Effects of early mycorrhization and colonized root length on low-soil-phosphorus resistance of West African pearl millet

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Abstract

Phosphorus (P) deficiency at early seedling stages is a critical determinant for survival and final yield of pearl millet in multi-stress Sahelian environments. Longer roots and colonization with arbuscular mycorrhizal fungi (AMF) enhance P uptake and crop performance of millet. Assessing the genotypic variation of early mycorrhization and its effect on plant growth is necessary to better understand mechanisms of resistance to low soil P and to use them in breeding strategies for low P. Therefore, in this study, eight pearl millet varieties contrasting in low-P resistance were grown in pots under low P (no additional P supply) and high P (+0.4 g P pot^{-1}) conditions, and harvested 2, 4, 6, and 8 weeks after sowing (WAS). Root length was calculated 2 WAS by scanning of dissected roots and evaluation with WinRhizo software. AM infection (%) and P uptake (shoot P concentration multiplied per shoot dry matter) were measured at each harvest. Across harvests under low P (3.3 mg Bray P kg^{-1}), resistant genotypes had greater total root length infected with AMF (837 m), higher percentage of AMF colonization (11.6%), and increased P uptake (69.4 mg P $plant^{-1}$) than sensitive genotypes (177 m, 7.1% colonization and 46.4 mg P $plant^{-1}$, respectively). Two WAS, resistant genotypes were infected almost twice as much as sensitive ones (4.1% and 2.1%) and the individual resistant genotypes differed in the percentage of AMF infection. AMF colonization was positively related to final dry matter production in pots, which corresponded to field performance. Early mycorrhization enhanced P uptake in pearl millet grown under P-deficient conditions, with the genotypic variation for this parameter allowing selection for better performance under field conditions.

Key words: acid soils / arbuscular mycorrhizal fungi / P deficiency / P-acquistion efficiency

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1 Introduction

In the West African Sahel, with annual rainfall above 300 mm, plant productivity is mainly limited by low phosphorus (P) availability (*Buerkert* et al., 2000). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the main staple crop grown in the predominantly acid sandy soils, with P deficiency in the early stages of life critically affecting plant survival and final yield (*Grant* et al., 2001). Root length density and symbiosis with arbuscular mycorrhizal fungi (AMF) determine total root absorption area, which for many species is critical under P limiting growth conditions (*Allen*, 2007; *Bucher*, 2007).

It has been shown for many species that AMF hyphae can mobilize insoluble inorganic P (*Tawara* et al., 2006) and transfer it to the host plant from soil beyond the rhizosphere depletion zone (*Smith* and *Read*, 2008), enhancing thereby P uptake (*Sorensen* et al., 2008; *Conversa* et al., 2013). Association with AMF is often considered the most common strategy adopted by plants to cope with low-P conditions (*Richardson* et al., 2009), and it is strongly influenced by P availability (*Covacevich* et al., 2007). In sorghum (*Sorghum bicolor* Moench), for example, P deficiency promotes the exudation of 5-deoxystrigol (*Yoneyama* et al., 2007), one of the strigolactone molecules responsible for the onset of symbiosis with AMF. The regulation of strigolactone exudation seems to be closely related to the shoot's P status (*Yoneyama* et al., 2012) and to the nutrient acquisition strategy of the plants (*Yoneyama* et al., 2008). The extent of AMF infection and the degree of benefit from AMF are both plant heritable traits selectable through plant breeding (*Manske*, 1990). Therefore, understanding the time-related interaction of root growth and AMF infection traits under P deficiency may help in the breeding of varieties more resistant to low P soils as well as the development of more efficient methods to apply P.

Ramos-Zapata et al. (2009) showed that AMF played a crucial role in seedling growth and P uptake of P-deficient *Desmoncus orthacanthos*. In their pioneering work, *Krishna* and *Lee* (1987) showed genotypic variability for AMF colonization and efficiency in pearl millet, but we are not aware of any work investigating the role of naturally occurring AMF on very young millet seedlings. For the Sudano-Sahelian soils it was found that the beneficial effect of cereal (sorghum, pearl mil-

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Table 1: Identification number, name, level of resistance to low P, and country of origin of eight pearl millet varieties grown in pots with and without P (0.4 g P pot^{-1} at sowing) for 8 weeks at ICRISAT Sahelian Centre, Sadoré, Niger, in 2012.

