Effects of phosphorus and water stress on shoot and root growth and on mycorrhization of different pearl millet (*Pennisetum glaucum* (L.) R. Br.) varieties from West Africa

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Dissertation

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by

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Hiermit versichere ich, dass ich die vorliegende Dissertation " **Effects of phosphorus and water stress on shoot and root growth and on mycorrhization of different pearl millet (*Pennisetum glaucum* (L.) R. Br.) varieties from West Africa**" selbständig, ohne unerlaubte Hilfe angefertigt und andere als die in der Dissertation angegebenen Hilfsmittel nicht benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden.

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(Francesca Beggi)

Preface

This thesis was prepared within the research project “Tackling abiotic production constraints in pearl millet and sorghum-based agricultural systems in the West African Sahel” funded by Bundesministerium fur wirtschaftliche Zusammenarbeit und Entwicklung (BMZ), implemented by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The thesis is submitted to the Faculty of Organic Agricultural Sciences to fulfil the requirements for the degree “Doktor der Agrarwissenschaften” (Dr. agr.). The dissertation is based on three papers as first author, destined to international refereed journals. The manuscripts are included in chapters 2, 3 and 4.

Chapter 2:

Beggi F, Haussmann BIG, Falalou H, Gemenet DC, Buerkert A (to be submitted): Early phosphorus efficiency of 102 pearl millet varieties from West Africa.

Chapter 3:

Beggi F, Falalou H, Hash CT, Buerkert A (to be submitted): Effect of early mycorrhization and colonized root length on low soil phosphorus tolerance of eight West African millet varieties.

Chapter 4:

Beggi F, Falalou H, Buerkert A, Vadez V (2014) Tolerant pearl millet (*Pennisetum glaucum* (L.) R. Br.) varieties to low soil P have higher transpiration efficiency and lower flowering delay than sensitive ones. Plant and Soil DOI 10.1007/s11104-014-2338-8.

To Isabella

That day of June 2010, sitting in front of home and wondering, you told me:

“Go ahead, you know that this is the path for you”

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Summary

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a major food crop for 50 millions of people in the African Sahel. Farming systems in this zone are predominantly low input cereal-based systems on acid, sandy and heavily weathered soils. Precipitation is very scattered temporally and the unimodal rainy season is often characterized by late onset of the rains and mid- or end-season droughts. Low soil phosphorus (P) and unpredictable water availability are known for decades to be the main interacting constraints to crop production in this area, crucial especially during plants early establishment. Phosphorus fertilizers are often too expensive for Sahelian subsistence farmers, limiting their use in this region, thereforeenhancing plant-based strategies to optimize P availability to crops and tolerance to drought is an important alternative to be undertaken. The necessity to understand the relative effect of these abiotic stresses, and their interaction as well as plants-related mechanisms of adaptation, led the present research work at the research station ICRISAT Sahelian Centre, Niger, with the following objectives:

1. Characterization of genotypic variation for P efficiency at early growth stages using a set of 102 pearl millet West African varieties (incl. landraces and modern varieties), and identification of screening parameters for high P efficiency.

2. Analysis of the effect of root length density and arbuscular mycorrhizal fungi (AMF) infection at early plant growth on tolerance to low P, and assessment of possible genotypic differences in these parameters.

3. Investigation of the relationship between water use and final yield under the combined effect of water and P stress.

The first experiment was carried out in pots to examine seedling growth and dry matter (DM) production of 102 pearl millet varieties for 37 days (June - August 2011) under low P (LP, no additional P supply) and high P (HP, addition of 0.4 g P pot^{-1}). The varieties represented the most promising material of the research centre and national breeding programmes from several West African countries. Phosphorus efficiency was calculated as the ratio of shoot DM produced at LP to that at HP, and it varied across

the 102 genotypes from 17.6 to 47.9%. Its components phosphorus acquisition efficiency (PAE, equal to total amount of P in the shoot) and P use efficiency (PUE, equal to the square of DM produced per unit of P uptake) ranged from 7 to 65 mg P pot^{-1} and from 2.1 to 7.1 g^2 DM mg P^{-1} at LP, respectively. PAE was slightly higher in landraces than in modern varieties. Phosphorus concentration in shoot was analysed by Near Infra-Red Spectroscopy (NIRS), and together with height at 4 weeks after sowing (WAS), was shown to be a good indicator of P efficiency. Phosphorus efficiency based on grain yield (LP/HP) of corresponding field trials in Niger and Burkina Faso, was only slightly correlated with shoot DM under LP (r=0.18) and under HP (r=0.34). The large genotypic variation in P efficiency indicates scope for breeding millet varieties more tolerant to P deficient soils.

Following on from this experiment, eight millet genotypes contrasting for their tolerance to low P were selected and planted in a second pot experiment during the following rainy season (July - August 2012). The aim was to test the hypothesis that longer roots per unit of volume and mycorrhization at early plant growth result in higher tolerance to soil P deficiency. Millet plants were grown under LP (no P supply) and HP (addition of 0.4 g P pot^{-1}) and were harvested four times: at two, four, six and eight WAS. Root length was calculated at two WAS by scanning of washed roots and evaluation with WinRhizo software, while AMF colonization was quantified using the “Gridline intersection method” at each harvest. This study partially confirmed the initial hypothesis, i.e., under LP, at two WAS tolerant genotypes were infected with AMF more than sensitive ones (4.1% and 2.1%) but no difference was found for root length. Nevertheless, tolerant genotypes showed greater total root length infected with AMF across harvests (83700 cm) and also higher average AMF colonization (11.6%) with consequent enhanced P uptake (69.4 mg P $plant^{-1}$) and DM production as compared with sensitive genotypes (17700 cm, 7.1% and 46.4 mg P $plant^{-1}$, respectively). It is possible that tolerant plants were able to interact earlier with AM fungi and/or to select symbiosis with fast colonizing AMF species. For instance, individual tolerant genotypes had the highest AMF infection at different harvest times, which suggested variety-

specific nutrition strategies including a possible role of organic acids within this time-shifted pattern of mycorrhization.

Two lysimeter trials were conducted in December 2010–March 2011 and September –December 2012. Two sets of fifteen millet varieties with contrasting response to low P soil were grown until maturity under a combination of P stress and terminal drought, in order to combine early plant growth observations with transpiration dynamics and final yield. The hypotheses of this work were that (i) P deficiency delays flowering, (ii) water availability is crucial for grain filling and (iii) P enhances transpiration efficiency (TE). Lysimeters are 1 m long PVC tubes filled with P poor Sahelian soil mimicking a soil profile and allowing the assessment of transpiration curves through a procedure of subsequent weighing and controlled re-watering. TE was calculated as DM produced per kg of water transpired. This study showed that TE was higher in varieties tolerant to low P (1.6 g DM kg water^{-1}) than in sensitive ones (1.45 g DM kg water^{-1}) across treatments and TE was enhanced by P supply. Delay in booting or flowering was not always caused by P deficiency, but in general it made plants more sensitive to P deficiency and terminal drought. Under P limiting soil, genotypes tolerant and sensitive to low P used similar total amounts of water (21.7 and 19.8 kg water plant^{-1}, respectively). However, tolerant genotypes transpired less water prior to anthesis (8.8 kg water plant^{-1}) leaving more water available for grain filling (11 kg water plant^{-1}); while sensitive genotypes used 14.4 kg water plant^{-1} during pre-anthesis, leaving only 7.2 kg water plant^{-1} for grain filling. Grain yield was positively correlated with water extracted after anthesis, strongly affected by P deficiency as it decreased seed size. This work provided an important new insight regarding the combined role of P and water for pearl millet productivity. The pattern of water use in this crop is critical to cope with terminal drought under different levels of P availability. For instance, a mechanism of water conservation during pre-anthesis can play a role in avoiding stress and assuring grain filling, and this beneficial effect appears to be amplified by P availability.

In conclusion, this work has contributed to the understanding of differences in P uptake strategies of pearl millet and relative plant biomass produced under different soil P conditions. The present research provides insights into the combined role of P and water for crop productivity. Breeding of multi-stress tolerant varieties and the design of improved agricultural practices will benefit the dryland cereal farmers of West Africa.

Zusammenfassung

Perlhirse (*Pennisetum glaucum* (L.) R. Br.) ist ein wichtiges Grundnahrungsmittel für ca. 50 Millionen Menschen des afrikanischen Sahels. Das vorherrschende Anbausystem dieser Zone kann als getreidebasiertes Anbausystem bezeichnet und durch sauren, sandigen und stark verwitterte Böden charakterisiert werden. Die zeitliche Variation der Niederschläge ist hoch und die Regenzeit unimodal verteilt mit oft deutlichen Verzögerungen der Regenzeit als auch Dürreperioden in der Mitte und zum Ende der Regenzeit. Niedrige Bodenphosphor(P)-Level und unvorhersehbare Wasserverfügbarkeiten gehören seit Jahrzehnten zu den wichtigsten interagierenden Beschränkungen im Pflanzenbau dieser Region, was vor allem in den frühen Pflanzenentwicklungsphasen von entscheidender Bedeutung ist. P-Dünger sind kaum verfügbar oder für die Bauern des Sahel unerschwinglich, was die Anwendung im Gebiet limitiert. Darüber hinaus werden die weltweiten P-Reserven im nächsten halben Jahrhundert wahrscheinlich aufgebraucht sein. Beide Faktoren machen eine optimierte Phosphornutzung und ein verbesserte Trockentoleranz durch Pflanzen in Zukunft unerlässlich, zwei Strategien demnach, die als wichtige und nachhaltige Alternativen betrachtet werden. Die Notwendigkeit, die jeweilige Wirkung dieser abiotischen Stressfaktoren und deren Interaktionen, sowie deren pflanzliche Anpassungsmechanismen zu verstehen, führte zu folgenden Untersuchungszielen, welche in der Forschungsstation des ICRISAT Sahelcenter, Niger durchgeführt wurden:

1. Genotypische Charakterisierung von 102 westafrikanischen Hirsesorten (inkl. Landrassen und modernen Sorten) hinsichtlich ihrer P-Effizienz in frühen Wachstumsstadien und Identifikation von Parametern, die auf eine hohe P-Effizienz hinweisen.

2. Analyse der Wirkung von Wurzellängendichte und arbuskulärer Mykorrhizapilz (AMP)-Infektion in frühen Pflanzenwachstumsstadien hinsichtlich der Toleranz gegenüber eines niedrigen Boden-P-Status und Bewertung möglicher genotypischer Unterschiede.

3. Untersuchung der Beziehung zwischen Wassernutzung und Endertrag unter der kombinierten Wirkung von Wasser- und P-Stress.

Der erste Versuch wurde in einem Topfexperiment durchgeführt, um das Wachstum der Keimlinge und die Trockenmasse(TM)-Produktion aller 102 Hirsesorten unter niedrigem P (NP, keine zusätzliche P-Gabe) und hohem P (HP, Zugabe von 0,4 g P $Topf^{-1}$) während 37 Tagen (Juni - August 2011) zu prüfen. Die Sorten repräsentierten das vielversprechendste Material des Forschungszentrums und nationaler Zuchtprogramme mehrerer westafrikanischer Staaten. Die P-Effizienz wurde als Verhältnis der TM, welche unter NP- und HP-Bedingungen aufgebaut wurde, berechnet und variierte über alle 102 Genotypen von 17,6 bis 47,9 %. Die Parameter P-Aufnahmeeffizienz (PAE, entspricht der Gesamtkonzentration von P im Spross) und P-Nutzungseffizienz (PNE, entspricht dem Quadrat der produzierten TM pro Einheit aufgenommenem P) variierte von 7 bis 65 mg P $Topf^{-1}$ bzw. von 2,1 bis 7,1 mg^{-1} unter LP. Die PAE-Werte waren bei Landrassen etwas höher als jene der modernen Sorten. Die P-Konzentration im Spross, gemessen mit Nah-Infrarot-Spektroskopie (NIRS) und die Pflanzenhöhe vier Wochen nach der Aussaat (WNA), erwiesen sich als gute Indikatoren, um die P-Effizienz zu bestimmen. Die P-Effizienzen basierend auf dem Kornertrag (LP/HP) von entsprechenden Feldversuchen in Niger und Burkina Faso, waren nur schwach korreliert (Spross-TM: LP, r = 0,18 und HP, r = 0,34). Die große genotypische Variabilität der P-Effizienz verdeutlicht das Potential mangeltolerante Hirsesorten zu züchten.

Im Anschluss an dieses Experiment wurden acht Hirsesorten hinsichtlich ihrer Toleranz gegenüber niedrigen Boden-P-Level für die folgende Regenzeit (Juli- August 2012) ausgewählt und in einem zweiten Topfexperiment gepflanzt. Es sollte die Hypothese getestet werden, dass längere Wurzeln pro Volumeneinheit und Mykorrhizierungsgrad in frühen Pflanzenwachstumsphasen zu einer höheren Toleranz gegenüber Boden-P-Mangel führt. Die Pflanzen wurden unter NP (keine zusätzliche P-Gabe) und HP (Zugabe von 0,4 g P $Topf^{-1}$) gezogen und viermal, nach zwei, vier, sechs und acht WNA, geerntet. Wurzellängen wurden nach zwei WNA durch das Scannen von gewaschenen Wurzeln und mit der WinRhizo Software ausgewertet, während die

AMP-Kolonisierung durch die "Rasterlinienschnittpunkt-Methode" nach jeder Ernte bestimmt wurde.

Diese Studie bestätigte teilweise die Ausgangshypothese, dass unter LP, tolerante Genotypen nach zwei WNA eher infiziert wurden als sensitive Genotypen (4,1% bzw. 2,1%), jedoch kein Unterschiede in der Wurzellänge aufwiesen. Dennoch zeigten tolerante Genotypen größere Gesamtwurzellängen mit AMP (83700 cm), eine erhöhte durchschnittliche AMP Kolonisierung (11,6%) mit einer konsequent erhöhten P-Aufnahme (69,4 mg P Pflanze^{-1}) und TM-Produktion im Vergleich zu empfindlichen Genotypen (17700 cm, 7,1% bzw. 46,4 mg P Pflanze^{-1}). Es scheint möglich, dass tolerante Pflanzen früher mit AMP interagierten und/oder schneller Symbiosen mit AMP-Arten eingingen. Einzelne tolerante Genotypen hatten z. B. die höchste AMP-Infektion zu unterschiedlichen Erntezeiten, was sortenspezifische Ernährungsstrategien nahelegt, einschließlich einer möglichen Rolle von organischen Säuren innerhalb dieses zeitversetzten Musters der Mykorrhizierung.

Zusätzlich wurden zwei Lysimeterversuche im Dezember 2010-März 2011 und September-Dezember 2012 durchgeführt. Zwei Gruppen von 15 unterschiedlich auf niedrigen Boden-P-Status reagierender Perlhirsensorten wurden bis zur vollen Reife unter einer Kombination von P-Stress und terminaler Trockenbedingung angebaut, um Keimlingsstadien mit Transpirationsdynamiken und Endertrag zu integrieren. Die Hypothesen dieser Arbeit war, dass (i) P-Mangel die Blütenbildung verzögert, (ii) die Verfügbarkeit von Wasser entscheidend für die Kornfüllung ist, und (iii) P die Transpirationseffizienz (TE) erhöht.

Die Lysimeter waren 1 m lange PVC-Rohre und wurden mit P-armem Sahelboden gefüllt, welche Bodenprofile imitieren und die Beurteilung von Transpirationskurven ermöglichen, wobei nach jeder Gewichtsmessung eine kontrollierte Auffüllung durch Wasser stattfand. Die TE wurde als produzierte TM pro kg transpiriertem Wasser berechnet. Diese Studie zeigte, dass TE in toleranten Sorten gegenüber niedrigem P (1,6 g TM kg_{Wasser}^{-1}) höher war als gegenüber sensiblen Sorten (1,45 g TM kg_{Wasser}^{-1}) und die TE durch P-Zugabe verbessert wurde. Verzögerungen beim Blütenshub bzw.

Blühvorgang wurden nicht immer durch P-Mangel verursacht, machten aber Pflanzen allgemein empfindlicher gegenüber P-Mangel und terminaler Trockenheit.

Unter P-Mangelbedingungen verbrauchten tolerante und sensitive Genotypen ähnliche Gesamtwassermengen (21,7 bzw. 19,8 kg Wasser Pflanze^{-1}). Tolerante Genotypen transpirierten jedoch weniger Wasser vor der Blüte (8,8 kg Wasser Pflanze^{-1}), so dass mehr Wasser für das Füllen des Getreidekorns (11 kg Wasser Pflanze^{-1}) verwendet werden konnte; sensible Genotypen hingegen verbrauchten 14,4 kg Wasser vor der Blüte, so dass nur 7,2 kg Wasser Pflanze^{-1} für die Kornfüllung bereitstanden. Der Kornertrag war mit dem extrahierten Wasser nach der Blüte positiv korreliert und stark beeinflusst durch P-Mangel, wie es geringere Samengrößen verrieten.

Diese Arbeit stellt wichtige und neue Erkenntnisse über den kombinierten Effekt von P und Wasser auf die Perlhirsenproduktivität dar. Das Muster der Wassernutzung dieser Feldfrucht unter einer terminalen Trockenbedingung und verschiedenen P-Verfügbarkeitsstufen muss als kritisch betrachtet werden. Der Mechanismus des Wassersparens vor der Blüte kann eine Rolle bei der Vermeidung von Stress und der gesicherten Kornfüllung spielen, und diese positive Wirkung scheint durch die Verfügbarkeit von P verstärkt zu werden.

Im Ergebnis hat diese Arbeit zum Verständnis unterschiedlicher P-Aufnahmestrategien der Perlhirsen unter verschiedenen Boden-P-Level beigetragen. Die vorliegende Untersuchung gab einen wichtigen Einblick in den interaktiven Effekt von P und Wasser auf die Feldfruchtproduktivität. Auf lange Sicht und bei vollständiger Umsetzung, könnte die Zucht von multi-stresstoleranten Sorten und die Entwicklung verbesserter landwirtschaftlicher Praktiken den Trockenlandbauern in Westafrika zugutekommen.

1 General introduction

The world´s attention is often drawn to food crises in the Sahel, where 11 millions of people are severely food insecure (FAO, 2013). A stable and productive agricultural sector is fundamental for the development of this region, where agriculture plays a prominent role in poverty reduction (FAO, 2005) and it accounts on average for one-third of the gross domestic product (Dixon *et al*., 2001). Sahelian farming systems are predominantly low input cereal-based systems and they are characterized by less and less fertile soils, due to heavy soil weathering, millennia old-nutrient exports through harvest removals and low use of mineral fertilizers. Large negative nutrient balances are the consequence (Storvoogel and Smaling, 1994), and this is true in particular for phosphorus (P).

1.1 Pearl millet

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), major subsistence crop for 50 millions of people in the African Sahel (FAO and ICRISAT, 1996), is strongly adapted to the climate and edaphic conditions of this area and it is crucial for food security. It is a highly cross-pollinated, diploid annual cereal. Although yields are low, averaging 500 to 600 kg per hectare, pearl millet yields are more reliable than maize (*Zea mays* L.) or sorghum (*Sorghum bicolor* L. Moench) in marginal areas (CGIAR, 2013). This cereal is grown on sandy soils with low water retention capacity, in hot environments characterized by high vapour pressure deficit and high temporal and spatial variability in rainfall (Payne *et al*., 1990). The Sahelian region is together with India one of the cradles of the domestication of pearl millet, which dates back to more than 3500 years ago and in Africa it is nowadays cultivated over an area of 16 million hectares. Since 1982, the productivity of pearl millet across the six major pearl millet producing countries of the Sahel (Niger, Nigeria, Mali, Chad, Burkina Faso and Senegal) has varied from 0.2% per year in Chad and Niger to 1.6% per year in Burkina Faso (ICRISAT, 2014). Yields of pearl millet have for millennia been limited by low soil P and unpredictable droughts (Manu *et al*., 1991).

1.2 Phosphorus deficiency

After nitrogen (N), P is quantitatively the most important nutrient for plant growth (Vance *et al*., 2003). It is a structural component of nucleic acids and phospholipids, and it is especially critical in establishing the enzymatic machinery in energy storage and transfer. The productivity of acidic sandy Sahelian soils is limited by the low concentration of plant-available P (Bray-P typically below 5 mg Kg^{-1}), mainly due to adsorption of P with Al/Fe oxides and hydroxides (Shen *et al*., 2011). The form of P readily accessed by plants is inorganic P (P_i, present mainly as $H_2PO_4^-$ below pH 6.0; Schachtman *et al*., 1998) but 20 to 80% of P in soils is found in the organic form, such as phytic acid (Richardson, 1994) which therefore must be mineralized before it can be taken up by plants (Horst *et al*., 2001).

Although an external input of P through mineral fertilizers would undoubtedly help sustaining the growing population of the Sahel (Bationo *et al*.,1992; Buerkert *et al*., 2000), the use of it is severely limited by the unstable prices of cereals, lack of capital and poorly developed infrastructures (Bationo *et al*., 1997). A successful solution consisting in the placement of NPK fertilizer at the rate of 4 Kg P ha^{-1} with the seed at planting ("micro-dosing") was proposed in the late 90's by Buerkert and Hiernaux (1998). This strategy led to average dry matter increases of 70% for millet as compared to broadcast application of P, and its benefits for cereal productivity have been confirmed in different countries of sub-Saharan Africa (Buerkert *et al*., 2001; Hayashi *et al*., 2007; Pender *et al*., 2008). Nevertheless, micro-dosing still implies a minimum availability of fertilizers and erroneous application in combination with a dry spell after planting can provoke the burning of the seeds with deleterious effects on crop productivity.

Developing farming systems that require less P becomes a challenge for future (Gilbert, 2009) and enhancing plant-based strategies to optimize P availability to crops is a sustainable alternative to be considered under smallholder farmers' conditions. The approach has been promisingly addressed for different cereals (Osborne and Rengel,

2002; Gahoonia & Nielsen, 2004; Rose *et al*., 2010; Rose and Wissuwa, 2012) but no study was undertaken for pearl millet.

1.3 Plant adaptation to low P

Plants have evolved a large and diverse array of morphological and physiological mechanisms to obtain adequate P supply under limiting conditions (Schachtman *et al*., 1998; Vance *et al*., 2003; Lambers *et al*., 2006). Plants need to increase the rate of P uptake by roots from the soil, retranslocate P from older leaves to younger leaves and growing roots, and deplete the vacuolar stores of P.

Typical responses of plants to improve P uptake under P-deficiency include increased carbon allocation to roots (Varadarajan *et al*., 2002), expansion of root surface area by increasing root length, lateral root formation (Williamson *et al*., 2001), length and number of root hairs (Lynch, 1995; Gilroy and Jones, 2000; Lynch and Brown, 2001; Ticconi *et al*., 2001), decreased root diameter (Borch *et al*., 1999; Schroeder and Janos, 2005), cluster-root formation (Skene, 1998; Lambers *et al*., 2006), secretion of phosphatases which catalyse the hydrolysis of organic phosphate compounds (Goldstein *et al*., 1988; Tadano and Sakai, 1991), up-regulation of high-affinity P transporters (Marschener *et al*., 1986; Smith *et al*., 1997; Liu *et al*., 1998; Lynch and Brown, 2001), exudation of organic acids such as carboxylates (Dinkelaker *et al*., 1989; Johnson *et al*., 1994; Neumann and Römheld, 1999; Raghothama and Karthikeyan, 2005) which can mobilize both inorganic P and organic P by complexing the metal cations that bind phosphate (Hayes *et al*., 2000; Jones *et al*., 2003). Phenolics and mucilage act similarly to carboxylates but they are less effective (Neumann and Römheld, 2001). The most prevalent evolutionary adaptation of terrestrial plants for acquiring P is though symbioses with arbuscular mycorrhizal fungi (AMF, Smith *et al*., 2000; Burleigh *et al*., 2002; Tibbett and Sanders, 2002). Varietal differences on nutrient efficiency have been found in different crops (Wissuwa and Ae, 2001; Hoffland *et al*., 2006) such as pearl millet (Bruck, 2003; Buerkert *et al*., 1997, 2001, 2002). To date there is little knowledge on the genetic diversity of pearl millet for P uptake and P use efficiency and on the mechanisms involved in it.

