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

3 Tiziano Caruso ^a, Rocco Mafrica ^b, Marcello Bruno ^c, Rosa Vescio ^b, Agostino Sorgonà ^{b,*}

4 ^a *Department of Agriculture, Food and Forest Science, University of Palermo, Palermo, Italy*

5 ^b *Department of Agriculture, University "Mediterranea" of Reggio Calabria, Reggio
6 Calabria, Italy*

7 ^c *Calabrian Agriculture Development Regional Agency (A.R.S.A.C.), Cosenza, Italy*

8
9 *Keywords:* Arbuscular mycorrhizal fungi, Root architecture, Root morphology, Fig,
10 Rooted cutting, Image analysis

11 12 **Abstract**

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

34 **Introduction**

35 The symbiosis between plant roots and arbuscular mycorrhizal fungi (AMF) is common in
36 nature (Kiers and van der Heijden, 2006). The mycorrhizas enhance in the hosting plants
37 the nutrient acquisition from the soil (Chen et al., 2016; Ruiz-Lozano, 2003), increase the
38 plant resistance against the biotic (Tchameni et al., 2012; D'Amelio et al., 2011) and abiotic
39 stresses (Latef et al., 2016) and contribute to maintain the nutrient cycling and the organic
40 matter in the soil (Azcon-Aguilar and Barea, 2015). In turns, the mycorrhizas receive
41 energy for growth and reproduction from the host plant. These benefits increase the plant
42 health and productivity of both annual (Ortas, 2012a) and fruit crop trees such as citrus
43 (Ortas and Ustuner, 2014; Ortas, 2018), and grape- vine (Trouvelot et al., 2015). The
44 growth improvements of mycorrhizal plants respect to the no-inoculated plants (Janos,
45 2007; Smith and Read, 2010; Hoeksema et al., 2010) has been defined as mycorrhizal
46 growth response (MGR). Although the ubiquity of the AMF symbiosis, the MGR largely varies
47 in relation to the species (Jones and Smith, 2004), cultivar (Tawaraya, 2003; Aguín et al.,
48 2004) and fungal characteristics (Maherali and Klironomos, 2007). The nursery industry
49 takes also ad- vantages from AM biotechnology improving the survival rates of
50 micropropagated plantlets, their quality and the performances once transplanted in the field
51 (Aguín et al., 2004).

52 Among fruit crops, fig (*Ficus carica* L.) is one of the crop species less studied for the effects of
53 AMF, although Yaseen et al. (2016) evidenced that the root system of fig trees grown under
54 orchards conditions were colonized by indigenous AMF. Furthermore, Comlekcioglu et al.
55 (2008) observed a positive effect on the root system growth in the fig cultivar 'Alkuden' in
56 responses to different *Glomus* species. Starting from the experiences above reported one of
57 the aims of this research has been to understand if the growth and development of fig trees
58 are affected by mycorrhizal treatments and if the effects depends on the fig cultivar. Root
59 architecture play a fundamental role for water and nutrients uptake from the soil and in
60 turn, plant productivity (Lynch, 1995) and improve its flexibility to adapt to the climate
61 change (Abenavoli et al., 2011). The root architecture influenced the plant dependency of
62 the mycorrhizal association for the nutrient foraging. Indeed, the coarse root architectures
63 are more susceptible than fine ones to the mycorrhizal inoculation inducing an improvement
64 of the plant growth (early hypothesis defined by Baylis, 1975). However, the root
65 architecture and mycorrhizal relationships are not enough clarified (Atkinson et al., 2003,
66 Maheraly, 2014), especially for fruit trees, likely due to genetic and environmental effects
67 (Sorgonà et al., 2007; Romano et al., 2013; Tellah et al., 2014; Abenavoli et al., 2016) but
68 also to the fungi species (Sikes et al., 2009; Jin et al., 2013). Furthermore, root traits

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

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

95

96 **Materials and methods**

97 *Cutting collection and rooting process*

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

103 centimeter above the bud at the upper end. Afterwards, cuttings were buried in heated bed
104 (1.0m × 4.0m, 22—24 °C) filled with perlite for one month for the rooting process. Then,
105 the rooted cuttings were transplanted into polyethylene pots having a volume of 0.60 L, and
106 filled with a substrate whose components were 1:1:1 (v:v:v) soil:peat:sand sterilized mixture. The
107 pots were placed for 20 days in air-conditioned glasshouse to facilitate root growth and the
108 self-rooted plants adaptation to the environmental conditions. Seventy two uniform self-
109 rooted plants for each cultivar were used for the experiment.

