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Babacar Thioye, Hervé Sanguin, Aboubacry Kane, Cheikh Ndiaye, Dioumacor Fall, et al.. Mycorrhizal inoculation increases fruit production without disturbance of native arbuscular mycorrhizal community in jujube tree orchards (Senegal). *Symbiosis*, 2021, 83 (3), pp.361-372. 10.1007/s13199-021-00757-5 . hal-03278843

HAL Id: hal-03278843

<https://hal.science/hal-03278843>

Submitted on 4 Jul 2022

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

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Abstract

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

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

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1 Introduction

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

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

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

2 Materials and methods

2.1 Experiment in nursery conditions

The soil used in nursery was collected from Sangalkam (14°46'N, 17°13'W), Senegal. It was a sandy soil with 88.8 % sand, 5.8 % silt, 5.4 % clay, 0.6 % organic matter, 0.3 % total C, 0.02 % 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 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 mixture (ratio 1:2, v/v). The soil was passed through a 2 mm sieve, sterilized for 4h in an autoclave oven system at 180 °C to eliminate native AMF, and bagged in plastic bags (1.5 kg soil per plastic bag).

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

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

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

2.2 Experiment in field conditions

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

of dry soil, by using maize as host plant and 1/4 (v/v) as dilution of the substrate. The experiment was set up as a 2 × 2 factorial design consisting of two jujube cultivars and inoculated or not with *R. irregularis* IR27. Plantings were established using a fully randomized block design with a surface area of 160 m² each, four treatments and ten replicates per treatment and block. During the dry season, the jujube trees were watered without fertilizer application three times a week until the end of the experiment.

