosseae*; Krüger et al. 2012) Gerd. & Trappe BEG12 (Mycagrolab, France) was performed by mixing the inoculum with sterile quartz sand (30% v/v). The plants were maintained in a growth chamber under 14 h light (2,500 lux) / 10 h dark at 23°C and watered twice a week: once with a modified Long-Ashton nutrient solution (Hewitt, 1966) containing 320 µM phosphate and once with water. Phosphate content in the nutrient solution was optimized so that non mycorrhizal plants were not suffering from phosphate starvation and did not show growth defects compared to mycorrhizal ones. To guarantee the establishment of a good mycorrhization level, virus inoculation was performed 4 weeks after AM fungus inoculation. Two groups of plants (V and MV) were inoculated with TYLCSV using the agroinoculation method with 20 µL of a suspension of *Agrobacterium tumefaciens* strain LBA4404 cells carrying infectious viral clone (Kheyr-Pour et al. 1991)**.** The remaining plants (C and M) were mock-inoculated with 20 µL of a suspension of *A. tumefaciens* cells strain LBA4404 cells containing the binary plasmid without viral insert.

To detect the virus presence a tissue print was performed 21 days post-infection (dpi). The petiole of a young leaf from each plant was printed on positively charged nylon membrane (Roche, Mannheim, Germany). Membranes were then

hybridized with a digoxigenin-labeled TYLCSV-specific probe (Accotto et al. 2000). Infected plants were retained for further analysis (data not shown).

Plants were harvested 28 dpi for analysis. First, shoot (the aboveground portion) and root (the underground portion) components were separately weighted for biomass evaluation. Roots from mycorrhizal plants were checked for the presence of extraradical mycelium under a stereomicroscope. A portion of the root system was then used to assess the mycorrhization level while the remaining portion of the roots, the apex and the second yongest leaf (leaf -2) of each plant were frozen in liquid nitrogen and stored at -80 C. Following the preliminary screening for virus infection and *F. mosseae* colonization, 15 plants per condition were considered for further analyses.

Mycorrhizal evaluation

Mycorrhizal roots were stained with 0.1 % (w/v) cotton blue overnight and then washed with lactic acid. Randomly selected roots segments were cut in 1 cm pieces and observed under a light microscope. According to Trouvelot et al. (1986), four parameters were considered: frequency of mycorrhization (F%): percentage of fragments showing intraradical mycelium; intensity of mycorrhization (M%): mean volume of individual root segments colonized by mycelium; percentage of arbuscules within infected areas (a%): arbuscules in the colonized portions of the root system; percentage of arbuscules in the root system (A%): presence of arbuscules in the whole roots system.

Estimation of viral infection and quantification of viral genomes

At 28 dpi the symptom severity was estimated by visual inspection using a Disease Severity Index (DSI) ranging from 1 (very mild symptoms) to 4 (severe symptoms), according to Lapidot and Friedman (2002).

To estimate the amount of viral genomes in infected plants qPCR assays were performed on shoot and root total DNA. About 100 mg of tissue were ground in liquid nitrogen, 800 µl of TLES (5% SDS, 150 mM LiCl, 50 mM Tris-HCl pH 9.0, 5 mM EDTA) were added, followed by 0.5 vol of phenol and 0.5 vol of chloroform. Following mixing and centrifugation, the aqueous phase was recovered and the DNA was precipitated with 2.5 vol of cold ethanol and 1/20 vol of 4 M Na-acetate. The DNA was finally resuspended in 200 µl of TE solution. TYLCSV DNA was amplified with primers TY2222(+) (5'- TTTTACTTGTATATTCGAAGTGTGCCA-3') and TY2371(-) (5'-ACAACTGCAAAATTAGAATCTAGTTGGTA-3'). PCR reactions were run in parallel with primers QTOMAPX-2818(+) (5'- TTTTACTTGTATATTCGAAGTGTGCCA-3') and QTOMAPX-2910(-) (5'-ACAACTGCAAAATTAGAATCTAGTTGGTA -3') for the reference plant gene APX encoding an ascorbate peroxidase. Quantitative PCR assay were carried out using EvaGreen Mix (Bio-Rad) in a StepOnePlus^{TM} apparatus (Applied Biosystems). The reactions were conducted in a total volume of 10 µl, containing 40 ng DNA, 5 µl Eva Green, 1 µl of each primer 3µM, 0.3 µl of ROX. The PCR cycling program was: 95 °C for 5 min, and 40 cycles each consisting of 95 °C for 15 s and 60 °C for 1 min. A melting curve was recorded at the end of each run to assess amplification production specificity. All reactions were performed with three technical replicates and three biological replicates. PCR efficiency was determined from standard curves constructed with serial dilutions of genomic DNA from TYLCSV-infected tomato. For each sample we calculated the relative viral amount using the ΔCt method where ΔCt is $|Ct_{virus} - Ct_{apx}|$. The fold change in viral amount between non mycorrhizal (V) and mycorrhizal (MV) samples was calculated as $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct = \Delta Ct_{MVplants} - \Delta Ct_{Vplants}$.