1.4 Water stress in the Sahelian conditions

In West Africa annual precipitation increases when moving south of the Sahara desert. The Sahelian and Sahelo-Sudanian zones are bordered by average annual rainfall limits of 250 mm year^{-1} in the north (northern limit of cultivation) and 600 mm year^{-1} in the south. Pearl millet grows mainly in the 250-750 mm rainfall year^{-1} zone (Hoogmoed and Klaij, 1990). The single rainy season goes from May to September with typical late onset of rains and mid- or end-season drought. Sahelian agriculture is restricted to this period because of lack of infrastructures and financial resources. The irregular rainfall with intense storms and the very low moisture-holding capacity of soil have strong implications for water conservation and crop production (Hoogmoed and Klaij, 1990). Thus, in these conditions at the high temperatures characterizing the Sahelian region (average max T varies between 36 °C and 42 °C and average min T between 15°C and 21°C), plant water management is key to growth. Plants transpiring less water but still able to maintain high yield are transpiration efficient and this feature depends on interactions between water and nutrient availability (Payne *et al*., 2000). Important contributions in the understanding of drought tolerance in pearl millet have been provided over the last years. A major quantitative trait locus (QTL) for terminal drought tolerance was initially determined (Yadav *et al*., 2002) by comparing yield performances under terminal drought and tolerant genotypes showed higher panicle harvest index (PNHI). Recently, it has been found that pearl millet genotypes introgressed with this QTL showed higher concentration of leaf abscisic acid, they maintained a lower transpiration rate even at low vapor pressure deficit (VPD) and they reacted to higher VPD (more severe water stress) by further decreasing transpiration rate. These traits contributed to water conservation, specifically to more water available during grain filling (Kholová *et al*. 2010a,b; Vadez *et al*., 2013).

A recently developed lysimeter system (Vadez *et al*., 2008; Karanam and Vadez, 2010) allows to study and compare the combinedeffects of water stress and low P by a dynamic measurement of water uptake. Such screening / phenotyping facility has being

installed at ICRISAT-Sadore and consists of 400 lysimiters (PVC tubes with a sufficient depth and diameter to represent a soil profile of the Sahel) and a crane balance (load cell for weighing) placed in a trench which is covered by a rain-out shelter.

1.5 Research objectives and hypotheses

Pearl millet almost certainly originated in tropical West Africa where the highest variation of wild and cultivated forms occurs (FAO, 1995). The studies conducted within this PhD research made use of a large collection of millet varieties selected throughout West Africa by farmers and scientists at ICRISAT Sahelian Centre in order to unravel available genetic diversity for tolerance to low P and water stress, and to clarify the underlying mechanisms.

The research activities were conducted in 2010–2012 in Niger at ICRISAT Sahelian Centre (13° 23' N, 02° 27' E, 206 m asl), 40 km from the capital Niamey, where the 30-year average annual rainfall is 542 mm and the daily average temperature peaks in May at 34ºC and drops to 25ºC in December (World Climate, 2008). The research has been subdivided in three parts, whose starting hypotheses and objectives are:

Study 1 – Hypotheses:

- Seedlings of different pearl millet varieties respond differently under soil P limiting conditions;
- Phosphorus efficiency depends on P concentration of plant tissues and seeds;
- Bigger seeds are more P efficient.

Study 1 – Objectives:

(i) Characterization of types of adaptation and existence of genetic variation for P efficiency of a set of 102 West African pearl millet varieties;

(ii) Identification of reliable early screening parameters for high P efficiency.

Study 2 – Hypotheses:

- Increased root length and association with AMF are adaptations to cope with low P soil;
- Early mycorrhization enhances P uptake.

Study 2 – Objectives:

(i) Verifying whether a set of low P tolerant varieties selected in the study 1 have longer roots and higher infection of AMF at early growth stage;
(ii) Establishing whether the peak of infection of AMF is critical for P uptake;
(iii) Assessing the existence of genetic variability for these mechanisms of tolerance to P deficiency.

Study 3 – Hypotheses:

- Phosphorus deficiency delays flowering;
- water availability during grain filling is crucial for final yield;
- Phosphorus improves TE.

Study 3 – Objectives:

(i) Evaluating the effect of low soil P on the agronomic parameters at maturity of a set of pearl millet genotypes selected in the study 1 and 2;
(ii) Analysing water use curves under combined P and water stress;
(iii) Assessing the effect of low soil P on the pre- and post-anthesis water use;
(iv) Studying the impact of low soil P on transpiration efficiency (TE).

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2 Early phosphorus efficiency of 102 pearl millet varieties from West Africa

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Abstract

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the most important staple crop in the West African Sahel and on the predominantly acid sandy soils its productivity is severely limited by phosphorus (P) deficiency. Assessing genotypic variation for P efficiency, and for its components P acquisition and P use efficiency, is crucial for breeding strategies to enhance growth and performance in P-deficient soils. To this end, a pot experiment with an acid Arenosol of pH 5.7 and 3.3 mg Bray-P kg^{-1} soil was conducted in Niger to study seedling development and dry matter (DM) production of 102 pearl millet varieties for 37 days under low P (LP, no P supply) and high P (HP, 0.4 g P pot^{-1}) conditions. Phosphorus concentration in root and shoot was measured with Near Infra-Red Spectroscopy (NIRS). Phosphorus efficiency, calculated as the ratio of shoot DM production under LP to that under HP, ranged from 18% to 48%. Phosphorus efficiency correlated positively with shoot P concentration (r=0.41). Across genotypes P acquisition varied more than P use efficiency and was lower in landraces than in modern varieties at HP. Plant height at four weeks after sowing correlated positively with P efficiency (r=0.43). Phosphorus efficiency based on grain yield (LP/HP) of corresponding field trials in Niger and Burkina Faso, was only slightly correlated with shoot DM under LP (r=0.18) and under HP (r=0.34) and with shoot P content under LP (r=0.23) of the present study. Also plant height in the pot trial weakly correlated with the harvest index (HI, r=0.31) in a field trial conducted at the same location during the previous rainy season.The large genotypic variation in P efficiency indicates scope for breeding millet varieties more tolerant to P deficient soils. Shoot height and shoot P concentration are simple and reliable criteria to assess P efficiency of millet germplasm at early growth stages, but screening results need verification in multi-location field trials.

Key words: Genotypic variation, NIRS, *Pennisetum glaucum*, Phosphorus acquisition

2.1 Introduction

Phosphorus (P) deficiency is a major limitation for plant growth on many highly P-deficient weathered soils throughout the world (Simpson *et al*., 2011), such as the largely acidic sandy soils (Arenosols) of sub-Saharan West Africa, where the little P contained in the soils is largely adsorbed to Al/Fe oxides and hydroxides (Shen *et al*., 2011). In most of these soils, plant available P (Bray-1) is below the critical level of 5 mg P kg^{-1} soil (Manu *et al*., 1991; Buerkert *et al*., 2000; Doumbia *et al*., 2003). Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a major staple food for millions of people in the semi-arid tropics of Africa (FAO and ICRISAT, 1996) whereby throughout much for Sahelian West Africa P is the most limiting nutrient from early growth stages onwards (Bationo *et al*., 1990; Kretzschmar *et al*., 1991; Buerkert *et al*., 2001).

The use of mineral fertilizers in the West African Sahel is among the lowest in the world, mainly due to high investment risks given unpredictable returns from staples such as pearl millet of which grain yields are low in absolute terms given low soil organic matter, heavily depend on rainfall and insect incidence, are largely auto-consumed, and of which market prices are subjected to seasonal speculation. Heavily weathered soils, low external input levels, and continuous extraction of plant nutrients with harvest removals have over millenia led to mining of P in the predominantly low input cereal-based farming systems (Rose *et al*., 2010). Under these conditions efforts to enhance input use in more profitable farming systems have to be complemented by selection of genotypes with high P acquisition and especially with high P utilization/translocation efficiency (Wang *et al*., 2010). This contributes to reduce costs associated with fertilizer application and may foster food security (Vance *et al*., 2003; Simpson *et al*., 2011), particularly in view of a likely rise in global P prices during the next decades.

There are numerous examples of plant species and genotypes within a given species with morpho-physiological adaptations to low P conditions (Brück, 2003) and it is well known that the ability to overcome mineral nutrient stresses at early growth stages may be critical for final yield (Cowie and Asher, 1986; Rebafka *et al*., 1993). Over four decades ago Menary (1967) reported that tomato yield (*Lycopersicon esculentum* L.) was reduced by 50% if P deficiency occurred at the seedling stage.

In the Sahelian region, vigorous early growth is particularly important as sand storms and cricket invasions often attack seedlings at the onset of the season (Rebafka *et al*., 1993, Buerkert and Hiernaux, 1995).

Phosphorus efficiency can be defined as P acquisition efficiency (PAE) or P utilization efficiency (PUE). Thereby PAE refers to plant´s strategies to take up P from soils as governed by root morphology, root exudation, and expression of high-affinity inorganic P transporters. PUE in contrast refers to the ability to produce biomass or yield using the acquired P, mainly through re-translocation and re-use of stored P (Marschner, 1995; Lambers *et al*., 2006). Enhanced P efficiency in plants can be achieved through improving P acquisition and/or utilization. However, the relative contribution of PAE or PUE to crop P efficiency varies with crop species and environmental conditions such as a soil's P status (Wang *et al*., 2010).

In recent years several tropical cereals have been studied for their P tolerance to low P and varietal differences have been reported for rice (*Oryza sativa* L., Saharawat *et al*., 2000; Rose *et al*., 2010), maize (*Zea mays* L., Horst, 2000), wheat (*Triticum aestivum* L., Korkmaz *et al*., 2009), and pearl millet (Bationo *et al*., 1992, 1993; Buerkert *et al*., 2002; Faye *et al*., 2006).

However, except for a study on P acquisition and utilization efficiency using 106 Australian cereal genotypes (Osborne and Rengel, 2002a, 2002b), we are unaware of large scale screening studies to determine phenotypic and genetic variation of traits determining P efficiency. To fill this gap of knowledge for West African pearl millet this study aimed at (i) determining genotypic variation for P efficiency in early growth and (ii) defining early screening parameters.

2.2 Materials and methods

2.2.1 Experimental site and investigation design

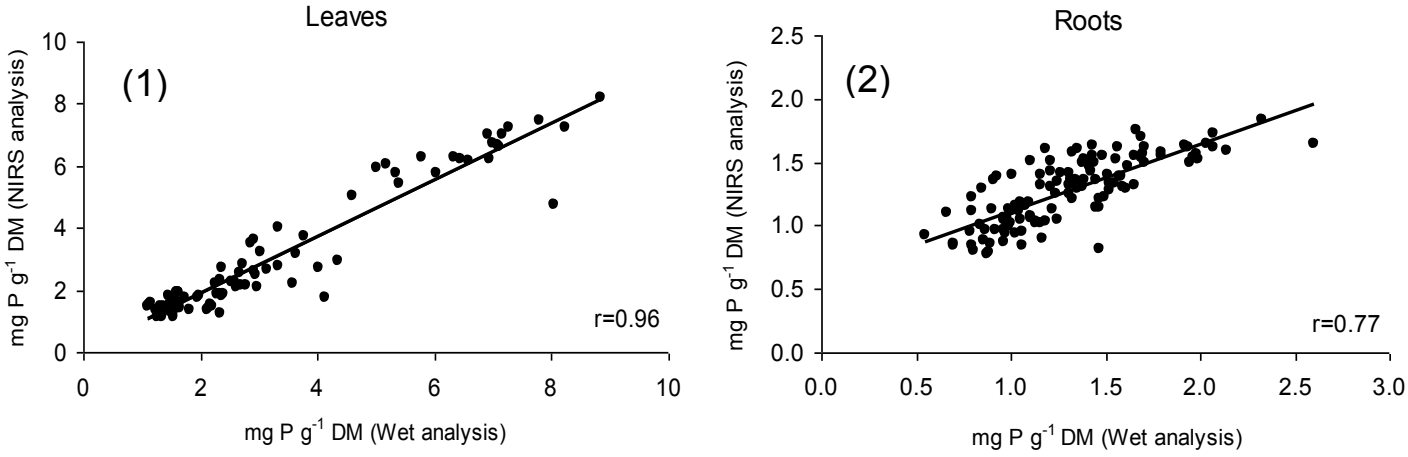
A set of 102 West African pearl millet varieties was grown in plastic pots at ICRISAT's research station in Sadore, Niger, for 37 days (June - August 2011). These varieties represented the most promising materials of ICRISAT Sahelian Centre and

national breeding programmes from Burkina Faso, Cameroun, Chad, Mali, Mauritania, Niger, Nigeria, and Senegal and comprised 46 landraces and 56 varieties (Suppl. Table 1). The pots of 23 cm diameter were filled with 500 g of gravel to facilitate drainage superimposed by 8 kg of a Psammentic Paleustalf (West *et al*., 1984) or Arenosol (FAO, 1988) obtained from a P deficient field at Sadoré (pH_{H20} 5.7, 3.3 mg Bray-P kg^{-1} soil, Corg 0.3% and 207 mg total N kg^{-1} soil; Buerkert *et al*., 1995). Advantages of such pot trials compared to field experiments in the Sahelian environment are the homogenization of the soil before filling the pots (reducing experimental error) and the possibility to separate the effects of low soil P and water stress, which often interact in field experiments. On the other hand the amount of soil in such pots does not allow to obtain meaningful data of final dry matter and grain yield that are comparable to field conditions. The local temperature regime is isohyperthermic (van Wambeke, 1982) with an average temperature of 31.7 °C during our trial (minimum of 20.2 °C and maximum of 43.1°C).

At the onset of the trial in each pot five seeds were placed at 2 cm depth in three central holes . Two levels of P were used: non-limiting P (HP) with an addition of 0.4 g P/pot as 2 g of Diammonium Phosphate (DAP 18-46-0) and a P unamended control (LP) to which 0.78 g of urea were added to compensate for the N contained in DAP. The fertilizers were placed at the time of sowing and split into three holes in alternate off-centre position with respect to the seeding holes. At the end the six holes were 2 cm distant from each other. The experimental layout was a randomized complete block design with five replicates. Pots from LP and HP were kept separately to avoid shading effects, so physically there were two trials: one LP and one HP side by side. All plants stands were thinned to three per pot ten days after sowing and pots were watered each two days to avoid water limitation during the duration of the trial.

The parameters measured for each entry were: 1000-seed weight before planting, date of emergence, height (measured by stretching the longest leaf of the most vigorous stem per pot) at 2, 3, and 4 weeks after sowing (WAS), number of leaves, tillers, and diameter of the main stem. At 37 days after sowing (DAS), shoots were harvested and

roots extracted by gently washing off all adherent soil on a sieve. Biomass was sun-dried to weight constancy in cotton bags during two weeks followed by determination of shoot and root dry matter (DM). The latter were analyzed for P concentration to calculate P acquisition (total P content in the plant). All leaves were ground together with stems to obtain average shoot P concentrations as P is high in expanding leaves, but substantially lower in mature leaves (Suzuki *et al*., 2001). Estimates of shoot P concentration were obtained through Near Infra-Red Spectroscopy (NIRS) analysis, which were strongly correlated with standard wet chemical analysis for a subset of genotypes for shoot (r=0.96) and root dry matter (r=0.77; Figures 1 and 2). There were 91 comparisons for leaves (69 genotypes LP and 22 genotypes HP) and 122 comparisons for roots (56 genotypes LP and 66 genotypes HP).



Figures 1-2. Relationship between P concentration (mg P g^{-1} DM) of leaves (1) and roots (2) obtained through Near Infra-Red Spectroscopy (NIRS) analysis and P concentration (mg P g^{-1} DM) obtained through standard wet analysis of the leaves of a set of pearl millet genotypes grown in pots at different level of available P for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger.

To estimate the relationship between performance under pot conditions and field performance, the parameters measured in pots were correlated to grain yield data from independent locations in West Africa (Gemenet *et al*., unpublished; Haussmann *et al*., unpublished). These trials were conducted side by side under HP and LP conditions at four locations: Sadoré, Niger (17 36' 28.04" N; 8 4' 53.99" W); Gampela, Burkina Faso (12 25' 51" N; 1 22' 18" W); Bambey, Senegal (14 42' 2.66" N, 16 27' 32.8" W); and Koporo, Mali (14 3' 49.9"N; 3 4' 31" W) in the rainy seasons 2010 and 2011 (Table 1).

Table 1. Site characterization for the field trials used to correlate with pot data showing pH, Bray-1 P, annual rainfall (RF), nitrogen fertilization (N Fertilization) and phosphorus fertilization (P fertilization) under low P (LP) and high P (HP) conditions. '-' = data unknown.

					N fertilization		**P fertilization**	
		pH-H_2O	**Bray-P**	**RF**	**LP**	**HP**	**LP**	**HP**
Year	Location	(1:2.5)	(mg P kg^{-1})	(mm)	(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})
2010	Sadore	5.00	3.50	646	50	50	0	20
	Koporo	-	4.20	519	50	50	0	20
	Gampela	-	7.45	823	50	50	0	20
	Bambey	-	2.98	601	50	50	0	20
2011	Sadore	4.60	3.35	466	50	50	0	20
	Koporo	4.95	3.35	308	50	50	0	20
	Gampela	6.45	6.95	893	50	50	0	20
	Bambey	6.00	5.00	584	50	50	0	20

2.2.2 Calculation and statistical analysis

Grain yield (GY) P efficiency was calculated as the ratio between grain yields produced under LP and HP. On a subset of 18 genotypes (previously selected as contrasting genotypes for LP tolerance) seeds were analyzed for P concentration with standard chemical wet analysis before sowing. Subsequently, we calculated:

- P efficiency (%) = g shoot DM pot^{-1} under LP / g shoot DM pot^{-1} under HP (Ozturk *et al*., 2005);
- PAE = total shoot P content (mg shoot P pot^{-1}) = mg P g shoot DM^{-1} * g shoot DM pot^{-1} (equals Total P Uptake, TPU; Gill *et al*., 1994; Fageria and Baligar, 1997);
- Specific P Uptake (SPU; mg shoot P g root DM^{-1})= mg shoot P pot^{-1} / g root DM pot^{-1} (Zhu *et al*., 2001) and
- PUE = shoot DM produced per unit of P taken up = (g shoot DM $pot^{-1})^2$ / (mg shoot P pot^{-1} + mg root P pot^{-1}) (Gourley *et al*., 1994).

Data were statistically analyzed by two-way ANOVA using R version 2.15.0 (R Development Core Team, 2012). Parameters were log-transformed whenever their residuals were not normally distributed. The levels of significance used were: $P<0.05$, $P<0.01$, and $P<0.001$.

2.3 Results

As expected, shoot growth (height, diameter, number of leaves, and number of tillers) and dry matter across genotypes was lower under LP than with P application. Phosphorus supply led on average to a three-fold increase in total shoot dry matter, from 10 g at LP to 31 g at HP (Suppl. Table 1). The same trend was observed for total root mass which increased from 7.6 g to 20 g, respectively (data not shown). At two WAS HP plants were on average twice as tall as LP plants (31 cm and 16 cm, respectively).

The P efficiency ratio (relative shoot growth) showed a large genotypic variation, even if values were all below 50% (Figure 3). It ranged from 17.6 (variety 73 - Bondabia_C1) to 47.9 (variety 56 - PE08030 'SounaMau'), with a mean value of 32.5 (Suppl. Table 1). The genotypes 91 (PE03012) and 102 (Local_check_2) were most P efficient and produced higher dry matter under LP than all other genotypes. Plants on LP soil had on average a 73% lower shoot P concentration (1.81 mg P g^{-1} DM) compared to HP (6.62 mg P g^{-1} DM) where shoot P concentrations ranged from 3.7 to

10.2 mg P g^{-1} DM. Contrastingly, roots had a similar average P concentration under LP (1 mg P g^{-1} DM) and HP (1.4 mg P g DM $^{-1}$; Figure 4). Phosphorus concentration was higher in pearl millet shoots than in roots, in particular at HP. Shoot P concentrations were almost 5-fold respective root values (6.62 and 1.44 mg P g^{-1}DM; Figure 4).

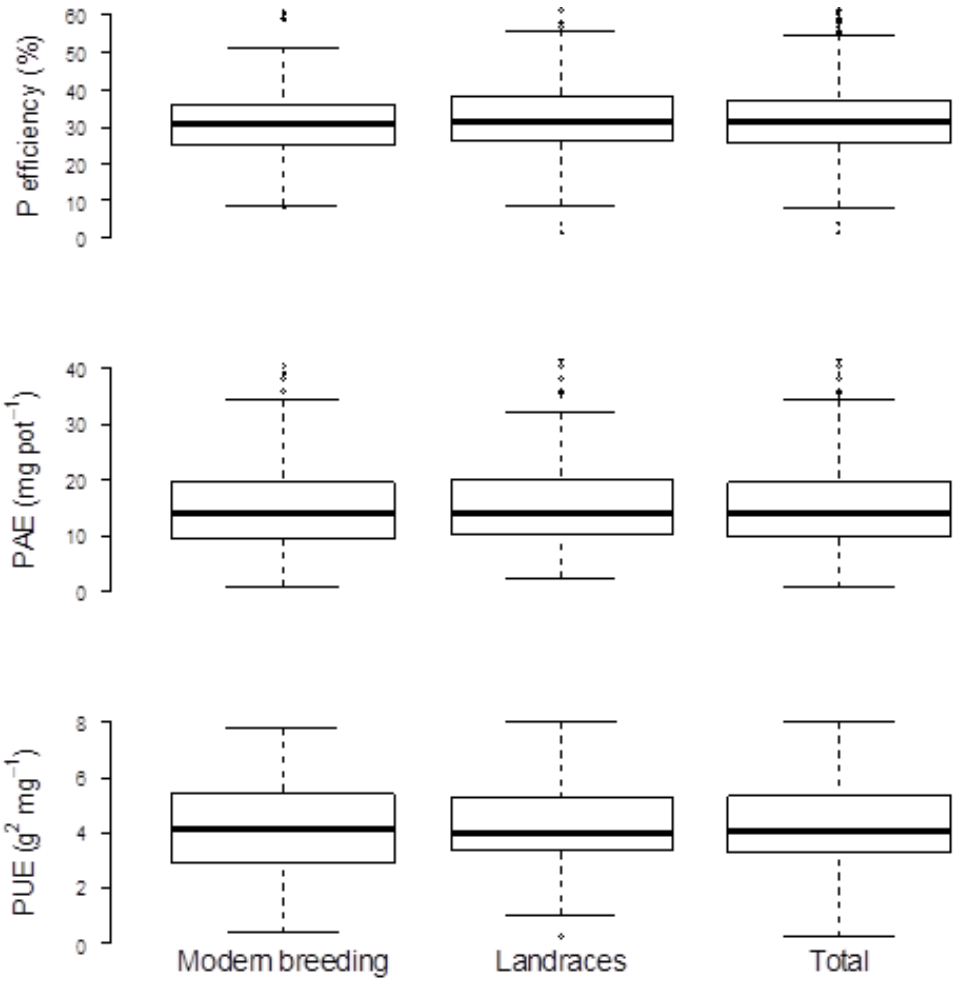


Figure 3. Variation of P efficiency (relative shoot growth), P acquisition efficiency (PAE) and P use efficiency (PUE) of 102 pearl millet genotypes ("Total") grown on a P poor acid Arenosol in pots for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger. The genotypes are 46 varieties bred by scientists and 56 farmer bred 'Landraces'.