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

2.3 DNA extraction, PCR and MiSeq Illumina sequencing

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

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

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

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

2.5 Statistics

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

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

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

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

3 Results

3.1 Experiment in nursery conditions: growth and mycorrhizal colonization

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

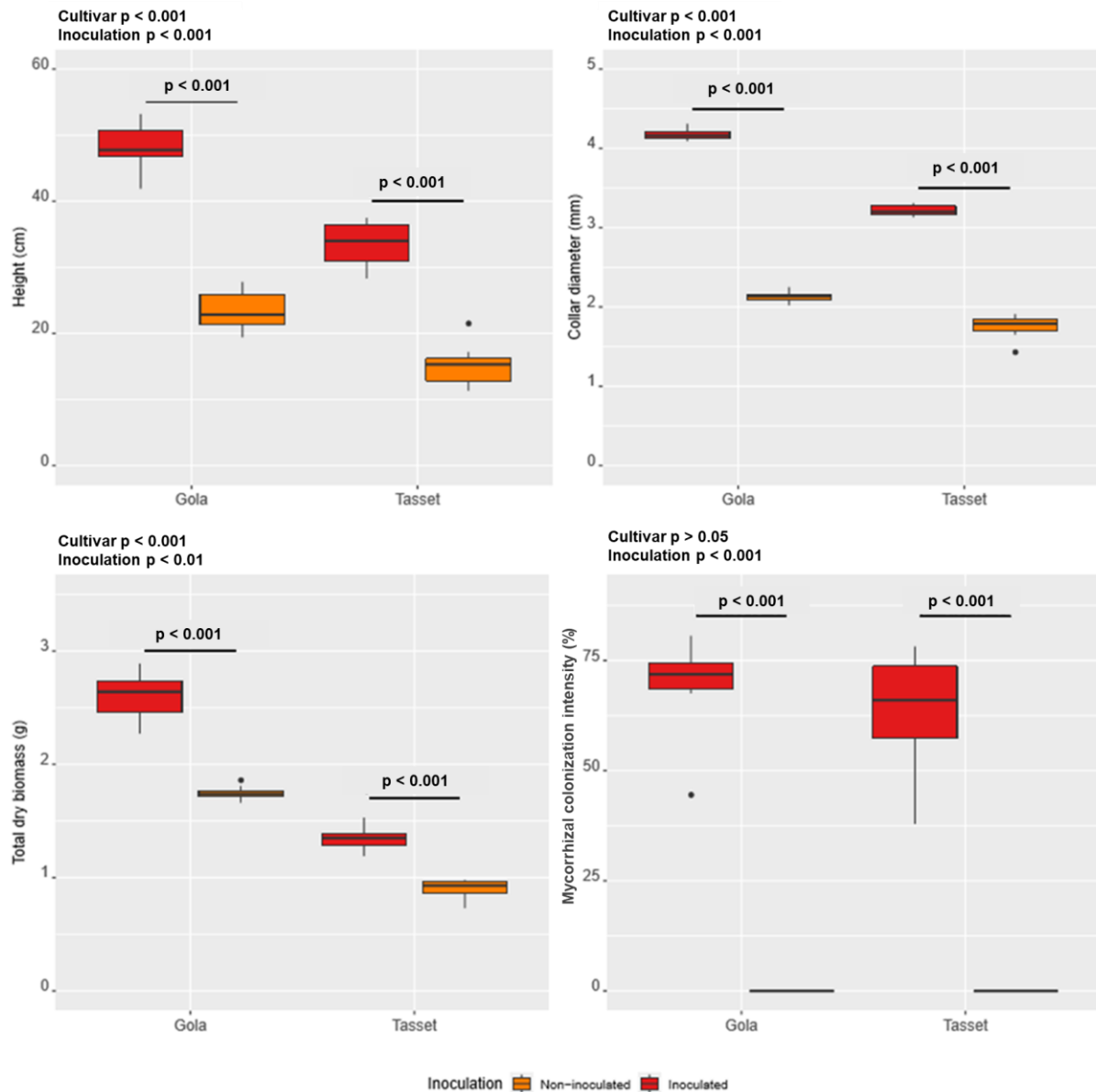


Fig 1 Effect of inoculation with *Rhizophagus irregularis* IR27 on growth parameters (height, collar diameter and total dry biomass) and mycorrhizal colonization intensity of *Z. mauritiana* cultivars 4 months under greenhouse conditions. The boxplot with p-values represent means of each treatment using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

3.2 Experiment in field conditions: growth, survival, mycorrhizal colonization and fruit production

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

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

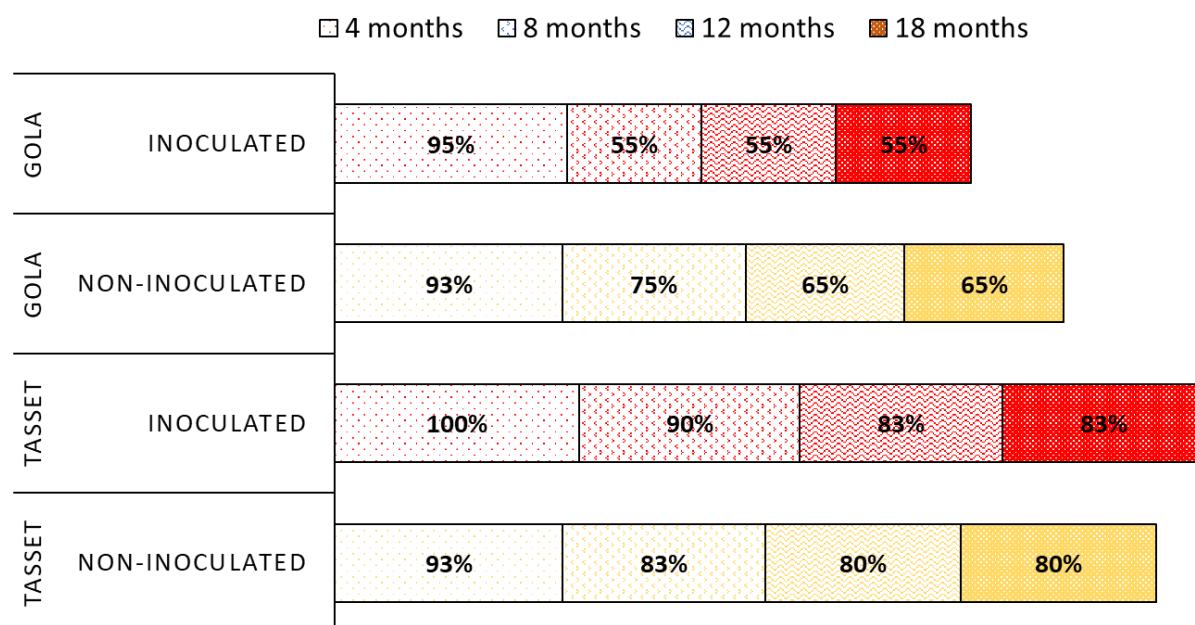


Fig. 2 Effect of inoculation with *Rhizophagus irregularis* IR27 on rate of survival of *Z. mauritiana* cultivars 4, 8, 12 and 18 months after transplanting. Data values represent the percentages of survival rates of each treatment (n=40).