110 *Experimental layout*

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

135

136 *Measurements*

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

146
147 *Evaluation of mycorrhizal colonization*

148 To determine the extent of AMF root colonization, was adopted the modified procedure of
149 Brundrett et al. (1996). In particular, the adventitious roots were first kept in a 10 %
150 potassium hydroxide solution for 4 days, at room temperature, autoclaved for 15 min at 120
151 °C in 10 % KOH, transferred to an alkaline hydrogen peroxide solution (0.05 % H₂O₂ and
152 0.5 % NH₄OH, v/v) at room temperature, and 30 min later transferred to a 2% HCl for two
153 hours at room temperature. The staining was done by immersion of the samples in a solution
154 containing 0.05 % (w/v) trypan blue in lactoglycerol (1:1:1, lactic acid:glycerol:water),
155 overnight at room temperature (Kormanik and McGraw, 1982). Per- centage of AMF root
156 colonization (F, %) was done using the gridline intersect method under a stereo microscope
157 (Giovannetti and Mosse, 1980). Mycorrhizal growth response (MGR) was calculated for
158 each cultivar as the variation in percent of plant dry biomass colonized with AM fungi
159 relative to non-colonized plants by the following equation (Janos, 2007):

160
161
$$MGR (\%) = \frac{PDWi - PDWn}{PDWn} \times 100$$

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

164
165 *Root architecture evaluation*

166 The remaining root systems of each cultivars and treatments were stained with 0.1 %
167 toluidine blue solution for 5 min and then scanned at a resolution of 600 dpi (WinRhizo STD
168 1600, Instruments Regent Inc., Canada). To measure the following parameters was used

169 WinRhizo Pro v. 4.0 software package (Instruments Regent Inc., Chemin Sainte-Foy,
170 Quebec, Canada): root length (RL, cm), surface area (RSA, cm²), average diameter (RD,
171 cm) and volume (RV, cm³). Moreover, was measured the distribution of root length among
172 the following root classes diameter (Bohm, 1979): very fine (VF, 0–0.5 mm), fine (F, 0.5–1 mm)
173 and large (L, >1 mm). The number of adventitious roots (NR, n.) were directly counted
174 from the images. Afterwards, the root fresh weight (RFW, g) and then the root dry weights
175 (RDW, g) were measured after oven-drying at 70 °C for 48 h. As reported by Ryser and
176 Lambers (1995), the followings ‘morphological components’ of the root length were
177 calculated: root length ratio (RLR, root length/whole plant dry weight, cm g⁻¹), root
178 mass ratio (RMR, root dry weight/whole plant dry weight, g g⁻¹), root fineness (RF, root
179 length/root volume, cm cm⁻³), root tissue density (RTD, root dry weight/root volume, g
180 cm⁻³) and the root average length (RAL, cm).

181

182 *Statistics*

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

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

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

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

206

207 **Results**

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

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

219 The root architecture traits were affected by cultivars and AMF formulation (Tables 1–3).
220 The root system of the two fig cultivars was only different for the root length and surface
221 area with the higher values in Natalese than Dottatto one (Table 1). However, this pattern
222 was observed in mycorrhizal plants only ($p < 0.05$ for the CVxT interaction, Table 1).

