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1 **Mycorrhizal inoculation increases fruit production without**  
2 **disturbance of native arbuscular mycorrhizal community in jujube**  
3 **tree orchards (Senegal)**

4  
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16  
17 **Abstract**

18 Arbuscular mycorrhizal (AM) fungi constitute promising biofertilizers for the deployment of a  
19 sustainable agriculture. However, the evaluation of mycorrhizal benefits in the field is poorly  
20 documented, notably in fruit tree orchards, and concerns exclusively growth performance and  
21 nutrition. Here, we evaluated the persistence of beneficial effects of AM fungal inoculation on  
22 two jujube (*Ziziphus mauritiana* Lam.) cultivars (Tasset and Gola) inoculated in nursery  
23 conditions and subsequently transferred in the field until fruit production. The growth-  
24 promoting effects on jujube observed in nursery was still maintained 18 months after planting,  
25 with significant higher rates of survival and an increase of fruit production for inoculated jujube  
26 cultivars. Nevertheless, AM-mediated responses were dependent on cultivar. No major  
27 disturbance of native AM fungal community was associated with AM fungal inoculation but a  
28 stimulation of AM fungal colonization is probable. The use of mycorrhizal inoculation with the  
29 *R. irregularis* IR27 strain hold promise for the development of efficient jujube orchards under  
30 semi-arid conditions.

31  
32 **Keywords:** *Ziziphus mauritiana*; Cultivars; *Glomeromycota*; Orchard; Fruit production

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## 36 **1 Introduction**

37 Indigenous fruit trees are an important biological resource in many agroecosystems all over the  
38 world (Awodoyin et al. 2015). They are important for the Sahelian and Sudanian populations  
39 in West African rural areas, both as food resources and source of incomes (Bonkougou et al.  
40 1998; Tchoundjeu et al. 2008). The species *Ziziphus mauritiana* Lam., commonly named  
41 jujube, is one of the most-sought indigenous fruit trees. The farmers are mainly interested in  
42 the fruit pulp (Ouédraogo et al. 2006), which has led to the domestication of Indian cultivars  
43 (e.g. Gola) producing earlier, larger and tastier fruits than local cultivars (e.g. Tasset from  
44 Senegal). Domestication of fruit trees can be achieved through different management practices  
45 combining the selection and multiplication of quality planting material, the mineral fertilization  
46 and irrigation, as well as the use of controlled mycorrhization (Guissou et al. 1998; Bâ et al.  
47 2003; Ouédraogo et al. 2006).

48 The controlled mycorrhization methods rely on the association between plant roots and  
49 arbuscular mycorrhizal (AM) fungi (Hart et al. 2015; Berruti et al. 2016). AM fungi are known  
50 for their ability to improve plant growth and notably to efficiently scavenge for soil phosphorus  
51 (P) resources (Smith and Read 2008). In West African soils, P deficiency represents one of the  
52 main limitation for the establishment of tree plantations and agriculture crops (Friesen et al.  
53 1997). It has been suggested that AM fungal root colonization of jujube seedlings in a nursery  
54 was an essential prerequisite enhancing the survival of outplanted jujube trees in the field (Bâ  
55 et al. 2001). Controlled mycorrhization in nursery highlighted the performance of the  
56 *Rhizophagus irregularis* IR27 strain (syn. *Glomus aggregatum* IR27; Bâ et al. 1996) on tree  
57 growth and nutrition for a wide diversity of jujube trees (Thioye et al. 2017). A field trial  
58 conducted on the route of Great Green Wall in an arid region of Senegal demonstrated higher  
59 jujube survival rates and a relative stability of beneficial effects observed in nursery on tree  
60 growth and nutrition for jujube cultivars inoculated with *R. irregularis* IR27 (Thioye et al.  
61 2019). However, no information was available on fruit production, as generally observed in the  
62 surveys for fruit tree orchards (Mercado-Blanco et al. 2018), whereas it constitutes one of the  
63 most valuable agronomic criteria for farmers. In addition, disturbance of native AM fungal  
64 community was observed, notably for the local jujube cultivar (Thioye et al. 2019). The  
65 intensity of environmental impacts due to AM inoculation may be related to the levels of  
66 environmental stress (Hart et al. 2015), and the investigation of different ecological context is  
67 thus crucial to robustly predict environmental impacts of mycorrhizal inoculation.

68

69 The current study investigated the sustainability of mycorrhizal inoculation benefits on jujube  
70 growth and fruit production of two cultivars (Tasset and Gola). The mycorrhiza-based  
71 inoculation strategy was in accordance with that of Thioye et al. (2019) but the field trial was  
72 set up in a semi-arid region of Senegal with less harsh conditions to evaluate if the disturbance  
73 of native AM fungal community associated with jujube trees occurred.

74  
75

## 76 **2 Materials and methods**

### 77 **2.1 Experiment in nursery conditions**

78 The soil used in nursery was collected from Sangalkam (14°46'N, 17°13'W), Senegal. It was a  
79 sandy soil with 88.8 % sand, 5.8 % silt, 5.4 % clay, 0.6 % organic matter, 0.3 % total C, 0.02  
80 % total N, ratio C/N = 14, 333.5 ppm total K, 41.4 ppm total P, 2.1 ppm P-Bray 1, 1.03 ppm  
81 Ca, 0.3 ppm Mg, pH = 6.0 of a soil/water mixture (ratio 1:2, v/v) and pH = 4.6 of a soil/KCl  
82 mixture (ratio 1:2, v/v). The soil was passed through a 2 mm sieve, sterilized for 4h in an  
83 autoclave oven system at 180 °C to eliminate native AMF, and bagged in plastic bags (1.5 kg  
84 soil per plastic bag).

85 The AM fungal inoculum, *Rhizophagus irregularis* isolate IR27 (syn. *Glomus aggregatum*  
86 IR27 Schenck & Smith) originated from the rhizosphere of *Acacia holosericea* growing in  
87 Burkina Faso (Bâ et al. 1996), was provided by the LCM laboratory (IRD, Dakar, Senegal,  
88 certified ISO 9001, version 2000). It was propagated on maize (*Zea mays* L.) for 3 months on  
89 sterilized sandy soil under greenhouse conditions. The sandy soil was autoclaved two times at  
90 120 °C for 2h, with an interval of 24h. Mycorrhizal inoculation of the soil was achieved by  
91 placing 20 g portions of a crude inoculum of AMF consisting of sand, spores, hyphae and  
92 colonized maize root fragments below the seeds during sowing. The inoculum density of *R.*  
93 *irregularis* IR27 was determined by the Most Probable Number (MPN) method (Adelman and  
94 Morton, 1986) as 1635 infective propagules [95% CI = 763–3483] per 20 g of inoculum, by  
95 using maize as host plant and ¼ (v/v) as a dilution of the substrate. Controls also received 20 g  
96 of autoclaved crude inoculum of AMF with a 10 ml water extract of the inoculum obtained by  
97 filtration (Whatman No.1 filter paper) as controls to balance composition of the microbial  
98 community between inoculated and non-inoculated plants (Guissou et al. 1998).