160

161 **RNA extraction and RT-qPCR assays**

162 About 100 mg of shoot and root samples were ground in liquid nitrogen and used for total RNA extraction. RNA
163 extractions were performed using the SIGMA RNA plant kit. DNA contaminations were removed using the Turbo DNA
164 free kit (Bio-Rad) following manufacturer's instructions. RNA quantification was carried out using a Picodrop 1000
165 spectrophotometer. The quality of RNAs was checked using the Experion automated electrophoresis station (BioRad)
166 following the manufacturer's instructions. Total RNA (500 ng) from each pool was transcribed into cDNA with the
167 High-Capacity cDNA reverse Transcription Kit (Applied Biosystems). All RT-qPCR assays were carried out using
168 EvaGreen Mix (Bio-Rad) in a StepOnePlus^{TM} apparatus (Applied Biosystems) as described above. The comparative
169 threshold cycle method (Rasmussen 2001) was used to calculate relative expression level using the tomato UBC
170 (ubiquitin conjugating enzyme, SGN-U582847) as reference gene. The list of analyzed genes and corresponding
171 primers are shown in Table 1. For each condition three biological replicates, each consisting of RNA extractions from
172 four plants, were analysed.

173

174 **RESULTS**

175 To study the impact of colonization by the AM fungus *Funneliformis mosseae* on TYLCSV infected tomatoes, four
176 biological conditions were considered: control plants (C), TYLCSV-infected plants (V), mycorrhizal plants (M),
177 TYLCSV-infected mycorrhizal plants (MV). At the time of sampling (28 dpi), shoot and root fresh weight was
178 measured.

179 No difference was observed in shoot and root biomass between C and M plants, confirming that the phosphate content
180 in the nutrient solution was optimized to avoid phosphate starvation in C plants. The only statistically significant
181 difference was a slight reduction of shoot and root biomass in MV plants (Fig. 1).

182 AM colonization level was also evaluated: of the four parameters considered, only the mycorrhization frequency was
183 moderately but significantly increased in TYLCSV-infected mycorrhizal plants compared to mycorrrhizal plants (Fig.
184 2). In their whole these data indicate that the viral infection has no major impact on the intraradical growth of the AM
185 fungus.

186 Viral symptoms on leaves were estimated according to the DSI (Fig. 3). Overall, the infected mycorrhizal plants (MV)
187 showed less severe symptoms than the infected non mycorrhizal plants (V): the majority (53%) of TYLCSV-infected
188 plants presented the most severe symptoms (DSI=4) while most (53%) of TYLCSV-infected mycorrhizal plants
189 presented the mildest symptoms (DSI=1). To gain a more accurate description of viral infection, the relative amount of
190 viral genomes in shoot and root tissues was estimated by qPCR assays with primers specific for a viral DNA sequence.
191 Investigations were performed on genomic DNA from both shoots and roots. Viral DNA was detected also in roots
192 confirming that the virus causes a systemic infection. The amount of viral DNA was higher in V plants compared to MV
193 plants, both in shoots (2.6 times) and in roots (1.9 times) (Fig. 4). Such differences were statistically significant ($p<0.05$,
194 ANOVA) . Interestingly, this result is in agreement with the observed symptoms, that were more severe in V plants. In
195 addition, the amount of viral DNA was higher ($p<0.05$, ANOVA) in shoots than in roots, irrespectively from the
196 presence of the AM fungus. In both V and MV plants, the difference in viral concentration between shoots and roots
197 remained similar, indicating that mycorrhization does not influence virus movement towards the roots.

198

199 **Expression profiles of genes considered markers of a functional AM symbiosis**

200 A molecular analysis was performed to analyse whether a functional AM symbiosis was active in mycorrhizal plants
201 infected by the virus. We first considered *LePT4*, a phosphate transporter encoding gene specifically induced in
202 arbusculated cells (Balestrini et al. 2007) and recognized as a molecular marker of a key function of the AM symbiosis.
203 The transcript was highly induced in mycorrhizal plants and, remarkably, *LePT4* induction was not affected by the
204 presence of the virus (Fig. 5). In addition, the expression profiles of a selection of genes (a kinesin-like protein, a β-
205 xylosidase β-l-arabinosidase, a cytochrome P450 CYP707A3 and a UDP-glucoronosyl transferase), previously
206 identified as up-regulated in tomato roots colonized by the AM fungus *F. mosseae* (Fiorilli et al. 2009), were also
207 investigated. All the genes showed an up-regulation in mycorrhizal roots compared to control roots. A similar level of
208 gene expression was found in TYLCSV-infected mycorrhizal plants.

209

210 **DISCUSSION**

211 Tomato plants are subjected to a number of viral diseases (Hanssen 2010). Since viruses cannot be eradicated by
212 chemical treatments alternative protection strategies need to be developed. Among beneficial microorganisms such as
213 PGPR (plant growth promoting rhizobacteria), tomato plants interact with AM fungi (Conrath et al. 2006; Beckers and
214 Conrath 2007) which, with their multifunctional roles, have also been proposed as sustainable alternative to chemicals
215 in pest management. The degree of protection seems highly dependent on the AM fungus involved (Kobra et al. 2009)
216 with *F. mosseae* in natural and agricultural systems, showing a stronger bioprotective role compared to other AM fungi
217 (Pozo et al. 2002; Utkhede 2006; Ozgonen and Erkilic 2007; Veresoglou and Rillig 2012).