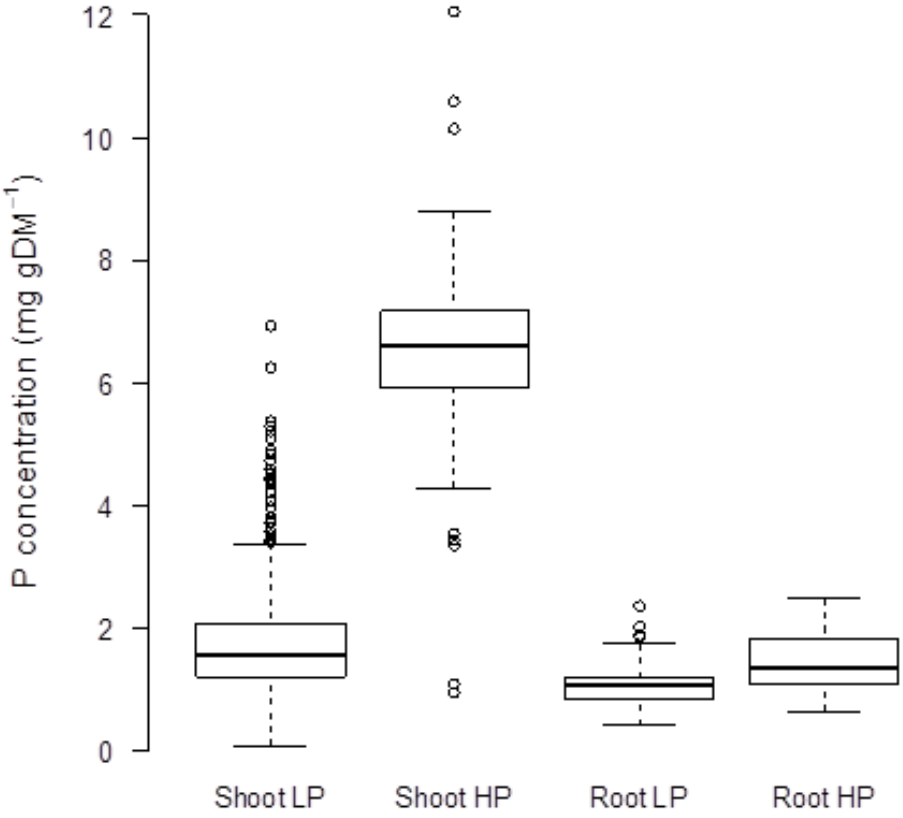
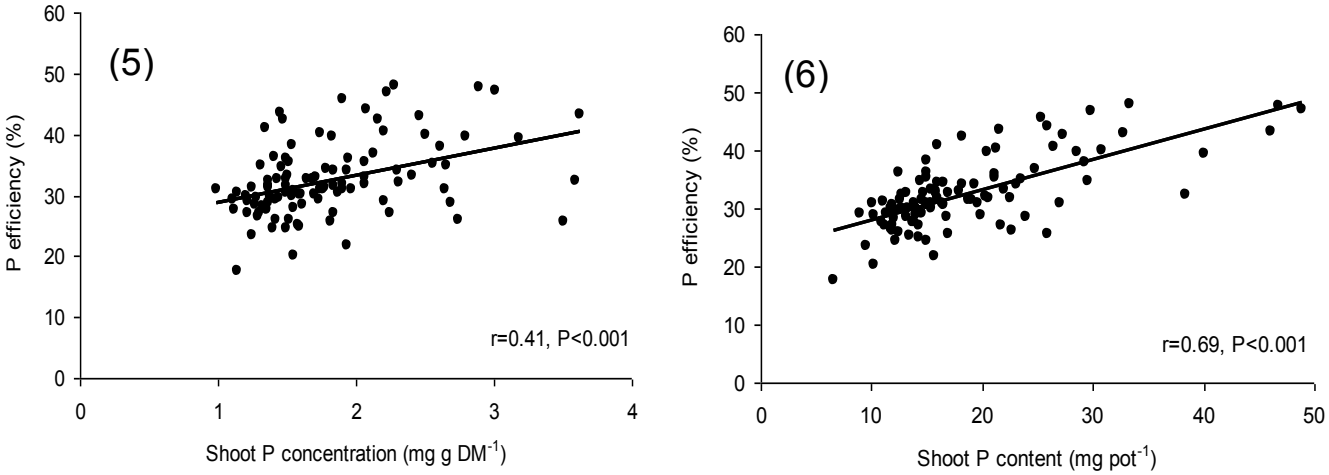


Figure 4. P concentration in the shoot (P accumulated in leaves and stems) and in the root under contrasting soil conditions (LP: no P supply, HP: supply of 0.4 g P pot^{-1} at sowing) of 102 pearl millet genotypes from West Africa and grown on a P poor acid Arenosol in pots for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger. The genotypes are 46 varieties bred by scientists and 56 farmer bred 'Landraces'.

The P efficiency ratio correlated with shoot P concentration at LP and shoot P content at LP ($r=0.41$ and $r=0.69$, respectively, both $P<0.001$; Figures 5 and 6), but not with shoot P concentration and content at HP, whereby the latter comprises a large autocorrelation between shoot DM and shoot DM in shoot P content. In most cases, genotypes showing higher P efficiency had higher shoot dry matter under LP. The mean root:shoot ratio was higher at LP (0.81) than at HP (0.67), and varied among genotypes.



Figures 5-6. Relationship between P efficiency (relative shoot growth) and shoot P concentration (5) and shoot P content (6) under low P conditions (no P supply) of 102 pearl millet genotypes grown on a P poor acid Arenosol in pots for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger.

Total shoot DM at LP correlated weakly with shoot P concentration at LP (r=0.33, P<0.001), indicating no major P dilution effect. Shoot P content and specific P uptake varied across genotypes at LP. Shoot P content varied from 7 to 65 mg P pot^{-1} at LP and from 116 to 298 mg P pot^{-1} at HP (Suppl. Table 2). Total shoot P content (PAE) was slightly higher in landraces (19.7 mg P pot^{-1}, p=0.08) than in breeder varieties (17.3 mg P pot^{-1}). Phosphorus use efficiency varied among varieties (p=0.07) from 2.1 (var. 65) to 7.1 g^2 mg P^{-1} (var. 78; Suppl. Table 2), with an average of 3.9 g^2 mg P^{-1}. At high P, in contrast, treatment differences were not significant (3.93 g^2 mg P^{-1} at LP and 4.19 g^2 mg P^{-1} at HP). In contrast to shoot P content, average PUE values did not vary between breeder varieties and landraces at LP (Figure 3), but they tended to be higher for breeder varieties at HP (Figure 7). At LP, 30 of the 50 most P efficient varieties had high P uptake (> 18.8 mg P $plant^{-1}$), 26 had high PUE (> 3.9 g^2 mg P^{-1}); 15 genotypes had

high PAE and high PUE. None of the growth parameters analyzed (height, number of leaves, and tillers at 2 and at 4 WAS) correlated to PAE and PUE.

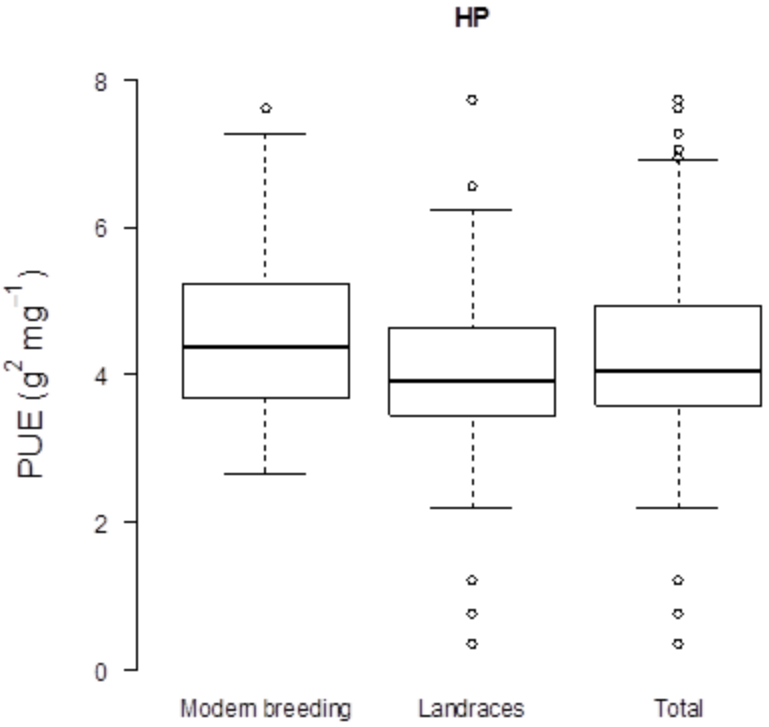


Figure 7. Variation of P use efficiency (PUE) under high P (supply of 0.4 g P pot^{-1} at sowing) of 102 pearl millet genotypes grown on a P-poor acid Arenosol in pots for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger. PUE at HP is equal to the square of shoot dry matter divided by total P content in the plant (shoot and root) under high P conditions. The genotypes are 46 varieties bred by scientists and 56 farmer bred 'Landraces'.

Variety 82 (Serkin_C2_Kandela_SMS) acquired much P at HP (10.2 mg P g^{-1} DM, data not shown), but it did not transform it efficiently (2.6 g^2 mg P^{-1}) while under P limitation plants took up a small quantity of P in the shoot (18.2 mg P pot^1) and produced relatively high biomass (PUE=5.8 g^2 mg P^{-1}, Suppl. Table 2). Early height values (at 2 and 4 WAS) were positively correlated with P efficiency (r=0.47 and r=0.43 respectively, both P<0.001).

In relation to field data, shoot height at 4 WAS in pots correlated with HI (ratio of grain yield to total plant weight, r=0.31, P<0.05) while total DM (sum of shoot and root dry matter) in pots weakly correlated with grain yield (r=0.19, p=0.07) of the field trial Sadore 2010. Shoot DM under LP and HP in pots correlated with GY P efficiency in Gampela 2011 (r=0.18, P<0.05 and r=0.30, P<0.01 respectively) whereas shoot DM under HP correlated with GY P efficiency in Sadore 2010 (r=0.34, P<0.001). Shoot P content under LP in pots was associated with GY P tolerance in Gampela 2010 (r=0.23, P<0.05).

Seed weight differed among genotypes, but made no major contribution to differential plant biomass production and P efficiency. In the subset of 18 genotypes the P concentration of seeds used for sowing ranged from 3.5 mg g^{-1} DM to 4.7 mg g^{-1} DM (data not shown). Phosphorus concentration as well as total seed P did barely contribute to early seedling vigor and differential P efficiency.

2.4 Discussion

The present study shows major genetic variation in early P efficiency of the examined 102 pearl millet varieties from West Africa. Phosphorus efficiency ratios were very small as DM under stress was always less than half one of that produced without P supply. Phosphorus concentrations were higher in pearl millet shoots than in roots, where P concentrations were also less variable. Phosphorus supply enhanced shoot P concentration reflecting rapid translocation from the roots which at 37 DAS had already colonized the entire pot. In our study shoot P concentration under LP was positively correlated with P efficiency suggesting its utility for early selection. For the same germplasm root parameters, in contrast, showed only limited genotypic variation. In a similar study with a large set of wheat varieties grown under greenhouse conditions only P content, not P concentration, was a reliable criterion to rank genotypes for P efficiency (Ozturk *et al*., 2005). Similar results have been found in rice (Fageria and Baligar, 1997) and wheat (Korkmaz *et al*., 2009).

Across varieties the shoot / root ratio decrease at LP as under such conditions carbohydrates are preferentially transported to the roots (Smith *et al*., 1990;

Vance *et al*., 2003), combined with a higher elongation rate of individual root cells (Anuradha and Narayanan, 1991).

PAE and PUE varied greatly across the 102 pearl millet varieties. The variation was stronger at LP than at HP similar to the results reported by Wissuwa and Ae for rice (2001). Therefore selection efficiency for these traits is expected to be higher under LP. Our data indicate that high P efficiency is slightly more related to an above average P acquisition than to an above average P use efficiency at LP. This suggests a more pronounced contribution of enhanced P acquisition capacity than use towards high P efficiency in the tested materials, but these results might be carefully interpreted as they are dependent on the formula used. Similarly in maize plants, P acquisition was far more important than P use efficiency in determining overall P efficiency on an acidic and calcareous low P soil (Corrales *et al*., 2007; Parentoni and Junior, 2008). This is in line with a recent review concluding that PAE has a dominant effect on P efficiency of crops grown under LP (Wang *et al*., 2010). Only a few of our pearl millet genotypes had above average P acquisition and use efficiency. Similar results were also found in a study with a large number of wheat (*Triticum aestivum* L), triticale (*Triticum aestivum* x *Secale cereale* L.) and rye (*Secale cereale* L.) varieties, where no genotype was efficient in both P uptake and utilization (Osborne and Rengel, 2002a). According to Wang and colleagues (2010) PUE becomes more relevant with increasing P availability, as it was in our case. This was probably due to an enhancing effect of P availability on P utilization efficiency. This effect was more pronounced in breeder varieties, probably due to preferential selection under fertilized conditions effected by scientists as compared to farmers.

The variation of PUE likely reflected the different P uptake capacities of the selected genotypes. According to Rose and colleagues (2012) a negative power function relating P use efficiency to shoot P accumulation indicated that PUE and PAE are closely correlated. These authors pointed out that under such conditions high PUE values may indicate P starvation, which was not the case in our study where dry matter at LP increased with increasing P concentration. Genotypes may have comparable PAE, but differ greatly in P efficiency (Figure 6). This may help in the selection of

genotypes for high biomass production and low P concentration (high PUE). Such combination of traits may allow to break the vicious cycle of further depleting a soil's P pool with increasing yields (Henry *et al*., 2010). Nonetheless such a selection at early growth may imply a smaller P source at later growth stages and lead to a poorer nutritional quality of crop residues (Maroko *et al*., 1999).

An alternative hypothesis is that P acquisition mechanisms are critical for P efficiency. Thus genotypes can be selected for their correlation between P efficiency and P content (Figure 6). In this case the selection focus is on PAE. Nevertheless the mechanisms involved in P uptake and utilization are closely associated and their interaction is complex. According to the theoretical model for rice elaborated by Rose and Wissuwa (2012), an increase in root PUE of 20% would lead to a 20% higher root biomass and a 20% enhanced soil P uptake that would have been taken up in a genotype without increased PUE. Experimental evidence supports the notion that a separation of PUE and P uptake is difficult since enhanced PUE would also increase P uptake (as biomass is allocated into root length) and depletion of soil P (Zhu and Lynch, 2004).

In our research the low P tolerant variety 26 (PE05387) had accumulated three times more P than the sensitive variety 85 (2898x92222_C1_Sad_Low_2009), and supplementary work showed that PE05387 plants grown in lysimeters under limiting P conditions had roots almost twice as long than the roots of variety 2898x92222_C1_Sad_Low_2009 at two WAS (46 cm and 26 cm, respectively) and longer total shoot length at five WAS (58 cm and 49 cm, respectively; unpublished data).

A genotype with higher P acquisition efficiency is likely to have higher relative root growth because the additional P taken up will allow for additional assimilate allocation also to grow neew roots. Grouping of our varieties shows that PAE was higher in landraces while PUE was higher in breeder varieties. The reason for this distinction is not clear, but analogous results were found in rice by Wissuwa and Ae (2001) and in sorghum (*Sorghum bicolor* M.), where the landraces group also had higher mycorrhizal infection (Leiser *et al*., unpublished data).

Apparently, the crop species so far investigated did not evolve a general mechanism conferring high P efficiency and the complexity of relation between PAE, PUE and their components such as root length, P translocation, expression of high-affinity P transporters and mycorrhizal infection confounds the identification of the causes of P efficiency. Further research on genotypes contrasting for their response to low P is requested to analyse genotypes-specific strategies of P uptake and low P tolerance. The variety 82 (Serkin_C2_Kandela_SMS) is of particular interest as it showed a very high capacity of P acquisition at HP which was coupled with a much lower uptake under LP conditions, but it was still able to produce high DM. This variety was among the 15 varieties with highest P efficiency ratio and P use efficiency (Table 3). In a consecutive pot trial conducted in Niger in 2011 on a subset of twenty genotypes selected for tolerance to LP, Serkin_C2_Kandela_SMS plants were within the three best performing varieties, with an average of 8.9 cm height at three WAS (unpublished data). This variety originated from participatory breeding in a woman farmer's field at Serkin Houssa (Niger), with typically poor soil conditions. Variety 78 (StrigaRes_2009_Sad_Cinz_comb) also stands out as it has high PAE and PUE.

Until now the majority of crop breeding research has focused on improved PAE to counteract the consequences of soils' low P status as encountered by many poor farmers worldwide (Richardson *et al*., 2011). This may be a reasonable option only for P application on soils with low P-sorption capacity. Given the low P concentration in shoots compared with seed the return of crop residues to the field will not help much to balance P losses from harvested grains. An alternative is to enhance P efficiency by selecting for strategies to mobilize soil P from less available fractions (Braum and Helmke, 1995) but these strategies are energetically expensive leading to reduced growth. Further research should address this as well as to improve P use in plants, which would successfully reduce the export of P from the soil at harvest.

In our pot study two genotypes showing high PUE at LP also had high DM production in lysimeters at maturity, but lower grain yield (r=0.35 and r=0.42, respectively) in LP soil and water stressed conditions (data not shown). This indicates that PUE efficiency for shoot and grain yield will need to be considered separately.

There is evidence supporting the hypothesis that enhanced P use in photosynthetic tissues leads to a down-regulation of high-affinity Pi-transporters in reproductive tissues, which may result in a decrease of grain P levels (Lambers *et al*., 2006; Rose *et al*., 2010). Gemenet and colleagues (unpublished) reported positive response to selection of early growth traits of pearl millet measured in pots (Sadore, Niger) in an index with grain yield under field conditions. The presence of low, but significant correlations between the traits measured in pots and GY under LP suggests some potential for indirect selection for higher GY under LP conditions in pot trials with young millet.

Seed size is a key determinant of evolutionary fitness in many species (Orsi and Tanksley, 2009) and seed reserves govern P accumulation and root development (Zhu and Smith, 2001), which is fundamental to determine plant performance at early growth stages. That also explains why seed P concentration is often high in species that evolved on P poor soils (Groom and Lamont, 2010). Higher seed P can contribute to higher P efficiency and this variable is often considered when evaluating genotypes for P efficiency (Teixeira *et al*.,1999; Liao and Yan, 1999; Zhu and Smith, 2001). Nonetheless such a relationship was not detected in the subset of 18 genotypes of our study where neither P concentration nor total P content in seeds affected early vigor nor P efficiency. This indicates that genotypic variation of P efficiency is rather unrelated to seed P concentration or seed size (Osborne and Rengel, 2002a). In a wild species this could be a strategy to the benefit of seed number, an ecologically important trait in an unpredictable environment such as the Sahel, but it is not likely to be the case of pearl millet whose bigger seeds are intentionally selected by farmers and breeders.

Finally, traits related to PUE should thoroughly complement high PAE traits in future breeding efforts and P fertilization practices if we want to foster P cycling, in particular in low or zero input agricultural systems, such as sub-Saharan West Africa.

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3 Effect of early mycorrhization and colonized root length on low soil phosphorus tolerance of eight West African millet varieties

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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) are well known to enhance P uptake and crop performance of millet. Assessing the genotypic variation of early mycorrhization and its effect on plant growth is necessary to clarify mechanisms of tolerance to low soil P. Therefore we in this study, eight pearl millet varieties contrasting in tolerance to low P were grown in pots under low P (no additional P supply) and high P (+0.4 g P pot^{-1}) conditions, and harvested at two, four, six and eight weeks after sowing (WAS). Root length was calculated at 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}), tolerant genotypes had greater total root length infected with AMF (83700 cm), higher percentage of AMF colonization (11.6%) and increased P uptake (69.4 mg P $plant^{-1}$) than sensitive genotypes (17700 cm, 7.1% colonization and 46.4 mg P $plant^{-1}$, respectively). At 2 WAS tolerant genotypes were infected almost twice as much than sensitive ones (4.1% and 2.1%) and the individual tolerant 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 enhances 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 use efficiency

3.1 Introduction

In the West African Sahel above 300 mm of annual rainfall, plant productivity is mainly limited by low P availability (Bationo *et al*., 1990, 1992; 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 (Bucher, 2006; Allen, 2007; Reinhardt, 2007). It has been shown across many species that AMF hyphae can solubilize 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 (Li *et al*., 1991; Liu *et al*., 2000; 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 the available soil P (Covacevich *et al*., 2007). In sorghum, 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). Ramos-Zapata and colleagues (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. On the Sudano-Sahelian soils it was found that the beneficial effect of cereal (sorghum, pearl millet) / legume (groundnut, cowpea) rotation on dry matter production of cereals could be explained by higher AM infection levels early in the season at 35 DAS (Bagayoko *et al*., 2000), but no information was reported about the effect of an earlier mycorrhization.

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 the low P tolerant varieties had longer roots and higher levels of AMF infection at early growth stages than the sensitive ones. We also intended to assess whether the peak of AMF infection at a certain harvest time is critical for P uptake. Both the extent of AMF infection and the degree of benefit from AMF are plant heritable traits selectable through plant breeding (Krishna *et al*., 1985; 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 tolerant to low P soils as well as the development of more efficient methods to apply P.

3.2 Material and methods

3.2.1 Study site and experimental layout

The 8-week experiment was carried out during the rainy season (July-August) 2012 in 18 liter pots at ICRISAT Sahelian Centre in Sadoré, 40 kilometers SE of Niger's capital Niamey (13° 23' N, 02° 27' E, 206 m asl). The local temperature regime is isohyperthermic (van Wambeke, 1982) 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_20:soil), 3.3 mg Bray-P kg^{-1} soil, C_{org} 0.3% and 207 mg total N kg^{-1} soil (Buerkert *et al*., 1995). The experimental design was a split plot with eight different genotypes as split-plot factors: four genotypes tolerant and four sensitive to low-P conditions. The tested materials originated from Mali, Niger and Senegal, with their tolerance 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 (control, '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.

Table 1. Identification number, name, level of tolerance 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 eight 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	tolerant	Mali
6	PE03089	tolerant	Senegal
7	Madougou5	tolerant	Mali
8	Serkin_C2_Kandela_SMS	tolerant	Niger

Half of the pots were fertilized with P 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 Hiernaux, 1995; Buerkert and Schlecht, 2013). The LP pots received 1.1 g of potassium sulphate (K_2SO_4) to compensate for K contained in KH_2PO_4 of the HP pots. Nitrogen was applied at 20 days after sowing (DAS) as top-dressed urea to all pots. At 14 DAS plants were thinned to one plant per pot. Plants were harvested at 17, 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). A total of 256 pots were sown (8 genotypes x 2 P soil conditions x 4 repetitions x 4 harvests).

3.2.2 Agronomic parameters

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 stem, shoot and root dry matter (DM) (oven dried at 60°C), root length density (RLD), AMF infection (%)and total P concentration in shoot and root. 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. P 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.

3.2.3 Determination of root length density

RLD was determined by scanning total fresh roots at 2 WAS. To this end we carefully washed samples and then sieved them 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 roots 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).

3.2.4 Arbuscular mycorrhizal fungi colonization and statistical analysis

Fresh root samples (corresponding to the entire root system) were preserved in 50% ethanol-water solution inside a 2 ml Eppendorf tube. Around 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 (Phillips and Hayman, 1970; 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 trial was conducted from mid October 2012 following exactly the same experimental set up 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 compare it with the outcome of the first trial.

Data was statistically analyzed by one- and two-way ANOVA using R software. Parameters were log-transformed when their residuals were not normally distributed. Significance levels were computed at: $P<0.05$, $P<0.01$ and $P<0.001$, whereas results at $P>0.05$ are shown as absolute numbers.