99 Two jujube cultivars (Tasset from Senegal and Gola from India) were used in this study and  
100 provided by the CNRF/ISRA, Senegal. Seeds of each jujube cultivar were surface-sterilized  
101 with 1 % NaOCl for 15 min, washed several times and soaked in sterile distilled water for 30

102 min before being planted in the sterilized soil as three per plastic bag. Seeds were transferred  
103 to the plastic bags such that they were in direct contact with the AM fungal inoculum in nursery.  
104 Seeds of both jujube cultivars were watered with tap water without fertilizer application and  
105 grown during 4 months in a nursery (35 °C day, 27 °C night, relative humidity 75 % and 14h  
106 photoperiod) located at research center ISRA/IRD, Bel Air, Senegal (14°44'N, 17°30'W). After  
107 emergence, the seedlings were thinned to one plant per plastic bag to obtain in all 200 seedlings  
108 (100 per jujube cultivar). The experiment was set up as a 2 × 2 factorial design consisting of  
109 two jujube cultivars with AM fungal inoculation or not. Experiment was arranged in a  
110 completely randomized design with 50 replicates per treatment combination.  
111 Four months after sowing, 40 seedlings (10 per treatment) were sampled to measure height,  
112 collar diameter and dry weight of shoots and roots (48h at 70 °C). For mycorrhizal root  
113 colonization measurement, a part of fresh fine roots was collected from the root system of each  
114 seedling. Root were gently washed under tap water, bleached (KOH, 10 %) at 80 °C during 30  
115 min, and stained in 0.05 % Trypan blue at 80 °C during 35 min following the method of Phillips  
116 and Hayman (1970). Percentage of root length colonized by AMF was assessed at ×40  
117 magnification using 100 fragments of lateral roots (approximately 1 cm length) on microscopic  
118 slides. Mycorrhizal root colonization was evaluated by using the method of Trouvelot et al.  
119 (1986).

120

## 121 **2.2 Experiment in field conditions**

122 The 160 remaining of the pre-inoculated seedlings and controls from jujube cultivars were  
123 transplanted to the field after 4 months of growth in the nursery. The experimental site is an  
124 orchard (5.5 ha) located near the village of Keur Mangari in the rural community of Tattaguine  
125 at 15 km from Fatick city (14°27'N, 16°38'W), Senegal. The orchard contains planted fruit  
126 trees such as mango, cashew, citrus and vegetable crops. The climate is semi-arid, with mean  
127 annual rainfalls varying from 450 to 600 mm and 30-35 °C monthly temperature. Each sample  
128 collected from the orchard consisted of 5 auger samples (0-20 cm depth), which were mixed in  
129 a plastic bag. Physical and chemical analyses of soil were performed in the Agricultural  
130 Chemistry Laboratory of EMBRAPA at Rio de Janeiro, Brazil. The soil was tropical  
131 ferruginous with 0.14 % total C, 0.04 mg.kg<sup>-1</sup> Al, 0.02% total N, C/N ratio 7, 19.62 mg.kg<sup>-1</sup>  
132 total K, 26.9 mg.kg<sup>-1</sup> P-Bray 1, Ca 0.52 mg.kg<sup>-1</sup>, Mg 0.17 mg.kg<sup>-1</sup>, and pH (of a soil/water  
133 mixture, ratio 1:2) 5.43. The mycorrhizal soil infectivity was determined by the MPN method  
134 (Adelman and Morton, 1986) as 23.87 infective propagules [95% CI = 11.19–50.81] per 100 g

135 of dry soil, by using maize as host plant and 1/4 (v/v) as dilution of the substrate. The experiment  
136 was set up as a 2 × 2 factorial design consisting of two jujube cultivars and inoculated or not  
137 with *R. irregularis* IR27. Plantings were established using a fully randomized block design with  
138 a surface area of 160 m<sup>2</sup> each, four treatments and ten replicates per treatment and block.  
139 During the dry season, the jujube trees were watered without fertilizer application three times  
140 a week until the end of the experiment.

141 Measurements were performed on ten plants for each treatment and block. They consisted of  
142 assessing height, collar diameter of the main stem and rate of survival at 4, 8, 12 and 18 months  
143 after transplanting. Crown diameter, mycorrhizal colonization and fruit production were only  
144 recorded at 18 months after transplanting. For each jujube cultivar, mature fruits were harvested  
145 per week for 2 months. The mean number, diameter and fresh weight of fruits were evaluated  
146 for each tree. Sub-samples of 20 fruits per treatment and per block were used to evaluate the  
147 mean diameter of fruits. Approximately 500 mg (fresh weight) of fine roots were randomly  
148 collected on three plants per treatment and per block. One part of the roots was preserved in  
149 ethanol 90 % to determine the percentage of AM fungal colonization (see experiment in  
150 greenhouse conditions).

151

### 152 **2.3 DNA extraction, PCR and MiSeq Illumina sequencing**

153 Roots collected at 18 months after planting were used for DNA extraction and MiSeq Illumina  
154 Sequencing. Forty milligrams (dry weight) of roots per sample (16 samples in total) were  
155 ground in a 1.5 mL tube using a pestle in liquid nitrogen and extracted using a FastPrep-24  
156 homogenizer (MP biomedical Europe, Illkirch, France) and the FastDNA® SPIN kit (MP  
157 biomedical, Europe) according to manufacturer's instructions. DNA extracts were then loaded  
158 onto PVPP (polyvinylpyrrolidone) Micro Bio-Spin® Columns (Bio-Rad, Marnes-la-  
159 Coquette, France) and eluted by centrifugation to improve DNA purity and avoid PCR  
160 inhibitors. Two replicates were done per root sample. DNA integrity was checked on 1.5 %  
161 agarose gel and DNA quantification was done by using QuantiT™ PicoGreen® dsDNA assay  
162 kit (Invitrogen, St. Aubin, France).

163 Molecular diversity of AM fungi (Glomeromycota) from plant DNA was assessed by 18S  
164 rRNA gene amplification with the primers NS31 and AML2 (Simon et al. 1992; Lee et al.  
165 2008). The forward and reverse primers were modified prior to amplification by adding the  
166 adaptor sequences 5'-CTTCCCTACACGACGCTCTTCCGATCT-3' and 5'-  
167 GGAGTTCAGACGTGTGCTCTTCCGATCT-3', respectively, which are needed for

168 multiplexing the PCR products before sequencing. PCR round was carried out in a final volume  
169 of 50 µl with NS31 and AML2 primers (0.6 µM each), 20 ng/µl DNA (2 extracted DNA  
170 replicates per sample), 200 µM of each dNTP, 200 ng/ml BSA, GoTaq® DNA Polymerase (1  
171 unit) and 1X Green GoTaq® Reaction Buffer (Promega, Charbonnières, France), with the  
172 following cycling conditions: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 58 °C for 60 s, 72°C  
173 for 80 s; a final elongation step at 72°C for 10 min. After PCR, the duplicate products were  
174 pooled per sample and purified by using illustra GFX PCR DNA and Gel Band Purification Kit  
175 (GE Healthcare Life Sciences, Velizy-Villacoublay, France) following manufacturer's  
176 guidelines.