Identification number	Variety	Material	Origin
1	GBx89305_YLD_2009	sensitive	Niger-ICRISAT
2	2898x92222_C1_Sad_Low_2009	sensitive	Niger
3	SOSAT_C88_Check_all	sensitive	Mali-IER-ICRISAT
4	Strigares_expvar_ep_long_noir	sensitive	Niger-ICRISAT
5	PE05387	resistant	Mali
6	PE03089	resistant	Senegal
7	Madougou5	resistant	Mali
8	Serkin_C2_Kandela_SMS	resistant	Niger

let) / legume (groundnut, cowpea) rotation on dry matter production of cereals could be partly explained by higher AM infection levels at 35 days after sowing (DAS) (*Bagayoko* et al., 2000a, b), but no information exists about the effects of even earlier mycorrhization on plant growth.

To fill this gap of knowledge, we selected eight varieties from a set of 102 millet genotypes from West Africa (Table 1) based on their contrasting response to low-P soil in Niger (unpublished data). The aim of our work was to test the hypothesis that low P-resistant varieties have longer roots and higher levels of AMF infection at early growth stages than sensitive ones. We also intended to assess whether the peak of AMF infection at a certain harvest time is critical for P uptake.

2 Material and methods

2.1 Experimental setup

The 8-week experiment was carried out during the rainy season (July–August) 2012 in 18-L pots at ICRISAT Sahelian Centre in Sadoré (ISC; 13°23′ N, 02°27′ E, 206 m asl), 40 km SE of Niger's capital Niamey. The local temperature regime is isohyperthermic with an average value of 31.7°C during our trial (minimum 20.2°C, maximum 43.1°C). Pots were filled with 27 kg of the 0–20 cm topsoil of an air-dry Psammentic-Paleustalf (*West* et al., 1984) or Arenosol (*FAO*, 1988) with pH 5.7 (1 : 2.5 H_2O : soil), 3.3 mg Bray-P kg^{-1} soil, 0.3% C_{org}, and 207 mg total N kg^{-1} soil (*Buerkert* et al., 1995). The experimental design with four replications was arranged in a split-plot design with eight different genotypes as split-plot factors: four genotypes resistant and four sensitive to low-P conditions. The tested materials originated from Mali, Niger, and Senegal, with their resistance to low P being assessed in 2011 based upon early biomass production in pots (Table 1). Two treatments were tested in the main plots: high P (HP; P application) and low P (LP; without additional P supply). In all pots, seeds were placed 2 cm below the surface in two separate pockets containing five seeds each. Before and after sowing, each pot was irrigated with 300 mL of water. Half of the pots were fertilized with P (thereby creating the HP environment) at sowing by applying 1.8 g of potassium dihydrogen phosphate (KH_2PO_4) in two different pockets alternating (3 cm) with the sowing pockets. This application rate was equivalent to 0.4 g P per pot or 4 kg P ha^{-1}, which is the recommended P microdose for West Africa (*Buerkert* and *Schlecht*, 2013). The LP pots received 1.1 g of potassium sulfate (K_2SO_4) to compensate for K contained in KH_2PO_4 of the HP pots. Nitrogen was applied 20 DAS as top-dressed urea to all pots. Plants were thinned to one plant per pot 14 DAS. Plants were harvested17, 31, 45, and 58 DAS to record AMF infection of the roots at different growth stages. For simplicity, we will denote the four harvest times as 2, 4, 6, and 8 weeks after sowing (WAS). Thus, a total of 256 pots were set up (8 genotypes × 2 P soil conditions × 4 repetitions × 4 harvests).

The following parameters were measured at each harvest: shoot height (taken by stretching the highest fully expanded leaf), number of leaves and tillers, diameter of main culm, shoot and root dry matter (DM, oven-dried at 60°C), root length density (RLD), AMF infection (%), and total P concentration of shoots and roots. In roots, P concentration was measured only for the last two harvests, as biomass was insufficient for both AMF and P analysis for the first two harvests. Phosphorus concentration was analyzed according to standard methods. 'Colonized root length' was root length (cm) multiplied by percentage of AMF infection. P uptake efficiency (PAE) was finally calculated as the amount of P accumulated in the shoot (P uptake) per unit of root biomass.

2.2 Determination of RLD

RLD was determined by scanning total fresh roots 2 WAS. To this end, we carefully washed the samples over a sieve to remove adherent soil and stone particles. Subsequently, roots were spread out on a transparent plexiglass tray in a few mm layer of water to keep them afloat. When the root biomass of one plant exceeded the capacity of a single tray, roots were separated in several trays and scanned consecutively. After scanning, the image was analyzed for root length with the WinRhizo software package (Regent Instruments Canada Inc., Quebec, Canada).