3.3 Results

3.3.1 Low P conditions

Shoot biomass differed among genotypes ($P<0.01$) and between sensitive and tolerant varieties ($P<0.05$) 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) at 2 WAS. Tolerant varieties tended to have higher RLD (0.022 cm cm^{-3}) than sensitive ones (0.018 cm cm^{-3}, Figure 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 ($r=-0.713$, $P<0.001$). Shoot P concentration varied from 1.54 to 6.81 mg P g DM^{-1}, which was the level reached at 2 WAS by tolerant variety 5. The tolerant genotypes had higher shoot P concentration (3.7 mg P g DM^{-1}) than sensitive ones (3.3 mg P g DM^{-1}, $P=0.078$). When each harvest time was considered separately, the differences among individual genotypes became apparent ($P<0.05$). Root P concentration ranged from 0.48 to 4.20 mg P g DM^{-1} across harvests at 6 and 8 WAS. In contrast to shoot P concentration, root P did not differ among genotypes irrespective of their P sensitivity. Tolerant 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, $P<0.01$). Total shoot P content increased on average from the first to the last harvest (0.44 to 59.70 mg P $plant^{-1}$,

Figure 2). At the third and fourth harvest tolerant genotypes had significantly higher shoot P, while individual genotypic differences were evident at 8 WAS, with varieties 8 and 7 outperforming the others (Figure 3).

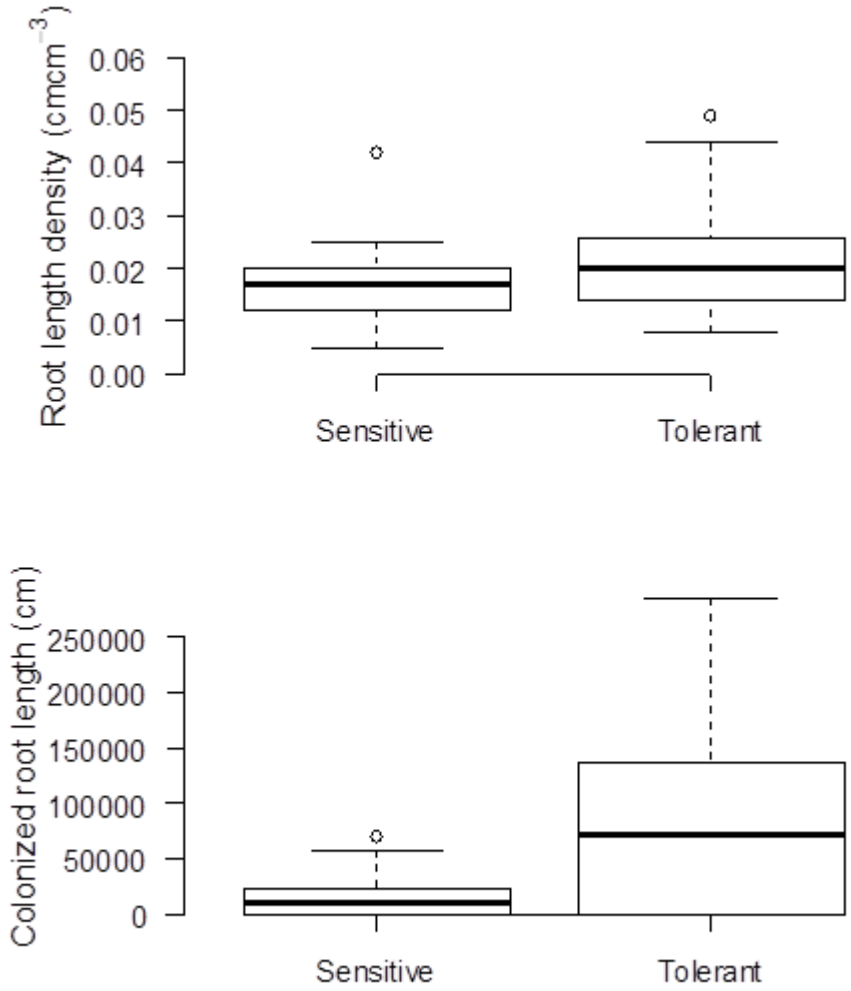

Figure 1. Root length density and root length colonized by arbuscular mycorrhizal fungi of eight pearl millet varieties grown under low P soil conditions and harvested at 2 weeks after sowing at ICRISAT Sahelian Centre, Sadoré, Niger. Varieties are grouped into 4 sensitive and 4 tolerant to low P soil in 2012. Colonized root length was higher in the tolerant varieties as compared to the sensitive ones ($P<0.001$).

Percentage of AMF colonization increased significantly across harvest times, averaging from 0.5 (genotype 4, sensitive) to 26.4% (genotype 5, resistant, Figure 2). This was strongly correlated with shoot dry matter ($r=0.55$, $P<0.001$) and total shoot P

(r=0.59, P<0.001), but not with P content 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 tolerant and sensitive genotypes (P<0.01).

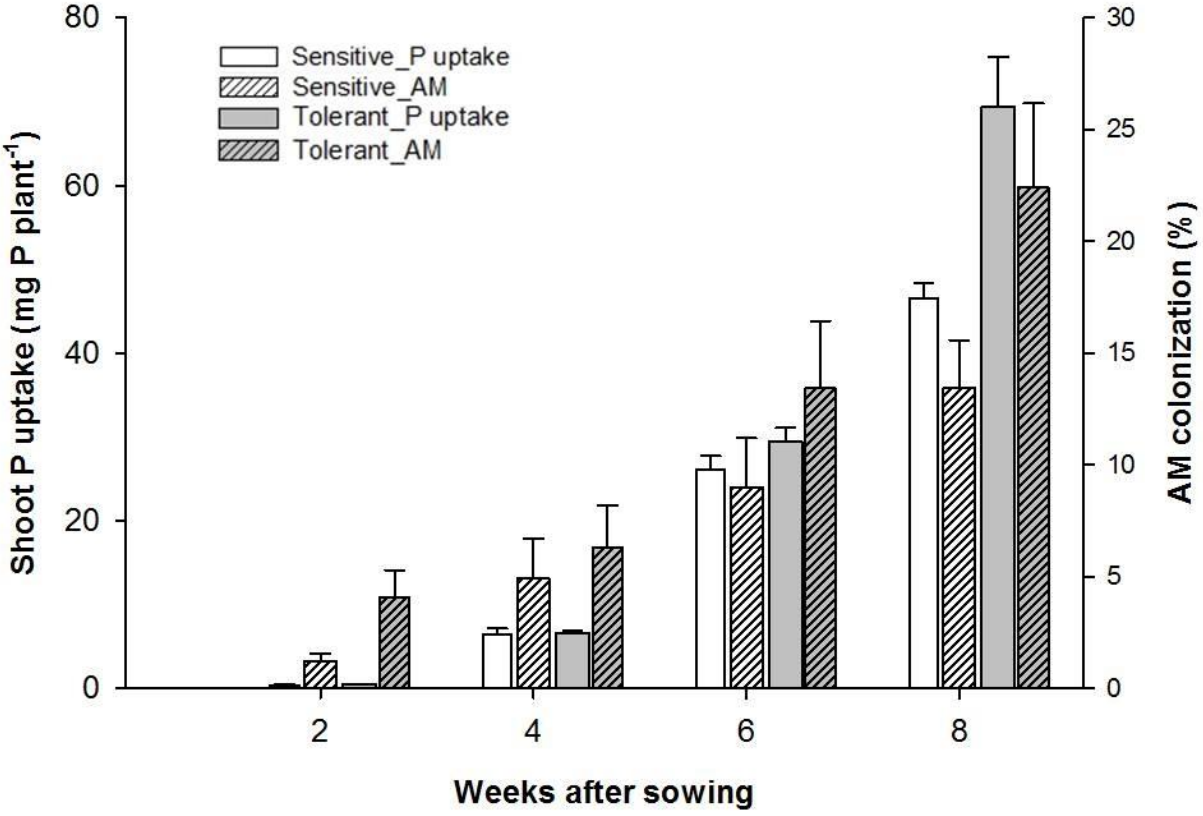

Figure 2. Shoot P uptake (total amount of P in the shoot) and arbuscular mycorrhizal colonization of eight pearl millet varieties grown under low P conditions at ICRISAT Sahelian Centre, Sadoré, Niger and harvested at 2, 4, 6 and 8 weeks after sowing. Pearl millet genotypes are grouped into sensitive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

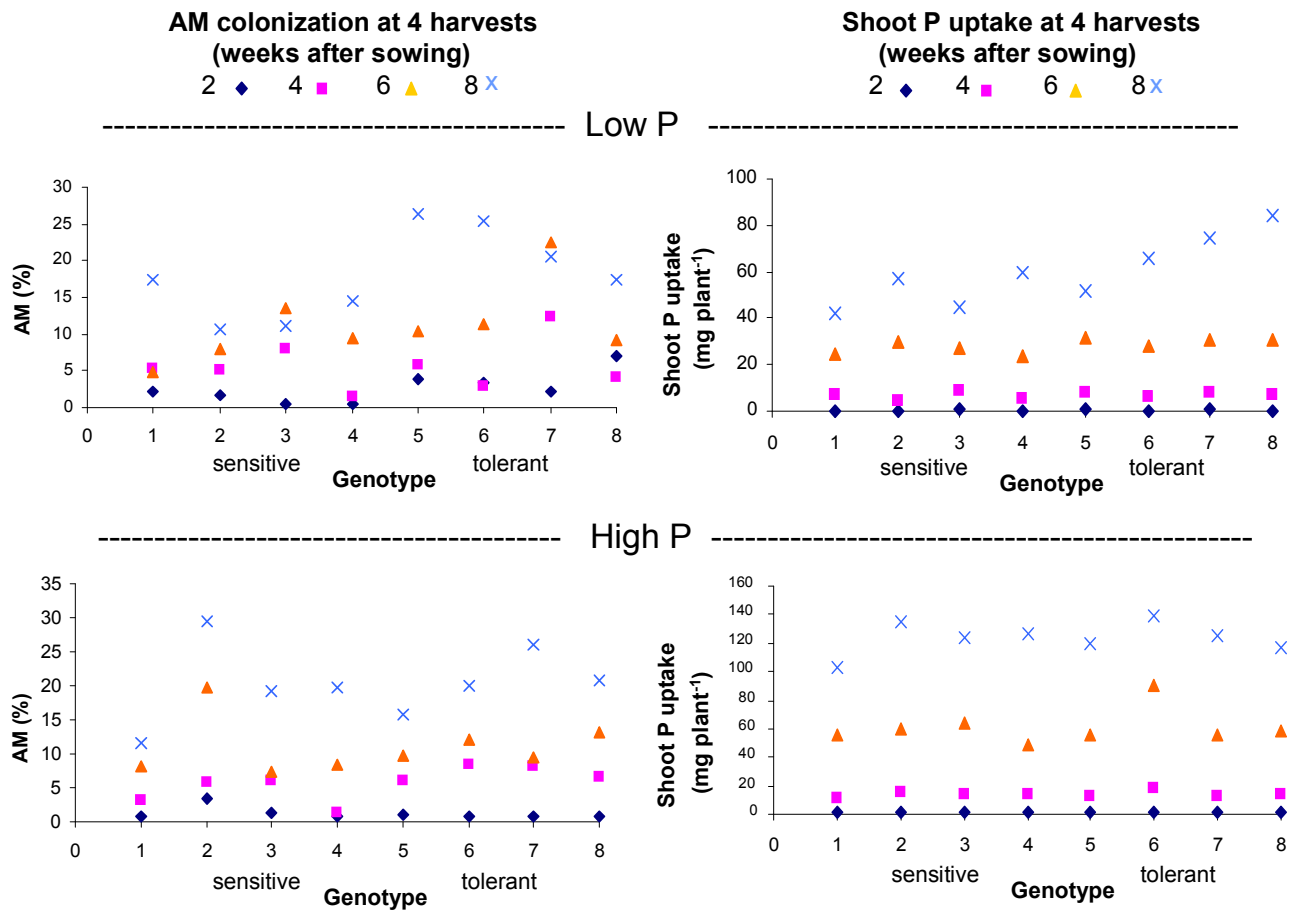

Figure 3. Arbuscular mycorrhizal (AM) colonization and shoot P uptake (total amount of P in the shoot) of eight pearl millet varieties grown under low P (no P application) and high P (supply of 0.4 g P pot^{-1}) conditions at ICRISAT Sahelian Centre, Sadoré, Niger and harvested at 2, 4, 6 and 8 weeks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

Tolerant genotypes had higher AMF infection (11.6%) and longer colonized root length (83700 cm) than sensitive ones (7.1% and 17700 cm respectively, Figure 1). At 2 and 8 WAS tolerant genotypes were significantly more colonized with AMF than sensitive ones ($P<0.05$). By 8 WAS, shoot P uptake differed between tolerant and sensitive genotypes (Figure 2), indicating the positive effect of an early discrimination between the two groups. The low P tolerant variety 7 had the greatest AMF infection across harvest times (14.5%) and sensitive variety 2 had the lowest (6.4%). At 2 WAS

there was significant genotypic variation in AMF infection within the group of tolerant varieties (ranging from 2.2% to 7.0%, $P<0.05$). Variety 8 had highest AM infection at 2 WAS, whereas variety 7 had highest infection at 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 at 2 WAS plants grown at LP had higher interaction with AMF than plants at HP (Figure 3). The sensitive variety 2 with lowest AMF colonization at LP had very high colonization at HP. In contrast, the tolerant variety 5 recorded greatest AMF infection at 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 ($r=0.26$, $P<0.05$). In both trials two varieties not matching were excluded from this calculation. No similar correlation was found for root dry matter.

3.3.2 High P conditions

RLD at 2 WAS ranged from 0.020 (genotype 1) to 0.049 cm cm^{-3} (genotype 3), and was on average twice as high as at LP ($P<0.001$). Also shoot P concentration was on average two-fold higher than at 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 at 6 and 8 WAS. AMF colonization across harvests was 9.4% on average, similar across P levels. At 2 WAS AMF colonization under HP was just half (1.2 %) of that under LP (2.6%), but from 4 WAS onwards both treatments were similarly infected. None of these parameters differed either between tolerant and sensitive genotype groups or among individual genotypes. In fact, the higher AMF infection at 2 and at 8 WAS and longer colonized root length of tolerant genotypes observed at LP were not found at HP. Examining AMF infection and shoot P uptake at each single harvest (Figure 3) yielded no significant differences between tolerant and sensitive genotype

groups; and no significant differences among the individual genotypes. This suggests that the tolerant varieties had specific adaptation strategies to low soil P.

At 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 $P<0.001$, respectively).

3.4 Discussion

Pearl millet varieties previously selected as tolerant 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 to discriminate between varieties tolerant and sensitive to P limited soil conditions. At LP, as early as at 2 WAS tolerant varieties were significantly more colonized with AMF than sensitive ones, which led to higher P uptake and shoot dry matter of tolerant varieties at the end of the trial. At 2 WAS, AMF infection under LP was twice that under HP. This suggests that, first, some tolerant varieties were better adapted to LP because of earlier physiological detection of P deficiency and subsequent consequent interaction with AMF. Second, the P microdose applied at sowing was high enough to keep the shoot P status at 2 WAS at a non-critical point to interact with AMF, even roots grew more and therefore likely interacted with more AMF spores in the soil. Later during the growth, added P must have decreased in the soil and at 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), whereby AMF colonization negatively related to availability of P in soil (Krishna *et al*., 1984).

Our data suggests that very early mycorrhization plays a pivotal role in tolerance to P stress and root colonization with AMF from 2 WAS onwards is and adaptative strategy of millet with lack of P. 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 among the tolerant genotypes. Out of the eight genotypes, variety 8 had of the highest

percentage of AMF infection at 2 WAS; variety 7 at 4 and at 6 WAS; and varieties 5 and 6 had the peak at 8 WAS (Figure 3).

One possible explanation for this varietal difference is that genotypes investing assimilates early in setting up a symbiosis with AMF such as genotypes 7 and 8 have later carboxylate production and that an early carboxylate production corresponds to a late infection with AMF. This hypothesis is advanced since mycorrhization and carboxylate exudation are a major carbon drains so there may well be a trade-off between these two strategies (Ryan *et al*., 2012). Carboxylates are well known for mobilizing inorganic P and organic P by complexing the metal cations that bind phosphate (Jones *et al*., 2003; 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 (Goldstein *et al*., 1988; Lambers *et al*., 1998; Ezawa *et al*., 2005). However, any understanding of the different P acquisition strategies in pearl millet must also be aware of possible specific interactions of plant genotype x AMF species. Recently 30 different AMF species have been identified in a soil sample collected 2012 in Mali on an Arenosol similar to the one from Niger used in this 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 data). This suggests an intriguing evolutionary host-symbiont interaction at a fine taxonomic level.

At 2 WAS RLD did not show any varietal difference as it was previously found at the flowering stage of pearl millet in Niger (Brueck *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. The relationships between AMF colonization, P uptake and shoot biomass of millet, confirm our initial hypothesis and are in agreement with the body of existing literature (Tinker *et al*., 1992; Raiesi and Ghollarata, 2006). The outcome of this pot trial was comparable to field conditions, as the biomass produced in pots at 2 and 8 WAS positively correlated with total dry matter at the end of the growing cycle and total

yield (leaves, stem 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 (Abbott and Robson, 1991; Luetter *et al.*, 1993; Bagayoko *et al*., 2000).

Further studies focusing on the regulation between early mycorrhization, carboxylate release and phosphatase excretion, and final grain and biomass yield are needed in order to develop a useful indirect screening method for tolerance 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. To foster early mycorrhization system components such as agroforestry, minimum soil disturbance at cultivation such as traditionally practiced by most farmers in the West African Sahel and crop rotation are more suitable alternatives than inoculation (Garcia *et al*., 2007; Cardoso and Kuyper, 2006).

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4 Tolerant pearl millet (*Pennisetum glaucum* (L.) R. Br.) varieties to low soil P have higher transpiration efficiency and lower flowering delay than sensitive ones

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Abstract

In the West African Sahel low soil phosphorus (P) and unpredictable rainfall are major interacting constraints to growth and grain yield of pearl millet. Investigating the relationship between transpiration and final yield under the combined effect of water and P stress is fundamental to understand the underlying mechanisms of tolerance and improve breeding programs. We conducted two lysimeter trials using 1 m long PVC tubes (35 cm diameter) filled with a P poor Sahelian soil mimicking soil profiles to assess grain and stover yield, and water use of 15 pearl millet genotypes grown under different P (no P supply or addition of 1.5 g P $tube^{-1}$) and water (well watered or terminal water stress) regimes. In experiment 2 transpiration was measured twice a week from tube weight differences, and transpiration efficiency (TE) was calculated as dry matter (DM) produced per kg of water transpired. Low soil P delayed flowering, and more so in sensitive genotypes. Later flowering of genotypes sensitive to low P made them more sensitive to terminal water stress. Under P limiting soil, genotypes tolerant and sensitive to low P used similar amounts of water (19.8 and 21.7 kg water $plant^{-1}$, respectively). However, tolerant lines transpired less water prior to anthesis (8.8 kg water $plant^{-1}$) leaving more water available for grain filling (11 kg water $plant^{-1}$) while sensitive lines used 14.4 kg water $plant^{-1}$ pre-anthesis, leaving only 7.2 kg water $plant^{-1}$ for grain filling. Low soil P decreased grain yield by affecting seed size at harvest and its damage during seed filling overrode the effect of seed size at sowing. Grain yield was positively correlated with water extracted after anthesis. TE was enhanced by P supply, especially in sensitive lines, and TE was higher in tolerant than in sensitive genotypes under low soil P. Pearl millet plants tolerant to low P were more resistant to the delay of flowering caused by low P soil and they presented higher transpiration efficiency. The pattern of transpiration was important to cope with terminal water stress under different levels of P availability. Higher transpiration after anthesis, resulting from conservative water mechanism pre-anthesis (higher TE) and possibly by a shorter delay in flowering under low soil P, enhanced grain yield.

Key words: Water stress; Lysimeter; Transpiration efficiency; Water use

4.1 Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the most important staple crop for millions of people in the semi-arid tropics of Africa (FAO and ICRISAT, 1996). Sahelian farming systems are characterized by heavily weathered soils, low external inputs and continuous extraction of plant nutrients which, over centuries, have produced an extremely phosphorus (P) poor soil, with values often below 5 mg Bray-P kg^{-1} soil (Bationo *et al*., 1990; Buerkert *et al*., 2000). In Niger the 4 months unimodal rainy season typically lasts from mid-May to mid-September, but precipitation is very scattered and often characterized by a late onset of the rains and mid- or end-season droughts. On the predominantly acid Arenosols dedicated to smallholder millet production, lack of rainfall and high temperatures (daily average temperature peaks in May at 34°C and drops in December to 25°C (World Climate, 2008)) quickly lead to major water stress for plants (Wallace *et al*., 1993). Low soil P and unpredictable water stress have for millennia been major interacting constraints to millet growth (Manu *et al*., 1991).

Phosphorus deficiency reduces leaf expansion (Fredeen *et al*., 1989), number of leaves (Lynch *et al*., 1991) and root development, which in turn affects the capacity of plants to uptake water from deeper soil horizons under low rainfall (Marschner, 1995). Nevertheless, plant roots have developed several strategies to enhance P uptake under low moisture conditions with subsequent reduced diffusion, such as increased root length, length and number of root hairs, symbiosis with arbuscular mycorrhizal fungi (Gahoonia and Nielsen, 2004; Liebersbach *et al*., 2004). In pearl millet, an enhancement of root hair formation was observed under P-deficiency (Faye *et al*., 2006). Phosphorus-deficient *Lotus japonicus* showed lower root hydraulic conductivity due to a decreased expression of genes encoding aquaporins which explained the reduction of epidermal cell (and leaf) expansion (Clarkson *et al*., 2000). Other adaptive plant development responses to P deficiency comprise delay of flowering (Nord *et al*., 2008), decreased number of flowers (Bould and Parfitt, 1973), inhibition of seed

formation (Barry and Miller, 1989) and premature leaf senescence, which all affect grain yield.

Despite numerous reports about the effects of P deficiency and water stress on millet growth (Manu *et al*., 1991; Buerkert, 1995; Bagayoko *et al*., 2000; Brueck *et al*., 2003; Valluru *et al*., 2009; Vadez *et al*., 2013), to our knowledge studies investigating the combination of both major stress factors on plant development are lacking, except few (Payne *et al*., 1990; 1992). The novelty of the present study is then in addressing this knowledge gap by using a lysimeter system consisting of large PVC tubes in which plants can be grown in a soil volume allowing soil exploration by the plant similar to field conditions, and where the effects of a factorial of P and water treatments can be investigated. The novelty of the approach is also in allowing to obtain highly relevant agronomic data in a system where the homogeneity of the soil can be controlled, whereas studies in low soil P field are often bound to face large field heterogeneity in P availability. Such a system has recently been used to assess water use throughout the cropping cycle until maturity in different crops (Ratnakumar and Vadez, 2011; Vadez *et al*., 2011; Zaman-Allah *et al*., 2011). It has also been tested to produce highly relevant agronomic data in low and high P soils (Karanam and Vadez, 2010). The setup allows to determine transpiration efficiency (TE, calculated as dry matter (DM) produced per kg of water transpired) to distinguish tolerant and sensitive genotypes (Ratnakumar and Vadez 2011; Vadez *et al*., 2011). It is well known that TE depends on interactions between water and nutrient availability (DeWit, 1958; Tanner and Sinclair, 1983; Clarkson *et al*., 2000; Vadez *et al*., 2014), and is particularly affected by nutrient deficiency (Payne, 2000). For Sahelian conditions, Sivakumar and Salaam (1999) reported a 84% increase of water use efficiency in millet (WUE, grain yield per mm rain), of which TE is an important component, due to the addition of mineral fertilizers. Therefore, under limited water supply, plant nutrients may play an important role in enhancing WUE (Waraich *et al*., 2011), and more research is required to understand genotypic differences in TE under various levels of P.