177 All amplicon products were analyzed using paired-end Illumina MiSeq sequencing (2×300 bp)  
178 performed by Get-PlaGe (Genotoul, Castanet-Tolosan, France) with single multiplexing using  
179 a home-made 6 bp index, which was added to the reverse primers for a second PCR with 12  
180 cycles, using the forward primer  
181 (AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC) and the reverse  
182 primer (CAAGCAGAAGACGGCATACGAGAT-index-GTGACTGGAGTTCAGA-  
183 CGTGT). The resulting PCR products were purified, spiked with PhiX (15 %), and loaded onto  
184 the Illumina MiSeq cartridge according to the manufacturer's instructions.

185

## 186 **2.4 Data treatment**

187 MiSeq Illumina sequencing, base calling and demultiplexing were carried out using RTA  
188 v1.18.54, MCS 2.6 and bcl2fastq2.17. Primer clipping of Illumina MiSeq R1 reads was  
189 performed with cutadapt v1.11 (Martin, 2011), allowing a 2/3rd-of-the-length partial match for  
190 the forward primer. Only reads containing the forward primer and with a final length ranging  
191 from 32 to 200 nucleotides were retained. For each trimmed read, the expected error was  
192 estimated with vsearch's command fastq\_filter and the option eeout. Each sample was then  
193 dereplicated, i.e. strictly identical reads were merged, using vsearch's command  
194 derep\_fulllength, and converted to FASTA format. To prepare clustering, samples were pooled  
195 and submitted to another round of dereplication with vsearch. The files containing expected  
196 error estimations were also dereplicated to retain only the lowest expected error for each unique  
197 sequence. Clustering was performed with swarm v2.2.1 (Mahé et al. 2015), using a local  
198 threshold of one difference and the fastidious option. Operational taxonomic unit (OTU)  
199 representative sequences were then searched for chimeras with vsearch's command

200 uchime\_denovo (Edgar et al. 2011). In parallel, representative sequences received taxonomical  
201 assignments using the stampa pipeline (<https://github.com/frederic-mahe/stampa>) and a custom  
202 version of the PR2 database (gb203) (Guillou et al. 2013) implemented with *Glomeromycota*  
203 reference sequences from Krüger et al. (2012). Clustering results, expected error values,  
204 taxonomic assignments and chimera detection results were used to build a raw OTUs (Table  
205 S1). Up to that point, were eliminated reads that could not be merged, reads without tags or  
206 primers, reads shorter than 32 nucleotides and reads with uncalled bases ("N"). To create the  
207 “cleaned” OTU table, additional filters were applied to retain non-chimeric OTUs, OTUs with  
208 an expected error divided by length below 0.0002, and OTUs containing more than three reads.

209

## 210 **2.5 Statistics**

211 Tables of sequencing data were transformed using the R tidyverse package version 1.2.1  
212 (Wickham, 2019) and the plots were generated using the R ggplot2 package version 3.0.0  
213 (Wickham, 2016). The sequencing data for the AM fungal community were subjected to a  
214 global rarefaction down to 38,811 reads per sample with with R vegan package version 2.5-6  
215 (Oksanen et al. 2019) using the rrarefy() function for downstream statistical tests. The rarefied  
216 data set with OTU abundance, frequency and taxonomic assignment is available in Table S1.  
217 The AM fungal community richness (number of OTUs) and diversity (Shannon, inverse  
218 Simpson [1/D]) were estimated using the R package vegan. Normality of data (plant growth  
219 and mycorrhizal parameters, AM fungal indices) distribution was verified using the Shapiro-  
220 Francia test as implemented in sf.test() function from the R nortest package version 1.0-4 (Gross  
221 and Ligges 2015).

222 The effect of treatments (cultivar and inoculation) on plant parameters was estimated using non-  
223 parametric Kruskal-Wallis test as implemented in kruskal.test() function from the R package  
224 stats (R Core Team, 2018) because of non-normality of data. Survival rates were calculated in  
225 percentages for each treatment. Mycorrhizal richness and diversity data were analyzed using a  
226 two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test.

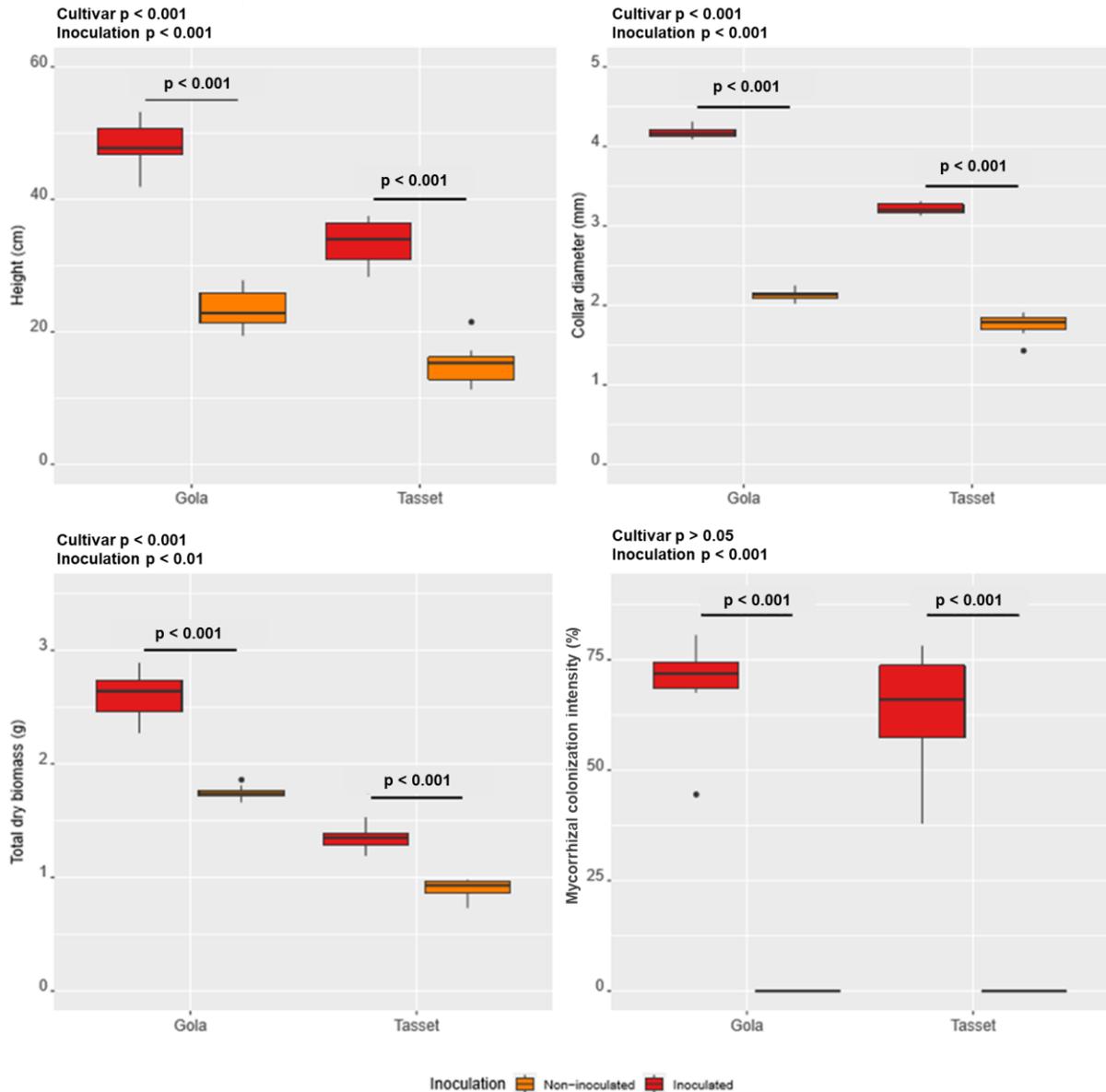
227 The differences in the AM fungal community structures among all treatments were based on  
228 the Bray-Curtis dissimilarity matrix and assessed using non-parametric permutational  
229 multivariate analysis of variance (PERMANOVA) as implemented in the *adonis()* function  
230 from the R package vegan. Multivariate dispersion was estimated for each treatment using the