223 Conversely to the cultivar, the influence of the AMF formulation to the whole root system
224 morphology was higher. Indeed, the root fresh and dry weight, total root length, surface
225 area and average diameter were positively affected by mycorrhizal inoculation. The
226 mycorrhizal-related increases varied between 37 % and 88 % respect to the non-
227 mycorrhizal plants with the larger effect obtained in the average root diameter (Table 1). No
228 differences were evidenced for the number of roots and root average length (Table 1).
229 Although this mycorrhizal- related pattern was maintained in root fresh and dry weight and
230 average diameter for both cultivars, conversely the variation of the root length and surface
231 area determined by mycorrhizal inoculation was different between the two cultivars ($p <$
232 0.05 for the CVxT interaction, Table 1): sharply increase in Natalese and no modification
233 in Dottato one (Table 1). Further, the average length was affected by CVxT inter- action
234 with increase in Natalese and decrease in Dottato one in response to the mycorrhizal
235 infection (Table 1).

236 Data reported in Table 2 showed the effects of the cultivars, mycorrhizal formulation and
237 their interaction on the 'morphological components' of the root length of the fig cultivars.
238 The cultivar affected the root fineness only: the Natalese pointed out a higher value of this
239 trait than Dottato one (647 vs 453 cm cm⁻³, Table 2). However, the significant CVxT
240 interaction indicated that this pattern was observed in mycorrhizal plants only (Table 2);
241 further, it is noted that the Natalese root system exhibited a higher tissue density respect
242 than Dottato one at non-mycorrhizal plants only ($p < 0.05$ CVxT interaction, Table 2).
243 Mycorrhizal formulation did not modified root traits as main factor but, in relation to the
244 cultivar, they promoted differences for the RLR and RTD ($p < 0.05$ of CVxT interaction) but
245 not for the biomass allocation to the root, the RMR (Table 2). In particular, the mycorrhizal
246 inoculation sharply increased the RLR in Natalese respect to the non-inoculated plants
247 (+136 %) but not in Dottato. Similar pattern was observed for the RTD but with decrease of
248 -36 % in Natalese and no modification was revealed in Dottato one (Table 2).
249 Deepening information on the fineness of the fig root system are highlighted by root length
250 partitioning among the different diameter classes (Table 3). The cultivar affected the VF
251 roots only with the Natalese exhibiting a higher length with very fine diameter than Dottato
252 one (Table 3) but this pattern is observed in mycorrhizal plants only ($p < 0.05$ CVxT
253 interaction, Table 3). Mycorrhizal formulation affected both VF and F roots with an
254 increase of 66.8 % and 41.6 %, respectively, respect to the non-mycorrhizal plants (Table
255 3). However, this outcome is observed in Natalese root system only (significant CVxT
256 interaction, Table 3). The length of the large roots (or coarse roots) are not modified by the
257 cultivar and mycorrhizal formulation (Table 3).
258 The principal component analysis permitted to reduce form 14 root traits in only 7 as
259 relevant to explain the 92 % of the total variability. The Kaiser-Meyer-Olkin Measure of
260 Sampling Adequacy (0.611) and the Bartlett's Test of Sphericity (0.001) supported this
261 PCA analysis. Further, the PCA analysis grouped the seven significant root traits into two
262 components (PCs). Total variability of the three dimensional space was efficiently
263 summarized by the two principal components (PCs), which accounted for 70 % and 22 %
264 of the variability, respectively (Table 4). The first component (PC1) consisted of high
265 positive loadings for RL, RAL, RLR, RF and VF (Table 4) which can be assumed to largely reflect
266 the "root morphology": positive values of this component result in thin and longer root
267 systems. The second principal component (PC2) had of high positive loadings for RFW
268 and RDW (Table 4), the "plant below-ground biomass or C allocation" which could be

