Root architectural traits of rooted cuttings of two fig cultivars: Treatments with arbuscular mycorrhizal fungi formulation

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Keywords: Arbuscular mycorrhizal fungi, Root architecture, Root morphology, Fig, Rooted cutting, Image analysis

Abstract

Many fruit tree species develop symbioses relationships with mycorrhizal fungi by which they improve their efficiency in water and nutrient uptake and, in turn, increase their vegetative growth and productivity, particularly under stressful environments. These benefits origin from the effects that mycorrhizal determined on the root architecture, morphology and physiology. Usually, few attentions has been devoted to the tree root structure and function, especially, in fig plants during their growth phase in the nursery. Recently, several root traits or phenes have been reported as fundamental for the root functions such as the root length ratio (plant's potential for the exploitation of soil resources); root mass ratio (allocation traits); the root fineness and tissue density (structural traits); the root very fine, fine and coarse (functional traits). Aim of the study was to test the effects of an arbuscular mycorrhizal fungi (AMF) on the root architecture traits of self-rooted cuttings of two fig (*Ficus carica* L.) cultivars: Dottato and Natalese. The root architecture traits were evaluated by image analysis system (WinRHIZO). Single root traits and rooting architecture models were statistically tested by univariate and multivariate analysis, respectively. This study confirmed that also the *Ficus carica* was positively responsiveness to the mycorrhizal inoculation but with cultivar-dependent patterns. Further, the fig with coarse root architecture is more responsive to the fungi inoculation and the AMF induced different root architecture models in Natalese and Dottato suggesting diverse root strategies for exploiting the soil resources.

Introduction

The symbiosis between plant roots and arbuscular mycorrhizal fungi (AMF) is common in nature (Kiers and van der Heijden, 2006). The mycorrhizas enhance in the hosting plants the nutrient acquisition from the soil (Chen et al., 2016; Ruiz-Lozano, 2003), increase the plant resistance against the biotic (Tchameni et al., 2012; D'Amelio et al., 2011) and abiotic stresses (Latef et al., 2016) and contribute to maintain the nutrient cycling and the organic matter in the soil (Azcon-Aguilar and Barea, 2015). In turns, the mycorrhizas receive energy for growth and reproduction from the host plant. These benefits increase the plant health and productivity of both annual (Ortas, 2012a) and fruit crop trees such as citrus (Ortas and Ustuner, 2014; Ortas, 2018), and grape- vine (Trouvelot et al., 2015). The growth improvements of mycorrhizal plants respect to the no-inoculated plants (Janos, 2007; Smith and Read, 2010; Hoeksema et al., 2010) has been defined as mycorrhizal growth response (MGR). Although the ubiquity of the AMF symbiosis, the MGR largely varies in relation to the species (Jones and Smith, 2004), cultivar (Tawaraya, 2003; Aguín et al., 2004) and fungal characteristics (Maherali and Klironomos, 2007). The nursery industry takes also ad- vantages from AM biotechnology improving the survival rates of micropropagated plantlets, their quality and the performances once transplanted in the field (Aguín et al., 2004).

Among fruit crops, fig (*Ficus carica* L.) is one of the crop species less studied for the effects of AMF, although Yaseen et al. (2016) evidenced that the root system of fig trees grown under orchards conditions were colonized by indigenous AMF. Furthermore, Comlekcioglu et al. (2008) observed a positive effect on the root system growth in the fig cultivar 'Alkuden' in responses to different *Glomus* species. Starting from the experiences above reported one of the aims of this research has been to understand if the growth and development of fig trees are affected by mycorrhizal treatments and if the effects depends on the fig cultivar. Root architecture play a fundamental role for water and nutrients uptake from the soil and in turn, plant productivity (Lynch, 1995) and improve its flexibility to adapt to the climate change (Abenavoli et al., 2011). The root architecture influenced the plant dependency of the mycorrhizal association for the nutrient foraging. Indeed, the coarse root architectures are more susceptible than fine ones to the mycorrhizal inoculation inducing an improvement of the plant growth (early hypothesis defined by Baylis, 1975). However, the root architecture and mycorrhizal relationships are not enough clarified (Atkinson et al., 2003, Maheraly, 2014), especially for fruit trees, likely due to genetic and environmental effects (Sorgonà et al., 2007; Romano et al., 2013; Tellah et al., 2014; Abenavoli et al., 2016) but also to the fungi species (Sikes et al., 2009; Jin et al., 2013). Furthermore, root traits

considered for the evaluation of the effects of the root architecture on the mycorrhizal inoculation and MGR are very few (root weight, length, surface area, specific root length and diameter, only) and they are not able to evidence the functional changes also. Indeed, further root traits or "phene" were identified for their functional role in the plant growth and development. For example, the root length ratio (RLR), the root length per unit of the plant's dry mass, and its 'morphological components', i.e. the allocation (RMR), root dry mass per unit of the plant's dry mass) and the structural components (root fineness, RF, root length per unit root volume; root tissue density, RTD, root dry mass per unit root volume) are very important root features for the water and nutrient uptake (Ryser, 1998) especially under stress conditions (Sorgonà et al., 2007; Romano et al., 2013; Tellah et al., 2014; Abenavoli et al., 2016). Moreover, the length of the roots partitioned in the various diameter classes, i.e. the functional component of the root length, are not in-depth investigated yet in the studies of the root-mycorrhiza association (Yao et al., 2009). Because in fig tree there is a lack of information on the effects of the AMF on root architecture, a second question addressed by this research is *"does AM fungi colonization change the root architecture traits of fig and is there any differences between the cultivars in the effects of root-fungi association?"*.

