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RESEARCH ARTICLE

Field arbuscular mycorrhizal inoculation increased plant performance without phosphorus fertilizer supply of four promoted upland rice varieties in Madagascar

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Summary

In Madagascar, upland rice cropping is constrained by soil acidity and low phosphorus (P) bioavailability. Given their role in plant P nutrition, arbuscular mycorrhizal fungi (AMF) may improve crop yield in nutrient-poor tropical soils. In the Vakinankaratra region, a field experiment was conducted at 908 m asl on an acidic Ferralsol during the 2019–2020 growing season. The aim was to test the ability of four promoted rice varieties to respond to AMF seed-coating inoculation with a commercial strain of *Rhizophagus irregularis* in the absence or presence of P fertilizer (20 kg ha^{-1} of P_2O_5) under no expected nitrogen (N) limitation. In absence of P fertilization, both at tillering and at maturity and irrespective of the rice varieties, AMF inoculation significantly improved plant performance and finally grain yield, grain N, and grain P amounts by an average of 28%, 30%, and 39%, respectively. In contrast, when P fertilizer was supplied, no significant effect of AMF inoculation was observed. Rice growth variables were significantly higher with the application of P fertilizer than with AMF inoculation both at tillering and at maturity. P fertilizer without inoculation provided an average grain yield improvement of 85%. At tillering, mycorrhizal parameters for root colonization assessment were not positively linked with rice growth variables suggesting an early effect of AMF inoculation. We concluded that, with no P fertilization, AMF seed coating inoculation at the field scale significantly improved upland rice plant performance in a limited soil P environment. Our rice genetic variability did not interfere significantly both with mycorrhizal parameters and crop AMF inoculation benefits.

Keywords: Acidic soil; Phosphorus deficiency; *Oryza* sp.; Plant nutrition; Seed inoculant; *Rhizophagus irregularis*; Root colonization

Introduction

Because of their high phosphorus (P) sorption ability, ferralsols contain low amounts of available P for plants. Crop yields are thus highly limited by P, which also reduces crop uptake of other nutrients (Haefele *et al.*, 2013; Bindraban *et al.*, 2015). In this context, soil P deficiency is exacerbated by small-scale farmers' limited access to mineral P fertilizers for financial reasons (Kochian, 2012). In Madagascar, upland rice is mainly grown on ferralsols and P is a main limiting nutrient (Andriamananjara *et al.*, 2018; Raminoarison *et al.*, 2020). Nutrient availability in these poor acidic soils drastically limits the increased use of improved varieties with high yield potential

(Rakotoson *et al.,* 2017). In this context, agronomic strategies are needed to cope with the severe P deficiency.

Improving arbuscular mycorrhizal fungal (AMF) symbiosis for plant P nutrition is one possible P-efficiency pathway to improve rice P uptake and yield and to reduce the need for P fertilizers (Smith and Read, 2008; Zhang *et al.,* 2020). Plants that are starved of mineral nutrients, particularly P, generally allow a higher degree of AMF colonization to improve their nutritional status (Feddermann *et al.,* 2010). A recent meta-analysis of major cereal crops reported an average 16% increase in yields due to AMF inoculation, along with a 17% increase in rice yield in both lowland and upland areas (Zhang *et al.,* 2019).

The mechanisms involved in AMF symbiosis crop benefits are complex, mainly depending on the soil environment and plant genetic traits. If the success of field AMF inoculation on crop yield requires an adequate level of available P, P fertilization is generally known to be detrimental for AMF symbiosis in a wide range of crops (Smith and Read, 2008). Thus, large amounts of available plant P reduce AMF taxonomical richness and AMF root colonization (Verbruggen *et al.,* 2013). For upland rice, rice root systems are expected to differ among cultivars and that these traits would induce differences in plant P uptake efficiency (Wissuwa *et al.*, 2020). Thus, selected new rice varieties could thus be more dependent on P fertilizer (Crusciol *et al.,* 2019) and favor P acquisition by the direct P uptake pathway instead of the mycorrhizal P uptake pathway (Zhang *et al.,* 2021). Further, upland rice genetic has been shown to be sensitive to AMF strains under fungus plant symbiosis development (Diedhiou *et al.*, 2016; Campo *et al.*, 2020).

In this study of upland rice (*Oryza* sp.) in Madagascar, we investigated whether the upland varieties recommended to farmers could enter efficient AMF symbiosis with a commercial inoculant based on a *Rhizophagus irregularis* strain, in interaction with P fertilization on a deficient P ferralsol. We hypothesized that P soil availability in interaction with rice genetics should drive AMF inoculation benefits and crop performance.

Materials and Methods

Study site

The study site is located in the middle-west of the Vakinankaratra region near Ivory (19°33′03″S; 46°24′37″E; 908 m asl) on mesozoic sedimentary rocks. Mean annual precipitation and temperature recorded from 2005 to 2020, with an automatic weather station (CIMEL, Electronique, Paris, France) located near the experimental field, were, respectively, 1,291 mm and 23.1 °C. Total annual precipitation from September 2019 to August 2020, corresponding to the cropping season in the present study, was 1,350 mm, showing a normal rainfall pattern. The soil is a sandy loam ferralsol (FAO) comprising 4% clay, 38% silt, and 58% sand. The soil contained 27.2 g kg^{-1} of C, 2.09 g kg^{-1} of total N, 540 mg kg^{-1} of total P and 11 mg kg^{-1} of available Olsen P. The cation exchange capacity (cobaltihexamine) was 136 cmolc kg^{-1}, and pH of water was 4.7.