Seed size is a also a key determinant of evolutionary fitness in many species (Orsi and Tanksley, 2009) and seed reserves govern P accumulation and root development (Zhu and Smith, 2001), which is fundamental to determine plant performance at early growth stages. That is why seed P concentration is often high in species that evolved on P poor soils (Groom and Lamont, 2010). Higher seed P can contribute to higher tolerance to low P and this variable is often considered when evaluating genotypes for P efficiency (Liao and Yan, 1999; Zhu and Smith, 2001).

Studies on millet genotypes contrasting for terminal water stress tolerance identified two water-saving mechanisms, which consist in maintaining a lower transpiration rate even at low vapour pressure deficit (VPD) and by further decreasing transpiration rate under higher VPD (Kholova *et al*., 2010a, 2010b). These mechanisms enhanced water availability during the reproductive and grain filling period, leading to higher grain yields under terminal stress (Vadez *et al*., 2013). Similar effects of water stress during periods critical for grain yield formation have been reported for chickpea (Zaman-Allah *et al*., 2011), sorghum (Hammer *et al*., 2006) and cowpea (Belko *et al*., 2012). Because low soil P condition are known to delay flowering, the combination of a water and a low P stress is therefore likely to modify the proportion of water used before and after anthesis, and then to have possible negative consequences on the grain filling.

The first hypothesis of this work was that the low soil P conditions would alter the phenological development and the water use efficiency of the crops in ways that could explain part of the genotypic differences in the yield under low soil P conditions. The other hypothesis was that differences in plant development and growth under low soil P would alter the kinetics of plant water use and would then have profound effects on the plant response to water limitation. The objectives of this work were then three-folds: (i) to assess the effect of low soil phosphorus on the agronomic attributes, including flowering time, of a set of pearl millet genotypes; (ii) to monitor the kinetics of plant water use in a factorial of P and water treatments and assess the effect of low soil P on the pre- and post-anthesis water use; (iii) to measure the impact of low soil P on transpiration efficiency (TE). These analyses were done in pearl millet genotypes that

turned out to contrast for the seed yield under low soil P conditions, allowing us to infer some general trends of plant attributes characterizing low soil P tolerance in pearl millet.

4.2 Material and methods

4.2.1 Experimental conditions

Two experiments with 15 West African pearl millet varieties each were carried out: Experiment 1 (Exp. 1) was conducted between December 2010 and March 2011 and Experiment 2 (Exp. 2) between September and December 2012 (Table 1). The fifteen genotypes grown in Exp. 1 were selected from a collection of 102 genotypes according to their contrasting vegetative biomass production at five weeks after sowing in pot trials during the rainy season of 2010. The same procedure was applied to select the 15 genotypes for Exp. 2, based on results of a pot trial run during the 2011 rainy season. In Exp. 1, seeds were sown on 23 December 2010 at a rate of 4-5 seeds per pocket and 3 pockets per tube. During the cropping period, the maximum and minimum temperatures ranged from 32.1-41.1°C and 16.0-24.0°C, respectively, and relative air humidity at 1 pm averaged 12% (January-March 2011). In Exp. 2, seeds were sown on 27 September 2012 at the same rate as in Exp. 1. During the cropping period, maximum and minimum temperatures ranged from 33.4 to 38.3°C and 17.2 to 23.3°C, respectively, and relative humidity of the air at 1 pm decreased from 90% (September) to 13% (December). The same lysimeters were used in Exp. 1 and Exp. 2, and soil was not replaced between trials, though first cereals (pearl millet and sorghum) and then a legume (cowpea) were grown in the cylinders between the two trials.

Table 1. Genotype identification number, full variety name, selection category and country of origin of the 15 pearl millet genotypes grown in lysimeters in 2010 and 2012. In bold: genotypes used in both trials. Category *Breeding variety* refers to improved varieties.

Trial	Genotype identification number	Variety name	Category	Origin
2010				
	1	PE06001	Landrace	Burkina Faso
	2	PE00077	Landrace	Cameroun
	3	PE00397	Landrace	Mali
	4	CZ Boboni-Sanougoula	Breeding variety	Mali
	5	SOSAT_C88_Check_all	Breeding variety	Mali-IER-ICRISAT
	6	PE08057	Landrace	Mauritania
	7	PE08058	Landrace	Mauritania
	8	PE02724	Landrace	Niger
	9	ICMV IS 94206	Breeding variety	Niger-ICRISAT
	10	Striga_res_expvar_epis_long_noir	Breeding variety	Niger-ICRISAT
	11	M66xSosat_C1_Sad_Low_2009	Breeding variety	NigerxMali
	12	PE03089	Landrace	Senegal
	13	PE03012	Landrace	Senegal
	14	GB8735xMoro_C1_PF_SAD	Breeding variety	SenegalxNiger
	15	Sadore Local_check_1	Landrace	Niger
2012				
	1	GB8735xMoro_C1_PF_SAD	Breeding variety	SenegalxNiger
	2	2898x92222_C1_Sad_Low_2009	Breeding variety	NigerxNiger
	3	PE05387	Landrace	Mali
	4	StrigaRes_2009_Sad_Cinz_comb	Breeding variety	Niger-ICRISAT
	5	SOSAT_C88_Check_all	Breeding variety	Mali-IER-ICRISAT
	6	PE03089	Landrace	Senegal
	7	Madougou5	Landrace	Mali
	8	Serkin_C2_Kandela_SMS	Breeding variety	NigerxNiger
	9	Striga_res_expvar_epis_long_noir	Breeding variety	Niger-ICRISAT
	10	ICMVIS94206	Breeding variety	Niger-ICRISAT
	11	PE08030("SounaMau")	Landrace	Mauritania
	12	Doga_C2_PF_comb	Breeding variety	Niger
	13	Tera_C2_PF_comb	Breeding variety	Niger
	14	Serkin_C2_Ali_SMS2	Breeding variety	Niger
	15	Ankoutess	Breeding variety	Niger-ICRISAT

4.2.2 Description of lysimeters and soil preparation

The lysimeters consisted of PVC cylinders filled with the topsoil of a severely P deficient Arenosol (Bray-P < 5 mg P kg^{-1} soil) from the ICRISAT Sahelian Centre at Sadoré, Niger. These tubes (35 cm diameter, 100 cm height) yielded a plant spacing of 4-5 plants m^{-2}. The bottom of the tube consisted in a PVC plate maintained on top of four screws. Water drainage could take place between the PVC plate and the inner wall of the tube, although soil could not slip through. All lysimeters were placed upright in 1 m deep trench, over which the weighing mechanism could be moved to select individual cylinders for weighing (Exp. 2 only). The tops of the cylinders were equipped with metal collars and chains to allow the lysimeters to be lifted and weighed. The lysimeter weighting procedure involved a crane balance (S-type load cell with a 200 kg load capacity; Mettler-Toledo, Geneva, Switzerland) connected to a block-chained pulley to lift the tubes. The scale allowed repeated measurements at an accuracy of ± 20 g.

In order to mimic a low P profile, we collected top soil (0-0.2 m) and subsoil (0.2-0.8 m) from a low P field. The top soil had the following characteristics: 5.5 pH_{H2O} (1:2.5), 3.7 mg Bray-P kg^{-1} soil, Corg 0.3%, 247.4 mg total N kg^{-1} soil. The bulk soil was characterized as follows: 5.8 pH_{H2O} (1:2.5), 3.6 mg Bray-P kg^{-1} soil, Corg 0.1%, 81 mg total N kg^{-1} soil. Both soil types were kept separate and brought back to the farm, air-dried and homogenized thoroughly prior to filling the tubes with 95 kg subsoil and then 25 kg topsoil, leaving the upper 0.15 m of the tubes empty to allow for the application of a layer of anti-evaporation beads and for watering.

4.2.3 Treatment application and water extraction measurements

Two P treatments were used in each of the two experiments. The high P (HP) treatment in Exp. 1 consisted of applying 300 mg DAP kg^{-1} topsoil, i.e. 7.5 g DAP $tube^{-1}$ applied in a circle 2-3 cm around the seedling area after emergence. The low P (LP) lysimeters did not receive any P application, but were supplied with urea to compensate for DAP nitrogen input into HP tubes (3.45 g urea applied in two doses: 2 g after emergence and 1.5 g 3-4 weeks after sowing). For Exp. 2 in 2012, no application of P

was necessary because the soil in the Exp. 1 HP tubes from 2010 was still rich in available P (32.8 mg P g^{-1} soil). A top dressing dose of 2.5 g urea was applied on all the cylinders in Exp. 2 at 23 day after sowing (DAS). Millet plants were grown until maturity in both experiments, in Exp. 1, only one well-watered (ww) treatment was applied, whereas in Exp. 2, both a ww treatment and a water stress (ws) treatment were used. Transpiration was measured in Exp. 2 only.

In both experiments, seedlings were thinned to three plants per tube at 14 DAS and to one plant per tube at 25 DAS. Soil was kept at 90% of field capacity by weighing the tubes every 4 days, measuring the amount of water lost by evapotranspiration and compensating by proper rewatering.

In Exp. 1 the experimental design was a randomized complete block design with LP and HP treatments at either side of the trench in which all the tubes were placed in order to avoid HP plants shading the LP plants. The 15 genotypes randomized within each of the five repetitions (blocks). In Exp. 2 we used a design with P treatment as the main plot and water treatment as the sub-plots, the 15 genotypes were randomized 6 times within each sub-plot giving a four treatment factorial: LPws, LPww, HPws and HPww, where: LP = low P, HP = high P, ww = well watered and ws = water stressed.

In Exp. 2, we assessed plant transpiration as a proxy for plant growth. Thus, at 35 DAS, the soil surface was covered with a round plastic sheet superposed with a 2-cm layer of low-density polyethylene beads to prevent soil evaporation. The lysimeters were weighted every 4 days from 36 DAS to 81 DAS yielding a total of 12 measurements (39, 43, 46, 50, 54, 57, 61, 64, 67, 70, 74 and 77 DAS). Water extraction related to plant transpiration was calculated from cylinder weight differences between consecutive weighings and additions of water. Transpiration data were assigned to the latest weighing so that e.g. transpiration at 50 DAS refers to the water transpired by the plant in the interval between 47 and 50 DAS.

In Exp.2 all plants were irrigated until 57 DAS. There were altogether 180 LP tubes and 180 HP tubes (15 genotypes by 12 replicated tubes per genotype). Then both HP

and LP treatments were split into a well watered (ww) and water stressed (ws) treatments. The ws treatment consisted in omitting irrigation in 90 LP and 90 HP tubes, from 57 DAS to 74 DAS. This gave a 17 days period of terminal stress consistent with similar situation in the field. However, the transpiration measurements between 57 and 74 DAS allowed us to monitor carefully the stress intensity (by assessing the ratio of transpiration values under WS and WW conditions). As such, at 74 DAS the transpiration of WS plants fell below 30% of that in WW plants and it was decided to apply a 2L watering per cylinder, which was also the final one. The 180 ww tubes (90 LP and 90 HP) were kept regularly watered until maturity of pearl millet.

4.2.4 Harvest procedure and statistical analysis

The seeds used for sowing were previously stored in the genebank of ICRISAT Sahelian Centre and produced under optimal conditions. The size of sown and harvested seeds was measured as the weight (g) of 100 seeds. Growth parameters were measured weekly and included height, number of tillers and number of leaves. Booting and flowering time (d) were recorded for the main stem in each tube. Phosphorus concentration (mg g^{-1} DM) in plant tissues was measured colorimetrically on the main stem's flag leaf at the time of its appearance in Exp. 2. Plants were harvested at maturity at soil level starting from 83 DAS onwards, and all the material was sun dried to constant weight in cotton bags.

Stover yield was calculated as the sum of leaves and stem; the total dry weight (TDM) was the stover yield plus the spike weight and total yield was the stover yield plus grain yield.The panicle harvest index (PHI) was calculated by dividing the grain yield (g) by the total panicle biomass (g). In Exp. 2, water uptake in the pre- and post-anthesis period was calculated for each plant by summing transpiration values before and after flowering. Transpiration efficiency (TE) was then calculated as the ratio of the total biomass produced (grain and stover) per kg of water transpired (g kg^{-1} WU). In this study, root biomass was not measured, that is why TE assessments were based only on shoot dry matter and thus slightly underestimated. However, in earlier studies, we

found that omitting the roots was not likely to alter the genotypic ranking (Vadez *et al*., 2011a,b).

Data were statistically analyzed by one- and two-way ANOVA using R at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***). As regression analysis we conducted a simple linear regression to assess the relationship between our variables.

Residual yields as a tool for measuring tolerance to low P

In case of lack of genotype-by-treatment interaction (GxTrt) for yield components, yield performance under LP could not be attributed to the P tolerance of genotypes alone, but to a grain or stover yield potential component plus a residual yield variation. This residual would then account for tolerance to low P *per se* plus an error component, and represent the part of variation in yield under LP that is not explained by grain or stover yield potential (Vadez *et al*., 2008, Bidinger *et al*., 1987). In this study these residuals were calculated as the difference between the observed yield values under LP and the predicted ones from the linear regression model (LP *vs.* HP) and they were used as proxy for tolerance to low P.

4.3 Results

4.3.1 Low P (LP) effect on agronomic traits under well watered (ww) conditions

The total yield of LPww plants was 44 and 41% lower than that of HPww plants in Exp 1 and 2, respectively (Table 2 a, b). Grain yields of LPww plants reached 39 and 66% of grain yields of HPww plants in Exp. 1 and Exp. 2, respectively. The LPww treatment dramatically reduced seed size (100-seed weight) from 0.72 (HPww) to 0.49 g (LPww) in Exp. 1 (Table 2a), and from 0.61 g (HPww) to 0.50 g (LPww) in Exp.2 (Table 2b). The onset of flowering was delayed by two weeks in the LP treatment in Exp. 1, which was not the case in the Exp. 2 trial, when flowering varied only among genotypes but not between treatments (Table 2b). This could be due to a forced flowering under short days in this September to December trial. In general, all yield components (except for HI and panicle HI in Exp. 2) decreased under LPww, and the majority of these traits

differed also among genotypes within treatment (Table 2 a, b). None of the parameters showed any genotype-by-treatment interaction.

In Exp. 2, the grain yield varied from 4.13 to 13.85 g among genotypes under LPww (Table 3). This genetic variation was taken into account to select contrasting genotypes: 4 low P tolerant (T) with significantly higher grain yield under LPww and 3 low P sensitive (S) with lower grain yield under LPww (Table 3), although genotype number 14 was different from the tolerant group at $p<0.1$. These groups of “tolerant” and “sensitive” genotypes (as we will refer to in this paper) were meant to analyse possible plant attributes explaining the difference in performance under low soil P.

Flag leaf P concentration did not discriminate the tolerant from the sensitive group of genotypes. Genotypic differences in flag leaf P concentration were not due to a dilution effect as there was no correlation between the P concentration and dry weight. Under HPww treatment, the flag leaf P concentration was positively related to transpiration efficiency ($r=0.520$, $p<0.05$) and to grain yield ($r=0.642$, $p<0.01$, data not shown).

Table 2. Means and two-way ANOVA for agronomic traits in Experiment 1 conducted at ICRISAT Sahelian Centre (Sadoré) in 2010 under well watered conditions (a) and Experiment 2 in 2012 under well watered (b) and water stressed (c) conditions. Traits include days to boot or flower (days after sowing), weight of 100 seeds at harvest (g), grain and stover yield (g), total dry matter (g), total yield (g, sum of grain and stover yield), Harvest Index (ratio between grain yield and total yield), panicle Harvest Index (ration between grain yield and total panicle biomass) and P concentration measured in the flag leaf (mg g^{-1} dry matter). Means (±SE) are calculated under two P treatments across 15 pearl millet genotypes (P) and compared among genotypes within treatment (Geno). GxP represents genotype-by-P treatment interaction. *n.s.* not significant

a) Exp. 1 - Well watered

	Days to boot	Days to flower	100-seed weight	Grain yield	Stover yield	Total DM	Total yield	Harvest Index	Panicle HI
LP	76±1.5	79±1.5	0.49±0.03	15.9±1.2	73.3±2.7	106.1±2.8	88.2±2.4	0.1±0.01	0.4±0.03
HP	61±1.0	65±1.0	0.72±0.02	40.5±1.6	116.2±3.4	181.4±3.5	157.7±1.9	0.24±0.01	0.62±0.01
G	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	p<0.05	p<0.01	p<0.01	n.s.
P	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p=0.1	p<0.05
GxP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

b) Exp. 2 - Well watered

	Days to boot	Days to flower	100-seed weight	Grain yield	Stover yield	Total DM	Total yield	Harvest Index	Panicle HI	Flag leaf P conc
LPww	55±0.6	59±0.6	0.50±0.02	8.4±0.3	14.0±0.3	28.6±0.4	22.4±0.3	0.37±0.01	0.52±0.01	0.3±0.009
HPww	55±0.6	59±0.6	0.61±0.02	12.7±0.6	24.9±0.7	49.4±0.9	37.7±0.8	0.33±0.01	0.46±0.02	0.5±0.008
Geno	p<0.05	p<0.05	p<0.05	p<0.1	n.s.	n.s.	n.s.	p<0.001	p<0.001	n.s.
P	n.s.	n.s.	p<0.1	p<0.001	p<0.001	p<0.001	p<0.001	n.s.	n.s.	p<0.001
GxP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

c) Exp. 2 - Water stressed

	Days to boot	Days to flower	100-seed weight	Grain yield	Stover yield	Total DM	Total yield	Harvest Index	Panicle HI	Flag leaf P conc
LPws	54±0.6	58±0.6	0.36±0.03	5.7±0.3	13.5±0.4	24.9±0.3	19.2±0.3	0.30±0.01	0.44±0.02	0.3±0.004
HPws	53±0.5	58±0.5	0.26±0.02	4.9±0.2	18.7±0.4	31.0±0.4	23.1±0.5	0.21±0.01	0.28±0.01	0.5±0.016
Geno	p<0.001	p<0.01	p<0.05	p<0.01	n.s.	n.s.	n.s.	p<0.05	p<0.05	p<0.01
P	n.s.	n.s.	p<0.05	p<0.1	p<0.001	p<0.001	p<0.01	p<0.001	p<0.001	p<0.001
GxP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.01

4.3.2 Low P (LP) effect on agronomic traits under water stressed (ws) conditions

In Exp. 2, grain yield varied from 2.24 to 11.86 g (Table 3). Grain yields under LPws were higher (5.7 g) than under HPws (4.9 g; Table 2 b,c). This was in part because the drought stress effect in the larger HPws plants was more severe. Under ws conditions, seed size, HI and panicle HI were also significantly lower in HPws than in LPws, which could indeed be seen in the increased failure in seed set. We observed a large genotypic variation under LP. Among the tolerant genotypes identified under LPww conditions, only genotype 3 and 7 were also the higher yielding under LPws conditions (Table 3). Under these conditions of combined low soil P and water stress, the group of sensitive genotypes produced just 40% of the grain yield of tolerant ones (Table 3).

Flag leaf P concentration did not discriminate tolerant from sensitive lines in any of the four treatments. Phosphorus concentration in the flag leaf was calculated in Exp. 2 where it ranged from 0.3 mg P g DM^{-1} (LP) to 0.5 mg P g DM^{-1} (HP) being then lower in the LPws than in the HPws treatment (Table 3 and Table 4). Flag leaf P concentration was positively associated with transpiration efficiency ($r=0.521$, $p<0.05$) and with grain yield ($r=0.730$, $p<0.01$, data not shown) under HPws.

Table 3. Yield components and water uptake traits of 15 pearl millets genotypes tested under LPww and LPws in Experiment 2 conducted at ICRISAT Sahelian Centre (Sadoré) in 2012. *T* and *S* are tolerant and sensitive genotypes selected according to their contrasting performance across LPww and LPws. Water use refers to transpiration. Parameters include P concentration measured in the flag leaf (mg g^{-1} dry matter), grain yield (g), total dry matter (g), total WU (total water used during the trial), pre-anthesis WU (water used before flowering), post-anthesis WU (water used after flowering), TE (transpiration efficiency) and PHI (panicle harvest index). One-way ANOVA was used to determine least significant differences (LSDs) and differences between T and S genotypes. Two-way ANOVA was used to test differences among genotypes (Geno), between water treatments (W) and genotype-by-W treatment (GxW) interaction. Different levels of significance ($p<0.05$ *, $p<0.01$ **, $p<0.001$ ***) have been considered. n.s.: not significant

	Genotype	Type	Flag leaf P conc	Grain yield	Total DM	Total WU	Pre-anthesis WU	Post-anthesis WU	TE	PHI
			(mg g^{-1} DM)	(g)	(g)	(kg $plant^{-1}$)	(kg $plant^{-1}$)	(kg $plant^{-1}$)	(g kg^{-1} WU)	
LPww	1		0.24	8.15	24.77	17.42	8.61	8.81	1.40	0.61
	2	T	0.21	13.85	37.18	24.60	12.28	12.32	1.50	0.64
	3	T	0.29	11.42	27.70	17.02	4.95	12.07	1.62	0.67
	4		0.21	7.73	27.47	19.89	12.11	7.78	1.36	0.57
	5		0.30	6.69	22.00	16.50	8.27	8.23	1.31	0.55
	6	T	0.23	11.87	33.45	21.24	12.78	8.46	1.52	0.62
	7	T	0.26	11.18	26.06	16.31	5.15	11.16	1.57	0.70
	8		0.22	7.79	29.84	20.03	13.14	8.03	1.47	0.43
	9	S	0.22	4.13	25.86	21.16	14.93	6.23	1.22	0.35
	10	S	0.24	5.22	26.34	23.29	16.50	6.28	1.14	0.46
	11		0.19	8.11	29.55	21.13	11.93	9.19	1.38	0.57
	12		0.21	10.58	27.33	16.89	7.46	9.43	1.65	0.53
	13		0.23	4.33	24.79	19.81	12.19	7.62	1.24	0.42
	14	S	0.20	6.42	28.97	20.77	11.71	9.07	1.38	0.41
	15		0.20	7.90	28.17	19.86	11.34	8.51	1.41	0.48
	LSD		0.09*	5.8*	n.s.	n.s.	4.16**	n.s.	n.s.	0.22***
	Mean	**T**	**0.25**	**12.08**	**31.09**	**19.79**	**8.79**	**11.00**	**1.55**	**0.66**
	Mean	**S**	**0.22**	**5.26**	**27.06**	**21.74**	**14.38**	**7.19**	**1.25**	**0.38**
			n.s.	p<0.001	n.s.	n.s.	p<0.001	p<0.05	p<0.001	p<0.001
LPws	1		0.25	5.83	26.72	19.41	11.64	7.84	1.37	0.48