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

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

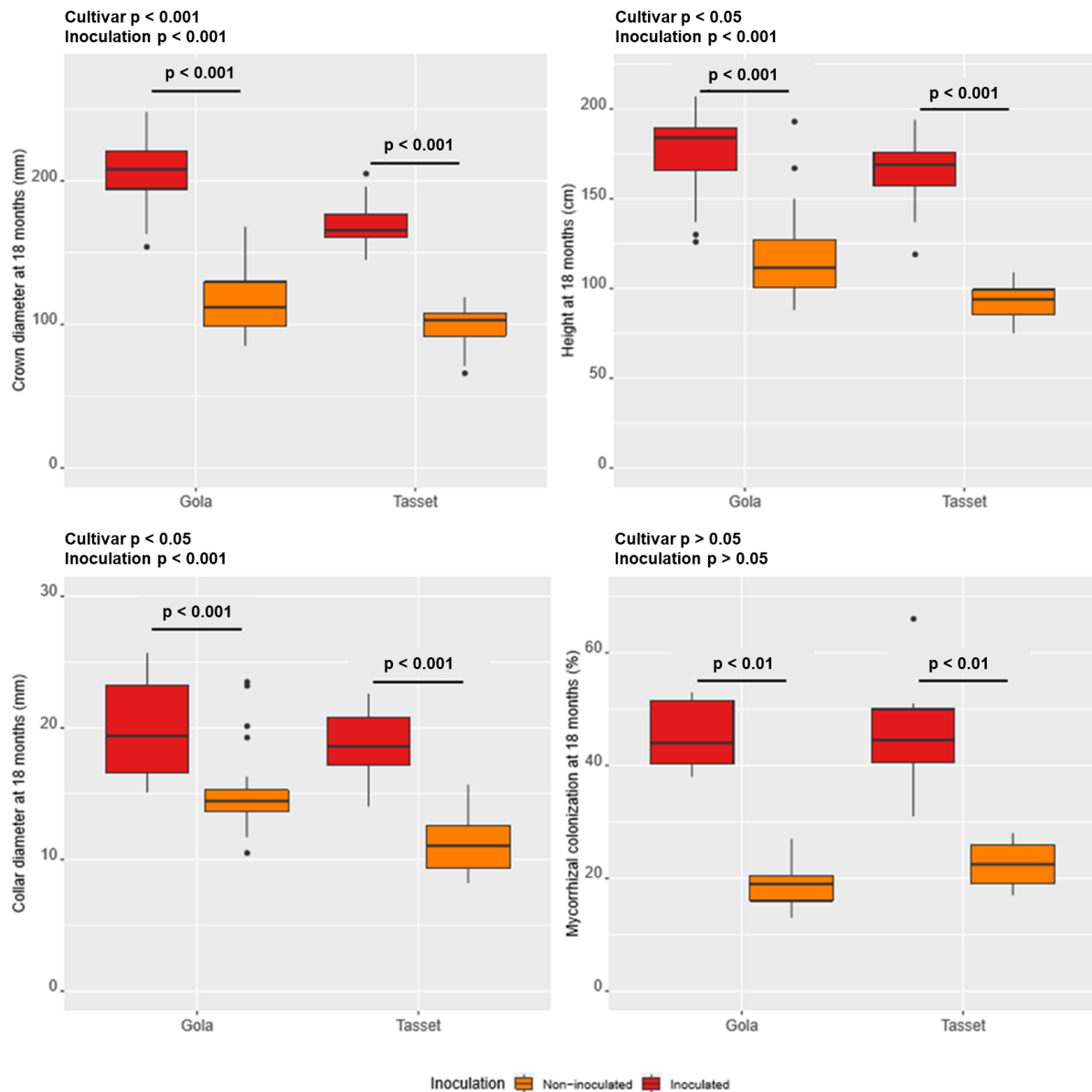


Fig. 3 Effect of inoculation with *Rhizophagus irregularis* IR27 on growth parameters (crown diameter, height and collar diameter) and mycorrhizal colonization of *Z. mauritiana* cultivars 18 months after transplanting. The boxplot with p-values represent means of each treatment using Kruskal-Wallis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

