Effects of two AM fungi on phytoplasma infection in the model plant *Chrysanthemum carinatum*

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Phytoplasmas are plant pathogenic bacteria, naturally transmitted by insects and confined in the phloem of the host plant, where they take up nutrients and eventually cause plant death. Their control is mainly based on insecticide treatments. The aim of this work was to study the effect of two AM fungi in modifying plant response to chrysanthemum yellows phytoplasma (CY) infection in chrysanthemum plants. Inoculation of *Glomus intraradices* BB-E and *G. mosseae* BEG12 reduced the damage caused by this plant pathogen in the aerial part of the plant, increased plant tolerance to the infection and reduced the severity of symptom expression, probably in a systemic way. Inoculation with *G. mosseae* did not alter CY multiplication and viability in young leaves, whilst the morphology of CY mature leaves was typical of senescent cells. Possible mechanisms involved are discussed.

Key words: Phythoplasma infection, *Glomus intraradices*, *G. mosseae*, *Chrysanthemum carinatum,* resistance, tolerance

Introduction

Mycorrhizal fungi are specialized members of a vast population of microorganisms that colonize the rhizosphere (Smith and Read 2008). Among mycorrhizal associations, arbuscular mycorrhizas (AMs) represent the most widespread and ancient type. They are formed by a wide range of land plants, including at least 80% Angiosperms, and fungi belonging to the Glomeromycota phylum (Schüßler et al. 2001). The location of the fungal symbiont in the root, and the hyphal connection with the soil, ensure the absorption of soil-derived nutrients, through the fine exploration of the rhizosphere and nearby soil, and the translocation of nutrients, otherwise not available, to the plant. This symbiotic association is known to carry out many critical ecosystem functions such as improvement of plant establishment, enhancement of plant nutrient uptake, improvement of soil structure (Smith and Read 2008), plant protection against cultural and environmental stresses (Mirasnari et al. 2010, Smith et al. 2010), including biotic stress (Berta et al. 2005). AMs, in fact, can induce resistance or increased tolerance to a number of root pathogens (Azcon-Aguilar and Barea 1996, Trotta et al. 1996, Cordier et al. 1996, Demir and Akkopru 2005, Akhtar and Siddiqui 2008). On the contrary, contrasting results have been reported on the role of AM against arial pathogens (Dehne 1982, Lindermenn 1994, Dugassa et al. 1996, Shaul et al. 1999, Guerrieri et al. 2004, Akhtar and Siddiqui 2008, Lopez-Raez et al. 2010).

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Phytoplasmas are plant pathogenic Mollicutes, naturally transmitted by insects and restricted to the phloem of the plant, where they live and multiply, taking up host nutrients and eventually causing plant death (McCoy et al. 1989, Deng and Hiruki 1991, Weintraub and Beanland 2006). They are associated with economically important diseases affecting fruit trees, vegetables, ornamentals and weeds, mostly causing proliferation, stunting, yellowing and virescence or phyllody (Bertaccini 2007). The control of phytoplasma diseases relies on insecticide treatments to reduce vector populations and, in some cases, rouging of infected plants. Insecticide treatments against insect vectors, often required by law, have a negative impact on the environment and human health, and, in addition, their efficacy depends on the adoption of this practice by all the farmers in an area. Hence, the presence of AM fungi associated with induced resistance against a number of pathogens (Ismail and Hijri 2010, Kempel et al. 2010) may represent a valid alternative to control phytoplasma diseases. Lingua et al. (2002) showed that symptoms induced by the Stolbur phytoplasma in tomato plants were less severe when colonized by AM fungi. AM fungal inoculum also improved morphology and flow cytometry parameters of both healthy and stolbur-infected plants, increasing root system development and inducing callose accumulation on the sieve pores (Lingua et al. 2002). Similar structural modifications occurred in sieve elements of elicitin-treated and Stolbur-infected tobacco plants (Delannoy 2003).

Chrysanthemum yellows phytoplasma (CY) is a strain of the "*Candidatus* Phytoplasma asteris" phytoplasma (16Sr-IB) which infects a variety of dicotyledonous plants and is transmitted with different efficiencies by a number of leafhopper species (Bosco et al. 2007). Efficient CY infection of chrysanthemum can be achieved under controlled conditions (Bosco and D'Amelio 2010) and several reagents are available to study phytoplasma multiplication and viability in the plant (Marzachì and Bosco 2005, Gamalero et al. 2010). In the present study we analyzed the ability of two AM fungi, *Glomus mosseae* and *G. intraradices*, to alleviate phytoplasma-specific symptoms, modify plant development, root architecture and cell morphology, and alter phytoplasma multiplication and viability in daisy (*Chrysanthemum carinatum)* as a model plant.