2	T	0.19	6.60	27.30	20.66	10.58	10.08	1.30	0.46
3	T	0.30	10.03	21.90	13.78	4.69	9.09	1.57	0.71
4		0.30	4.09	23.92	18.17	13.53	4.65	1.30	0.26
5		0.27	7.57	23.27	17.07	9.13	7.94	1.36	0.62
6	T	0.22	5.26	24.97	17.93	11.09	6.84	1.40	0.40
7	T	0.27	11.86	29.20	18.48	5.26	13.21	1.59	0.65
8		0.24	3.35	27.63	20.88	12.97	7.91	1.32	0.28
9	S	0.24	2.24	26.00	19.72	14.76	4.96	1.32	0.23
10	S	0.21	3.90	28.30	21.76	15.46	6.30	1.32	0.31
11		0.23	7.19	29.13	18.80	10.77	8.04	1.54	0.50
12		0.27	4.40	15.51	12.36	7.94	4.42	1.27	0.51
13		0.26	6.77	26.60	19.79	12.32	7.47	1.33	0.46
14	S	0.29	3.84	21.71	20.94	15.01	4.71	1.06	0.50
15		0.24	3.31	21.56	16.25	11.74	4.51	1.40	0.32
LSD		n.s	5.2*	n.s	n.s.	4.74**	4.11**	n.s	n.s
Mean	**T**	**0.24**	**8.44**	**25.84**	**17.71**	**7.90**	**9.81**	**1.46**	**0.55**
Mean	**S**	**0.25**	**3.33**	**25.34**	**20.81**	**15.08**	**5.32**	**1.23**	**0.29**
		n.s.	p<0.05	n.s.	n.s.	p<0.05	p<0.05	p<0.001	p<0.001
LPww		0.3±0.009	8.4±0.3	28.6±0.4	19.7±0.5	10.80±0.5	8.92±0.4	1.41±0.03	0.52±0.01
LPws		0.3±0.004	5.7±0.3	24.9±0.3	18.4±0.4	11±0.5	7.2±0.4	1.4±0.03	0.44±0.02
G		n.s.	p<0.05	n.s.	n.s.	p<0.05	p<0.05	p<0.1	p<0.05
W		p<0.1	p<0.001	p<0.005	p<0.05	n.s.	p<0.05	n.s.	p<0.05
GxW		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4. Yield components and water uptake traits of 15 pearl millets genotypes tested under HPww and HPws in Experiment 2 conducted at ICRISAT Sahelian Centre (Sadoré) in 2012. *T* and *S* are tolerant and sensitive genotypes selected according to their contrasting performance across LPww and LPws. Water use refers to transpiration. Parameters include P concentration measured in the flag leaf (mg g^{-1} dry matter), grain yield (g), total dry matter (g), total WU (total water used during the trial), pre-anthesis WU (water used before flowering), post-anthesis WU (water used after flowering), TE (transpiration efficiency) and PNHI (panicle harvest index). One-way ANOVA was used to determine least significant differences (LSDs) and differences between T and S genotypes. Two-way ANOVA was used to test differences among genotypes (Geno), between water treatments (W) and genotype-by-W treatment (GxW) interaction. Different levels of significance ($p<0.05$ *, $p<0.01$ **, $p<0.001$ ***) have been considered. n.s.: not significant

	Genotype	Type	Flag leaf P conc	Grain yield	Tot DM	Total WU	Pre-anthesis WU	Post-anthesis WU	TE	PNHI
			(mg g^{-1} DM)	(g)	(g)	(kg $plant^{-1}$)	(kg $plant^{-1}$)	(kg $plant^{-1}$)	(g kg^{-1} WU)	
HPww	1		0.65	23.38	59.92	27.04	12.09	14.95	2.12	0.69
	2	T	0.45	6.31	52.03	29.31	15.16	14.15	1.73	0.28
	3	T	0.53	15.07	38.05	19.90	7.32	12.57	1.92	0.64
	4		0.49	9.89	49.32	28.71	19.94	8.77	1.77	0.43
	5		0.59	19.46	50.24	25.39	11.48	13.91	1.91	0.72
	6	T	0.44	8.15	35.41	19.90	12.59	7.31	1.78	0.44
	7	T	0.50	17.16	52.76	26.92	8.66	18.26	1.85	0.50
	8		0.41	8.08	48.01	26.45	20.80	5.65	1.80	0.37
	9	S	0.51	4.93	38.09	21.89	15.93	5.96	1.82	0.23
	10	S	0.55	13.14	66.09	35.48	21.12	14.36	1.80	0.40
	11		0.68	13.53	45.98	24.58	11.55	13.03	1.86	0.50
	12		0.44	11.79	36.41	19.33	7.33	12.00	1.85	0.57
	13		0.52	18.90	59.41	29.81	17.06	12.75	1.97	0.59
	14	S	0.47	9.60	58.54	31.77	17.34	14.42	1.80	0.30
	15		0.54	11.64	51.18	29.60	16.49	13.11	1.66	0.38
	LSD		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Mean	**T**	**0.48**	**11.67**	**44.56**	**24.01**	**10.93**	**13.07**	**1.82**	**0.47**

	Mean	**S**	**0.51**	**9.23**	**54.24**	**29.71**	**18.13**	**11.58**	**1.80**	**0.31**
			n.s.	n.s.	n.s.	n.s.	p<0.001	n.s.	n.s.	n.s.
HPws	1		0.66	8.97	40.11	22.74	11.86	10.88	1.70	0.52
	2	T	0.62	6.48	32.78	20.14	11.08	9.07	1.61	0.32
	3	T	0.68	7.37	28.83	18.32	7.57	10.74	1.54	0.41
	4		0.49	2.31	31.08	19.72	15.36	4.36	1.49	0.14
	5		0.56	6.20	30.24	19.35	10.97	8.39	1.55	0.30
	6	T	0.48	3.12	30.43	19.25	12.28	6.96	1.58	0.18
	7	T	0.51	6.18	26.34	16.56	8.33	8.23	1.56	0.39
	8		0.57	3.27	31.99	20.19	12.72	7.47	1.59	0.22
	9	S	0.42	0.71	26.39	19.62	16.89	2.73	1.35	0.06
	10	S	0.64	5.04	38.29	23.34	13.46	9.88	1.64	0.32
	11		0.48	6.24	39.40	23.64	13.31	10.33	1.62	0.30
	12		0.56	4.36	28.29	18.98	11.05	7.93	1.47	0.33
	13		0.53	4.33	34.48	20.55	14.55	6.00	1.67	0.24
	14	S	0.42	N	32.36	21.07	14.67	5.64	1.53	N
	15		0.44	3.55	28.87	19.01	12.70	6.31	1.50	0.23
	LSD		0.22***	6.28**	n.s.	n.s.	7.21*	6.99*	n.s.	0.35*
	Mean	**T**	**0.57**	**5.79**	**29.59**	**18.57**	**9.82**	**8.75**	**1.57**	**0.32**
	Mean	**S**	**0.49**	**2.87**	**32.35**	**21.34**	**15.01**	**6.08**	**1.50**	**0.19**
			n.s.	p<0.05	n.s.	n.s.	p<0.001	n.s.	n.s.	p<0.05
	HPww		0.52±0.02	12.7±1.03	49.4±2.39	26.4±1.05	14.3±0.78	12.1±0.72	1.84±0.03	0.47±0.03
	HPws		0.54±0.02	4.5±0.54	32.0±1.34	20.2±0.58	12.5±0.48	7.7±0.5	1.56±0.03	0.28±0.27
	G		p<0.05	p=0.06	n.s.	n.s.	p<0.05	n.s.	n.s.	p<0.01
	W		n.s.	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	p<0.001	p<0.001
	GxW		p<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

4.3.3 Low P tolerance index and relationship to flowering delay under low P

A significant linear relationship was found between the grain yield in LP and HP treatments in Exp. 1 under ww conditions and in Exp. 2 under ws conditions (Figure 1a, c), and also between stover yield in LP and HP treatments in Exp. 1 and in Exp. 2 under both ws and ww conditions (Figure 1a,b,c) (Supplementary Table 1 for detailed flowering and booting data). As there was no genotype-by-treatment interaction (GxTrt) for these traits, the residuals calculated from the linear regression model between grain yield under LP and grain yield under HP (Figure 1a,b) were used as proxy for tolerance to low P. In Exp. 1 LPww and Exp. 2 LPws higher residuals were in fact related to increasing absolute grain yield values ($r=0.710$ and $r=0.759$ respectively, $p<0.001$). However, genotypes with highest residual grain yield did not correspond to those with the highest residual stover yield because both trials showed a clear negative association between seed and total biomass production ($r=0.862$, $p<0.001$ in Exp. 1 and $r=0.630$, $p<0.05$ in Exp. 2, data not shown). In relation to the residual grain yield in Exp. 2 water stressed (Figure 1c), because of the strong effect of the water stress in grain yield under HP conditions, these residual were likely to account for a combination of low soil P tolerance and drought tolerance, and not only low soil P tolerance as in Exp.1

In Exp. 1 delay in booting or flowering under LP was then negatively and highly significantly correlated with the residual grain yield (Figure 2). In Exp. 2, there was also a negative relationship between the delay in flowering under ws conditions, although this could have been related in part to late flowering entries having lower yield under stress (data not shown).

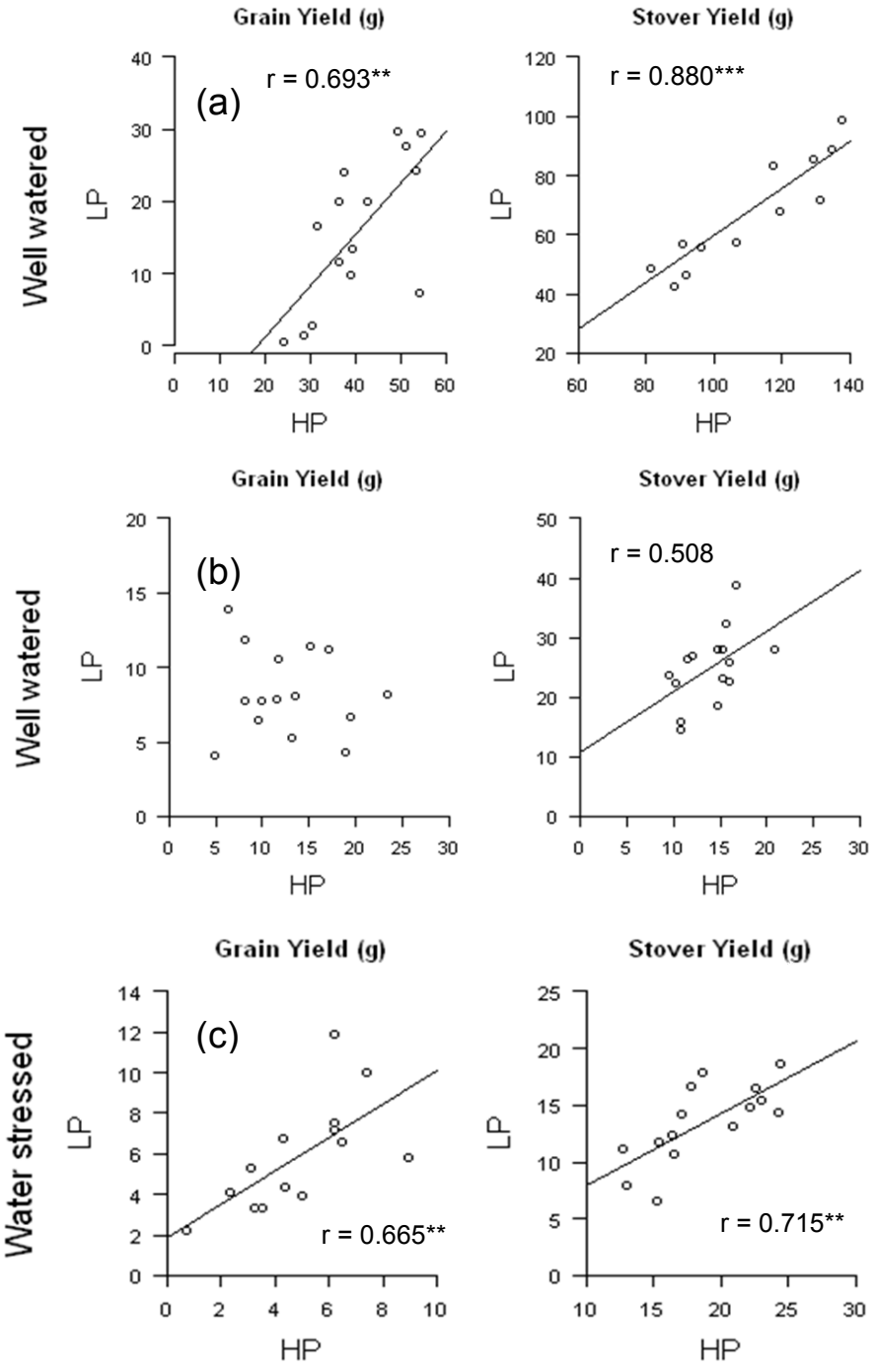

Figure 1. Relationship between yield under low P (LP) and high P (HP) in Experiment 1 conducted at ICRISAT Sahelian Centre (Sadoré) in 2010 under well watered (a) conditions and Experiment 2 in 2012 under well watered (b) and water stressed (c) conditions. The values are the means of 15 pearl millet genotypes across repetitions.

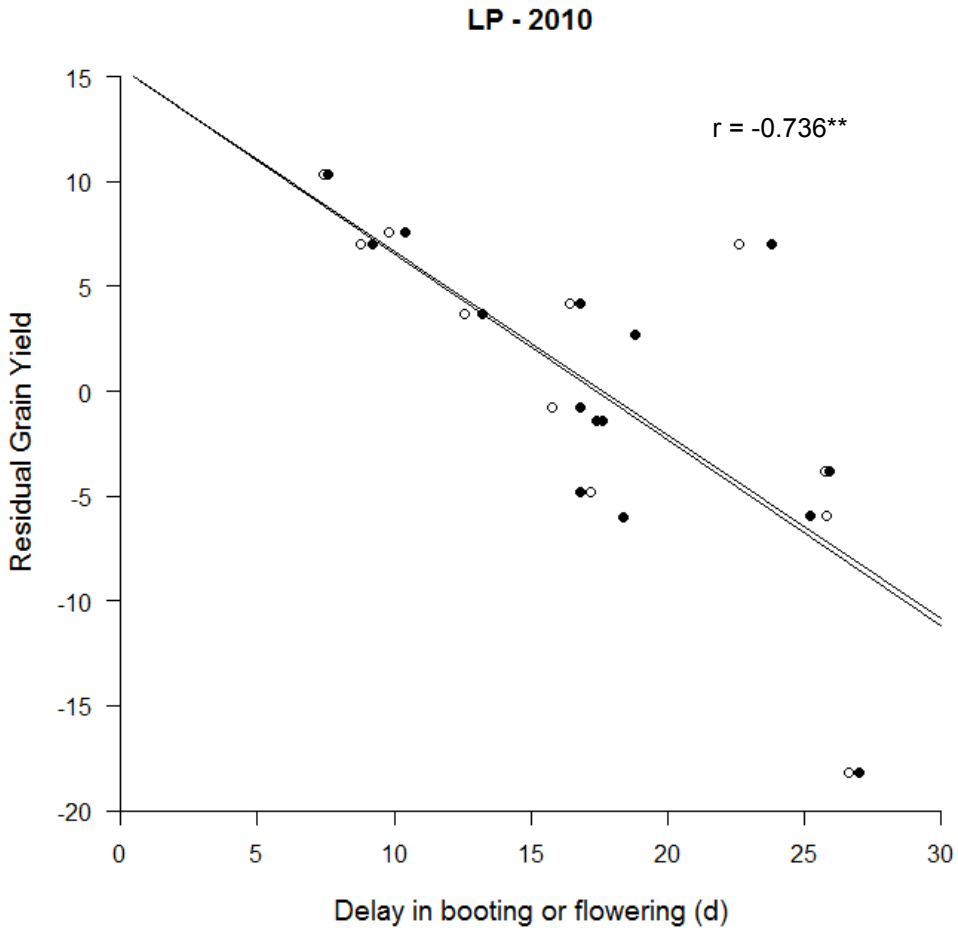

Figure 2. Relationship between residual grain yield and delay in 50% booting (*closed symbols*) or flowering (*open symbols*) for Exp. 1 in 2010. Residual grain yield is the difference between the observed value under LP and the predicted value by the regression line LP vs HP. High residuals are used as proxy for high plant tolerance to low P. Delay in booting or flowering was calculated as the difference between the number of days to boot or flower in LP and those in HP treatments.

4.3.4 Relationship between water uptake and yield components

Total water transpired, measured from 39 DAS to maturity (77 DAS), differed significantly among the four treatments and varied from 18.4 kg plant^{-1} in the LPws treatment to 26.4 kg plant^{-1} in the HPww treatment ($p<0.001$). The decrease in water transpiration after water stress imposition was more pronounced under HP than under

LP conditions because of the larger size of HP plants (Figure 3). Under both ww and ws conditions, the water use over time of sensitive lines was generally above that of tolerant lines, with greater differences under ww conditions (Figure 3). Despite this, the total water use did not differ significantly between tolerant and sensitive genotypes in any of the four treatments combinations (Table 3 & 4). Nevertheless, tolerant genotypes 3 and 7 that had the lowest plant water use across water treatment.

In fact, large differences were found between the tolerant and sensitive groups of genotypes in the water use before and after flowering (Figure 4). Under LPww conditions, sensitive genotypes used 14.4 kg water per plant pre-anthesis while tolerant genotypes transpired almost 40% less, thus leaving more water in the soil for use post-anthesis (Table 3). Under LPww conditions, the total water use of tolerant and sensitive groups was similar, although the post-anthesis water use value of the tolerant group was about 4L above that of the sensitive group. A similar situation occurred under LPws, where the tolerant group used significantly lower amount of water in the pre-anthesis period than the sensitive group and, reversely, the tolerant group used significantly higher amount of water in the post-anthesis period than the sensitive group (Table 3). Hence under both LPww and LPws, the pre- and post-anthesis water use differed between tolerant and sensitive genotypes (Table 3, Figure 4), although this could have been related to the later flowering of sensitive genotypes (Suppl. Table 1). During the post-anthesis period in Exp. 2, sensitive lines used 35% less water under LPww and 45% less water under LPws than in tolerant lines, which reflected the flowering time differences in the sensitive lines. Sensitive varieties indeed flowered 8 days later under HP conditions and then 10 days later under LP than tolerant ones (Table 3). Tolerant genotypes transpired less before flowering mainly because of earlier flowering under low P. This was especially the case for genotypes 3 and 7, which transpired the lowest amount of water of all genotypes at this stage (4.7 kg $plant^{-1}$ and 5.3 kg $plant^{-1}$, respectively) and transpired the most after flowering (9.1 kg $plant^{-1}$ and 13.2 kg $plant^{-1}$, Table 3). The water transpired post-anthesis was related to higher grain yield, HI and panicle HI in LP (Figure 5 and 6), further highlighting the importance of water availability for grain filling. The HP treatment did not change this observation:

tolerant genotypes still extracted less water in the pre-anthesis period than sensitive ones, but the HP treatment aggravated the effect of water stress in sensitive genotypes and led to a lower water extraction under water stress (20.2 kg) than under well watered conditions (26.4 kg). In contrast to LP, no genotypic variation in post-anthesis water use was observed under HP (Table 4).

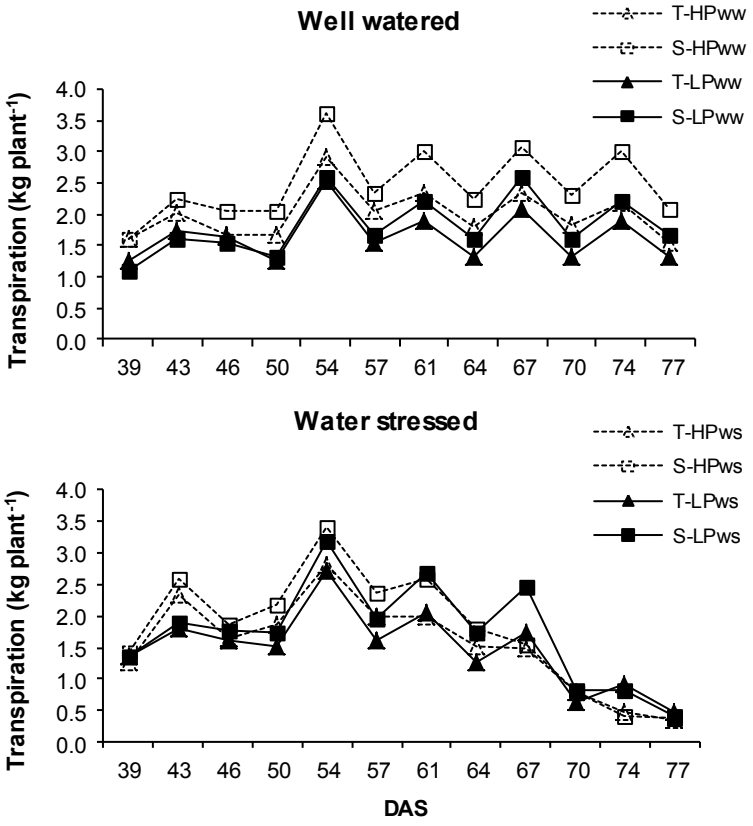

Figure 3. Water uptake over time (days after sowing, DAS) of pearl millet under well watered (above) and water stressed (below) conditions. Different lines represent LP and HP treatments of 4 low P tolerant (T) and 3 low P sensitive (S) genotypes. Irrigation was suspended in ws treatments at 56 DAS and restarted at 74 DAS.

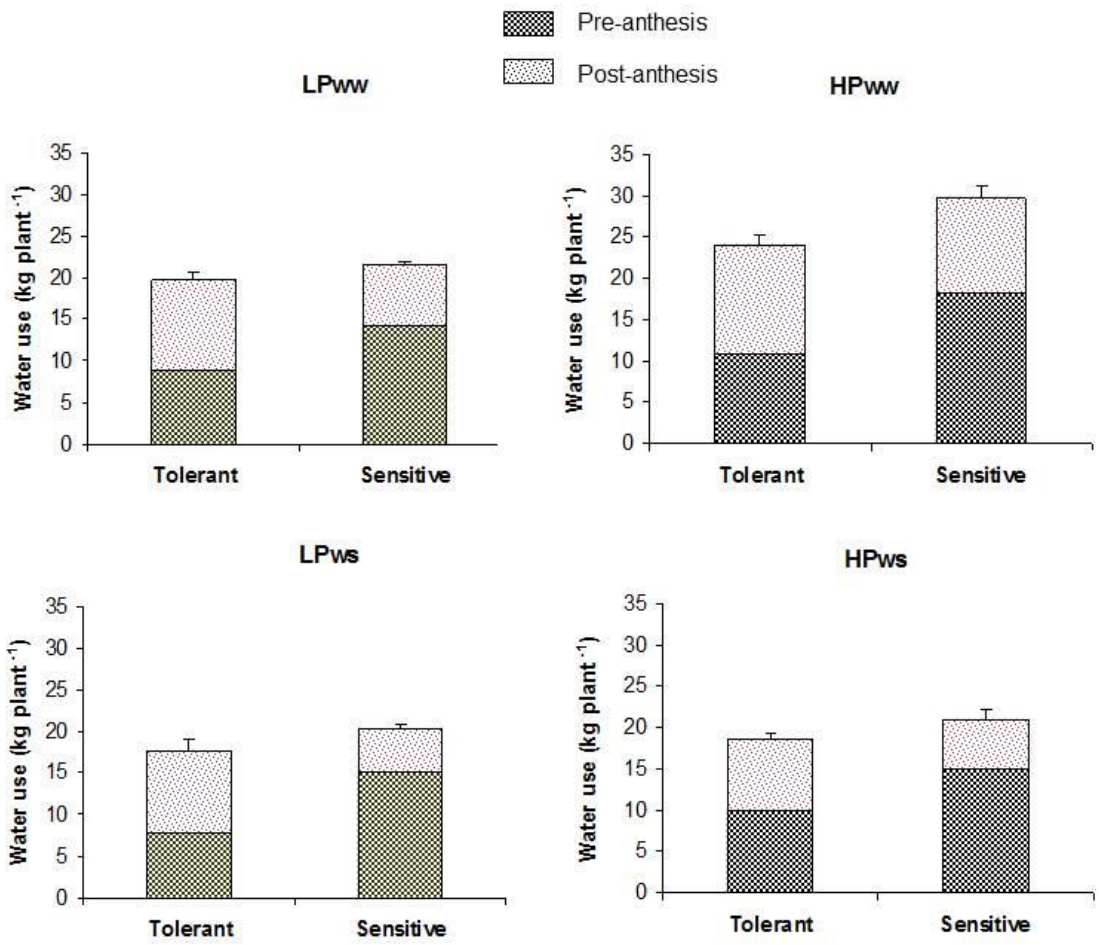

Figure 4. Water use in the pre-anthesis and post-anthesis period in Exp.2. Dat are the mean of the values for 4 tolerant and 3 sensitive pearl millet genotypes, selected from their yield difference contrast under LPww conditions. Plants were grown in lysimeters under ww (well watered, above) and ws (water stressed, below) treatments. No significant differences were observed when comparing total water use (pre- plus post-antithesis water use).