2.3 AMF colonization

Fresh root samples (corresponding to the entire root system) were preserved in a 50% ethanol–water solution inside a 2-mL Eppendorf tube. A 1 g sub-sample of fine roots was selected randomly from the root system to determine the level of AMF infection. The procedure involved root staining with Pelican Blue ink and 5% acetic acid solution at a ratio 1 : 20 (*Vierheilig* et al., 1998). AM colonization was quantified with a video-microscope and a grid-plate following the gridline intersection method (*Giovannetti* and *Mosse*, 1980).

A second experiment was conducted from mid October 2012 following exactly the same experimental setup except that plants were grown for 6 weeks instead of 8 weeks and one of the eight varieties was different. This trial was conducted to determine final root and shoot dry matter production and to compare it with the outcome of the first experiment.

Data were statistically analyzed with one- and two-way ANOVA using R software. Parameters were log-transformed whenever their residuals were not normally distributed. Significance levels were computed at: $P < 5\%$, $P < 1\%$, and $P < 0.1\%$; results at $P > 5\%$ are shown as absolute numbers.

3 Results

3.1 Low-P conditions

Shoot biomass differed among genotypes ($P < 1\%$) and between sensitive and resistant varieties ($P < 5\%$) at final harvest, but these differences were not statistically significant for root DM (data not shown). RLD ranged from 0.010 (genotype 1) to 0.029 cm cm^{-3} (genotype 7) 2 WAS. Resistant varieties tended to have higher RLD (0.022 cm cm^{-3}) than sensitive ones (0.018 cm cm^{-3}; Fig. 1), but this numerical difference was not statistically significant. No genotypic variation was found for this parameter.

Phosphorus concentration in the shoot increased until 4 WAS and then declined as the plants grew, resulting in a negative relationship between shoot P concentration and shoot DM ($r = -0.713$, $P < 0.1\%$). Shoot P concentration varied from 1.54 to 6.81 mg P g DM^{-1} which was the level reached 2 WAS by resistant variety 5. The resistant genotypes had higher shoot P concentration (3.7 mg P g DM^{-1}) than sensitive ones (3.3 mg P g DM^{-1}, $P = 7.8\%$). When each harvest time was considered separately, the differences among individual genotypes became apparent ($P < 5\%$). Root P concentration ranged from 0.48 to 4.20 mg P g DM^{-1} across harvests 6 and 8 WAS. In contrast to shoot P concentration, root P did not differ among genotypes irrespective of their P sensitivity. Resistant genotypes had higher PAE (4.5 mg shoot P g^{-1} root DM) than sensitive ones (3.3 mg shoot P g^{-1} root DM, $P < 1\%$). Total shoot P content increased on average from the first to the last harvest (0.44–59.70 mg P plant^{-1}; Fig. 2). At the third and fourth harvest, resistant genotypes had significantly higher shoot P, while individual genotypic differences were evident 8 WAS, with varieties 8 and 7 outperforming the others (Fig. 3).

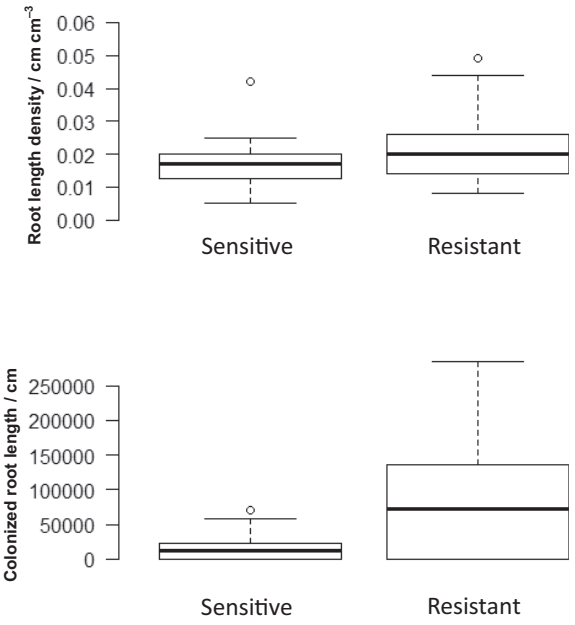

Figure 1: Root length density and root length colonized by arbuscular mycorrhizal fungi of eight pearl millet varieties grown under low-P soil conditions and harvested 2 weeks after sowing at ICRISAT Sahelian Centre, Sadoré, Niger. Varieties are grouped into four sensitive and four low-P resistant ones in 2012. Colonized root length was higher in the resistant varieties as compared to the sensitive ones ($P < 0.1\%$).