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

277

278 **Discussion**

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

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

304

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

306 Although very few studies experimentally confirmed the relation- ships between root
307 architecture traits and AMF on fruit trees (Berta et al., 1995; Aguín et al., 2004; Yao et al.,
308 2009), the results of this study revealed for the first time the changes induced by AMF on
309 the root architecture of fig plants. Indeed, the mycorrhizal treatment increased several root
310 morphological traits (root fresh and dry weight, total root length, surface area and average
311 diameter) (Table 1). The increased root length and surface area by mycorrhizal inoculation
312 in fig could improve the nutrient and water acquisition as observed in citrus (Sorgonà and
313 Cacco, 2002; Sorgonà et al., 2005, Ort`as, 2012b) and temperate tree species (Eissenstat et
314 al., 2015). But in the face of the increase of the fig root size (length, surface area and
315 biomass), no change in number roots and average root length are observed (Table 1)
316 suggesting a no clear response of the root architecture to the mycorrhizal inoculation as
317 also confirmed from literature (Atkinson et al., 2003). Specifically for the fruit trees, the
318 root responses to the mycorrhizae are inconsistent. Indeed, the citrus seedlings pointed out
319 a reduction of the root length and surface area but an increase of the lateral roots also (Yao
320 et al., 2009); the *Annona cherimola* increased the root length and number only (Padilla and
321 Encina, 2005); the total root length and the length of the first order lateral roots are
322 improved in *Prunus cerasifera* but not the higher order lateral roots (Berta et al., 1995);
323 three grapevine rootstocks increased the number of first-order lateral roots but only one
324 rootstock was responsive for the second-order lateral roots (Aguín et al., 2004). These
325 contrasting results are probably due to the higher root plasticity in response to the
326 environmental conditions (Sorgonà et al., 2007; Romano et al., 2013; Tellah et al., 2014;
327 Abenavoli et al., 2016) but to the fungi identity also (Sikes et al., 2009; Jin et al., 2013).

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

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

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

364

365 *Mycorrhizal treatment induced different rooting architecture patterns between fig cultivars*

366 Maherali (2014) observed a no clear relationships between the single root traits and the
367 mycorrhizal growth response by meta-analysis of data from literature. Conversely, Yang
368 et al. (2014) conducting a meta-analysis with higher number of peer-review
369 publications than Maherali study and using the “rooting type” instead than “single root
370 traits”, demonstrated a robust and consistent response of the root architecture model to the
371 mycorrhizal treatments. Indeed, they pointed out that the “taprooted” plants were more
372 responsiveness than “fibrous root system” to the mycorrhizal inoculation (Yang et al.,
373 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 hierarchial
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;

409 Roumet et al., 2016).

410

411 **Conclusion**

412 Overall, these results permitted the following conclusions:

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

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

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

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

428

429 It is important recognize the limitations to scale-up the results of this study from greenhouse
430 to the field due to the different behavior of the AM fungi among the soils (Carrenho et al.,
431 2007), the high diversity of the rhizosphere organisms (Larimer et al., 2014) and the
432 different effects on plant productivity in relation to AM fungi identity (Sikes et al., 2009; Jin
433 et al., 2013) and diversity (Sharma et al., 2009). However, a study conducted in citrus
434 rootstocks in a mature field planting, showed that the rootstocks with higher MGR observed
435 in pot experiments were more rapidly colonized by mycorrhizal fungi in field (Graham et
436 al., 1991). Nonetheless, the results of this study could be relevant for the commercial
437 growing plants in containers, such as in the nursery industry, in which the ensuring the
438 high-quality of the planting materials is important.

439

440 **Author contributions**

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

444

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448 **CRedit authorship contribution statement**

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

453

454 **Declaration of Competing Interest**

455 The authors report no declarations of interest.

456

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462

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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	Treatments (T)			CV average
		CV	M	nM	
Root fresh weight (g)	CV ^{NS}	N	198 ^a	144 ^b	178 ^x
	T ^{***}	D	189 ^a	138 ^b	167 ^x
	CVxT ^{NS}	<i>T average</i>	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}	<i>T average</i>	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 ^{**}	<i>T average</i>	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 ^{**}	<i>T average</i>	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}	<i>T average</i>	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}	<i>T average</i>	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 [*]	<i>T average</i>	1194 ^A	1017 ^A	

689 #Statistic analysis: two-way ANOVA with 6 replicates (CV: cultivar; T: treatments; CVxT: cultivar x treatments interaction);
690 *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.

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

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698 **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
 699 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
 700 cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*.

Parameters	#Statistics	CV	Treatments		CV average
			M	nM	
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	

701 #Statistic analysis: two-way ANOVA with 6 replicates (CV: cultivar; T: treatments; CVxT: cultivar x treatments interaction);
 702 *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.
 703 Different letters in lower case within column indicated significant difference at P<0.05 (test of Fisher). Different letters in
 704 uppercase within rows indicated significant difference at P<0.05 (test of Fisher).
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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	

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#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).