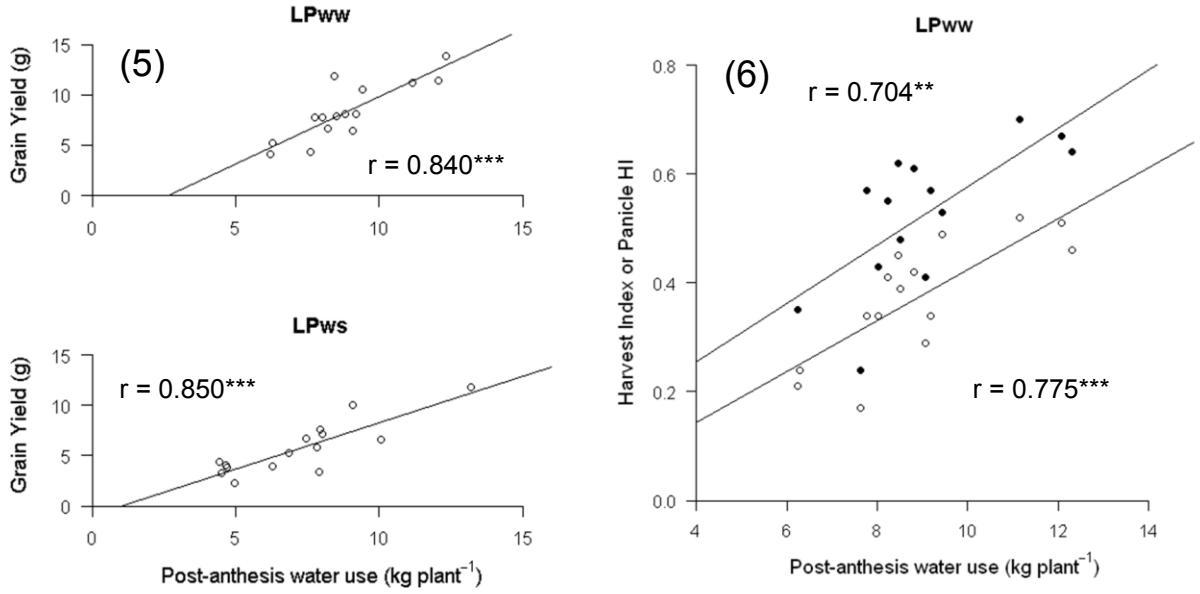

Figure 5. Relationship between grain yield and water use in the post-anthesis period. Data are the means of 15 pearl millet genotypes grown in lys ime ters under LPww (low P well watered) conditions (above) and under LPws (low P water stressed) conditions (below) in Exp. 2

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Figure 6. Relationship between water use in the post-anthesis period and the harvest index (HI) (*open symbols*) or panicle HI (*closed symbols*). Data are the means of 15 pearl millet genotypes grown in lysimeters under LPww (low P well watered) in Exp. 2.

4.3.5 Transpiration efficiency (TE)

Phosphorus supply increased TE. Transpiration efficiency values increased from LP conditions to HP, from 1.4 to 1.8 under ww conditions ($p<0.05$) and from 1.4 to 1.6 under ws conditions ($p<0.05$). Average TE of sensitive genotypes was significantly more affected by P deficiency (LP) than average TE of tolerant genotypes under both ww and ws conditions (Figure 7). A negative relationship was present between the ratio TE under HP/TE under LP and grain yield under ww conditions ($r=0.591$, $p<0.05$). This

indicated that a lower TE decrease in the LP treatment was related to a higher grain yield under LP. Under LPww and LPws, TE was positively correlated with grain yield, highlighting again genotypes 3 and 7 with highest TE and high yield (Figure 8, Table 3).

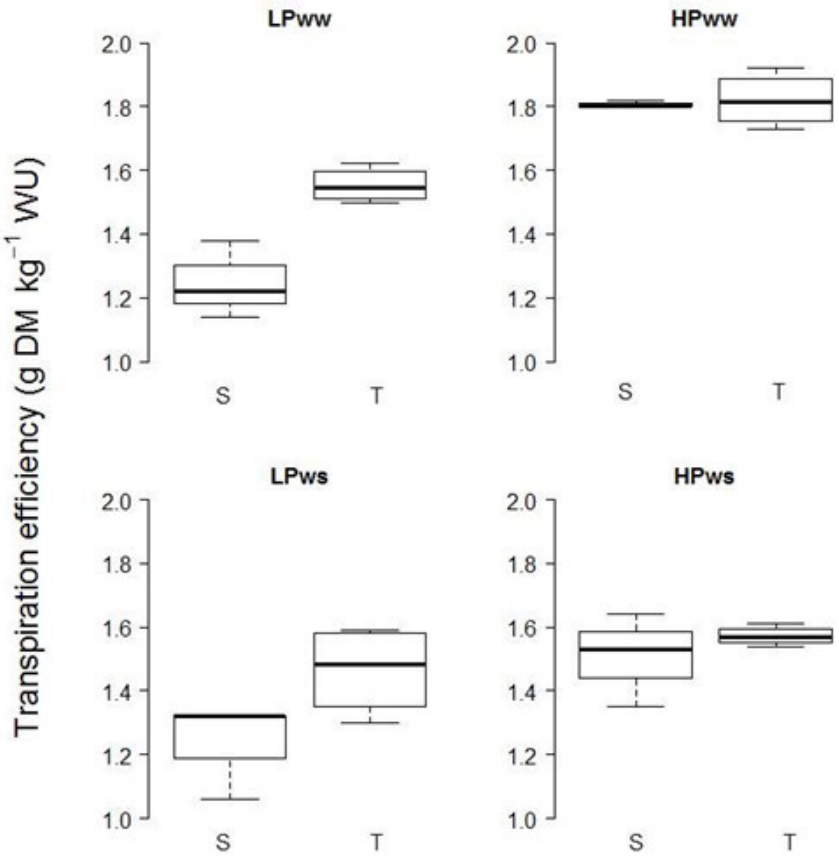

Figure 7. Transpiration efficiency of four tolerant (T) and three sensitive (S) pearl millet genotypes grown in lysimeters under four treatments with different availability of P (LP: low P and HP: high P) and water (ws: water stressed and ww: well watered) in Exp. 2.

4.3.6 Seed size

Grain yield was positively related to seed size in Exp. 1: LP ($r=0.840$, $p<0.001$) and HP ($r=0.781$, $p<0.001$); and in Exp. 2: LP ($r=0.835$, $p<0.001$) and HP ($r=0.767$,

$p<0.001$), across water treatments. Again, P played a major role in seed filling as P stressed plants produced smaller seeds. Moreover, the seeds size of water stressed HP plants (100 seed weight = 0.26 g) was half as large as that of well watered HP plants (100 seed weight = 0.61 g) (Table 2 b,c). At LPww, the 100-seed weight of genotypes 3 and 7 was twice as much as the average of the other genotypes. This did not happen under HPww, where the 100-seed weight of genotypes 12, 5 and 1 was higher or similar to that of genotypes 3 and 7, which indicate a specific effect of the low P treatment on the filling of the seeds. Under combined stresses (LPws) TE was positively related to seed size, i.e. high TE genotypes were able to better fill the grains ($r=0.680$, $p<0.05$, data not shown). This relationship was less clear in the other treatments.

The ratio between the size of the seeds that were sown and the size of harvested seeds under LPww revealed a 28-fold variation among the 15 genotypes. Moreover, this ratio was strongly and negatively associated with grain yield under LPww ($r=0.839$, $p<0.001$, Figure 9), indicating that sensitivity to LP treatment was related to an important decrease in seed filling of the low yielding genotypes.

In summary, the low P treatment decreased the size of the seeds, especially in sensitive genotypes. The water stress treatment further decreased the seed size, and more so in the HP treatment where the water stress had a more severe effect.

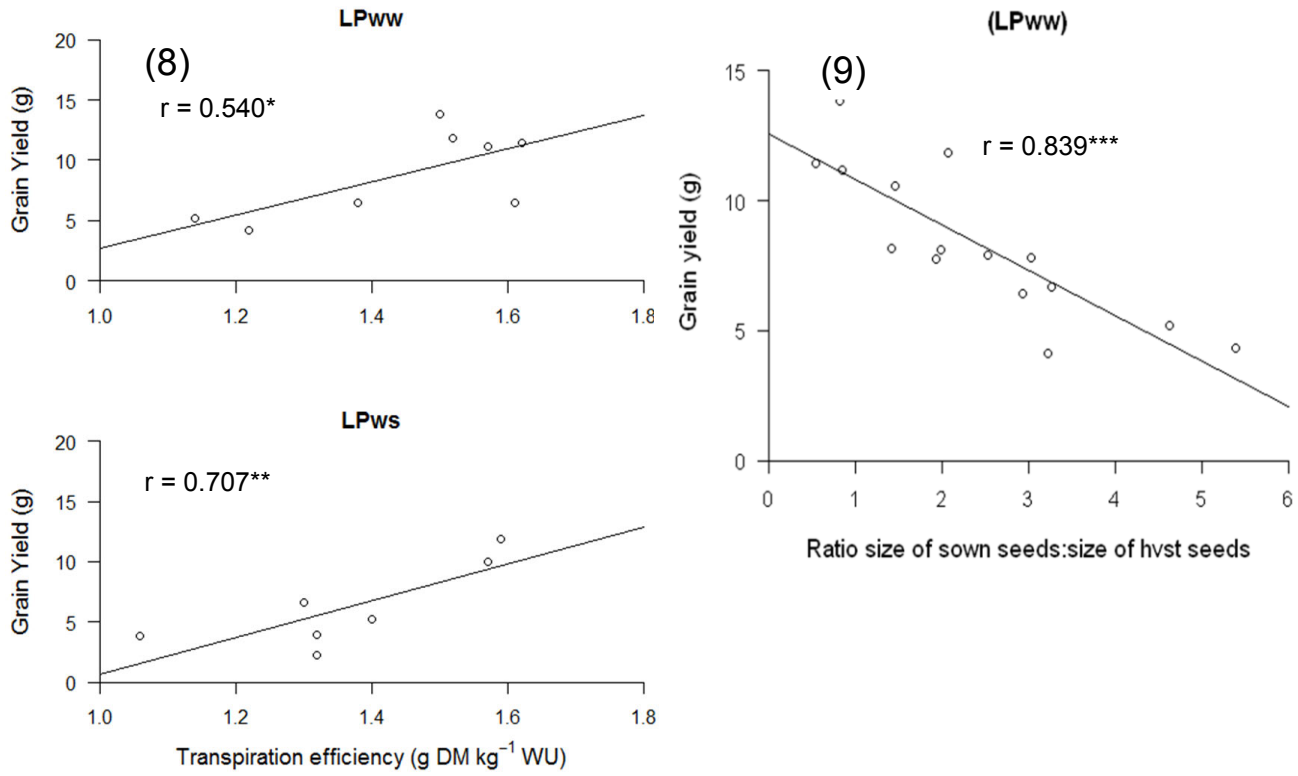

Figure 8. Relationship between grain yield and transpiration efficiency of 7 pearl millet genotypes (4 tolerant and 3 sensitive) grown in lysimeters under LPww (low P well watered) and LPws (low P water stressed) conditions in Exp. 2.

Figure 9. Relationship between grain yield and the ratio between the size of seeds that were sown and harvested under LPww (low P well watered) conditions. Data are the means of 15 pearl millet genotypes grown in lysimeters in Exp. 2.

4.4 Discussion

4.4.1 Agronomic aspects

As expected, P deficiency had a deleterious effect on plant growth and biomass production, which ultimately affected seed size at harvest. Smaller seed size means that fewer reserves are stored for plant establishment and less food is produced since grain

yield was positively correlated with seed size in our trials. The LP treatment effect during seed filling overrode the effect of the size of planted seeds, i.e. at a given seed size at sowing the most tolerant genotypes were those that maintained a seed size closer to the one in the HP treatment (genotypes 3 and 7; Figure 9). This in itself could be taken as a simple proxy for selecting tolerant and sensitive genotypes, and this proxy would be independent of the water stress effect since the relationship of Figure 9 takes place under WW conditions.

The positive effect of P supply on grain yield was only evident under ww conditions. Under water stressed conditions, P fertilized plants were larger and they would have run short of water quicker, being therefore more exposed to terminal water stress. In this way their final seed/biomass production did not differ from LPws plants. The lysimeter system we used magnified an effect that might have been different under field conditions, where larger plant spacing would have made more soil available to the plants to explore for water. Water was identified as the key factor for the grain production, because at both P levels the watering regime explained the largest difference in grain yield. In contrast, the P treatment was the most important determinant of vegetative biomass production (final dry matter and total yield).

Interestingly, genotypes 2, 3 and 7 confirmed the outcome of the pot trial that was run to identify low P sensitivity or tolerance and from which these lines were originally selected before being planted in lysimeters. In particular, the genotype 3 had one of the highest P efficiency (43.2%, i.e. ratio between vegetative biomass produced under low P and biomass produced under high P) (unpublished data). Previous work indicated that the tolerant genotypes had different strategies to cope with low P: genotypes 3 and 7 had significantly longer roots than genotype 2 at 5 WAS (Beggi *et al.*, unpublished data). Genotype 3 accumulated three times more P than lines 2 or 7, showing a peak in arbuscular mycorrhizal (AM) colonization at 4 WAS, whereas line 2 did not show any important infection of AM (unpublished data). The sensitive varieties used in this study also had a low P efficiency when grown in pots, as well as variety 6 which was here selected as tolerant.

4.4.2 Flowering delay

Severe water deficit during the period of panicle development or P deficiency are known to delay flowering in pearl millet (Mahalakshmi and Bidinger, 1985; Karanam and Vadez, 2010). Phenological delay has often been reported as an adaptive response of annual plants to P-deficiency because it increases the duration of nutrient uptake (Nord and Lynch, 2008). This, however, assumes there is no water limitation later in the growing season. For pearl millet grown under Sahelian conditions, delayed flowering would indeed increase the risk of the plant encountering water deficient conditions during grain filling at the end of the rains and of grain filling occuring solely under residual moisture. That is why it is generally accepted that annual plants flower and mature earlier to avoid late-season water stress (Thies *et al*., 1995; Dorn *et al*., 2000; Gungula *et al*., 2003). In contrast to previous findings, the data from our first trial show that genotypes sensitive to low P had a larger delay in flowering under LP. This indicates that a delay in booting or flowering decreased pearl millet's tolerance to low P conditions and the greatest reproductive success was observed in pearl millet genotypes that flowered earlier under P deficiency. This could not be tested adequately in Experiment 2 that was carried out under short days and would require additional research on a potentially quite important result.

4.4.3 Water use kinetics

Varieties tolerant to low P – i.e. having higher yields under LPww- used the same total amount of water during the cropping cycle as low P sensitive ones, which yielded less. Thus the total water extraction capacity of the root system did not seem to be responsible for the tolerance differences, which is in agreement with a similar study in chickpea (Zaman-Allah *et al.*, 2011). Rather, what differed between tolerant and sensitive varieties in our experiment was the pre- and post-anthesis pattern of water use. Tolerant varieties had higher post-anthesis water use while sensitive ones transpired more water during vegetative stage, leaving less water available for the grain filling stage. As tolerant genotypes flowered earlier, the relative shorter duration of the low P tolerant than the low P sensitive genotypes could have explained in part a

stronger effect of the water stress in the sensitive genotypes. However, the second experiment, set in short days, did not allow us to confirm the results from Exp.1 of a delayed flowering being related to lower yield under low soil P (Fig.1a). This confirms very recent findings in pearl millet hybrids, of a strong positive relationship between soil available water under water stress and grain yield (Vadez *et al*., 2013). However, more research would be needed to assess whether low soil P can aggravate this by delaying flowering further in low P sensitive genotypes. Previous results stress the decisive role of water availability during grain filling (Merah, 2001; Kato *et al*., 2008). We observed this trend in both low P and high P treatments, indicating no major role of P in the trend, although the delay in booting under low soil P in sensitive material would simply exacerbate that trend. Differences between pre- and post-anthesis water extraction were independent of water availability, suggesting that a constitutive mechanism (not activated by stress) giving an advantage in terms of stress avoidance might have played a role there, such as previously found for pearl millet in India (Kholova *et al*., 2010).

4.4.4 Transpiration efficiency

Improved water use efficiency represents one major avenue to enhance crop productivity under limited water supply. In our study, the application of P improved TE, thus confirming what was reported in literature (Payne *et al*., 2000) and what was hypothesized here. Different authors (Tanner and Sinclair, 1983; Gregory, 1989; Schmidhalter and Studer 1998) have concluded that TE is mostly affected by atmospheric evaporative demand and the CO_2 pathway, while TE was only affected by severe nutrient limitation, which is unfortunately the case for P under on-farm conditions of the West African Sahel. Payne and colleagues (1992) found similar results by growing pearl millet in pots, i.e. TE increased for increasing levels of P availability. Our findings showed that tolerant genotypes produced higher yields under combined P and water stress, had higher TE under low P conditions, while the total amount of water used was similar compared with sensitive genotypes. These results add to a recent review that argues that high TE can indeed be related to higher grain yield (Vadez et al.,

2014). More research would be needed to understand the mechanisms underlying the TE differences occuring between tolerant and sensitive genotypes under low soil P.

Both of our trials were conducted under high evaporative demand and it is known that there are genotypic differences in pearl millet for their transpiration response to high VPD (Kholova *et al*., 2010). At this stage we can only speculate how the low P conditions could have altered the way in which transpiration responds to high VPD. This deserves additional detailed work. Upon these considerations and according to the water use kinetics that we have seen, P also plays a role as it increases the amount of dry matter produced per unit water transpired, and so it could be that P amplifies the beneficial effect of water uptake at a key stage in plant development

4.5 Conclusions

Our results showed that tolerant pearl millet genotypes had more conservative water use (they transpired relatively more water during the post-anthesis period), their delay in flowering under low soil P was less, their seed size was less decreased under low soil P, and they had a lower TE decrease under low soil P than in sensitive genotypes. This leaves us with a basket of option for pre-screening of potentially interesting materials for low soil P conditions. In addition, it opens necessary research actions, especially on the need to understand / confirm a possible effect of low soil P on the delay in flowering (longer phyllochron? Increase in leaf number?), and on TE (differences in the response of transpiration to high VPD between low and high soil P conditions?). These questions are currently the object of additional studies.

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5 General discussion

5.1 Nutrient efficiency

Selection of genotypes with high nutrient efficiency is attracting increasing attention (White and Brown, 2010), but multiple definitions of nutrient efficiency are found in the literature (Fageria and Baligar, 2008), often creating ambiguity instead of enhancing a conceptual and in-depth understanding of the underlying mechanisms in plants (Marschner, 2011). In agronomic terms, nutrient efficiency needs to be used in the context of yield / biomass differences of crop genotypes (among or within species) growing in a soil with limiting amounts of nutrients (Graham, 1984; Marschner, 2011). Generally, high nutrient efficiency depends on root traits, but it may also be due to the transport from the roots to the shoot (Läuchli, 1976). Overall it is assumed that two components contribute to nutrient efficiency: *acquisition efficiency,* which reflects the absorbed nutrient and *use efficiency*, which represents the capacity of plants to "transform" the absorbed nutrients into biomass and yield. The relative importance of these two components depends mainly on nutrient availability. Generally, the most limiting the nutrient supply the more important becomes acquisition efficiency (Marshner, 2011). The main breeding efforts done until now focused on increasing P acquisition efficiency (PAE) rather than P use efficiency (PUE), even if improving crops that require fewer P inputs would help reducing the impact of grain crops in the P cycle (Rose and Wissuwa, 2012). Some of the reasons raised recently to explain the limited progress in exploitation of PUE are (i) ambiguous definition, (ii) inappropriate discussion of the level of P stress, and (iii) inadequate methods for screening genotypes (Rose and Wissuwa, 2012).

Two equations are commonly used to refer to PUE (Gourley *et al*., 1994; Vadez and Drevon, 2001): the ratio of biomass to the total content of P and the ratio of biomass to the concentration of P. The first ratio represents the reciprocal of P concentration in the biomass and tends to increase at very low P because P concentration tends to decrease. Thus under very limiting P soil conditions, like the Sahelian situation, the first formula risks to express a dilution effect of P rather than a real P use efficiency. The

second equation reflects the PUE used in our work and it is considered more reliable as it takes into account the nutrient concentration as well as plant growth (Siddiqui and Glass, 1981; Vadez and Drevon, 2001). Our research gave a first insight on the differences in P acquisition and relative biomass produced under different soil P conditions for a large set of varieties from Subsaharan West Africa, nevertheless PAE and PUE are closely related in traditional screening studies (Rose and Wissuwa, 2012). To avoid the confounding effect of PAE on PUE, millet plants could be grown in nutrient solution supplied with a specific low amount of soluble P to uniform PAE, as proposed by Rose and colleagues (2010). It is, however, important to consider that P efficient plants may store more P in the form of phytate in the seed which could have adverse effects on human mineral (Fe/Zn) nutrition, and this effect is amplified by P fertilizer application (Buerkert *et al*., 1998). A solution to this constraint and to the low availability of P in the Sahel (in the soil and in the form of fertilizer) could be the selection of millet plants able to access less available P fractions and translocate less P to seeds.

5.2 How to estimate the extent of AMF infection?

The assessment of percent root length colonized by AMF is a relatively inexpensive and technically undemanding methodology but it is not the only measure of AMF abundance (Vierheilig *et al*., 2005). Some scientists used percent root length colonized by arbuscules since they are primary sites of nutrient and C exchange between plant and fungus (Smith and Gianinazzi-Pearson, 1990; Ezawa *et al*., 2002; Smith and Read, 2008) but they are difficult to observe (Brundrett, 2009). However, Treseder (2013) reviewed the measurements of AMF abundance and stressed the importance of estimating root length and not just the percentage of infection, as we have done in our work for roots at 2 WAS. Extraradical hyphae are responsible for nutrient uptake far away from root surface, therefore standing hyphal length in soils is another common index of AMF biomass (Schweiger and Jakobsen, 2000; Hart and Reader, 2002). Other approaches used are quantitative PCR of AMF-specific DNA from roots or soil (Alkan *et al*., 2006; Gamper *et al*., 2008), which is relatively expensive, and measurement of phospholipids or neutral lipid fatty acids (Allison and Miller, 2005). They measure

biomass but not necessarily the function or activity of AMF. In a meta-analysis of agricultural systems, Lekberg and Koide (2005) observed that increases of percent root length colonized were related to higher crop yield, but the suitability of this parameter as indicator of AMF effectiveness is still a matter of debate (Smith and Read, 2008).

5.3 Trait interactions and trade-offs

Root strategies for P acquisition may be synergistic or antagonistic (Richardson *et al*., 2011). Better understanding the trait interactions and the eco-physiology of the rhizosphere is necessary in breeding programs to evaluate the potential and limitations of metabolical and ecological trade-offs associated with traits expression (George and Richardson, 2008). For instance, plants colonized by AMF are known to display altered root traits such as lower root hairs frequency and length, and less branching (Kothari *et al*., 1990; Hetrick, 1991), although the impact of this on the efforts to improve root systems is unknown (Richardson *et al*., 2011). We have seen that individual millet genotypes tolerant to low P had the highest AMF infection at different harvest times, therefore we hypothesized the existence of variety-specific nutrition strategies and the possible role of organic acids as alternative strategy to mycorrhization. In some plant species such as *Lupinus albus, Brassica napus* and *Cicer aretinum*, a common strategy for mobilizing sparingly soluble P forms in the soil consists in fact in an abundant release of carboxylates into the rhizosphere (Zhang *et al*., 1997; Neumann and Römheld, 1999; Neumann and Römheld, 2001). It is conceivable that differences in patterns of carboxylate production exist among millet genotypes as previously found in cultivars of chickpea (Wouterlood *et al*., 2004), and that this explains some of the observed differences in mycorrhization timing. Organic anion and AM fungal strategies are high energy-requiring ways to acquire P (Richardson *et al*., 2011) and the assumption of a temporal trade-off would avoid the dilemma on carbon allocation. For many species, the tendency is to either have cluster roots with organic anion secretion or to be infected by AM fungi, but a limited number of species displays both P-efficiency traits (Lambers *et al*., 2006). How single traits interact in a plant is not yet understood and our work opens an intriguing arena of research.