Material and methods

Seed sterilization

Chrysanthemum carinatum (Schousboe) seeds were sterilized by gently shaking in 5% (v/v) sodium hypochlorite for 3 min. The seeds were then rinsed six times for 5 min and four times for 20 min in sterile deionized water, placed in Petri-dishes on moist sterile filter paper and incubated in the dark at 24 °C for 3 days. Twenty daisy seeds were placed on nutrient agar (NA-Fluka) for 3 days to verify the efficacy of the sterilization procedure.

Plant growth conditions and treatments

C. carinatum plants were grown in 8×8 cm pots containing a mixture of 0.6−1.2 mm coarse grade quartz sand sterilized at 200 °C for 2 h. Inocula of the two AM fungi, *Glomus mosseae* (Gerd. and Nicol.) Gerdemann and Trappe BEG 12, and *G. intraradices* (Schenck and Smith) BB-E were supplied by Biorize (Dijon, France) on a sand carrier. In a preliminary experiment to select the AM fungus to be used in the main experiment (based on the symptom severity induced by phytoplasma infection on *C. carinatum* L.), *G. mosseae* and *G. intraradices* were introduced by mixing 25% (v/v) of the fungal inoculum to the sterile quartz sand at sowing. Control plants were sowed in the sterile quartz sand without the inoculum. In this

experiment, 6 treatments (15−20 replicates) were compared: control plants (C), CY-infected plants (CY), *G. mosseae* plants (Gm), *G. intraradices* plants (Gi), CY-infected plants with *G. mosseae* (GmCY) and CY-infected plants with *G. intraradices* (GiCY).

In the main experiment, to investigate the possible role of AM fungi in modifying plant response to phytoplasma infection, only the AM fungus *G. mosseae* was used. Plants were grown in a greenhouse (25 °C, photoperiod 16 hours light, 8 hours dark) and watered to saturation with a modified Long Ashton nutrient solution containing 32 µM phosphate three times per week (Trotta et al. 1996). Four treatments (15 replicates per treatment) were compared: control plants (C), CY-infected plants (CY), *G. mosseae* plants (Gm), and CY-infected plants with *G. mosseae* (GmCY).The experiment was repeated twice.

Phytoplasma and vector insect

The Chrysanthemum yellows (CY) strain of the "*Candidatus* Phytoplasma asteris" originally isolated from *Argyranthemum frutescens* (L.) Schultz-Bip plants in Liguria, Italy (Conti et al. 1987), was maintained on daisy (*C. carinatum*) by vector transmission.

Healthy colonies of *Macrosteles quadripunctulatus* (Kirschbaum) were reared on potted oat plants inside plexiglas and nylon cages in growth chambers at 25 °C, photoperiod 16 hours light, 8 hours dark and checked by PCR assays to verify phytoplasma absence.

For transmission experiments, third-fifth in star nymphs were fed for 1 week on CY-infected plants, transferred on to healthy oat for 2 weeks to complete latency and then transferred singly to 10/15 daisies for each elicitor and control treatment for an inoculation access period (IAP) of 3 days inside glass cylinders. Inoculation with single vectors was performed in order to assure a low initial concentration of the phytoplasmas in the plant. Test plants were exposed to vectors 2 months after sowing. All the plants were then treated with insecticides and maintained in the greenhouse for one month. Daisies treated with water and exposed to CY-infective vectors were used as transmission controls and plants inoculated with AM fungi (*G. mosseae* and *G. intraradices)* not exposed to vectors were used as treatment controls. The experiment was repeated twice.

Apical leaves of the same 5 inoculated plants from each treatment were sampled at 17 and 24 days post inoculation (dpi), and total DNA was extracted for quantification of CY DNA. The quantification of phytoplasma DNA was repeated three times. At 17 and 24 dpi total RNA was extracted from the same 5 inoculated plants from each treatment for the quantification of CY 16S rDNA transcripts.