Experimental design

In the previous cropping season, the field was cultivated with homogenous cowpea crops with no fertilization. The legume residues after grain harvesting were left on the soil. Our field experiment was conducted in the 2019–2020 cropping season with a complete split-plot design with four blocks and three factors, rice variety (V), phosphorus fertilization (P), and AMF inoculation (I). To make field operations easier, PI treatments combining P supply and AMF inoculation were applied in the main plots using four randomly allocated treatments: control (00), inoculation alone (0I), P alone (P0), and inoculation and P (PI). The V factor was applied in subplots with four randomly allocated varieties. The experiment totaled 64 plots. The plot size was 10.8 m^2 (width 3.6 m and length 3 m).

Table 1 Main characteristics of the four rice varieties tested in the experiment

Rice variety	Origin	Mean 1000 grain weight (g)	Mean crop cycle at 900 asl (days)
N4: Nerica 4	Popular variety; interspecific breeding with *Oryza sativa* group *japonica* × *O. glaberrima* parents; AFRICARICE center.	25.5	116
F182: FOFIFA 182	New variety improved from N4; SCRID 09110-1-3-2-5-3; FOFIFA CIRAD centers	27.5	118
F185: FOFIFA 185	New variety from *Oryza sativa* group *japonica*; Botramaintso x CT 134–32; SCRID 111-1-4-3-3-5; FOFIFA CIRAD centers.	31.0	120
WAB: WAB 880-1-32-1-1-P2-HB-1	New variety improved from N4; AFRICARICE center.	27.5	115

Plant and AMF materials

Four rice varieties promoted by national research (Rakotoson *et al.,* 2017) were used with the following main characteristics (Table 1): The Nerica 4 variety (N4), an interspecific crossbreeding of *Oryza sativa* group japonica × *O. glaberrima* parents. This variety is popular and used in different African countries (Diedhiou *et al.,* 2016; Wissuwa *et al.,* 2020). FOFIFA 182 (F182) and WAB 880-1-32-1-1-P2-HB-1 (WAB) were new varieties, both improved from Nerica 4. FOFIFA 185 is an intraspecific variety with *Oryza sativa* group japonica parents.

The commercial inoculant AGTIV PTB297 © was provided by Premier Tech company. This inoculant contained a *Rhizophagus irregularis* strain with an expected colony formation of $6,400\,g^{-1}$ units in an inert kaolinite matrix.

Soil and crop management

No pesticide was applied so as not to interfere with either native or inoculated AMF. The plots were plowed by hand to a depth of 15 cm, from November 4–9, 2019. The rice was sowed on November 19 after a cumulated rainfall of 36.5 mm measured starting on November 10. Rice holes were dug by hand with an *angady* (local spade) at 20 cm intervals using a string to be sure that both the holes and the rows were evenly spaced.

In the P0 and PI treatments, $20\,kg\,ha^{-1}$ of P_2O_5 was applied in the form of a triple superphosphate fertilizer (46% P_2O_5, 14% Ca). Six granules of triple superphosphate fertilizer were carefully placed in each hole, corresponding to around 0.18 g per hole and 47 g per plot. Seven seeds were then placed in each hole. In the 0I and PI treatments, the inoculant was adjusted with weight grain by seed coating to obtain 16 spores per grain. For each variety, 70 g of seeds per plot were mixed with 5 ml of tap water.

A farmyard cattle manure previously homogenized by thorough mixing was applied in all the treatments using a standard quantity per hole to reach a quantity of 5 tons per ha based on dry matter (DM) weight (75% DM at application time, 7 kg per plot). The nutrient contents of the cattle manure were 0.83% N, 9.88% C, 0.22% P, 1.47% K, 0.70% Ca, and 0.38% Mg.

In order to characterize the environment of the bioinoculant, *in situ* soil pH was recorded 7 days after sowing (DAS) using a Hanna Instruments 99121 portable meter and an electrode inserted to a depth of 5 cm after saturating the soil with deionized water. In rice holes, an increase of around 0.6 pH unit was observed, i.e. 5.3 in rice holes thanks to localized manure application compared to rice inter-rows in inter-rows without manure (pH of 4.7).

Three applications of urea (46% N) were manually and gently made in all the treatments 40, 65, and 88 DAS each at a rate of 30 N units (total of $90\,kg\,ha^{-1}$). Hand weeding was performed 35 and 58 DAS. Rice was harvested at maturity on March 20 (F185), March 28 (N4 and F182), and April 1 (WAB).

Plant analysis

At 60 DAS corresponding to tillering, plants (shoot and roots) were sampled from two rice holes chosen at random in rows bordering the central plot to measure the number of tillers, shoot DM, and shoot nitrogen (N) and P content on representative samples. At maturity, i.e., from 110 to 125 DAS, we recorded the total number of tillers, the DM in the straw and grain on a 4.84 m^2 area in the central part of the plot. Subsamples of straw and grain were kept for N and P analysis. Total N content was measured with a CHNS microanalyzer (Flash 2000 Series, CHNS/O 122 Analyzers Thermo Scientific, IRCOF, France). Total P content was also measured following mineralization in a microwave oven using 65% nitric acid. Colorimetric dosage was then performed using the malachite green method (Ohno and Zibilske, 1991).