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

	Attribute loadings	
	PC1	PC2
<i>Statistics</i>		
<i>Eigenvalue and variability</i>		
Eigenvalue	4.31	2.15
Proportion of variability (%)	70.17	22.16
<i>Variable</i>		
<i>Eigenvectors</i>		
Root fresh weighth	.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

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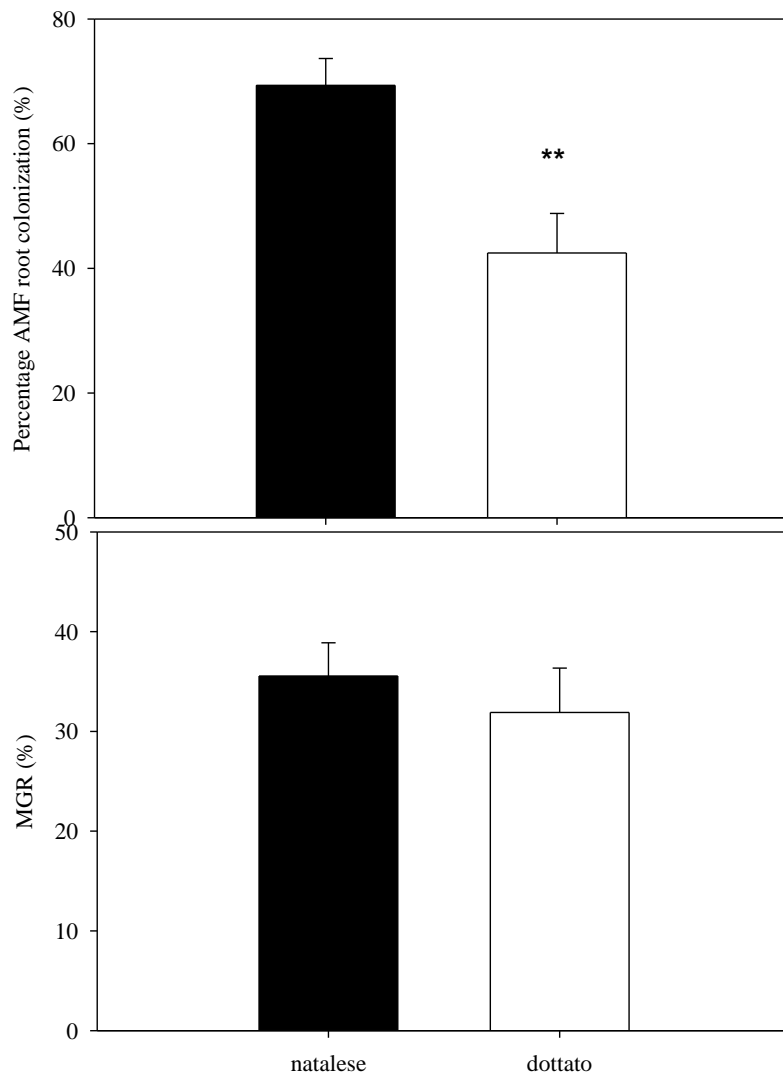


Figure 1 - Percentage root infected (A) and mycorrhizal growth response of self-rooted plants of the fig cultivars, Dottato and Natalese inoculated with a *Glomus intraradices*. Asterisk indicated significant difference between the two fig cultivars ($<0.01p<0.001$; t-test).