In searching correlations between MGR and root architecture, Yang et al. (2014) conducted a meta-analysis with 943 peer-review publications observing that the "taprooted" plants, characterized by coarse roots and lower branching density, are more responsive to the mycorrhizae. This study suggested a different approach to analyze the root architecture-mycorrhizal association based on the "rooting model" instead than on "single root traits". This approach is also stressed by the fact that is well-documented the synergisms among different root traits for water and nutrient uptake (York et al., 2013). Unfortunately, no researches have been conducted for understanding the effects of mycorrhizal on rooting model. In this respect, *"which rooting architecture model explains the mycorrhizal growth responses of the fig cultivars?"* was the last question discussed in this work.

Materials and methods

Cutting collection and rooting process

In January 2018 at Bisignano (South Italy - 39°31'09.39"N 16°14'49.36"E), the portions leafless hardwood were collected from the median part of one-year-old branch adult fig trees of two cultivars [Natalese (N) and Dottato (D)]. The wood portions have been temporarily stored in a cold room with a temperature of 3 °C and relative humidity of 90 %. In February, 20 cm long cuttings were taken, with cuts at the base just below a bud and about one

centimeter above the bud at the upper end. Afterwards, cuttings were buried in heated bed (1.0m × 4.0m, 22—24 °C) filled with perlite for one month for the rooting process. Then, the rooted cuttings were transplanted into polyethylene pots having a volume of 0.60 L, and filled with a substrate whose components were 1:1:1 (v:v:v) soil:peat:sand sterilized mixture. The pots were placed for 20 days in air-conditioned glasshouse to facilitate root growth and the self-rooted plants adaptation to the environmental conditions. Seventy two uniform self-rooted plants for each cultivar were used for the experiment.

Experimental layout

The experimental layout consisted of pots (30 cm height ×20 cm Ø) filled with a substrate whose composition was above reported. Thirty six selfrooted plants for each fig cultivars [Natalese mycorrhizal plants (Nm), Dottato mycorrhizal plants (Dm)] were inoculated with the following commercial microbial formulation adding it to the substrate: Mycor (IF TECH, Les Ponts de C´e, France), containing *Glomus intra- radices* (treatment m). The experiment also included the no-inoculated plants [Natalese non-mycorrhizal plants (Nnm) and Dottato non- mycorrhizal plants (Dnm)]. One selfrooted plants per cultivar, and mycorrhizal treatments were transplanted in each pot. Trials involved hundred forty-four selfrooted plants (thirty six for each cultivar and each treatment). Finally, the pots were placed in a shade house covered with a green shading net constituted by a high density polyethylene monofilament sized 2 × 1.6 mm mesh and a shade value of 35 %. The pots were arranged inside the shade house in rows, with a spacing of 30 cm intra-row and 100 cm between rows, as a randomized complete block design with six blocks, and each treatment had six plants per block. During the experimental period (late March – start November), the pots were irrigated by a drip irrigation system with one emitter per pot and a flow rate of 4 L h^{-1}. Pots were daily irrigated 3–4 times depending on the leaves surface of selfrooted plants and climate. Mineral nutrition was ensured by two weekly fertigation with the following nutrient solution (mg l^{-1}): N (130), P (11), K (42), Ca (36), Mg (6), Fe (3), Mn (0.1), Cu (0.03), Zn (0.4), B (0.05), Mo (0.02). The EC values were kept within the range of 1.8–2.0 dS m^{-1}, while the pH of the nutrient solution was maintained between 5.8 and 6.3. The amount of nutrient solution supplied to each plant for each fertigation was linked to plant development stages and it varied from a minimum of 0.5 L $plant^{-1}$ (in the early stages of growth) to a maximun of 1 L $plant^{-1}$ (in the final stages of growth).

Measurements

Two hundred twenty days after mycorrhizal inoculation, six self- rooted plants for each cultivar and treatment were collected and partitioned in leaf, shoot, cutting axes and root system. For the aboveground part of the plant, the following parameters were measured: fresh and dry shoot weight (g), fresh and dry leaves weight (g), and fresh and dry cutting weight (g). The dry weights were measured after drying samples in a heated oven at 80 °C for the time required to obtain a constant weight. The root systems were carefully washed from the substrate and one adventitious root, representative of the whole root system, was collected for studying mycorrhizal colonization, while the remaining root system was used for the 2-D root architectural analysis.

Evaluation of mycorrhizal colonization

To determine the extent of AMF root colonization, was adopted the modified procedure of Brundrett et al. (1996). In particular, the adventitious roots were first kept in a 10 % potassium hydroxide solution for 4 days, at room temperature, autoclaved for 15 min at 120 °C in 10 % KOH, transferred to an alkaline hydrogen peroxide solution (0.05 % H_2O_2 and 0.5 % NH_4OH, v/v) at room temperature, and 30 min later transferred to a 2% HCl for two hours at room temperature. The staining was done by immersion of the samples in a solution containing 0.05 % (w/v) trypan blue in lactoglycerol (1:1:1, lactic acid:glycerol:water), overnight at room temperature (Kormanik and McGraw, 1982). Per- centage of AMF root colonization (F, %) was done using the gridline intersect method under a stereo microscope (Giovannetti and Mosse, 1980). Mycorrhizal growth response (MGR) was calculated for each cultivar as the variation in percent of plant dry biomass colonized with AM fungi relative to non-colonized plants by the following equation (Janos, 2007):

$$MGR\ (\%) = \frac{PDWi - PDWn}{PDWn} \times 100$$

where PDWi was plant dry weight for the AM treatment and cultivar and PDWn was the plant dry weight mean values for each non-inoculated cultivar.