Symptom evaluation

Presence and severity of CY symptoms on test plants (15 per treatment) was evaluated 3 times a week starting from their first appearance until 28 days after the beginning of inoculation (dpi) and plants were classified in 5 classes of severity: 0 = no symptoms, 1 = yellowing of the apex, 2 = yellowing and distortion of the apex, 3 = apex growth stunt, 4 = severe yellowing and dwarfing of the whole plant, 5 = plant death.

Evaluation of mycorrhizal colonization

Thirty root pieces (1 cm long each) per root system were sampled. These pieces were cleared for 30 min at 60 °C in 10% KOH, stained with 1% methyl blue in lactic acid and finally mounted on slides. The percent-

ages of root fragments colonized by the fungus (F%) and the intensity of colonization of the root cortex (M%) in all fragments, and arbuscle (A%) and vesicle (V%) abundance in the mycorrhizal root cortex were evaluated microscopically using the scale described by Trouvelot et al. (1986). M%, A% and V% were determined by calculating weighted indices based on scoring frequencies of root area colonized by the fungus and from 0 to 5 according to the root area colonized by the fungus and from 0 to 3 according to the number of arbuscules, respectively.

Evaluation of plant development and root architecture

Ten plants per treatment were collected 3 months after sowing (at 30 dpi). Shoot growth was assessed by evaluating shoot fresh and dry weight, number of dead leaves and root growth by quantifying fresh and dry weight and root branching degree (T/TL), represented by root tip number divided by total root length. For this purpose, whole root systems were placed in a transparent water container and digitized by using a dedicated Desk Scan II scanner, equipped with a special lighting system for root measurement, connected to a Power Macintosh 4400/200 computer. Digitized root images were analysed by WinRhizo software (Régent Instruments, Canada) and morphometric parameters were evaluated.

Phytoplasma concentration: DNA extraction and quantitative real time PCR

Samples of 200 mg were collected from apical leaves (5 plant per treatment) at 17 and 24 days post inoculation (dpi), and total DNA was extracted from 100 mg with the Pure Link Plant Total DNA Purification Kit (Invitrogen Carlsbad, CA, USA) according to the manufacturer's protocol. The same plants were sampled at both dates. Concentration of DNA extracts was estimated using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Wilmington, DE, USA). Diluted samples (1ng/µL in sterile double-distilled water) were analysed in triplicate in quantitative real time PCR (Q-PCR) assays. CY DNA in each sample was measured as the number of CY genome units (GU) per ng of plant DNA as described by Marzachì and Bosco (2005). Q-PCR reactions were performed with Platinum Quantitative Supermix-UDG (Invitrogen, UK) with 300 nM primers (CYS2Fw/ Rv for CY; ChrysFw/Rv for daisy) and 200 nM probes (CYS2Probe for CY; ChrysProbe for daisy), together with 5 µL of the corresponding template, in a final volume of 25 µL. Reactions were run in 96-well plates in a BioRad iCycler (BioRad) using cycling conditions described by Marzachì and Bosco (2005). DNA from healthy plants and PCR mix devoid of template were used as negative controls. Threshold cycles and standard curves were automatically calculated by the BioRad iCycler software (version 3.06070). CY and host DNA from the same sample were quantified in the same plate.

Phytoplasma viability: total RNA extraction and quantitative reverse transcription real time PCR

Total RNA was extracted with the Trizol Reagent (Invitrogen, UK) following the manufacturer's recommendations from 0.1 g of CY infected daisy apical leaves, ground to powder under liquid nitrogen. DNA contamination was removed by RQI RNase-free DNase (Promega, Italy) digestion at 37 °C for 30 min. Total RNA, extracted twice with a mixture of phenol and chloroform and precipitated with ethanol and ammonium acetate (0.5 M), was then resuspended to a final concentration of 5 ng/ µL and 1 µL was loaded as template in reverse transcription (RT) PCR experiments. Absolute quantification of phytoplasmas 16Sr transcripts was achieved with a standard curve method as detailed in Gamalero et al. (2010). In control reactions, RNA samples were not supplemented with reverse transcriptase to rule out DNA contamination. RNA from healthy plants and PCR mix devoid of template were used as negative controls.

The RT-PCR samples were amplified in triplicate in 96-well plates in a BioRad iCycler (BioRad) and the cycle parameters were as in Gamalero et al. (2010). Phytoplasma 16S rDNA transcript copy number per 100 mg of fresh leaf tissue was then derived. The viability of CY (expressed as 16S rDNA transcripts per phytoplasma cell) was estimated by dividing the transcript copy number by the number of CY cells in 100 mg of the same sample (measured as detailed above).