AMF root colonization

Root systems of rice plants harvested in the field at 60 DAS were washed with tap water. All coronal roots were cut and 30 fragments of lateral roots were randomly selected, carefully washed and fixed in 20 ml of 70% ethanol before transport to the laboratory. The fragments were then incubated for 12 hours in a 10% KOH solution, rinsed twice with tap water and immediately stained with 8% of blue Shaefer ink diluted in vinegar (5% acetic acid) (Wilkes *et al.,* 2020). Endomycorrhizal structures were stained by immersion in a water bath for 30 minutes at 70° C. Roots were de-stained with acetic-glycerol for 30 minutes. Five mycorrhizal parameters of 1-cm long root segment were assessed under the microscope (Olympus BX 41), using a semi-subjective scoring method at a magnification ranging from ×40 to ×200. Each root segment required 10 observations. The structures of AMF infection (intracellular hyphae, vesicles, arbuscules) were assessed using six scores with their percentages in parentheses, 0 (0%), 1 (0–1%), 2 (1–10%), 3 (10–50%), 4 (50–90%), 5 (90–100%), with $n1$ = number of fragments rated 1, $n2$ = 2, $n3$ = 3, $n4$ = 4, $n5$ = 5. Arbuscular abundance was assessed using four scores, 0 (no arbuscular colonization), 1 (low abundance), 2 (medium abundance), 3 (high abundance), with $A1$ = number of fragments scored 1, $A2$ = 2, $A3$ = 3. The five mycorrhizal parameters were computed as % according to the methodology used by Vallino *et al.,* 2014 and Campo *et al.,* 2020:

- The AMF frequency of the root system,

$$F\ (\%) = (MF/30)100 \tag{1}$$

- The AMF Intensity of the root system,

$$M\ (\%) = (95n5 + 70n4 + 30n3 + 5n2 + n1)/30 \tag{2}$$

- m, as the AMF Intensity of the mycorrhized fragments,

$$m\ (\%) = M\ (\%)(30/MF) \tag{3}$$

- The arbuscular abundance of the mycorrhized fragments,

$$a\ (\%) = (100mA3 + 50mA2 + 10mA1)/100 \tag{4}$$

- A, as the arbuscular abundance in the root system,

$$A\ (\%) = a(M/100) \tag{5}$$

where:

MF: number of mycorrhized fragments

$$mA3 = (95n53 + 70n4A3 + 30n3A3 + 5n2A3 + n1A3)/MF100/m \tag{6}$$

$$mA2 = (95n5A2 + 70n4A2 + 30n3A2 + 5n2A2 + n1A2)/MF100/m \qquad (7)$$

$$mA1 = (95n5A1 + 70n4A1 + 30n3A1 + 5n2A1 + n1A1)/MF100/m \qquad (8)$$

Data analysis

All statistical analyses were performed in R (R-4.1.1). The packages lme4 and lmerTest were used with a p-value threshold set at 5% for tests of linear mixed effects model fit. Means were compared according to the least significant difference (LSD) with Fisher's LSD test with a probability level of 5% with the stats package. Normality and variance assumptions were tested with the Shapiro test (stats package) and the Levene test (car package). ANOVAs were used with the V, P, and I factors as fixed effects, and the Block and PI × block (constraint of the split-plot design) as random effects. For the mycorrhizal parameters expressed in percentages, data were transformed using arcsin square root function. The packages FactoMineR and factoextra were used to perform a principal component analysis (PCA) with rice growth variables as active (n = 11), and mycorrhizal parameters (n = 5), as additional variables.

Results

AMF root colonization at tillering

At tillering, 60 DAS, any significant effect of the rice variety factor (V) was revealed as well as for the AMF inoculation factor (I), while the phosphorus fertilization factor (P) was highly significant for the five mycorrhizal parameters, F, M, m, a, and A, expressed in %. The VI interaction was not significant for any variable while the VP interaction was significant for M, m, and A, and the PI interaction was significant for F, M, m, and A. The VIP interaction of these three factors was not significant for the five mycorrhizal parameters. In comparing mean treatments, the effect of AMF inoculation with no P supply (00 *versus* 0I) was not significant for all parameters. In contrast, with P supply (P0 *versus* PI), there was a significant decrease in F, M, m, and A with AMF inoculation 76.6 *versus* 56.2%, 5.9 *versus* 2.4%, 7.8 *versus* 3.9%, 4.6 *versus* 1.5%, respectively. The interaction between the rice variety factor (V) and the phosphorus fertilization factor (P) appeared for M, m, and A. The WAB variety had the lowest mycorrhizal parameters values compared to the other varieties, specifically in the presence of P fertilizer. Without P supply compared with P supply, the average values were there 79.1 *versus* 66.5% for F (AMF frequency of the root system), 8.4 *versus* 4.2% for M (AMF intensity of the root system), 10.1 *versus* 5.9% for m (AMF intensity of the mycorrhized fragments), 74.8 *versus* 57.8% for a (the arbuscular abundance of the mycorrhized fragments), and 6.5 *versus* 3.1% for A (the arbuscular abundance in the root system) (Table 2).

Plant growth variables at tillering

At tillering, 60 DAS, the VI, VP, and VPI interactions were not significant, while the PI interaction was significant on the number of tillers, DM shoot biomass, and shoot P amount (Table 3). There was a significant effect of the rice variety only for the number of tillers m^{-2}. The inoculation factor alone was not significant for the four plant growth variables while the phosphorus fertilization factor (P) was highly significant for all of these (Table 3). Without P fertilizer average values with AMF inoculation (0I) compared without AMF inoculation (00) were significantly higher, 236 *versus* 162 tillers m^{-2} (Figure 1A), 2.4 t ha^{-1} *versus* 1.5 t ha^{-1} shoot biomass (Figure 1B), 4.5 kg ha^{-1} *versus* 2.7 kg ha^{-1} shoot P amount (Figure 1D), respectively. No significant effect of AMF inoculation without P fertilizer appeared for shoot N, 26.1 *versus* 19.7 kg ha^{-1} (Figure 1C). In the presence of P fertilizer, the values did not differ between the treatments P0 and PI, 283 and 266 for tillers m^{-2}, 3.6 and 3.5 t ha^{-1} for shoot biomass and 6.5 and 6.3 kg ha^{-1} shoot P amount. All these