Percentage of AMF colonization increased significantly across harvest times, averaging from 0.5 (genotype 4, sensitive) to 26.4% (genotype 5, resistant; Fig. 2). This was strongly correlated with shoot dry matter ($r = 0.55$, $P < 0.1\%$) and total shoot P ($r = 0.59$, $P < 0.1\%$), but not with P content

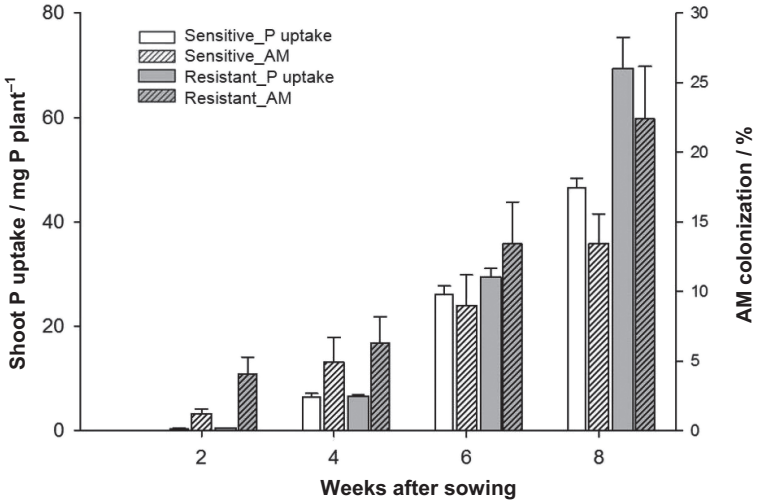

Figure 2: Shoot P uptake (total amount of P in the shoot) and arbuscular mycorrhizal colonization of eight pearl millet varieties grown under low P conditions at ICRISAT Sahelian Centre, Sadoré, Niger, and harvested at 2, 4, 6, and 8 weeks after sowing. Pearl millet genotypes are grouped into sensitive and low-P resistant ones according to the biomass production under P-limiting soil conditions in previous trials in pots and field-like (lysimeter) conditions.