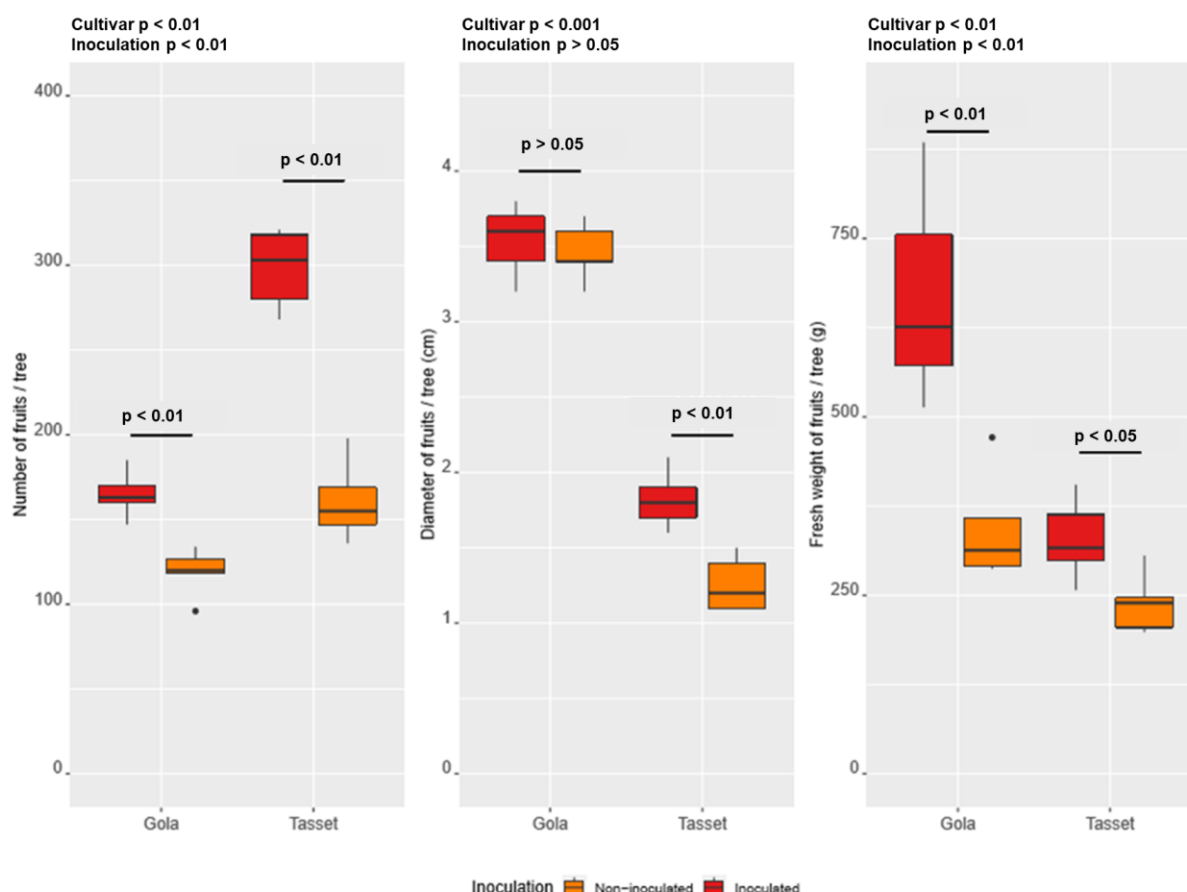


Fig. 4 Effect of inoculation with *Rhizophagus irregularis* IR27 on fruit parameters (fresh weight, diameter of fruits and number of fruits) and mycorrhizal colonization of *Z. mauritiana* cultivars 18 months after transplanting. The boxplot with p-values represent means of each treatment using Kruskal-Wallis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