Table 2 Results of ANOVAs of mycorrhizal parameters at tillering and means for treatments ± standard error; F (%) is the Arbuscular Mycorrhizal (AM) frequency of the root system, M (%) is the AM intensity of the root system, m (%) is AM Intensity of the mycorrhized fragments, a (%) is the arbuscular AM abundance of the mycorrhized fragments and A (%) is the AM arbuscular abundance of the root system

	Chi-square values				
Source of variation	F (%)	M (%)	m (%)	a (%)	A (%)
Rice variety (V)	2.14	2.52	1.72	1.55	0.84
Phosphorus (P)	7.95**	18.3***	14.24**	7.58**	15.79***
Inoculation (I)	1.37	0.58	0.38	0.20	0.29
VP	1.16	13.07**	14.25**	1.04	9.75*
VI	1.48	1.45	0.77	0.54	1.00
PI	7.09**	10.36**	7.40**	1.24	9.43**
VIP	0.67	1.43	0.62	0.40	0.65
Treatments P			Means and standard errors		
0	79.1 ± 9.4 a	8.4 ± 3.0 a	10.1 ± 3.3 a	74.8 ± 9.7 a	6.5 ± 2.7 a
P	66.5 ± 10.6 b	4.2 ± 2.0 b	5.9 ± 2.9 b	57.8 ± 15.1 b	3.1 ± 1.9 b
Treatments VP			Means and standard errors		
N4 0	83.8 ± 6.6	8.2 ± 2.0 ab	9.5 ± 2.2 ab	77.1 ± 9.9	6.4 ± 2.1 a
N4 P	71.3 ± 7.1	4.6 ± 1.1 ab	6.5 ± 1.6 ab	59.8 ± 14.2	2.8 ± 1.0 ab
F182 0	83.7 ± 5.8	9.8 ± 2.4 a	12.1 ± 3.4 a	69.1 ± 12.9	7.1 ± 2.2 a
F182 P	69.2 ± 8.8	4.0 ± 1.8 ab	5.3 ± 2.1 ab	56.9 ± 13.2	2.8 ± 1.5 ab
F185 0	67.9 ± 13.6	5.0 ± 2.7 ab	6.4 ± 2.4 ab	77.9 ± 6.6	4.1 ± 2.3 ab
F185 P	64.6 ± 14.5	6.5 ± 3.1 ab	9.4 ± 4.7 ab	65.3 ± 19.4	5.8 ± 3.1 ab
WAB 0	80.8 ± 8.8	10.4 ± 4.1 a	12.4 ± 4.3 a	74.9 ± 9.7	8.3 ± 3.8 a
WAB P	60.8 ± 11.9	1.5 ± 0.5 b	2.3 ± 0.6 b	49.2 ± 14.5	0.9 ± 0.3 b
Treatments PI			Means and standard errors		
00	75.6 ± 9.5 a	6.9 ± 2.0 a	8.7 ± ab	72.2 ± 8.5	5.1 ± 1.5 ab
0I	82.5 ± 3.0 a	9.7 ± 1.0 a	11.5 ± 1.1 ab	77.2 ± 4.2	7.7 ± 0.9 a
P0	76.6 ± 3.6 a	5.9 ± 1.4 a	7.8 ± 2.2 b	62.0 ± 12.2	4.6 ± 1.4 b
PI	56.2 ± b	2.4 ± 2.2 b	3.9 ± 4.6 c	53.1 ± 12.5	1.5 ± 4.1 c

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; letters (a, b, c) indicate significant differences between treatments.

values of P fertilizer treatments (P0 and PI) were significantly higher than treatments without P fertilizer (00 and 0I) for the number of tillers (Figure 1A), DM shoot biomass (Figure 1B), and shoot P amount (Figure 1D). For shoot N, the phosphorus fertilization factor was significantly 22.9 kg ha^{-1} without P *versus* 34.5 kg ha^{-1} with P (Figure 1C). Concerning the variety factor, the smallest number of tillers (Figure 1A) was recorded for F185 (189), i.e. different from the variety N4 (284) but not the variety WAB (225) or the variety F182 (248).

Plant growth variables at maturity

On straw variables at maturity, we did not show any significant VI, VP, and VPI interactions and a significant PI interaction on shoot P amount. There was a significant effect of the rice variety on the number of tillers m^{-2} and DM straw biomass. The inoculation factor alone was not significant for the four growth variables while the phosphorus fertilization factor (P) alone was highly significant for the number of tillers m^{-2} and DM straw biomass (Table 3). Without P fertilizer, average values with AMF inoculation (0I) compared without AMF inoculation (00) were significantly higher 1.3 *versus* 1.7 kg ha^{-1} shoot P amount, respectively (Figure 2D). In the presence of P fertilizer, the values did not differ between the treatments P0 and PI, 276 and 260 for tillers m^{-2} (Figure 2A), 3.5 and 3.3 t ha^{-1} for shoot biomass (Figure 2B), 35.7 and 34.5 kg ha^{-1} for shoot N amount (Figure 2C) and 6.5 and 6.3 kg ha^{-1} shoot P amount. For till number and shoot biomass, the phosphorus fertilization factor was significant, without P *versus* with P fertilizer, 213 and 268, 2.7 and 3.4 t ha^{-1}, respectively. The rice variety factor had a significant effect on the number of tillers (Figure 2A) and DM straw biomass (Figure 2B), and straw P amount (Figure 2D), but no