Root architecture evaluation

The remaining root systems of each cultivars and treatments were stained with 0.1 % toluidine blue solution for 5 min and then scanned at a resolution of 600 dpi (WinRhizo STD 1600, Instruments Regent Inc., Canada). To measure the following parameters was used

WinRhizo Pro v. 4.0 software package (Instruments Regent Inc., Chemin Sainte-Foy, Quebec, Canada): root length (RL, cm), surface area (RSA, cm^2), average diameter (RD, cm) and volume (RV, cm^3). Moreover, was measured the distribution of root length among the following root classes diameter (Bohm, 1979): very fine (VF, 0–0.5 mm), fine (F, 0.5–1 mm) and large (L, >1 mm). The number of adventitious roots (NR, n.) were directly counted from the images. Afterwards, the root fresh weight (RFW, g) and then the root dry weights (RDW, g) were measured after oven-drying at 70 °C for 48 h. As reported by Ryser and Lambers (1995), the followings 'morphological components' of the root length were calculated: root length ratio (RLR, root length/whole plant dry weight, cm g^{-1}), root mass ratio (RMR, root dry weight/whole plant dry weight, g g^{-1}), root fineness (RF, root length/root volume, cm cm^{-3}), root tissue density (RTD, root dry weight/root volume, g cm^{-3}) and the root average length (RAL, cm).

Statistics

For statistical analysis has been used the SPSS Statistics v. 15.0 software (IBM Corp., Armonk, NY). Graphics have been prepared by using the SigmaPlot v. 8.0 software (Jandel Scientific, San Rafael, CA). All data have been tested for normality (Kolmogorov Smirnoff test) and homogeneity of variance (Levene Median test) and, where required, the data have been transformed.

The *t*-test ($p < 0.05$) has been applied to test the effect of cultivar on the mycorrhizal inoculation and mycorrhizal growth response. Two-way ANOVA has been performed to test the effects of the mycorrhizal formulations (T), cultivar (CV) and TxCV interaction on single root architecture traits. Post hoc mean comparisons has been done by the Tukey's test ($p < 0.05$).

To evaluate the relationships between multiple root traits and cultivar and mycorrhizal treatment, a multivariate statistical approach has been performed. In particular, the root dataset has been subjected to a principal components analysis (PCA), based on a correlation matrix of all the measured root parameters (Afifi et al., 2004). The PCA produced uncorrelated multivariate axes that might be interpreted as representing a given fig rooting architecture model in response to the microbial formulation. The use of the correlation matrix standardizes differences among variables due to the measurement scale. The importance of different root traits in a given axis is indicated by the relative loading of the traits in the eigenvector. Finally, the cluster analysis was carried out to measure the

hierarchical similarity among the single fig cultivars for each mycorrhizal treatments. In particular, from the PCA scores, a squared Euclidean distance matrix is established to obtain a relative dendrogram. The entries are clustered using Ward's minimum-variance method (Afifi et al., 2004).

Results

Fig. 1A showed the percentage of root length infected of the root systems of both fig cultivars. The mycorrhizal formulation infected more than 40 % of root length of the fig plants. However, difference in fungi- infected root length between the fig cultivars were reported: Natalese cultivar was statistically more susceptible to the microbial infection than Dottato one (69 % vs 42 %, Fig. 1A). No contamination of the mycorrhizal formulations was found in non-mycorrhizal plants (data no re- ported). The mycorrhizal vesicles and hyphaes, indicative of the success of the fungi infection of the fig root, are showed in Fig. 2.

The fig plants positively responded to the mycorrhizal infection by increasing their growth. Indeed, the mycorrhizal growth response of plants (MGR), i.e. increased value of the fig growth in response to the mycorrhizal inoculation, varied between +31 % and +35 %, but no statistically difference between the two fig cultivars was observed (Fig. 1B).

The root architecture traits were affected by cultivars and AMF formulation (Tables 1–3). The root system of the two fig cultivars was only different for the root length and surface area with the higher values in Natalese than Dottatto one (Table 1). However, this pattern was observed in mycorrhizal plants only ($p < 0.05$ for the CVxT interaction, Table 1). Conversely to the cultivar, the influence of the AMF formulation to the whole root system morphology was higher. Indeed, the root fresh and dry weight, total root length, surface area and average diameter were positively affected by mycorrhizal inoculation. The mycorrhizal-related increases varied between 37 % and 88 % respect to the non-mycorrhizal plants with the larger effect obtained in the average root diameter (Table 1). No differences were evidenced for the number of roots and root average length (Table 1). Although this mycorrhizal- related pattern was maintained in root fresh and dry weight and average diameter for both cultivars, conversely the variation of the root length and surface area determined by mycorrhizal inoculation was different between the two cultivars ($p < 0.05$ for the CVxT interaction, Table 1): sharply increase in Natalese and no modification in Dottato one (Table 1). Further, the average length was affected by CVxT inter- action with increase in Natalese and decrease in Dottato one in response to the mycorrhizal infection (Table 1).

Data reported in Table 2 showed the effects of the cultivars, mycorrhizal formulation and their interaction on the 'morphological components' of the root length of the fig cultivars. The cultivar affected the root fineness only: the Natalese pointed out a higher value of this trait than Dottato one (647 *vs* 453 cm cm^{-3}, Table 2). However, the significant CVxT interaction indicated that this pattern was observed in mycorrhizal plants only (Table 2); further, it is noted that the Natalese root system exhibited a higher tissue density respect than Dottato one at non-mycorrhizal plants only ($p < 0.05$ CVxT interaction, Table 2). Mycorrhizal formulation did not modified root traits as main factor but, in relation to the cultivar, they promoted differences for the RLR and RTD ($p < 0.05$ of CVxT interaction) but not for the biomass allocation to the root, the RMR (Table 2). In particular, the mycorrhizal inoculation sharply increased the RLR in Natalese respect to the non-inoculated plants (+136 %) but not in Dottato. Similar pattern was observed for the RTD but with decrease of -36 % in Natalese and no modification was revealed in Dottato one (Table 2).