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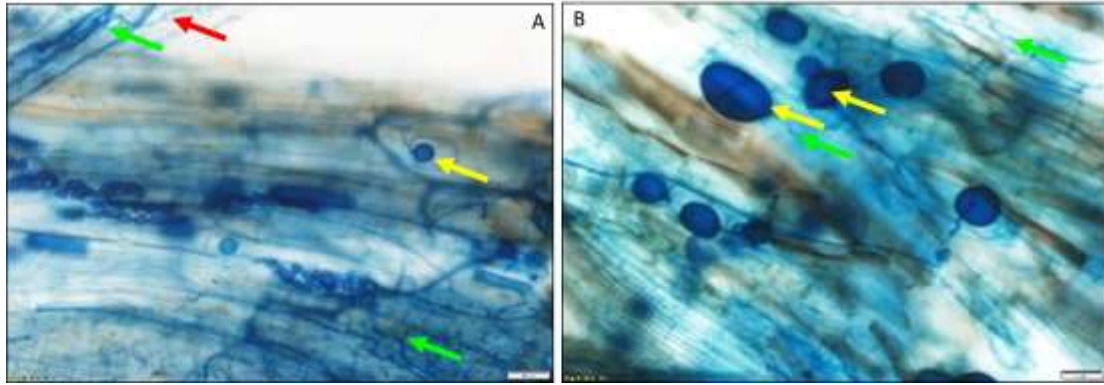


Figure 2 – Microscopic visualization of arbuscular mycorrhizal fungi showing vesicles (yellow arrows) and hyphae within- (green arrows) and outside-root (red arrows) of fig rooted cuttings.

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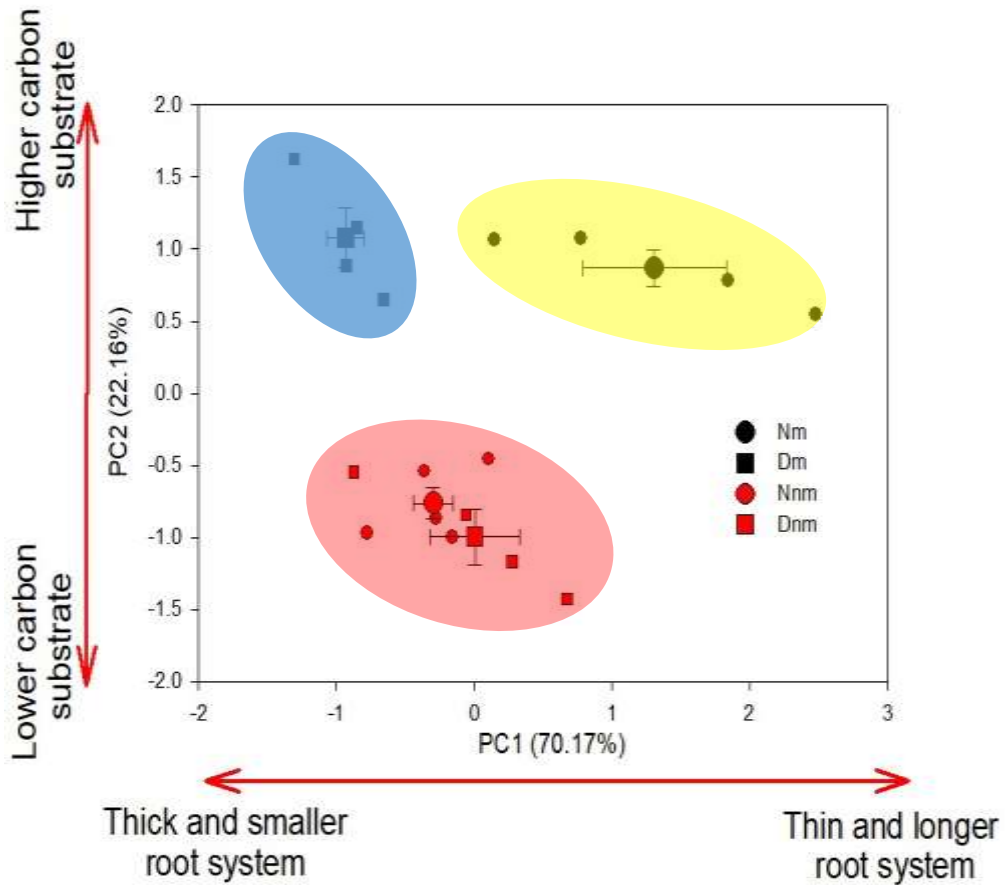


Figure 3 – Scores (means and error standard bars) of the principal components 1 and 2 of the root architectural traits of self-rooted plants of the fig cultivars (CV), Dottato (D) and Natalese (N) inoculated (M) and non-inoculated (nM) with a *Glomus intraradices*. The arrows indicate the biological 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).