Table 3 Results of ANOVAs of rice growth variables at tillering and at maturity; shoot and straw dry matter (DM) biomass; amounts of N, P; grain yield expressed at 13% of humidity

| | Chi-square values | | | | | | | | | | |
| | At tillering | | | | At maturity | | | | | | |
Source of variation	Till m^{-2}	Shoot DM (t ha^{-1})	Shoot N (kg ha^{-1})	Shoot P (kg ha^{-1})	Till m^{-2}	Straw DM (t ha^{-1})	Straw N (kg ha^{-1})	Straw P (kg ha^{-1})	Grain yield (t ha^{-1})	Grain N (kg ha^{-1})	Grain P (kg ha^{-1})
Rice variety (V)	17.24***	2.80	4.23	4.00	23.88***	7.98**	4.66	7.94*	0.97	1.79	2.50
Phosphorus (P)	20.42***	26.03***	11.23***	19.12***	13.01***	21.73***	0.01	1.39	77.06***	82.36***	32.86***
Inoculation (I)	2.92	1.93	0.53	1.68	0.80	0.03	2.11	0.95	2.30	2.54	1.07
VP	7.01	0.41	0.20	0.73	0.63	1.07	1.21	1.56	0.96	0.99	1.79
VI	3.21	1.00	1.52	2.06	0.86	0.54	0.80	0.11	0.87	1.23	5.38
PI	7.25**	4.19*	1.22	3.74*	1.68	1.29	0.27	4.33*	5.77*	6.00*	12.81***
VIP	1.87	1.98	0.91	2.43	1.33	1.43	3.01	1.98	3.37	3.90	0.88

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; letters (a, b, c) indicate significant differences between treatments.
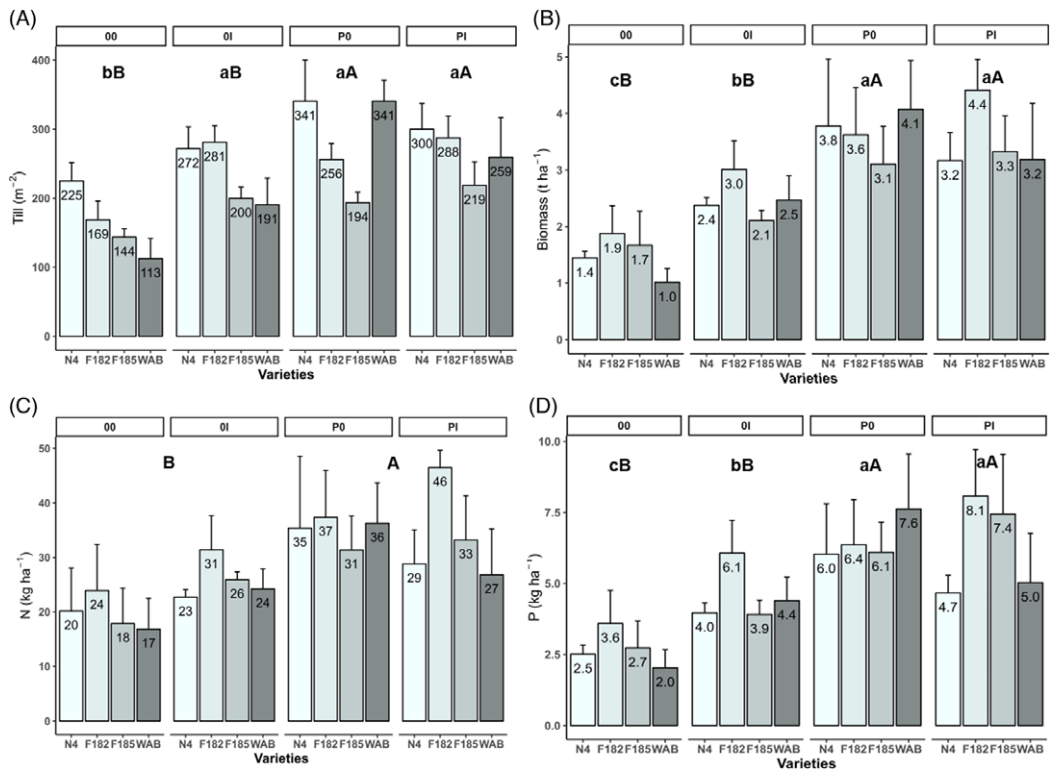
Figure 1. Means and standard error for the number of tillers (A), dry matter biomass (B), nitrogen (C), and phosphorus (D) amounts of rice shoots at tillering in the four varieties under the four PI treatments; 00=no fertilizer and no inoculation; 0I = no fertilizer and inoculation; P0 = fertilizer and no inoculation; PI = fertilizer and inoculation; ± standard error; different lowercase letters represent significant differences related to the P and I interaction while uppercase letters represent significant differences related to the P factor at the 5% level.

effect on straw N amount (Figure 2C). The lowest number of tillers was found in F185 and F182 (200 and 207), which differed significantly from the number of tillers in N4 and WAB (276 and 278). DM straw biomass values ranged from 2.7 in N4 to 3.3 in F182 (significant difference), with intermediate values found in F185 and WAB, respectively, 3.1 and 3.0 t ha^{-1}. Straw P amount was significantly higher in F182 than in the other varieties.

On grain variables at maturity, we did not show any significant VI, VP, and VPI interactions. Significant PI interaction on grain yield, grain N, and P amounts variables were observed. For these grain variables, they were no significant effects of the rice variety factor nor for the inoculation factor. The phosphorus fertilization factor (P) alone was highly significant for the same variables (Table 3). The treatments 00 and 0I produced significant contrasting grain yields (Figure 3A), grain N (Figure 3B), and grain P (Figure 3C) amount values, 2.1 *versus* 2.8 t ha^{-1}, 34.9 *versus* 45.6 kg ha^{-1}, and 2.8 *versus* 3.9 kg ha^{-1}, respectively. With a supply of P, no effect of inoculation was found for any of the variables. P0 and PI values were higher compared to 00 and 0I for grain yield: 3.9 and 3.8 t ha^{-1}, grain N amount 65.3 and 63.0 kg ha^{-1}, grain P amount 5.1 and 4.5 kg ha^{-1}, respectively.