Deepening information on the fineness of the fig root system are highlighted by root length partitioning among the different diameter classes (Table 3). The cultivar affected the VF roots only with the Natalese exhibiting a higher length with very fine diameter than Dottato one (Table 3) but this pattern is observed in mycorrhizal plants only ($p < 0.05$ CVxT interaction, Table 3). Mycorrhizal formulation affected both VF and F roots with an increase of 66.8 % and 41.6 %, respectively, respect to the non-mycorrhizal plants (Table 3). However, this outcome is observed in Natalese root system only (significant CVxT interaction, Table 3). The length of the large roots (or coarse roots) are not modified by the cultivar and mycorrhizal formulation (Table 3).

The principal component analysis permitted to reduce form 14 root traits in only 7 as relevant to explain the 92 % of the total variability. The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (0.611) and the Bartlett's Test of Sphericity (0.001) supported this PCA analysis. Further, the PCA analysis grouped the seven significant root traits into two components (PCs). Total variability of the three dimensional space was efficiently summarized by the two principal components (PCs), which accounted for 70 % and 22 % of the variability, respectively (Table 4). The first component (PC1) consisted of high positive loadings for RL, RAL, RLR, RF and VF (Table 4) which can be assumed to largely reflect the "root morphology": positive values of this component result in thin and longer root systems. The second principal component (PC2) had of high positive loadings for RFW and RDW (Table 4), the "plant below-ground biomass or C allocation" which could be

considered as the root mass available to 'model or shape" the root system. Fig. 3 showed the biplot graph obtained plotting each fig cultivar and mycorrhizal treatment by means of their component scores. By Hierarchical Cluster Analysis (Ward's method with distance measure by squared Euclidean distance), three well-defined and –separated clusters are highlighted (Fig. 3). In particular, the cluster I (red one) grouped the non- mycorrhizal plants of both cultivars; the cluster II (the blue one) involved the mycorrhizal plants of Natalese while the cluster III (pink one) revealed the fungi-inoculated plants of Dottato cultivar (Fig. 3).

Discussion

The fig pointed out a higher mycorrhizal-induced growth but cultivar- dependent infectivity

The fig root systems are mostly infected by mycorrhizal treatment with the Natalese cultivar more susceptible to the microbial infection than Dottato one (Figs. 1A and 2). These results confirmed the responsiveness of the self-rooted fig plants to the root inoculation by *Glomus* species (Comlekcioglu et al., 2008), but for the first time, evidenced the cultivar dependency in *Ficus carica*. The cultivar-dependent root colonization has been also highlighted in grapevine (Aguín et al., 2004), *Prunus* (Calvet et al., 2004) and citrus rootstocks (Graham and Syvertsen, 1985). In order to understand the higher infectivity of the root system of Natalese respect to the Dottato one, it is need to consider the mechanisms of the root-AMF association. The AMF colonizes the plants via the fungi germination by the root exudates (Akiyama et al., 2005) and subsequently penetration and spread of the fungi hyphae mainly in the root cortex (Gutjahr and Paszkowski, 2013) indicating that the AM fungi preferentially colonize the coarse and dense roots, such as the large lateral roots of the rice. (Gutjahr et al., 2009). Already in 1975, Baylis (1975) hypothized the strictly relationship between root architecture and mycorrhizal dependency and, subsequently, Hetrick (1991) and Smith and Read (2010) demonstrated that coarse root architecture are more dependent to mycorrhiza than fine root ones. This result is also confirmed by this research: the higher responsiveness (higher colonization percentage per root length) of the Natalese cultivar to the than that of the average of 26 tree species (79 %) but similar to the olive tree (27 %), the only fruit crop reported in Tawaraya (2003). Considering that the mycorrhizal symbiosis enhanced the crop growth and development especially in stressful environments, the lower MGR of fig cultivars observed in this work could be underestimated. Overall, these data confirmed that the *Ficus carica*, as other fruit trees (Ortas, 2018), is responsive to the mycorrhizal symbiosis by a significant increase of the growth but the infectivity degree is dependent on the cultivars: Natalese better than Dottato.

Fig root architecture traits are modified by mycorrhizal colonization but cultivar-dependent

Although very few studies experimentally confirmed the relation- ships between root architecture traits and AMF on fruit trees (Berta et al., 1995; Aguín et al., 2004; Yao et al., 2009), the results of this study revealed for the first time the changes induced by AMF on the root architecture of fig plants. Indeed, the mycorrhizal treatment increased several root morphological traits (root fresh and dry weight, total root length, surface area and average diameter) (Table 1). The increased root length and surface area by mycorrhizal inoculation in fig could improve the nutrient and water acquisition as observed in citrus (Sorgonà and Cacco, 2002; Sorgonà et al., 2005, Ort`as, 2012b) and temperate tree species (Eissenstat et al., 2015). But in the face of the increase of the fig root size (length, surface area and biomass), no change in number roots and average root length are observed (Table 1) suggesting a no clear response of the root architecture to the mycorrhizal inoculation as also confirmed from literature (Atkinson et al., 2003). Specifically for the fruit trees, the root responses to the mycorrhizae are inconsistent. Indeed, the citrus seedlings pointed out a reduction of the root length and surface area but an increase of the lateral roots also (Yao et al., 2009); the *Annona cherimola* increased the root length and number only (Padilla and Encina, 2005); the total root length and the length of the first order lateral roots are improved in *Prunus cerasifera* but not the higher order lateral roots (Berta et al., 1995); three grapevine rootstocks increased the number of first-order lateral roots but only one rootstock was responsive for the second-order lateral roots (Aguín et al., 2004). These contrasting results are probably due to the higher root plasticity in response to the environmental conditions (Sorgonà et al., 2007; Romano et al., 2013; Tellah et al., 2014; Abenavoli et al., 2016) but to the fungi identity also (Sikes et al., 2009; Jin et al., 2013).