218 In this work we have analysed in tomato plants the impact of *F. mosseae* colonization on infection by TYLCSV, a
219 devastating viral pathogen responsible of the tomato yellow leaf curl disease in the Mediterranean region. Since a well
220 established symbiosis prior the challenge with the pathogen is considered a requirement for bioprotection (Rosendahl
221 1985; Cordier et al. 1998; Slezack et al. 2000; Khaosaad et al. 2007), plants were first inoculated with the AM fungus,
222 then 28 days later with TYLCSV. Four weeks were then allowed before sampling and analyzing plants, to let systemic
223 viral infection with the typical symptoms. Considering plant biomass, a month of viral infection was probably not
224 sufficient to affect plant growth, a typical systemic symptom of TYLCSV, although a slight reduction of shoot and root
225 biomass was observed in MV plants (Fig. 1), probably due to the simultaneous presence of the two microorganisms.

226 Morphological observation of roots (for fungal colonization) and young leaves (for yellow leaf curling) showed that the
227 colonization by *F. mosseae* attenuates TYLCSV symptoms. In line with this, the amount of viral DNA in both shoots
228 and roots was reduced in TYLCSV-infected mycorrhizal (MV) vs TYLCSV-infected non-mycorrhizal (V) plants. These
229 results indicate that, at least under our experimental conditions, the AM symbiosis appears to confer some protection
230 against TYLCSV in tomato.

231 Interestingly, the viral infection does not affect mycorrhization: analysis of AM colonization levels showed no major
232 difference between M and MV plants, indicating that the onset and spread of TYLCSV in the whole plant (including the
233 roots colonized by *F. mosseae*) does not interfere with the fungal intraradical development. Beside these morphological
234 observations, we detected the up-regulation of a selected group of genes previously described as mycorrhiza-responsive
235 and found as preferentially expressed in arbuscule-containing cells of tomato-*F. mosseae* mycorrhizal roots (Fiorilli et

al. 2009). With the exception of the well known AM-marker *LePT4* (Nagy et al. 2005; Balestrini et al. 2007; Xu et al. 2007; Gomez-Ariza et al. 2009), these genes have not been characterized yet. Based on sequence similarity their functions might be related to reorganization of cell components (kinesin-like protein and β-xylosidase β-l-arabinosidase), abscisic acid catabolism (cytochrome P450 CYP707A3) and control of carbon flux (UDP-glucoronosyl transferase protein). Remarkably, their expression profiles in mycorrhizal plants were not modified by TYLCSV infection (compare M vs MV in Fig. 5). These genes can be proposed for the role of novel markers of functional AM symbiosis in tomato.

Although the mycorrhization develops only in roots, it clearly has long-distance effects on the non colonized aboveground parts of the plants. Molecular evidences of this systemic effect have been shown in gene expression studies, where up- or down-regulation of several genes has been detected not only in leaves (Taylor and Harrier 2003; Liu et al. 2007; Fiorilli et al. 2009) but also in fruits (Salvioli et al. 2012). Transcript profiles of the shoots of mycorrhizal plants indicated the systemic induction of many genes predicted to be involved in stress or defense responses in *Medicago truncatula* (Liu et al. 2007) colonized by *G. intraradices* or *G. versiforme*, while down-regulation of many defence-related genes was detected in shoots of tomato colonized by *F. mosseae* (Fiorilli et al. 2009). In spite of these apparently contradictory reports, it is clear that a systemic effect exists, and probably different plants and/or different AM fungi-plant combinations may lead to distinctive reactions. Specific efforts will be necessary to better elucidate the phenomenon and understand the mechanisms and signals involved.

The reduction in virus amount and disease symptomatology in mycorrhizal plants is a novel and interesting result. In the limited literature, where the influence of mycorrhization on virus infection was studied, the AM fungal colonization in roots did not reduce virus infection, but rather increased it (Daft and Okusanya 1973; Dehne 1982; Shaul et al. 1999). This increase in virus amount was observed with several mycorrhizal plant/virus combinations, including tomato infected by TMV and Potato virus X. In our previous work (Miozzi et al. 2011) with the same tomato cultivar, AM fungus, growth condition (with the exception of Pi nutritional levels), we found that a different RNA virus, TSWV, multiplied better in mycorrhizal plants. All these data suggest that the reason of the different reaction that we observed with TYLCSV is likely due to the particular nature of this virus. In fact, this and other geminiviruses are phloem-limited and colonize the nucleus of cells (Morilla et al. 2004), and encode proteins capable to interact with plant hormone pathways.

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), are plant signaling molecules involved, among others, in plant defense (Penninckx et al. 1998; Schenk et al. 2000; Heil and Ton 2008). JA is involved in the process of mycorrhiza formation, as demonstrated in several host-fungus interactions (Hause et al. 2007; López-Ráez et al. 2010) as well as specifically in tomato (Herrera-Medina et al. 2007), and it has been implicated in priming plant defenses (Jung et al. 2012). In a previous work we demonstrated that mycorrhization induces in tomato roots a significant increase of the JA level (Miozzi et al. 2011). Therefore, it is possible that a higher JA level renders the mycorrhizal plants a less favourable environment for TYLCSV, limiting its replication and reducing the severity of symptoms. In favour of this hypothesis there are some lines of evidence. First, in the case of another geminivirus, *Beet curly top virus*, it was recently demonstrated in *Arabidopsis* that application of exogenous MeJA results in milder symptoms and lower viral DNA accumulation, indicating a disruption of the geminivirus infection by this compound (Lozano-Duran et al. 2011). Second, using another geminivirus, *Cabbage leaf curl virus*, repression of jasmonate responsive genes was

reported (Ascencio-Ibañez et al. 2008). Third, geminiviruses have evolved proteins able to interfere with JA metabolism and response, repressing the JA pathway (Ascencio-Ibañez et al. 2008; Yang et al. 2008).