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

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

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

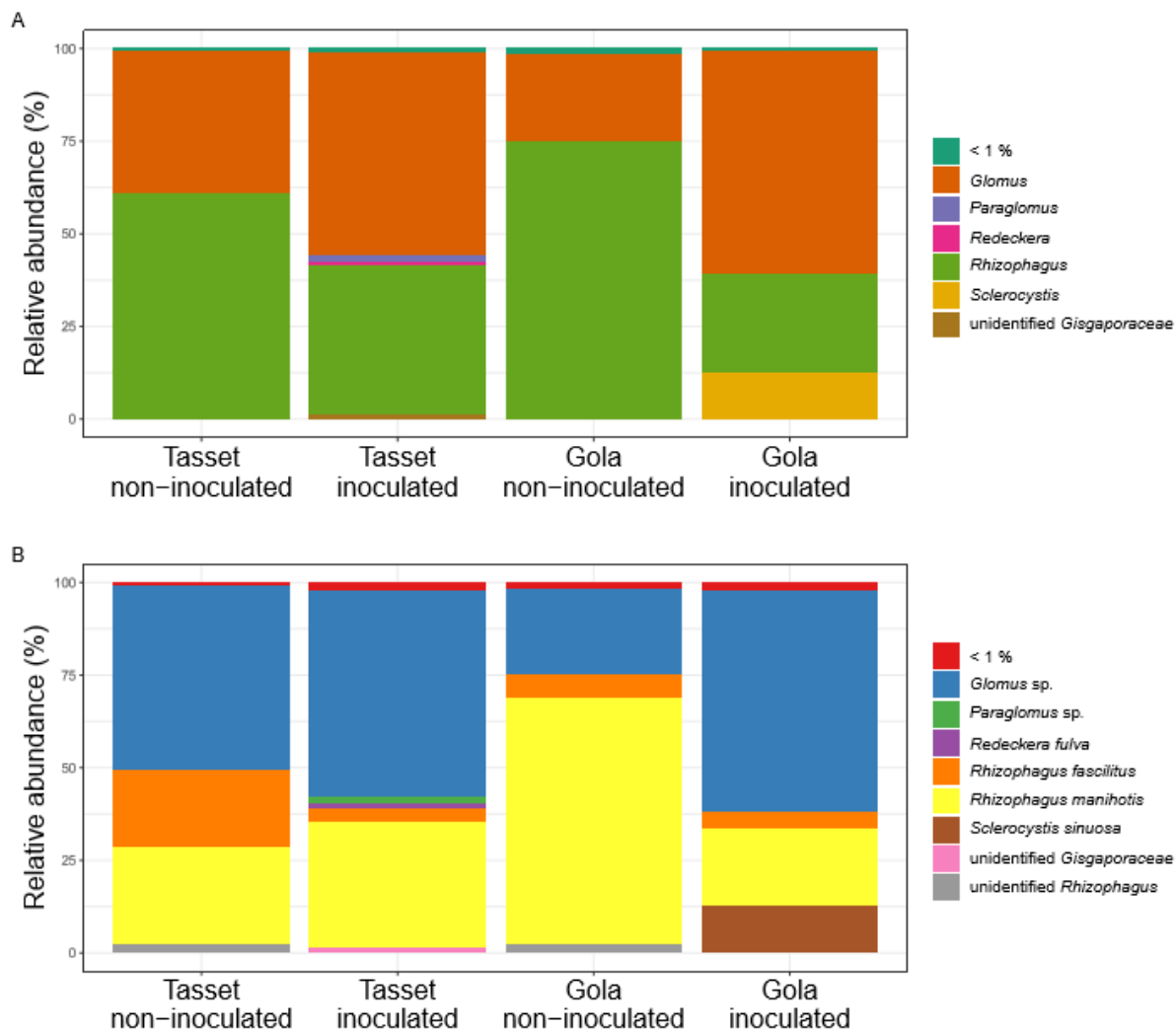


Fig. 5 Taxonomic distribution of AM fungal (A) genera and (B) species associated with non-inoculated and inoculated (+ Ri) jujube cultivars (Tasset and Gola).