The mycorrhizal-induced increases of the fig root size (length, sur- face area, average diameter and biomass) are different in relation to the cultivars. Indeed, the mycorrhizal treatment increased the root length, surface area, biomass and average length in the Natalese cultivar only (Table 1). Conversely, the Dottato root architecture traits are lesser modified by mycorrhizal inoculation with increase of the biomass only (Table 1). These cultivar-dependent root responses to the mycorrhizal inoculation are already highlighted in other fruit crops such as grape- vine (Aguín et al., 2004), olive (Tawaraya, 2003) and citrus (Ortas, 2012b).

As argued by Yao et al. (2009) and Gutjahr and Paszkowski (2013), the root-AMF interactions are very complex and an in-depth understandings are needed. As above observed, for example, why equal mycorrhizal-induced increases on the fresh and dry

biomass between fig cultivars corresponded a higher root length and surface area and average root length in Natalese but not in Dottato cultivar? This result can be explained by the investigation of the mycorrhizal-induced variations of the 'morphological components' of the root length which, as suggested by Ryser (1998), are the allocation (root mass ratio) and the structural components (root fineness and tissue density). In this respect, although the same biomass allocation (RMR) is exhibited in both inoculated and uninoculated plants, the Natalese increased the root length in response to the mycorrhizal treatment thanks to a decrease in tissue density (Table 2). Further, the root architecture of inoculated plants of Natalese is mainly constituted by very fine and fine diameter (Table 3).Conversely, the Dottato cannot achieve these root responses (Tables 2 and 3). In other words, the Natalese manages to better model a certain biomass to obtain a longer root system in response to mycorrhizal treatment than Dottato one. The mycorrhizal-induced root architecture changes could be due to a modified nutritional status coordinate or in- dependent by complex phytohormonal signaling network (Gutjahr and Paszkowski, 2013). This physiological mechanism could be evoked in the root responses of the Natalese cultivar to the mycorrhizal treatment. Indeed, both root tissue density and very fine roots, the root traits modified by mycorrhizal treatment in Natalese cultivar, are negatively correlated with the nutrient fertility (Kramer-Walter et al., 2016). Further, the success of the AMF-plant symbiosis is based on the cost-benefit related to the trade between fungus-delivered nutrients (mainly N and P) and plant-delivered carbon (Kiers et al., 2011) suggesting a threshold value which triggers the mycorrhiza-mediated physiological mechanism of the root architecture changes (Yang and Paszkowski, 2011). In this respect, we can speculate that probably there is a different threshold value of cost-benefit AMF symbiosis which pro- duce different mycorrhizal-mediated root architecture responses be- tween Dottato and Natalese cultivars.

Mycorrhizal treatment induced different rooting architecture patterns between fig cultivars

Maherali (2014) observed a no clear relationships between the single root traits and the mycorrhizal growth response by meta-analysis of data from literature. Conversely, Yang et al. (2014) conducting a meta-analysis with higher number of peer-review publications than Maherali study and using the "rooting type" instead than "single root traits", demonstrated a robust and consistent response of the root architecture model to the mycorrhizal treatments. Indeed, they pointed out that the "taprooted" plants were more responsiveness than "fibrous root system" to the mycorrhizal inoculation (Yang et al., 2014). This study, together to the importance of the synergism among the different root

374 traits for understanding the influences plant function in diverse environments (York et
375 al., 2013), suggested us to use a different approach for comparing the different fig root
376 architectures observed in presence of mycorrhizal treatment which is based on the "rooting
377 architecture model". In this respect, the principal component analysis (PCA), as
378 multivariate analysis, permit an efficient and meaningful "multi-trait classifiers" of the
379 root systems (Bodner et al., 2013) helping to identify the rooting strategy in terms of traits
380 and mechanisms which operate independently or jointly to enable the fig growth by the
381 mycorrhizal infection. The PCA was able to reduce and group the root architecture traits
382 into two components (PC1 and PC2) according to their ability to describe most of the
383 variability of the fig cultivars responses to the mycorrhizal treatment (Table 4). The PC1
384 involving the RL, RAL, RLR, RF and VF, could represents the "root morphology":
385 positive values of this component resulted in thin and longer root systems and,
386 consequently, more soil volume could be explored for the soil resources capture. The PC2
387 grouping the RFW and RDW, that is the "plant below-ground biomass or C allocation",
388 which could represent the biomass for the construction the root system but also the carbon
389 substrate for the AM fungi. Hence, the positive values of PC1 could indicate more carbon
390 for the root and mycorrhizal growth and function. Plotting the single fig cultivars of each
391 mycorrhizal treatments by means of their component scores and subsequently hyerarchial
392 cluster analysis separated three different clusters (Fig. 3) which permitted to point out the
393 following considerations. The mycorrhizal inoculation produced an increase of the below
394 carbon substrate but the cultivars determined the ultimately form of root architecture
395 suggesting a different rooting architecture model between the AMF-inoculated plants of the
396 two fig cultivars. Indeed, the Natalese cultivar exhibited rooting architecture model
397 characterized by higher length and fineness (Fig. 3). This rooting strategy, typical of the
398 "fast growth species" with high uptake rate over a short lifespan could be better performant
399 for the plant nutrient foraging especially in environments characterized by high competitive
400 and heterogeneous-distributed nutrient such as the agricultural soils (Eissenstat et al., 2000;
401 Bouma et al., 2001; Kong et al., 2014; Roumet et al., 2016). Conversely, the root systems
402 of AMF-inoculated Dottato cultivar did not changes the root architecture model respect to
403 the uninoculated plants exhibiting coarser and smaller root axis (Fig. 3). This rooting
404 architecture model suggest a more conservative strategy typical of the "slow growth
405 species" with low uptake rate over a long lifespan and characterized by more C and
406 nutrients per unit area (or length) devote to root construction, maintenance, and persistence
407 compensated by living longer, and by having better chemical defense and thus less tissue
408 loss as a result of herbivory (Eissenstat et al., 2000; Bouma et al., 2001; Kong et al., 2014;

Roumet et al., 2016).