231 *betadisper()* function and *permutest()* as it can affect PERMANOVA results. Nonmetric multi-  
232 dimensional scaling (NMDS) implemented in the *metaMDS()* function, based on Euclidean  
233 distances was used to display AM fungal community structure associated with inoculated and  
234 non-inoculated jujube cultivars.

### 235 **3 Results**

#### 236 **3.1 Experiment in nursery conditions: growth and mycorrhizal colonization**

237 Statistical analysis showed significant effects of both factors on all parameters (Fig. 1). Pre-  
238 inoculation with *R. irregularis* IR27 significantly increased plant growth and mycorrhizal  
239 colonization compared with non-pre-inoculated controls in nursery conditions. Height, collar  
240 diameter, total dry biomass and mycorrhizal colonization of plants were found to be  
241 significantly superior ( $P < 0.001$ ) to non-inoculated controls four months after sowing (Fig. 1),  
242 with higher rates of plant growth for Gola cultivar compared to Tasset cultivar. However, no  
243 difference of AM colonization was observed between the cultivars ( $P > 0.6$ ).



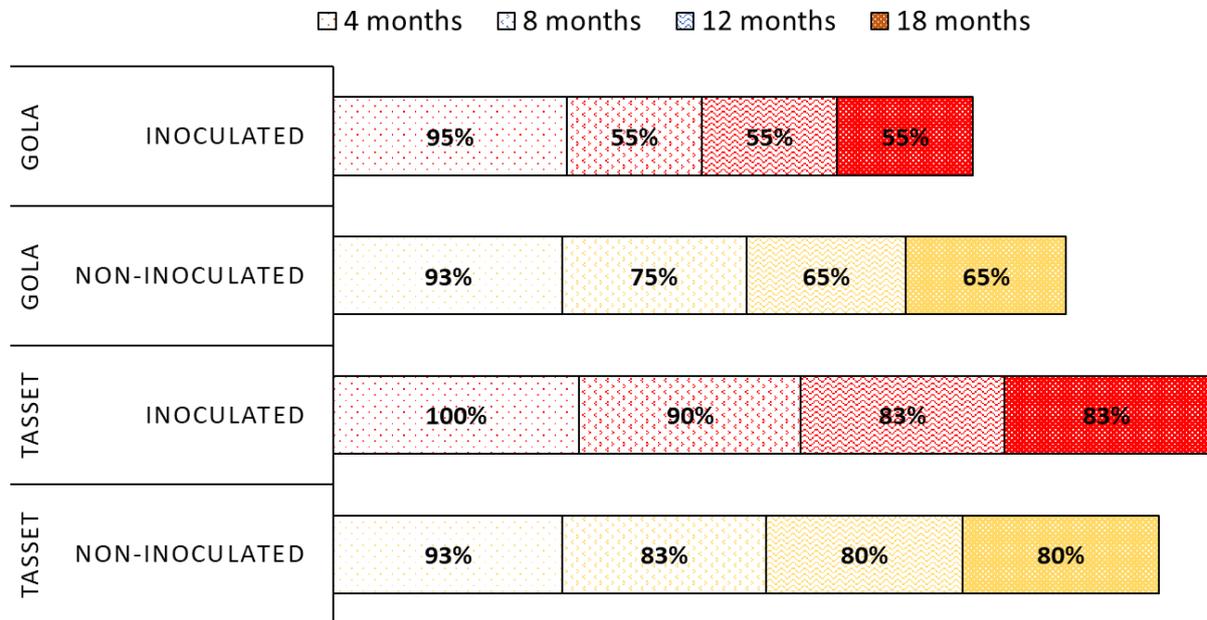
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**Fig 1** Effect of inoculation with *Rhizophagus irregularis* IR27 on growth parameters (height, collar diameter and total dry biomass) and mycorrhizal colonization **intensity** of *Z. mauritiana* cultivars 4 months under greenhouse conditions. The boxplot with p-values represent means of each treatment using Kruskal-Wallis test (n=10); significant levels:  $P < 0.05$ .

### 250 **3.2 Experiment in field conditions: growth, survival, mycorrhizal colonization and fruit** 251 **production**

252 The positive effect of mycorrhizal pre-inoculation with *R. irregularis* IR27 on growth of both  
253 jujube cultivars was monitored for 18 months after planting. The differences between non-  
254 inoculated and inoculated *jujube* trees in terms of height and collar diameter were still  
255 maintained after 18 months (Fig. S1). The survival rates dropped for Gola cultivar from 8  
256 months after planting, regardless of treatment inoculated or non-inoculated, whereas it  
257 remained relatively high for Tasset (Fig. 2). At 18 Months after planting, the survival rates for

258 Tasset was still high (> 80%). At contrary, the survival rates for Gola reached rather low values  
 259 (< 65%). In addition, mycorrhizal inoculation negatively impacted the survival rates of Gola  
 260 cultivar.  
 261