We are aware that the mycorrhizosphere is a complex environment where other microbes associates to mycorrhizal fungi and roots and may influence plant growth and health (Bonfante and Anca, 2009). However, to our knowledge, this is the first time that an amelioration of a viral disease severity in plants colonized by an AM fungus has been observed, at least under experimental conditions. If this will be confirmed in other plant/virus/fungus combinations, and in field conditions, the use of mycorrhizal inocula might help to limit the damage caused by *Geminiviridae*, a family of viruses which counts hundreds of species causing severe crop losses worldwide.

ACKNOWLEDGEMENTS

We thank Fondazione CRT for funding in part the PhD fellowship of GM. This work was supported by the CISIA Project (CNR, Italy) and by the ‘‘GenoPom’’ project (MIUR, Italy) to GPA and by the “60% University grant” to LL. We are grateful to M. Vecchiati for technical assistance.

REFERENCES

Accotto GP, Navas-Castillo J, Noris E, Moriones E, Louro D (2000) Typing of Tomato yellow leaf curl viruses in Europe. Eur J Plant Pathol 106(82):179-186

Aroca R, Vernieri P, Ruiz-Lozano JM (2008) Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. J Exp Bot 59:2029-2041

Ascencio-Ibáñez JT, Sozzani R, Lee TJ, Chu TM, Wolfinger RD, Cella R, Hanley-Bowdoin L (2008) Global analysis of *Arabidopsis* gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. Plant Physiol 148(1):436-454

Balestrini R, Gómez-Ariza J, Lanfranco L, Bonfante P (2007) Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. Mol Plant Microbe Interact 20(9):1055-1062

Bekers GJ, Conrath U (2007) Priming for stress resistance: from the lab to the field. Curr Opin Plant Biol 10:425-431

Bonfante P, Anca I-A (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol 63:363-383

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nature Commun 1-11

Conrath U, Beckers GJM, Flors V, Gracia-Agustin P, Jakab G, Mauch F, Newman MA, Pieterse CMJ, Poinssot B, Pozo MJ et al (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062-1071

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microbe Interact 11(10):1017-1028

Czosnek H (2007) Tomato yellow leaf curl virus disease. Management, molecular biology, breeding for resistance. Springer, Netherlands ISBN: 978-1-4020-4768-8

Daft MJ, Okusanya BO (1973) Effect of *Endogone* mycorrhiza on plant growth. V. Influence of infection on the multiplication of viruses in tomato, petunia, strawberry. New Phytol 72:975-983

D'Amelio R, Massa N, Gamalero E, D'Agostino G, Sampo S, Berta G, Faoro F, et al. (2007) Preliminary results on the evaluation of the effects of elicitors of plant resistance on chrysanthemum yellows phytoplasma infection. B Insectol 60(2):317-318

Dehne HW (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72(8):1115-1119

de la Peña E, Echeverría SR, van der Putten WH, Freitas H, and Moens M (2006) Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytol 169:829-840

Fiorilli V, Catoni M, Miozzi L, Novero M, Accotto GP, Lanfranco L (2009). Global and cell-type gene expression profiles in tomato plants colonized by an arbuscular mycorrhizal fungus. New Phytol 184:975-987

Fiorilli V, Catoni M, Francia D, Cardinale F, Lanfranco L (2011) The arbuscular mycorrhizal symbiosis reduces disease severity in tomato plants infected by *Botrytis cinerea*. J Plant Pathol 93:237- 242

Fritz M, Jakobsen I, Lyngkjaer MF, Thordal-Christensen H, Pons-Kuehnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza 16(6):413-419

García-Chapa M, Batlle A, Laviña A, Camprubí A, Estaún V, Calvet C (2004) Tolerance increase to pear decline phytoplasma in mycorrhizal OHF-333 pear rootstock. Acta Hort 657:437-441

Gernns H, Von Alten H, Poehling HM (2001) Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen - is a compensation possible? Mycorrhiza 11:237-243

Gomez-Ariza J, Balestrini R, Novero M, Bonfante P (2009) Cell-specific gene expression of phosphate transporters in mycorrhizal tomato roots. Biol Fert Soils 45:845-853

Hanssen IM, Lapidot M, Thomma BPHJ (2010) Emerging viral disease of tomato crops. Mol Plant Microbe Interact 23(5):539-548

Hause B, Mrosk C, Isayenkov S, Strack D (2007) Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 68(1):101-110

Heil M, Ton J (2008) Long-distance signalling in plant defence. Trends Plant Sci 13(6):264-272

Herrera-Medina MJ, Steinkellner S, Vierheilig H, Bote JAO, Garrido JMG (2007) Abscisic acid determines arbuscule development and functionality in the tomato arbuscular mycorrhiza. New Phytol 175:554-564