Plant growth variables and AMF root colonization

The two main axes of the PCA (Figure 4) explained 73.5% of total inertia with 11 active plant variables and 5 mycorrhizal parameters as additional variables. The first axis of the PCA is related
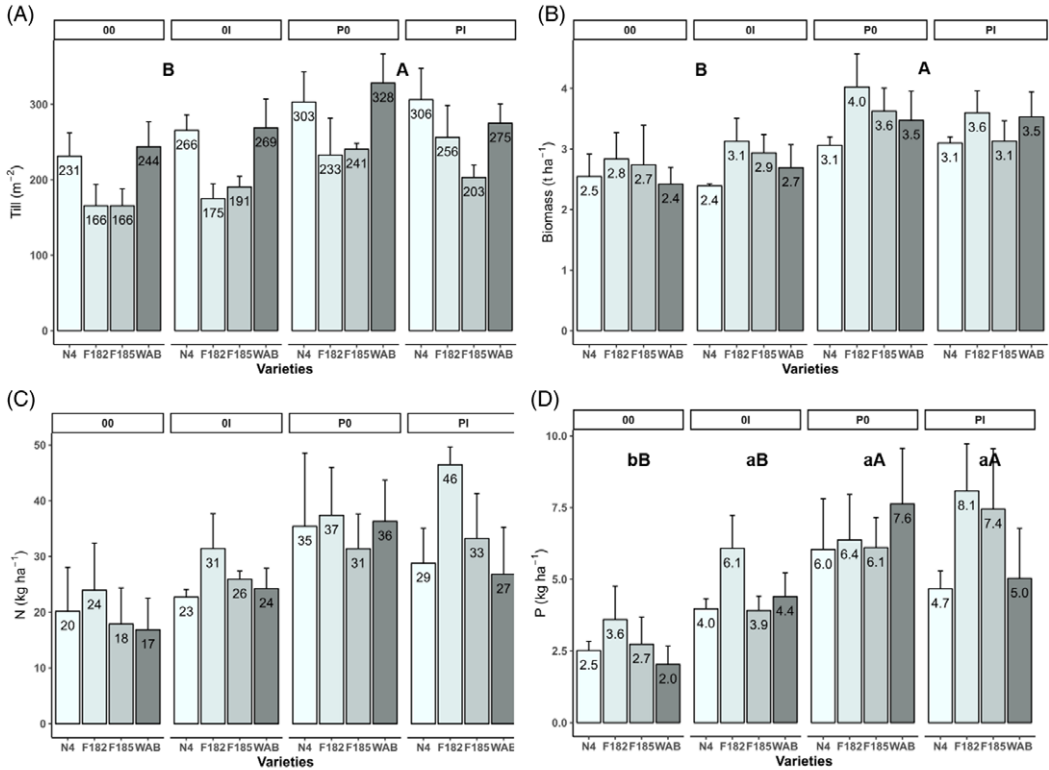
Figure 2. Means and standard error for number of tillers (A), dry matter straw biomass (B), nitrogen (C), and phosphorus (D) amounts in rice straw at maturity in the four varieties under the four PI treatments; 00 = no fertilizer and no inoculation; 0I = no fertilizer but with inoculation; P0 = with fertilizer but no inoculation; PI = fertilizer and inoculation; ± standard error; different lowercase letters represent significant differences related to the P and I interaction while uppercase letters represent significant differences related to the P factor at the 5% level.

to the 11 active plant variables, especially grain-based variables such as yield, grain N, and P. Similarly, the number of tillers at maturity and all tillering-based variables (i.e., number of tillers, DM straw biomass, straw N amount, and straw P amount) are closely associated with this first axis. At maturity, straw biomass, straw N and P amounts are differently oriented toward the second axis. The five mycorrhizal variables (F, M, m, a, and A in %) are orthogonal to the plant variables and poorly related to the first axis. The four treatment groups, each comprising 16 plots, are distributed along the first axis from the positive to the negative scores. The 00 and 0I treatment groups have the lowest plant variable values and the highest mycorrhizal values, whereas the P0 and PI treatment groups have the highest plant variable values and the lowest mycorrhizal values.