Conclusion

Overall, these results permitted the following conclusions:

1)the mycorrhizal inoculation by *Glomus intraradices* infected the root system of the fig self-rooted plants but with cultivar-dependent response: the Natalese cultivar was more infected than Dottato one; this different infectivity between the fig cultivars is dependent of the root architecture: coarser roots of uninoculated plants of the Natalese cultivar were more susceptible to the mycorrhizal inoculation;

2)the mycorrhizal treatments produced an increase of the growth in terms of biomass of the fig plants at similar levels between the two cultivars;

3)the fig root architecture was modified by mycorrhizal inoculation mainly in the Natalese cultivar which exhibited higher root length and surface area and length of the very fine roots determined by a lower root tissue density. These mycorrhizal-mediated root responses are not highlighted in Dottato cultivar;

4)the mycorrhizal inoculation also produced the different changes in rooting architecture models between the fig cultivars: finer and longer root axis in Natalese respect the Dottato culivars; this different root architecture model could underlying diverse rooting strategies typical of the fast- and slow-growth species for the soil resource acquisition.

It is important recognize the limitations to scale-up the results of this study from greenhouse to the field due to the different behavior of the AM fungi among the soils (Carrenho et al., 2007), the high diversity of the rhizosphere organisms (Larimer et al., 2014) and the different effects on plant productivity in relation to AM fungi identity (Sikes et al., 2009; Jin et al., 2013) and diversity (Sharma et al., 2009). However, a study conducted in citrus rootstocks in a mature field planting, showed that the rootstocks with higher MGR observed in pot experiments were more rapidly colonized by mycorrhizal fungi in field (Graham et al., 1991). Nonetheless, the results of this study could be relevant for the commercial growing plants in containers, such as in the nursery industry, in which the ensuring the high-quality of the planting materials is important.

Author contributions

TC: critical revision of the text; RM: designed and carried out experiment and collected aboveground data; MB: carried out experiment; RV: collected root architecture data; AS: analyzed data, prepared tables and figures, interpreted the results, and written manuscript.

Funding

This work was supported by the grant of the Calabria Region within the Rural Development Program (Project RDP, Measure 124 "Innovation of Cosenza's fig industry" n. 94752168222).

CRediT authorship contribution statement

Tiziano Caruso: Writing - review & editing, Supervision. **Rocco Mafrica:** Conceptualization, Investigation, Writing - review & editing. **Marcello Bruno:** Investigation. **Rosa Vescio:** Investigation. **Agostino Sorgona`:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The authors would like to thank the San Paolo Nursery of Bisignano and the farm personnel for their support and patience when performing the experiment. The authors also thank the Drs Di Domenico, D'Onghia and Filippelli of the ARSAC for the support provided in the nutrient solution management and water supply.

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Table 1 – Morphological traits of the root systems of self-rooted plants of the fig cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*.

Parameters	[#]Statistics	CV	Treatments (T) M	Treatments (T) nM	*CV average*
Root fresh weight (g)	CV^{NS}	**N**	198[a]	144[b]	***178x***
	T***	**D**	189[a]	138[b]	***167x***
	$CVxT^{NS}$	***T average***	***193[A]***	***141[B]***	
Root dry weight (g)	CV^{NS}	**N**	61[a]	43[b]	***52[x]***
	T*	**D**	58[a]	40[b]	***49[x]***
	$CVxT^{NS}$	***T average***	***60[A]***	***42[B]***	
Root total length (cm)	***CV****	**N**	105206[a]	30160[b]	***70468[x]***
	T*	**D**	37626[b]	53502[b]	***45564[y]***
	CVxT*	***T average***	***71416[A]***	***44864[B]***	
Root total surface area (cm^2)	***CV****	**N**	13175[a]	4671[b]	***9298[x]***
	T*	**D**	6926[b]	8175[b]	***7550[y]***
	CVxT*	***T average***	***10050[A]***	***6864[B]***	
Root average diameter (mm)	CV^{NS}	**N**	129[a]	41[ab]	***88[x]***
	T*	**D**	63[ab]	55[b]	***58[x]***
	$CVxT^{NS}$	***T average***	***96[A]***	***51[B]***	
Number of roots (n.)	CV^{NS}	**N**	69[a]	73[a]	***71[x]***
	T^{NS}	**D**	68[a]	50[a]	***59[x]***
	$CVxT^{NS}$	***T average***	***69[A]***	***62[A]***	
Root average length (cm)	CV^{NS}	**N**	1759[a]	534[b]	***1257[x]***
	T^{NS}	**D**	630[b]	1386[a]	***942[x]***
	CVxT*	***T average***	***1194[A]***	***1017[A]***	

[#]Statistic analysis: two-way ANOVA with 6 replicates (CV: cultivar; T: treatments; CVxT: cultivar x treatments interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.

Different letters in lower case within column indicated significant difference at P<0.05 (test of Fisher). Different letters in uppercase within rows indicated significant difference at P<0.05 (test of Fisher).

Table 2 – Components of the root length [RLR, root length per unit of the plant's dry mass; RMR, root mass per unit of the plant's dry mass; RF, root length per unit root volume; RTD, root dry mass per unit root volume) of self-rooted plants of the fig cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*.