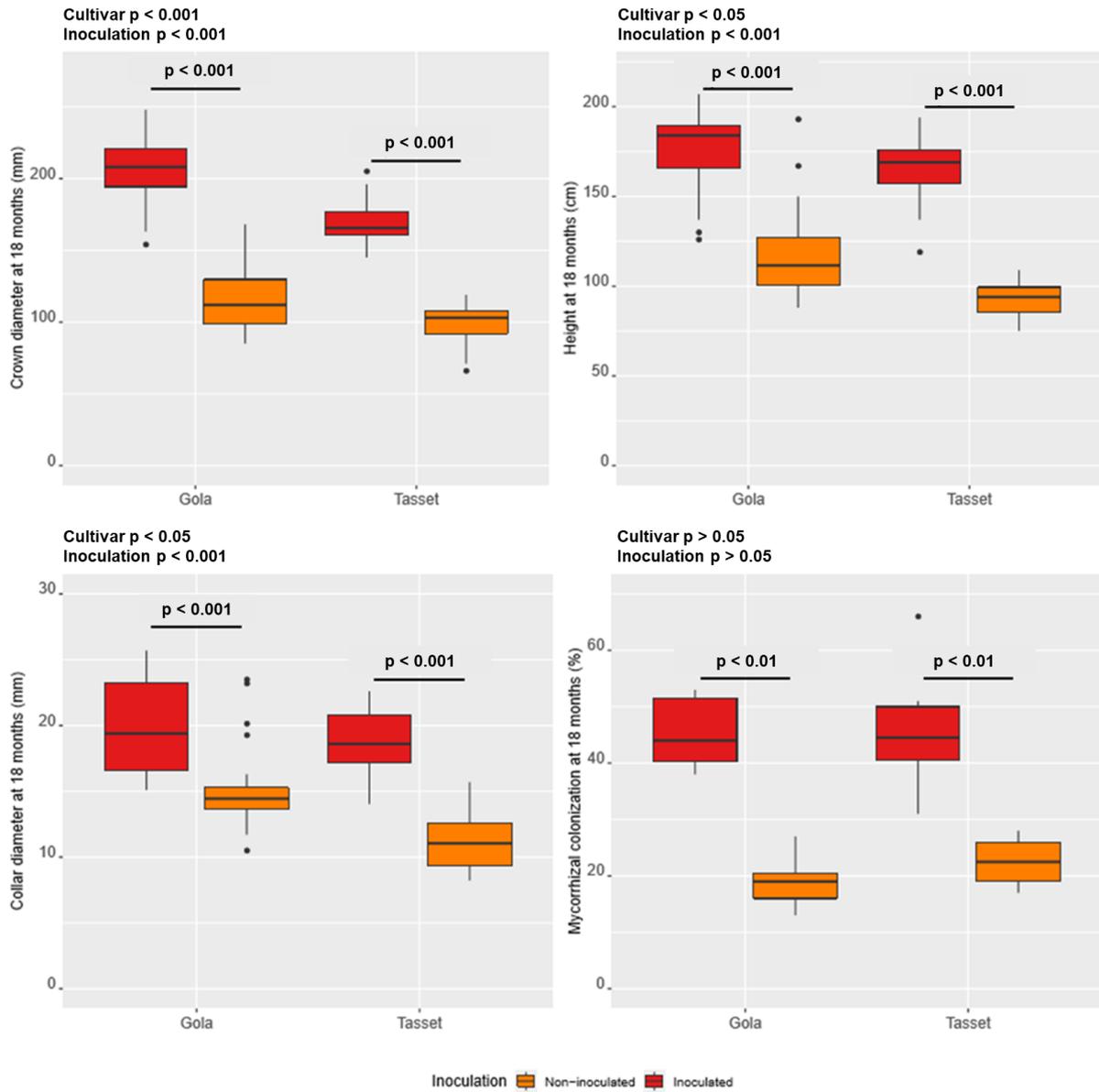


262  
 263  
 264 **Fig. 2** Effect of inoculation with *Rhizophagus irregularis* IR27 on rate of survival of *Z. mauritiana*  
 265 cultivars 4, 8, 12 and 18 months after transplanting. Data values represent the percentages of survival  
 266 rates of each treatment (n=40).

267  
 268 The monitoring of tree growth parameters and mycorrhizal colonization at 18 months after  
 269 planting still showed positive impacts of mycorrhizal inoculation ( $P < 0.001$ ) for both jujube  
 270 cultivars (Fig. 3), with higher levels for Gola cultivar notably for the crown diameter. As  
 271 observed in nursery, no significant difference ( $P > 0.6$ ) was observed for the mycorrhizal  
 272 colonization between the two jujube cultivars, reaching *c.a.* 45% and 20% for inoculated and  
 273 non-inoculated, respectively.

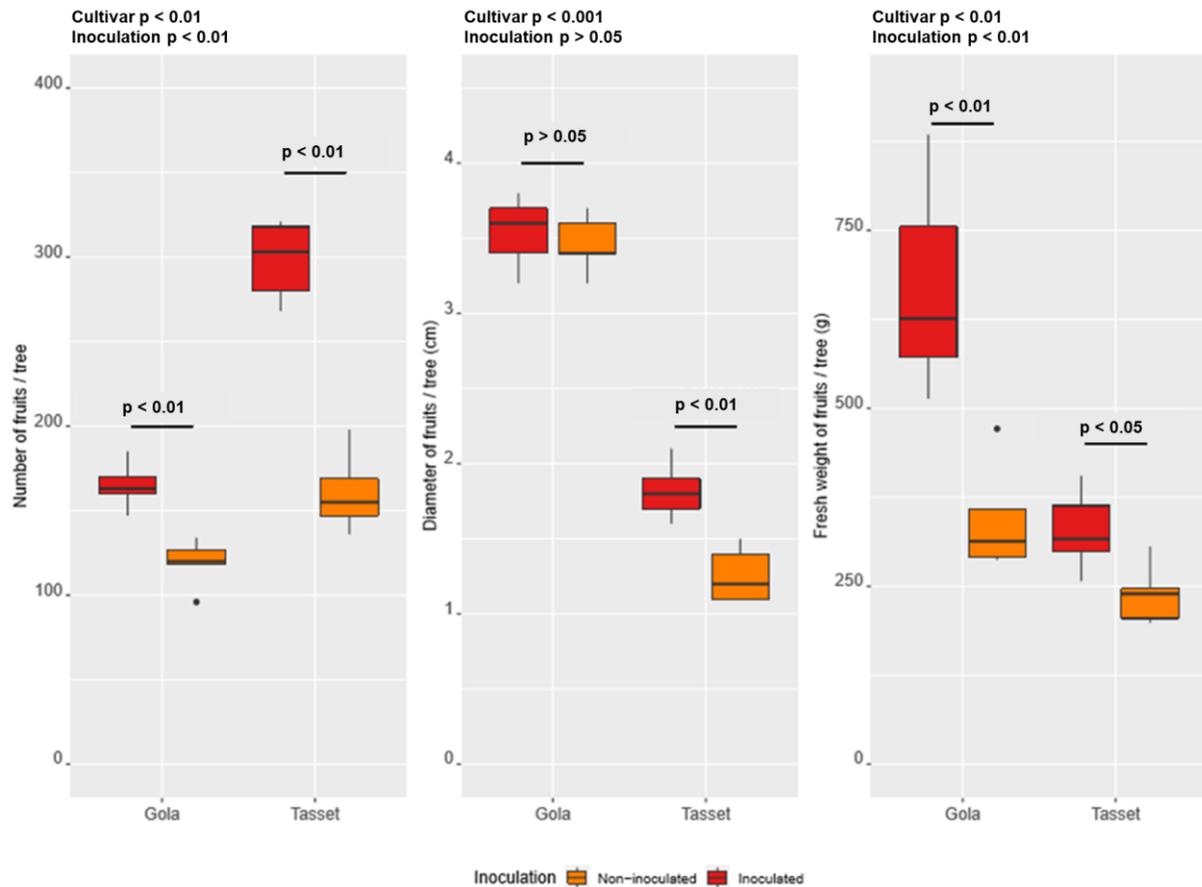
274 The analysis of the fruit production data revealed significant differences between the two  
 275 cultivars ( $P < 0.01$ ) and the treatments ( $P < 0.01$ ), inoculated and non-inoculated ( $P < 0.05$ ),  
 276 except for the diameter of fruits for Gola cultivar (Fig. 4). Inoculated Tasset cultivar gave the  
 277 highest mean number of fruits (*c.a.* 300 and per tree; Fig. 4), twice the amount of that obtained  
 278 for Gola, whereas highest mean fresh weight of fruits were obtained for inoculated Gola cultivar  
 279 (*c.a.* 670 g per tree; Fig. 4).