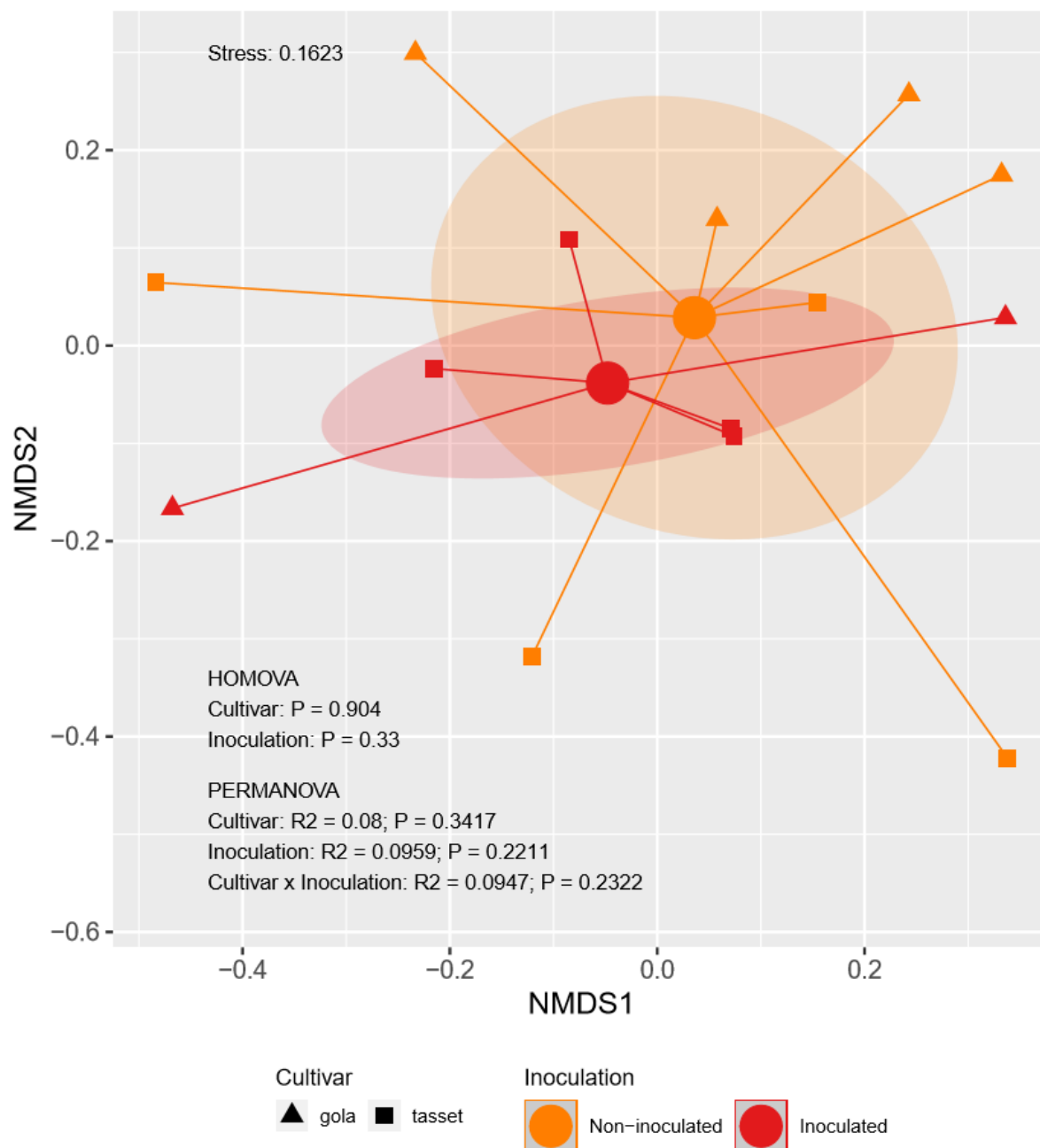


Fig. 6 Two-dimensional nonmetric multidimensional scaling (NMDS) ordination of native AM fungal communities based on the Bray-Curtis coefficient. Each point represents AM fungal composition in inoculated (red) and non-inoculated (orange) jujube cultivars (Tasset and Gola); significant levels: $P < 0.05$.

Table 1 Effect of jujube cultivar and mycorrhizal pre-inoculation on richness and diversity of the jujube 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

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

4 Discussion

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

4.1. Environmental-dependent AM inoculation-mediated tree benefits

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

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

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

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

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

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

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

4.4. Fruit production

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

5. Conclusion

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

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

Acknowledgements

This work was funded by the Africa-Brazil-France tripartite research project entitled “Fight against desertification in Africa” (2014-2016) and supported by AIRD, CNPq and APGMV. Dr. Babacar Thioye received grants from the Institut de Recherche pour le Développement (IRD), the Institut Sénégalais de Recherches Agricoles (ISRA) and from the French Embassy in Senegal (SCAC). The authors thank the Genotoul sequencing facility (Get-PlaGe, Toulouse, France) for MiSeq sequencing and Frédéric Mahé (CIRAD, UMR BGPI, Montpellier, France) for MiSeq data treatment.

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