Light and electron microscopy

Three fully developed and mature leaves for each treatment were harvested for microscopical analyses. The median zone of each leaf was cut, fixed overnight in 3% glutaraldehyde in 0.15 M phosphate buffer (pH 7.2) for 3 h at 4 °C, post-fixed in 1% OsO_4 in the same buffer for 15 min at room temperature, stained in 1% uranyl acetate in water for 12 h at 4 °C, dehydrated in ethanol series (30−100 °C) and embedded in Epon-araldite resin at 60 °C. Ultrathin sections were cut with a diamond knife in a Leica Ultracut UCT ultramicrotome (Milan, Italy) stained with lead citrate and examined through a Philips CM 10 (Eindhoven, Netherland*).*

Statistical analysis

Symptom evaluation. At each rating date severity class means and standard errors were calculated for CY infected plant colonized or not with *G. mosseae* and *G. intraradices*. Pairwise t-tests were performed to compare symptom severity between treatments, according to Alexander et al. (1993).

Evaluation of mycorrhizal infection, plant development and root architecture. All data means were analysed for significance aims by analysis of variance followed by Fisher's least significance difference test ($p \leq 0.05$) using Statview ® software (SAS Institute, Cary, NC, USA).

Phytoplasma concentration. Raw data (obtained as log) were used and the concentration of CY in each plant was expressed as the difference between the logarithm concentration of CY and that of daisy DNAs. To compare the phytoplasma titre measured at different dpi, in elicited and control plants, two-way ANOVA for treatment and date was performed (Jandel SigmaPlot 11.0, Systat Software Inc San Jose, CA USA).

Phytoplasma viability. Raw data (CY transcripts/ CY cells) were transformed into logarithms, since the standard deviation appeared a function of the mean. To compare the number of 16S rDNA transcripts per CY cell (as an estimator of phytoplasma viability) at different dpi in elicited and control plants, two-way ANOVA for treatment and date was performed.

Results

Symptom evaluation

Starting from 21 days post inoculation (dpi) GmCY plants showed less symptom severity than GiCY and CY plants. At the first observation dates the difference in median values of symptom severity was not significant, but from 21 dpi until the end of the experiment GmCY plants exhibited significantly less symptoms than CY (21 dpi p= 0.001, 25 dpi p =0,017, and 28 dpi p =0.004) and GiCY plants (21 dpi p = 0.002, 25 dpi p =0,044, and 28 dpi p =0.002) (data not shown). At the end of experiment, none of the plants inoculated with Gm or Gi was dead (5 class), 30% of GmCY plants were symptomless, and all GiCY plants were in 4 class (severe yellowing and dwarfing of the whole plant). In contrast plants not inoculated with either. Gm or Gi were in 3 and 4 class and 40% of CY plants were dead (5 class) (Fig. 1).

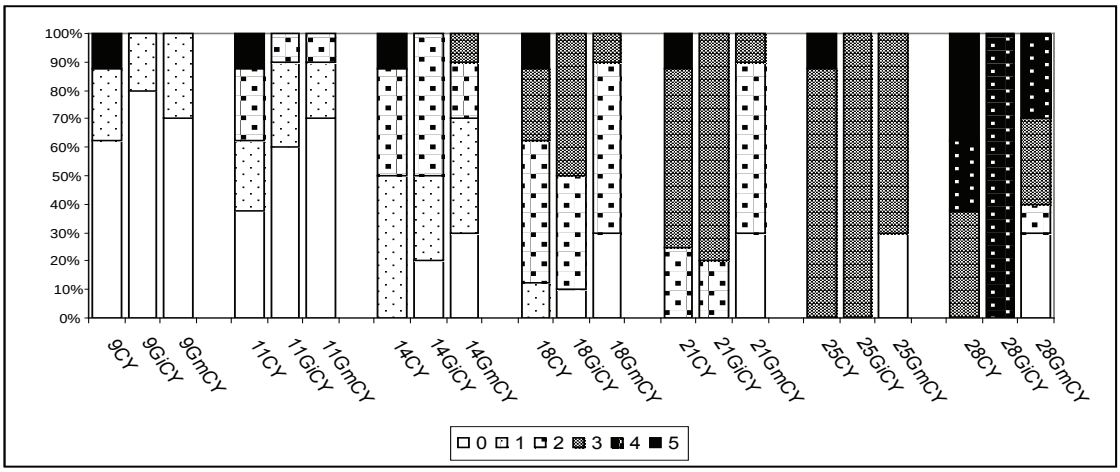

Fig. 1. CY symptom severity for control (CY) and elicited plants (GiCY and GmCY) observed starting from 9 until 28 days post inoculation (dpi). Plants were classified in 5 classes of severity: 0 = no symptoms, 1 = yellowing of the apex, 2 = yellowing and distortion of the apex, 3 = apex growth stunt, 4 = severe yellowing and dwarfing of the whole plant, 5 = plant death.