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**Fig. 3** Effect of inoculation with *Rhizophagus irregularis* IR27 on growth parameters (crown diameter, height and collar diameter) and mycorrhizal colonization of *Z. mauritiana* cultivars 18 months after transplanting. The boxplot with p-values represent means of each treatment using Kruskal-Wallis test (n=10); significant levels:  $P < 0.05$ .



289  
290

291 **Fig. 4** Effect of inoculation with *Rhizophagus irregularis* IR27 on fruit parameters (fresh weight,  
292 diameter of fruits and number of fruits) and mycorrhizal colonization of *Z. mauritiana* cultivars 18  
293 months after transplanting. The boxplot with p-values represent means of each treatment using Kruskal-  
294 Wallis test (n=10); significant levels:  $P < 0.05$ .

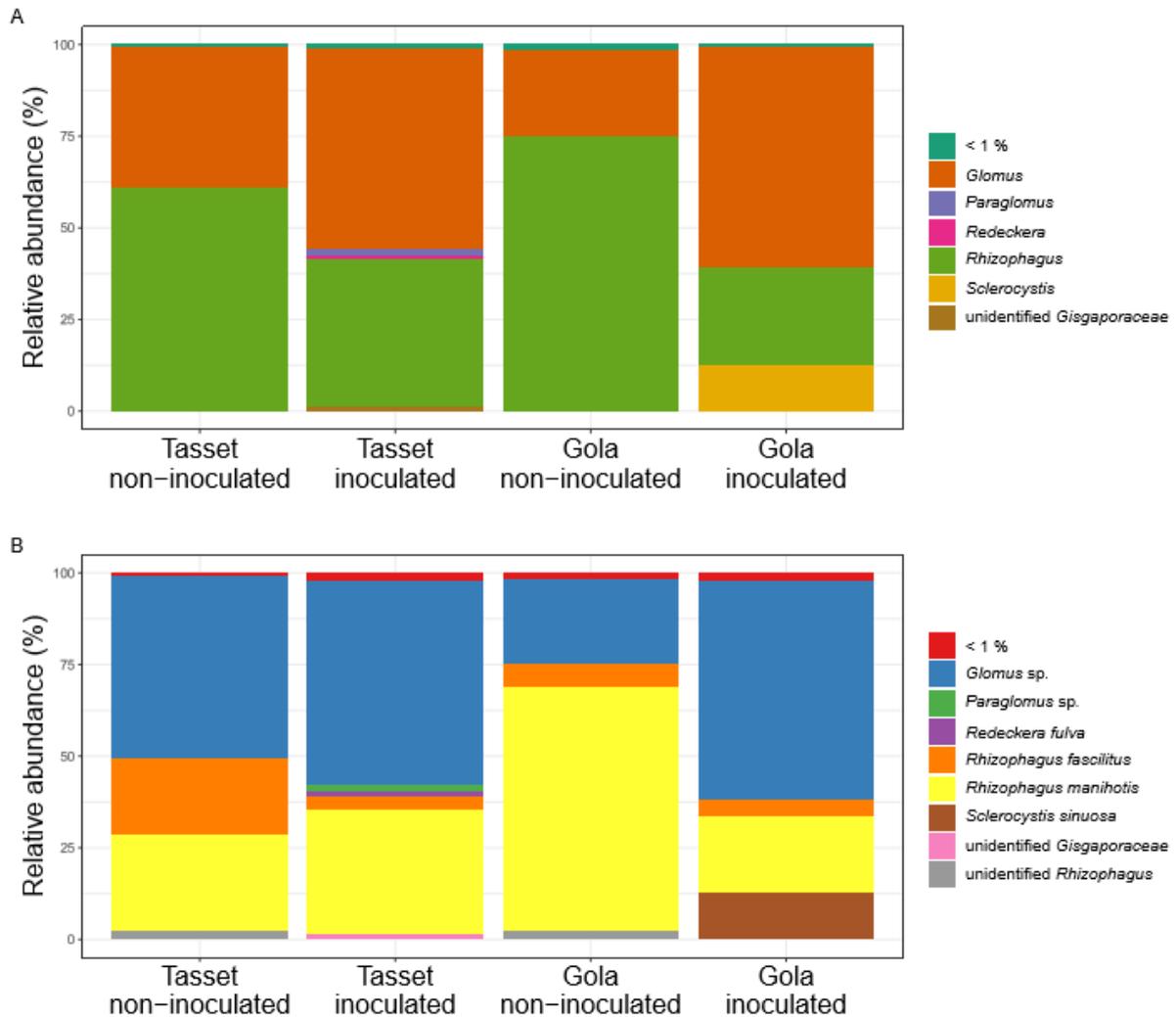
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### 297 **3.3 Experiment in field conditions: effect of AM fungal pre-inoculation on the native AM** 298 **fungal communities**