Discussion

No effect of rice varieties linked with AMF inoculation

Contrary to our hypothesis, the weight of the rice variety factor was low in the expression of plant responses to AMF inoculation with and without a P supply. This low varietal impact could be related to a low genetic differentiation of our four cultivars. In the same way, Rakotoson *et al.* (2017) also showed low interaction between genetics and N use efficiency in a range of 13 japonica rice varieties including N4 and WAB. Furthermore, in this same context, this low genetic differentiation on AMF inoculation could be linked with the selection of resistant ecotypes to the hemi-
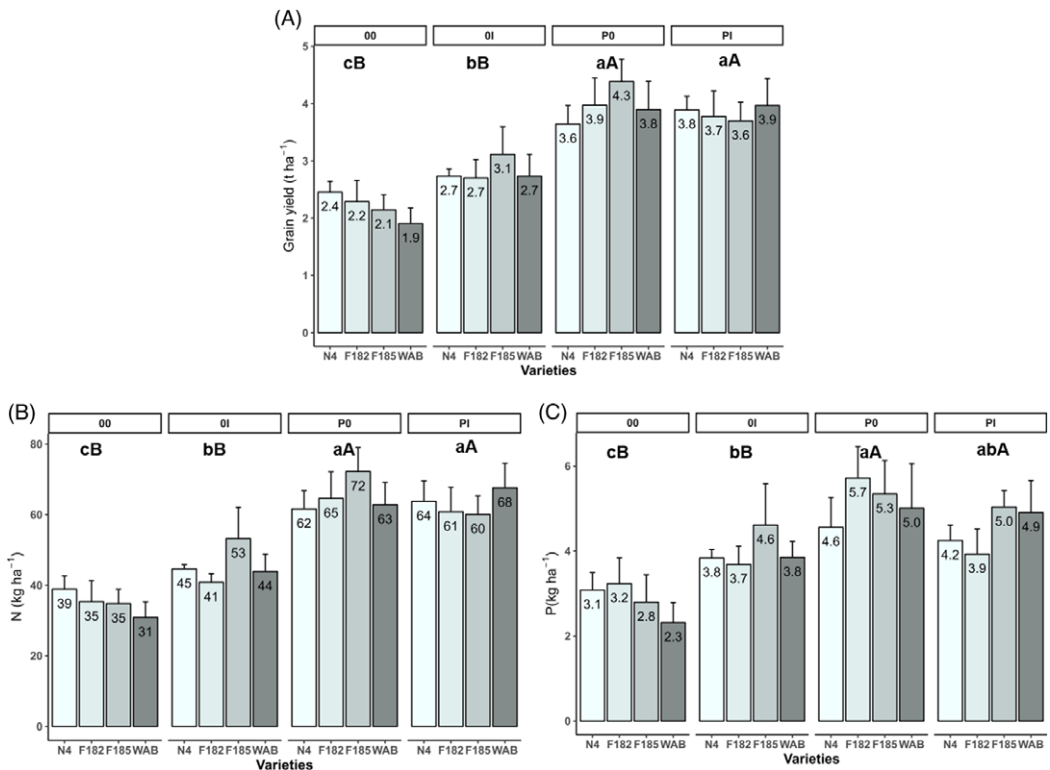
Figure 3. Means and standard error for grain yield (13% humidity) (A), nitrogen (B), and phosphorus (C) grain amounts of rice at maturity in the four varieties under the four PI treatments; 00 = no fertilizer and no inoculation; 0I = no fertilizer but with inoculation; P0 = fertilizer but no inoculation; PI = fertilizer and inoculation; ± standard error; different lowercase letters represent significant differences related to the P and I interaction while uppercase letters represent significant differences related to the P factor at the 5% level.

parasitic weed *Striga asiatica* during the research process, which is one of the main objectives of our local breeding research programs (Randrianjafizanaka *et al.*, 2018). This selection could eliminate varieties that are more able to favor rapid colonization of AMF thanks to strigolactone emission compounds also known to stimulate striga seed germination (Jamil *et al.*, 2011).

The benefits of AMF inoculation

The other new finding for our agroecological area, with an altitude range between 800 and 1300 m, is the significant positive effect of AMF inoculation in the absence of P fertilization (0I) on grain yield (+28%), grain N (+30%), and grain P (+39%) in the four varieties tested, compared to the control (00). The positive impacts of AMF inoculation on plant growth variables were observed at rice tillering (60 DAS). In all four varieties at 60 DAS, we found a mean 45% increase in the number of tillers, a 60% increase in DM shoot biomass and a 66% increase in shoot P amount when AMF were inoculated in the absence of P fertilization. Other studies also reported that these effects of AMF inoculation on rice were highest during the vegetative growth period both in the greenhouse and in the field (Zhang *et al.*, 2019; Campo *et al.*, 2020). These results are of importance for two reasons. First, they underline the fact that, in the context of a nutrient-deficient tropical soil, AMF inoculation can improve rice yield, irrespective of the rice cultivar used. These results are in agreement with other field studies on a wide range of crops, with 37% more yield reported after AMF inoculation (Smith and Read, 2008), and for rice, 17% more yield (Zhang

et al., 2019). Similar responses to AMF inoculation both during the vegetative stage and at maturity have been recorded as growth variables for the upland rice variety N4 (Diedhiou *et al.*, 2016). Second, these results show that the availability of soil P is a main driver of the efficiency of AMF inoculation for plant growth. The low P application rate (20 kg ha^{-1}) but seed-localized supply of fertilizer affected AMF inoculation, as no significant difference was observed between P0 and PI treatments. The beneficial effects of AMF inoculation on plant growth thus disappeared with a supply of P fertilizer and were associated with a decrease in mycorrhizal parameters (Table 2). This negative effect of P fertilizer on AMF colonization has also been described by other authors (Smith and Read, 2008; Verbruggen *et al.*, 2013). In addition, the significant increase in grain N with AMF inoculant was obtained with a high supply of N fertilizer (90 kg ha^{-1}). Improvement of crop N nutrition thanks to efficient AMF symbiosis was also reported by Ryan and Graham (2018). Interestingly, these effects were achieved by seed coating, which has been shown to be a suitable method for inoculating AMF fungi with a low spore rate (Rillig *et al.*, 2019).

These beneficial effects of AMF inoculation for rice were still much lower than the benefits of P fertilization. The effect of this moderate supplying fertilizer (20 kg ha^{-1} of P$_2$O$_5$) but close to the seed on rice yields was high, irrespective of the presence of AMF, i.e. the yields increased by 85% (P0) and 35% (PI) following the supply of P compared to in the 00 and 0I treatments, respectively. High rice yield responses to moderate P fertilizer supply associated with farmyard manure were also recorded in the same location (Andriamananjara *et al.*, 2018).