			Treatments		
Parameters	**#Statistics**	**CV**	**M**	**nM**	***CV average***
Root Length Ratio ($cm*g^{-1}$)	CV^{NS}	**N**	323^{a}	137^{b}	***238^{x}***
	T^{NS}	**D**	121^{b}	273^{ab}	***197^{x}***
	CVxT**	***T average***	***222^{A}***	***213^{A}***	
Root Mass Ratio ($g*g^{-1}$)	CV^{NS}	**N**	0.19^{a}	0.19^{a}	***0.19^{x}***
	T^{NS}	**D**	0.19^{a}	0.20^{a}	***0.19^{x}***
	$CVxT^{NS}$	***T average***	***0.19^{A}***	***0.20^{A}***	
Root Fineness ($cm*cm^{-3}$)	***CV*****	**N**	779^{a}	460^{ab}	***657^{x}***
	T^{NS}	**D**	378^{b}	527^{ab}	***452^{y}***
	CVxT**	***T average***	***578^{A}***	***531^{A}***	
Root Tissue Density ($g*cm^{-3}$)	CV^{NS}	**N**	0.49^{b}	0.77^{a}	***0.60^{x}***
	T^{NS}	**D**	0.58^{ab}	0.39^{b}	***0.49^{x}***
	CVxT**	***T average***	***0.56^{A}***	***0.54^{A}***	

#Statistic analysis: two-way ANOVA with 6 replicates (CV: cultivar; T: treatments; CVxT: cultivar x treatments interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.

Different letters in lower case within column indicated significant difference at P<0.05 (test of Fisher). Different letters in uppercase within rows indicated significant difference at P<0.05 (test of Fisher).

Table 3 – Root length distribution among the diameter classes (very fine, VF: 0–0.5 mm; fine, F: 0.5–2.0 mm; large, L: >2.0 mm) of self-rooted plants of the fig cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*.

Parameters	[#]Statistics	CV	Treatments		Average cultivar
			M	nM	
Very Fine Root (cm)	***CV****	**N**	87775[a]	23257[b]	***57667[x]***
	T*	**D**	24342[b]	39408[b]	***31874[y]***
	CVxT*	***T average***	***56058[A]***	***33602[B]***	
Fine root (cm)	*CV[NS]*	**N**	16301[a]	5551[b]	***11570[x]***
	T*	**D**	11967[ab]	12894[ab]	***12430[x]***
	CVxT*	***T average***	***14134[A]***	***9980[B]***	
Large root (cm)	*CV[NS]*	**N**	1082[a]	1331[a]	***1195[x]***
	T[NS]	**D**	1309[a]	1184[a]	***1246[x]***
	CVxT[NS]	***T average***	***1196[A]***	***1264[A]***	

[#]Statistic analysis: two-way ANOVA with 6 replicates (CV: cultivar; T: treatments; CVxT: cultivar x treatments interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.
Different letters in lower case within column indicated significant difference at P<0.05 (test of Fisher). Different letters in uppercase within rows indicated significant difference at P<0.05 (test of Fisher).

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Table 4 – Principal components of the 2-D root architectural traits of self-rooted plants of the fig cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*.

	Attribute loadings	
	PC1	***PC2***
Statistics		
Eigenvalue and variability		
Eigenvalue	4.31	2.15
Proportion of variability (%)	70.17	22.16
Variable		
Eigenvectors		
Root fresh weigth	.215	**.960**
Root dry weight	.146	**.973**
Total length of root system	**.910**	.364
Average length of the root system	**.890**	.183
RLR	**.962**	.087
Root fineness	**.915**	.064
VF	**.928**	.324

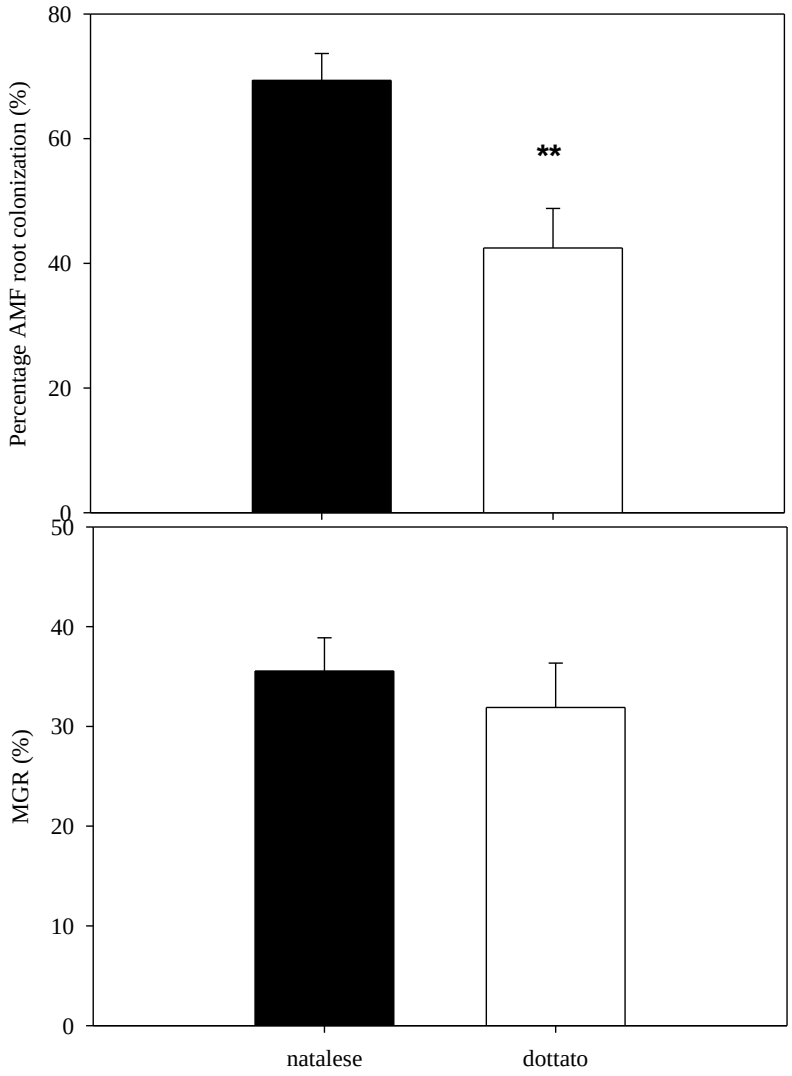

Figure 1 - Percentage root infected (A) and mycorrhizal growth response of self-rooted plants of the fig cultivars, Dottato and Natalese inoculated with a *Glomus intraradices*. Asterisk indicated significant difference between the two fig cultivars (<0.01p<0.001; t-test).