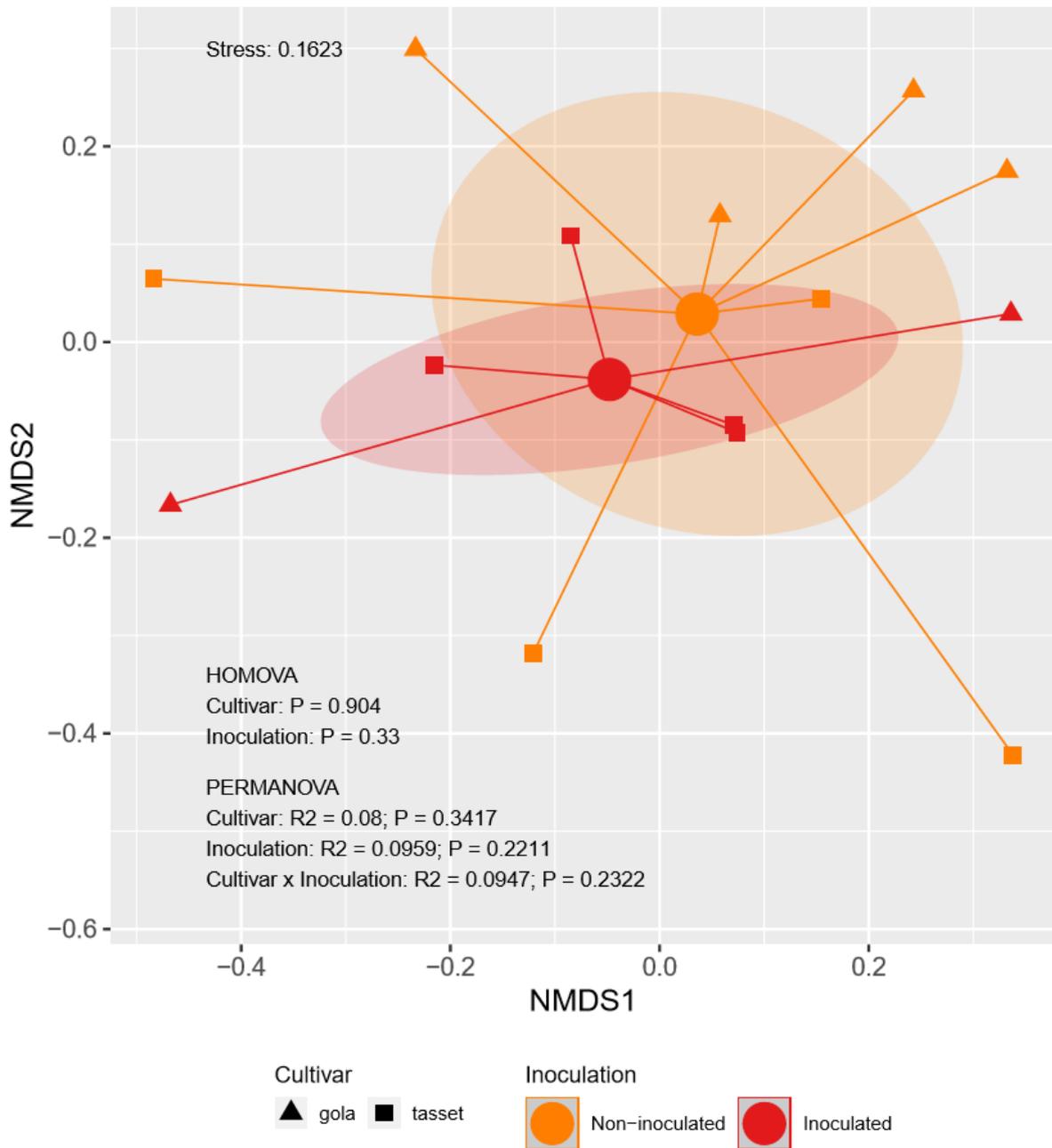
299 The majority of OTUs belonged to the *Glomeraceae* (89.7%) accounting to 99% to 97% of  
300 reads for Gola and Tasset cultivar, respectively. Only few belonged to the *Gigasporaceae*  
301 (3.9%) and *Diversisporaceae* (2.2%), with < 1% for the rest of other families  
302 (*Acaulosporaceae*, *Ambisporaceae*, *Archaeosporaceae*, *Claroideoglomeraceae*,  
303 *Geosiphonaceae*, *Paraglomeraceae*). The most predominant genera were *Rhizophagus* (57%  
304 and 49% of total reads) and *Glomus* (35% and 48%) for Gola and Tasset cultivars respectively  
305 (Fig. 5A). However, mycorrhizal inoculation mostly affected the taxonomic proportion of AM  
306 fungal community with a decrease of the ratio *Rhizophagus* / *Glomus*, and an increase of notably  
307 *Sclerocystis* in Gola cultivar. At the species level, the impact of mycorrhizal inoculation

308 observed on *Rhizophagus* was mainly due to a strong decrease of *Rhizophagus fascilitus* in  
 309 Tasset cultivar and *Rhizophagus manihotis* in Gola cultivar (Fig. 5B).  
 310 However, no significant impact of AM fungal inoculation or the type of cultivar was observed  
 311 on AM fungal community richness and diversity (Table 1). Similarly, the analysis of AM fungal  
 312 community structure associated with jujube trees do not revealed an impact of mycorrhizal  
 313 inoculation, nor of the cultivar (Fig. 6).  
 314



315  
 316  
 317 **Fig. 5** Taxonomic distribution of AM fungal (A) genera and (B) species associated with non-inoculated  
 318 and inoculated (+ Ri) jujube cultivars (Tasset and Gola).

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**Fig. 6** Two-dimensional nonmetric multidimensional scaling (NMDS) ordination of native AM fungal communities based on the Bray-Curtis coefficient. Each point represents AM fungal composition in inoculated (red) and non-inoculated (orange) jujube cultivars (Tasset and Gola); significant levels:  $P < 0.05$ .

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339 **Table 1** Effect of jujube cultivar and mycorrhizal pre-inoculation on richness and diversity of the jujube  
 340 root-associated AM fungal community in field.

Treatment	Number of OTUs (Richness)	Chao (Richness)	Shannon (Diversity)	Invsimpson (Diversity)
Gola	178 ± 15	255.6 ± 28	1.9 ± 0.3	4.2 ± 1.2
Gola + Ri	153 ± 17	235.7 ± 19	1.7 ± 0.4	4.5 ± 1.3
Tasset	160 ± 16	224.2 ± 26	1.9 ± 0.4	4.5 ± 1.9
Tasset + Ri	168 ± 12	229.6 ± 24	1.9 ± 0.2	4.4 ± 1.6
Factors				
Cultivar (C)	ns	ns	ns	ns
Inoculation (I)	ns	ns	ns	ns
C × I	ns	ns	ns	ns

341 The values represent means of each treatment using ANOVA test (n=10); Ri: *Rhizophagus irregularis*;  
 342 ns: not significant.