Mycorrhizal colonization

No significant difference in mycorrhizal colonization (arbuscules, vesicles and percentage of root fragments colonized) was measured. The highest values, mainly in CY treated plants, occurred in Gm (Table 1).

Table 1. Effect of the two AM fungi and phytoplasma infection on plant growth, root architecture and mycorrhizal colonization. Control plants (C), phytoplasma infected plants (CY) *Glomus intraradices* (Gi) and *G. mosseae* (Gm) inoculated plants, inoculated and infected plants (GiCY and GmCY). Mycorrhizal colonization evaluated microscopically as percentages of root fragments colonized by the fungus (F%), intensity of colonization of the root cortex (M%), arbuscular (A%) and vesicles (V%) abundance in the mycorrhizal root. Means with the same letter are not significantly different ($p \leq 0.05$) according to Fisher's least significant difference test.

TL, total length; T, number of tips; T/TL, root branching degree; AD, average diameter.

	C	CY	Gi	GiCY	Gm	GmCY
Fresh shoot weight (g)	8.56±0.89 d	5.88±0.80 e	12.06±0.98 ab	934±0.67 cd	12.71±0.76 a	10.59±0.70 bc
Fresh root weight (g)	4.44±0.69 ab	2.44±0.38 c	5.51±0.43 a	2.54±0.35 c	4.25±0.28 b	2.36±0.41 c
Number of dead leaves	10.67±2.59 a	9.00±1.19 a	10.00±0.76 a	7.37±0.82 a	11.45±1.46 a	5.07±1.37 b
TL (cm)	2310.49±291.65 b	842.65±144.67 d	3377.45±469.70 a	900.17±140.80 d	3281.29±491.75 a	1378.37±302.13 c
T	6357.60±1358.05 ab	1504.20±430.21 d	8463.80±1575.08 a	2014.80±400.91 d	4679.88±1477.79 bc	3702.33±1050.39 c
T/TL	2.72±0.32 a	1.70±0.23 b	2.41±0.28 a	2.24±0.30 a	1.30±0.24 b	2.68±0.23 a
AD (mm)	0.55±0.02 ab	0.59±0.02 a	0.55±0.02 ab	0.57±0.02 a	0.49±0.02 c	0.50±0.02 bc
F%	-	-	80.46±19.53 a	90.46±4.12 a	94.67±1.70 a	94.67±2.26 a
M%	-	-	59.30±15.11 a	50.74±7.89 a	59.41±8.15 a	56.81±3.80 a
A%	-	-	22.82±5.80 a	30.26±4.97 a	24.25±6.30 a	29.62±6.97 a
V%	-	-	19.50±5.00 a	18.61±4.88 a	16.29±5.73 a	21.57±4.15 a

Plant development and root architecture

Fresh shoot weights were significantly reduced in CY plants in comparison to all the treatments. The two fungi, as well as the presence of Gm together with CY, significantly increased fresh shoot weight in comparison to the C plants. The fresh root weights of C, Gi and Gm plants were significantly higher than CY, GiCY and GmCY plants. The number of dead leaves was lower in GmCY plants than in the other treatments (Table 1). Root architecture parameters were affected by the two *Glomus* species. Total root length (TL) was increased respect to C plants. Both TL and number of tips (T) was higher in GmCY plants than in GiCY and CY ones. The degree of root branching was significantly reduced in CY and in Gm plants; it was similar to C plants in all the other treatments (Table 1).

CY titre and viability

CY concentration in *G. mosseae* inoculated plants (GmCY) sampled at 17 dpi was similar to that of the CY plants (Fig. 2a). CY concentration increased significantly between 17 and 24 dpi in CY plants (P = 0.019) but not in the GmCY ones.

No difference in phytoplasma viability was recorded at both sampling dates between GmCY plants by Q-RT-PCR analysis. A significant reduction of phytoplasma viability overtime was measured only for CY plants (P = 0,005) (Fig. 2b).