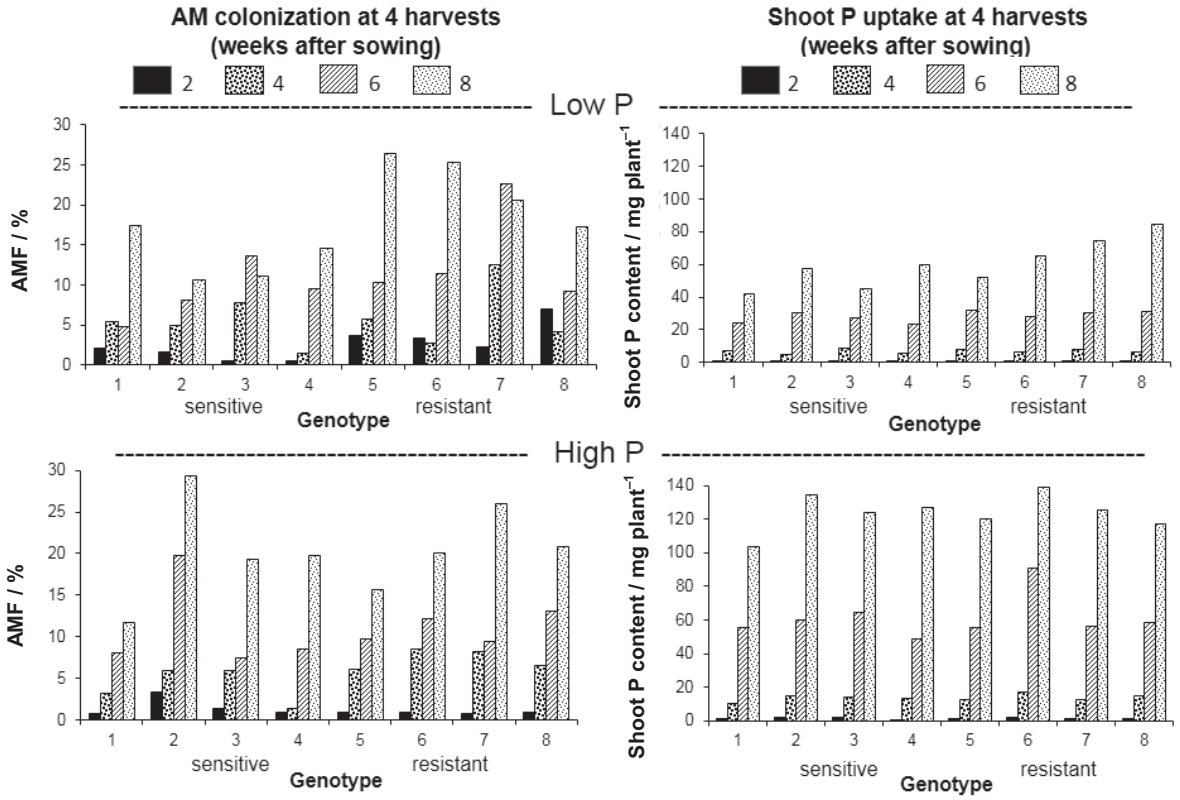

Figure 3: Arbuscular mycorrhizal (AM) colonization and shoot P uptake (total amount of P in the shoot) of eight pearl millet varieties grown under low P (no P application) and high P (supply of 0.4 g P pot^{-1} at ICRISAT Sahelian Centre, Sadoré, Niger, and harvested at 2, 4, 6, and 8 weeks after sowing) in 2012.

per unit root length. The combination of shoot dry matter, P concentration in the shoot and percentage of AMF at final harvest allowed us to discriminate between resistant and sensitive genotypes ($P < 1\%$).

Resistant genotypes had higher AMF infection (11.6%) and longer colonized root length (837 m) than sensitive ones (7.1% and 177 m respectively; Fig. 1). Two and 8 WAS, resistant genotypes were significantly more colonized with AMF than sensitive ones ($P < 5\%$). Eight WAS, shoot P uptake differed between resistant and sensitive genotypes (Fig. 2), indicating the positive effect of an early discrimination between the two groups. The low-P-resistant variety 7 had the greatest AMF infection across harvest times (14.5%) and sensitive variety 2 had the lowest (6.4%). Two WAS, there was significant genotypic variation in AMF infection within the group of resistant varieties (ranging from 2.2% to 7.0%, $P < 5\%$). Variety 8 had highest AM infection 2 WAS, whereas variety 7 had highest infection 4 WAS (12.5%). Varieties 7 and 8 took up most P at the end of the trial (86.2 and 74.4 mg P, respectively).

Average AMF colonization was similar between LP and HP treatments (9.4% and 9.6%, respectively), but 2 WAS plants grown under LP had higher interaction with AMF than plants under HP (Fig. 3). The sensitive variety 2 with lowest AMF colonization under LP had very high colonization under HP. In contrast, the resistant variety 5 recorded greatest AMF infection 8 WAS under LP, while its interaction with AMF was low under HP, suggesting that varieties adapted to LP had different strategies in terms of mycorrhization timing to optimize P uptake under P-limited conditions. Shoot dry matter in the first and the second trial correlated weakly, but significantly ($r = 0.26$, $P < 5\%$). In both trials two varieties not matching were excluded from this calculation. No similar correlation was found for root dry matter.

3.2 High-P conditions

Two WAS, RLD ranged from 0.020 (genotype 1) to 0.049 cm cm^{-3} (genotype 3) and was on average twice as high as under LP ($P < 0.1\%$). Also shoot P concentration was on average two-fold higher under HP than under LP (6.91 and 3.53 mg P g DM^{-1}, respectively) ranging from 3.49 (genotype 2 at harvest 4) to 10.25 mg P g DM^{-1} (genotype 5 at harvest 1, data not shown). Root P concentration varied from 1.40 to 1.87 mg P g DM^{-1} across harvests 6 and 8 WAS. AMF colonization across harvests was 9.4% on average, similar across P levels. Two WAS, AMF colonization under HP was just half (1.2%) of that under LP (2.6%), but plant roots from both treatments were similarly infected from 4 WAS onwards. None of these parameters differed either between resistant and sensitive genotype groups or among individual genotypes. The higher AMF infection 2 and at 8 WAS and longer colonized root length of resistant genotypes observed under LP were not found under HP. Examining AMF infection and shoot P uptake at each single harvest (Fig. 3) yielded no significant differences between resistant and sensitive genotype groups and no significant differences among the individual geno-

types. This suggests that the resistant varieties had specific adaptation strategies to low soil P. Under HP, the second trial clearly confirmed the outcome of the first, namely the linear relationship between shoot dry matter in the first trial and shoot dry matter or root dry matter in the second trial was high ($r = 0.77$ and $r = 0.76$, both $P < 0.1\%$, respectively).