Soil habitat as a driver of AMF inoculation benefits

Soil AMF habitat was defined as the different biological and abiotic factors that drive AMF symbiosis, including available P, a non-limiting N environment, and an acidity soil status above critical thresholds (Smith and Read 2008, Oehl *et al.*, 2010; Mäder *et al.*, 2000). According to our own data, different sources of bioavailable P than fertilizer could be provided and contribute to soil enrichment: (i) a carry-over effect of the preceding crops, i.e. 11 mg P kg^{-1} as P Olsen a level above the threshold for upland cultivation (Raboin *et al.*, 2016), (ii) P from manure supply, i.e. about 11 kg P ha^{-1} (Andriamananjara *et al.*, 2018), and (iii) an indirect effect of P bioavailability due to increased pH (Smith and Smith, 2012). For upland rice critical soil, P Olsen values may range from 10 to 20 mg kg^{-1} (Six *et al.*, 2013). Our soil available P status of 11 mg P Olsen kg^{-1} would be in this lower part of this range, confirming both our AMF inoculation and P fertilizer plant our high response of rice under P fertilizer. Further, the conditions required for the beneficial effects of AMF inoculation on plant growth and nutrition may be optimized by the N supply from organic manure at sowing time and from split mineral fertilizer until rice flowering. In a P-limited system, according to the observed high impact on rice growth of a moderate P fertilizer supply i.e. 20 kg ha^{-1} of P$_2$O$_5$, a rich supply source of N benefited both the plant and the fungus as mentioned by other studies (Thirkell *et al.*, 2016; Ryan and Graham 2018).

Relationship between rice variables at tillering and maturity, and AMF root colonization parameters

The PCA analysis showed that rice growth variables both at tillering and maturity strongly explained the variance induced by the treatments and were well correlated on the main axis. Thus, a strong relationship was observed between shoot plant variables at 60 DAS and grain yield at maturity (Figure 4). In contrast, the five mycorrhizal parameters are poorly correlated on the PCA axes. These results suggest that plant root mycorrhizal colonization is not necessarily good predictor of plant nutrient uptake by AMF (Smith and Read, 2008).

Different mechanisms could explain this gap in field conditions. First, AMF structure could vary weekly in root colonization as there are driven by C, N, and P exchanges at the plant level (Vallino *et al.*, 2014), with a known lifetime of arbuscules of 2–3 days (Mbodj et al, 2018). Thus, we
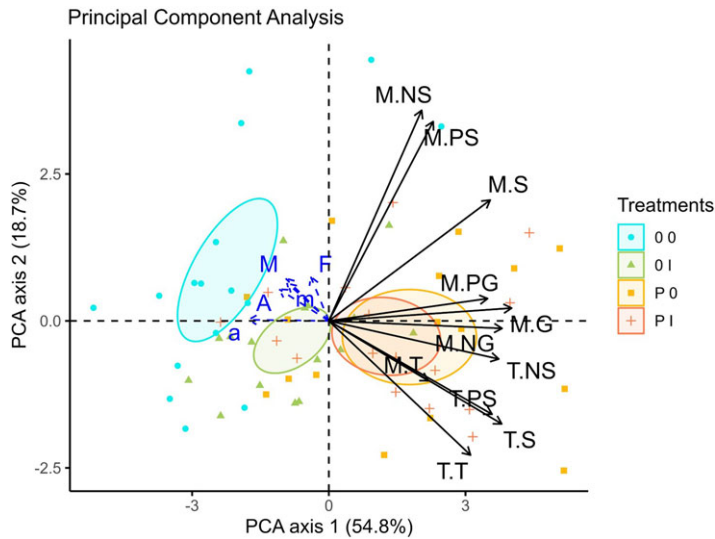
Figure 4. Principal component analysis (PCA) with the 11 growth plant active variables (red arrows); at tillering, number of tillers per m^{-2} (T.T), dry matter straw biomass in t ha^{-1} (T.S), straw nitrogen amount in kg ha^{-1} (T.NS), straw phosphorus amount in kg ha^{-1} (T.PS); at rice maturity, number of tillers per m^{-2} (M.T), dry matter straw biomass in t ha^{-1} (M.S), straw nitrogen amount (M.NS), straw phosphorus amount (M.PS), grain yield at 13% humidity (M.G), nitrogen (M.NG), and phosphorus (M.PG) grain amounts in kg ha^{-1}; the five AMF parameters nonactive variables are in blue arrows, F (% of AM frequency of the root system), M (% of AM intensity of the root system), m (% of AM intensity of the mycorrhized fragments), a (% of arbuscular abundance of the mycorrhized fragments), A (% of arbuscular abundance in the root system); the 4 different 16 colored individuals and 4 different ellipses at the 95% level represent the 4 PI treatments; 00 (no phosphorus and no inoculation), 0I (no phosphorus but with inoculation), P0 (with phosphorus but no inoculation), and PI (inoculation and phosphorus).

suppose that our plant response to AMF inoculation applied at sowing time was effective earlier than 60 days before when we realized both our plant and mycorrhizal assessment. Wissuwa *et al.* (2020) on a P-deficient soil observed that the development of AMF symbiosis on upland rice started to develop from 3 weeks after sowing. Also, the dispersal of mycorrhizal intraradical structures of rice in field conditions was highlighted by Campo *et al.,* (2020). This could create variability among the calculated mycorrhizal parameters and thus reduce the possibility of detecting significant differences among treatments. Indeed, our global parameters, at the entire root system level (M and % A), were low, ranging from 2.4 to 9.7 and from 1.5 to 7.7, respectively. In pot experiments, Vallino *et al.,* (2014) recorded M and A values that could reach more than 50% with rice during the vegetative period.

Conclusion

Our field study in Madagascar showed that AMF inoculation improved crop performance of promoted upland rice varieties grown on an acidic tropical soil without P fertilizer supply. Contrary to our hypothesis, any interaction between the rice variety factor with mycorrhizal parameters and AMF inoculation benefits was revealed. As this AMF inoculation improvement disappeared with P fertilizer supply without N expected limitation, we suppose that this increase was induced by more P plant uptake thanks to AMF symbiosis. It is nevertheless important to note that the yields obtained with P fertilization remained higher than those obtained with AMF inoculation. The absence of a relationship between AMF parameters and rice growth variables at tillering suggests an early effect of seed coating AMF inoculation and a complementary and positive effect of the introduced *Rhizophagus irregularis* AMF strain with the native AMF populations.

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