Hewitt EJ, Bureaux CA (1966) Sand and water culture methods used in the study of plant nutrition. Cambridge Univ Press

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139-146

Ismail Y, Hijri M (2012) Arbuscular mycorrhisation with *Glomus irregulare* induces expression of potato PR homologues genes in response to infection by *Fusarium sambucinum*. Funct Plant Biol 39(3):236-245

Jeske H (2009) Geminiviruses. Curr Top Microbiol Immunol 331:185-226

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming of plant defenses. J Chem Ecol 38(6):651-64

Kheyr-Pour A, Bendahmane M, Matzeit V, Accotto GP, Crespi S, Gronenborn B (1991) Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucleic Acids Res 19(24):6763-6769

Khaosaad T, García-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39(3):727-734

384 Kobra N, Jalil K, Youbert G (2009) Effects of three *Glomus* species as biocontrol agents against verticillium-induced
385 wilt in cotton. J Plant Prot 49(2):185 189
386
387 Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and
388 phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970-984
389
390 Lanfranco L, Young JPV (2012) Genetic and genomic glimpses of the elusive arbuscular mycorrhizal fungi. Curr Opin
391 Plant Biol 15(4):454-461
392
393 Lapidot M, Friedmann M (2002) Breeding for resistance to whitefly-transmitted geminiviruses. Ann Appl Biol
394 140(2):109-127
395
396 Li HY, Yang GD, Shu HR, Yang YT, Ye BX, Nishida I, Zheng CC (2006) Colonization by the arbuscular mycorrhizal
397 fungus *Glomus versiforme* induces a defense response against the root-knot nematode *Meloidogyne* incognita in the
398 grapevine (*Vitis amurensis* Rupr.), which includes transcriptional activation of the class III chitinase gene VCH3. Plant
399 Cell Physiol 47:154-163
400
401 Lingua G, D'Agostino G, Massa N, Antosiano M, Berta G (2002) Mycorrhiza-induced differential response to a yellows
402 disease in tomato. Mycorrhiza 12:191-198
403
404 Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal
405 symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in
406 the shoots. Plant J 50(3):529-544
407
408 López-Ráez JA, Verhage A, Fernández I, Garciá JM, Azcón-Aguilar C, Flors V, Pozo MJ (2010) Hormonal and
409 transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the
410 regulation of the oxylipin pathway. J Exp Bot 61:2589-2601
411
412 Lozano-Durán R, Rosas-Díaz T, usmaroli G, Luna AP, Taconnat L, Deng XW, Bejarano ER (2011) Geminiviruses
413 subvert ubiquitination by altering CSN-mediated derubylation of SCF E3 ligase complexes and inhibit jasmonate
414 signaling in *Arabidopsis thaliana*. Plant Cell 23(3):1014-1032
415
416 Mansoor S, Zafar Y, Briddon RW (2006) Geminivirus disease complexes: the threat is spreading. Trends Plant Sci
417 11(5):209-212
418
419 Miozzi L, Catoni M, Fiorilli V, Philip MM, Accotto GP, and Lanfranco L (2011) Arbuscular mycorrhizal symbiosis
420 limits foliar transcriptional responses to viral infection and favors long-term virus accumulation. Mol Plant Microbe
421 Interact 24(12):1562-1572
422
423 Morilla G, Krenz, B, Jeske H, Bejarano ER, Wege C (2004) Tete a tete of tomato yellow leaf curl virus and Tomato
424 yellow leaf curl Sardinia virus in single nuclei. J Virol 78(19):10715-10723
425
426 Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht M, Jakobsen I, Levy AA, Amrhein N, and Bucher M
427 (2005) The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and
428 *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. Plant J
429 42:236-250
430
431 Ozgonen H, Erkilic A (2007) Growth enhancement and *Phytophthora* blight (*Phytophthora capsici* Leonian) control by
432 arbuscular mycorrhizal fungal inoculation in pepper. Crop Protection 26(11):1682-1688
433
434 Penninckx I, Thomma B, Buchala A, Metraux JP, Broekaert WF (1998) Concomitant activation of jasmonate and
435 ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10:2103-2113
436
437 Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea, JM, Azcon-Aguilar C (2002) Localized *versus* systemic
438 effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J Exp Bot
439 53(368):525-534
440
441 Pozo JM, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393-398
442
443 Pozo MJ, Jung SC, Lòpez-Ràez J, Azcón-Aguilar C (2010) Impact of arbuscular mycorrhizal symbiosis on plant
444 response to biotic stress: The role of plant defence mechanisms. In: Koltai H, Kapulnik Y (ed) Arbuscular Mycorrhizas:

445 Physiology and Function, 2nd edn. Springer-Verlag, Heidelberg, Germany,193-207
446
447 Rasmussen R (2001) Quantification on the LightCycler instrument. In: Rapid Cycle Real-Time PCR: Methods and
448 Applications. Meuer S, Wittwer C, Nakagawara K (ed) Springer-Verlag, Heidelberg, Germany 21-34
449
450 Rosendahl S (1985) Interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* and
451 *Aphanomyces euteiches* root rot of peas. J Plant Physiol 144(1):31-40
452
453 Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-induced changes in disease
454 severity and PR protein expression in tobacco leaves. Mol Plant Microbe Interact 12:1000-1007.
455
456 Salvioli A, Zouari I, Chalot M, Bonfante P (2012) The arbuscular mycorrhizal status has an impact on the transcriptome
457 profile and amino acid composition of tomato fruit. BMC Plant Biol 12(44):1-12
458
459 Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM (2000). Coordinated plant
460 defence responses in *Arabidopsis* revealed by microarray analysis. PNAS USA 97:11655-11660
461
462 Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, and Kapulnik Y (1999) Mycorrhiza-induced changes in disease
463 severity and PR protein expression in tobacco leaves. Mol Plant Microbe Interact 12:1000-1007.
464
465 Slezack S, Dumas-Gaudot E, Paynot M, and Gianinazzi S (2000) Is a fully established arbuscular mycorrhizal
466 symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*? Mol Plant Microbe
467 Interact 13(2):238-241
468
469 Taylor J, Harrier LA (2003) Expression studies of plant genes differentially expressed in leaf and root tissues of tomato
470 colonised by the arbuscular mycorrhizal fungus *Glomus mosseae*. Plant Mol Biol 51:619-629
471
472 The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution.
473 Nature 485: 635–641
474
475 Trouvelot A, Kough K, Gianinazzi-Pearson V (1986) Measure du taux de mycorrhization VA d'un système radiculaire.
476 Proceedings of the 1st ESM 217-221
477
478 Utkhede R (2006) Increased growth and yield of hydroponically grown greenhouse tomato plants inoculated with
479 arbuscular mycorrhizal fungi and *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Biocontrol 51(3):393-400
480
481 Van der Heijden MGA, Sanders IR (2002) Mycorrhizal ecology. Berlin: Springer
482
483 Varma A, Malathi VG (2003) Emerging geminivirus problems: A serious threat to crop production. Ann Appl Biol
484 142(2):145-164
485
486 Veresoglou SD, Rillig MC (2012) Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal
487 fungi. Biol Lett 8(2):214-217
488
489 Vos C, Schouteden N, van Tuinen D, Chatagnier O, Elsen, A, De Waele D, Panis B, Gianinazzi-Pearson V (2013)
490 Mycorrhiza-induced resistance against the root-knot nematode *Meloidogyne incognita* involves priming of defense gene
491 responses in tomato. Sol Biol Biochem 60:45-54
492
493 Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198-1227
494
495 Xu GH, Chague V, Melamed-Bessudo C, Kapulnik Y, Jain A, Raghothama KG, Levy AA, Silber A (2007) Functional
496 characterization of LePT4: a phosphate transporter in tomato with mycorrhiza-enhanced expression. J Exp Bot 58:2491-
497 2501
498
499 Yang JY, Iwasaki M, Machida C, Machida Y, Zhou X, Chua NH (2008) βC1, the pathogenicity factor of TYLCCNV,
500 interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. Genes Dev 22: 2564-2577
501

502 Table 1: Primers designed on selected genes used in RT-qPCR

Gene name and annotation	Primer sequence	Amplicon size (bp)
SGN-U223227 Cytochrome p450	C450F: GGAATCAACTTAGCCAAACTGG	241
	C450R: ACAGCACCATGGTTATTTTTCC	
SGN-U222911 Putative kinesin-like protein	911F: CAAGAAATCAGAAGGGGACAAC	187
	911R: GAACCATCTCTTTCCGCTCTTA	
SGN-U214669 UDP-gluconorosyl transferase family protein	UDPF: GTTCAATGTTGTTGTTACGCCTTCA	228
	UDPR: TAGCTAATCCCCAAGCAGTCTC	
SGN-U236747 Beta-xylosidase α-l-arabinosidase	βxylF: GATGGTAATCCAAAAAGCCGTA	152
	βxylR: ATGGCAGTCGGAGTTAAAGGTA	
LePT4 phosphate transporter	LePT4F: GAAGGGGAGCCATTTAATGTGG	182
	LePT4R: ATCGCGGCTTGTTTAGCATTTCC	

503
504

505
506
507 **FIGURE LEGENDS**

508 **Fig. 1**

509 Biomass of the aboveground (a, shoot) and underground (b, root) portion of plants in the four conditions tested (C, M, V
510 and MV), measured 28 days after virus inoculation. Vertical lines on each bar represent standard error. Different letters
511 indicate statistically significant differences ($p<0.05$, Kruskal-Wallis test).

512 **Fig. 2**

513 Mycorrhization levels of mycorrhizal (M) and TYLCSV-infected mycorrhizal (MV) plants. F%, frequency of
514 mycorrhization; M%, intensity of mycorrhization; a%, percentage of arbuscules within infected areas; A%, percentage
515 of arbuscules in the root system. Vertical lines on each bar represent standard error. Different letters indicate statistically
516 significant differences ($p<0.05$, Kruskal-Wallis test).

517 **Fig. 3**

518 Evaluation of symptoms caused by TYLCSV in mycorrhizal (MV) and non-mycorrhizal (V) tomato plants. Disease
519 severity index (DSI) ranging between 1 (mild symptoms) and 4 (severe symptoms). a) graphic representation
520 (percentage of plants scored 1 to 4 DSI in the two sets of plants; b) example of a plant with DSI=2 on the left and one
521 with DSI=4 on the right.