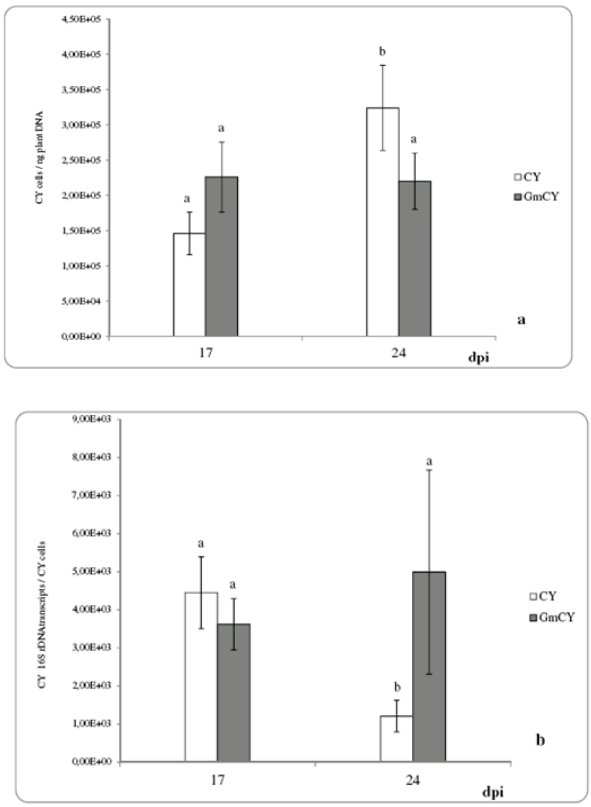

Fig. 2. CY concentration (a) measured as mean CY cells per ng plant DNA (± standard error) and CY viability (b) measured as mean copy number of CY 16SrDNA transcripts per phytoplasma cell (± standard error) in mycorrhized (GmCY) and not (CY) daisies sampled at 17 and 24 days post CY inoculation (dpi). Values followed by different letters are significantly different ($p \leq 0.05$).

Microscopical observations

No phytoplasma cells was detected in uninfected control, Gm and Gi plants (Figs. 3a, 3b, 3c). Phytoplasmas were observed in CY, GmCY and GiCY plants (Figs. 3d, 3e, 3f).

In the phloem vessel of CY plants, CY cells were well preserved, bounded by a well-defined unit membrane and contained a matrix of varying electron densities; some phytoplasmas were observed through the pores of the sieve plate. The whole lumen of phloematic vessel was often filled with phytoplasma cells of different shape and size and at different stage of development (Fig. 3d). The host cells of CY plants showed symptoms not observed in the healthy controls, such as distorted cell walls, deposition of callose near the sieve pores and filaments of PR (Pathogenesis-Related) proteins (Fig. 3d). Few and degenerated phytoplasmas were constantly found in infected plants inoculated with the AM fungi, especially in Gm plants (Fig. 3e).