4 Discussion

Pearl millet varieties, previously selected as resistant to LP, were characterized by adaptive mechanisms such as greater root length colonized with AMF and higher percentage of colonized root length. These two parameters allowed discriminating varieties as resistant and sensitive to P-limited soil conditions. Under LP, as early as 2 WAS, resistant varieties were significantly more colonized with AMF than sensitive ones, which led to higher P uptake and shoot dry matter of resistant varieties at the end of the trial. 2 WAS, AMF infection under LP was twice that under HP. This suggests that (1) some resistant varieties were better adapted to LP because of earlier physiological detection of P deficiency and subsequent consequent interaction with AMF. (2) The P microdose applied at sowing was high enough to keep the shoot P status 2 WAS at a non-critical point to interact with AMF, thereby roots grew larger and therefore likely interacted with more AMF spores in the soil. Later during growth, added P likely decreased in the soil, and 8 WAS HP plants ended up having similar percentage of AMF infection as LP plants. Recently, a similar trial showed that AMF colonization in pearl millet decreased only with P concentrations six times higher than the one in our HP treatment (*Gutbub* and *Szell*, unpublished data).

Our data suggest that very early mycorrhization plays a pivotal role in resistance to P stress and confirms that root colonization with AMF is an adaptative strategy of millet under low soil P from 2 WAS onwards. At the same time our study demonstrates that there is an important genetic variation of AMF infection during the first 8 weeks of plant growth even among the selected resistant genotypes. Out of the eight genotypes, variety 8 had the highest percentage of AMF infection 2 WAS, variety 7 at 4 and 6 WAS, and varieties 5 and 6 had their peak colonization 8 WAS (Fig. 3).

One possible explanation for this varietal difference, which merits further verification, is that genotypes invest assimilates early in setting up a symbiosis with AMF such as genotypes 7 and 8 have later carboxylate production and *vice versa*. Both P acquisition strategies are major carbon drains, so there may well be a tradeoff between them (*Ryan* et al., 2012). Carboxylates are well known for mobilizing inorganic P and organic P by complexing the metal cations that bind phosphate (*Lambers* et al., 2013). Across varieties this time-shifted pattern might be further combined with other P acquisition strategies such as the secretion of acid phosphatases (*Ezawa* et al., 2005).

However, any understanding of the different P acquisition strategies in pearl millet should also take into account possible specific interactions of plant genotype × AMF species. Recently, 30 different AMF species have been identified from a soil sample collected 2012 in Mali on an Arenosol similar to the one from Niger used in our study. In that sample the most abundant species belonged to the *Glomus* genus (33%) and the most common spores were *G. arborense, G.* (*Claroideoglomus) etunicatum*, and *G. intraradices* (*Leiser* et al., unpublished). This suggests an intriguing evolutionary host-symbiont interaction at a fine taxonomic level.

Two WAS, RLD did not show any varietal difference as it was previously found at the flowering stage of pearl millet in Niger (*Brück* et al., 2003). This difference may be due to the limited rooting volume in the pots, to high plant-to-plant variation within varieties of this highly out-crossing species, and to the unavoidable error in handling extremely fine millet roots at sample preparation.

The relationships between AMF colonization, P uptake and shoot biomass of millet confirm our initial hypothesis and are in agreement with the existing literature (*Raiesi* and *Ghollarata*, 2006). The outcome of this pot trial was comparable to field conditions, as the biomass produced in pots 2 and 8 WAS positively correlated with total dry matter at the end of the growth cycle and total yield (leaves, culm and grains) produced in lysimeters (Pearson coefficients $r = 0.65$ and $r = 0.38$, respectively; unpublished data). Results from this study also support earlier work that very early AMF colonization enhances grain yields under low-P conditions despite comparatively large possible costs (*Bagayoko* et al., 2000a, b).

Further studies focusing on the regulation between early mycorrhization, carboxylate release and phosphatase secretion, and final grain and biomass yields are needed in order to develop an effective indirect screening method for resistance to low P and refined management practices. *Abbott* and *Robson* (1991) had already postulated that if an early and rapid infection with AMF is positively related to yield response, early AMF infection might be a useful parameter for screening genotypes for P uptake efficiency. This may be far more advantageous to farmers in the West African Sahel than relying on artificial inoculation approaches (*Garcia* et al., 2007).

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