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#### 345 **4 Discussion**

346 The current study proposed one of the most extended monitoring of mycorrhizal inoculation  
 347 impacts in fruit tree orchards, providing new evidences of AM fungal-mediated benefits until  
 348 fruit tree production. The results obtained in nursery emphasized the benefits of mycorrhizal  
 349 inoculation previously observed on different jujube cultivars or provenances (Guissou et al.  
 350 1998; Sidibé et al. 2012; Guissou et al. 2016; Thioye et al. 2017). The field survey also  
 351 confirmed the lasting significant benefits on tree growth as observed in Thioye et al. (2019),  
 352 and consequently the suitability of the current strategy in different soil and climate conditions.  
 353 However, differences were noted regarding the response of jujube cultivars on field and the  
 354 impacts on the native AM fungal community.

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##### 356 **4.1. Environmental-dependent AM inoculation-mediated tree benefits**

357 After one-year plantation, the benefits of mycorrhizal inoculation on tree growth was still  
 358 significant but contrary to results in Thioye et al. (2019), the differences between the two  
 359 cultivars was less significant. This discrepancy between both studies may be due to less harsh  
 360 conditions in the current study with semi-arid conditions compared to arid conditions in Thioye  
 361 et al. (2019). AM fungi were identified as the main actors of plant tolerance to stress as drought  
 362 (Porter et al. 2019) and it has been reported that plant response to AM fungi was stronger in  
 363 extreme conditions (Hart et al. 2017). In addition, the survival rates, which was highly improved  
 364 by mycorrhizal inoculation in arid conditions (Thioye et al. 2019), was even negatively

365 impacted by inoculation for Gola cultivar, and unclear benefit was obtained for Tasset cultivar.  
366 Nevertheless, a better survival of the local cultivar (Tasset) was obtained compared to exotic  
367 cultivar (Gola), confirming the probable better adaption of Tasset to local conditions (Thioye  
368 et al. 2019).

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#### 370 **4.2. AM inoculation-mediated implication in mycorrhizal colonization rates in the field**

371 The level of mycorrhization was still significantly higher for pre-inoculated jujube trees,  
372 suggesting potential links between increased mycorrhizal colonization and improvement of tree  
373 growth, and notably fruit production. However, no difference in colonization rates was  
374 observed between the cultivars. The relationship between increasing colonization rates and an  
375 increase in yields was also demonstrated in a two year-long forage field (Pellegrino et al. 2012)  
376 but whereas biomass and mycorrhizal colonization is generally positively correlated (87% of  
377 field trials; Lekberg and Koide, 2005), a lower correlation significance is observed with yield  
378 suggesting that increase in mycorrhizal colonization cannot be the sole predictor of increase in  
379 yield. The differences in mycorrhizal colonization between inoculated and non-inoculated  
380 jujube trees at 18 months may indicate a higher colonization of *R. irregularis* IR27 compared  
381 to the native AM fungal community because of a potential priority effect due to the pre-  
382 colonization of jujube roots in the nursery (Werner and Kiers, 2015), but also to a higher  
383 competitiveness compared to native AM fungi. However, this hypothesis was not corroborated  
384 by the very low abundance of *R. irregularis* populations detected on jujube roots compared to  
385 other *Rhizophagus* spp. at 18 months after planting into the field and the low persistence of *R.*  
386 *irregularis* IR 27 estimated at only *c.a.* 15% of *R. irregularis* populations (Thioye et al. 2020).  
387 A second hypothesis may be the positive effect of *R. irregularis* IR27 pre-colonization on the  
388 native AM fungal community colonization (Rodriguez and Sanders, 2015; Werner and Kiers,  
389 2015). Moreover, beneficial interactions between AM fungal inoculants and native AM fungal  
390 community have been suggested in field trials with *Olea europaea* in arid and semi-arid  
391 degraded lands (Alguacil et al. 2011). The identity of AM inoculants has been shown to even  
392 determine the AM fungal assembly within roots (Mummey et al. 2009)

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#### 394 **4.3. AM inoculation-mediated impacts on root-associated mycorrhizal diversity**

395 Disentangling the mechanisms that support plant growth responses to mycorrhizal inoculation  
396 remains challenging in the field. Indeed, direct effects of the pre-inoculated AM fungus and/or  
397 indirect effects through the modification of the native AM fungal community may occur.  
398 However, in the current study, no major evidence was obtained demonstrating that *R.*

399 *irregularis* IR27 inoculation affected the native AM fungal community. The use of inoculants  
400 such as *R. irregularis* was however suggested as a potential threat to native soil and plant  
401 diversity because of probable negative environmental impacts (Hart et al. 2017). Deeper  
402 investigations of the impact of the use of *R. irregularis* IR 27 strain on soil and root-associated  
403 biodiversity should be performed to fully evaluate the sustainability of the strategy. It  
404 constitutes a major challenge, rarely investigated, since the modifications of root-associated  
405 microbiota can have major implications for plant growth and health (de la Fuente Canto et al.,  
406 2020).

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#### 408 **4.4. Fruit production**

409 The pre-mycorrhizal inoculation of jujube trees significantly improved the fruit production but  
410 at different level depending on the cultivar. An increase of yields due to AM fungal inoculation  
411 was widely observed for some annual species, notably vegetables (Douds et al. 2007; Regvar  
412 et al. 2003; Salvioli et al. 2012; Baum et al. 2015; Hijri 2016; Pellegrino et al. 2020), but rarely  
413 demonstrated on perennal species (Kapulnik et al. 2010). In the present experiment, the  
414 increased fruit production achieved may be due to the fact that the orchard soil was found to be  
415 deficient in P and, AM inoculation may be, in this context, an important factor contributing fruit  
416 production of jujubes. However, other factors (i.e. fertilization, irrigation) can also increase  
417 fruit production of jujube trees irrespective with AM status of jujube trees. For example,  
418 Ouédraogo et al. (2006) reported that jujube trees responded positively to irrigation and  
419 fertilization with rock phosphate in P-deficient soil by displaying the highest fruit production  
420 with Gola cultivar compared to the local cultivar.

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#### 422 **5. Conclusion**

423 The current results demonstrated the major benefits of mycorrhizal pre-inoculation on jujube  
424 trees until the evaluation of fruit production, a goal rarely achieved. Differences in tree  
425 responses were noticed but the local jujube cultivar Tasset appeared to be better adapted to local  
426 conditions. Results provide important insights to develop and improve the ecological  
427 management of jujube orchards, strengthening the importance of using AM fungi as sustainable  
428 inoculants for increased food security (Verbruggen et al. 2013; Rodriguez and Sanders, 2015;  
429 Berruti et al. 2016). However, further investigations should be implemented to assess the long-  
430 term plant impact of such mycorrhiza-based ecological engineering strategy, as well as the

431 impacts on soil and root-associated biodiversity. Understanding how introduced AM fungal  
432 strains interact and coexist with the native microbiota community and whether this directly  
433 leads to changes in plant productivity remains a main challenge to promote the use of AM fungi  
434 in agriculture, particularly in arid area where plant productivity sustainability is critical.

435

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