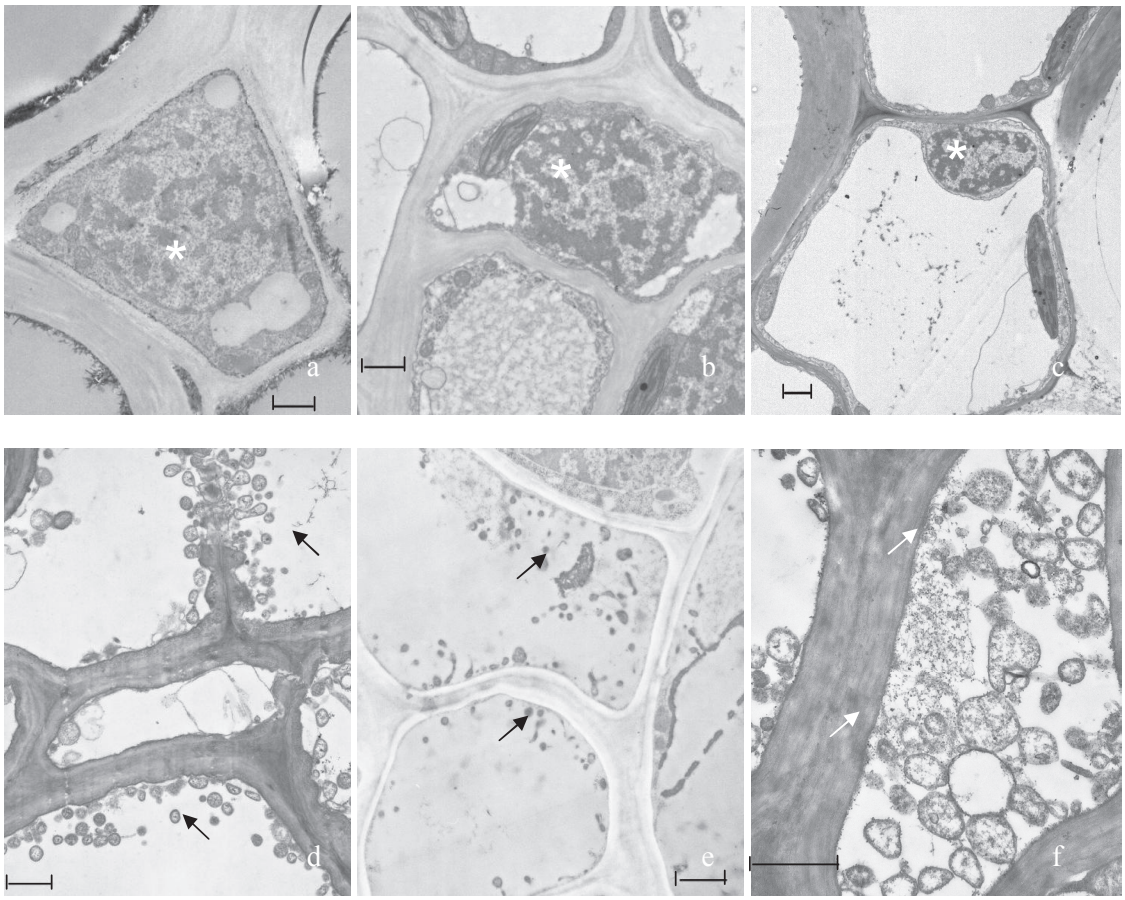

Fig. 3. Phloem vessels and companion cells in leaves of *Chrysanthemum carinatum*. No phytoplasma cells were detected in uninfected control (a) and *Glomus mosseae* (b) and *G. intraradices* (c) plants. Phytoplasmas were observed in CY (d), in GmCY (e) and in GiCY (f) plants (arrows). In the phloem of CY plants, the CY cells were well preserved (a), bounded by a well-defined unit membrane and contained a matrix of varying electron densities; some phytoplasmas were observed through the sieve plate. In the phloem of plants infected with CY and inoculated with the AM fungi, few and degenerated forms of CY were evident, especially in GmCY. Companion cells with well preserved nucleus of the reticulate type are visible (*) (Bar represent 1 μm).

Discussion

Phytoplasma infection depends on the interactions among three partners: the plant, the pathogen and the vector. The introduction of a fourth partner increases the complexity of the system. For this reason the model system employed in this experiment is an important tool to study the effects of elicitors against phytoplasma infection.

The first experiment here described was performed to select the AM fungus to be subsequently used. For this purpose, plant growth (evaluated as plant biomass and root development) was measured as an estimate of symptom severity induced by phytoplasma infection on the model plant *Chrysanthemum carinatum*.

Evaluation of plant development and root architecture showed that inoculation with the two *Glomus* species (Gm and Gi) had an impact on the development of the disease reducing by up to 40%. Moreover, GmCY plants showed less severe symptom than GiCY during the whole experiment. For this reason *G. mosseae* was selected for the subsequent experiment, in which phytoplasma multiplication and viability in GmCY and CY plants was compared. *G. mosseae* induced beneficial morpho-physiological effects on the plant, especially on root morphogenesis, in agreement with Berta et al. (2002). In addition, phytoplasma disease appeared less severe when the infected plants harboured AM fungi; morphological parameters were also similar to those of healthy plants, in agreement with Lingua et al. (2002) and Kaminska et al. (2010).