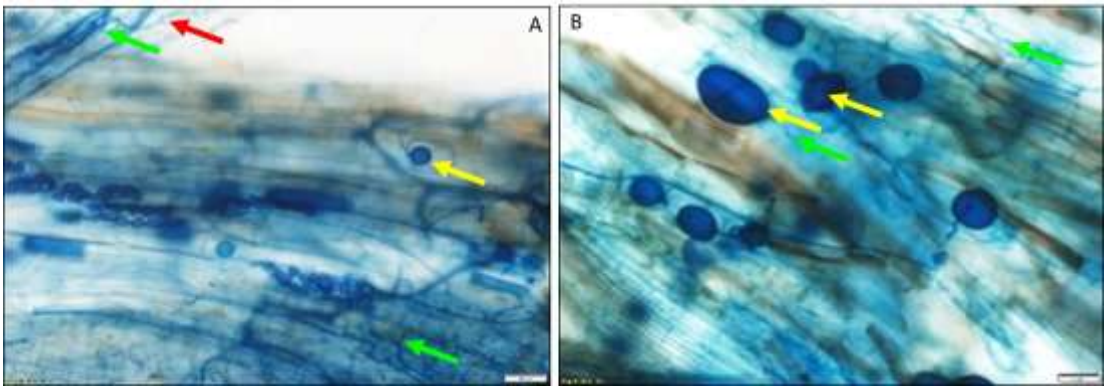

Figure 2 – Microscopic visualization of arbuscular mycorrhizal fungi showing vesicles (yellow arrows) and hyphae within- (green arrows) and outside-root (red arrows) of fig rooted cuttings.

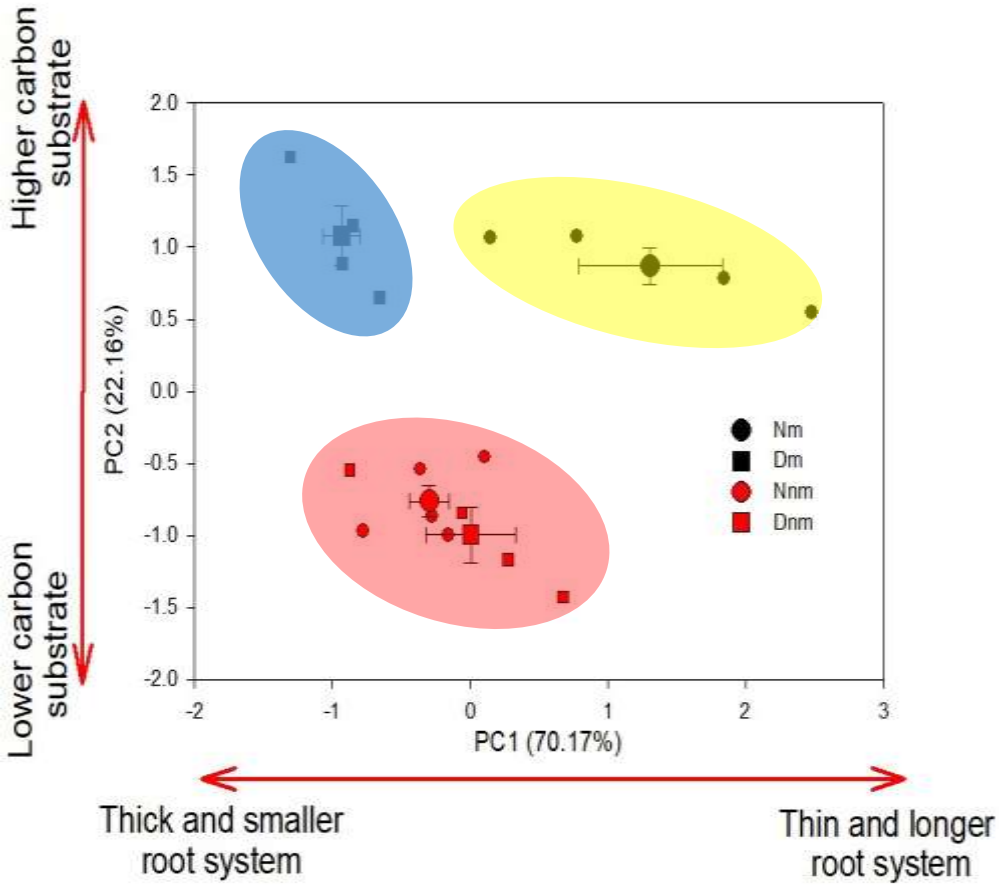

Figure 3 – Scores (means and error standard bars) of the principal components 1 and 2 of the root architectural traits of self-rooted plants of the fig cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*. The arrows indicate the biological interpretation of the principal component and the proportion of explained variability is given within the bracket. Circles denote the grouping of the single fig cultivars of each mycorrhizal treatments after Hierarchical Cluster Analysis (Ward's method with distance measure by squared Euclidean distance).