522 **Fig. 4**

523 Virus concentration in shoots and roots. ΔCt values on the vertical axis represent the difference between Ct (threshold
524 cycle) of TYLCSV and Ct of reference gene, and measure viral concentration in the four conditions. Vertical lines on
525 each bar represent standard error. Different letters indicate statistically significant differences within the same organ
526 ($p>0.05$, ANOVA).

527 **Fig. 5**

528 Expression of selected AM symbiosis-responsive genes (Kin: kinesin-like protein, β-xyl: β-xylosidase β-l-
529 arabinosidase; Cyt P450: cytochrome P450 CYP707A3; UDP-gluc: UDP-glucoronosyl UDP-glucosyl transferase; PT4:
530 phosphate transporter. Expression values (bars ± standard error) for each gene are given as $\log_2$ of fold change (FC)
531 relative to C. Different letters indicate statistically significant differences ($p<0.05$, ANOVA).

532

533

Figure 1

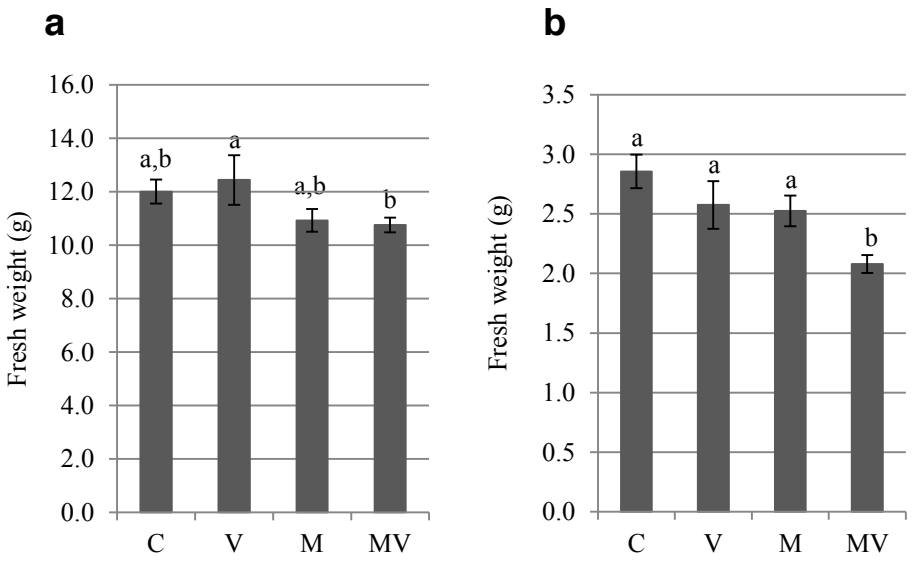

FIG 1

Figure 2

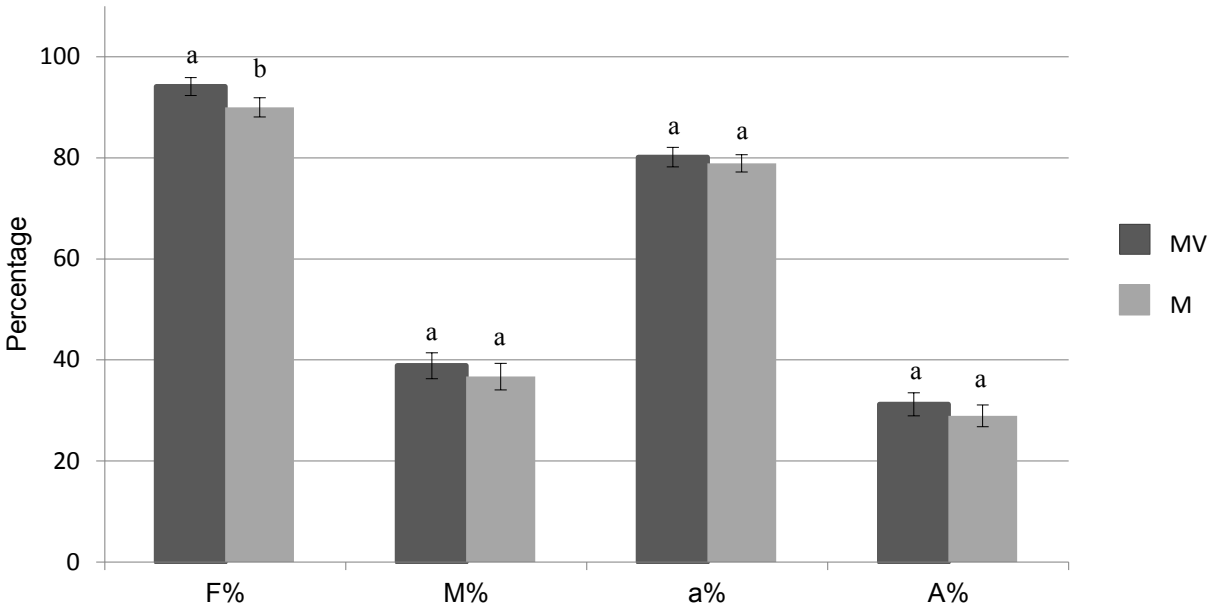

FIG 2

Figure 3

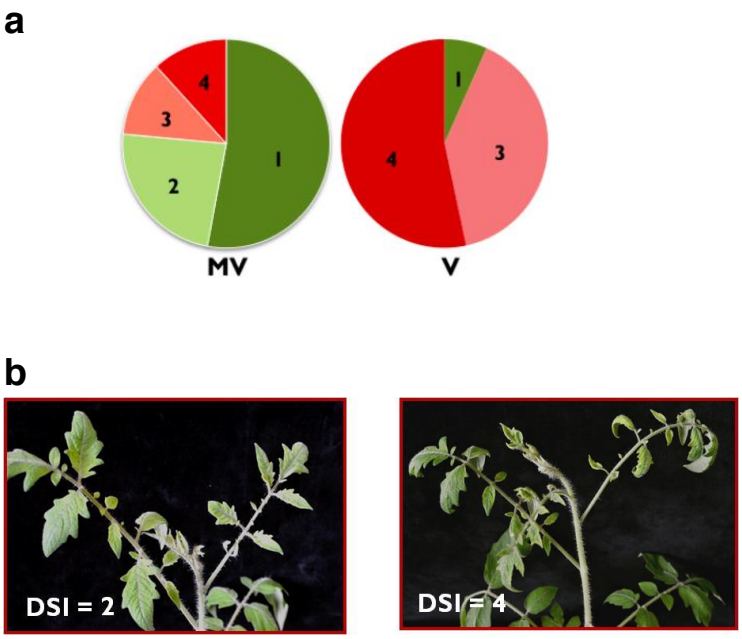

FIG 3

Figure 4

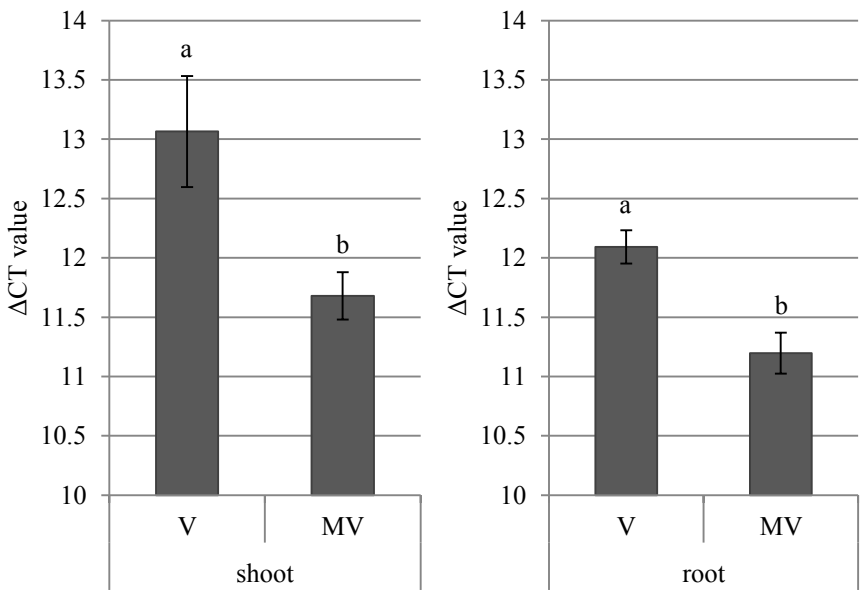

FIG.4

Figure 5

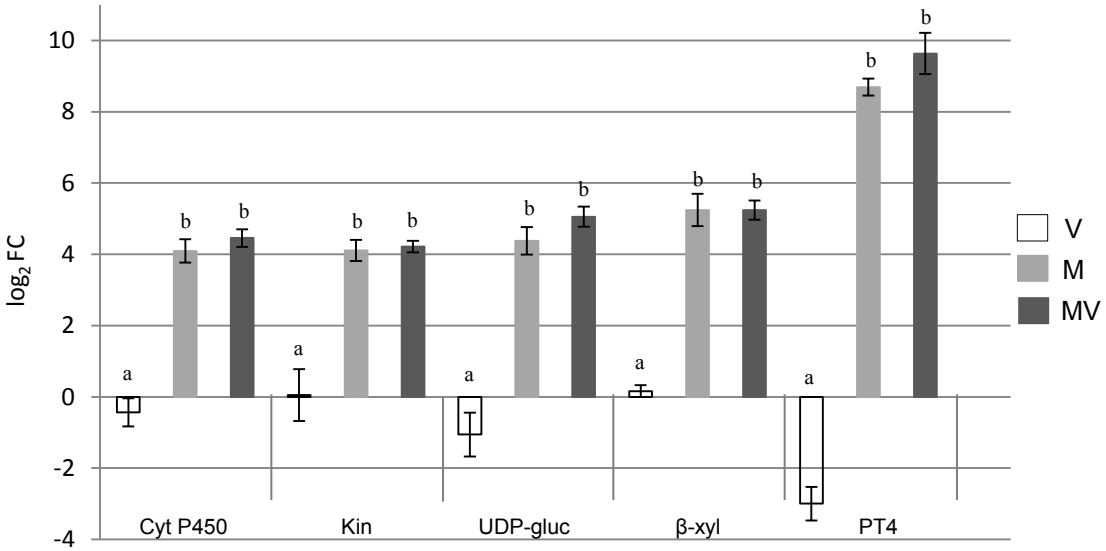

FIG. 5