In our model system the phytoplasma strain CY had negative effect on both shoot and root growth of plants. This is in agreement with other reports on phytoplasma infected herbaceous plants (Lingua et al. 2002, Favali et al. 2004, Ambrožič-Dolinšek et al. 2008, Hoshi et al. 2009, Kaminska et al. 2010, Gamalero et al. 2010) where important reduction of plant, shoot and root biomass, have also been reported. As observed in the pathosystem Stolbur phytoplasma /*Lycopersicon esculentum* Mill. (Lingua et al. 2002), CY infection decreased root branching, as generally observed in pathogen infected roots, leading to a root system less developed and therefore less efficient for water and nutrient uptake (Berta et al. 1993, Hodge et al. 2009). Since the total root length of the infected plants was significantly reduced, the total soil volume explored is expected to be lower than that explored by the root system of healthy plants. This difference could account for the reduced growth of the diseased plants as measured by plant weight. Plant weights in Gm plants were not significantly different to C plants. In presence of CY, mycorrhization improved daisy growth compared to the control plants and alleviated the deleterious impact of CY on the weight of the aerial part of daisy. The beneficial effect of *G. mosseae* in infected daisies was also supported by the measurement of CY symptom development. Symptom severity in the mycorrhizal and infected plants were delayed up to 21 days post infection under our experimental conditions. Beneficial effects of mycorrhizal symbiosis were also observed on the growth of pear (Garcia-Chapa et al. 2004). Our study also shows that this beneficial microorganism was effective in reducing transmission efficiency of CY by its leafhopper vector, as at the end of the experiment a higher number of mychorryza colonized plants was protected from phytoplasma infection compared to the non mychorryzed ones.

By contrast, the AM fungus did not induce beneficial effects at the root level, as total root length was significantly reduced both in CY and in GmCY plants compared to C and Gm plants. In our model system the response of chrysanthemum colonized by *G. mosseae* to the pathogen seems generally less marked than in other plant species. Colonization by AM fungi normally can induce several changes in root organization, resulting in a more-branched root system and increased longevity (Berta et al. 1993, Hooker et al. 1994). Actually, in roots, CY titre is higher compared to that of leaves (Saracco et al. 2006), probably due to a more active multiplication in root tissues and/or translocation of the pathogen to roots following the

phloem stream after multiplication in other plant organs. This could explain the inability of *G. mosseae* to alleviate phytoplasma damage at the root level. In addition, a general decrease in soluble carbohydrates and starch has been observed in tobacco roots infected by phytoplasmas (Lepka et al. 1999). Therefore, strong competition for nutrients is expected to occur at the root level, where both the fungus and the phytoplasma are present.

Phytoplasma titre was not affected by *G. mosseae* colonization. CY viability decreased over time only in the control plants. This result suggests that, irrespective of the presence of the AM fungus, CY infected plants loose the capability to fully support phytoplasma metabolic activity with time (D'Amelio et al. 2011). This hypothesis is consistent with the alteration of photosyntate transport efficiency in phloematic vessel, due to callose deposition on the sieve elements, observed through electron microscopy in CY plants. Phytoplasmas were present and viable only inside the youngest leaves (molecular techniques failed to check them in the oldest ones). Therefore, it is likely that *G. mosseae* acts by improving the tolerance of the plant to CY infection. AM fungi are known to affect plant growth and health by increasing resistance or tolerance to biotic (Cordier et al. 1996, Trotta et al. 1996, Berta et al. 2005, Sampò et al. 2007, Daei et al. 2009, Hodge et al. 2009) and abiotic stress (Joner and Leyval 2003) and improving mineral nutrition (Clark and Zeto 2000, Smith and Read 2008). Furthermore, the beneficial microorganisms could induce a mechanism of systemic defence (ISR) improving plant resistance against phytoplasma pathogen, but this ISR activation is depending on the pathosystems considered. A decreased plant tolerance to phytoplasma infection was also observed in AM inoculated periwinkle plant infected with two aster yellow strains (Kaminska et al. 2010).

Altered levels of IAA, cytokinin (Torelli et al. 2001) and abscisic acid (Esch et al. 1994) have been reported in AM-colonized plants. Moreover, IAA and indole-3-butyric acid (IBA) are involved in recovery of phytoplasma-infected periwinkle (Ćurković Perica 2008). It has been recently shown that expression of a "*Candidatus* Phytoplasma asteris" virulence factor (TENGU) in transgenic tobacco plants down regulates auxin signaling and biosynthesis pathway genes, resulting in altered plant growth (Hoshi et al. 2009). We therefore speculate that reduced levels of endogenous IAA in phytoplasma-infected *Chrysanthemum* plants could be partially relieved by *G. mosseae*.

Overall, these results indicate that *G. mosseae* induced a delay in symptom expression and a reduction of their severity, which we speculate could be predicted in part through IAA.

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