One more trade-off which deserves attention in the Sahelian conditions is between topsoil foraging of crops under P-deficiency and deep roots profusion under water stress. Some native plant species adapted to environments characterized by both stresses have evolved dimorphic root systems (Jeshke and Pate 1995; Schane *et al*., 2005), but no information is available for pearl millet. Our work did not allow to analyse root partitioning between top- and bulk-soil in the lysimeters, but similar yield performance of genotypes tolerant to low P between well-watered and water stress conditions leave the hypothesis open. Available evidence suggests that mycorrhizal diversity and symbiosis do not only positively contribute to nutrient use efficiency, but also to water uptake and use efficiency (Brussaard *et al*., 2007). This was confirmed by Boomsma and Vyn (2008) who reported encouraging results regarding the improvement of maize drought tolerance through promotion of AM symbiosis.

5.4 Management of indigenous versus exotic AMF

AM fungi play a pivotal role as an ecosystem service provider (Gianinazzi *et al*., 2010). The development and management of self-sustaining agro-ecosystems where soil stability and fertility are enhanced needs to take into account the factors responsible for the maintenance of AMF (Cardoso and Kuyper, 2006). Bielders and colleagues (2010) showed how indigenous AMF present in poor P soil from ICRISAT research station in Sadore, Niger, maintained their potential to contribute to millet P nutrition, irrespective of the soil degradation status. Studies on pearl millet showed that the use of exotic strains of AMF does not produce a time-efficient answer from the plant (Plenchette *et al*., 2005). Our experience with artificial inoculation of AMF species which are potentially present in Niger, *Glomus mossae* and *G. intraradices,* both in the greenhouse in Germany and in Niger suggested that in a soil stored for more than 15 years colonization of *G. intraradices* increased millet early dry matter and root length density, but no clear benefit was evident when fresh soil was used. Previous experiments showed that the trend was mainly dependent on the genotype used (unpublished data). Inoculation of crops with introduced AMF strains is an alternative to

the management of indigenous AMF for example when the local AM fungal populations are relatively ineffective or have been damaged by fungicides (Plenchette *et al*., 2005).

Generally, to predict the success of introduced AMF, it is important to determine whether AM fungi are limiting to processes in agricultural systems (Verbruggen *et al*., 2013). Limitation can be in terms of abundance and / or diversity. Abundance of AMF has been found to be negatively associated with intensive agricultural production (Smith and Read, 2008). Tillage practices, high nutrient inputs (particularly P) and frequent fallow periods are all predicted to negatively impact the survival of AM fungal propagules, such as spores and infective mycelium (Kabir, 2005). If stable and diversified communities of AMF are present, inoculation is not predicted to be successful (Verbruggen *et al*., 2013). The presented conditions indicate that maintaining native AMF is a better option for the cereal-based cropping systems of West African Sahel. The low AMF colonization percentages that we have found may be due to the typically low mycorrhizal symbiosis of Monocotyledons (Plenchette, in person), to the modern breeding approach which is often conducted under high P, and to long fallow period (15 years without cultivation in the case of the soil used in our work). Proper agronomical practices such as cereal / legume rotation, reduced soil disturbance, agroforesty maintain a good functioning of the symbiosis and avoid the problems and costs of large-scale inoculum production, which in the long term is not feasible for Sahelian farmers.

5.5 Transpiration efficiency

Having crops capable to produce more food for less amount of water used has never been as important as it is nowadays, especially in the arid and semi-arid regions. Transpiration efficiency (TE) is an important component of water use efficiency (WUE = grain yield produced per water received on a plot) and it consists of the water productivity evaluated at a plant level, i.e. biomass produced per water transpired. The measurements of TE have relied mostly on surrogate traits (Carbon-Isotope Discrimination, Specific Leaf Area measurement, Soil plant Analysis Development chlorophyll meter readings) because TE is difficult to measure, in particular under field

conditions (Vadez *et al*., 2014). The lysimeter approach used in the present research work allows the monitoring of plant water use and biomass production from very early plant stages until maturity, and it allows very robust and accurate gravimetric measurements of TE (Vadez *et al*., 2008, 2011a, 2011b). This system is versatile and suits many crops, such as chickpea, sorghum, maize, cowpea, and groundnut. Main disadvantages are that (i) sowing pockets are 4-5 times closer than in the traditional millet sowing density (one pocket ha^{-1}), (ii) this approach does not allow to mimick root to root competition, and (iii) the height of the tubes (1 m) risks to underrepresent the effective extension of millet roots, which can reach 2 m.

Passiura equation, quite popular among breeders indicates that ‘‘water uptake (WU), water use efficiency (WUE), and harvest index (HI) are drivers of yield’’, i.e. Yield = WU x WUE x HI (Passiura, 1977). According to Blum (2009), WU (transpiration, T) and HI are determining final yield but T alone is indeed the main driver of yield under drought stress, as HI (in terms of assimilate partitioning and reproductive success under drought) is also largely influenced by transpiration and plant water status. It has been discussed that selection for high TE might also select plants with low yield and, hence according to the TE formula, very low T (WU term of the equation; Condon *et al*., 2002; Blum, 2009). On the contrary, our work clearly showed how closely yield iscorrelated to TE but not to the T term, demonstrating that high TE is not necessarily related to low water use (Vadez *et al*., 2011a, 2011b). What is new to the Passiura equation is that the effect of T is not linear during the cropping cycle in pearl millet, as seen in our work as well as in India (Vadez *et al*., 2013), and the same was reported for chickpea (*Cicer arietinum* L., Zaman-Allah *et al*., 2011) and groundnut (*Arachis hypogea* L., Ratnakumar *et al*., 2009).

5.6 References

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6 General conclusions

The combination of agronomic and physiological experiments allowed to unravel the available genotypic diversity for tolerance to low P and water stress of West African pearl millet varieties and enhanced the understanding of a few related mechanisms.

Phosphorus efficiency at early growth presented a large variation in the set of 102 pearl millet genotypes even if values were all below 50% due to the extreme low P conditions. This variation indicated an important point for breeding purposes, as the ability of millet seedlings to overcome P stress is crucial in the unfavourable Sahelian conditions. The excellent correlation between NIRS analysis used to measure plant P concentration and standard chemical wet analysis strengthened the opportunity of using the cheap and time saving NIRS technique for further studies on millet. Phosphorus concentration in the shoot and height at 4 weeks after sowing (WAS) under unfertilized conditions, but not P concentration in the seeds, were shown to be early reliable indicators of P efficiency. Phosphorus acquisition efficiency (PAE) and P use efficiency (PUE) presented higher variation at low P (LP) being P availability more heterogeneous as compared to high P (HP).

The millet varieties selected out of the original collection for their high tolerance to low P presented longer roots infected with arbuscular mycorrhizal fungi (AMF) as early as at 2 WAS and better plant growth as compared to sensitive varieties. Our data showed that precisely at two, four, six and eight WAS the tolerant varieties had higher AMF infection and, interestingly, individual tolerant genotypes had the highest AMF infection at different harvest times. The mechanism of tolerance could thus rely on the capacity of tolerant plants to interact earlier with AM fungi and/or to specifically start the symbiosis with fast colonizing AM fungi (such as Glomaceae). Despite the well-known effect of roots surface for better P uptake, in this study it was not possible to identify differences in root length among the genotypes. Based on the results, it is advisable to substitute the root washing procedure with a technique which avoids roots manipulation, such as the installation of a transparent surface along the soil profile.

The tolerant varieties to LP presented higher transpiration efficiency and lower flowering delay than sensitive ones when they were grown in lysimeters. Phosphorus deficiency decreased millet transpiration efficiency and this effect was more severe for sensitive varieties to LP. Lack of P affected grain yield - by affecting seed size at harvest - and its damage during seed filling overrode the effect of seed size at sowing. At LP, the greatest reproductive success was observed in genotypes flowering earlier, particularly under terminal drought conditions. Anyhow, delay in booting or flowering decreased millet´s tolerance to LP conditions regardless of water regime. The varieties tolerant to LP showed a pattern of water use pre- and post-anthesis different from sensitive varieties, whereas they used similar amount of water. This trend was mainly independent from P and water availability, suggesting that a mechanism responsible for stress avoidance could be already present in the plant.

In this research work, a few pearl millet varieties were indentified including the two landraces PE05387 and Madougou5 from Mali, which performed better than all the others under low P and terminal drought conditions. However, in the long term, selection of multi-stress tolerant varieties from multi-location field trials should integrate crop management techniques in order to enhance resource use efficiency of smallholder Sahelian farmers and contribute to ensuring food security. To meet the challenge of food production with decreasing P resources and climate change, it is advisable to keep a focus on sustainable agriculture with participatory research methodologies, innovative phosphorus research, knowledge transfer and education.

7 Supplementary materials

Supplementary Table 1. Shoot dry matter production under contrasting soil conditions (LP: no P supply, HP: supply of 0.4 g P pot^{-1} at sowing) and P efficiency of 102 pearl millet genotypes from West Africa and grown on a P-poor acid Arenosol in pots for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger. Phosphorus efficiency is the ratio of LP to HP dry matter. Genotypes are divided into landraces (L) and varieties selected by breeders (B); they are ranked according to P efficiency

Genotype ID	Full name	Group	Shoot dry matter		P efficiency
			LP	HP	
			(g pot^{-1})		(%)
56	PE08030("SounaMau")	L	14.6	30.5	47.9
91	PE03012	L	16.2	34.0	47.6
102	Local_check_2	L	16.3	34.5	47.1
22	PE00576	L	13.4	28.6	46.9
62	PE02898	L	13.3	29.1	45.7
76	Strigares_expvar_ep_court	B	12.5	28.3	44.2
78	StrigaRes_2009_Sad_Cinz_comb	B	14.9	34.2	43.5
26	PE05387	L	12.7	29.4	43.2
79	Doga_C2_PF_comb	B	13.3	31.0	42.9
75	KBH_Dwarf	B	12.6	29.7	42.5
82	Serkin_C2_Kandela_SMS	L	12.4	29.2	42.5
59	PE02769	L	11.9	29.0	40.9
45	CZ_Syn00_06	B	12.0	29.6	40.5
6	Kapelga_Check_BF	L	12.2	30.4	40.2
31	Djiguifa	L	12.3	30.8	40.0
9	PE00017	L	10.2	25.7	39.7
87	F8xM1_C2_15FS_SAD_DM	B	11.2	28.3	39.6
88	KolalaxPE2987C1_Sad_DM_2009	B	12.6	32.0	39.4
40	CZ_NKK	B	9.7	25.4	38.2
25	PE05645	L	11.2	29.6	37.8
54	PE08057	L	11.6	31.5	36.8
39	CZ_Indiana_05	B	10.6	29.3	36.3
18	PE05607	L	8.3	23.0	36.1
5	PE05980	L	10.9	30.3	35.9
23	PE00456	L	10.2	28.8	35.4
1	PE00967	L	9.9	28.0	35.4
17	PE05660	L	9.2	26.2	35.1
3	PE05908	L	11.0	31.6	34.8
68	ICMVIS89305	B	11.1	32.0	34.8
96	F1xF8_C1_SAD_DM_2009	B	10.9	31.6	34.5
10	IP19415	B	9.3	27.1	34.3
29	PE00397	L	10.0	29.3	34.1
83	Tera_C2_PF_comb	L	9.4	27.6	34.1
74	KBH_Normal	B	10.5	30.9	34.0
49	SosatxToronio_C1_Sad_DM_2009	L	10.3	30.9	33.3

7	PE00077	L	9.1	27.5	33.2
27	PE11298	L	9.3	28.3	32.9
44	CZ_Syn00_02	B	8.7	26.5	32.8
20	PE05344	L	9.4	28.7	32.8
85	2898x92222_C1_Sad_Low_2009	B	9.0	27.5	32.7
66	PE02724	L	9.2	28.2	32.6
69	ICMVIS92222	B	11.4	35.0	32.6
84	M66xSosat_C1_Sad_DM_2009	B	10.7	33.1	32.3
30	PE05663	L	9.3	28.8	32.3
53	PE0353xKBHC1_Sad_DM_2009	B	8.8	27.4	32.1
60	PE00711	L	10.9	34.2	31.9
64	PE02770	L	10.8	34.0	31.8
67	Ankoutess	B	10.7	33.8	31.7
8	PE00012	L	10.2	32.4	31.5
63	PE02853	L	9.3	29.6	31.4
99	GBx92222_YLD_2009	B	10.7	34.1	31.4
93	ISMI9301	B	10.7	34.1	31.4
2	PE05887	L	8.8	28.1	31.3
80	FalC2_PF_Comb	B	10.3	33.2	31.0
16	PE05572	L	10.2	32.9	31.0
41	CZ_NKOxTC1	B	7.9	25.5	31.0
28	PE05665	L	9.3	30.1	30.9
61	PE02983	L	8.0	25.9	30.9
89	AON378	L	10.0	32.4	30.9
95	Souna_3_Check_Senegal	L	10.0	32.5	30.8
36	PE05578_C2	B	8.8	28.8	30.6
4	PE06001	L	10.1	33.1	30.5
72	BazagomeC2_SAD_DM_2009	B	10.4	34.1	30.5
13	PE00404	L	9.1	30.2	30.1
38	CZ_ICMV88102	B	8.7	28.9	30.1
24	PE05539	L	8.8	29.3	30.0
21	PE05346	L	9.6	32.0	30.0
50	F4xM1_C1_SAD_DM_2009	B	9.8	32.7	30.0
33	Intilène	L	8.3	27.7	30.0
51	5638x5344_C1_Sad_Low_2009	B	9.3	31.3	29.7
12	PE00437	L	8.4	28.4	29.6
43	CZ_Syn00_01	B	8.1	27.7	29.2
37	CZ_BoboniSanogola	B	8.0	27.4	29.2
97	P1xP2_C1_Sad_DM_2009	B	8.4	28.7	29.2
42	CZ_Sanioba_03	B	8.4	29.0	29.0
55	PE08011	L	9.0	31.1	28.9
48	437x92222_C1_Sad_Low_2009	B	10.2	35.4	28.8
47	CZ_Toroniou_C1_check_Mali	B	8.9	31.1	28.6
34	Madougou5	L	10.4	36.4	28.6
35	ICRI_Tabi	B	10.4	36.6	28.4
14	PE00253	L	9.1	32.3	28.2
19	PE05638	L	9.0	32.4	27.8
71	ICMVIS99001_Check_Niger	B	9.8	35.4	27.7
98	GBx89305_YLD_2009	B	10.1	36.5	27.7

46	CZ_Syn03_II	B	7.8	28.7	27.2
52	SOSAT_C88_Check_all	L	9.8	35.9	27.2
81	Serkin_C2_Ali_SMS2	L	9.7	35.8	27.1
57	PE08058	L	8.6	31.9	27.0
101	Local_check_1	L	9.1	34.3	26.5
32	Guifoué16	B	8.4	32.2	26.1
100	GBx99001_YLD_2009	B	8.3	31.7	26.1
11	PE05458	L	8.2	31.6	25.9
65	Strigares_expvar_ep_long_noir	L	8.4	32.7	25.6
77	ICMVIS94206	B	9.3	36.4	25.5
70	IBMV8402_Check_Senegal	B	8.5	33.7	25.2
92	PE02604	B	9.0	36.1	24.9
58	PE00320	L	10.0	40.8	24.5
15	F8xM10_C1_Sad_DM	L	8.7	35.5	24.5
86	PE02830	B	7.7	32.8	23.5
90	PE03089	L	8.1	37.2	21.8
94	ISMI9507	B	6.6	32.7	20.2
73	Bondabia_C1	B	5.8	32.7	17.6
	Mean		**10.04**	**31.08**	**32.5**
	SD (±)		1.82	3.16	6.12
	SE (±)		0.18	0.31	0.61

[1] SD: standard deviation; [2] SE: standard error

Supplementary Table 2. Shoot P content (total amount of P accumulated in leaves and stems, used as a measure of P acquisition efficiency, PAE) under low P (no P supply), P efficiency and P use efficiency of 102 pearl millet genotypes originated from West Africa and grown on a P-poor acid Arenosol in pots for 37 days at ICRISAT Sahelian Centre, Sadoré, Niger. Shoot P content (P acquisition efficiency, PAE) was calculated as shoot P concentration multiplied by shoot dry matter. P efficiency was calculated as ratio between dry matter produced under low P and dry matter produced under high P. P use efficiency (PUE) at LP is equal to the square of shoot dry matter divided by total P content in the plant (shoot and root) under low P conditions. Genotypes are divided into landraces (L) and varieties selected by breeders (B); they are ranked according to P efficiency

ID	Genotype - full name	Group	Shoot P content (mg pot^{-1})	P efficiency (%)	P use efficiency (g^2 mg P^{-1})
56	PE08030("SounaMau")	L	33.3	47.9	5.1
91	PE03012	L	46.8	47.6	3.6
102	Local_check_2	L	48.9	47.1	4.5
22	PE00576	L	29.8	46.9	4.6
62	PE02898	L	25.3	45.7	5.5
76	Strigares_expvar_ep_court	B	25.9	44.2	4.6
78	StrigaRes_2009_Sad_Cinz_comb	B	21.6	43.5	7.1
26	PE05387	L	46.0	43.2	2.9
79	Doga_C2_PF_comb	B	32.8	42.9	4.6
75	KBH_Dwarf	B	27.3	42.5	4.2
82	Serkin_C2_Kandela_SMS	L	18.2	42.5	5.8
59	PE02769	L	16.0	40.9	6.0
45	CZ_Syn00_06	B	26.5	40.5	3.9
6	Kapelga_Check_BF	L	21.2	40.2	4.7
31	Djiguifa	L	30.8	40.0	3.9
9	PE00017	L	28.5	39.7	3.0
87	F8xM1_C2_15FS_SAD_DM	B	20.5	39.6	4.2
88	KolalaxPE2987C1_Sad_DM_2009	B	40.1	39.4	3.2
40	CZ_NKK	B	14.9	38.2	4.2
25	PE05645	L	29.2	37.8	3.2
54	PE08057	L	24.7	36.8	4.4
39	CZ_Indiana_05	B	14.9	36.3	N
18	PE05607	L	12.4	36.1	3.8
5	PE05980	L	21.1	35.9	4.0
23	PE00456	L	21.1	35.4	3.4
1	PE00967	L	15.0	35.4	4.1
17	PE05660	L	23.6	35.1	3.0
3	PE05908	L	14.4	34.8	4.5
68	ICMVIS89305	B	29.6	34.8	3.4
96	F1xF8_C1_SAD_DM_2009	B	16.0	34.5	5.0
10	IP19415	B	16.6	34.3	3.7
29	PE00397	L	23.0	34.1	3.3
83	Tera_C2_PF_comb	L	18.2	34.1	3.3
74	KBH_Normal	B	19.3	34.0	4.2
49	SosatxToronio_C1_Sad_DM_2009	L	14.0	33.3	4.2
7	PE00077	L	21.9	33.2	2.9
27	PE11298	L	15.9	32.9	3.8
44	CZ_Syn00_02	B	17.9	32.8	3.1
20	PE05344	L	15.9	32.8	3.8
85	2898x92222_C1_Sad_Low_2009	B	14.8	32.7	3.8
66	PE02724	L	13.2	32.6	3.6
69	ICMVIS92222	B	17.0	32.6	5.3
84	M66xSosat_C1_Sad_DM_2009	B	38.4	32.3	2.4
30	PE05663	L	12.7	32.3	4.7
53	PE0353xKBHC1_Sad_DM_2009	B	20.3	32.1	2.8
60	PE00711	L	22.5	31.9	4.0

64	PE02770	L	20.6	31.8	4.2
67	Ankoutess	B	16.0	31.7	5.5
8	PE00012	L	18.8	31.5	3.7
63	PE02853	L	12.7	31.4	4.0
99	GBx92222_YLD_2009	B	14.6	31.4	5.5
93	ISMI9301	B	19.0	31.4	3.8
2	PE05887	L	11.0	31.3	4.1
80	FalC2_PF_Comb	B	10.1	31.0	5.9
16	PE05572	L	27.0	31.0	2.9
41	CZ_NKOxTC1	B	15.0	31.0	2.3
28	PE05665	L	16.4	30.9	3.5
61	PE02983	L	13.9	30.9	N
89	AON378	L	19.7	30.9	3.9
95	Souna_3_Check_Senegal	L	15.4	30.8	4.2
36	PE05578_C2	B	16.5	30.6	3.4
4	PE06001	L	15.0	30.5	3.8
72	BazagomeC2_SAD_DM_2009	B	11.9	30.5	5.4
13	PE00404	L	15.4	30.1	3.5
38	CZ_ICMV88102	B	14.0	30.1	3.5
24	PE05539	L	14.0	30.0	3.2
21	PE05346	L	13.2	30.0	4.2
50	F4xM1_C1_SAD_DM_2009	B	11.8	30.0	5.1
33	Intilène	L	12.7	30.0	2.9
51	5638x5344_C1_Sad_Low_2009	B	11.9	29.7	4.0
12	PE00437	L	12.1	29.6	3.5
43	CZ_Syn00_01	B	9.0	29.2	3.6
37	CZ_BoboniSanogola	B	11.4	29.2	2.8
97	P1xP2_C1_Sad_DM_2009	B	14.5	29.2	3.6
42	CZ_Sanioba_03	B	10.2	29.0	N
55	PE08011	L	19.8	28.9	3.2
48	437x92222_C1_Sad_Low_2009	B	14.0	28.8	5.1
47	CZ_Toroniou_C1_check_Mali	B	23.9	28.6	2.7
34	Madougou5	L	16.8	28.6	3.8
35	ICRI_Tabi	B	13.2	28.4	4.1
14	PE00253	L	12.1	28.2	4.2
19	PE05638	L	13.9	27.8	3.8
71	ICMVIS99001_Check_Niger	B	10.9	27.7	4.7
98	GBx89305_YLD_2009	B	13.7	27.7	N
46	CZ_Syn03_II	B	14.3	27.2	2.9
52	SOSAT_C88_Check_all	L	11.9	27.2	5.0
81	Serkin_C2_Ali_SMS2	L	21.8	27.1	3.1
57	PE08058	L	11.2	27.0	3.9
101	Local_check_1	L	11.7	26.5	6.4
32	Guifoué16	B	11.9	26.1	3.7
100	GBx99001_YLD_2009	B	22.7	26.1	2.3
11	PE05458	L	12.4	25.9	3.4
77	Strigares_expvar_ep_long_noir	B	16.9	25.5	3.7
70	ICMVIS94206	B	13.5	25.2	3.7
92	IBMV8402_Check_Senegal	B	14.3	24.9	3.7

58	PE02604	L	14.9	24.5	4.5
15	PE00320	L	12.1	24.5	3.5
86	F8xM10_C1_Sad_DM	B	9.6	23.5	3.3
65	PE02830	L	25.9	22.6	2.1
90	PE03089	L	15.7	21.8	3.3
94	ISMI9507	B	10.2	20.2	2.7
73	Bondabia_C1	B	6.6	17.6	2.3
	Mean		**18.6**	**32.5**	**3.9**
	SD (±)		8.2	6.2	0.9
	SE (±)		0.8	0.6	0.1
	Mean B		**17.3**	**31.5**	**3.9**
	SD (±) B		7.4	5.8	1.1
	SE (±) B		1.1	0.9	0.2
	Mean L		**19.7**	**33.3**	**3.9**
	SD (±) L		8.7	6.3	0.8
	SE (±) L		1.2	0.8	0.1

[1] N: missing value; [2] SD: standard deviation; [3] SE: standard error

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